# Assessment of plant growth promoting rhizobacteria for plant growth enhancement and biocontrol activity against *Fusarium pseudograminearum* on wheat

by

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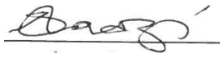
# DECLARATION

I, Renolda Ipeleng Mulaudzi declare that the thesis, which I hereby submit for the degree Master of Science at the University of Pretoria is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

SIGNITURE: 
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# SUMMARY

Plant growth promoting rhizobacteria (PGPR) are those bacteria that colonise the rhizosphere of various plants and promote growth either directly by improving nutrient uptake by the plant roots or indirectly through the control of pathogens. Due to the negative effects associated with the prolonged use of chemical fertilizers and fungicides, a lot of emphasis is now being given to research that investigates an alternative, sustainable and environmentally friendly method of crop production and protection. In the current study, a collection of rhizobacterial isolates from the University of Pretoria- Plant Growth Promoting Rhizobacteria (UP-PGPR) culture collection were screened for plant growth promotion and biocontrol activity against crown rot caused by *Fusarium pseudograminearum* on wheat (*Triticum aestivum*).

A seedling tray bioassay was utilised as a rapid small-scale method to screen the rhizobacterial isolates for biocontrol activity against wheat crown rot in the greenhouse. The same method was also used to screen the isolates for direct plant growth promotion of wheat. Of all the isolates (113) screened for wheat crown rot control, 52% (59 isolates) significantly increased the shoot dry weight of the seedlings, 41% (46 isolates) increased the root dry weight of the seedlings, and the total seedling dry weight was increased by 32% (36 isolates) of the isolates. A seedling bioassay was also used to screen the isolates for direct plant growth promotion of wheat. Of the 113 isolates screened, 12% (14 isolates) increased the shoot dry weight of the seedlings, 22% (25 isolates) increased the dry weight of the roots; while the total dry weight of the seedlings was increased by 32% (36 isolates) of the isolates.

Subsequent to the seedling bioassay in the greenhouse, the isolates were also assessed *in vitro* for selected traits associated with biocontrol activity and plant growth promotion. To test for a broad spectrum of biocontrol activity, in addition to *F. pseudograminearum,* the isolates were also screened for inhibition of *Rhizoctonia solani*, *Phytophthora capsici* and *Macrophomina phaseolina*. Almost 50% of the isolates displayed broad-spectrum activity against the pathogens on three different media. Some notable isolates in this regard were *Bacillus* sp. strain N54 and *Pseudomonas* sp. strain N59, N67 and N69. All isolates screened displayed multiple traits associated with biocontrol activity such as the production of antibiotic enzymes, volatiles ($NH_3$ and HCN) and the production of siderophores. The isolates also displayed multiple traits associated with direct plant growth promotion (nitrogen fixation, phosphate solubilization, IAA and ACC deaminase).

Based on the results obtained from the seedling bioassays in the greenhouse and the *in vitro* screening, a scoring system was developed, and the isolates were awarded points. *Bacillus* sp. strain A09AC, A17, A20, N02, N28, N54 *Stenotrophomonas* sp. strain A45, *Pseudomonas* sp. strain N04AC, N44 and N59A were selected for pot trials to confirm their *F. pseudograminearum* biocontrol efficacy (Figure 1.1). *Bacillus* sp. strain A10AC, *Stenotrophomonas* sp. strain A33, A43, A45, *Paenibacillus* sp. strain KBS1F3, *Pseudomonas* sp. strain N29, N69, N67, N76 and *Pantoea* sp. strain N34 were selected for use in pot trials in the greenhouse to confirm their efficacy as wheat growth promoters.

The selected isolates were further assessed for biocontrol activity and plant growth promotion in greenhouse experiments. KBS1F3 (*Paenibacillus alvei*) showed the best results for wheat growth promotion while A17 (*Bacillus cereus*) gave the best results for biocontrol activity. The effect of temperature, pH, NaCl and different carbon sources on the growth of the isolates was also assessed *in vitro*. The optimum temperature of all isolates was observed to be between 26$^{o}$C and 35$^{o}$C while KBS1F3 was able to grow at 47$^{o}$C and A17 at 50$^{o}$C. The growth of KBS1F3 decreased with an increase in NaCl concentration while A17 still grew well at 4% NaCl concentration. All isolates grew optimally at pH 7. KBS1F3 still grew well at pH 8 while A17 showed good growth at all pH values except pH 4. All isolates showed the ability to utilise a variety of carbon sources.

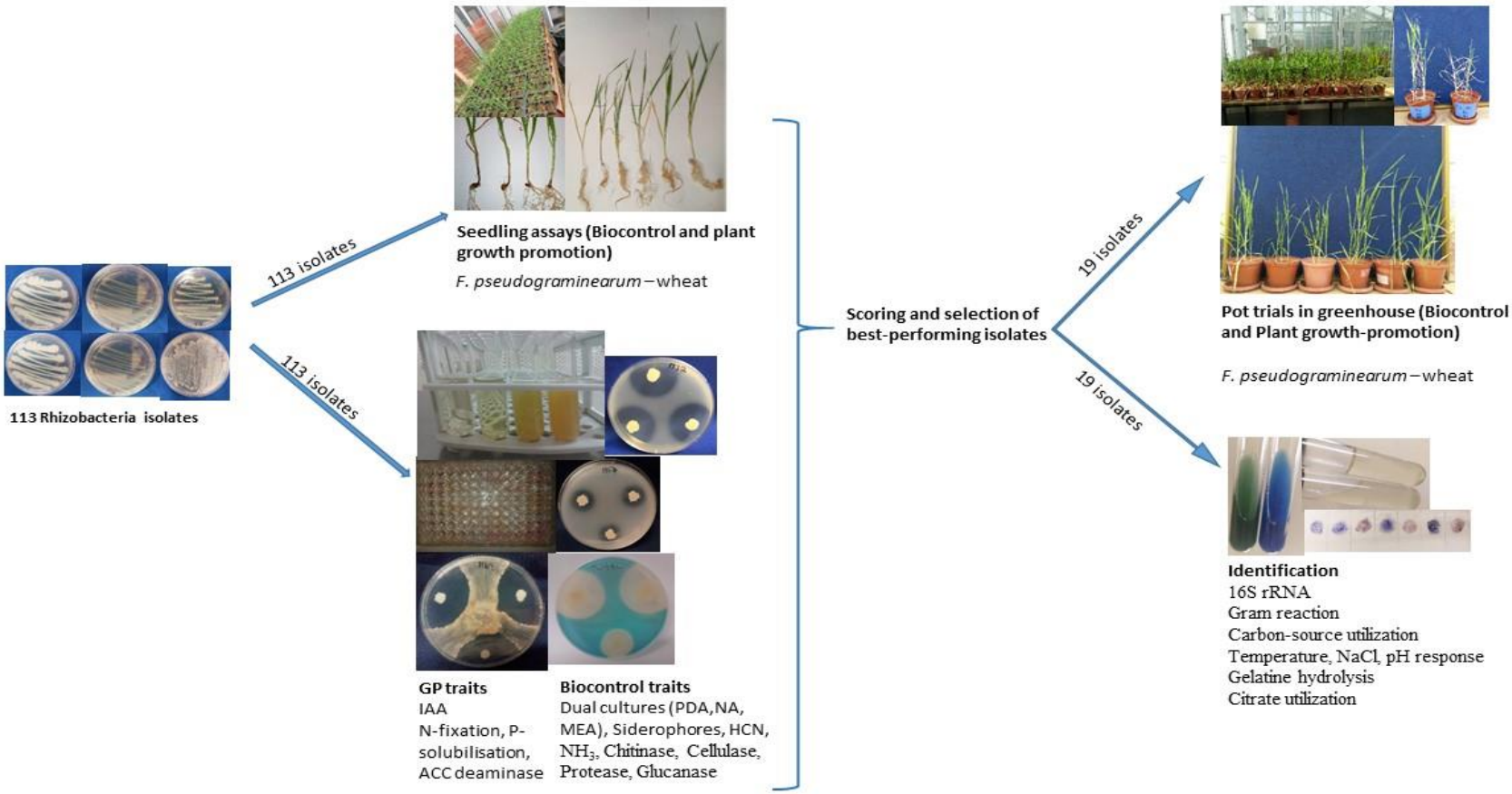


**Figure 1.1** Graphical summary of the study

Rhizobacterial isolates were screened both *in vitro* and under greenhouse conditions. Best performing isolates were selected for further greenhouse screening and subsequent characterization using biochemical and molecular methods.

# CHAPTER 1

# General introduction

## 1.1 Background

The world population is currently over 7.7 billion, up from 6.9 billion in 2010 and is estimated to reach 8 billion by 2024 (Worldometers, www.worldometers.info). In South Africa, the total population was almost 57.73 million in 2018 (Statistics South Africa 2018). A 1.55% increase in the SA population was recorded between 2017 and 2018 (Statistics South Africa 2018). The increase in the world human population has intensified the demand for food. In 2003, FAO stats predicted the need to double food production as the world population increases (FAO 2003).

Despite measures taken to boost productivity, several factors are currently exerting a huge pressure that limits food production. Some of these factors include climate change, water availability, soil nutrient status and pathogens. Losses incurred due to pathogens occur at all levels of the supply chain and the focus of the current study will be at the level of crop production. Different measures are taken to manage plant diseases such as the use of resistant cultivars, cultural and physical methods amongst others. Presently, the use of synthetic chemicals is still the main method of managing plant diseases and improving crop growth. However, due to the adverse effects of some of these agrichemicals on human health and the environment, other sustainable and environmentally friendly means of plant disease control are sought (Adesemoye and Kloepper 2009).

The soil is populated by a wide diversity of microorganisms, some of which are beneficial for plant growth and suppression of plant diseases. The use of soil microorganisms as a means to enhance plant growth started in 1886 when rhizobia were discovered to fix nitrogen. After observing that specific bacteria isolated from the rhizosphere improved the growth of radish, Kloepper and Schroth (1978) termed these bacteria “plant growth-promoting rhizobacteria”. Plant growth-promoting rhizobacteria (PGPR) are a group of free-living bacteria that colonize the rhizosphere and promote the growth of plants. In addition to promoting plant growth, PGPR also exhibit biocontrol activity, suppressing plant pathogens.

The use of PGPR for plant disease control and plant-growth promotion is a natural and environmentally friendly alternative to the use of synthetic chemicals. PGPR have thus become a popular topic of research in recent years. Pii et al. (2015) showed that the yield of some crops

has not increased proportionally with the increase in fertilizer use, resulting in pollution and contamination of the environment by synthetic fertilizers. However, PGPR research has shown an increase in nutrient uptake by incorporation of PGPR into the soil (Adesemoye et al. 2010; Adesemoye and Kloepper 2009; Saber et al. 2015). This increase in nutrient uptake efficiency has the benefit of reduced requirement of chemical fertiliser application. Previous investigations revealed that different PGPR are successful under different conditions and thus emphasized the need for screening of different PGPR strains with different modes of action that can work under different agro-ecological conditions (Ahemad and Kibret, 2014).

### 1.2 Aim

The main aim of this study was to identify new rhizobacteria strains with plant-growth promotion and biocontrol activity against *F. pseudograminearum* on wheat, by screening/assessing a total of 113 rhizobacterial isolates from the University of Pretoria's PGPR collection.

### 1.3 General objectives

To achieve the above aim, the following objectives were pursued:

- A literature study was undertaken to serve as a base of current knowledge.
- Assess the ability of the rhizobacterial isolates to control crown rot of wheat caused by *F. pseudograminearum b*y means of a seedling bioassay (Chapter 3).
- Assess the ability of the rhizobacterial isolates to enhance wheat growth in the absence of a pathogen by means of a seedling bioassay (Chapter 4).
- Determine the traits associated with biocontrol activity and direct plant growth promotion of the rhizobacterial isolates by means of appropriate *in vitro* bioassays (Chapter 5).
- Select the best-performing biocontrol and plant growth-promoting isolates, based on the results obtained from chapter 3, 4 and 5 (Chapter 6).
- Confirm efficacy of the selected best performing rhizobacterial isolates for biocontrol activity against *F. pseudograminearum* on wheat as well as direct growth promotion of wheat in subsequent greenhouse trials (Chapter 7).
- Assess the effect of temperature, pH, NaCl and different carbon sources on the growth of the selected best performing rhizobacterial isolates (Chapter 7).

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# CHAPTER 2

# Literature Review

## 2.1 Introduction

Wheat (*Triticum aestivum*) is a staple food around the world including South Africa where the major producing areas include Western Cape (50%), Northern Cape (16%), Free State (15%) and little amounts in the other provinces (Beukes et al. 2017). About 1.3- 2 million tons of wheat is produced annually. However, this amount is less than the 2.7 million tons needed to feed a growing population of over 57 million people (Department of Agriculture, Forestry & Fisheries 2016). Wheat is mainly produced for human consumption and animal feed. Some of the factors limiting wheat production include water availability, soil nutrient status and disease-causing phytopathogens.

One such pathogen of wheat is *Fusarium pseudograminearum*, the causal agent of crown and root rot of mainly wheat and barley crops that causes devastating losses in yield (Yli-Mattila & Gagkaeva 2016). This pathogen can cause pre-emergence and post-emergence damping-off of infected seedlings (Kazan and Gardiner 2018). Plants that survive damping-off can have stunted growth and unfilled kernels as translocation of water and nutrients in the plants is blocked. The infected seedlings mainly have dark brown to black crowns due to the rot. These symptoms are more apparent during water limiting conditions.

The pathogen is widely distributed throughout the world and also occurs in South Africa, especially in the main wheat-producing fields in Western Cape and Free State (Van Coller et al. 2013; Ferreira et al. 2015; Lamprecht et al. 2006; Pretorius et al. 2013). The control of this pathogen is very challenging as it survives in previous crop stubble and soil. It can also be harboured by certain weed grasses. *F. pseudograminearum* is thus mainly controlled through stubble management and crop rotation. To boost wheat yield, farmers tend to use fertilisers and pesticides to limit losses due to pathogen infestations. However, due to the adverse effects of some of these agrichemicals on human health and the environment, other non-chemical means of plant disease control are sought (Adesemoye and Kloepper 2009).

The soil is home to a wide diversity of microorganisms, of which most of the active microbes that interact with the plant roots are found in the rhizosphere. The rhizosphere is characterized by a relatively high concentration of nutrients exuded by plant roots (Bhattacharyya and Jha 2012). which are very essential for their survival and proliferation as carbon and energy source (Bhattacharyya and Jha 2012; Bishnoi 2015). Some of the microorganisms found in the rhizosphere can enhance plant growth and suppression of plant diseases. The use of soil microorganisms as a means to enhance plant growth started in 1886 when rhizobia which fix atmospheric nitrogen were discovered (Bishnoi 2015). After observing that specific bacteria isolated from the rhizosphere improved the growth of radish, Kloepper and Schroth (1978) termed these bacteria plant growth-promoting rhizobacteria (PGPR), a group of free-living bacteria that colonize the rhizosphere and promote the growth of plants.

Research has shown that several groups of rhizobacteria can enhance the growth of various plant species as well as aid in the control of pathogens (Muis and Quimio 2006; Verma et al. 2013; Zohara et al. 2016). Table 2.1 shows a few examples in which PGPR have been used successfully in different crops. Some of the most popular PGPR belong to the *Bacillus* and *Pseudomonas* group (Jetiyanon et al. 2003; Zohara et al. 2016). Studies have also shown that yield has not proportionally increased with an increase in fertiliser use (Pii et al. 2015). However, PGPR research has shown an increase in nutrient uptake by incorporation of PGPR into the soil (Adesemoye et al. 2010; Adesemoye and Kloepper 2009; Saber et al. 2015). This increase in nutrient uptake efficiency has the benefit of reduced requirement of chemical fertiliser application. The use of PGPR for plant disease control and plant-growth promotion is a natural and an environmentally friendly alternative to the use of synthetic chemicals and therefore PGPR have become a very popular topic of research in recent years.

**Table 2.1** PGPR that have shown significant control of different pathogens on different crops.

| PGPR | Disease/Pathogen | Crop | Reference |
|---|---|---|---|
| *Pseudomonas flourescens*<br>*Pseudomonas aurefaciens*<br>*Pseudomonas patudi* | *Collectotrichum orbiculare* (Anthracnose) | Cucumber | (Wei et al. 1991) |
| *Bacillus amyloliquefaciens*<br>*Bacillus pumulis* | *Sclerotium rolfsii* (Southern blight) | Tomato | (Jetiyanon et al. 2003) |
| | *Colletotrichum gloeosporioides* (Anthracnose) | Long cayenne pepper | |
| | *Cucumber mosaic virus* (CMV) | Cucumber | |
| *Bacillus cepacian* | *Fusarium oxysporum*<br>*Fusarium culmorum* | Potato | (Recep et al. 2009) |
| *Pseudomonas flourescens*<br>*Pseudomonas syringae*<br>*Pseudomonas chlororaphis* | *Rhizoctonia solani*<br>*Pythium ultimum* | Wheat | (Mavrodi et al. 2012) |
| *Bacillus subtilis* BR23 | *Rhizoctonia solani* (Banded sheath blight) | Corn | (Muis and Quimio 2006) |
| *Pseudomonas flourescens* | *Rhizoctonia solani* (Rice sheath blight) | Rice | (Kumar et al. 2009) |
| *Pseudomonas aeruginosa* | *Rhizoctonia solani* (Root rot)<br>*Fusarium oxysporum* f. sp. *cicero* (wilt) | Chickpea | (Verma et al. 2013) |
| *Streptomyces placates* B4-7 | *Phytophthora capsici*<br>*Phytophthora cinnamomi*<br>*Phytophthora palmivora*<br>*Phytophthora parasitica* | Bell pepper | (Chen et al. 2016) |
| *Pseudomonas* spp. | *Phytophthora capsici* (Damping-off) | Cucumber | (Zohara et al. 2016) |
| *Bacillus vallismortis* BS07 | *Phytophthora capsici* | Chili pepper | (Park et al. 2013) |

## 2.2 *Fusarium pseudograminearum* on wheat

*Fusarium* spp. have been regarded as the most important toxigenic fungi globally that reduce crop yield and quality of cereals (Yli-Mattila & Gagkaeva 2016). The pathogens are economically important as they affect the global staple food group by causing a variety of diseases including seedling blight, root and crown rot, head blight and ear rot in a wide range of cereal crops (Fernandez et al. 2009; Popovski and Celar 2013). *Fusarium* spp. are also known to produce mycotoxins which are toxic to humans and animals (Marin et al. 2012; Meca et al. 2010). These toxins include deoxynivalenol (DON), zearalenone (ZEA), nivalenol (NIV), fumonisins (FUM), T-2 and HT-2 (Cheli et al. 2017).

*Fusarium pseudograminearum* was formerly known as *Fusarium graminearum* Group 1 and later separated due to morphological and molecular differences (Kazan and Gardiner 2018). *F. pseudograminearum* has a narrow host range and mainly infects wheat and barley crops. Other cereals such as emmer (Burgess 1967), rye (Atanasov 1920), oats (Aoki and O'Donnell 1999) and maize (Atanasov 1920) have been reported as hosts of the pathogen. In addition, certain grass species, i.e. common wheatgrass, barley grass, harding grass, carany grass (Burgess 1967) and millet (Simmonds 1966) have been found susceptible to *F. pseudograminearum*. It often occurs in association with other *Fusarium* species such as *Fusarium culmorum*, *Fusarium graminearum* and *Fusarium avenaceum* (Paulitz et al. 2002). This pathogen usually colonizes the stem and crown of crops (Singh et al. 2009). Paulitz et al. (2002) described foot rot pathogens as unspecialised as they can infect any plant tissue of a susceptible plant if conditions are favourable for infection. There have also been reports of this pathogens causing head blight of wheat (Obanor et al. 2013; Xu et al. 2015).

Another means of controlling this pathogen is through the use of resistant cultivars though no fully resistant cultivar is available (Fernandez et al. 2009). *F. pseudograminearum* can infect the plant, but the plant is able to withstand the infection and grow. The infection reduces the growth and yield of the crop as the translocation of water and nutrients is disturbed. Crop rotation and stubble management are also implemented to reduce the level of inoculum in the field. The pathogen is more aggressive during water limiting conditions (Popovski and Celar 2013). PGPR can help control the pathogen and alleviate stress on the crop thus allowing it to defend itself and grow.

### 2.2.1 Disease cycle

The primary source of inoculum is mycelium or spores from infected grass and cereal residues which can infect the plant at all stages of growth given optimal conditions for infection (Figure 2.1). *F. pseudograminearum* is strictly residue-borne and depend on stubble for survival between cropping cycles (Dyer et al. 2009). The pathogen infects the coleoptile, progresses into the sub-crown internode and leaf sheaths and subsequently into the stem epidermal tissues (Kazan and Gardiner 2018). The pathogen frequently infects the plant via the stomatal openings. It then moves into hypodermis where it induces browning of the stem and enters the vascular tissues. The mycelium blocks the vascular tissues which interferes with the translocation of water and nutrients up and down the plant. This leads to the development of dead or partially filled 'white-heads'. The development of these 'white-heads' is exacerbated by water stress. Pink hyphal growth may also be visible under the leaf sheaths and around the nodes during the later stages of crop maturity. The pathogen can colonise up to 6 lower internodes of the plant (Alahmad et al. 2018). The colonisation of the plant has been shown to be greater under water limiting conditions as the physiological processes of the plant are compromised thus making the plant more susceptible to *F. pseudograminearum* (Chekali et al. 2016).

*F. pseudograminearum* has also been reported to cause head blight during anthesis during warm humid conditions which is characterised by necrotic or blotched spikelets contaminated with the fungal toxin, deoxynivalenol (DON), which is similar to head blight caused by *Fusarium graminearum* (Xu et al. 2015). The pathogen overwinters as mycelium in cereal and grass stubble and relies on the undecomposed plant tissue for protection (Paulitz et al. 2002). The pathogen is considered monocyclic, resulting in little or no secondary crown rot infection thus disease incidence in a growing season is directly influenced by incidence in the previous season (Alahmad et al. 2018). The hyphae of the pathogen are reported to survive up to 728 days in wheat stubble (Alahmad et al. 2018).

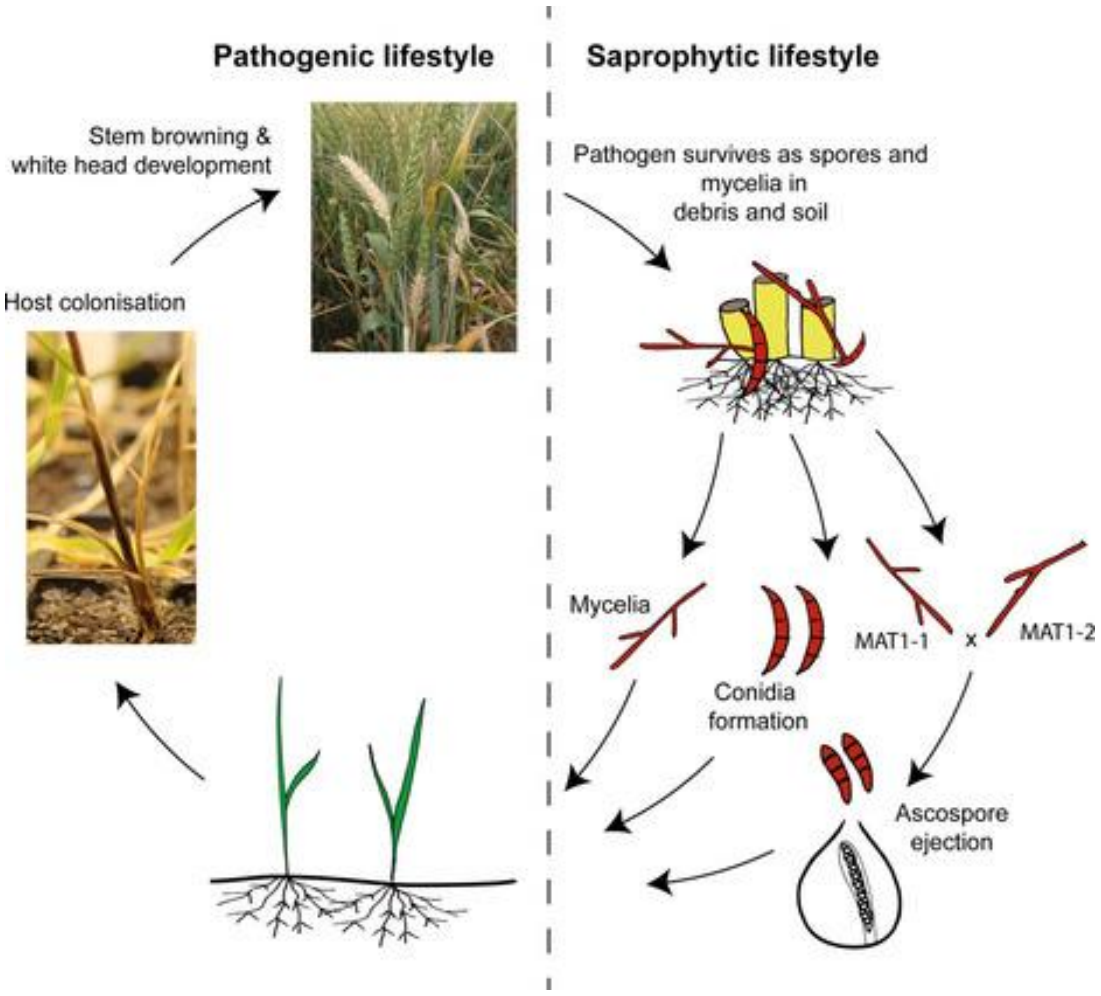


**Figure 2.1** Disease cycle of *F. pseudograminearum* on wheat (Source: Kazan and Gardiner 2018).

### 2.2.2 Control

#### 2.2.2.1 Cultural control

As previously mentioned, stubble from the previous season is the primary source of inoculum, thus, control of *F. pseusograminearum* is mainly based on previous crop stubble management. Stubble management can decrease inoculum load in the field. The stubble can be incorporated into the soil using tillage practices or burnt to kill the pathogen. Tillage reduces inoculum level in the soil by burying crop residues from the previous season and promoting degradation of the infected crop residues (Evans et al. 2010). The number of tillage operations has also been shown to greatly reduce the levels of inoculum in the soil (Bailey et al. 2001, 2000; Paulitz et al. 2002; Windels and Wiersma 1992). Bailey et al. (2001) also found that reduced tillage resulted in an increased

incidence of *Fusarium* species on wheat roots but had no effect on the overall root rot severity and yield. Lack of tillage leaves crop residues on the soil and does not get broken down, thus providing a source of inoculum in the subsequent growing season. However, removing stubble negatively impacts on soil moisture content as evaporation is increased which in turn favours disease development. In addition to this, soil moisture plays an important role in the breakdown of crop residues (Evans et al. 2010).

Crop rotation with non-host crop species is another method that is effective in reducing inoculum load in the field. Reports have shown that continuous cultivation of wheat season after season increases the incidence of *F. pseudograminearum* on wheat (Bailey et al. 2001; Lamprecht et al. 2006). As such, crops that can be cultivated in rotation with wheat and reduce inoculum in the field before wheat planting are of importance. Chekali et al. (2016) reported that the use of cereal crops before the cultivation of wheat increased disease incidence while the use of legumes such as chickpea and faba bean decreased the disease incidence.

Evans et al. (2010) also found that wheat rotation with non-cereal crops reduced the incidence of *F. pseudograminearum* and *F. culmorum* DNA in the soil. In addition, they found that leaving the land fallow also reduced the incidence of the pathogen on durum wheat roots. Tillmann et al. (2017) explored the potential of sugar beet and oil radish as rotation crops and reported that colonisation frequency by *Fusarium* species was higher when wheat was grown after sugar beet and lower when grown after oil radish. Bailey et al. (2001) found that the cultivation of wheat with pea and flax resulted in higher yields than continuous wheat cultivation. However, they (Bailey et al. 2001) also found that growing wheat after flax increased the incidence of *Fusarium* species such as *F. equiseti, F. culmorum*, *F. acuminatum, F. graminum* and *F. graminearum* in wheat roots.

Sanitation has also proved effective. Weed grasses that are known to be *F. pseudograminearum* hosts must also be removed before the cultivation. Durum wheat is also more susceptible to *F. pseudograminearum* than bread wheat and can cause an increase in inoculum levels if planted before bread wheat (Evans et al. 2010). Changing of planting date may also help in avoiding infection. Planting dates can be selected in such a way that the occurrence of dry conditions during the grain filling stage, which are known to favour the disease development, can be avoided during maturity of the crop. In addition, the nutrient status of the soil has an impact on disease

development. The use of nitrogen fertilizers can increase the incidence and severity of *Fusarium* root diseases in wheat (Kazan and Gardiner 2018). Zinc has been shown to increase wheat yield in wheat with crown rot.

#### 2.2.2.2 Chemical control

Chemical control is one of the most effective methods of plant disease control. However, the control of *F. pseudograminearum* using fungicides on wheat is very limited and does not provide protection against the pathogen throughout the growing season (Alahmad et al. 2018; Moya-Elizondo and Jacobsen 2016). In addition, there are no registered fungicides for the control of crown rot on wheat in South Africa (Beukes et al. 2017). Research has, however, gone into finding fungicides that could help control the disease. Lamprecht et al. (1990) reported that the application of triadimenol to wheat seeds reduced seedling damping-off but did not provide long term protection against crown rot beyond the seedling stage. The study by these researchers suggested the use of seed treatment with thiram and a combination of carboxin and thiram in integrated control programmes as they resulted in less damping-off and improved the emergence of wheat plants. Moya-Elizondo and Jacobsen (2016) found that the use of difenoconazole-mefenoxam as a seed treatment reduced crown rot severity by 29 to 50% in the greenhouse. Their finding also indicated that the combination of the fungicide with cultivar resistance and systemic acquired resistance reduced crown rot and disease severity more than the use of the individual methods in the field. This highlights the importance of an integrated disease management strategy.

## 2.3 Plant growth-promoting rhizobacteria (PGPR)

The use of plant beneficial rhizobacteria has been in place for decades. However, their mode of action and how they can be used successfully to maximise production is not fully understood. In order for PGPR to be effective, they must possess three properties, i.e. they must be able to colonise the root surface, they must be able to survive and compete with other microbiota in the rhizosphere in order to express their plant growth promotion and biocontrol activities, and they must ultimately be able to promote plant growth (Kloepper 1994).

The use of PGPR has provided successful plant growth promotion and disease control on several crops against different pathogens. For example, Jetiyanon et al. (2013) found that *Bacillus*

*amyloliquefaciens* strain IN937a and *Bacillus pumilus* strain IN937b can induce systemic resistance in tomato, cucumber and long cayenne pepper. In addition to this, the use of *B. amyloliquefaciens* strain IN937a and *B. pumilus* strain IN937b as a mixture provided the crops with increased protection against the pathogens tested (Jetiyanon et al. 2003). *Streptomyces placates* B4-7 is another example of PGPR that has provided significant control against *Phytophthora capsici*, *Phytophthora cinnamomi*, *Phytophthora palmivora* and *Phytophthora parasitica* on bell pepper (Chen et al. 2016). Cucumber plants that were grown from seeds treated with *Pseudomonas* spp. were more resistant to damping-off caused by *P. capsici*, than those that were grown from untreated seeds (Zohara et al. 2016).

PGPR possess different traits that allow them to enhance plant growth. Such PGPR traits include the production of phytohormones, the ability to fix atmospheric nitrogen and solubilise phosphate in the soil, production of siderophores for iron acquisition, ACC deaminase which aids with stress tolerance and production of the phytohormone indole-3-acetic acid (IAA). In addition, they indirectly enhance plant growth by the control of pathogens through competition of nutrients and space, the production of antibiotic substances and the induction of defence systems in the plant. However, PGPR are faced with different challenges in the soil that may hinder their growth. Some of these challenges include the availability of nutrients in the soil, harsh temperatures, soil water content, pH and salt concentration.

These factors are some of the things that need to be considered when screening isolates for field application. They determine the success or failure of the PGPR when applied on a plant. Another challenge facing the application of PGPR in the field or for commercial use is the formulation used. PGPR cannot be applied as a cell suspension and must be applied as seed treatments of formulated inoculants in carriers. The carriers used must support the survival of PGPR for a considerable period of time during storage (Sharma et al. 2015).

### 2.3.1 Root colonization and biofilm formation by PGPR

#### 2.3.1.1 Root colonisation

Root colonisation can be defined as the ability of bacteria to establish on or in the plant roots, to propagate, survive and disperse along the growing root in the presence of indigenous microflora

(Choudhary and Johri 2009). For this to happen, plant root exudates must apply a positive chemotaxis effect on the bacteria in the soil which allows them to find and colonize the rhizosphere and plant roots (Dutta and Podile 2010; Silva et al. 2003). Chemotaxis towards root exudates and plant roots can be affected by the amount and type of exudates produced (Hassen and Labuschagne 2010) as well as flagella and pili activity (Dutta and Podile 2010). Though root colonization is important, it does not translate to good disease control and cannot be used solely as a method of selection of rhizobacteria as PGPR potentials. Good PGPR are good root colonizers but not all good root colonizers are good PGPR (Mavrodi et al. 2012).

Soil-borne pathogens infect plants through the roots thus PGPR that are employed to control these pathogens must be able to colonize the rhizosphere. Rhizobacteria compete with pathogens for space and sites of infection. The community of rhizobacteria on the root of the plant acts as a barrier between the plant and pathogens. It has been shown that the formation of biofilms on the surface of the roots limits the release of root exudates into the soil, thereby limiting the growth of pathogens (Herrera et al. 2016). Good root colonization also allows for contact with the plant and communication between the plant and bacteria. Molecules produced by PGPR can travel directly to the plant while the released root exudates can be directly accessed by PGPR. This leads to the growth of both the plant and PGPR.

Hassen and Labuschagne (2010) reported efficient root colonization of wheat and tomato by *Paenibacillus alvei* NAS6G-6. Mavrodi et al. (2012) also reported efficient root colonization of wheat by *Pseudomonas* spp. However, there were inconsistencies in the level of disease suppression (Mavrodi et al. 2012). Though results found in a study by Zheng and Sinclair (2000) were inconsistent as well, the greatest root colonization resulted in reduced severity of *Rhizoctonia* root rot on soybean and they concluded that root colonization and disease suppression are dependent on a number of factors including soil type and microorganisms present in the soil (Zheng and Sinclair 2000). Good colonization does not always result in good disease suppression. However, when coupled with other modes, it could provide better plant protection against plant pathogens (Pal et al. 2001). It is therefore quite evident that root colonization as a trait cannot be used on its own as a criterion for selection of potential PGPR.

2.3.1.2 Biofilm formation

One of the other ways by which PGPR can protect plants from phytopathogens and promote their growth is through the formation of biofilms (Fallis 2013). Some PGPR produce exopolysaccharides which are important in the formation of biofilms (Gupta et al. 2015). Similar to root colonization, biofilm formation does not directly influence the growth of plants or act against pathogens, but PGPR that possess the trait offer an additional advantage over those that do not. Biofilms have been suggested to play a part in protecting colonization sites through which pathogens would otherwise colonize and infect plants. In addition to this, biofilms are associated with increased nutrient uptake of plants and also aid with their adaptation to changing environmental conditions (Liu 2015). The importance of biofilms extends to the binding of $Na^+$, holding free phosphorus and other essential nutrients, allowing the plant to access them (Gupta et al. 2015).

*Pseudomonas* spp. owe some of their efficacy as biocontrol agents to their ability to form a biofilm on the surface of plant roots. *Paenibacillus* sp. is also very efficient as a biocontrol agent due to its ability to form a biofilm on plant roots in addition to its spore-forming ability (Chauhan et al. 2015; Herrera et al. 2016). Herrera et al. (2016) observed that *Paenibacillus* spp. have biocontrol activity against *F. graminearum* and that in combination with *Pantoea* spp., they resulted in greater chlorophyll content of barley seedlings. Another promising PGPR is *Qchrobactrum intermedium* which Paulucci et al. (2015) showed to form a biofilm on the roots of peanut plants and is also able to survive under high concentrations of NaCl. The ability of biofilms to bind to $Na^+$ reduces the amount of the cation available to the plant for absorption thereby alleviating salinity stress of the plant.

Tewari and Aurora (2014) reported that at lower concentrations, exopolysaccharides function as biocontrol agents while at higher concentrations they function as an osmoprotective or stress alleviating metabolite. Kavamura et al. (2013) reported that *P. patudi* produces an exopolysaccharide, alginate, which influences biofilm formation in response to water-limiting conditions. Biofilm formation allows bacteria to escape water stress conditions by maintaining a hydrated microenvironment, thus protecting the bacteria from dehydration (Kavamura et al. 2013). As the biofilm is formed on the surface of roots or in the rhizosphere, the plant is also in turn protected from dehydration.

### 2.3.2 Mode of action

As mentioned before, PGPR promote plant growth directly and indirectly. Plant growth is promoted directly through the production of phytohormones, nitrogen fixation, phosphate solubilisation, production of siderophores; and restricting by hindering pathogen growth through the production of antibiotics and competition for space and nutrients (Arruda et al., 2013). PGPR must satisfy the criteria of being able to successfully colonize the root surface, compete with other microorganisms for survival and proliferation in the rhizosphere and promote the growth of the plant (Bishnoi, 2015). PGPR can be classified into four groups: i.e. biofertilizers, phytostimulators, biopesticides as well as phytoremediation and stress control agents (De-la-Peña & Loyola-Vargas 2014; Perez-Montano et al. 2014).

PGPR that have been developed into biofertilizers provide nutrients to the plant thereby promoting its growth and some examples include species belonging to the genera *Allorhizobium*, *Bacillus*, *Pseudomonas*, *Rhizobium* and *Trichoderma* (Kumar et al. 2014; Pal et al. 2001; Pii et al. 2015; Singh et al. 2009; Verma et al. 2013). Phytostimulators stimulate plant growth through the production of phytohormones such as IAA, with notable examples being some species belonging to *Azotobacter*, *Bacillus*, *Enterobacter* as well as *Pseudomonas* (Kavamura et al. 2013; Kumar et al. 2014; Macleod et al. 2015). Biopesticides are those that inhibit the growth of pathogens through the production of antibiotics and volatile compounds and some examples include *Bacillus*, *Pseudomonas* and *Streptomyces* spp. (Ali et al. 2015; Beneduzi et al. 2012; Kurabachew and Wydra 2013). Finally, phytoremediation and stress control agents are those that can degrade soil pollutants and produce ACC deaminase which assists in stress tolerance with *Pseudomonas* spp. as examples (Magnucka and Pietr 2015).

Figure 2.3 summarizes the different mode of action of PGPR. Many of these modes of action include the fixation of atmospheric nitrogen, solubilization of insoluble forms of phosphorus, production of phytohormones (auxins, cytokinin, gibberellins), production of ACC deaminase, proline synthesis, quaternary amine synthesis, antioxidative enzymes, antifreeze proteins, production of antibiotics (siderophores, HCN, antifungal metabolite), competitive exclusion, production of cellulolytic enzymes and the induction of systemic resistance (Adesemoye et al. 2010; Adesemoye and Kloepper 2009; Choubane et al. 2016; Son et al. 2014). All modes of action ultimately lead to the promotion of plant growth. PGPR that possess multiple plant growth promotion traits have a better potential than those that possess a single trait (Shahzad et al. 2013).

The need for selection of PGPR with multiple modes of action and for their evaluation under greenhouse conditions has been emphasised (Kavamura et al. 2013). Kumar et al. (2014) further supported this by highlighting the use of combinations of PGPR for control of pathogens and plant growth promotion. A combination of *Arthrobacter chlorophenolicus*, *B. megaterium* and *Enterobacter* sp. resulted in increased growth, yield and nutrient content of wheat (Kumar et al. 2014). As one PGPR may not possess all desirable plant growth promotion traits, the use of combinations of PGPR which have a synergistic effect on one another can lead to a combined mode of action leading to enhanced plant growth. There are many modes of action of PGPR and for the purpose of this study, only a few will be discussed.

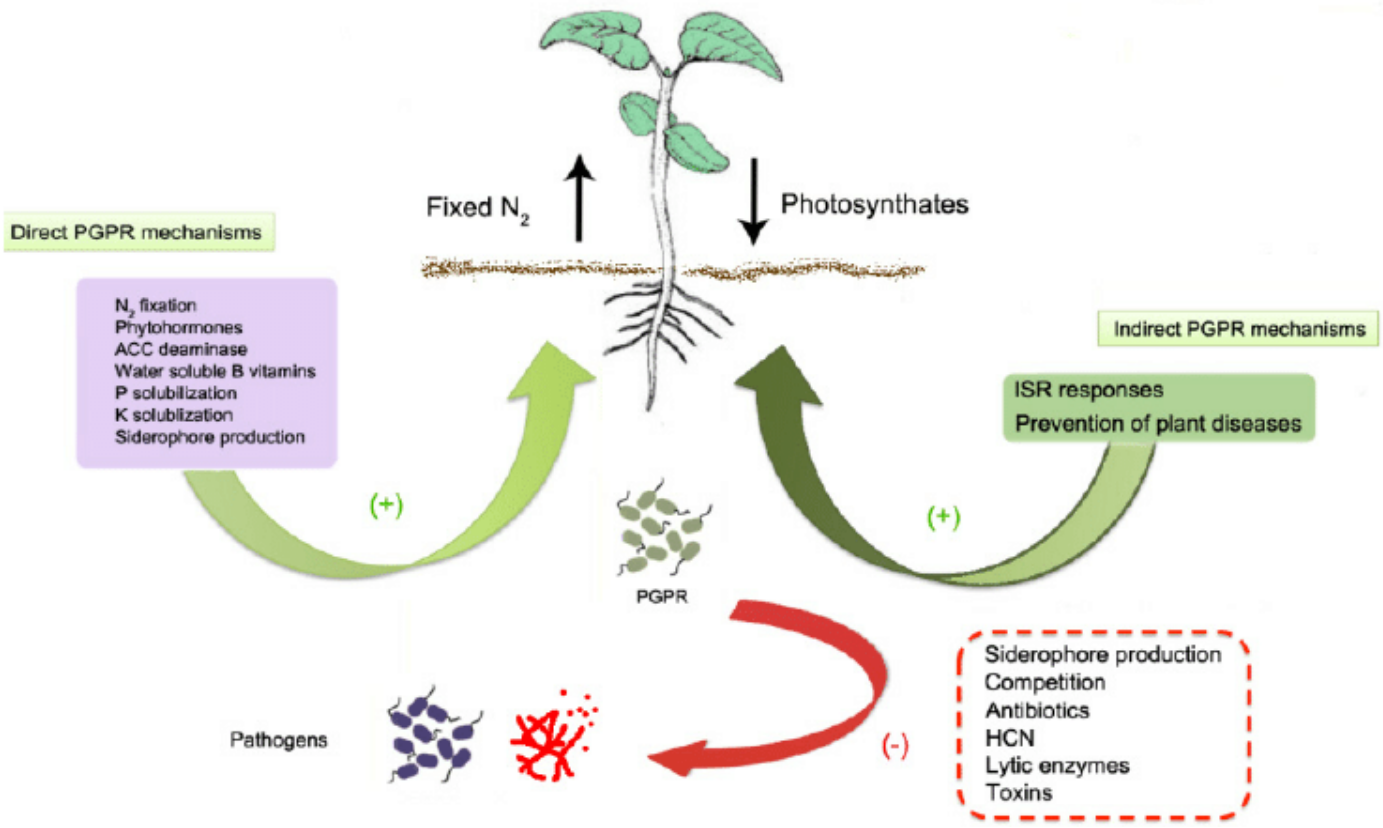


**Figure 2.2** The different modes of action of PGPR (Source: García-Fraile et al. 2015).

#### 2.3.2.1 Plant growth-promotion associated traits

##### 2.3.2.1.1 Nitrogen fixation

Nitrogen is one of the most important elements used by plants. It is incorporated into peptides and leads to the overall growth of plants. As important as nitrogen is to plants for their growth and survival, nitrogen is a limiting nutrient in the soil. Plants are unable to fix atmospheric nitrogen and rely on microorganisms that have evolved methods to fix atmospheric nitrogen. These nitrogen-fixing bacteria possess the *nif* gene cluster which codes for nitrogenase enzyme responsible for fixing nitrogen (Goswami et al. 2016). The presence of nitrogen-fixing bacteria in the soil means that there is more nitrogen available for plants thus reducing the amount of artificial fertilizer needed to be applied to the soil as a supplement (Dawwam et al. 2013). Popular free-living nitrogen fixers belong to the *Azospirillum*, *Azotobacter*, *Bacillus* and *Pseudomonas* species (Goswami et al., 2016). Verma et al. (2013) observed that *Mesorhizobium* sp. BHURC03 and *P. aeruginosa* BHUPSB02 are effective for use as biofertilizers due to their nitrogen-fixing ability. Adesemoye et al. (2010) observed that the treatment of the soil with *B. amyloliquefaciens* strain IN937a and *B. pumilis* strain T4 can increase the uptake of N by the plant. This also alleviates the negative effects the application of fertilizers poses to the environment.

##### 2.3.2.1.2 Phosphate solubilisation

After nitrogen, phosphate is the next important but limiting nutrient in the soil for plant growth. It is highly abundant in the soil but like nitrogen, it is also not readily available for plant use (Goswami et al. 2016). Phosphate is not readily available to plants as most of it is bound to other molecules which cannot be absorbed by plants. To increase the amount of phosphate available for plant use, fertilizers are applied to the soil. However, it has been reported that up to 90% of phosphorus applied to the soil in fertilizers can be precipitated by metal complexes (Adesemoye and Kloepper 2009; Pii et al. 2015). Phosphate forms complexes with iron, aluminium and calcium which makes it less soluble and unavailable for plant use (Pii et al. 2015).

Microorganisms are effective at solubilising these complexes thus making phosphate more available to plants for absorption. Rhizobacteria such as *Azobacter* spp., *Azospirillum* spp., *Bacillus* spp., *Pseudomonas* spp., *Rhizobium* spp. and *Serratia* spp. produce inorganic acids (acetate, oxalate, succinate, citrate and gluconate) which solubilize phosphate from mineral complexes (Pii et al. 2015). The application of PGPR that can solubilize phosphate increase the amount of available phosphate for plant use. As in the case of nitrogen, less fertilizer is needed to be applied to the soil as it is used by the plant more efficiently (Dawwam et al. 2013). This leaves less fertilizer available for leaching, running off into surface water or other ways that may affect the environment in a negative way.

2.3.2.1.3 ACC deaminase production

Under stress conditions, plants produce ethylene which regulates plant homeostasis resulting in reduced root and shoot growth (Vurukonda et al. 2016). The production of ethylene occurs in two phases, one of which leads to the initiation of defence mechanisms and the other to abscission, senescence and chlorosis (Glick 2014). The second phase inhibits the growth of the plant and may also lead to death. During the synthesis of ethylene, 1-aminocyclopropane-1-carboxylate (ACC), which is the precursor of ethylene, is produced. PGPR produce ACC deaminase which can hydrolyse ACC, thus lowering the production of ethylene by the plant (Singh et al. 2015). This ensures that, as environmental stress conditions continue, plant growth also continues.

During the hydrolysis of ACC by ACC deaminase, ammonia is released which is utilized by the rhizobacteria as a source of nitrogen and energy (Grobelak et al. 2015; Magnucka and Pietr 2015). This nitrogen can also be available to the plant during nitrogen-limiting conditions (Grobelak et al. 2015). *Pseudomonas* species have been reported to increase the biomass of wheat through the production of ACC deaminase (Magnucka and Pietr, 2015). Glick et al. (2013) found that *Bacillus* and *Pantoea* spp. can promote the growth of maize under stress conditions through the reduction of ethylene production. Some (Glick 2014) have argued that this may be the most important trait of PGPR as plants are often exposed to stress conditions which lead to increased production of ethylene.

#### 2.3.2.1.4 IAA production

One of the key phytohormones in the growth and development of plants is auxin. In the presence of L-tryptophan in root exudates, some PGPR can produce a form of auxin, indole-3-acetic acid (IAA), which leads to the enhancement of cell elongation and division, tissue differentiation and apical dominance (Goswami et al. 2016; Kavamura et al. 2013). This increase in the cell division results in increased length of roots which in turn increases their surface area and allows more nutrients to be absorbed from the soil, leading to more growth of the plant. In addition, IAA loosens cell walls of plants which leads to an increase in the release of root exudates which leads to more proliferation of PGPR in the rhizosphere (Etesami et al. 2015).

IAA has also been linked to the induction of the production ACC synthase which catalyses the formation of ACC in plants (De-Sen et al. 2002; Glick 2014). It has been argued that the induction of ACC production by exogenous IAA may increase the levels of ethylene in the plants which in turn reduces the growth of plants. IAA has also been linked to the production of reactive oxygen species which also lead to the reduction of plant growth (De-Sen et al. 2002). In addition, IAA has been reported to play an important role in salt stress tolerance and the induction of resistance against root rot diseases (Egamberdieva et al. 2015). It is not clear what PGPR use IAA for. Kavamura et al. (2013) reported that *Bacillus* and *Pantoea* spp. promote the growth of maize under water stress conditions, though the promotion of maize plant growth, although this was not clearly linked to the production of IAA by the PGPR. This suggests that there are other modes of action in play and their combined interaction lead to the enhancement of plant growth.

### 2.3.2.2 Biocontrol related traits

#### 2.3.2.2.1 Antibiosis

Plant growth can be promoted through the inhibition of pathogen activity. This is done through the production of inhibitory substances such as volatiles. These volatiles are also produced by plants in response to wounding by herbivores. The volatile compound produced by PGPR may also deter herbivors from feeding on plants. Examples of such inhibitory volatiles are hydrogen cyanide (HCN) and ammonia ($NH_3$).

HCN has been reported as a potential inhibitor of many enzymes involved in major metabolic processes. This compound inhibits the growth of fungal pathogens by inhibiting the activity of cytochrome c oxidase in the electron transport chain, thereby inhibiting mitochondrial respiration (Kumar et al. 2014). *P. patudi* PP255 has been found to produce HCN (Kurabachew and Wydra 2013). Kavamura et al. (2013) stated that most of the *Bacillus* spp. used in experiments produced HCN which were followed by *Pseudomonas* spp., *Rhizobium* spp. and finally *Azotobacter* spp. In addition to these species, Kumar et al. (2014) found that *Enterobacter* sp. produced HCN. Some other examples that have been reported successful in the control pathogens through the production of HCN include *P. aureofaciens*, *P. fluorescens* and *P. patudi* (Wei et al. 1991). These were successful in the inhibition of *Colletotrichum orbiculare* (anthracnose) on cucumbers. Santiago et al. (2015) found that the production of HCN by PGPR could delay the growth of *Rhizoctonia solanacearum* by two days at the lowest concentrations ($10^{-2}$- $10^{-4}$) and completely inhibits its growth at the highest concentration ($10^{-1}$).

Some PGPR produce enzymes such as chitinase, protease, urease, pectinase, cellulase, catalase and amylase which can inhibit the growth of pathogens and degrade organic matter (Choubane et al. 2016). These enzymes degrade cell walls of pathogens and help to inhibit their growth. For example, cellulase and chitinase inhibit the growth of fungal pathogens which have cellulose and chitin as major components of their cellular wall (Kavamura et al. 2013). The destruction of fungal cell wall results in the leakage of cellular components thus leading to the death of the pathogen. *Bacillus mycoides* B38V and *P. riograndesis* SBR5 have been found to produce cellusases, chitinases and proteases while *B. cepacia* 89 was found to produce proteases, chitinases as well as lipases (Bach et al. 2016). PGPR also produce biosurfactants which are primarily for the reduction of surface tension but also have antibiotic properties (Agaras et al. 2015; Bach et al. 2016). Some of these biosurfactants include fengycin, iturins, mycosubtilins and bacillomycins which are produced by *Bacillus* spp. (Fernando et al. 2006).

PGPR also produce antibiotics to inhibit the growth of pathogens. *Streptomyces* are widely known for their production of antibiotics (Kang et al. 2016). Examples of antibiotics produced by PGPR include pantocin, pyrrolnitrin, phenazine-1-carboxylic acid, colistin, polymyxin, 2,4-diacetyl phloroglucinol, kanosamine, pyoluteorin, circulin, zwittermycin-A as well as oomycin (Beneduzi et al. 2012; Fernando et al. 2006). These antibiotics may also be extracted and used to inhibit the growth of pathogens without inoculating the plant with the antibiotic-producing rhizobacteria.

Other PGPR that have been shown to produce antibiotics are *Serratia marcescens* KBS9-R, *B. laterosporus* KBS2-12, *B. cereus* KBS9-H and *B. subtilis* NAE1-7 (Hassen et al. 2008). These were shown to be effective in the control of *P. ultimum* which causes root rot on sorghum (Hassen et al. 2008).

2.3.2.2.2 Siderophores

Plants use iron (Fe) for the formation of chlorophyll molecules which play an important role in the growth of plants. The available iron in the soil can be bound to other molecules. To utilize the iron in the soil, microorganisms and plants have evolved similar strategies for the mobilization of iron though microorganisms are more competitive than plants (Pii et al. 2015). To do this, they make use of siderophores which have a strong affiliation to bind $Fe^{3+}$ ions. Siderophores can be defined as small peptide molecules containing side chains and functional groups that can provide a higher affinity set of ligands to coordinate ferric ions ($Fe^{3+}$) (Beneduzi et al. 2012).

Siderophores produced by bacteria are essential as they have a higher affinity for $Fe^{3+}$ than pathogens chelators thus, making $Fe^{3+}$ less available to pathogens and more available to plants (Grobelak et al. 2015). In addition to this, fungal pathogens are unable to absorb the iron-siderophore complex (Goswami et al. 2016). This slows the proliferation of pathogens as they have less $Fe^{3+}$ available to them. However, other studies have not been able to link siderophore production with pathogen inhibition (Ribeiro and Cardoso 2012). This further suggests the importance of the selection of PGPR with multiple mode of action or the use of more than one strain with a different mode of action. Interestingly, most of the isolates that are reported to produce siderophores can also solubilize phosphate (Arruda et al. 2013; Ribeiro and Cardoso 2012; Son et al. 2014). Siderophores production is usually linked to *Pseudomonas* spp. (Arruda et al. 2013; Goswami et al. 2016; Saber et al. 2015). *Burkholderia* spp. and *Ochrobactrum intermedium* have also been reported to produce siderophores (Arruda et al. 2013; Paulucci et al. 2015).

2.3.2.2.1 Induced Systemic Resistance

Another way in which PGPR can control pathogens is through the induction of plant defences. Necrosis is necessary for the induction of systemic resistance (Wei et al. 1991). It is accepted that PGPR can only lead to induced systemic resistance (ISR) while pathogens lead to systemic acquired resistance (SAR). The pathways followed by the two are different and rely on different signalling molecules, ISR relies on ethylene and jasmonic acid while SAR relies on salicylic acid as signalling molecules (Beneduzi et al. 2012). SAR leads to the production of pathogenesis-related (PR) proteins while ISR does not. This induction of systemic resistance can lead to the control of several pathogens that the crop may be faced with.

Recent research has shown that PGPR can make use of the SAR pathway and lead to the accumulation of PR proteins (Park et al. 2013). Park et al. (2013) found that the inoculation of chilli pepper with *B. vallismortis* BS07 induced the salicylic acid-dependent pathway and lead to an up-regulation of *PR1a* gene expression. Akram et al. (2013) used *B. subtilis* IAGS162 and *B. fortis* IAG174 on tomatoes for the control of *Fusarium* wilt which induced systemic resistance in the crop and gave good disease control in pot trials as under field conditions. Wei et al. (1991) also observed that seed bacterization with these PGPR strains can induce systemic resistance. Ran et al. (2005) reported that *P. putida* WCS358, *P. fluorescens* WCS374 as well as *P. fluorescens* WCS417 can induce systemic resistance in several plant-pathogen systems. This merits the need for research to explore this field as it leads to the control of multiple pathogens with the use of a single PGPR strain.

## 2.4 Factors affecting the survival and activity of PGPR in the soil

With all the research that has been done on rhizobacteria over the years, promising isolates for PGP use have been found (Dinesh et al. 2015; Lucas et al. 2014; Thokchom et al. 2014). However, not all rhizobacteria with PGP and biocontrol traits can be used for commercialization. Results observed in field trials differ from those obtained *in vitro* and in greenhouse trials (Kumar et al. 2014). No direct relationship is found between biocontrol activity and PGP *in vitro* and field trials.

The differences observed are due to the differences in environments (Prakamhang et al. 2014). Laboratory and greenhouse studies are done under controlled environments which do not pose stress on the PGPR, thus they are active and can proliferate. When moving to field application, PGPR are faced with more challenges that may hamper their growth and survival.

These factors can be divided into two groups, abiotic and biotic factors. They include competition with other microorganisms for space and resources, soil type, texture, moisture, pH, density, nutrient status, salinity, temperature and composition of root exudates. Maheshwari (2012) stated that inoculation of the soil with PGPR will not result in significant plant growth or disease suppression unless the environmental conditions support the growth and survival of the inoculants.

As with all living organisms, even a slight change in these factors can have a detrimental effect on the survival and activity of PGPR in the soil which may lead to the reduction in their ability to control pathogens and promote plant growth. For this reason, one of the desirable features of PGPR is high adaptability to new environments. In addition to being highly adaptable to new environments, PGPR must be resistant or be able to withstand stress environmental conditions. Rhizobacteria have developed ways which allow them to escape stress conditions. For example, *Bacillus* and *Paenibacillus* spp. produce endospores while *Pseudomonas* spp. form a biofilm which allow them to escape these stress environmental conditions (Chauhan et al. 2015; Dutta and Podile 2010). The ability to escape or withstand a change in or harsh environmental conditions allows for effective disease control and plant growth promotion.

### 2.4.1 Temperature

Temperature can have a major impact on the survival and activity of most living organisms. Extreme temperatures have been shown to have a negative impact on cell walls and protein function of organisms. Egamberdieva (2012) observed that bacterial strains isolated from loamy sandy soil resulted in more pea, wheat and maize growth at 16$^{o}$C while no plant growth stimulation occurred at 26$^{o}$C. Mao et al. (1997) also observed increased plant height and weight at 25$^{o}$C than at a lower temperature of 18$^{o}$C when *Burkholderia cepacia* (*Pseudomonas cepacia*) was inoculated on corn seeds. This suggests that changes in temperatures could result in different levels of disease suppression and plant growth as the weather or seasons change. However, studies have shown that

there are PGPR that can withstand high temperatures. In an investigation by Paulucci et al. (2015), *Ochrobactrum intermedium* was found to tolerate temperatures as high as 37$^{o}$C by modifying its cell wall composition. In addition to tolerating high temperatures, *O. intermedium* was able to promote the growth of the peanut plant (Paulucci et al. 2015). Careful consideration is thus needed when selecting PGPR to be applied to a plant during a season.

### 2.4.2 Soil and plant properties

Upon incorporation of PGPR into the soil, several soil factors may hamper its growth. These include soil type, texture, pore size, pH, moisture content, nutrient status and salinity. In addition to these factors, the composition of root exudates released in the rhizosphere also affects the growth and activity of PGPR. For this reason, it is important to carefully study soil properties where a particular PGPR strain will be used as well as the composition of root exudates of the host crop.

Root exudates may contain inhibitory substances that may hamper the growth of a particular PGPR strain (Dutta and Podile, 2010). Emmer and Handelsman (1999) emphasized the need for the examination of seed chemistry when selecting PGPR to be used with a particular crop as seeds and plants may contain PGPR inhibitors. The presence of inhibitory compounds in root exudates results in a decrease in growth or survival and activity of a particular PGPR which will lead to little or no disease suppression and plant growth enhancement. These inhibitory substances in root exudates also deter PGPR and prevent root colonization which is an important trait in pathogen control and plant growth promotion.

Soil type and texture can also hamper the survival and activity of PGPR. Bacterial strains that were isolated from loamy sand resulted in increased growth of wheat, pea plants and maize by up to 45% as compared to the control (Maheshwari, 2012). Adhya et al. (2015) found that the survival of inoculated *P. fluorescens* was better in silt loam than in sandy soils. This agrees with White et al. (1994) who observed more persistence of *P. fluorescens* cells in soil with smaller pores than soil with larger pores. As sandy soil is characterized by larger pore sizes, this suggests that the use of *P. fluorescens* in soils composed mainly of sand may result in decreased efficacy as compared to soils composed of a small percentage of sand such as loam and clay soils. However, other studies have shown that other PGPR genera are not affected by soil properties and have been isolated from different soil types (Arruda et al. 2013). These PGPR belong to the divisions *beta proteobacteria*

and *gammaproteobacteria* such as *Burkholderia* and *Klebsiella* species, respectively (Arruda et al. 2013). This agrees with previous studies by Reis et al. (2004) who reported the isolation of *Burkholderia* spp. from plants grown in regions with climates ranging from temperate sub-humid to hot-humid. Roesch et al. (2007) also reported the isolation of *betaproteobacteria* and *gammaproteobacteria* from different types of soil and different geographical regions. Another factor that affects the success of PGPR is their requirement of aerobic or anaerobic conditions (Handelsman et al. 1990). In saturated conditions, PGPR that require aerobic conditions may not survive thus not controlling a specific pathogen that thrives in anaerobic conditions.

Salinity level in the soil also affects the activity of PGPR. High salt concentrations affect the plant as well as any life forms found in the soil by disrupting cell membranes and water potential within cells. A decrease in bacterial growth rate with an increase in salt concentration has been reported (Membre and Burlot 1994). However, some *Pseudomonas* spp. have been described to tolerate high salinity levels (Maheshwari 2012). Tolerance of up to 2000mM NaCl has been reported for *P. aeruginosa* (Tewari and Arora 2014). Tewari and Arora (2014) also found that with an increase in soil salinity, *P. aeruginosa* produced more exopolysaccharides (EPS) which result in the formation of a sheath around the bacterial cells, protecting them from stress. The formation of this sheath protects both the bacteria and the plant from osmotic stress (Tewari and Arora 2014). *Ochrobactrum intermedium* has also been found to survive in environments of high salt concentration (Paulucci et al. 2015).

### 2.4.3 Competition with other microorganisms

When PGPR are introduced in the field, they have to compete with other microflora already established in the rhizosphere. The presence of other microorganisms, including pathogens, in the soil, affects the survival and activity of PGPR. These microorganisms compete for resources such as nutrients and space. Introduced PGPR thus face the challenge of establishing themselves and colonizing the rhizosphere in the presence of native microflora. Successful PGPR must be able to outcompete other microorganisms for the available resources and space (Lugtenberg and Kamilova 2009). As some of the resident microflora may be better adapted to survive in that particular

environment, the introduced PGPR must be applied in sufficient numbers to compete with the native microflora (Maheshwari 2012).

Studies have looked at different environments that can be used to find potential PGPR. Two sources of potential PGPR have been suggested. Yang et al. (2012) observed that the isolation of rhizobacteria from soils with healthy plants provided good antagonists for *Ralstonia* wilt on ginger. The rationale for isolating from soil with healthy plants is that in the presence of PGPR, pathogens are unable to grow which results in healthy plants with improved growth. However, Huang et al. (2013) tested the hypothesis that potential PGPR can be isolated from a pathogen-prevalent environment. Their findings showed that the pathogen-infested rhizosphere of tomato plants is a reservoir of biocontrol bacteria (Huang et al. 2013). They suggested that this is due to the adaptation of the rhizobacteria to harsh environmental conditions, including those imposed by the pathogen.

Considerations regarding the effect of these different factors on the survival and activity of PGPR when selecting PGPR for application under field conditions cannot be over-emphasised. These factors directly influence the activity of the introduced PGPR and determine the success or failure of PGPR. This warrants the need for soil tests before the application of the PGPR to determine if they are suitable for application in that particular field or environment. More research regarding the effect of changes in environmental conditions on the activity of PGPR is needed. The response of PGPR to these changes needs to be carefully examined to determine how the environment can be manipulated to better suit the survival and activity of PGPR of interest. This information can also be helpful during the selection process of PGPR to be applied in a certain environment as well as on a particular plant.

## 2.5 Selection and commercialization of PGPR

### 2.5.1 Selection of potential PGPR from a collection of rhizobacteria

Many rhizobacteria can be isolated from the soil and some may not be beneficial for plant growth promotion and biocontrol. Thus, there is a need for rapid screening methods that allow the selection of only those that can control pathogens and improve plant growth. Most studies on PGPR

screening start with a rapid *in vitro* pure culture screening for plant growth promotion and biocontrol activity (Liu 2015; Maheshwari et al. 2015; Yang et al. 2012). As important as this step may be, Cardinale et al. (2015) reported that this method alone is not enough for the selection of best plant growth promotion strains and further studies on their activity in the presence of the plant must be done. In doing so, the activity of the PGPR under natural conditions can be evaluated. Starting with *in vitro* screening results in the identification of strains that may be of no benefit when it comes to biocontrol and plant growth promotion in the greenhouse or field. The latter may also be costly and time-consuming especially when many traits are to be evaluated.

Seedling bioassays can be done to allow for the selection of rhizobacteria which have biocontrol activity and promote the growth of a crop in the presence or absence of a pathogen. When developing a seedling bioassay, it is important to mimic the conditions of the natural environment. Two important requirements for the development of bioassays have been emphasized, i.e. a) the bioassay has to be simple and rapid in order to screen a large number of potential PGPR and b) the bioassay has to have some relevance to natural conditions in order to identify PGPR that may be useful for disease suppression and plant growth promotion in the field (Handelsman et al., 1990). They further suggested that screening of pathogen inhibition *in vitro* is inferior to conducting seedling bioassays as results obtained *in vitro* have no direct relationship to disease suppression in the greenhouse or field (Handelsman et al. 1990).

This lack of correlation is due to the difference in the conditions *in vitro* and in the greenhouse or field when all three subjects (plant, pathogen and PGPR) are present in the same environment. The mechanism of induced systemic resistance, for instance, cannot be detected by *in vitro* screening (such as the dual culture assay) but can only be detected in tests involving plants. Handelsman et al (1990) for example, reported that *Bacillus cereus* was successful in suppressing damping-off of alfalfa caused by *Phytophthora* in seedling bioassays but did not inhibit the growth of the pathogen on agar plates.

In Table 2.2, some of the studies that have screened a large collection of PGPR for either biocontrol, plant growth promotion ability or both are indicated. Selection of the best PGPR isolates is different from one study to another. Some studies place more importance on the selection of isolates that can inhibit the highest percentage of pathogen growth (Dinesh et al. 2015; Kurabachew and Wydra 2013; Liu 2015) while others placed more importance on plant growth

promotion traits (Lucas et al. 2014; Son et al. 2014) and others selected isolates that can inhibit pathogen growth and also possess plant growth promotion traits (Thokchom et al. 2014).

**Table 2.2** Screening of a large collection of PGPR and the basis of selection of the best isolates.

| Number of isolates | Biocontrol | PGP | Assays | Basis of selection | Reference |
|---|---|---|---|---|---|
| **198** | ✓ | ✓ | Antibiosis (9 pathogens)<br>N-fixation, P-solubilization, IAA and siderophore production<br>Biofilm formation, biosurfactant activity<br>Growth chamber plant growth promotion and biocontrol trials.<br>Use of mixtures | Isolates that inhibited at least 8 pathogens in antibiosis assays were used in plant growth promotion assays. The selected PGPR were also used in growth chamber trials. | (Liu 2015) |
| **100** | ✓ | ✓ | IAA, $NH_3$ and HCN production<br>Solubilization of P, K, Zn and Si<br>Production of hydrolytic enzymes (cellulose, pectinase, protease, α-amylase)<br>Greenhouse and field experiments | All isolates were screened for both biocontrol and PGP traits. Only isolates that inhibited more than 70% of the pathogen in *in vitro* assays were used in greenhouse and field experiments. | (Dinesh et al. 2015) |
| **292** | | ✓ | IAA and siderophore production<br>P-solubilization<br>Plant growth promotion assay | From *in vitro* assays, strains were selected based on their phylogeny, sampling site and previously evaluated PGP traits. | (Arruda et al. 2013) |
| **900** | ✓ | ✓ | AHL and siderophore production<br>P-solubilization<br>ACC degradation<br>Greenhouse plant growth promotion assay<br>Biocontrol assay | From *in vitro* assays, those that possessed at least one PGRP trait were used in a plant growth promotion assay.<br>Isolates that resulted in significant plant growth were used in the biocontrol assay. | (Lucas et al. 2014) |
| **126** | ✓ | ✓ | IAA and siderophore production,<br>P-solubilization<br>Plant growth promotion assay<br>Induced systemic resistance assay | Isolates that were positive for all three PGPR traits were used in the plant growth promotion and induced resistance assays. | (Son et al. 2014) |

**Table 2.2 continued** Screening of a large collection of PGPR and the basis of selection of the best isolates.

| Number of isolates | Biocontrol | PGP | Assays | Basis of selection | Reference |
|---|---|---|---|---|---|
| **150** | ✓ | ✓ | Antibiosis<br>AHL, IAA, HCN and siderophore production | Isolates that inhibited the growth of the pathogen were tested for plant growth promotion traits. The isolates were also used in plant growth and biocontrol activity in pot trials. | (Kurabachew and Wydra 2013) |
| **299** | | ✓ | Indolic compounds and siderophore production<br>P-solubilization<br>N-fixation<br>Plant growth promotion assay | Isolates that had multiple PGP traits (Indolic compounds and siderophore production<br>P-solubilization) and taxonomic identification were then tested for N-fixation and used in the *in vivo* plant growth promotion assay. | (Ambrosini et al. 2012) |
| **217** | ✓ | ✓ | N-fixation, IAA production, P-solubilization, *in vitro* antibiosis<br>ACC deaminase activity<br>Pot trials | Isolates that showed all three PGP traits were used in antibiosis tests. Isolates were then ranked as per the quantity of PGP attribute produced and those with the highest rank were used in subsequent trials. | (Thokchom et al. 2014) |
| **48** | | ✓ | Exopolysaccharide, IAA, $NH_3$, HCN and cellulose production<br>P-solubilization<br>Plant growth promotion assay | Isolates not lacking more than one of the PGP traits were used in the plant growth promotion assay. | (Kavamura et al. 2013) |
| **420** | ✓ | | Cellulolytic enzymes and siderophore production<br>Biocontrol activity in the greenhouse | Isolates were grouped into clusters and those that produced at least one enzyme and siderophore were used in the greenhouse biocontrol assay. | (Yang et al. 2012) |
| **585** | ✓ | ✓ | Antibiosis, P-solubilization, ACC deaminase, IAA, siderophore, enzyme, HCN and $NH_3$ production<br>Plant growth promotion in the growth room | From the *in vitro* antibiosis tests, isolates that inhibited either one of the pathogens selected were tested for plant growth promotion traits and were also used in plant growth promotion experiments in the growth room. | (Ghyselinck et al. 2013) |

**Table 2.2 continued** Screening of a large collection of PGPR and the basis of selection of the best isolates.

| Number of isolates | Biocontrol | PGP | Assays | Basis of selection | Reference |
|---|---|---|---|---|---|
| **13** | ✓ | ✓ | *In vitro* antagonistic test, HCN, siderophore, β-1,3-glucanases, chitinases, proteases, IAA and salicylic acid production, P-solubilization, N-fixation, Disease suppression and plant growth promotion under greenhouse condition | All isolates were used in all *in vitro* assays and based on the results, some were selected for use in greenhouse trials. | (Zhang et al. 2015) |
| **373** | ✓ | ✓ | Antagonistic activity<br>Protease, cellulose and chitinase activity, P-solubilization, N-fixation<br>Biocontrol and plant growth promotion under greenhouse conditions | Isolates that showed antagonistic activity against the pathogen were screened for enzymes and PGP traits. Following *in vitro* assays, isolates were ranked and selected to be screened for biocontrol activity under greenhouse conditions. A further selection of the best isolates was done and were tested for plant growth promotion. | (Zheng et al. 2011) |
| **128** | ✓ | ✓ | *In vitro* antagonism<br>Exoprotease, phospholipase, HCN, IAA, ACC deaminase production, P-solubilization, presence of genes responsible for the production of antibiotics<br>Greenhouse plant growth promotion assay | Isolates that resulted in the inhibition of two or more of the pathogens tested were chosen to be used in subsequent assays. Isolates were also ranked through the calculation of biocontrol trait, biocontrol potential and direct growth-promoting indices. | (Agaras et al. 2015) |

### 2.5.2 Commercialisation of PGPR

The use of PGPR and the development of new strains is a difficult task which stems from the inconsistent results from trails. Excellent results may be obtained in *in vitro* and/or greenhouse screening but when the strains are taken a step further in greenhouse and/or field trials, they may offer little to no protection against pathogens and may also not increase plant growth. Kumar et al. (2014) conducted pot and field trials which supported this. In their trials (Kumar et al. 2014), there was no correlation in the results obtained in pot trials and field trials. As discussed above, the success of PGPR depends on several factors and these factors change and increase considerably between *in vitro* and field trials.

As has been discussed, the success of PGPR as plant growth-promoting and biocontrol agents is determined by several factors. Their success is dependent on a number of climatic, soil as well as other biological factors (Agaras et al. 2015; Ali et al. 2015). One of the reasons owing to the lack of much success with the use of PGPR, especially new strains may be difficulty adapting to a new habitat. As such PGPR must be easily adaptable to new environments and be good root colonizers of the host plant they are being used for (Agaras et al. 2015; Chauhan et al. 2015). Adaptability problems can be overcome by using native PGPR strains (Mercado-Flores et al. 2014). It has also been found that isolating PGPR from an area that is infested with pathogens can provide strains that are efficient disease controllers as they have evolved to survive in the harsher conditions (Huang et al. 2013).

One of the challenges involved in the commercialization of PGPR is the formulation of strains. For application in the field, PGPR cannot be applied as a cell suspension and must be applied in carriers. Carriers used must support the survival of PGPR for a considerable period of time (Sharma et al. 2015). Some of the carriers that can be used include sawdust, charcoal, liquid waste, talc, lignite, vermiculite, sand and manure (Akram et al. 2013; Maheshwari et al. 2015; Sharma et al. 2015), while the most commonly solid carriers being peat and/or perlite formulations. Maheshwari et al. (2015) observed that vermiculite was the best carrier for *B. licheniformis* KRB1 and *P. aeruginosa* KRP1 when compared to sand, sawdust and liquid waste and still provided viable inoculants after 180 days of storage. Some PGPR are difficult to formulate and do not survive for a long period of time in storage (Goswami et al. 2016).

There are many formulations of *Bacillus* spp. due to their ability to survive in formulations for a very long time and form endospores that allow them to withstand unfavourable conditions (Figueiredo et al. 2010; Kumar et al. 2011; Sharma et al. 2015). After *Bacillus* spp.,

*Pseudomonas* spp. are also formulated in high numbers. Saharan and Nehra (2011) listed the advantages of using *Pseudomonas* spp. as PGPR. These include the ability to synthesise antibiotic metabolites, they compete aggressively with other microorganisms, they grow at a fast rate and colonize the rhizosphere and root surfaces and they adapt to stressful conditions (Saharan and Nehra 2011). However, unlike *Bacillus*, *Pseudomonas* spp. do not form endospores. More research is still needed for the development of different PGPR that can survive for a considerable period in different formulations.

Even with all the factors that need to be taken into consideration when using PGPR for plant disease control and plant growth promotion, the positives still outweigh the negatives. There are several PGPR that are available commercially for use on a wide range of crops. PGPR have many different modes of action and are an attractive choice for organic, integrated plant disease management. In addition to the PGPR listed on the table, there are others which are available under tradenames such as BioYield, Companion, EcoGuard, HiStick N/T, Kodiak, Mepplus, Serenade, Sonata, Subtilex, Yield- Shield which are formulations of *Bacillus* spp., Deny and Intercept which are formulations of *Burkholderia cepacia* and AtEze, Bio-save, BlightBan, Frostban, Cedomon, Spot-Less which are formulations of *Pseudomonas* spp. (do Vale Barreto Figueiredo et al. 2010).

The use of PGPR mixtures has also been shown to have increased efficacy in plant disease control and plant growth enhancement (Pal et al. 2001). As one PGPR strain does not possess all plant growth promotion and biocontrol traits, it makes sense to use a few compatible PGPR strains in cropping systems. Akram et al. (2013) found that the use of a combination of *B. fortis* IAGS162 and *B. subtilis* IAGS174 provided better suppression of fusarium wilt on tomato plants under field conditions than using individual species for the control.

Past PGPR research has mainly focused on the discovery of PGPR and their modes of action. Current research is directed towards exploring the use of endophytic rhizobacteria in plant growth promotion and biocontrol; improved methods of encapsulating PGPR; as well as the use of PGPR in bioremediation. Bioremediation of contaminated sites with rhizobacteria has become a topic of interest due to their ability to sequester heavy metals and degrade pollutants such explosives, herbicides or hydrocarbons (Bishnoi 2015). Bioremediation allows for the restoration of environments. Encapsulation of PGPR refers to the technology of creating a barrier that protects, controls release and functionality of PGPR which allows for improved performance in the field (John et al. 2011). Encapsulation allows for increased efficacy due to high surface area, improved systemic activity due to smaller particle size and high mobility,

and minimise toxicity as the use of organic solvents is eliminated (Sasson et al. 2007). Improved methods of encapsulating PGPR could potentially be used in conjunction with ‘microbial precision production systems’; where, change in soil conditions (pH, water content, minerals, nutrients, PGPR and plant signal molecules and inoculant applications) can be monitored in order to decided when to perform additional PGPR applications (Welbaum et al. 2004). The use of endophytic PGPR offer the advantage of being translocated in the plant resulting in them being carried in seed. Endophytic PGPR are also less influenced by the surrounding environment.

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# CHAPTER 3

## Screening of rhizobacterial isolates for biocontrol activity against *Fusarium pseudograminearum* on wheat by means of seedling bioassays

**Abstract**

A small-scale greenhouse seedling assay was developed as a rapid method for screening rhizobacteria. The method was developed for the wheat-*Fusarium pseudograminearum* host-pathogen system and used to screen a collection of 113 rhizobacterial isolates for biocontrol activity against the disease. In the presence of *F. pseudograminearum*, fifty-two per cent of the isolates significantly increased the shoot dry weight of the seedlings, 41% increased the root dry weight of the seedlings, and the total seedling weight was increased by 32% of the isolates screened. The total dry seedling weight increase ranged between 39.13% and 123.61% while 14.58% to 106.41% of this was due to plant growth promotion effect of the isolates on the seedlings. The results obtained show that the rhizobacterial isolates have a potential for controlling crown rot of wheat and directly promoting the growth of the crop. Further screening and study of these isolates is required to develop these isolates for use on a larger scale.

### 3.1 Introduction

*Fusarium pseudograminearum*, the causal agent of crown/root/foot rot of wheat may cause up to Aus$80 million from lost production to yield quality (Murray and Brennan 2009). Most of the production of wheat in South Africa takes place in Western Cape (50%), Northern Cape (16%), Free State (15%) and little amounts in the other provinces (Beukes et al. 2017). Annually, 1.3- 2 million tons of wheat is produced, which is less than the 2.7 million tons needed (Department of Agriculture, Forestry & Fisheries 2016). The wheat produced is mainly for human consumption and animal feed. *F. pseudograminearum* has been found in fields in Western Cape and Free State (Van Coller et al. 2013; Ferreira et al. 2015; Lamprecht et al. 2006; Pretorius et al. 2013). The infected seedlings can die before or after emergence. Symptoms of infected seedlings include browning of the coleoptile, sub-crown internode, lower leaf sheaths and adjacent stems and nodal tissues (Kazan and Gardiner 2017). The symptoms can appear anywhere from a few weeks after planting or throughout plant development. These symptoms are more apparent during water limiting conditions. The disease

causes a decrease in yield which may lead to more wheat being imported into the country, affecting the economy of the country.

The use of chemical pesticides for the control of plant diseases is common. Examples of registered fungicides in South Africa for use on cereals include Baytan 15 DS, Royalcab FS, Galmano Plus, Apron XL, Celest XL and Latitude (AVCASA 2018). However, these are not sustainable and have adverse effects on the environment. Some of the dangers associated with the use of chemical pesticides include danger to human and animal health. In addition to this, pathogens often develop resistance against chemical pesticides (Chakwiya et al. 2015). As resistance increases, more chemicals are used which in turn increases costs involved. Consumers have also developed a negative attitude towards produce that have been treated with pesticides. Thus, alternative measures of plant disease control are needed. One such alternative method that has shown great potential is the use of plant growth-promoting rhizobacteria (PGPR) (Perez-Montano et al. 2014).

Using biocontrol agents such as PGPR is a sustainable alternative that can help decrease the dependence on chemical fertilizers (Cortivo et al. 2017). PGPR possess traits that allow them to control plant diseases either directly or indirectly. Some of these traits include the production of antibiotic enzymes (Bach et al. 2016; Choubane et al. 2016), siderophore (Paulucci et al. 2015; Pii et al. 2015), antibiotic volatile compounds (Kavamura et al. 2013; Kumar et al. 2014), competition for space and nutrients (Huang et al. 2013; Lugtenberg and Kamilova 2009) and induction of systemic resistance of the plant (Farina et al. 2012; Park et al. 2013). The search for PGPR with novel modes of action is important. However, determining the specific mode(s) of action of isolates in the field is difficult. Performing *in vitro* studies gives a good indication of the potential and inherent traits of the isolates but does not tell us which traits are activated when applied to the plant in the greenhouse or the field.

Due to lack of correlation between *in vitro* and *in vivo* performances of isolates (Mavrodi et al. 2012), performing a seedling assay as a rapid method of screening could help determine whether the isolates will be successful *in vivo*. Using seedling assays as a screening method before proceeding to large-scale greenhouse trials ensures the selection of efficient isolates (Sang et al., 2008). Performing seedling assays offer several advantages over conducting pot trials. Seedling assays are quicker to conduct, the seedlings can be easily inoculated and handled, and the experiments require less space and thus allow for high throughput. Furthermore, it allows for uniform infection and disease development (Ishiga et al. 2011) and

gives an easy indication of how the isolate may perform in bigger greenhouse trials or in the field.

The aim of this chapter is to use seedling assays in which a collection of rhizobacteria can be screened for biocontrol activity against *F. pseudograminearum* on wheat and direct growth promotion in the presence of the pathogen. This will assist in the selection of best-performing isolates to be used in subsequent pot trials.

## 3.2 Materials and methods

### 3.2.1 Bacterial culture preparation

All bacterial cultures were obtained from the University of Pretoria-PGPR culture collection. A total of 113 isolates which have been maintained in Microbank™ beads (Pro-Lab Diagnostics) and preserved at -70°C were used for this study. The frozen cultures were thawed and plated onto nutrient agar (NA) medium (Biolab), incubated at 25°C for 48h and checked for purity. Working cultures were prepared by streaking the bacteria onto the surface of nutrient agar slants in McCartney bottles. These were stored at room temperature and streaked onto NA plates when needed. The storage cultures were also preserved in glycerol. A 65% glycerol solution was prepared and 500µl was dispensed into sterile Eppendorf tubes. Bacteria were grown in nutrient broth and incubated in a shake-incubator at 25°C at 150rpm for 48h. An equal volume (500 µl) of bacterial broth was transferred to the Eppendorf tubes containing glycerol. These tubes containing the bacteria were then frozen.

### 3.2.2 Fungal inoculum preparation

The fungal pathogen, *Fusarium pseudograminearum* M7816N, was supplied by Dr S. Lamprecht, Agriculture Research Council (ARC), Stellenbosch, South Africa. A spore suspension of the fungus was prepared as described in the protocol by Bai & Shaner (1996). Briefly, forty grams of organic mung beans (Earth Products Store, 103 Komatie Road, Emmarentia, Johannesburg) were added to a 1L Erlenmeyer flask containing distilled water. This was autoclaved for 20 minutes and filtered using double filter papers to remove the solids. The resulting (brown coloured) solution was autoclaved two more times on two consecutive days and inoculated with a block of a freshly prepared *F. pseudograminearum* culture. This was placed in a shaking incubator at 25°C for 4 days at 150rpm. The solution was then filtered again with an autoclaved filter paper to remove the mycelium. A hemocytometer was used to

determine the spore concentration. Sterile $dH_2O$ was used to adjust the suspension to the required concentration.

### 3.2.3 Pathogenicity test

Wheat seeds of the cultivar Duzi, obtained from Klein Karoo Seed (P.O. Box 159, Oudtshoorn, 6620, South Africa) were used in pathogenicity test. The seeds were surface disinfected with 70% ethanol for one minute and then washed five times with sterile $dH_2O$. They were subsequently submerged in 1% sodium hypochlorite solution for 30 seconds and rinsed five times in sterile $H_2O$. The seeds were pre-germinated for three days in sterile petridishes containing moist filter paper. The pre-germinated seeds were transferred into 9-cup-multipot seedling trays containing 50ml/cup of steam-pasteurised soil (sand 87%, clay 7% and silt 6%) at a rate of 2 seeds per cell. Seven days after transplanting, the seedlings were thinned to 1 seedling/cell. *F. pseudograminearum* spore suspension was applied to the seedlings at the rates $10^3$, $10^4$, $10^5$ and $10^6$ spores/ml of suspension.

One millilitre of the spore suspension was applied at the base of each seedling. The seedling trays with seedlings were kept in the greenhouse at an average temperature of 25$^o$C for a further 21 days. Untreated seedlings served as the negative control. Seven days post-inoculation, the seedlings were watered every second day with tap water to restore water soil water capacity. This allowed the plants to become stressed to promote infection. Each seedling tray represented one replicate with 9 sub-replicates. Each treatment had 4 replicates. Twenty-one days after incubation, the seedlings were removed from the trays and the soil was washed off the roots. The roots were excised, and fresh root and shoot weight determined by weighing. The separated shoots and roots were then placed in brown paper bags and placed in an oven at 50$^o$C for 48 hours to dry. The dry root and shoot weight were determined by weighing. The experiment was repeated.

### 3.2.4 Screening of rhizobacteria for biocontrol activity against *F. pseudograminearum*

The wheat seeds were prepared as described above. Due to the large number of isolates, the bacterial collection was screened in four batches. The isolates were streaked onto NA plates from working cultures. After 3 days of incubation, a loop full of the bacterial cells were transferred to 30ml of autoclaved nutrient broth in 50ml centrifuge tubes. These were incubated in a dark shaking incubator (150rpm) for 48 hours at 25$^o$C. One millilitre of the bacterial

suspension was pipetted at the top of the seeds at planting. Fourteen days following bacterial inoculation, the seedlings were inoculated with 1ml of $1\times10^6$ spores/ml of *F. pseudograminearum* by pipetting it at the base of the seedling. This concentration was selected based on the pathogenicity trial results. The negative control was treated with uninoculated NB while the positive control was treated with the spore suspension of the pathogen only. Each treatment comprised three replicates with three sub-replicates. The plants were grown for a further 21 days at an average temperature of 25$^o$C in the greenhouse. The seedlings were watered every second day (to induce stress) with municipal tap water to restore soil water capacity. Upon harvesting, soil was washed from the roots, roots were excised, and the fresh root and shoot weight of the seedlings were determined by weighing. Roots and shoots were placed in brown paper bags and dried in an oven for 48 hours at 50$^o$C. Dry root and shoot weight were determined by weighing. This screening experiment was done twice.

### 3.2.5 Experimental design and statistical analysis

Both the pathogenicity trials and the screening of rhizobacteria for biocontrol against *F. pseudograminearum* experiments were set up according to a completely randomized design. Analysis of variance (ANOVA) was performed using SAS 9.2 software (SAS Institute, Cary, NC, USA) for the analysis of variance (ANOVA). Fischer's Protected Least significant difference (LSD) was used to separate means was at a significance level of 5% ($p=0.05$).

## 3.3 Results

### 3.3.1 Pathogenicity test

The two trials showed a similar trend in terms of the treatment effects but the data (average values per treatment) differed thus they were assessed separately (Table 3.1). In both trials, the uninoculated control showed no symptoms of disease associated with *F. pseudograminearum*. Additionally, in both trials, the inoculum concentration of $10^3$ spores/ml was not enough to cause disease on the seedlings. Generally, the weight of the seedlings decreased with increase in the inoculum concentration. This trend was more observable in the dry weight data than the wet weight data. Overall the results were ambiguous. In the first trial, there we no statistical differences between treatments in terms of the shoot fresh weight of the seedlings. In the second trial, the highest inoculum concentration ($1\times10^6$ spores/ml) significantly ($p<0.0001$) reduced shoot fresh weight (0.49g compared to 0.79g for the control). In both instances, all treatments

significantly reduced the root fresh weight of the seedling; however, the concentration of $1\times10^6$ spores/ml greatly reduced the fresh weight of the seedlings compared to all the other treatments.

A concentration of $1\times10^6$ spores/ml also showed great effect on shoot dry weight of the seedlings compared to the control but did not differ significantly from $1\times10^5$ spores/ml and $1\times10^3$ spores/ml treatments. Surprisingly, in the repeat trial, none of the treatments resulted in a significant reduction of the shoot dry weight of the seedlings. However, the mean root dry weight of the treatments decreased with increasing inoculum concentration. In both instances, $1\times10^6$ spores/ml reduced the root dry weight of the seedlings more than all the other treatments. Significant reduction of total dry weight was recorded with inoculum concentrations of $1\times10^6$ spores/ml, $1\times10^5$ spores/ml and $1\times10^4$ spores/ml compared to the control in the first trial; however, this reduction was not observed in the second trial. An inoculum concentration of $1\times10^6$ spores/ml was thus selected for use in the screening of the isolates.

### 3.3.2 Screening of rhizobacteria for biocontrol activity against *F. pseudograminearum*

With the first batch of bacterial isolates tested, significant differences between treatments and the controls were evident in the dry weight data (Table 3.2). In the presence of *F. pseudograminearum*, over half (62%), of the bacterial isolates caused an increase in shoot dry weight of wheat, whereas 17% of the isolates caused an increase in root dry weight compared to the pathogen inoculated control. Fifty-five % of the isolates increased the overall growth of the seedlings in the presence of *F. pseudograminearum* compared to the pathogen-inoculated control. Some of the isolates that resulted in the highest significant shoot dry weight compared to the pathogen-inoculated control included isolates N19, A26, A21, N37, A33, N44, T11, N54, A34, N20, T19AC and T22. However, the weight of these treatments did not differ significantly from one another. Only isolates N19 and A26 caused a significant increase in shoot dry weight compared to the uninoculated control. Isolates that resulted in significant root dry weight compared to the pathogen-inoculated control were N44, N28, N54, N65 and N59A. Of these isolates, only N44 resulted in statistically significant root dry weight compared to the uninoculated control. Significant total dry weight increase was observed with isolates N44, N19, A26, N37, N28, A21, T19AC, A33, N54, N65, N59A, A34, N20, T22, A14Y and T11 in the presence of the pathogen. These isolates caused an increase of 44.74% to 85.53% on the total seedling weight compared to the diseased control while 14.58% to 46.88% of this was due to plant growth promotion effect on the seedlings. Again, only N44 significantly increased the total dry weight of the seedlings compared to the uninoculated control.

In the second batch (Table 3.3), 88% of the isolates significantly increased the shoot dry weight of the seedlings in the presence of *F. pseudograminearum* compared to the pathogen inoculated control. These isolates were A28, N66, A36, A45, A25, A35, N77Y, A06, KBS1F3, N04AC, A08, N30, N67, A46, A07, N16W, N28, A29, N33, N01, N31 and N03. All these isolates, except A46, A07, N16W, N28, A29, N33, N01, N31 and N03, significantly increased the shoot dry weight of the seedlings compared to the uninoculated control. Of the 25 isolates in batch two, 20% were able to significantly increase root dry weight in the presence of the pathogen. These isolates were N71, N66, N67, N04AC and N56. The isolates also significantly increased root dry weight compared to the uninoculated control. Overall, 84% of the isolates significantly increased the total dry weight of wheat seedling in the presence of the pathogen compared to the inoculated control. These isolates were N71, N66, A28, N67, N04AC, A45, A25, N16W, N77Y, A36, N30, N01, N28, A08, A35, N56, A06, KBS1F3, A46, N33 and N69. All isolates, except N56, A06, KBS1F3, A46, N33 and N69, also differed significantly from the uninoculated control. These isolates caused an increase of 48.61% to 123.61% on the total seedling weight compared to the diseased control while 37.18% to 106.41% of this was due to plant growth promotion effect on the seedlings.

In the third batch (Table 3.4), the shoot dry weight of the wheat seedlings was significantly increased by 44.83% of the isolates compared to the pathogen inoculated control. Some of the isolates that resulted in the highest shoot weight included N63, N12, A20, A47, N38, N51, N59B, N27, N53 and A09, N60, A39 and N39. These isolates also significantly increased the shoot dry weight of the seedlings compared to the uninoculated control. Compared to the pathogen inoculated control, the root dry weight of the seedling was increased by 20.69% of the isolates; namely, N51, N60, A20, N63, N27 and A09. All isolates, except N27 and A09, significantly increased the root dry weight of the seedlings compared to the uninoculated control. The total dry weight of the wheat seedlings was increased significantly by 37.93% of the isolates in the batch. These were N63, N51, A20, N27, N60, N38, A09, A47, N53, N39 and N59B. The isolates also significantly increased the total seedling weight compared to the uninoculated control. These isolates caused an increase of 39.13% to 69.57% on the total seedling weight compared to the diseased control while 33.33% to 62.50% of this was due to plant growth promotion effect on the seedlings.

In the fourth batch, the dry shoot was increased by 30% of the isolates (Table 3.5). These isolates included N02, A14W, T29AC, N32, NAS6G6, A17, N55B, N29 and A16. In addition to this, they were also different from the uninoculated control. The root dry weight was

increased by 10% of the isolates. These were N74, A17 and N02. However, only N74 was significantly different from the uninoculated control. The total dry weight of the seedlings was significantly increased by 16.7% of the isolates. These were N02, N74, A17, T29AC and N55B. These isolates caused an increase of 40.74% to 54.32% on the total seedling weight compared to the diseased control while 32.56% to 45.35% of this was due to plant growth promotion effect on the seedlings. Only N02, N74, A17 and T29AC significantly increased the total dry weight of the seedlings compared to the uninoculated control.

### 3.4 Discussion

The purpose of this chapter was to screen a collection 113 rhizobacterial isolates for biocontrol activity against crown rot caused by *F. pseudograminearum* on wheat and enhancement of growth of wheat seedlings in the presence of the pathogen. Percentage change in weight compared to the negative control gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight compared to the positive control reflects a combination of direct growth promotion and biocontrol activity. Subtracting the former values from the latter values gives an indication of biocontrol effect (Table 3.2 to 3.5). It was observed that some of the isolates were only able to increase the growth of diseased seedlings while others could suppress the disease and improve plant growth. Due to the inconsistencies observed with the fresh weight of the seedlings caused by the handling of the seedlings from harvesting, washing and time taken to record the weight, only the dry weight was used for the selection of effective isolates.

Crown rot is highly favoured by drought stress on the plant. The application of PGPR to wheat could help alleviate the effects of drought on the crop while still providing protection against pathogens. Singh and Jha (2017) found that the application of the PGPR *Stenotrophomonas maltophilia* SBP-9 under wheat salt stress could increase the fresh or dry weight of the plant by up to 42% and increase the plant chlorophyll content by up to 59%. They also found that the application of the PGPR to wheat increased the levels of β-1,3-glucanase, phenylalanine ammonia-lyase (PAL) and polyphenol oxidase, which are involved in the breakdown of fungal cell walls and synthesis of phytoalexins and phenolics respectively and provide protection against pathogens (Singh and Jha 2017). Similarly, Baffoni et al. (2015) found that the application of *Lactobacillus plantarum* SLG17 and *Bacillus amyloliquefaciens* FLN13 on wheat from the heading period until anthesis of the plants provided better protection against Fusarium head blight in the field.

The effects of drought stress on crops can also be reduced by isolates that possess PGPR traits associated with plant growth promotion. These traits may assist the plant escape infection by the pathogen. In the current study, we found that the increase in plant growth of the seedlings was due to the plant growth-promoting ability of the isolates in addition to disease control. At most 106.41% of the observed seedling growth was due to the plant growth ability of the isolates. This growth may be due to nitrogen fixation, phosphate solubilisation or phytohormone production of the isolates (Goswami et al. 2016; Lwin et al. 2017; Vejan et al. 2016). This was confirmed by the *in-vitro* tests done on the isolates (chapter 5). In addition to its plant growth stimulating ability, IAA has also been reported to induce systemic resistance against root rot in cotton (Egamberdieva et al. 2015). According to these workers, root rot disease incidence went down from 70% to 41-56% upon inoculation with low concentrations of IAA. In a study conducted by Mavrodi et al. (2012), the application of PGPR increased shoot length and root weight of wheat and plant growth promotion was correlated with disease suppression.

Other methods to rapidly screen large collections of rhizobacteria can be explored. Ishiga et al. (2011) developed a petri dish seedling assay to study the plant-bacteria interaction between *Arabidopsis thaliana* and *Pseudomonas syringae*. Comby et al. (2017) tested wheat pathogenicity on wheat spikelets, growth capacity and the antagonistic activities of strains on the targeted plant. These types of tests allow for a rapid *in vitro* way of testing the efficacy of rhizobacterial isolates on a smaller scale. They suggested that it could allow for more relevant selection of rhizobacterial isolates to be retained for larger scale trials on whole plants. However, they are limited in that they do not assess the activity of the isolates in the natural environment in the presence of soil and unstable conditions.

As the numbers of applied PGPR rapidly decline in the soil overtime, multiple applications can be done to supplement the first application thus increasing the efficacy of the PGPR (Bull et al. 1991; Georgakopoulos et al. 2002; Milus and Rothrock 1997). Further research is needed to assess the efficacy of the isolates that gave significant results from the control on a larger scale. The efficacy of the isolates that performed the best including those that did not perform the best could be screened on other crops besides wheat and against other pathogens besides *F. pseudograminearum*. The capability of the isolates should also be assessed in the greenhouse using unsterilized soil to mimic soil in the field. In addition, the capability of the isolates can be assessed in the field exposed to the natural environment.

### 3.5 Tables and Figures

**Table 3.1** Effect of *Fusarium pseudograminearum* inoculum concentration on wheat seedlings in seedling trays in the greenhouse.

| Trial 1 | Seedling weight (g) | | | | | | Percentage change in weight compared to un-inoculated control | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[1] | | | Mean dry weight[1] | | | Mean fresh weight[1] | | | Mean dry weight[1] | | |
| **Inoculum concentration (spores/ml)** | **Shoot** | **Root** | **Total** | **Shoot** | **Root** | **Total** | **Shoot** | **Root** | **Total** | **Shoot** | **Root** | **Total** |
| 0 (control) | 0.49$^{ab}$ | 0.29$^{a}$ | 0.57$^{a}$ | 0.21$^{ab}$ | 0.21$^{a}$ | 0.29$^{a}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $10^3$ | 0.51$^{a}$ | 0.27$^{b}$ | 0.58$^{a}$ | 0.22$^{a}$ | 0.19$^{ab}$ | 0.29$^{a}$ | 4.08 | 6.90 | 1.75 | 4.76 | -9.52 | 0 |
| $10^4$ | 0.49$^{ab}$ | 0.26$^{b}$ | 0.55$^{ab}$ | 0.20$^{bc}$ | 0.18$^{b}$ | 0.27$^{b}$ | 0 | -10.34 | -3.51 | -4.76 | -14.29 | -6.87 |
| $10^5$ | 0.48$^{ab}$ | 0.26$^{b}$ | 0.55$^{ab}$ | 0.19$^{bc}$ | 0.16$^{b}$ | 0.27$^{b}$ | -2.04 | -10.34 | -3.51 | -9.52 | -23.81 | -6.87 |
| $10^6$ | 0.45$^{b}$ | 0.23$^{c}$ | 0.51$^{b}$ | 0.19$^{c}$ | 0.17$^{c}$ | 0.25$^{b}$ | -8.16 | 20.69 | -10.53 | -9.52 | -19.05 | -13.79 |
| LSD[2] | 0.04 | 0.02 | 0.04 | 0.02 | 0.01 | 0.01 | | | | | | |
| CV[2] (%) | 5.95 | 5.20 | 5.46 | 4.89 | 5.16 | 4.71 | | | | | | |
| **Trial 2** | | | | | | | | | | | | |
| 0 (control) | 0.79$^{a}$ | 0.64$^{a}$ | 1.02$^{a}$ | 0.28$^{c}$ | 0.19$^{a}$ | 0.34$^{d}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| $10^3$ | 0.78$^{a}$ | 0.53$^{b}$ | 0.94$^{ab}$ | 0.36$^{a}$ | 0.18$^{a}$ | 0.40$^{b}$ | -1.27 | -17.19 | -7.84 | 28.57 | -5.26 | 17.65 |
| $10^4$ | 0.77$^{a}$ | 0.36$^{c}$ | 0.85$^{bc}$ | 0.34$^{a}$ | 0.18$^{a}$ | 0.38$^{bc}$ | -2.53 | -43.79 | -16.67 | 21.43 | -5.26 | 11.76 |
| $10^5$ | 0.71$^{a}$ | 0.36$^{c}$ | 0.80$^{c}$ | 0.33$^{ab}$ | 0.18$^{a}$ | 0.45$^{a}$ | -10.13 | -43.79 | -21.57 | 17.86 | -5.26 | 32.35 |
| $10^6$ | 0.49$^{b}$ | 0.28$^{d}$ | 0.57$^{d}$ | 0.31$^{b}$ | 0.16$^{b}$ | 0.35$^{cd}$ | -37.97 | -56.25 | -44.12 | 10.71 | -15.79 | 2.94 |
| LSD[2] | 0.09 | 0.06 | 0.11 | 0.03 | 0.013 | 0.04 | | | | | | |
| CV[2] (%) | 9.11 | 9.44 | 8.62 | 5.36 | 4.70 | 6.33 | | | | | | |

[1]Means have been square-root transformed. Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100]. Negative values imply weight that is less than that of the un-inoculated control and positive values imply weight higher than that of the un-inoculated control.
[2]LSD= significant difference. CV= coefficient of variance expressed as a percentage.

**Table 3.2** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 1).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.38$^{abcd}$ | 0.29$^{a}$ | 0.67$^{ab}$ | 0.055$^{bcdefgh}$ | 0.042$^{bcdef}$ | 0.096$^{bcdef}$ | 11.76 | 7.41 | 9.83 | 27.91 | 23.53 | 26.32 | 0 | 0 | 0 |
| PC[1] | 0.34$^{abcde}$ | 0.27$^{a}$ | 0.61$^{ab}$ | 0.043$^{h}$ | 0.034$^{ef}$ | 0.076$^{f}$ | 0 | 0 | 0 | 0 | 0 | 0 | -21.82 | -19.05 | 20.83 |
| A05A | 0.34$^{abcde}$ | 0.25$^{a}$ | 0.59$^{ab}$ | 0.060$^{abcdefgh}$ | 0.041$^{bcdef}$ | 0.099$^{bcdef}$ | 0 | -7.41 | -3.28 | 39.53 | 20.59 | 30.26 | 9.09 | -2.38 | 3.13 |
| A10 | 0.29$^{de}$ | 0.30$^{a}$ | 0.57$^{ab}$ | 0.050$^{gh}$ | 0.037$^{def}$ | 0.085$^{def}$ | -14.71 | 11.11 | -6.56 | 16.28 | 8.82 | 11.84 | -9.09 | -11.90 | 11.46 |
| A14Y | 0.41$^{abc}$ | 0.33$^{a}$ | 0.74$^{a}$ | 0.063$^{abcdefg}$ | 0.047$^{bcde}$ | 0.110$^{abcde}$ | 20.59 | 22.22 | 23.31 | 46.51 | 38.24 | 44.74 | 14.55 | 11.90 | 14.58 |
| A21 | 0.38$^{abcd}$ | 0.29$^{a}$ | 0.67$^{ab}$ | 0.073$^{ab}$ | 0.047$^{bcde}$ | 0.120$^{abc}$ | 11.76 | 7.41 | 9.83 | 69.77 | 38.24 | 57.89 | 32.73 | 11.90 | 25 |
| A24 | 0.36$^{abcde}$ | 0.29$^{a}$ | 0.65$^{ab}$ | 0.062$^{abcdefg}$ | 0.045$^{bcdef}$ | 0.107$^{bcdef}$ | 5.88 | 7.41 | 6.56 | 44.19 | 32.35 | 40.78 | 12.73 | 7.14 | 11.46 |
| A26 | 0.35$^{abcde}$ | 0.26$^{a}$ | 0.61$^{ab}$ | 0.077$^{a}$ | 0.049$^{bcde}$ | 0.125$^{ab}$ | 2.94 | -3.70 | 0 | 79.07 | 44.12 | 64.47 | 40 | 16.67 | 30.21 |
| A32 | 0.34$^{abcde}$ | 0.29$^{a}$ | 0.63$^{ab}$ | 0.060$^{abcdefgh}$ | 0.039$^{cdef}$ | 0.098$^{bcdef}$ | 0 | 7.41 | 3.28 | 39.53 | 14.71 | 28.95 | 9.09 | -7.14 | 2.08 |
| A33 | 0.37$^{abcde}$ | 0.30$^{a}$ | 0.67$^{ab}$ | 0.071$^{abc}$ | 0.045$^{bcdef}$ | 0.117$^{abcd}$ | 8.82 | 11.11 | 9.83 | 65.11 | 32.35 | 53.95 | 29.09 | 7.14 | 21.88 |
| A34 | 0.38$^{abcd}$ | 0.30$^{a}$ | 0.68$^{ab}$ | 0.069$^{abcdef}$ | 0.043$^{bcdef}$ | 0.114$^{abcd}$ | 11.76 | 11.11 | 11.48 | 60.47 | 26.47 | 50 | 25.45 | 2.38 | 18.75 |
| A40 | 0.27$^{e}$ | 0.30$^{a}$ | 0.57$^{ab}$ | 0.052$^{fgh}$ | 0.045$^{bcdef}$ | 0.096$^{bcdef}$ | -20.59 | 11.11 | -6.56 | 20.93 | 32.35 | 26.32 | -5.45 | 7.14 | 0 |
| N13 | 0.32$^{bcde}$ | 0.29$^{a}$ | 0.62$^{ab}$ | 0.059$^{abcdefgh}$ | 0.040$^{cdef}$ | 0.098$^{bcdef}$ | -5.88 | 7.41 | 1.64 | 37.21 | 17.65 | 28.95 | 7.27 | -4.76 | 2.08 |
| N17 | 0.34$^{abcde}$ | 0.29$^{a}$ | 0.63$^{ab}$ | 0.064$^{abcdef}$ | 0.044$^{bcdef}$ | 0.107$^{bcdef}$ | 0 | 7.41 | 3.28 | 48.84 | 29.41 | -42.11 | 16.36 | 4.76 | 11.46 |
| N19 | 0.42$^{a}$ | 0.32$^{a}$ | 0.74$^{a}$ | 0.077$^{a}$ | 0.049$^{bcde}$ | 0.125$^{ab}$ | 23.53 | 18.52 | 23.31 | 79.07 | 44.12 | 64.47 | 40 | 16.67 | 30.21 |
| N20 | 0.36$^{abcde}$ | 0.30$^{a}$ | 0.66$^{ab}$ | 0.069$^{abcdef}$ | 0.047$^{bcde}$ | 0.114$^{abcd}$ | 5.88 | 11.11 | 8.19 | 60.47 | 38.24 | 50 | 25.45 | 11.90 | 18.75 |
| N28 | 0.37$^{abcde}$ | 0.35$^{a}$ | 0.72$^{a}$ | 0.064$^{abcdefg}$ | 0.056$^{abc}$ | 0.120$^{abc}$ | 8.82 | 29.63 | 18.03 | 48.84 | 64.71 | 57.89 | 16.36 | 33.33 | 25 |
| N34 | 0.34$^{abcde}$ | 0.33$^{a}$ | 0.67$^{ab}$ | 0.052$^{cdefgh}$ | 0.039$^{cdef}$ | 0.091$^{cdef}$ | 0 | 22.22 | 9.83 | 20.93 | 14.71 | 19.74 | -5.45 | -.7.14 | -5.21 |
| LSD[2] | 0.095 | 0.11 | 0.19 | 0.019 | 0.016 | 0.032 | | | | | | | | | |
| CV[2] (%) | 23.73 | 33.12 | 25.40 | 27.00 | 31.37 | 25.78 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.2**. The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 1 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.38$^{abcd}$ | 0.29$^{a}$ | 0.67$^{ab}$ | 0.055$^{bcdefgh}$ | 0.042$^{bcdef}$ | 0.096$^{bcdef}$ | 11.76 | 7.41 | 9.83 | 27.91 | 23.53 | 26.32 | 0 | 0 | 0 |
| PC[1] | 0.34$^{abcde}$ | 0.27$^{a}$ | 0.61$^{ab}$ | 0.043$^{h}$ | 0.034$^{ef}$ | 0.076$^{f}$ | 0 | 0 | 0 | 0 | 0 | 0 | -21.82 | -19.05 | 20.83 |
| N37 | 0.41$^{ab}$ | 0.33$^{a}$ | 0.74$^{a}$ | 0.072$^{ab}$ | 0.050$^{bcde}$ | 0.122$^{abc}$ | 20.59 | 22.22 | 23.31 | 67.44 | 47.06 | 60.53 | 30.91 | 19.05 | 27.08 |
| N43 | 0.33$^{bcde}$ | 0.31$^{a}$ | 0.64$^{ab}$ | 0.052$^{efgh}$ | 0.041$^{bcdef}$ | 0.093$^{cdef}$ | -2.94 | 14.81 | 4.92 | 20.93 | 20.59 | 22.37 | -5.45 | -2.38 | -3.13 |
| N44 | 0.40$^{abc}$ | 0.32$^{a}$ | 0.71$^{a}$ | 0.071$^{abcd}$ | 0.069$^{a}$ | 0.141$^{a}$ | 17.65 | 18.52 | 16.39 | 65.11 | 102.94 | 85.53 | 29.09 | 64.29 | 46.88 |
| N54 | 0.35$^{abcde}$ | 0.31$^{a}$ | 0.66$^{ab}$ | 0.064$^{abcdefg}$ | 0.053$^{abc}$ | 0.116$^{abcd}$ | 2.94 | 14.81 | 8.19 | 48.84 | 55.88 | 52.63 | 16.36 | 26.19 | 20.83 |
| N59A | 0.36$^{abcde}$ | 0.32$^{a}$ | 0.68$^{ab}$ | 0.064$^{abcdefg}$ | 0.050$^{bcd}$ | 0.115$^{abcd}$ | 5.88 | 18.52 | 11.48 | 48.84 | 47.06 | 51.32 | 16.36 | 19.05 | 19.79 |
| N59C | 0.31$^{cde}$ | 0.29$^{a}$ | 0.61$^{ab}$ | 0.051$^{fgh}$ | 0.047$^{bcde}$ | 0.098$^{bcdef}$ | -8.82 | 7.41 | 0 | 18.60 | 38.24 | 28.95 | -7.27 | 11.91 | 2.08 |
| N65 | 0.38$^{abcd}$ | 0.34$^{a}$ | 0.72$^{a}$ | 0.062$^{abcdefg}$ | 0.053$^{bcd}$ | 0.115$^{abcd}$ | 11.76 | 25.93 | 18.03 | 44.19 | 55.88 | 51.32 | 12.73 | 26.19 | 19.79 |
| N70 | 0.35$^{abcde}$ | 0.26$^{a}$ | 0.60$^{ab}$ | 0.056$^{bcdefgh}$ | 0.043$^{bcdef}$ | 0.10$^{bcdef}$ | 2.94 | -3.70 | -1.64 | 30.23 | 26.47 | 31.58 | 1.81 | 2.38 | 4.17 |
| N76 | 0.33$^{abcde}$ | 0.32$^{a}$ | 0.65$^{ab}$ | 0.056$^{bcdefgh}$ | 0.030$^{f}$ | 0.097$^{bcdef}$ | -2.94 | 18.52 | 6.56 | 30.23 | -11.76 | 27.63 | 1.81 | 28.57 | 1.04 |
| N77W | 0.28$^{e}$ | 0.24$^{a}$ | 0.52$^{b}$ | 0.052$^{defgh}$ | 0.039$^{cdef}$ | 0.083$^{ef}$ | -17.65 | -11.11 | -14.75 | 20.93 | 14.71 | 9.21 | -5.45 | -7.14 | 13.54 |
| T11 | 0.38$^{abcd}$ | 0.26$^{a}$ | 0.64$^{ab}$ | 0.071$^{abcd}$ | 0.039$^{cdef}$ | 0.110$^{abcde}$ | 11.76 | -3.70 | 4.92 | 65.11 | 14.71 | 44.74 | 29.09 | -7.14 | 14.58 |
| T22 | 0.35$^{abcde}$ | 0.35$^{a}$ | 0.70$^{ab}$ | 0.065$^{abcdef}$ | 0.049$^{bcde}$ | 0.114$^{abcde}$ | 2.94 | 29.63 | 14.75 | 51.16 | 44.12 | 50 | 18.18 | 16.67 | 18.75 |
| T19AC | 0.40$^{abc}$ | 0.31$^{a}$ | 0.72$^{a}$ | 0.066$^{abcdefg}$ | 0.048$^{bcde}$ | 0.119$^{abc}$ | 17.65 | 14.81 | 18.03 | 53.49 | 41.18 | 56.58 | 20 | 14.59 | 23.96 |
| LSD[2] | 0.095 | 0.11 | 0.19 | 0.019 | 0.016 | 0.032 | | | | | | | | | |
| CV[2] (%) | 23.73 | 33.12 | 25.40 | 27.00 | 31.37 | 25.78 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.3** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 2).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.251$^{g}$ | 0.233$^{cde}$ | 0.485$^{g}$ | 0.044$^{gh}$ | 0.034$^{h}$ | 0.078$^{de}$ | -12.85 | 0.43 | -6.73 | 25.71 | -8.11 | 8.33 | 0 | 0 | 0 |
| PC[1] | 0.288$^{defg}$ | 0.232$^{de}$ | 0.520$^{cdefg}$ | 0.035$^{h}$ | 0.037$^{gh}$ | 0.072$^{e}$ | 0 | 0 | 0 | 0 | 0 | 0 | -20.45 | 8.82 | -7.69 |
| A06 | 0.374$^{abcd}$ | 0.332$^{abc}$ | 0.706$^{ab}$ | 0.069$^{bcdef}$ | 0.043$^{efgh}$ | 0.112$^{bcd}$ | 29.86 | 43.10 | 35.77 | 97.14 | 16.22 | 55.56 | 56.82 | 26.47 | 43.59 |
| A07 | 0.357$^{abcde}$ | 0.309$^{abcde}$ | 0.666$^{abcdef}$ | 0.062$^{cdefg}$ | 0.046$^{defgh}$ | 0.104$^{bcde}$ | 23.96 | 33.19 | 28.08 | 77.14 | 24.32 | 44.44 | 40.91 | 35.29 | 33.33 |
| A08 | 0.353$^{abcde}$ | 0.311$^{abcde}$ | 0.663$^{abcdefg}$ | 0.065$^{bcdef}$ | 0.049$^{defgh}$ | 0.113$^{bc}$ | 22.57 | 34.05 | 27.50 | 85.71 | 32.43 | 56.94 | 47.73 | 44.12 | 44.87 |
| A25 | 0.359$^{abcde}$ | 0.314$^{abcd}$ | 0.674$^{abcde}$ | 0.071$^{bcde}$ | 0.047$^{defgh}$ | 0.119$^{bc}$ | 24.65 | 35.34 | 29.62 | 102.86 | 27.03 | 65.28 | 61.36 | 38.24 | 52.56 |
| A28 | 0.395$^{ab}$ | 0.315$^{abcd}$ | 0.706$^{ab}$ | 0.108$^{a}$ | 0.048$^{defgh}$ | 0.156$^{a}$ | 37.15 | 35.18 | 35.77 | 208.57 | 29.73 | 116.67 | 145.45 | 41.18 | 100 |
| A29 | 0.272$^{efg}$ | 0.241$^{bcde}$ | 0.513$^{defg}$ | 0.059$^{cdefg}$ | 0.039$^{fgh}$ | 0.098$^{cde}$ | -5.56 | 3.88 | -1.35 | 68.57 | 5.41 | 36.11 | 34.09 | 14.71 | 25.64 |
| A35 | 0.398$^{a}$ | 0.302$^{abcde}$ | 0.700$^{abc}$ | 0.071$^{bcde}$ | 0.042$^{fgh}$ | 0.113$^{bc}$ | 38.19 | 30.17 | 34.62 | 102.86 | 13.51 | 56.94 | 61.36 | 23.53 | 44.87 |
| A36 | 0.357$^{abcde}$ | 0.329$^{abcd}$ | 0.686$^{abcd}$ | 0.074$^{bc}$ | 0.041$^{fgh}$ | 0.115$^{bc}$ | 23.96 | 41.81 | 31.92 | 111.43 | 10.81 | 59.72 | 68.18 | 20.59 | 47.43 |
| A45 | 0.372$^{abcd}$ | 0.337$^{ab}$ | 0.709$^{ab}$ | 0.072$^{bcd}$ | 0.056$^{cdefg}$ | 0.128$^{abc}$ | 29.17 | 45.26 | 36.35 | 105.71 | 51.35 | 55.56 | 63.64 | 64.71 | 64.10 |
| A46 | 0.348$^{abcdef}$ | 0.281$^{abcde}$ | 0.629$^{abcdefg}$ | 0.062$^{cdefg}$ | 0.049$^{defgh}$ | 0.111$^{bcd}$ | 20.83 | 21.12 | 20.96 | 77.14 | 32.43 | 54.17 | 40.91 | 44.12 | 42.31 |
| KBS1F3 | 0.387$^{abc}$ | 0.297$^{abcde}$ | 0.683$^{abcde}$ | 0.066$^{bcdef}$ | 0.046$^{defgh}$ | 0.112$^{bcd}$ | 34.38 | 28.12 | 31.34 | 88.57 | 24.32 | 55.56 | 50 | 35.29 | 43.59 |
| N01 | 0.324$^{abcdef}$ | 0.297$^{abcde}$ | 0.620$^{abcdefg}$ | 0.057$^{cdefg}$ | 0.057$^{bcdef}$ | 0.114$^{bc}$ | 12.5 | 28.12 | 19.23 | 62.86 | 54.05 | 58.33 | 29.55 | 67.65 | 46.15 |
| N03 | 0.299$^{cdefg}$ | 0.287$^{abcde}$ | 0.585$^{abcdefg}$ | 0.055$^{defg}$ | 0.051$^{defgh}$ | 0.105$^{bcde}$ | 3.82 | 23.71 | 12.5 | 57.14 | 37.84 | 45.83 | 25 | 50 | 34.62 |
| N04AC | 0.368$^{abcd}$ | 0.319$^{abcd}$ | 0.653$^{abcdefg}$ | 0.065$^{bcdef}$ | 0.063$^{bcd}$ | 0.128$^{abc}$ | 27.78 | 37.50 | 25.58 | 85.71 | 70.27 | 77.78 | 47.73 | 85.29 | 64.10 |
| N16W | 0.348$^{abcdef}$ | 00.358$^{a}$ | 0.707$^{ab}$ | 0.062$^{cdefg}$ | 0.056$^{cdefg}$ | 0.118$^{bc}$ | 20.83 | 54.31 | 35.96 | 77.14 | 51.35 | 63.89 | 40.91 | 64.71 | 51.28 |
| LSD[2] | 0.093 | 0.099 | 0.180 | 0.019 | 0.019 | 0.035 | | | | | | | | | |
| CV[2] (%) | 24.19 | 29.65 | 25.16 | 26.77 | 32.60 | 26.38 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.3** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 2 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.251$^{g}$ | 0.233$^{cde}$ | 0.485$^{g}$ | 0.044$^{gh}$ | 0.034$^{h}$ | 0.078$^{de}$ | -12.85 | 0.43 | -6.73 | 25.71 | -8.11 | 8.33 | 0 | 0 | 0 |
| PC[1] | 0.288$^{defg}$ | 0.232$^{de}$ | 0.520$^{cdefg}$ | 0.035$^{h}$ | 0.037$^{gh}$ | 0.072$^{e}$ | 0 | 0 | 0 | 0 | 0 | 0 | -20.45 | 8.82 | -7.69 |
| N28 | 0.378$^{abcd}$ | 0.339$^{ab}$ | 0.717$^{ab}$ | 0.060$^{cdefg}$ | 0.053$^{cdefgh}$ | 0.113$^{bc}$ | 31.25 | 46.12 | 37.88 | 71.43 | 43.24 | 56.94 | 36.36 | 55.88 | 44.87 |
| N30 | 0.316$^{abcdef}$ | 0.274$^{abcde}$ | 0.590$^{abcdefg}$ | 0.064$^{cdef}$ | 0.051$^{defgh}$ | 0.115$^{bc}$ | 9.72 | 18.10 | 13.46 | 82.86 | 37.84 | 59.72 | 45.45 | 50 | 47.43 |
| N31 | 0.292$^{defg}$ | 0.213$^{e}$ | 0.505$^{efg}$ | 0.057$^{cdefg}$ | 0.046$^{defgh}$ | 0.103$^{bcde}$ | 1.39 | -8.19 | -2.88 | 62.86 | 24.32 | 43.06 | 29.55 | 35.29 | 32.05 |
| N33 | 0.258$^{fg}$ | 0.283$^{abcde}$ | 0.541$^{bcdefg}$ | 0.058$^{cdefg}$ | 0.051$^{cdefgh}$ | 0.110$^{bcd}$ | -10.42 | 21.98 | 4.04 | 65.74 | 37.84 | 52.78 | 31.82 | 50 | 41.03 |
| N56 | 0.246$^{g}$ | 0.243$^{bcde}$ | 0.488$^{fg}$ | 0.051$^{fgh}$ | 0.061$^{bcde}$ | 0.113$^{bcd}$ | -14.58 | 4.74 | -6.15 | 45.71 | 64.86 | 56.94 | 15.91 | 79.41 | 44.87 |
| N66 | 0.410$^{a}$ | 0.315$^{abcd}$ | 0.722$^{a}$ | 0.084$^{b}$ | 0.076$^{b}$ | 0.160$^{a}$ | 42.36 | 35.78 | 38.85 | 140 | 105.41 | 122.22 | 90.91 | 123.53 | 105.13 |
| N67 | 0.338$^{abcdefg}$ | 0.317$^{abcd}$ | 0.656$^{abcdefg}$ | 0.063$^{cdef}$ | 0.071 | 0.134$^{ab}$ | 17.36 | 36.64 | 26.15 | 80 | 91.89 | 86.11 | 43.18 | 108.82 | 71.79 |
| N69 | 0.304$^{bcdefg}$ | 0.297$^{abcde}$ | 0.601$^{abcdefg}$ | 0.052$^{efgh}$ | 0.055$^{cdefg}$ | 0.107$^{bcd}$ | 5.56 | 28.02 | 15.58 | 48.57 | 48.64 | 48.61 | 18.18 | 61.76 | 37.18 |
| N71 | 0.297$^{cdefg}$ | 0.245$^{bcde}$ | 0.542$^{bcdefg}$ | 0.052$^{defgh}$ | 0.109$^{a}$ | 0.161$^{a}$ | 3.13 | 5.60 | 4.23 | 48.57 | 194.59 | 123.61 | 18.18 | 220.59 | 106.41 |
| N77Y | 0.389$^{abc}$ | 0.298$^{abcde}$ | 0.687$^{abcd}$ | 0.069$^{bcdef}$ | 0.049$^{defgh}$ | 0.117$^{bc}$ | 35.07 | 28.45 | 32.12 | 97.14 | 32.43 | 62.50 | 56.82 | 44.12 | 50 |
| LSD[2] | 0.093 | 0.099 | 0.180 | 0.019 | 0.019 | 0.035 | | | | | | | | | |
| CV[2] (%) | 24.19 | 29.65 | 25.16 | 26.77 | 32.60 | 26.38 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants

**Table 3.4** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 3).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.279$^{ghij}$ | 0.294$^{abcdef}$ | 0.573$^{bcdef}$ | 0.037$^{hij}$ | 0.035$^{defghijk}$ | 0.072$^{fghijkl}$ | -0.36 | 35.48 | 15.06 | -2.63 | 9.38 | 4.35 | 0 | 0 | 0 |
| PC[1] | 0.280$^{ghij}$ | 0.217$^{fghi}$ | 0.498$^{fghi}$ | 0.038$^{hij}$ | 0.032$^{fghijklm}$ | 0.069$^{ghijkl}$ | 0 | 0 | 0 | 0 | 0 | 0 | 2.70 | -8.57 | -4.17 |
| A01 | 0.306$^{cdefghi}$ | 0.245$^{cdefgh}$ | 0.551$^{cdefg}$ | 0.046$^{defghi}$ | 0.032$^{fghijklm}$ | 0.078$^{defghijk}$ | 9.29 | 12.90 | 10.64 | 21.05 | 0 | 13.04 | 24.32 | -8.57 | 8.33 |
| A05B | 0.229$^{j}$ | 0.137$^{j}$ | 0.367$^{i}$ | 0.038$^{hij}$ | 0.017$^{n}$ | 0.056$^{jkl}$ | -18.21 | -40.10 | -26.31 | 0 | -46.88 | -18.84 | 2.70 | -51.43 | -22.22 |
| A05C | 0.291$^{fghij}$ | 0.211$^{ghij}$ | 0.502$^{fghi}$ | 0.039$^{hij}$ | 0.028$^{hijklmn}$ | 0.067$^{hijkl}$ | 3.93 | -2.76 | 0.80 | 2.63 | -12.5 | -2.89 | 5.40 | -20 | -6.94 |
| A07AC | 0.259$^{ij}$ | 0.135$^{j}$ | 0.395$^{hi}$ | 0.036$^{hij}$ | 0.025$^{ijklmn}$ | 0.062$^{ijkl}$ | -7.5 | 45.16 | -20.68 | -5.26 | -21.88 | -10.14 | -2.70 | -25.57 | -13.89 |
| A09 | 0.326$^{abcdefghi}$ | 0.255$^{bcdefgh}$ | 0.581$^{abcdef}$ | 0.056$^{abcde}$ | 0.048$^{abcde}$ | 0.104$^{abcd}$ | 16.43 | 17.51 | 16.67 | 47.37 | 50 | 50.72 | 51.35 | 37.14 | 44.44 |
| A10AC | 0.271$^{hij}$ | 0.253$^{bcdefgh}$ | 0.524$^{efgh}$ | 0.043$^{efghi}$ | 0.035$^{efghijkl}$ | 0.078$^{efghijk}$ | -3.21 | 16.59 | 5.22 | 13.16 | 9.38 | 13.04 | 16.22 | 0 | 8.33 |
| A20 | 0.349$^{abcdefg}$ | 0.315$^{abc}$ | 0.665$^{abcde}$ | 0.064$^{ab}$ | 0.051$^{ab}$ | 0.115$^{ab}$ | 24.64 | 45.16 | 33.53 | 68.42 | 59.38 | 66.67 | 72.97 | 45.71 | 59.72 |
| A22 | 0.288$^{fghij}$ | 0.272$^{bcdefg}$ | 0.491$^{fghi}$ | 0.026$^{j}$ | 0.021$^{lmn}$ | 0.047$^{l}$ | -18.57 | 25.35 | -1.41 | -31.57 | -34.38 | -31.88 | -29.73 | -40 | -70.83 |
| A37 | 0.281$^{ghij}$ | 0.134$^{j}$ | 0.415$^{ghi}$ | 0.039$^{hij}$ | 0.019$^{mn}$ | 0.059$^{jkl}$ | -21.07 | -38.25 | -16.67 | 2.63 | -40.63 | -14.49 | 5.41 | -45.71 | -18.06 |
| A39 | 0.380$^{abcd}$ | 0.287$^{abcdefg}$ | 0.667$^{abcde}$ | 0.055$^{abcdef}$ | 0.037$^{cdefghij}$ | 0.092$^{abcdefgh}$ | 35.71 | 32.26 | 33.94 | 44.74 | 15.63 | 33.33 | 48.65 | 5.71 | 27.78 |
| A47 | 0.366$^{abcde}$ | 0.318$^{abc}$ | 0.684$^{abcd}$ | 0.063$^{ab}$ | 0.038$^{bcdefgh}$ | 0.101$^{abcde}$ | 30.71 | 46.54 | 37.35 | 65.79 | 18.75 | 46.38 | 70.27 | 8.57 | 40.28 |
| N05 | 0.336$^{abcdefgh}$ | 0.253$^{bcdefgh}$ | 0.589$^{abcdef}$ | 0.051$^{bcdefg}$ | 0.037$^{cdefghijk}$ | 0.087$^{cdefghi}$ | 20 | 16.59 | 18.27 | 34.21 | 15.63 | 26.09 | 37.84 | 5.71 | 20.83 |
| N07 | 0.358$^{abcdef}$ | 0.306$^{abcd}$ | 0.664$^{abcde}$ | 0.050$^{bcdefgh}$ | 0.039$^{bcdefgh}$ | 0.089$^{bcdefgh}$ | 27.86 | 41.01 | 33.33 | 31.58 | 21.88 | 27.54 | 35.14 | 11.43 | 23.61 |
| N08 | 0.318$^{bcdefghi}$ | 0.237$^{defgh}$ | 0.552$^{cdefg}$ | 0.045$^{defghi}$ | 0.027$^{hijklmn}$ | 0.079$^{defghij}$ | 13.57 | 9.22 | 10.84 | 18.42 | -15.63 | 14.49 | 21.62 | -22.86 | 9.72 |
| N12 | 0.339$^{abcdefgh}$ | 0.283$^{abcdefg}$ | 0.622$^{abcdef}$ | 0.066$^{a}$ | 0.029$^{ghijklmn}$ | 0.094$^{abcdefg}$ | 21.07 | 30.41 | 24.89 | 76.68 | -9.38 | -7.24 | 78.37 | -17.14 | 30.56 |
| N16 | 0.325$^{abcdefghi}$ | 0.234$^{defgh}$ | 0.559$^{bcdefg}$ | 0.045$^{defghi}$ | 0.029$^{ghijklmn}$ | 0.074$^{fghijk}$ | 16.07 | 7.83 | 12.25 | 18.42 | -9.38 | 7.25 | 21.62 | -17.14 | 2.78 |
| LSD[2] | 0.075 | 0.078 | 0.15 | 0.015 | 0.014 | 0.026 | | | | | | | | | |
| CV[2] (%) | 20.51 | 27.16 | 23.08 | 25.83 | 34.93 | 27.06 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.4** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 3 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.279$^{ghij}$ | 0.294$^{abcdef}$ | 0.573$^{bcdef}$ | 0.037$^{hij}$ | 0.035$^{defghijk}$ | 0.072$^{fghijkl}$ | -0.36 | 35.48 | 15.06 | -2.63 | 9.38 | 4.35 | 0 | 0 | 0 |
| PC[1] | 0.280$^{ghij}$ | 0.217$^{fghi}$ | 0.498$^{fghi}$ | 0.038$^{hij}$ | 0.032$^{fghijklm}$ | 0.069$^{ghijkl}$ | 0 | 0 | 0 | 0 | 0 | 0 | 2.70 | -8.57 | -4.17 |
| N27 | 0.328$^{abcdefghi}$ | 0.312$^{abcd}$ | 0.643$^{abcdef}$ | 0.061$^{abc}$ | 0.048$^{abcd}$ | 0.109$^{abc}$ | 17.14 | 43.78 | 29.12 | 60.53 | 50 | 57.97 | 64.86 | 37.14 | 51.39 |
| N38 | 0.395$^{a}$ | 0.306$^{abcde}$ | 0.701$^{abc}$ | 0.062$^{abc}$ | 0.043$^{abcdef}$ | 0.105$^{abc}$ | 41.07 | 41.01 | 40.76 | 63.18 | 34.38 | 52.17 | 67.57 | 22.86 | 45.83 |
| N39 | 0.345$^{abcdefgh}$ | 0.268$^{bcdefgh}$ | 0.613$^{abcdef}$ | 0.054$^{abcdefg}$ | 0.043$^{abcdef}$ | 0.097$^{abcdef}$ | 23.21 | 23.50 | 23.09 | 42.11 | 34.38 | 40.58 | 45.95 | 22.86 | 34.72 |
| N41 | 0.318$^{bcdefghi}$ | 0.191$^{hij}$ | 0.509$^{fghi}$ | 0.048$^{cdefgh}$ | 0.025$^{ijklmn}$ | 0.072$^{fghijkl}$ | 13.57 | -11.98 | 2.21 | 26.32 | -21.88 | 4.35 | 29.73 | -28.57 | 0 |
| N51 | 0.368$^{abcd}$ | 0.361$^{a}$ | 0.729$^{a}$ | 0.062$^{abc}$ | 0.054$^{a}$ | 0.116$^{a}$ | 31.43 | 66.36 | 46.39 | 63.18 | 68.75 | 68.12 | 67.57 | 54.29 | 61.11 |
| N53 | 0.334$^{abcdefgh}$ | 0.297$^{abcde}$ | 0.631$^{abcdef}$ | 0.058$^{abcd}$ | 0.042$^{abcdefg}$ | 0.100$^{abcde}$ | 19.29 | 36.87 | 26.71 | 52.63 | 31.25 | 44.92 | 56.76 | 20 | 52.78 |
| N58 | 0.306$^{defghi}$ | 0.218$^{fghi}$ | 0.523$^{efgh}$ | 0.040$^{ghij}$ | 0.023$^{klmn}$ | 0.063$^{ijkl}$ | 9.29 | 0.46 | 5.02 | 5.26 | 28.13 | -8.69 | 8.11 | -34.29 | 12.5 |
| N59 | 0.301$^{efghij}$ | 0.234$^{defgh}$ | 0.536$^{defgh}$ | 0.041$^{fghi}$ | 0.026$^{hijklmn}$ | 0.067$^{hijkl}$ | 7.5 | 7.83 | 7.63 | 7.89 | -18.75 | -2.89 | 10.81 | -25.71 | -6.94 |
| N59B | 0.302$^{efghij}$ | 0.236$^{defgh}$ | 0.538$^{defgh}$ | 0.061$^{abc}$ | 0.035$^{efghijkl}$ | 0.096$^{abcdef}$ | 7.86 | 8.76 | 8.03 | 60.53 | 9.38 | 39.13 | 64.86 | 0 | 33.33 |
| N60 | 0.380$^{abc}$ | 0.325$^{ab}$ | 0.706$^{ab}$ | 0.056$^{abcdef}$ | 0.051$^{ab}$ | 0.107$^{abc}$ | 35.71 | 49.77 | 41.77 | 47.37 | 59.38 | 55.07 | 51.35 | 45.71 | 48.61 |
| N62 | 0.286$^{fghij}$ | 0.228$^{efghi}$ | 0.514$^{fghi}$ | 0.046$^{defghi}$ | 0.029$^{fghijklmn}$ | 0.075$^{efghijk}$ | 2.15 | 5.07 | 3.21 | 21.05 | -9.38 | 8.69 | 24.32 | -17.14 | 4.17 |
| N63 | 0.385$^{ab}$ | 0.318$^{abc}$ | 0.702$^{ab}$ | 0.068$^{a}$ | 0.049$^{abc}$ | 0.117$^{a}$ | 37.5 | 46.54 | 40.96 | 78.95 | 53.13 | 69.57 | 83.78 | 40 | 62.5 |
| N64 | 0.272$^{hij}$ | 0.149$^{ij}$ | 0.421$^{ghi}$ | 0.033$^{ij}$ | 0.019$^{mn}$ | 0.052$^{kl}$ | -2.86 | -31.34 | -15.46 | -13.16 | -40.63 | -24.64 | -10.81 | -5.71 | -27.78 |
| LSD[2] | 0.075 | 0.078 | 0.15 | 0.015 | 0.014 | 0.026 | | | | | | | | | |
| CV[2] (%) | 20.51 | 27.16 | 23.08 | 25.83 | 34.93 | 27.06 | | | | | | | | | |

[1]NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.5** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 4).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean fresh weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.337$^{bcdefg}$ | 0.262$^{cdefg}$ | 0.598$^{abcde}$ | 0.047$^{efghi}$ | 0.039$^{bcdef}$ | 0.086$^{defghi}$ | 5.31 | 13.04 | 13.04 | 4.44 | 8.33 | 6.17 | 0 | 0 | 0 |
| PC[1] | 0.320$^{bcdefg}$ | 0.209$^{gh}$ | 0.529$^{def}$ | 0.045$^{fghi}$ | 0.036$^{def}$ | 0.081$^{fghij}$ | 0 | 0 | 0 | 0 | 0 | 0 | -4.26 | -7.69 | -5.81 |
| A12 | 0.323$^{bcdefg}$ | 0.252$^{cdefg}$ | 0.575$^{bcde}$ | 0.056$^{abcdefgh}$ | 0.050$^{abcd}$ | 0.106$^{abcdefg}$ | -27.50 | 20.57 | 8.69 | 24.44 | 38.89 | 30.86 | 19.15 | 28.21 | 23.56 |
| A14W | 0.326$^{bcdefg}$ | 0.241$^{defg}$ | 0.567$^{bcdef}$ | 0.069$^{ab}$ | 0.034$^{efg}$ | 0.103$^{abcdefgh}$ | 1.88 | 15.31 | 7.18 | 53.33 | -5.56 | 27.16 | 46.81 | -12.82 | 19.77 |
| A16 | 0.386$^{abcd}$ | 0.242$^{cdefg}$ | 0.628$^{abcde}$ | 0.063$^{abcde}$ | 0.045$^{bcdef}$ | 0.108$^{abcdefg}$ | 20.63 | 15.79 | 18.71 | 40 | 25 | 33.33 | 34.04 | 15.38 | 25.58 |
| A17 | 0.368$^{abcde}$ | 0.246$^{cdefg}$ | 0.615$^{abcde}$ | 0.065$^{abcd}$ | 0.053$^{ab}$ | 0.118$^{abc}$ | 15 | 17.70 | 16.26 | 44.44 | 47.22 | 45.68 | 38.29 | 35.89 | 37.21 |
| A19 | 0.352$^{bcdefg}$ | 0.267$^{cdefg}$ | 0.691$^{abcde}$ | 0.061$^{abcdefgh}$ | 0.045$^{bcde}$ | 0.106$^{abcdefgh}$ | 10 | 27.75 | 30.62 | 35.56 | 25 | 30.86 | 29.79 | 15.38 | 23.26 |
| A27 | 0.353$^{bcdef}$ | 0.246$^{cdefg}$ | 0.599$^{abcde}$ | 0.056$^{abcdefgh}$ | 0.038$^{bcdef}$ | 0.094$^{bcdefghi}$ | 10.31 | 17.70 | 13.23 | 24.44 | 5.56 | 16.05 | 19.15 | -2.56 | 9.30 |
| A38 | 0.307$^{bcdefg}$ | 0.257$^{cdefg}$ | 0.564$^{bcdef}$ | 0.045$^{ghi}$ | 0.039$^{bcdef}$ | 0.083$^{efghij}$ | -4.06 | 22.97 | 6.62 | 0 | 8.33 | 2.47 | -4.26 | 0 | -3.49 |
| A41 | 0.271$^{gf}$ | 0.227$^{fgh}$ | 0.499$^{ef}$ | 0.044$^{hi}$ | 0.035$^{def}$ | 0.078$^{ghij}$ | -15.31 | 8.61 | -5.67 | -2.22 | -2.78 | -3.70 | -6.38 | -10.26 | -9.30 |
| A42 | 0.339$^{bcdefg}$ | 0.240$^{defg}$ | 0.579$^{bcde}$ | 0.056$^{abcdefgh}$ | 0.034$^{def}$ | 0.090$^{cdefghi}$ | 5.94 | 14.83 | 9.45 | 24.44 | -5.56 | 11.11 | 19.15 | -12.82 | 4.65 |
| A43 | 0.322$^{bcdefg}$ | 0.248$^{cdefg}$ | 0.569$^{bcdef}$ | 0.047$^{efghi}$ | 0.028$^{fg}$ | 0.076$^{hij}$ | 0.63 | 18.66 | 7.56 | 4.44 | -22.22 | -6.17 | 0 | -28.21 | -11.63 |
| A44 | 0.378$^{abcde}$ | 0.258$^{cdefg}$ | 0.636$^{abcde}$ | 0.062$^{abcdefg}$ | 0.045$^{bcde}$ | 0.107$^{abcdefg}$ | 18.13 | 23.44 | 20.23 | 37.78 | 25 | 32.09 | 31.91 | 15.38 | 24.42 |
| A48 | 0.293$^{defg}$ | 0.238$^{efg}$ | 0.532$^{def}$ | 0.047$^{defghi}$ | 0.033$^{efg}$ | 0.081$^{fghij}$ | 22.81 | 13.88 | 0.57 | 4.44 | -8.33 | 0 | 0 | -15.38 | -5.81 |
| N02 | 0.377$^{abcde}$ | 0.269$^{bcdefg}$ | 0.646$^{abcde}$ | 0.073$^{a}$ | 0.052$^{abc}$ | 0.125$^{a}$ | 17.81 | 28.71 | 22.12 | 62.22 | 44.44 | 54.32 | 55.32 | 33.33 | 45.35 |
| N10 | 0.285$^{efg}$ | 0.255$^{cdefg}$ | 0.520$^{def}$ | 0.051$^{cdefghi}$ | 0.033$^{efg}$ | 0.084$^{efghij}$ | -10.94 | 22.01 | -1.70 | 13.33 | -8.33 | 3.70 | 8.51 | -15.38 | -2.33 |
| N14 | 0.321$^{bcdefg}$ | 0.250$^{cdefg}$ | 0.571$^{bcdef}$ | 0.054$^{bcdefgh}$ | 0.034$^{def}$ | 0.089$^{cdefghi}$ | 0.31 | 19.62 | 7.94 | 20 | -5.56 | 9.87 | 14.89 | -12.82 | 3.49 |
| N15II | 0.323$^{bcdefg}$ | 0.244$^{cdefg}$ | 0.568$^{bcdef}$ | 0.046$^{efghi}$ | 0.037$^{bcdef}$ | 0.083$^{efghij}$ | -27.50 | 16.75 | 7.37 | 2.22 | 2.78 | 2.47 | -2.13 | -5.13 | -3.49 |
| LSD[2] | 0.093 | 0.076 | 0.156 | 0.018 | 0.016 | 0.031 | | | | | | | | | |
| CV[2] (%) | 24.15 | 25.85 | 22.93 | 27.96 | 35.22 | 28.03 | | | | | | | | | |

[1]NC - Negative control (uninoculated), PC - Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 3.5** The effect of rhizobacterial isolates on weight of *Fusarium pseudograminearum* inoculated wheat seedlings in seedling trays in the greenhouse (batch 4 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[4] | | | | | | Percentage change in weight B[5] | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.337$^{bcdefg}$ | 0.262$^{cdefg}$ | 0.598$^{abcde}$ | 0.047$^{efghi}$ | 0.039$^{bcdef}$ | 0.086$^{defghi}$ | 5.31 | 13.04 | 13.04 | 4.44 | 8.33 | 6.17 | 0 | 0 | 0 |
| PC[1] | 0.320$^{bcdefg}$ | 0.209$^{gh}$ | 0.529$^{def}$ | 0.045$^{fghi}$ | 0.036$^{def}$ | 0.081$^{fghij}$ | 0 | 0 | 0 | 0 | 0 | 0 | -4.26 | -7.69 | -5.81 |
| N21 | 0.312$^{bcdefg}$ | 0.231$^{efgh}$ | 0.544$^{cdef}$ | 0.047$^{efghi}$ | 0.037$^{bcdef}$ | 0.084$^{efghij}$ | -2.5 | 10.53 | 2.84 | 4.44 | 2.78 | 3.70 | 0 | -5.13 | -2.33 |
| N26 | 0.299$^{cdefg}$ | 0.237$^{efg}$ | 0.537$^{def}$ | 0.044$^{hi}$ | 0.036$^{cdef}$ | 0.080$^{fghij}$ | -6.56 | 13.39 | 0.95 | -2.22 | 0 | -1.23 | -4.26 | -7.69 | -6.98 |
| N29 | 0.356$^{abcdef}$ | 0.268$^{cdefg}$ | 0.623$^{abcde}$ | 0.063$^{abcde}$ | 0.039$^{bcdef}$ | 0.103$^{abcdefgh}$ | 11.25 | 28.23 | 17.77 | 40 | 8.33 | 27.16 | 34.04 | 0 | 19.77 |
| N32 | 0.448$^{a}$ | 0.306$^{abcde}$ | 0.754$^{a}$ | 0.068$^{abc}$ | 0.041$^{bcdef}$ | 0.109$^{abcdef}$ | 40 | 46.41 | 42.53 | 51.11 | 13.89 | 34.57 | 44.68 | 5.13 | 26.74 |
| N42 | 0.336$^{bcdefg}$ | 0.220$^{gh}$ | 0.557$^{bcdef}$ | 0.045$^{fghi}$ | 0.026$^{fg}$ | 0.072$^{ij}$ | 5 | 5.26 | 5.29 | 0 | -27.78 | -11.11 | -4.26 | -33.33 | -16.28 |
| N45 | 0.259$^{g}$ | 0.157$^{h}$ | 0.417$^{f}$ | 0.036$^{i}$ | 0.018$^{g}$ | 0.054$^{j}$ | -19.06 | -24.88 | -21.17 | -20 | -50 | -33.33 | -23.40 | -53.85 | -37.21 |
| N55A | 0.397$^{ab}$ | 0.298$^{abcdef}$ | 0.695$^{abc}$ | 0.061$^{abcdefgh}$ | 0.045$^{bcde}$ | 0.106$^{abcdefgh}$ | 24.06 | 42.48 | 31.38 | 35.56 | 25 | 30.86 | 29.79 | 15.38 | 23.26 |
| N55B | 0.376$^{abcde}$ | 0.318$^{abc}$ | 0.694$^{abc}$ | 0.065$^{abcd}$ | 0.048$^{abcde}$ | 0.114$^{abcde}$ | 17.50 | 52.15 | 31.19 | 44.44 | 33.33 | 40.74 | 38.29 | 23.08 | 32.56 |
| N68 | 0.273$^{fg}$ | 0.225$^{fgh}$ | 0.497$^{ef}$ | 0.051$^{cdefgh}$ | 0.041$^{bcdef}$ | 0.092$^{cdefghi}$ | 14.69 | 7.66 | -6.05 | 13.33 | 13.89 | 13.58 | 8.51 | 5.13 | 6.98 |
| N72 | 0.323$^{bcdefg}$ | 0.348$^{a}$ | 0.671$^{abcd}$ | 0.054$^{bcdefghi}$ | 0.047$^{abcde}$ | 0.101$^{abcdefghi}$ | 0.94 | 66.51 | 26.84 | 20 | 30.56 | 24.69 | 14.89 | 20.51 | 17.44 |
| N74 | 0.361$^{abcdef}$ | 0.345$^{ab}$ | 0.700$^{abc}$ | 0.062$^{abcdefg}$ | 0.061$^{a}$ | 0.123$^{ab}$ | 12.81 | 65.07 | 32.33 | 37.78 | 69.44 | 51.85 | 31.91 | 56.41 | 43.02 |
| N78 | 0.371$^{abcde}$ | 0.224$^{fgh}$ | 0.612$^{abcde}$ | 0.063$^{abcdef}$ | 0.036$^{def}$ | 0.099$^{abcdeghi}$ | 15.94 | 7.18 | 15.69 | 40 | 0 | 22.22 | 34.04 | -7.69 | 15.12 |
| NAS6G6 | 0.355$^{abcdef}$ | 0.318$^{abc}$ | 0.673$^{abcd}$ | 0.066$^{abc}$ | 0.042$^{bcdef}$ | 0.108$^{abcdefg}$ | 10.94 | 52.15 | 27.22 | 46.67 | 16.67 | 33.33 | 40.43 | 7.69 | 25.58 |
| T29AC | 0.386$^{abc}$ | 0.316$^{abcd}$ | 0.705$^{ab}$ | 0.068$^{abc}$ | 0.047$^{abcde}$ | 0.115$^{abcd}$ | 20.63 | 51.19 | 33.27 | 51.11 | 30.56 | 41.98 | 44.68 | 20.51 | 33.72 |
| LSD[2] | 0.093 | 0.076 | 0.156 | 0.018 | 0.016 | 0.031 | | | | | | | | | |
| CV[2] (%) | 24.15 | 25.85 | 22.93 | 27.96 | 35.22 | 28.03 | | | | | | | | | |

[1]NC - Negative control (uninoculated), PC - Positive control (pathogen inoculated).

[2]LSD = least significant difference. CV = coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[4]Percentage change in weight A = [(treatment-positive control)/positive control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control.

[5]Percentage change in weight B = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.


**Figure 3.1** Disease severity observable as lesions on the crown and stem of wheat seedlings inoculated with *F. pseudograminearum* at inoculum concentrations of: A= 0 (control), B= $10^3$, C= $10^4$, D= $10^5$ and E= $10^6$spores/ml.

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# CHAPTER 4

## Screening of rhizobacterial isolates for plant growth enhancement of wheat by means of seedling bioassays

**Abstract**

In screening experiments for PGPR growth promotion or biocontrol activity, a lack of correlation is often observed between the results of *in vitro* and *in planta* assays. The main aim of the current study is therefore to use a small-scale greenhouse seedling assay in which to screen a collection of rhizobacterial isolates for wheat growth enhancement and concomitantly do *in vitro* screening of the isolates for plant growth-promoting traits. The developed seedling assays were used to screen a collection of 113 rhizobacterial isolates for promotion of wheat seedling growth. Of the 113 isolates screened, 12% increased the shoot dry weight of the seedlings, 22% increased the dry weight of the roots; while the total dry weight of the seedlings was increased by 32% of the isolates. Increase in total seedling weight ranged from 32.35% to 87.95%. N69 resulted in the greatest increase in both shoot (67.35%) and root (117.65%) weight. Isolates that resulted in significant increase in wheat dry mass belonged to the *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas* and *Stenotrophomonas* genera. These isolates have the potential to be developed as biofertilisers in wheat cropping systems and potentially other crops as well.

### 4.1 Introduction

Several environmental factors (both biotic and abiotic) limit the growth of plants. Some of these factors include environmental stress, limited of nutrients in the soil and pathogen infection that affect the physiological processes in plants leading to reduced plant growth. For this reason, the use of chemical fertilizers to enhance plant growth has become common practice for the past many decades. Such prolonged and excessive use of chemical fertilisers is, however, not sustainable and has adverse consequences on the environment. Excessive use of fertilisers alters the pH of the soil (Savci 2012), disturbs soil biodiversity (Barabasz et al. 2002), leaches into rivers and dams and disturbs marine ecosystems (Chauhan et al. 2012). Chemical fertilisers can cause the emission of greenhouse gases which lead to global warming and acid rain (Adesemoye and Kloepper 2009). Therefore, there is a huge focus by researchers

to find an alternative, sustainable ways for plant growth enhancement without harming the environment.

One such alternative to chemical fertiliser use is the application of plant growth-promoting rhizobacteria (PGPR) (Perez-Montano et al. 2014; Cortivo et al. 2017). The use of PGPR could help decrease the dependence on chemical fertilizers (Cortivo et al. 2017). PGPR possess traits such as the ability to produce phytohormones that assist in shoot and root development (Paul and Sarma 2006), fix atmospheric nitrogen (Parada et al. 2016), solubilize phosphate (Bumunang and Babalola 2014), produce ACC deaminase that is involved in stress alleviation (Magnucka and Pietr 2015), Fe(III) chelation (Visca et al. 1993) and defence against plant pathogens (Baffoni et al. 2015; Egamberdieva et al. 2015). Together, these traits allow PGPR to enhance plant growth either directly or indirectly through control plant diseases.

The search for PGPR with novel modes of action is ongoing. However, determining the exact mode of action of isolates in the field is difficult. Performing *in vitro* studies gives a good indication of the potential and traits of the isolates but does not reveal which traits are activated *in vivo*/*in planta* or compatibility and interaction between the PGPR and plant. A number of studies have employed a strategy of screening isolates *in vitro*, selecting isolates which possess desired traits followed by screening of the isolates *in planta* either in the greenhouse or the field (Agaras et al. 2015; Kavamura et al. 2013; Zheng et al. 2011).

The fresh and dry weight of wheat has also been found to increase due to rhizobacterial inoculation (Gontia-Mishra et al. 2017; Kudoyarova et al. 2017; Saleemi et al. 2017). Khan et al. (2016) found that the amendment of soil with rhizobacteria enhanced water holding capacity of sandy soil, thereby alleviating drought stress. In addition to this, they also found that the dry root and shoot weight was higher in plants inoculated with rhizobacteria as compared to those that were not. Dal Cortivo et al. (2017) found that the inoculation of wheat with *Azospirillum Azoarcus* and *Azorhizobium* spp. increased the number of root tips as well as the volumetric root length density of wheat. This also contributes to better attainment of water by the crop. Performing seedling assays offer several advantages over conducting pot trials such as shorter growth and incubation period, allows for easy handling of seedlings, requires less space thus allowing for screening large numbers of isolates at a time (Ishiga et al. 2011). Performing seedling assays provides a rapid method of screening isolates to give an indication of how the isolates may perform under field conditions.

In the previous chapter, the collection of rhizobacteria were screened for plant growth enhancement in seedlings inoculated with *Fusarium pseudograminearum*. The aim of the current chapter is to use a seedling assay to screen the collection of rhizobacteria for direct plant growth promotion of wheat in the absence of a pathogen. This will assist in the selection of best-performing isolates to be used in subsequent pot trials.

## 4.2 Materials and methods

### 4.2.1 Culture preparation

All bacterial cultures were obtained from the University of PRetoria-PGPR culture collection. A total of 113 isolates was used. The isolates were maintained in Microbank™ beads (Pro-Lab Diagnostics) and preserved at -70$^{o}$C. To recover them, they were plated onto nutrient agar (NA) medium (Biolab), incubated at 25$^{o}$C for 48h and checked for purity. Working cultures were prepared by streaking the bacteria onto the surface of nutrient agar slants in McCartney bottles. These were stored at room temperature and streaked onto NA plates when needed. The storage cultures were also preserved in glycerol. A 65% glycerol solution was prepared and 500µl was dispensed into sterile Eppendorf tubes. Bacteria were grown in nutrient broth and incubated at 25$^{o}$C and 150rpm for 48h. An equal volume (500 µl) of bacterial broth was transferred to the Eppendorf tubes containing glycerol. These were then frozen.

### 4.2.3 Seed preparation

The wheat seeds (Duzi cultivar, Klein Karoo Seed, P.O. Box 159, Oudtshoorn, 6620, South Africa) were surface disinfected with 70% ethanol for one minute and then washed five times with sterile $dH_2O$. Following this, they were submerged in 1% sodium hypochlorite solution for 30 seconds and washed again five times in sterile $dH_2O$. The seeds were pre-germinated in sterile petri dishes containing moist filter paper, for three days prior to planting.

### 4.2.4 Screening of rhizobacterial isolates for plant growth promotion of wheat

The wheat seeds were prepared as described above. Due to a large number of bacterial isolates, the collection was screened in batches. The isolates were streaked onto NA plates from working cultures. After three days of incubation, a loop full of the bacterial culture was inoculated into 30ml sterile nutrient broth in 50ml centrifuge tubes and incubated in a dark shaking incubator (150rpm) for 48 hours at 25$^{o}$C. The pre-germinated seeds were transferred into 30-cup-multipot

seedling trays containing 50ml/cup of steam-pasteurised soil (sand 87%, clay 7% and silt 6%) at a rate of 1 germinated seed per cell. The seeds were inoculated with 1ml of the 48h $1\times10^8$cells/ml bacterial suspension at planting. The suspension was pipetted at the top of the seeds. The negative control was inoculated with uninoculated NB. Each treatment was composed of three replicates with three sub-replicates per replicate. These were incubated for a further 21 days at an average temperature of 25$^o$C in the greenhouse. The seedlings were watered daily with municipal tap water. Upon harvesting, excess soil was rinsed from the roots with tap water. The shoots were excised, and fresh root and shoot weight of the seedlings were recorded. The roots and shoots were placed in brown paper bags and dried in an oven at 50$^o$C for 48 hours. Following this, the dry root and shoot weight were determined by weighing. This screening experiment was repeated.

4.2.5 Experimental design and statistical analysis

The experiment was set up according to a completely randomized design. The seedling trays on the benches inside the greenhouse compartment were rotated at three-day intervals to mitigate any variation in greenhouse conditions. Analysis of variance (ANOVA) was performed using SAS 9.2 software (SAS Institute, Cary, NC, USA). Fischer's Protected Least significant difference (LSD) was used to separate the means at a significance level of 5% (p=0.05).

## 4.3 Results

4.3.1 Screening of rhizobacterial isolates for plant growth promotion of wheat

In the first batch of bacterial isolates tested, some statistically significant differences between bacterial treatments and the untreated control were observed in terms of both fresh and dry weight of the seedlings (Table 4.1). The shoot dry weight was increased by 9.4% of the isolates namely N76, A45 and A33. The root dry weight was increased by 59.4% of the isolates with the top 10 best performing isolates being A20, A33, N76, A24, A34, A16, A47, A26, A45 and N20. Significant increases in the dry total weight of the seedlings was observed with 25% of the isolates, namely A33, A20, N76, A45, A26, A47, N02 and A24. Increase in total seedling dry weight ranged from 42.50% (isolate A24) to 66.25% (isolate A33).

As in the first batch, some significant differences between the uninoculated control and treatments was observed with respect to both the fresh and dry weight of the seedlings in the

second batch (Table 4.2). Only N69, N34 and N67 (12.5% of the isolates) increased the shoot dry weight of wheat seedlings in comparison to the uninoculated control. The root dry weight of the wheat seedlings was increased by 24% of the isolates, namely N69, N67, N34, A07, N13 and N01, while the total dry weight of wheat seedlings was increased by 16% of the isolates namely N69, N67, N34 and A07. Increase in total seedling dry weight ranged from 43.37% (isolate A07) to 87.95% (isolate N69).

In the third batch of isolates (Table 4.3), no significant difference between the shoot dry weight of the seedlings was observed. The root dry weight was only increased by two of the isolates, (A25 and N59C) whereas the total dry weight of the seedlings was significantly increased by only one isolate, A25, by 33.72%. In the fourth batch (Table 4.4), the shoot dry weight of the seedlings was increased by 16.7% of the isolates (KBS1F3, A05C, A10AC, N27 and A43). The root dry weight was significantly increased by just one isolate (KBS1F3) whereas the overall dry weight of the seedlings was significantly increased by three of the isolates, (KBS1F3, A10AC and A43). Increase in total seedling dry weight ranged from 32.35% (A43) to 46.08% (KBS1F3).

### 4.4 Discussion

In this study, a collection of rhizobacterial isolates were screened for wheat growth promotion by means of a seedling assay. The rhizobacterial isolates that were used in this study were selected based on their plant growth-promoting ability in previous studies (Makgolane 2016; Pretorius 2012; Rudolph 2014). Thirty-two per cent of the isolates significantly increased the total weight of wheat seedlings, with the greatest increase (87.95%) recorded with isolate N69. Isolate N69 also resulted in the highest increase in dry shoot (67.35%) and root (117.65%) weight. These isolates could be employed in integrated strategies aimed at decreasing the dependence on chemical fertilizers and adopting the use of more sustainable and environmentally friendly farming methods.

PGPR possess several types of traits that affect plant growth in a variety of ways. Some isolates may produce phytohormones that induce either shoot or root growth of plants or both (Agrawal and Agrawal 2013; Bumunang and Babalola 2014; Egamberdieva et al. 2015). The results obtained in the current study showed that 12% of the isolates significantly increased the shoot dry weight of the seedlings while 22% increasing just the root dry weight of the seedling. Isolates that promote root growth may be targeted for use in water-limited environments, where

increased water or nutrient absorption is desired. Kudoyarova et al. (2017) found that the treatment of plants with PGPR increased plant mass in accordance with the increased level of auxin in the treated plant. However, they found that the increase in plant mass did not correlate with the potential ability of PGPR strains for the production of auxins or phosphate mobilization *in vitro*. They observed that the introduction of bacteria in the soil increased phosphorus content in the plants and thus suggest increased capacity for efficient uptake of phosphorus from the soil. Increase in phosphorus content in the absence of plants was observed (Kudoyarova et al. 2017).

Isolates that significantly increased the total dry weight of wheat belong to the *Bacillus*, *Paenibacillus*, *Pantoea*, *Pseudomonas* and *Stenotrophomonas* genera. There have been multiple reports of the efficacy of species belonging to these genera to promote the growth of various plants. *Pseudomonas* sp. have been found to produce ACC deaminase (Agaras et al. 2015) fix nitrogen, produce siderophores, auxins (Ahmad et al. 2008), gibberellins and solubilise phosphate (Toribio-Jiménez et al. 2017). *Pantoea* sp. have also been reported as nitrogen fixers, siderophore and auxin producers as well as phosphate solubiliser (Toribio-Jiménez et al. 2017). Amongst others, *Bacillus* sp. can produce IAA and solubilise phosphate (Ahmad et al. 2008) while *Stenotrophomonas* sp. produce IAA, ACC deaminase, fix nitrogen and solubilise phosphate (Ahemad and Kibret 2014). As such, these traits may have allowed these isolates to aid in the growth of wheat.

The phytohormone, ethylene performs many functions in the plant, from assisting in seed germination to fruit ripening; however, at high concentrations, the phytohormone can inhibit shoot and root growth of plants (Tsukanova et al. 2017; Vejan et al. 2016). Drought accentuates the production of ethylene which inhibits plant growth through several mechanisms. Thus, the application of rhizobacterial isolates that produce ACC deaminase to plants could be one of the strategies that can be employed to minimize the effects of stress on the plant. To further enhance the effect of PGPR on plants, mixtures of isolates could have an enhanced effect in terms of plant growth promotion (Cortivo et al. 2017; Delshadi et al. 2017).

During other stress conditions such as high $Na^+$ concentrations, PGPR have the ability to protect plants in saline soil environment through the production of ACC deaminase which lowers the levels of harmful ethylene (Parray et al. 2016). Khan et al. (2016) found that *Bacillus subtilis* could increase shoot and root mass as well as chlorophyll content of tomato plants. Some of the traits that allowed the PGPR to promote tomato growth was its ability to solubilize

phosphate and produce ACC deaminase and IAA (Khan et al. 2016). An additional advantage of this PGPR is the fact that it is endophytic and can be carried in plant seeds.

*Bacillus cereus*, *Planomicrobium chinense* and *Pseudomonas flourescens* have also been found to enhance the water holding capacity of sandy soils through the production of exopolysaccharides that form soil aggregates around plant roots (Khan et al. 2016). According to this report, the inoculation of wheat with these isolates could increase the water content of leaves by 80% while fresh root mass was increased by 80% and the total dry weight of the plants by 68% (Khan et al. 2016). In another study, Cortivo et al. (2017) reported that species of *Azoarcus*, *Azobispirillum*, and *Azorhizobium* increased the number of root tips and the volumetric root length density in the field.

Using a seedling bioassay as a means of screening a large number of PGPR was effective as significant differences between the treatments could be observed. However, as the rhizobacteria diminish in numbers over time, more than one application would be beneficial for the observation of differences between the treatments. Future studies involving screening of rhizobacterial isolates could investigate other methods of screening the isolates at an even smaller scale for a short period of time but still allowing for the observation of significant differences between the treatments. The current study showed that many of the isolates screened have the potential to be studied further under field conditions and developed as biofertilizer.

**Tables and Figures**

**Table 4.1** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 1).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.208$^{e}$ | 0.089$^{e}$ | 0.298$^{e}$ | 0.049$^{cdef}$ | 0.031$^{gh}$ | 0.080$^{fg}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| A05A | 0.409$^{abcd}$ | 0.379$^{abc}$ | 0.789$^{abcd}$ | 0.063$^{abcde}$ | 0.046$^{bcdefg}$ | 0.109$^{abcdef}$ | 96.63 | 325.84 | 161.75 | 28.57 | 48.39 | 36.25 |
| A06 | 0.338$^{cd}$ | 0.384$^{ab}$ | 0.723$^{abcd}$ | 0.052$^{bcdef}$ | 0.051$^{abcdef}$ | 0.103$^{abcdef}$ | 62.5 | 331.46 | 142.62 | 6.12 | 64.52 | 25.75 |
| A08 | 0.442$^{abc}$ | 0.306$^{abcd}$ | 0.748$^{abcd}$ | 0.067$^{ab}$ | 0.044$^{bcdefgh}$ | 0.111$^{abcdef}$ | 112.50 | 243.82 | 151.01 | 36.73 | 41.94 | 43.75 |
| A16 | 0.360$^{abcd}$ | 0.271$^{cd}$ | 0.631$^{cd}$ | 0.056$^{abcdef}$ | 0.053$^{abcd}$ | 0.109$^{abcdef}$ | 73.08 | 204.49 | 111.75 | 14.29 | 70.97 | 36.25 |
| A20 | 0.466$^{a}$ | 0.399$^{a}$ | 0.865$^{a}$ | 0.067$^{abc}$ | 0.065$^{a}$ | 0.13$^{ab}$ | 124.04 | 348.31 | 190.27 | 36.73 | 109.68 | 62.50 |
| A21 | 0.341$^{cd}$ | 0.267$^{d}$ | 0.608d | 0.051$^{bcdef}$ | 0.041$^{cdefgh}$ | 0.092$^{cdefg}$ | 63.94 | 200 | 104.03 | 4.08 | 32.26 | 15 |
| A22 | 0.352$^{bcd}$ | 0.296$^{abcd}$ | 0.648$^{bcd}$ | 0.049$^{cdef}$ | 0.037$^{efgh}$ | 0.086$^{defg}$ | 69.23 | 232.58 | 117.45 | 0 | 19.35 | 7.5 |
| A24 | 0.393$^{abcd}$ | 0.381$^{abc}$ | 0.774$^{abcd}$ | 0.058$^{abcdef}$ | 0.056$^{abc}$ | 0.114$^{abcde}$ | 88.94 | 328.09 | 159.73 | 18.37 | 80.65 | 42.50 |
| A26 | 0.402$^{abcd}$ | 0.362$^{abcd}$ | 0.764$^{abcd}$ | 0.066$^{abc}$ | 0.052$^{abcde}$ | 0.118$^{abcd}$ | 93.27 | 306.74 | 156.38 | 34.69 | 67.74 | 47.50 |
| A27 | 0.444$^{abc}$ | 0.384$^{ab}$ | 0.829$^{abc}$ | 0.065$^{abcd}$ | 0.045$^{bcdefgh}$ | 0.109$^{abcdef}$ | 113.46 | 331.46 | 178.19 | 32.65 | 45.16 | 36.25 |
| A28 | 0.386$^{abcd}$ | 0.302$^{abcd}$ | 0.689$^{abcd}$ | 0.058$^{abcdef}$ | 0.041$^{cdefgh}$ | 0.099$^{bcdef}$ | 85.58 | 239.33 | 131.21 | 18.37 | 35.26 | 23.75 |
| A29 | 0.385$^{abcd}$ | 0.316$^{abcd}$ | 0.701$^{abcd}$ | 0.057$^{abcdef}$ | 0.046$^{bcdefg}$ | 0.104$^{abcdef}$ | 85.09 | 225.06 | 135.23 | 16.33 | 48.39 | 30 |
| A32 | 0.314$^{de}$ | 0.318$^{abcd}$ | 0.632$^{cd}$ | 0.057$^{abcdef}$ | 0.049$^{abcdef}$ | 0.106$^{abcdef}$ | 50.96 | 257.30 | 112.08 | 16.33 | 58.06 | 32.50 |
| A33 | 0.385$^{abcd}$ | 0.331$^{abcd}$ | 0.715$^{abcd}$ | 0.071$^{a}$ | 0.063$^{a}$ | 0.133$^{a}$ | 85.09 | 271.91 | 139.93 | 44.89 | 103.23 | 66.25 |
| A34 | 0.342$^{cd}$ | 0.349$^{abcd}$ | 0.692$^{abcd}$ | 0.057$^{abcdef}$ | 0.056$^{abc}$ | 0.112$^{abcdef}$ | 55.77 | 292.13 | 111.07 | 16.33 | 80.65 | 40 |
| A35 | 0.427$^{abcd}$ | 0.370$^{abcd}$ | 0.798$^{abcd}$ | 0.047$^{def}$ | 0.039$^{defgh}$ | 0.091$^{cdefg}$ | 105.29 | 315.73 | 167.79 | -4.08 | 25.81 | 13.75 |
| A36 | 0.343$^{cd}$ | 0.296$^{abcd}$ | 0.638$^{cd}$ | 0.043$^{f}$ | 0.029$^{h}$ | 0.065$^{g}$ | 64.90 | 232.58 | 114.09 | 12.24 | -6.45 | -18.75 |
| A44 | 0.349$^{bcd}$ | 0.299$^{abcd}$ | 0.648$^{bcd}$ | 0.052$^{bcdef}$ | 0.043$^{bcdefgh}$ | 0.094$^{bcdef}$ | 67.79 | 235.96 | 117.45 | 6.12 | 38.71 | 17.50 |
| LSD[2] | 0.114 | 0.109 | 0.204 | 0.019 | 0.016 | 0.032 | | | | | | |
| CV[2] (%) | 26.00 | 29.55 | 25.21 | 28.15 | 30.05 | 27.08 | | | | | | |

[1]NC= negative control (uninoculated).

[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100]. Negative values signify treatment values that are less than those of the control and positive values are treatments with a higher weight than the control.

Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.1** Effect of rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 1 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| A45 | 0.431$^{abc}$ | 0.364$^{abcd}$ | 0.795$^{abcd}$ | 0.071$^{a}$ | 0.052$^{abcde}$ | 0.123$^{abc}$ | 107.21 | 308.99 | 166.78 | 44.89 | 67.74 | 53.75 |
| A47 | 0.463$^{ab}$ | 0.381$^{ab}$ | 0.843$^{ab}$ | 0.063$^{abcde}$ | 0.053$^{abcde}$ | 0.116$^{abcd}$ | 122.59 | 328.09 | 182.89 | 28.57 | 70.97 | 45 |
| A48 | 0.349$^{bcd}$ | 0.312$^{abcd}$ | 0.662$^{abcd}$ | 0.052$^{bcdef}$ | 0.039$^{defgh}$ | 0.091$^{cdefg}$ | 67.79 | 250.56 | 122.15 | 6.12 | 25.81 | 13.75 |
| N02 | 0.426$^{abcd}$ | 0.307$^{abcd}$ | 0.733$^{abcd}$ | 0.066$^{abc}$ | 0.049$^{abcdef}$ | 0.115$^{abcde}$ | 104.81 | 244.94 | 145.97 | 34.69 | 58.06 | 43.75 |
| N04 | 0.377$^{abcd}$ | 0.304$^{abcd}$ | 0.682$^{abcd}$ | 0.057$^{abcdef}$ | 0.040$^{cdefgh}$ | 0.097$^{cdefg}$ | 81.25 | 241.57 | 128.86 | 16.33 | 29.03 | 21.25 |
| N10 | 0.356$^{abcd}$ | 0.304$^{abcd}$ | 0.661$^{bcd}$ | 0.050$^{bcdef}$ | 0.036$^{fgh}$ | 0.086$^{defg}$ | 71.15 | 241.57 | 121.81 | 2.04 | 16.13 | 7.50 |
| N17 | 0.385$^{abcd}$ | 0.367$^{abcd}$ | 0.752$^{abcd}$ | 0.060$^{abcdef}$ | 0.045$^{bcdefg}$ | 0.104$^{abcdef}$ | 85.09 | 312.36 | 143.29 | 22.45 | 45.16 | 30 |
| N20 | 0.365$^{abcd}$ | 0.329$^{abcd}$ | 0.697$^{abcd}$ | 0.057$^{abcdef}$ | 0.051$^{abcdef}$ | 0.109$^{abcdef}$ | 75.48 | 269.66 | 133.89 | 16.33 | 64.52 | 36.25 |
| N29 | 0.365$^{abcd}$ | 0.310$^{abcd}$ | 0.676$^{abcd}$ | 0.061$^{abcdef}$ | 0.049$^{abcdef}$ | 0.110$^{abcdef}$ | 75.48 | 248.31 | 126.85 | 24.49 | 58.06 | 37.50 |
| N53 | 0.416$^{abcd}$ | 0.331$^{abcd}$ | 0.747$^{abcd}$ | 0.058$^{abcde}$ | 0.050$^{abcdef}$ | 0.108$^{abcdef}$ | 100 | 271.91 | 150.67 | 18.37 | 61.29 | 35 |
| N65 | 0.402$^{abcd}$ | 0.313$^{abcd}$ | 0.715$^{abcd}$ | 0.061$^{abcdef}$ | 0.049$^{abcdef}$ | 0.110$^{abcdef}$ | 93.27 | 251.69 | 139.93 | 24.49 | 58.06 | 37.50 |
| N76 | 0.444$^{abc}$ | 0.353$^{abcd}$ | 0.797$^{abcd}$ | 0.072$^{a}$ | 0.058$^{ab}$ | 0.130$^{ab}$ | 113.46 | 296.63 | 167.45 | 46.94 | 87.09 | 62.50 |
| N77W | 0.349$^{bcd}$ | 0.285$^{bcd}$ | 0.634$^{cd}$ | 0.045$^{ef}$ | 0.039$^{defgh}$ | 0.084$^{efg}$ | 67.79 | 220.22 | 112.75 | -8.16 | 25.81 | 5 |
| T29AC | 0.428$^{abc}$ | 0.375$^{abcd}$ | 0.802$^{abcd}$ | 0.066$^{abc}$ | 0.045$^{bcdefgh}$ | 0.110$^{abcdef}$ | 105.77 | 321.35 | 169.13 | 34.69 | 45.16 | 37.50 |
| LSD[2] | 0.114 | 0.109 | 0.204 | 0.019 | 0.016 | 0.032 | | | | | | |
| CV[2] (%) | 26.00 | 29.55 | 25.21 | 28.15 | 30.05 | 27.08 | | | | | | |

[1]NC= negative control (uninoculated).
[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.
[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.
Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.2** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 2).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.239$^{ij}$ | 0.096$^{gh}$ | 0.334$^{jk}$ | 0.049$^{cde}$ | 0.034$^{fg}$ | 0.083$^{efghi}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| A07 | 0.376$^{bcde}$ | 0.298$^{bcd}$ | 0.673$^{bcdefg}$ | 0.064$^{abc}$ | 0.055$^{bcd}$ | 0.119$^{bcd}$ | 57.32 | 210.42 | 101.49 | 30.61 | 61.76 | 43.37 |
| A14W | 0.379$^{abcde}$ | 0.303$^{bcd}$ | 0.682$^{bcdef}$ | 0.057$^{bcde}$ | 0.043$^{bcdefg}$ | 0.100$^{bcdefghi}$ | 58.58 | 215.63 | 104.19 | 16.33 | 26.47 | 20.48 |
| A46 | 0.313$^{efghi}$ | 0.176$^{efg}$ | 0.4897$^{hi}$ | 0.048$^{cde}$ | 0.044$^{bcdefg}$ | 0.092$^{defghi}$ | 30.96 | 83.33 | 46.41 | -2.04 | 29.41 | 10.84 |
| N01 | 0.388$^{abcde}$ | 0.306$^{abcd}$ | 0.694$^{bcdef}$ | 0.062$^{bc}$ | 0.053$^{bcde}$ | 0.115$^{bcdef}$ | 62.34 | 218.75 | 107.78 | 26.53 | 55.88 | 35.55 |
| N11 | 0.159$^{j}$ | 0.064$^{h}$ | 0.223$^{k}$ | 0.042$^{de}$ | 0.034$^{fg}$ | 0.076$^{hi}$ | -33.47 | -33.33 | -41.02 | -14.29 | 0 | -8.43 |
| N13 | 0.413$^{abcd}$ | 0.342$^{abc}$ | 0.756$^{abcd}$ | 0.063$^{bc}$ | 0.054$^{bcde}$ | 0.117$^{bcde}$ | 72.80 | 256.25 | 126.35 | 28.57 | 58.82 | 40.96 |
| N14 | 0.363$^{cdef}$ | 0.279$^{cd}$ | 0.643$^{cdefg}$ | 0.054$^{bcde}$ | 0.035$^{fg}$ | 0.089$^{defghi}$ | 51.88 | 190.63 | 92.51 | 10.20 | 2.94 | 7.23 |
| N16W | 0.281$^{fghi}$ | 0.275$^{cd}$ | 0.556$^{fghi}$ | 0.043$^{de}$ | 0.042$^{bcdefg}$ | 0.084$^{efghi}$ | 17.57 | 160.46 | 66.47 | -12.24 | 23.53 | 1.20 |
| N19 | 0.339$^{defgh}$ | 0.266$^{cde}$ | 0.606$^{defgh}$ | 0.051$^{cde}$ | 0.046$^{bcdefg}$ | 0.097$^{bcdefghi}$ | 41.84 | 177.08 | 81.44 | 4.08 | 35.29 | 16.87 |
| N28 | 0.397$^{abcde}$ | 0.311$^{abcd}$ | 0.728$^{abcde}$ | 0.060$^{bcd}$ | 0.047$^{bcdefg}$ | 0.107$^{bcdefgh}$ | 66.11 | 223.96 | 117.96 | 22.45 | 38.24 | 28.92 |
| N30 | 0.287$^{fghi}$ | 0.153$^{fgh}$ | 0.439$^{ij}$ | 0.049$^{cde}$ | 0.042$^{bcdefg}$ | 0.091$^{defghi}$ | 20.08 | 59.38 | 31.44 | 0 | 23.53 | 9.64 |
| N31 | 0.262$^{hi}$ | 0.176$^{efg}$ | 0.437$^{ij}$ | 0.039$^{e}$ | 0.033$^{g}$ | 0.072$^{i}$ | 9.62 | 83.33 | 30.84 | -20.41 | -.2.94 | -13.25 |
| N34 | 0.423$^{abc}$ | 0.385$^{ab}$ | 0.814$^{ab}$ | 0.071$^{ab}$ | 0.057$^{bc}$ | 0.128$^{abc}$ | 76.99 | 301.04 | 143.71 | 44.89 | 67.65 | 54.22 |
| N37 | 0.349$^{cdefg}$ | 0.305$^{abcd}$ | 0.653$^{cdefg}$ | 0.058$^{bcde}$ | 0.046$^{bcdefg}$ | 0.103$^{bcdefghi}$ | 46.02 | 217.71 | 95.51 | 18.37 | 35.29 | 24.09 |
| N43 | 0.272$^{ghi}$ | 0.294$^{bcd}$ | 0.562$^{fghi}$ | 0.047$^{cde}$ | 0.035$^{fg}$ | 0.082$^{ghi}$ | 13.81 | 206.25 | 68.26 | -4.08 | 2.94 | -1.20 |
| N55B | 0.357$^{cdefg}$ | 0.298$^{bcd}$ | 0.655$^{cdefg}$ | 0.048$^{cde}$ | 0.038$^{efg}$ | 0.087$^{defghi}$ | 49.37 | 210.42 | 96.11 | -2.04 | 11.76 | 4.82 |
| N59B | 0.357$^{cdefg}$ | 0.304$^{bcd}$ | 0.662$^{cdefg}$ | 0.056$^{bcde}$ | 0.039$^{defg}$ | 0.095$^{cdefghi}$ | 49.37 | 216.67 | 98.20 | 14.29 | 11.71 | 14.46 |
| LSD[2] | 0.085 | 0.0955 | 0.149 | 0.019 | 0.017 | 0.033 | | | | | | |
| CV[2] (%) | 21.64 | 30.82 | 21.32 | 29.99 | 32.09 | 28.56 | | | | | | |

[1]NC= negative control (uninoculated).

[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.

Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.2** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 2 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| N66 | $0.286^{fghi}$ | $0.245^{def}$ | $0.532^{ghi}$ | $0.064^{abc}$ | $0.049^{bcdef}$ | $0.113^{bcdefg}$ | 16.67 | 155.21 | 59.28 | 30.61 | 44.12 | 36.14 |
| N67 | $0.462^{a}$ | $0.399^{a}$ | $0.862^{a}$ | $0.070^{ab}$ | $0.058^{ab}$ | $0.128^{ab}$ | 93.31 | 315.63 | 158.08 | 42.86 | 70.59 | 54.22 |
| N69 | $0.452^{ab}$ | $0.325^{abcd}$ | $0.777^{abc}$ | $0.082^{a}$ | $0.074^{a}$ | $0.156^{a}$ | 89.12 | 238.54 | 132.63 | 67.35 | 117.65 | 87.95 |
| N71 | $0.341^{defgh}$ | $0.257^{cde}$ | $0.598^{efgh}$ | $0.055^{bcde}$ | $0.046^{bcdefg}$ | $0.101^{bcdefghi}$ | 42.68 | 167.71 | 79.04 | 12.24 | 35.29 | 21.69 |
| T11 | $0.423^{abcd}$ | $0.321^{abcd}$ | $0.745^{abcde}$ | $0.057^{bcde}$ | $0.045^{bcdefg}$ | $0.102^{bcdefghi}$ | 76.99 | 234.38 | 132.05 | 16.33 | 32.35 | 22.89 |
| T19AC | $0.314^{efghi}$ | $0.246^{def}$ | $0.559^{fghi}$ | $0.051^{cde}$ | $0.041^{cdefg}$ | $0.091^{defghi}$ | 31.38 | 156.25 | 67.37 | 4.08 | 20.59 | 9.64 |
| T22 | $0.386^{abcde}$ | $0.338^{abcd}$ | $0.724^{abcde}$ | $0.057^{bcde}$ | $0.044^{bcdefg}$ | $0.098^{bcdefghi}$ | 61.51 | 252.08 | 116.77 | 16.33 | 29.41 | 18.07 |
| LSD[2] | 0.085 | 0.0955 | 0.149 | 0.019 | 0.017 | 0.033 | | | | | | |
| CV[2] (%) | 21.64 | 30.82 | 21.32 | 29.99 | 32.09 | 28.56 | | | | | | |

[1]NC= negative control (uninoculated).
[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.
[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.
Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.3** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 3).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.219$^{i}$ | 0.168$^{f}$ | 0.388$^{f}$ | 0.051$^{abcde}$ | 0.036$^{cd}$ | 0.086$^{bcde}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| A09AC | 0.385$^{abcde}$ | 0.309$^{abcde}$ | 0.694$^{abcde}$ | 0.051$^{abcde}$ | 0.045$^{abc}$ | 0.097$^{abcde}$ | 75.79 | 83.93 | 78.87 | 0 | 25 | 12.79 |
| A12 | 0.328$^{defgh}$ | 0.283$^{abcde}$ | 0.611$^{bcde}$ | 0.048$^{bcde}$ | 0.038$^{bcd}$ | 0.086$^{bcde}$ | 49.77 | 68.45 | 57.47 | -5.88 | 5.56 | 0 |
| A17 | 0.339$^{cdefgh}$ | 0.324$^{abcde}$ | 0.663$^{abcde}$ | 0.049$^{abcde}$ | 0.043$^{abcd}$ | 0.093$^{abcde}$ | 54.79 | 92.86 | 70.88 | -3.92 | 19.44 | 8.14 |
| A19 | 0.422$^{abc}$ | 0.342$^{abcde}$ | 0.764$^{abc}$ | 0.058$^{abcd}$ | 0.039$^{abcd}$ | 0.097$^{abcde}$ | 92.69 | 103.57 | 96.91 | 13.73 | 8.33 | 12.79 |
| A25 | 0.460$^{a}$ | 0.347$^{abcde}$ | 0.807$^{a}$ | 0.063$^{a}$ | 0.052$^{a}$ | 0.115$^{a}$ | 110.05 | 106.55 | 107.99 | 23.53 | 44.44 | 33.72 |
| A40 | 0.399$^{abcd}$ | 0.379$^{a}$ | 0.778$^{ab}$ | 0.055$^{abcd}$ | 0.048$^{abc}$ | 0.102$^{abc}$ | 82.19 | 125.59 | 100.52 | 7.84 | 33.33 | 18.60 |
| N03 | 0.332$^{defgh}$ | 0.323$^{abcde}$ | 0.655$^{abcde}$ | 0.046$^{cde}$ | 0.036$^{bcd}$ | 0.082$^{cde}$ | 51.59 | 92.26 | 68.81 | -9.80 | 0 | -4.65 |
| N04AC | 0.372$^{bcdefg}$ | 0.350$^{abcd}$ | 0.722$^{abcd}$ | 0.054$^{abcd}$ | 0.048$^{abc}$ | 0.102$^{abc}$ | 69.86 | 108.33 | 86.08 | 5.88 | 33.33 | 18.60 |
| N12 | 0.333$^{defgh}$ | 0.321$^{abcde}$ | 0.654$^{abcde}$ | 0.051$^{abcde}$ | 0.039$^{abcd}$ | 0.090$^{bcde}$ | 52.05 | 91.07 | 68.56 | 0 | 8.33 | 4.65 |
| N44 | 0.276$^{hi}$ | 0.259$^{cdef}$ | 0.536$^{ef}$ | 0.045$^{cde}$ | 0.039$^{abcd}$ | 0.085$^{bcde}$ | 26.03 | 113.69 | 38.14 | -11.76 | 8.33 | -1.16 |
| N51 | 0.298$^{fghi}$ | 0.279$^{abcde}$ | 0.577$^{de}$ | 0.045$^{cde}$ | 0.035$^{cd}$ | 0.080$^{cde}$ | 37.07 | 66.07 | 48.71 | -11.76 | -2.78 | -6.98 |
| N54 | 0.321$^{defgh}$ | 0.326$^{abcde}$ | 0.647$^{abcde}$ | 0.055$^{abcd}$ | 0.047$^{abc}$ | 0.102$^{abc}$ | 46.58 | 94.05 | 66.75 | 7.84 | 30.56 | 18.60 |
| N55A | 0.359$^{bcdefgh}$ | 0.268$^{bcde}$ | 0.627$^{bcde}$ | 0.063$^{a}$ | 0.046$^{abc}$ | 0.092$^{abcde}$ | 63.93 | 59.52 | 61.59 | 23.53 | 27.78 | 6.98 |
| N56 | 0.362$^{bcdefg}$ | 0.372$^{ab}$ | 0.734$^{abcd}$ | 0.053$^{abcd}$ | 0.045$^{abc}$ | 0.098$^{abcd}$ | 65.29 | 121.43 | 89.18 | 3.92 | 25 | 13.95 |
| N58 | 0.299$^{fghi}$ | 0.239$^{ef}$ | 0.539$^{ef}$ | 0.050$^{abcde}$ | 0.039$^{abcd}$ | 0.089$^{bcde}$ | 36.53 | 42.26 | 38.92 | -1.96 | 8.33 | 3.49 |
| N59A | 0.341$^{cdefgh}$ | 0.314$^{abcde}$ | 0.655$^{abcde}$ | 0.055$^{abcd}$ | 0.045$^{abc}$ | 0.099$^{abcd}$ | 55.71 | 86.90 | 68.81 | 7.84 | 25 | 15.11 |
| N59C | 0.385$^{abcdef}$ | 0.344$^{abcde}$ | 0.729$^{abcd}$ | 0.056$^{abcd}$ | 0.049$^{ab}$ | 0.105$^{abc}$ | 75.79 | 104.76 | 87.89 | 9.80 | 36.11 | 22.09 |
| N63 | 0.315$^{defgh}$ | 0.263$^{cdef}$ | 0.575$^{de}$ | 0.055$^{abcd}$ | 0.043$^{abcd}$ | 0.098$^{abcd}$ | 43.84 | 56.55 | 48.19 | 7.84 | 19.44 | 13.95 |
| LSD[2] | 0.085 | 0.109 | 0.170 | 0.014 | 0.013 | 0.025 | | | | | | |
| CV[2] (%) | 21.61 | 31.52 | 22.95 | 23.23 | 26.93 | 23.27 | | | | | | |

[1]NC= negative control (uninoculated).

[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the control.

Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.3** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 3 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| N64 | $0.366^{bcdefg}$ | $0.355^{abc}$ | $0.720^{abcd}$ | $0.062^{a}$ | $0.044^{abcd}$ | $0.107^{ab}$ | 67.12 | 111.31 | 85.57 | 21.57 | 22.22 | 24.41 |
| N68 | $0.315^{defgh}$ | $0.302^{abcde}$ | $0.617^{bcde}$ | $0.044^{de}$ | $0.037^{bcd}$ | $0.081^{cde}$ | 43.84 | 79.76 | 59.02 | -14.73 | 2.78 | -5.81 |
| N70 | $0.376^{abcdef}$ | $0.304^{abcde}$ | $0.679^{abcde}$ | $0.047^{bcde}$ | $0.038^{bcd}$ | $0.085^{bcde}$ | 71.69 | 80.95 | 75 | -7.84 | 5.56 | -1.16 |
| N72 | $0.336^{defgh}$ | $0.271^{abcdef}$ | $0.607^{cde}$ | $0.048^{bcde}$ | $0.037^{bcd}$ | $0.085^{bcde}$ | 53.42 | 61.31 | 56.44 | -5.88 | 2.78 | -1.16 |
| N74 | $0.305^{efghi}$ | $0.239^{ef}$ | $0.544^{ef}$ | $0.044^{de}$ | $0.032^{d}$ | $0.076^{de}$ | 39.27 | 42.26 | 40.21 | -13.73 | -11.11 | -11.62 |
| N77Y | $0.299^{fghi}$ | $0.242^{def}$ | $0.541^{ef}$ | $0.038^{e}$ | $0.035^{cd}$ | $0.073^{e}$ | 36.53 | 44.05 | 39.43 | -25.49 | -2.78 | -15.12 |
| N78 | $0.442^{ab}$ | $0.359^{abc}$ | $0.802^{a}$ | $0.061^{ab}$ | $0.043^{abcd}$ | $0.104^{abc}$ | 101.82 | 113.69 | 106.70 | 19.61 | 19.44 | 20.93 |
| LSD[2] | 0.085 | 0.109 | 0.170 | 0.014 | 0.013 | 0.025 | | | | | | |
| CV[2] (%) | 21.61 | 31.52 | 22.95 | 23.23 | 26.93 | 23.27 | | | | | | |

[1]NC= negative control (uninoculated).
[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.
[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.
Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.4** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 4).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| NC[1] | 0.229$^{hi}$ | 0.176$^{hi}$ | 0.406$^{jk}$ | 0.056$^{ef}$ | 0.046$^{bcdef}$ | 0.102$^{de}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| A01 | 0.393$^{abcde}$ | 0.332$^{abcde}$ | 0.724$^{bcdefg}$ | 0.070$^{abcde}$ | 0.059$^{abcd}$ | 0.129$^{abcd}$ | 71.62 | 88.64 | 78.33 | 25 | 28.26 | 26.47 |
| A05B | 0.423$^{abcd}$ | 0.359$^{abc}$ | 0.782$^{abcd}$ | 0.074$^{abc}$ | 0.057$^{abcd}$ | 0.130$^{abcd}$ | 84.72 | 103.98 | 92.61 | 32.14 | 23.91 | 27.45 |
| A05C | 0.395$^{abcde}$ | 0.305$^{bcdef}$ | 0.699$^{bcdefgh}$ | 0.074$^{ab}$ | 0.054$^{abcd}$ | 0.128$^{abcd}$ | 72.49 | 73.29 | 72.17 | 32.14 | 17.39 | 25.49 |
| A07AC | 0.375$^{bcdef}$ | 0.309$^{abcdef}$ | 0.684$^{bcdefgh}$ | 0.064$^{bcdef}$ | 0.050$^{abcde}$ | 0.114$^{bcde}$ | 63.76 | 75.56 | 68.47 | 14.29 | 8.69 | 11.76 |
| A10AC | 0.412$^{abcde}$ | 0.274$^{cdefgh}$ | 0.686$^{bcdefgh}$ | 0.073$^{abcd}$ | 0.060$^{abc}$ | 0.140$^{ab}$ | 79.91 | 55.68 | 68.97 | 30.36 | 30.43 | 37.25 |
| A37 | 0.403$^{abcde}$ | 0.343$^{abcde}$ | 0.746$^{abcdef}$ | 0.071$^{abcde}$ | 0.053$^{abcde}$ | 0.124$^{abcd}$ | 75.98 | 94.89 | 83.74 | 26.79 | 15.22 | 21.57 |
| A38 | 0.420$^{abcd}$ | 0.289$^{bcdefg}$ | 0.709$^{bcdefg}$ | 0.060$^{bcdef}$ | 0.054$^{abcde}$ | 0.113$^{bcde}$ | 83.41 | 64.20 | 74.63 | 7.14 | 17.39 | 10.78 |
| A39 | 0.356$^{cdefg}$ | 0.266$^{cdefghi}$ | 0.616$^{cdefgh}$ | 0.063$^{bcdef}$ | 0.048$^{abcdef}$ | 0.111$^{bcde}$ | 55.46 | 51.14 | 51.72 | 12.5 | 4.35 | 8.82 |
| A41 | 0.209$^{i}$ | 0.180$^{ghi}$ | 0.389$^{k}$ | 0.039$^{g}$ | 0.030$^{f}$ | 0.067$^{f}$ | -8.73 | 2.27 | -4.19 | -30.36 | -34.78 | -34.31 |
| A42 | 0.359$^{cdefg}$ | 0.315$^{abcdef}$ | 0.674$^{bcdefgh}$ | 0.057$^{cdef}$ | 0.044$^{cdef}$ | 0.101$^{de}$ | 56.77 | 78.98 | 66.01 | 1.79 | -4.35 | -0.98 |
| A43 | 0.351$^{cdefg}$ | 0.367$^{abc}$ | 0.718$^{bcdefg}$ | 0.073$^{abcd}$ | 0.062$^{ab}$ | 0.135$^{abc}$ | 53.28 | 108.52 | 76.85 | 30.36 | 35.78 | 32.35 |
| KBS1F3 | 0.490$^{a}$ | 0.415$^{a}$ | 0.905$^{a}$ | 0.084$^{a}$ | 0.065$^{a}$ | 0.149$^{a}$ | 113.97 | 135.79 | 122.91 | 50 | 41.30 | 46.08 |
| N05 | 0.345$^{cdefg}$ | 0.296$^{bcdef}$ | 0.642$^{bcdefgh}$ | 0.063$^{bcdef}$ | 0.051$^{abcde}$ | 0.114$^{bcde}$ | 50.66 | 68.18 | 58.13 | 12.5 | 10.87 | 11.76 |
| N07 | 0.330$^{defgh}$ | 0.249$^{defghi}$ | 0.579$^{efghij}$ | 0.062$^{bcdef}$ | 0.044$^{bcdef}$ | 0.107$^{cde}$ | 44.10 | 41.48 | 42.61 | 10.71 | -4.35 | 4.90 |
| N08 | 0.362$^{cdefg}$ | 0.309$^{abcdef}$ | 0.671$^{bcdefgh}$ | 0.068$^{abcdef}$ | 0.052$^{abcde}$ | 0.121$^{abcd}$ | 58.08 | 75.57 | 65.27 | 21.43 | 13.04 | 18.63 |
| N15ii | 0.354$^{cdefg}$ | 0.284$^{bcdefgh}$ | 0.638$^{bcdefgh}$ | 0.062$^{bcdef}$ | 0.052$^{abcde}$ | 0.114$^{bcde}$ | 54.59 | 61.36 | 57.14 | 10.71 | 13.04 | 11.76 |
| N16 | 0.330$^{defgh}$ | 0.239$^{efghi}$ | 0.569$^{fghij}$ | 0.058$^{bcdef}$ | 0.046$^{bcdef}$ | 0.103$^{de}$ | 44.10 | 35.79 | 40.15 | 3.57 | 0 | 0.98 |
| N21 | 0.273$^{fghi}$ | 0.282$^{bcdefgh}$ | 0.555$^{ghijk}$ | 0.057$^{def}$ | 0.045$^{bcdef}$ | 0.102$^{de}$ | 19.21 | 60.23 | 36.69 | 1.79 | -2.17 | 0 |
| N26 | 0.401$^{abcde}$ | 0.349$^{abcd}$ | 0.750$^{abcde}$ | 0.061$^{bcdef}$ | 0.044$^{cdef}$ | 0.105$^{cde}$ | 75.11 | 98.29 | 84.73 | 8.93 | -4.35 | 2.94 |
| N27 | 0.356$^{cdefg}$ | 0.239$^{efghi}$ | 0.595$^{efghi}$ | 0.073$^{abcd}$ | 0.045$^{bcdef}$ | 0.119$^{abcd}$ | 55.46 | 92.61 | 46.55 | 30.36 | -2.17 | 16.67 |
| LSD[2] | 0.104 | 0.109 | 0.179 | 0.017 | 0.018 | 0.030 | | | | | | |
| CV[2] (%) | 25.06 | 32.39 | 23.83 | 22.95 | 32.09 | 23.18 | | | | | | |

[1]NC= negative control (uninoculated).

[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.

[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.

Highlighted figures indicate those that are significantly different from the applicable control.

**Table 4.4** Effect of different rhizobacterial isolates on the growth of wheat seedlings in seedling trays in the greenhouse (batch 4 continued).

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight[3] | | | Mean dry weight[3] | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| N32 | $0.268^{ghi}$ | $0.161^{i}$ | $0.429^{ijk}$ | $0.052^{fg}$ | $0.035^{ef}$ | $0.087^{ef}$ | 60.69 | -8.52 | 5.67 | -7.14 | -23.91 | -14.71 |
| N33 | $0.313^{efghi}$ | $0.217^{fghi}$ | $0.529^{hjik}$ | $0.061^{bcdef}$ | $0.043^{cdef}$ | $0.104^{de}$ | 36.68 | 23.29 | 30.29 | 8.93 | -6.52 | 1.96 |
| N39 | $0.338^{cdefg}$ | $0.268^{cdefghi}$ | $0.606^{defghi}$ | $0.060^{bcdef}$ | $0.050^{abcde}$ | $0.110^{bcde}$ | 47.59 | 52.27 | 49.26 | 7.14 | 8.69 | 7.84 |
| N41 | $0.383^{bcde}$ | $0.354^{abcd}$ | $0.737^{abcdef}$ | $0.064^{bcdef}$ | $0.059^{abc}$ | $0.124^{abcd}$ | 67.25 | 101.14 | 81.53 | 14.29 | 28.26 | 21.57 |
| N42 | $0.366^{cdefg}$ | $0.307^{abcdef}$ | $0.673^{bcdefgh}$ | $0.062^{bcdef}$ | $0.041^{def}$ | $0.103^{de}$ | 59.83 | 74.43 | 65.76 | 10.71 | -10.87 | 0.98 |
| N45 | $0.349^{cdefg}$ | $0.326^{abcde}$ | $0.675^{bcdefgh}$ | $0.062^{bcdef}$ | $0.053^{abcde}$ | $0.115^{bcde}$ | 52.40 | 85.23 | 66.26 | 10.71 | 15.22 | 12.75 |
| N59 | $0.471^{ab}$ | $0.272^{cdefgh}$ | $0.743^{abcdef}$ | $0.069^{abcde}$ | $0.050^{abcde}$ | $0.119^{abcd}$ | 105.68 | 54.55 | 83.00 | 23.21 | 8.69 | 16.67 |
| N60 | $0.359^{cdefg}$ | $0.315^{abcdef}$ | $0.675^{bcdefgh}$ | $0.060^{bcdef}$ | $0.046^{bcdef}$ | $0.106^{cde}$ | 56.77 | 78.98 | 66.26 | 7.14 | 0 | 3.92 |
| N62 | $0.400^{abcde}$ | $0.386^{ab}$ | $0.787^{abc}$ | $0.067^{bcdef}$ | $0.049^{abcde}$ | $0.116^{bcde}$ | 74.67 | 199.32 | 93.84 | 19.64 | 6.52 | 13.73 |
| NAS6G6 | $0.441^{abc}$ | $0.361^{abc}$ | $0.802^{ab}$ | $0.071^{abcde}$ | $0.059^{abcd}$ | $0.130^{abcd}$ | 92.58 | 105.11 | 97.54 | 26.79 | 28.26 | 27.45 |
| LSD[2] | 0.104 | 0.109 | 0.179 | 0.017 | 0.018 | 0.030 | | | | | | |
| CV[2] (%) | 25.06 | 32.39 | 23.83 | 22.95 | 32.09 | 23.18 | | | | | | |

[1]NC= negative control (uninoculated).
[2]LSD= least significant difference. CV= coefficient of variance expressed in percentages.
[3]Means within a column followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05. The percentage change in weight= [(treatment-control)/control×100] negative values are treatments that are less than the control and positive values are treatments with a higher weight than the control.
Highlighted figures indicate those that are significantly different from the applicable control.

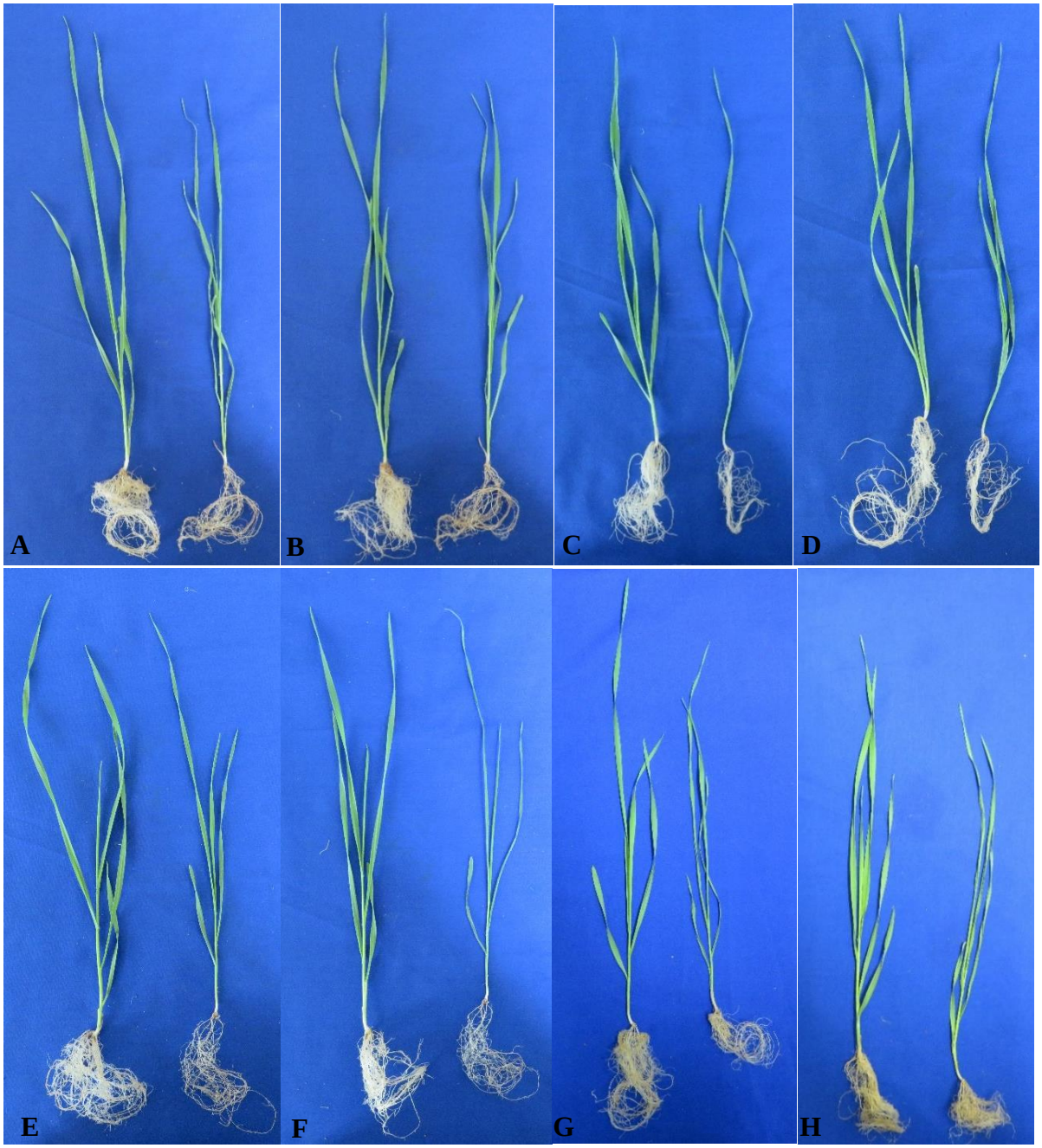


**Figure 4.1** Some representative examples of the effect of PGPR inoculation with selected best performing PGPR isolates (left) in comparison with the uninoculated control (right) on growth of wheat seedlings. A= A33, B=, A20, C= N76, D= A45, E= A08, F= A24, G= KBS1F3, H= N69.

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# CHAPTER 5

## *In vitro* assessment of PGPR traits associated with biocontrol and direct plant growth enhancement

**Abstract**

Plant growth-promoting rhizobacteria with multiple modes of action give the best results in the greenhouse and in the field. In the current chapter, we report the *in vitro* screening of 113 rhizobacterial isolates for a number of PGPR traits. The isolates were screened for antibiosis activity using the dual-culture assay on potato dextrose agar (PDA), nutrient agar (NA) and malt extract agar (MEA) media. Traits such as the production of siderophores, cellulolytic enzymes (cellulase, chitinase, β-1,3-glucanase and protease) and volatile compounds ($NH_3$ and HCN) were also tested. The isolates were also screened for traits associated with direct plant growth promotion such as N-fixation, P-solubilisation, IAA and ACC-deaminase production. Eleven of the isolates inhibited mycelial growth of the phytopathogens *Rhizoctonia solani*, *Fusarium pseudograminearum*, *Phytophthora capsici* and *Macrophomina phaseolina*, while 41 isolates inhibited 3 of the pathogens on either PDA, NA or MEA. These bacterial isolates thus exhibit a broad spectrum of activity. Sixty-eight per cent of the isolates were found to possess at least four of the seven traits associated with biocontrol activity. Of the 113 isolates screened for pathogen inhibition, 13.27% showed 50% or more inhibition of mycelial growth against *R. solani*, 16.81% against *M. phaseolina* and 3.54% against *P. capsici in vitro.* Twenty-one of the isolates tested positive for all four of the PGP traits assessed. These results confirm that several of the isolates possess multiple traits that are desirable in PGPR.

### 5.1 Introduction

The use of plant growth-promoting rhizobacteria began decades ago. However, the search for isolates with novel mode of action is ongoing (Delshadi et al. 2017; Misra et al. 2017; Toribio-Jiménez et al. 2017; Yanti et al. 2017). Various traits of PGPR that may be associated with their mode of action have been studied and PGPR with multiple traits have shown great success in the greenhouse and the field (Arruda et al. 2013; Díaz-Zorita and Fernández-Canigia 2009; Fernandez et al. 2009; Tewari and Arora 2014). In the previous chapters (Chapters 3 and 4), all 113 isolates were screened for plant growth promotion in the presence of *F.*

*pseudograminearum* and direct plant growth promotion in the absence of the pathogen, in seedling assays.

Since *in vitro* studies give an indication of the possible mode of action of PGPR, many studies involving the screening of PGPR start with *in vitro* studies and then proceed to test the efficacy of the isolates in the greenhouse and field (Agaras et al. 2015; Liu 2015; Lucas et al. 2014; Son et al. 2014). It is important to bear in mind that isolates behave differently under different conditions, thus careful consideration is needed when choosing screening methods. In addition to this, the parameters of the assays must be considered.

PGPR promote plant growth directly by increasing nutrient uptake, shoot and root growth leading to increased crop yield or indirectly through the control of plant diseases. Several studies demonstrated that the application of PGPR in crop cultivation enhances N and P uptake from the soil (Adesemoye et al. 2010; Magnucka and Pietr, 2015; Thokchom et al. 2014). Several modes of action are used by PGPR in the direct and indirect plant growth promotion. One of these PGPR mechanisms include the production of ACC deaminase plays a major role in stress alleviation in plants such as drought stress (Vurukonda et al. 2016). PGPR also produce the phytohormone IAA which directly promotes plants growth through the enhancement of cell elongation and division, tissue differentiation and apical dominance (Goswami et al. 2016; Kavamura et al. 2013). This increase in the cell division results in increased length of roots and allows more nutrients to be absorbed from the soil leading to more growth of the plant.

The production of volatiles such as HCN and $NH_3$ also suppress and inhibit the growth of pathogens (Kumar et al. 2014; Kurabachew and Wydra 2013). Production of iron-chelating siderophores is used by PGPR isolates as both a biocontrol and a plant growth promotion trait. Siderophores bind to iron in the soil making it unavailable to pathogens but available for plant use (Paulucci et al. 2015). In addition to this, PGPR can produce cellulolytic enzymes such as glucanases, chitinase, protease and cellulase which degrade fungal cell walls thus inhibiting fungal growth (Ghyselinck et al. 2013). These are just some of the many traits associated with PGPR. Studying these traits contributes to a better understanding of these beneficial microbes.

Screening isolates *in vitro* allows for the assessment of the potential of the isolates under favourable or unfavourable conditions. There are, however, several reports on the lack of correlation between the performance of inoculated isolates *in vitro* and their performance in the greenhouse or the field. A good example is demonstrated by Broadbent et al. (1971), where

out of 3500 isolates screened, 40% could inhibit at least one of nine pathogens on agar plates whilst only 4% were effective in soil. Other examples of this are research conducted by Nguyen & Ranamukhaarachchi (2010) and Ran et al. (2005) where antagonists showed high antagonism *in vitro* but were not very effective in the greenhouse where no disease suppression was observed when isolates were applied either individually or as mixtures.

Performing laboratory screening allows for the detection of traits that might be part of possible modes of action that could be activated in vivo under different conditions. This information is essential as the response of the isolate to different environmental conditions can determine its success or failure as a PGPR. The more screening assays performed the better the mode of action of the isolates may be predicted.

In this chapter, we will assess different PGPR isolates for their ability to demonstrate biocontrol activity as well as plant growth promotion. The major objective is to test a collection of 113 rhizobacteria isolates for biocontrol and plant growth promotion traits such as the production of lytic enzymes (chitinase, glucanase, cellulose and protease), volatiles, siderophores and salicylic acid. Direct plant growth promotion related traits such as the production of indole-3-acetic acid (IAA), 1-aminocyclopropane-1-carboxylate (ACC) deaminase, asymbiotic nitrogen fixation and phosphate solubilization was also assessed. The results obtained from this chapter will be used in combination with those from chapter 3 and 4 (screening by means of seedling assays) to identify the overall best-performing isolates.

## 5.2 Materials and methods

### 5.2.1 Cultures

All bacterial cultures were obtained from the UP-PGPR culture collection. One-hundred and thirteen (113) isolates were used. The isolates are maintained in Microbank™ beads (Pro-Lab Diagnostics) and preserved at 70$^{o}$C. These were plated onto freshly prepared nutrient agar (NA, Biolab) medium and incubated at 25$^{o}$C for 48h.

Working cultures were prepared by streaking bacteria onto the surface of NA slants in McCartney bottles. These were stored at room temperature and streaked onto freshly prepared NA plates when needed. The back-up bacterial cultures were preserved in glycerol. A 65% glycerol solution was prepared and 500µl was dispensed into sterile Eppendorf tubes. Bacteria were grown in a shake-incubator in nutrient broth at 25$^{o}$C and 150rpm for 48h.

An equal volume (500 µl) of bacterial broth was transferred to the Eppendorf tubes containing glycerol. These cultures were then stored at -72$^{o}$C in a freezer.

Fungal pathogen cultures were obtained from different sources. *F. pseudograminearum* was obtained from storage cultures previously used in studies conducted by Makgolane (2016). Storage cultures were prepared by transferring a 5mm diameter fungal culture from a half-strength potato dextrose agar (Biolab) (½ PDA) plate to the centre of another sterile ½ PDA plate. Filter paper was cut into small pieces and autoclaved twice for 30 minutes. The autoclaved filter papers were placed on the surface of ½ PDA plates together with the fungal cultures. The plates were sealed with parafilm and incubated at 25$^{O}$C for 14 days. The fungi colonised filter papers were then aseptically removed from the agar plates and placed on empty sterile petri dishes and allowed to dry in the laminar flow for 3 days. The dried colonized filter paper discs were then placed in sterilised 30ml bottles and placed in the refrigerator. A piece of filter paper was taken out and placed on a ½ PDA plate when needed.

*R*hizoctonia *solani* (AG 2.2 ll) and *Macrophomina phaseolina* was obtained from the Potato Culture Collection of the University of Pretoria. Both pathogens were preserved by placing a block of fungal culture on PDA slants in 30ml McCartney bottles and stored at room temperature. *Phytophthora capsici* (PPRI 17610) was obtained from the Agricultural Research Council (ARC-LNR, PPRI). This pathogen was preserved in 30ml McCartney bottles each containing 20ml sterile (autoclaved) distilled water, 2 wooden toothpicks and cuttings of lawn grass. The bottles were then stored at room temperature.

### 5.2.2 Biocontrol-related traits

#### 5.2.2.1 Antibiosis test

The fungal isolates were initially grown on PDA for 7 days after which a 5mm diameter disc of the actively growing fungal pathogen was placed in the centre of a 9cm diameter petri dish containing PDA. A single bacterial colony of the test isolate was stab-inoculated onto the PDA plate using an inoculation needle as shown in the figure below (Figure 5.1). The controls were prepared in the same manner but were not inoculated with any bacteria. Three replicates were used per treatment with one PDA plate representing one replicate. The plates were incubated at 25$^{o}$C for 7 days or until pathogen growth in the control reached the edge of the plate after which they were assessed for fungal inhibition. The actual zone of inhibition of the pathogen was measured as explained below. This test procedure was repeated using NA and malt extract

agar (MEA, Biolab) respectively. The percentage inhibition of the pathogen was calculated according to the following equation:

$$\% \text{ of pathogen growth inhibition} = [(R2-R1)/R2] \times 100$$

Where, R2 is the maximum radial growth of pathogen on the control plate and R1 is the growth of the pathogen between the edge of the fungal disc and the margins of bacterial growth.

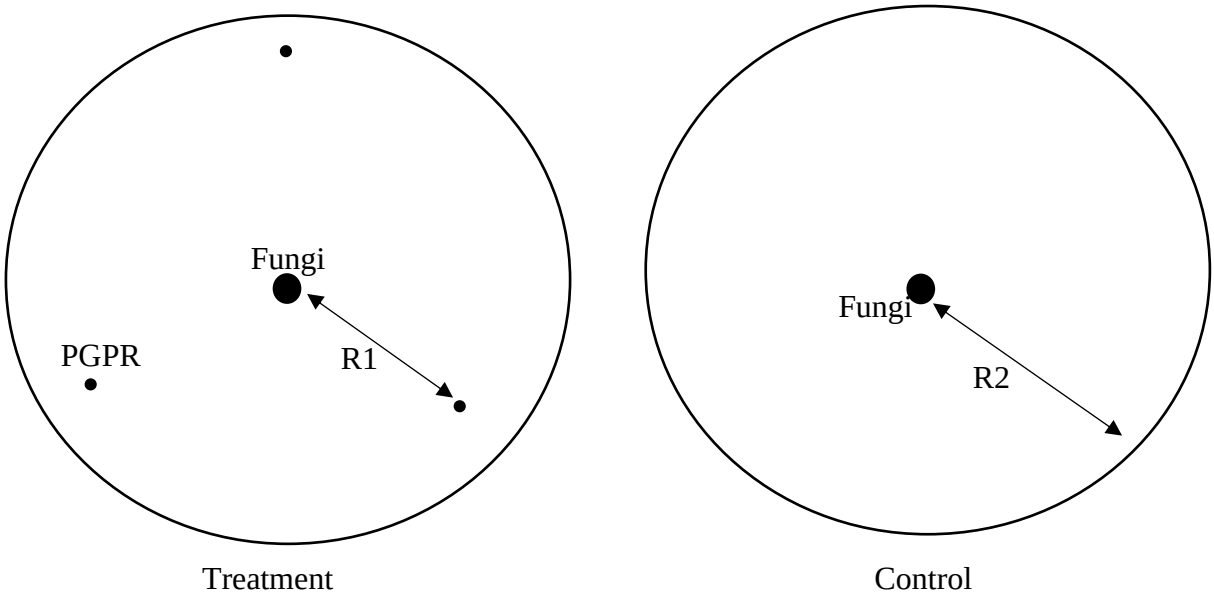


**Figure 5.1** Demonstration of the set-up of dual-culture assays. Treatment plate was stabbed in three different areas with a single PGPR isolate, with the block of fungi in the centre of the plate. The control plate had only the fungal block in the centre of the plate.

5.2.2.2 Production of volatiles

The production of hydrogen cyanide (HCN) was determined using HCN indicator paper (Castric and Castric 1983). Isolates were streaked on NA amended with and 4.4g/l glycine. To record the effect of glycine on the production of HCN, the isolates were also grown on NA without glycine. To prepare the HCN indicator paper, a Whatman no. 1 filter paper was soaked in HCN detection reagent and allowed to dry. The reagent was composed of a solution of 5mg copper (II) ethyl acetoacetate and 5mg 4,4'-methylenebis-(*N*,*N*-dimethylaniline) in 2 ml of chloroform (Merck). The indicator paper was then placed on the lid of the petri plates. The

plates were sealed with parafilm and incubated for 3 days at 25ºC. Following the 3 days of incubation, the change in colour of the filter paper was recorded. Colour change from pale aqua to dark blue was indicative of a positive reaction.

The production of $NH_3$ was tested using the method described by Cappuccino and Sherman (1992). Freshly grown cultures of the bacteria were inoculated into 10ml of autoclaved peptone water (15g/1L) in 15ml test tubes of three replicates and incubated at 25ºC for 72h. Following incubation, 0.5ml of Nessler's reagent (10% $HgI_2$; 7% KI; 50% aqueous solution of NaOH (32%)) was added to each test tube. The development of a yellow to brown colour was indicative of a positive result for ammonia production (Cappuccino and Sherman 1992). The development of a light-yellow colour was indicative of a small amount of ammonia while a deep yellow to brown colour was indicative of maximum ammonia production.

5.2.2.3 Siderophore production

The production of siderophores was assessed using chrome azurol S (CAS) agar (Alexander and Zuberer 1991) as described by Hassen (2007). CAS media was prepared by separately preparing CAS indicator, basal solutions and 20ml sterile 50% glucose solution. The CAS indicator comprised of 60.5mg chrome azurol S in 50ml $ddH_2O$ mixed with 10ml $Fe^{3+}$. A solution of 72mg HDTMA in 40ml $ddH_2O$ was added to the above mixture resulting in a total volume of 100ml. CAS basal medium was prepared by dissolving 30g MOPS, 0.1g $NH_4Cl$ and 0.3g $KH_2PO_4$ in 880ml $ddH_2O$. The pH was adjusted to 6.8 with 6M NaOH. Fifteen grams of agar was added to the solution while stirring. The two solutions were autoclaved, cooled to 50ºC and then mixed. The CAS indicator solution was added to the basal medium by carefully pouring it along the wall of the flask. The agar solution was poured into petri dishes. After setting, the CAS-agar was overlaid with a thin layer (5mm) of NA. The plates were stab inoculated with bacterial isolates from NA in triplicate and incubated at 25ºC for 7 days. An uninoculated plate served as the negative control. After incubation, isolates that could separate the Fe-CAS complex caused a change in the colour of the media from blue to yellow-orange, indicating siderophore production (Hassen 2007). The halo diameter was also measured.

5.2.2.4 Chitinase, protease, cellulase and β-1,3-glucanase production

The production of chitinase was assessed using chitin agar (Roberts and Selitrennikoff 1988). Choloidal chitin was prepared by grinding 20g crab shell chitin (Sigma) in a coffee grinder and

digesting for 24h with 350ml concentrated hydrochloric acid at 4ºC. Following this, the digested chitin was filtered into 2L ethanol with rapid stirring and centrifuged at 2000g for 20 minutes. The pellet was washed with distilled water until the pH of the solution was around 4.0 after which it was adjusted to 6.0 using 2N NaOH. This solution was centrifuged again, and the pellet collected. The colloidal chitin was stored at -20ºC. The chitin agar comprised of 1g $NH_4H_2PO_4$, 0.2g KCl, 0.2g $MgSO_4 \cdot 7H_2O$, 1% (w/v) colloid chitinolytic, 15g agar in 1000mL distilled water and the pH adjusted to 7.0. The media was then autoclaved and poured into Petri plates. A bacterial colony was spot inoculated on the plate at three places equidistance from each other in triplicates and incubated at 30ºC for 7 days. Development of clear halo zones around the bacterial culture was indicative of positive production of chitinase (Murthy 2012). The diameter of the halos was measured in millimetre (mm).

Protease activity was assessed on skimmed milk plates (Zheng et al. 2011). The bacterial isolates were spot inoculated onto NA plates containing 1% (W/V) skimmed milk (10g in 1000mL $dH_2O$). After autoclaving, the two solutions were mixed while still hot. The prepared medium was poured into Petri plates and inoculated with the respective rhizobacterial isolates at three places equidistance from each other. This was done in triplicate and the plates were incubated at 25ºC for 7 days. Following incubation, clear zones around the bacterial colonies were indicative of the production of protease. The diameter of the clear zones was recorded.

The production of cellulase was assessed in 1% (W/V) carboxymethyl-cellulose (10g in 1000mL) (Sigma-Aldrich) basal medium containing 1g $NaNO_3$, 1g $K_2HPO_4$, 1g KCl, 0.5g $MgSO_4$, 0.5g yeast extract, 1g glucose and 15g agar in 1000ml distilled water. The medium was autoclaved and poured into Petri plates. The respective bacterial isolates were spot inoculated onto the plate at three places equidistance from each other and incubated at 25ºC for 7 days. Each isolate was done in triplicate. Following incubation, the plates were flooded with 0.01% Congo red and allowed to stand for 15 minutes. De-staining was done using 1% NaCl solution and plates were allowed to stand for 5 minutes. A clear zone against the red background was indicative of the production of cellulase (Dinesh et al. 2015). The diameter of the clear zones was recorded.

The production of β-1,3-glucanase was tested for by assessing the ability of the isolates to grow on medium supplemented with laminarin as the sole carbon source (Kumar 2012). The medium used comprised of 0.8g $K_2HPO_4$, 0.2g $KH_2PO_4$, 0.5g $(NH_3)_2SO_4$, 0.2g $MgSO_4 \cdot 7H_2O$, 10mg $CaCl_2 \cdot 2H_2O$, 10mg $FeC1_3 \cdot 6H_2O$, 1mg $ZnSO_3 \cdot 7H_2O$, 500mg laminarin and 15g agar in 1L distilled water. The media was autoclaved and poured into Petri plates. The respective bacterial

isolates were streaked onto the plates. Three plates were used for each isolate. The plates were incubated at 30°C for 3 days. After incubation, growth on the medium was indicative of the production of β-1,3-glucanase (Kumar 2012).

### 5.2.3 Plant growth promotion traits

#### 5.2.3.1 Nitrogen fixation

The rhizobacteria were tested for their ability to grow on nitrogen-free semisolid media (JNFb) as described by Olivares et al. (1996) and glucose nitrogen free mineral medium (GNFMM) (Ahmad et al. 2013). JNFb comprised of 0.6g $K_2HPO_4$, 5g malic acid, 1.8g $KH_2PO_4$, 0.2g $MgSO_4 \cdot 7H_2O$, 0.1g NaCl, 0.02g $CaCl_2 \cdot 2H_2O$, 0.5% bromothymol blue in 2ml 0.2N KOH, 1ml vitamin solution, 2ml micronutrient solution, 1.64% Fe-ethylene diaminetetraacetic acid solution (4ml), 4.5g KOH per l000mL $dH_2O$. The micronutrient solution contained 0.4g $CuSO_4 \cdot 5H_2O$, 0.12g $ZnSO_4 \cdot 7H_2O$, 1.4g $H_2BO_3$, 1.0g $Na_2MoO_4 \cdot 2H_2O$, 1.5g $MnSO_4 \cdot H_2O$ per 1L $H_2O$ while the vitamin solution contained 10mg biotin and 20mg pyridoxol-HCl in 100ml. Ten milliletres were poured into test tubes and autoclaved. A loop full of one bacterial isolate from a single colony was transferred into the test tube containing the media. The control was inoculated with sterile distilled water. The tubes were incubated at 25°C for 4 days. After incubation, the presence of the formation of white pellicles were indicative of the possible ability to fix atmospheric nitrogen (Pretorius 2012). GNFMM comprised of 20g glucose, 1g $K_2HPO_4$, 0.1g CaCl, 0.5g NaCl, 0.25g $MgSO_4 \cdot 7H_2O$, 0.01g $FeSO_4 \cdot 7H_2O$, 0.01g $MnSO_4 \cdot H_2O$, 20g agar, 2ml bromothymol blue (0.5g BTB in 100ml ethanol) per 1000ml distilled water. The respective bacterial isolates were streaked onto the plates and incubated at 25°C for 4 days. Three replicate plates were used per isolate. After incubation, growth on the media was indicative of the ability to fix nitrogen (Ahmad et al. 2013).

#### 5.2.3.2 Phosphate solubilization

To test the ability of the rhizobacterial isolates to solubilize phosphate, the Pikovskaya (PVK) and its variation, the National Botanical Research Institute's Phosphate (NBRIY) growth media were used (Nautiyal 1999). The PVK growth medium comprised of 10g glucose, 5g $Ca_3(PO_4)_2$, 0.5g $(NH4)_2SO_4$, 0.2g NaCl, 0.1g $MgSO_4 \cdot 7H_2O$, 0.2g KCl, 0.002g $MnSO_4 \cdot H_2O$, 0.002g $FeSO_4 \cdot 7H_2O$, 0.5g yeast extract and 15g agar per 1000ml of distilled water. The NBRIY growth media contained 10g glucose, 5g $Ca_3(PO_4)_2$, 0.5g $(NH4)_2SO_4$, 0.2g NaCl, 0.1g $MgSO_4 \cdot 7H_2O$,

0.2g KCl, 0.002g $MnSO_4 \cdot H_2O$, 0.002g $FeSO_4 \cdot 7H_2O$ and 15g agar per 1000ml of distilled water. After preparation, the media was autoclaved for 20 min at 120$^oC$ and upon cooling decanted into 90mm petri dishes. The respective rhizobacterial isolates were spot inoculated onto the plates at three places equidistance from each other. The control plates were inoculated with sterile distilled water. Three plates were used per isolate. Plates were incubated at 25$^oC$ for seven days. After incubation, clear zones or halos around the bacterial colonies was indicative of phosphate solubilization (Pretorius 2012). The size of the halo was measured.

5.2.3.3 IAA production

The ability of the rhizobacteria to produce IAA was assessed using Salkowski's reagent (Glickmann and Dessaux 1995). A single colony of PGPR isolate was inoculated into 10ml of NB in a test tube and incubated in a shaking incubator at 150rmp at 25$^oC$ for 72h. Fifty millilitres of the sample was centrifuged at 12000g for 6 minutes after which 1ml of the supernatant was transferred into a test tube and mixed with 2ml of Salkowski's reagent (4.5g of $FeCl_3$ per litre in 10.8M $H_2SO_4$). A negative control was prepared by adding 2ml of Salkowski reagent to 1ml of NB. The mixtures were left in the dark at room temperature for 30min. The change in colour of the mixture to pink-red was indicative of the production of indolic compounds (Bumunang and Babalola 2014). A standard curve was prepared by using a series of IAA concentrations ranging from 0 to 200µl in sterile nutrient broth. The samples were transferred into microtitre plates and measured spectrophotometrically at an absorbance of 530nm and the concentration of IAA produced was determined using the standard curve.

5.2.3.4 ACC deaminase production

The production of ACC deaminase was tested for as per Kejela et al. (2016) on Dworkin and Foster (DF) minimal medium. The medium was comprised of 4g $KH_2PO_4$, 15g $Na_2HPO_4 \cdot 12H_2O$, 0.2g $MgSO_4 \cdot 7H_2O$, 1mg $FeSO_4 \cdot 7H_2O$, 10µg $H_3BO_3$, 11µg $MnSO_4 \cdot H_2O$, 125µg $ZnSO_4 \cdot 7H_2O$, 78µg $CuSO_4 \cdot 5H_2O$, 17µg$Na_2MO_4 \cdot 2H_2O$ and 15g agar in 1L $dH_2O$. The medium was autoclaved and poured into Petri dishes and allowed to set. ACC solution was prepared by dissolving 0.007g ACC powder in 25ml 10% ethanol to bring the concentration to 3mM. The prepared ACC solution was spread onto the surface of the medium and allowed to dry in the laminar flow for 10 minutes. The plates were then inoculated with the respective bacteria and incubated at 30$^oC$ for 3 days. After incubation, bacterial growth on the plate was

considered as evidence for the ability to utilize ACC as the sole source of nitrogen through the production of ACC deaminase (Kejela et al. 2016). To confirm the utilization of ACC, the isolates were also streaked on medium without ACC. Inability of isolates to grow on this medium confirmed the production of ACC deaminase.

## 5.3 Results

### 5.3.1 Biocontrol related traits

The mycelial growth of *M. phaseolina* was inhibited by only seven bacterial isolates, namely, N53 (36%), N54 (43%), N59 (37%), N63 (50%), N67 (50%), N69 (45%) and T29AC (40%) on PDA. Because *M. phaseolina* is a fast-growing fungus, this trial was repeated under different conditions (the bacterial isolates were plated 3 days before plating the fungus). In addition to the seven isolates, N26 (35%) could inhibit the growth of the pathogen. With the different incubation style, the inhibition of the pathogen by the isolates was greater: N53 (54%), N54 (63%), N59 (49%), N63 (50%), N67 (70%), N69 (64%) and T29AC (57%) (Table A1 and A2 in Appendix A and B, Figure 5.1). Actual inhibition zones caused by the isolates were 1, 7, 4, 6, 7, 12, 10 and 8mm for N26, N53, N54, N59, N63, N67, N69 and T29AC respectively. Forty-six per cent of the isolates inhibited the growth of the pathogen on NA. Isolates that resulted in the largest inhibition zones were N54, N69 and T29AC at 13, 11 and 12mm respectively. On MEA, 12% of the isolates inhibited pathogen growth. Maximum inhibition was observed with N56(59%), and N59(68%) rendering inhibition zones of 9 and 11mm respectively. Isolate N69 also resulted in an inhibition zone of 8mm.

Only A19 (33%), A22 (51%), A28 (39%), N59 (45%), N67 (67%), N69 (47%) and T29AC (35%) were able to inhibit the growth of *R. solani* on PDA (Table A1, Figure 5.2). However, some of these isolates did not result in large inhibition zones. Inhibition zones of 15, 5 and 8mm were produced by the bacterial isolates A22, N59 and N69 respectively (Table A2). Where isolates did not inhibit the growth of the pathogen mycelia grew over the bacterial colonies at the point of inoculation. Where inhibition did occur, the margins of the fungal colonies showed a change in colour. Isolate N54 (53%) and T29AC (59%) produced inhibition zones of 5 and 9mm respectively on NA whereas 13% of the bacterial isolates inhibited the growth of the pathogen on MEA. Notable examples with the largest zone of inhibition were N12 (50%) and N69 (59%) producing inhibition zones of 12 and 8mm, respectively.

The growth of *P. capsici* on PDA was inhibited by 17% of the isolates. Isolate N56 and N59 resulted in the greatest inhibition. Isolates that resulted in the largest inhibition zones were N56, N59 and N69 producing inhibition zones of 9, 10 and 8mm, respectively (Table A1, A2 and Figure 5.3). On NA, 17% of the bacterial isolates inhibited the growth of the pathogen with the highest inhibition by isolates N42 (48%), N63 (57%) and T29AC (40%) with inhibition zones of 9, 12 and 11mm respectively whereas 5% of the bacterial isolates were able to inhibit the growth of the pathogen on MEA. These isolates were A12 (30%), A28 (51%), N28 (25%), N53 (32%), N54 (28%) and T29AC (34%). Isolate A12 (4mm), N28 (1mm), N53 (4mm), N54 (3mm) and T29AC (5mm) produced in inhibition zones.

None of the bacterial isolates inhibited the growth of *F. pseudograminearum* on PDA. The pathogen either touched the margins of the bacterial colonies or grew over it. On NA, A41 (38%) and N63 (32%) inhibited the pathogen producing inhibition zones of 1 and 6mm respectively (Figure 5.4). In contrast, on MEA, the bacterial isolates A48 (19%), N28 (25%), N31 (9%), N32 (3%), N54 (36%), N59 (26%), N67 (7%), N69 (18%), T11 (32%) and T29AC (21%) inhibited the pathogen (Table A1). However, no distinct inhibition zones were observed with either of these isolates.

Sixty-five per cent of the bacterial isolates tested positive for protease production (Table 5.1). Isolates such as N01, N16W, N28, N59C, N60, N71 and NAS6G6 produced the maximum amount of protease and totally cleared the plates as shown in Figure 5.5. Out of all the isolates that were screened, 24 resulted in clear zones after staining of CMC agar indicating cellulase production (Figure 5.6). N17 and N54 resulted in the maximum clear zones with an average of 25 and 21mm diameters respectively. Twenty-one of the isolates (19%) tested positive for chitinase production and formed the largest clear zones amongst which were isolates N03 (22mm), N53 (21 mm), A09 (19mm), N41 (19mm), A14Y (18mm) and A41 (18mm) (Figure 5.7). Of the 113 isolates that were screened for glucanase production, only eight were found unable to grow on the medium containing laminarin as the sole carbon source (Figure 5.8) indicating a lack of production of glucanase.

Out of all 113 bacterial isolates that were assessed, 23 showed no production of siderophores while 51 showed little production, 54 showed moderate and 12 the highest production of siderophores. The 12 isolates that showed the highest production of siderophores were A22, A39, N19, N21, N44, N59A, N59C, N69, N70, N74, T22 AND T29AC (Figure 5.9). All isolates were found to produce $NH_3$. Of all the isolates tested, 6 changed the colour of the solution from colourless to brown which was indicative of high production of $NH_3$ (Figure

5.10). Fifty-nine isolates were found to produce moderate levels while 51 were found to produce the least amount of the volatile compound. Twenty-two isolates (19%) tested positive for HCN production (Figure 5.11).

### 5.3.2 Plant growth promotion-related traits

Possible ability for nitrogen fixation was assessed on two different media namely JNFb and GNFMM (Figure 5.12). All the isolates tested positive for growth on nitrogen-free medium (JNFb) except A48. It was also observed that even though some isolates (T22 and N77W) did not change the colour of the medium, growth could be observed. Out of 113 isolates, 31 did not grow on GNFMM.

Phosphate solubilisation was tested on two different media namely PVK that contained yeast extract while NBRIY did not. A significant percentage, 73%, of the bacterial isolates showed an ability to solubilize phosphate on NBRIY while only 65% of the isolates showed the ability on PVK (Table 5.1). Isolates that resulted in the maximum halo diameter were A16, N03, N42, N53, N67, N69 and N78 with 20, 21, 21, 24, 23, 26 and 22mm halo diameters respectively. Isolates such as A06, A46, KBS1F3, N20, N26, N37, N45, N59B and N68 did not grow on NBRIY which does not contain yeast extract. The maximum halo diameter on PVK was observed with N69 (19mm), N67 (19mm), N42 (18mm), N39 (17mm), N26 (17mm) A39 (18mm) and A35 (18mm) while A46, N20, N68 and NAS6G6 did not grow on this medium (Figure 5.13). A few isolates that showed the ability to solubilize phosphate on NBRIY did not do so on PVK and *vice versa*.

All the bacterial isolates tested positive for IAA production but produced very small amounts (Figure 5.14), the concentration of which could not be determined using the IAA standard curve. After 3 days of incubation 26 of the isolates grew on the medium supplemented with ACC (Figure 5.15) while 15 of the isolates also grew on medium without ACC.

## 5.4 Discussion

The aim of the current study was to screen a collection of 113 rhizobacterial isolates to determine their biocontrol and plant growth promotion traits. Of the 113 rhizobacterial isolates screened during the current study, 11 inhibited the growth of all four pathogens while 41 inhibited 3 of the pathogens on either of the media used. Some notable isolates in this regard include N54, N59, N67 and N69. All bacterial isolates were found to produce volatile $NH_3$ while 19% of the isolates tested positive for HCN production. Twenty-seven of the 113 bacterial isolates produced at least three of the enzymes tested for while 90 of the bacterial isolates produced siderophores. All bacterial isolates tested in the current study exhibited at least two of the four direct plant growth promotion associated traits that were assayed for (nitrogen fixation, phosphate solubilization, IAA and ACC deaminase) while most isolates tested positive for three of the traits.

Plant growth can be promoted indirectly through biocontrol of plant pathogens. There are several different mechanisms of biological control of plant pathogens namely competition for nutrients and space, production of antibiotic compounds, volatiles and siderophores. This indicates broad-spectrum antibiotic activity by the isolates. Careful consideration needs to be taken on the type of medium to be used for dual-culture assays. The type of medium used should ideally support the growth of both the pathogen and PGPR. The use of a number of different media in dual cultures allows for the study of the behaviour and capability of isolates under different nutrient status.

In many PGPR related studies, screening for biocontrol activity is mainly done on PDA medium which provides optimal conditions for fungal growth and not bacterial growth (Agaras et al. 2015; Dinesh et al. 2015; Khalili et al. 2016; Rais et al. 2016). This creates a bias in favour of the fungi and to the detriment of the bacteria. Some of the factors that may influence the production and diffusion of antibiotics in artificial growth media include pH, composition of the medium and the thickness of the medium (Muiru 2015). Thus, the bacterial isolates may be able to inhibit the growth of a pathogen on one medium and not another. Generally, most of the bacterial isolates in the current study were able to inhibit the growth of the pathogens on NA medium. The pH or other components of the medium used could contribute to the slower growth rate of the pathogen and this can be confused with the effect of the PGPR on the fungi. Inhibition zones observed on a plate could be due to inhibitory pH or depletion of nutrients resulting in the decreased growth rate of either the bacteria, fungi or even both organisms. This would then lead to false-positive results. Gas chromatography–mass spectrometry analysis of

cell-free culture filtrates can be used to confirm the production of specific antibiotics by isolates (Hassen et al. 2008; Zalila-kolsi et al. 2016; Zhang et al. 2015).

The production of cellulytic enzymes could contribute to the antibiosis activity of the isolates. Only soil-borne fungal pathogens were included in the study, and the major cell wall components of fungi are chitin and glucans (Legrand et al. 2017). The production of enzymes such as chitinase and glucanases may have contributed to the antifungal activity of the bacteria. In addition to the enzymes produced, some of the isolates produced siderophores which can assist in the control of pathogens. Siderophores are iron chelators that bind iron ($Fe^{3+}$), making it unavailable to fungal pathogens, thereby limiting the growth of the pathogens. However, iron bound to siderophores is available to plants as they possess mechanisms that allow them to use the bound iron (Parray et al. 2016).

All the rhizobacterial isolates assessed produced $NH_3$ while 19% produced HCN. Santiago et al. (2015) found that the production of HCN could delay the growth of *Rhizoctonia solanacearum* by two days at the lowest concentrations and completely inhibits its growth at the highest concentration. Bacterial isolate A19 inhibited three pathogens whilst producing the maximum amount of $NH_3$ and showing strong production of cellulose and protease (large halos on test medium). This isolate also tested positive for the production of siderophores and chitinase. In contrast, isolate A28 which also inhibited three of the pathogens only tested positive for the production of $NH_3$ and protease.

As previously stated, rhizobacteria behave differently under different conditions. The presence or absence of certain traits is not enough to determine the performance of a bacterium either in the greenhouse or in the field (Batista et al. 2017). Similar results were obtained with bacterial isolate N26 that only tested positive for the production of $NH_3$ and siderophores and none of the lytic enzymes but could still inhibit the growth of three of the pathogens on either of the media. Isolate N54 on the other hand inhibited all four of the pathogens. This isolate was found to produce a moderate amount of $NH_3$, siderophores, cellulase as well as protease. Isolate N59 also inhibited four pathogens whilst producing a moderate amount of $NH_3$, high level of HCN and siderophores but none of the enzymes. Surprisingly, isolate N69, which inhibited all four of the pathogens only produced $NH_3$ and siderophores but none of the antibiotic enzymes. Isolates such as A34 and A07AC inhibited the growth of just two of the pathogens but produced high levels of chitinase, protease, cellulose, siderophores and $NH_3$.

One of the characteristics of a good PGPR is the ability to compete with other microorganisms for space and nutrients. Some of the bacterial isolates in the current study did not produce inhibition zones during the dual culture assays (unable to inhibit pathogen growth). However, the isolates occupied most of the space on the plate indicating excellent ability to compete for space and nutrients (Koutb and Ali 2010). Past research has shown that the ability of PGPR to grow fast and use nutrients that other microbes cannot use affords them the ability to compete and proliferate better than the other microbes (Hao et al. 2012; Kim et al. 2012). Thus, the ability to compete for resources and niche is another important factor to consider when screening for PGPR. Though competition was not directly assessed in this study, one cannot ignore the results observed and what they could mean (Adhya et al. 2015). As Mavrodi et al. (2012) stated, good PGPR are good root colonizers, however, good root colonizers are not always good PGPR. The ability of PGPR to compete for space is an excellent quality but does not guarantee control of pathogens.

All the biocontrol traits assessed above act together to contribute towards fungal control by the isolates. In this study, 68% of the isolates exhibited at least four of the seven biocontrol traits assayed. It was also observed that amongst these isolates, there were isolates that resulted in the highest percentage of inhibition of the pathogens. Batista et al. (2017) highlighted the need for the investigation of PGP with multiple traits. However, it must be kept in mind that their performance can differ with different conditions. Success or failure *in vitro* does not automatically translate to success or failure *in planta*. The behaviour of the isolates in different situations cannot be predicted. This, however, does not negate the importance of performing *in vitro* screening. Performing *in vitro* tests allow for the investigation of possible mode of action of plant growth promotion or biocontrol activity of the isolates.

Apart from promoting plant growth through the control of pathogens, PGPR can also promote plant growth by directly increasing nutrient availability for plant growth (Ghignone et al. 2012). Biological N-fixation plays an important role in plant growth as it accounts for two-thirds of the nitrogen fixed in the world (Parray et al. 2016). Amongst other microorganisms, PGPR have been shown to increase the amount of available nitrogen and phosphate in the soil thus allowing for reduction in the need for agrochemicals (Agaras et al. 2015). In the current study 109 of the bacterial isolates were able to grow on nitrogen-free media indicating possible ability to fix atmospheric nitrogen. This fixed nitrogen is not only available for PGPR growth but available for plant use as well. N-fixation was assayed on different media to also assess the effect of different medium composition on the ability of the isolates to fix atmospheric nitrogen.

Most of the phosphate in the soil is insoluble as it is present as aluminium, iron or calcium phosphate making it unavailable for plant use (Bhattacharyya and Jha 2012). Phosphate solubilizing bacteria can break the bond between these molecules to make the nutrient available for plant use. The use of PGPR that solubilize phosphate can, therefore, reduce the amount of phosphate fertilizers applied to the soil (Kaur and Sudhakara Reddy, 2014). Eighty-seven of the 113 bacterial isolates tested in the current study could solubilize phosphate. Again, phosphate solubilization was assayed on two different media to assess the effect of medium composition on the ability of the isolates to solubilize phosphate. The use of PGPR that fix nitrogen and solubilise phosphate could decrease the reliance of farmers on chemical fertilisers which pose a danger to the environment and human health (Adesemoye and Kloepper 2009; Barlog and Grzebisz 2004; Kaur and Reddy 2014; Kumar et al. 2009).

IAA has been shown to loosen plant cell walls and increase the amount of root exudates (Kavamura et al. 2013). The increase of root exudates, in turn, promotes the proliferation of PGPR in the rhizosphere. Although the amount of IAA produced by the bacterial isolates in the current study was too small to be estimated using a standard curve, all the isolates showed the ability to produce this important phytohormone. This corresponds with the work of other authors who have also found a high number of IAA producers in their collections of rhizobacteria (Arruda et al. 2013; Batista et al. 2017; Farina et al. 2012; Ji et al. 2014; Passos et al. 2014). IAA has also been shown to be directly related to pathogen control through priming of plant defence responses (Egamberdieva et al. 2015; Petti et al. 2012).

1-aminocyclopropane-1-carboxylate (ACC) deaminase alleviates drought and salinity related stress through the inhibition of the lethal phytohormone ethylene (Yang et al., 2009). Ethylene is a phytohormone produced in response to environmental and abiotic stress in plants. Overproduction of this signalling phytohormone is however detrimental to the plant (Magnucka and Pietr 2015). ACC is the precursor of ethylene. ACC deaminase hydrolyses ACC, thus halting the production of ethylene. Through the production of ACC deaminase, PGPR can upregulate and downregulate genes responsible for drought response (Vurukonda et al. 2016). In the current study, 26 bacterial isolates grew on medium supplemented with ACC as the sole source of carbon thus indicating the ability to produce ACC deaminase.

These results indicate that application of these bacterial isolates could potentially protect plants against salinity and drought stress. However, results also showed growth in a medium that was not supplemented with any nitrogen source (ACC). Some PGPR species are known to be effective nitrogen scavengers and this might have been the reason why some of the isolates

could grow in a medium that did not contain the nitrogen source ACC (Ambrosini et al. 2012; Baldani 2014). N-fixation by PGPR is highlighted in this regard as well. The production of ACC deaminase by rhizobacteria has also been shown in studies conducted by Yue et al. (2007) to enhance the availability and absorption of nutrients such as nitrogen, phosphate, potassium and calcium while decreasing the absorption of sodium. The hydrolysis off ACC also leads to the production of $NH_4^+$, which can also be used as a nitrogen source by the plant thereby promoting plant growth. The benefits of ACC deaminase can hardly be emphasized enough, thus making strains with this trait highly desirable

In this study, the bacterial isolates A14W, A14Y, A41, A45, N04, N04AC, N10, N12, N14, N15ii, N29, N30, N31, N42, N43, N44, N56, N59, N64, N67 and N74, tested positive for all four of the PGP traits that were assayed. N-fixation, P-solubilization, IAA and ACC deaminase production all contribute to the promotion of plant growth. Therefore, the bacterial isolates exhibiting these traits have promising potential as efficient plant growth promoters both in the greenhouse and in the field. Biofertilizer strains can also be used in combinations to improve soil fertility and plant growth.

The results of the current study show that some of the isolates have multiple PGPR traits associated with plant growth promotion and biocontrol activity. The isolates show huge potential as PGPR and thus warrant further studies both under greenhouse and field conditions. It was also noted that some of the isolates showed the potential as direct plant growth promoters and could be used to supplement organic fertilizers while others showed great potential as biocontrol agents and some showed potential as both plant growth promoters and biocontrol agents.

## 5.5 Tables and Figures

**Table 5.1** Specific PGPR traits exhibited by the rhizobacterial isolates (– being negative and + being positive for the particular trait).

| Isolate | Biocontrol trait | | | | | | | | Direct plant growth promotion trait | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | HCN | | Siderophore | Cellulase | Protease | Chitinase | β-1,3-Glucanase | N-fixation | | P-solubilisation | | IAA | ACC-deaminase |
| | | NA | Glycine-NA | | | | | | JNFb | GNFMM | NBRIY | PVK | | |
| A05A | + | - | - | - | - | + | - | + | + | + | - | - | + | + |
| A05B | + | - | - | - | - | + | - | + | + | + | - | - | + | - |
| A05C | + | - | - | - | - | + | - | + | + | + | - | - | + | - |
| A06 | + | - | - | - | - | + | - | + | + | - | - | - | + | - |
| A07 | + | - | + | + | - | + | - | + | + | + | + | - | + | - |
| A07AC | + | - | - | + | + | + | + | + | + | + | + | - | + | - |
| A08 | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| A09 | + | - | - | + | - | + | + | + | + | + | + | + | + | - |
| A1 | + | - | - | - | - | + | - | + | + | - | - | - | + | + |
| A10 | + | - | - | + | + | + | - | + | + | + | + | + | + | - |
| A10AC | + | - | - | + | + | - | - | + | + | + | + | + | + | - |
| A12 | + | - | - | - | - | + | - | + | + | + | + | + | + | - |
| A14W | + | - | - | + | + | + | - | + | + | + | - | + | + | + |
| A14Y | + | - | - | + | + | + | + | + | + | + | + | + | + | + |
| A16 | + | - | - | + | - | + | - | + | + | + | + | - | + | - |
| A17 | + | - | - | - | + | + | - | + | + | - | - | + | + | - |
| A19 | + | - | - | + | + | + | + | + | + | - | + | + | + | - |
| A20 | + | - | - | + | - | + | + | + | + | + | + | - | + | - |
| A21 | + | - | - | - | + | + | + | + | + | + | - | - | + | + |
| A22 | + | - | - | + | - | + | - | + | + | + | - | - | + | + |
| A24 | + | - | - | + | - | + | - | - | + | - | - | - | + | - |
| A25 | + | - | - | + | - | + | - | + | + | + | - | - | + | - |

**Table 5.1 continued** Specific PGPR traits exhibited by the rhizobacterial isolates (– being negative and + being positive for the particular trait).

| Isolate | Biocontrol trait | | | | | | | | Direct plant growth promotion trait | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | HCN | | Siderophore | Cellulase | Protease | Chitinase | β-1,3-Glucanase | N-fixation | | P-solubilisation | | IAA | ACC-deaminase |
| | | NA | Glycine-NA | | | | | | JNFb | GNFMM | NBRIY | PVK | | |
| A26 | + | - | - | + | + | + | - | + | + | + | + | - | + | - |
| A27 | + | - | - | + | + | + | - | + | + | + | - | - | + | - |
| A28 | + | - | - | - | - | + | - | + | + | + | + | + | + | - |
| A29 | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| A32 | + | - | - | - | - | + | - | + | + | - | - | - | + | - |
| A33 | + | - | - | + | - | + | - | + | + | - | - | - | + | - |
| A34 | + | - | - | + | + | + | + | + | + | - | + | + | + | - |
| A35 | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| A36 | + | - | - | + | - | + | - | + | + | - | - | - | + | - |
| A37 | + | - | - | - | - | + | - | + | + | - | + | - | + | - |
| A38 | + | - | - | + | - | + | - | + | + | + | - | - | + | - |
| A39 | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| A40 | + | - | - | - | - | - | - | + | + | - | + | - | + | - |
| A41 | + | - | - | + | - | + | + | + | + | + | + | - | + | + |
| A42 | + | - | - | + | - | + | - | + | + | + | + | - | + | - |
| A43 | + | - | - | - | - | - | - | + | + | + | + | + | + | - |
| A44 | + | - | - | + | + | - | - | + | + | + | + | + | + | - |
| A45 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| A46 | + | - | - | - | + | - | - | + | + | - | - | - | + | - |
| A47 | + | - | - | + | + | + | - | + | + | + | + | + | + | - |
| A48 | + | - | - | + | - | + | - | + | - | + | + | + | + | - |
| KBS IF3 | + | - | - | + | + | - | - | + | + | + | - | - | + | - |

**Table 5.1 continued** Specific PGPR traits exhibited by the rhizobacterial isolates (– being negative and + being positive for the particular trait).

| Isolate | Biocontrol trait | | | | | | | | Direct plant growth promotion trait | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | HCN | | Siderophore | Cellulase | Protease | Chitinase | β-1,3-Glucanase | N-fixation | | P-solubilisation | | IAA | ACC-deaminase |
| | | NA | Glycine-NA | | | | | | JNFb | GNFMM | NBRIY | PVK | | |
| N01 | + | + | + | - | - | + | - | - | + | + | + | + | + | - |
| N02 | + | - | - | - | + | + | - | + | + | - | + | + | + | - |
| N03 | + | - | - | + | - | + | + | + | + | + | + | + | + | - |
| N04 | + | + | + | + | - | + | - | + | + | + | + | + | + | + |
| N04AC | + | + | + | + | - | + | - | + | + | + | + | + | + | + |
| N05 | + | - | - | - | - | - | - | + | + | + | + | + | + | - |
| N07 | + | + | + | + | - | - | - | + | + | - | + | + | + | - |
| N08 | + | - | - | + | - | - | - | + | + | + | + | + | + | - |
| N10 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N11 | + | + | + | + | - | - | - | + | + | + | + | + | + | - |
| N12 | + | - | - | + | - | - | - | + | + | + | + | + | + | + |
| N13 | + | - | - | + | - | - | - | + | + | + | + | + | + | - |
| N14 | + | + | + | + | - | - | - | - | + | + | + | + | + | + |
| N15ii | + | + | + | + | - | + | - | + | + | + | + | + | + | + |
| N16 | + | - | - | + | - | - | + | + | + | + | + | + | + | - |
| N16W | + | - | - | - | - | + | - | + | + | + | - | + | + | - |
| N17 | + | - | - | + | + | + | - | + | + | + | + | + | + | - |
| N19 | + | - | - | + | - | - | - | - | + | - | - | + | + | - |
| N20 | + | - | - | + | - | - | - | - | + | - | - | - | + | - |
| N21 | + | - | - | + | + | - | - | + | + | + | + | + | + | - |
| N26 | + | - | - | + | - | - | - | - | + | + | - | - | + | - |
| N27 | + | - | - | + | - | - | + | + | + | + | + | + | + | - |
| N28 | + | - | - | + | - | + | - | + | + | + | + | + | + | - |

**Table 5.1 continued** Specific PGPR traits exhibited by the rhizobacterial isolates (– being negative and + being positive for the particular trait).

| Isolate | Biocontrol trait | | | | | | | | Direct plant growth promotion trait | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | HCN | | Siderophore | Cellulase | Protease | Chitinase | β-1,3-Glucanase | N-fixation | | P-solubilisation | | IAA | ACC-deaminase |
| | | NA | Glycine-NA | | | | | | JNFb | GNFMM | NBRIY | PVK | | |
| N29 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N30 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N31 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N32 | + | - | - | + | - | + | - | + | + | + | + | - | + | - |
| N33 | + | - | - | + | - | - | - | + | + | + | + | - | + | - |
| N34 | + | - | - | + | + | - | - | + | + | + | + | - | + | - |
| N37 | + | - | - | + | - | - | - | + | + | - | - | - | + | - |
| N38 | + | - | - | + | - | - | - | + | + | + | + | + | + | - |
| N39 | + | - | + | - | - | - | + | + | + | + | + | + | + | - |
| N41 | + | - | - | + | - | + | + | + | + | + | + | + | + | - |
| N42 | + | - | - | + | - | - | - | + | + | + | + | + | + | + |
| N43 | + | + | + | + | - | + | - | + | + | + | + | + | + | + |
| N44 | + | + | + | + | - | - | - | + | + | - | + | + | + | + |
| N45 | + | - | - | + | - | + | - | + | + | - | - | - | + | + |
| N51 | + | - | - | + | + | + | - | + | + | + | + | + | + | - |
| N53 | + | - | - | - | - | + | + | + | + | + | + | + | + | - |
| N54 | + | - | - | + | + | + | - | + | + | - | + | + | + | - |
| N55A | + | - | - | + | - | - | - | + | + | + | + | - | + | - |
| N55B | + | - | - | + | - | + | - | + | + | + | + | - | + | - |
| N56 | + | - | - | + | - | - | - | + | + | + | + | + | + | + |
| N58 | + | - | - | - | - | + | + | + | + | + | + | + | + | - |
| N59 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N59A | + | + | + | + | - | + | - | + | + | + | + | - | + | - |

**Table 5.1 continued** Specific PGPR traits exhibited by the rhizobacterial isolates (– being negative and + being positive for the particular trait).

| Isolate | Biocontrol trait | | | | | | | | Direct plant growth promotion trait | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | $NH_3$ | HCN | | Siderophore | Cellulase | Protease | Chitinase | β-1,3-Glucanase | N-fixation | | P-solubilisation | | IAA | ACC-deaminase |
| | | NA | Glycine-NA | | | | | | JNFb | GNFMM | NBRIY | PVK | | |
| N59B | + | - | - | + | - | + | - | + | + | - | - | - | + | - |
| N59C | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| N60 | + | - | - | + | + | + | - | + | + | - | - | + | + | - |
| N62 | + | - | - | + | - | - | - | + | + | - | + | + | + | - |
| N63 | + | + | + | + | - | + | - | + | + | - | + | + | + | - |
| N64 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N65 | + | - | - | + | - | - | - | + | + | + | + | + | + | - |
| N66 | + | - | - | + | - | - | - | - | + | - | + | + | + | - |
| N67 | + | - | - | + | - | + | - | + | + | - | + | + | + | + |
| N68 | + | - | - | + | - | - | - | - | + | + | - | - | + | - |
| N69 | + | - | - | + | - | - | - | + | + | + | + | + | + | - |
| N70 | + | - | - | + | + | + | + | + | + | - | + | + | + | - |
| N71 | + | - | - | + | - | + | - | + | + | - | + | + | + | - |
| N72 | + | - | - | + | - | + | + | + | + | + | + | + | + | - |
| N74 | + | + | + | + | - | - | - | + | + | + | + | + | + | + |
| N76 | + | - | - | + | - | - | + | + | + | - | + | + | + | - |
| N77W | + | - | - | + | - | + | - | + | + | + | + | + | + | - |
| N77Y | + | + | + | + | - | + | + | + | + | + | + | + | + | - |
| N78 | + | - | - | - | - | + | + | + | + | + | + | + | + | - |
| NAS696 | + | - | - | + | + | + | - | + | + | - | - | - | + | - |
| T11 | + | - | - | + | - | + | + | + | + | + | + | + | + | - |
| T19AC | + | - | - | + | - | - | - | + | + | + | - | - | + | - |
| T22 | + | - | - | + | + | - | - | + | + | - | + | + | + | - |
| T29AC | + | - | - | + | - | - | - | + | + | + | + | + | + | - |

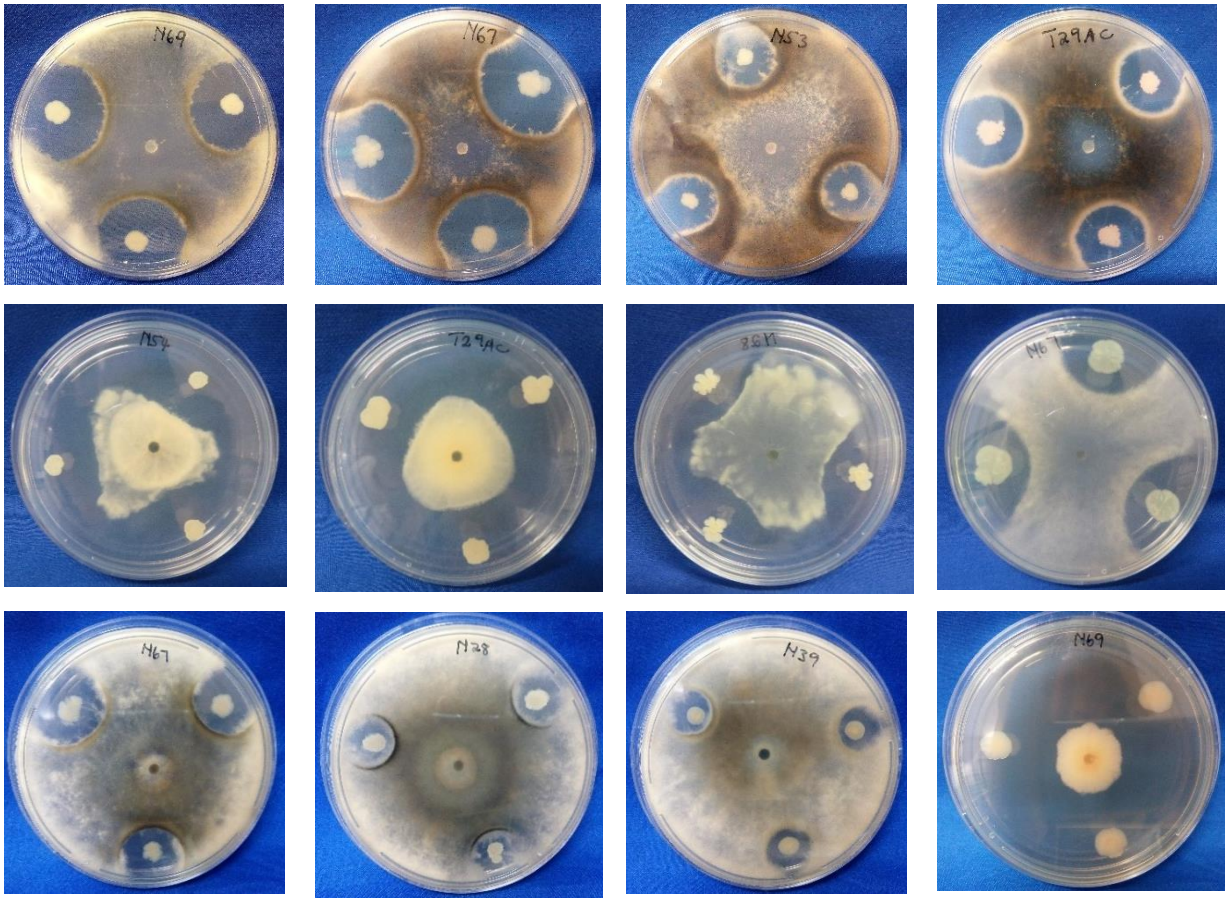

**Figure 5.2** Results of dual culture assay of *M. phaseolina* challenged with selected rhizobacterial isolates on PDA (1st row), NA (2nd row) and MEA (3rd row). From left to right: N69, N67, N53, T29AC, N54, T29AC, N28, N67, N67, N28, N39 and N69.

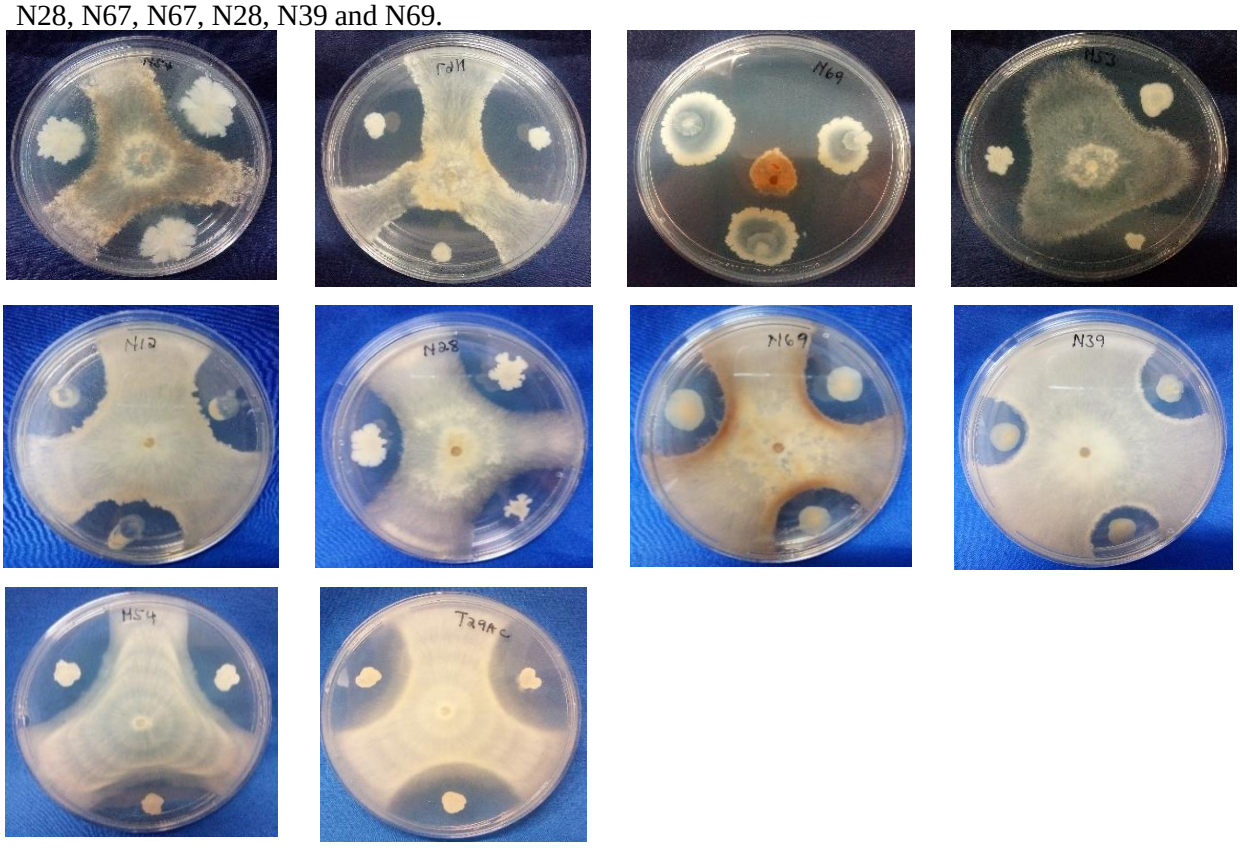

**Figure 5.3** Results of dual culture assay of *R. solani* challenged with selected rhizobacterial isolates on PDA (1st row), MEA (2nd row) and NA (3rd row). Isolate codes from left to right: N54, N67, N69, N53, N12, N28, N69, N39, N54 and T29AC.

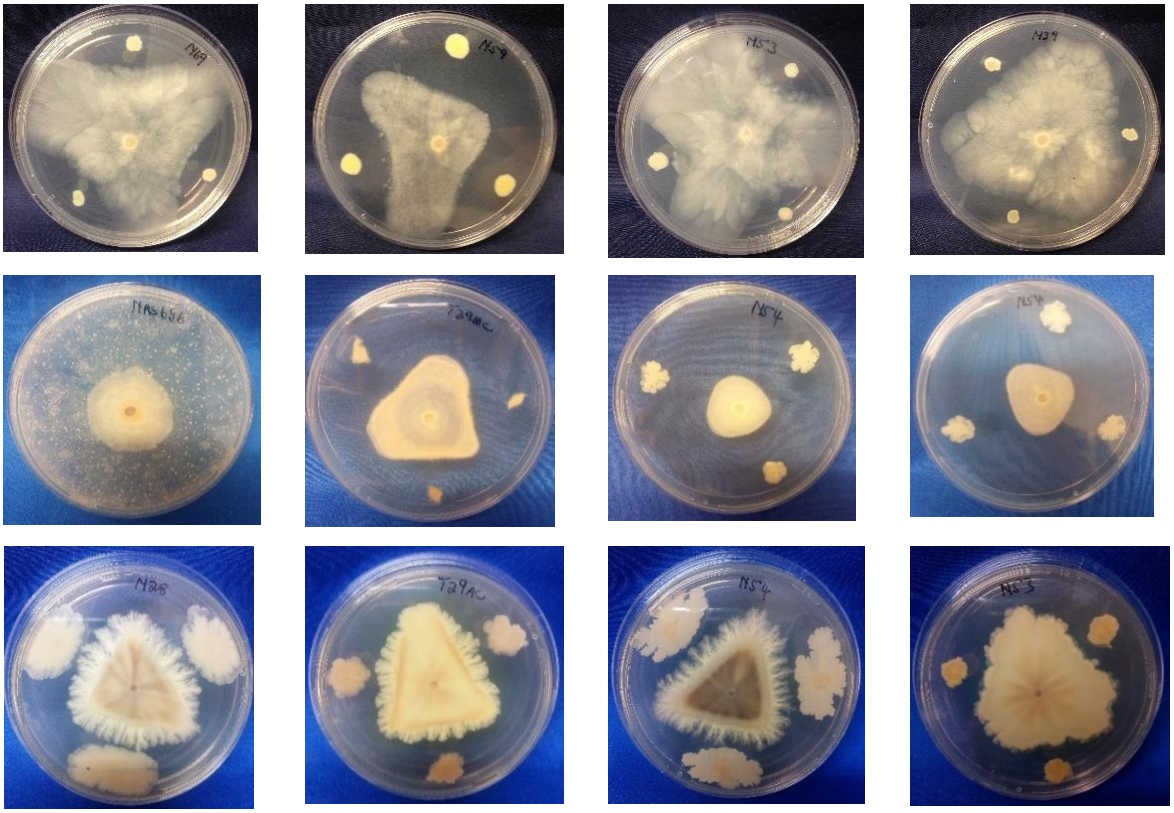

**Figure 5.4** Results of dual culture assay of *P. capsici* challenged with selected rhizobacterial isolates on PDA (1st row), NA (2nd row) and MEA (3rd row). Isolate codes from left to right: N69, N54, N53, N29, NAS6G6, T29AC, N54, N54, N28, T29AC, N54, N53.

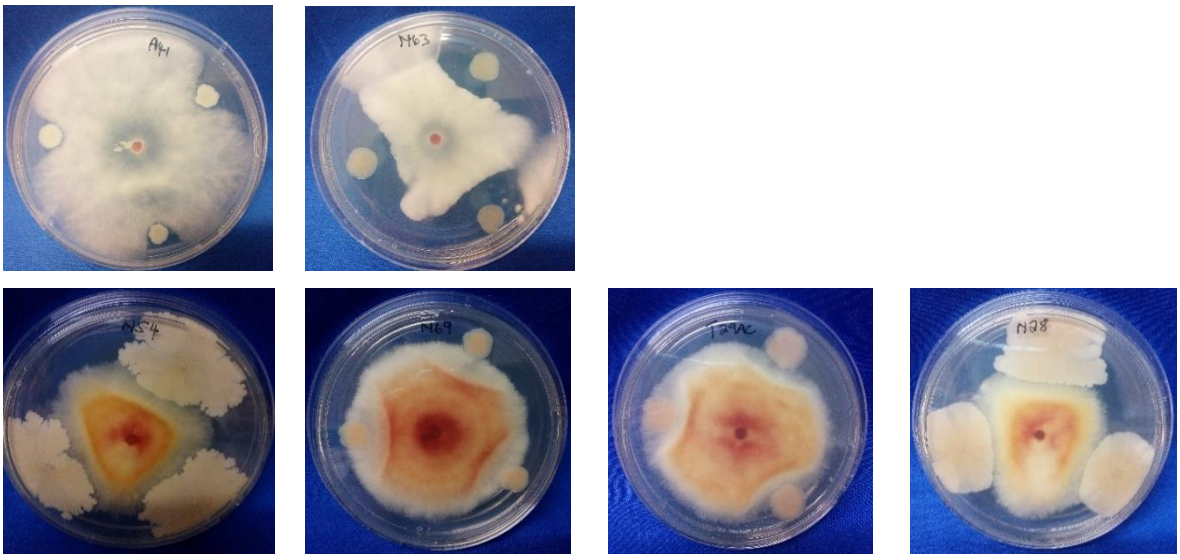

**Figure 5.5** Results of dual culture assay of *F. pseudograminearum* challenged with selected rhizobacterial isolates on NA (1st row) and MEA (2nd row). Isolate codes from left to right: A41, N63, N54, N69, T29AC and N28.

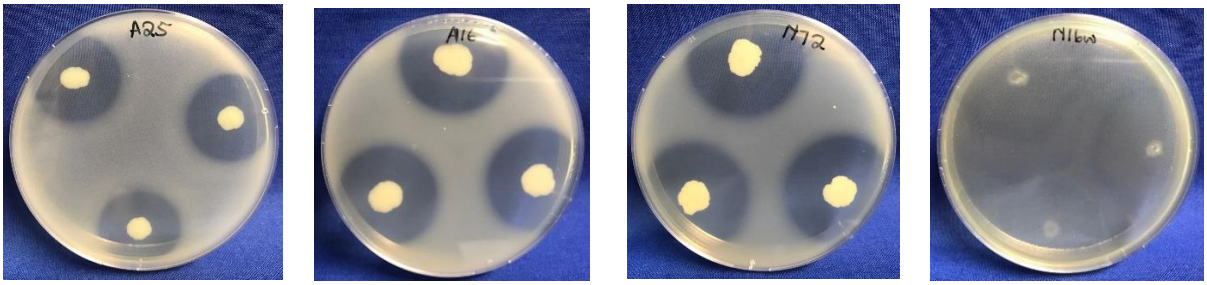


**Figure 5.6** Protease production by rhizobacterial isolates on skimmed milk amended nutrient agar. Clear zones around the bacterial colonies are indicative of the ability of the isolates to degrade the protein in the media through protease production. Isolate codes from left to right: A25, A16, N72 and N16W.

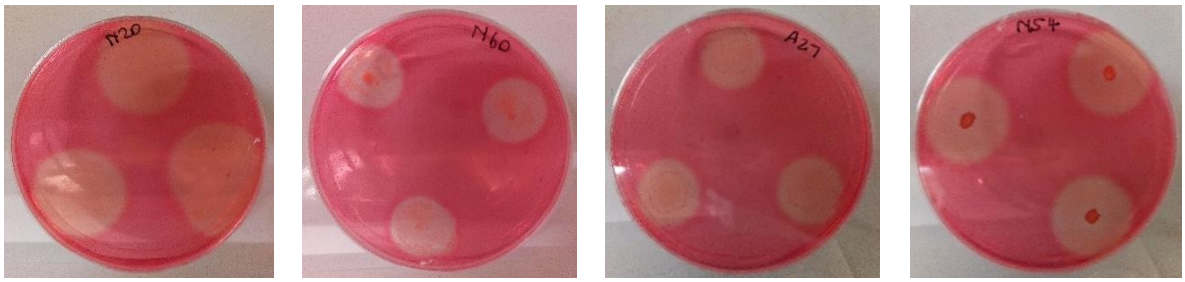


**Figure 5.7** Cellulase production on carboxymethyl-cellulose amended plates. Clear zones indicative of the ability of the isolates to produce cellulases. Isolate codes from left to right N20, N60, A27 and N54.

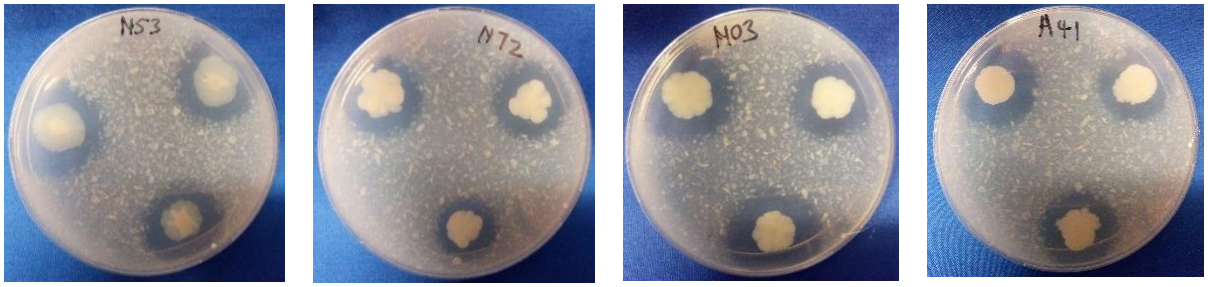


**Figure 5.8** Chitinase production on chitin-amended medium. Clear zones around the bacterial culture are indicative of the ability of the isolates to degrade chitin in the media through chitinase production. Isolate codes from left to right N53, N72, N03 and A41.

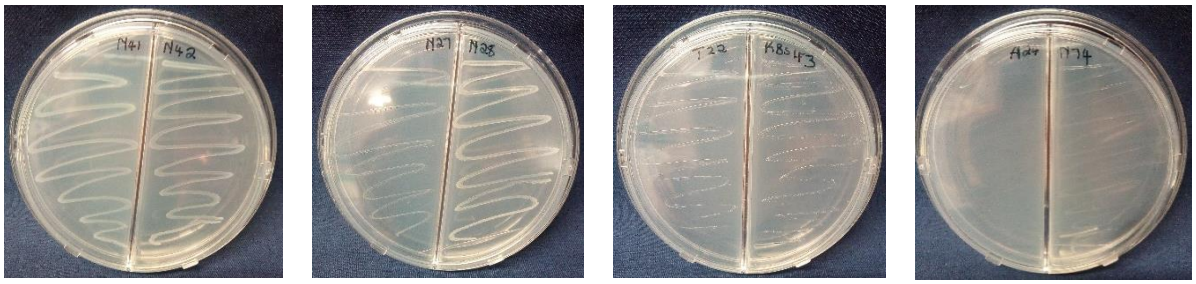


**Figure 5.9** β-1,3-glucanase production. Growth of the different isolates on medium supplemented with laminarin as the sole carbon source. Isolate codes from left to right N41, N42, N27, N28, T22, KBS1F3, A24 and N74.

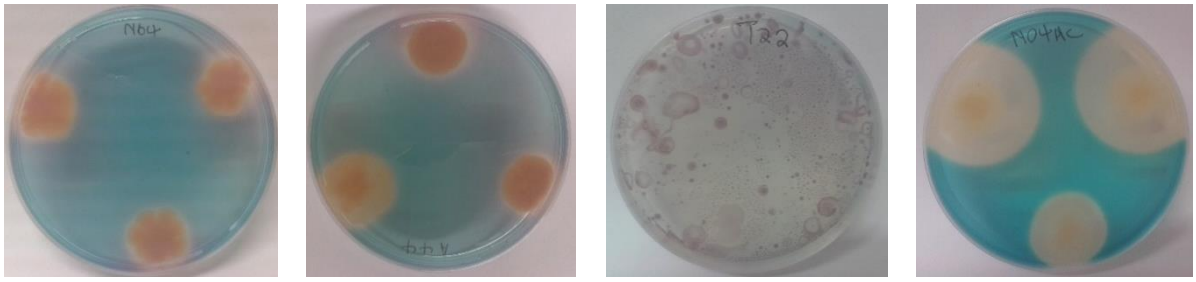


**Figure 5.10** Siderophore production on CAS agar. The colour change in the medium and the clear zones result when iron is bound to bacterial siderophores. Isolate code from left to right N, A44, T22 and N04AC.

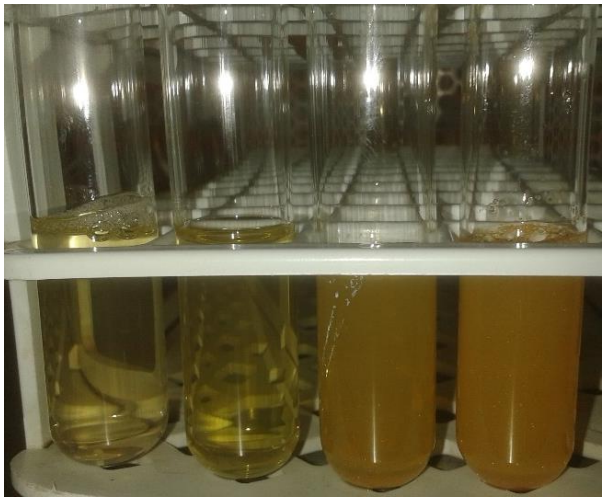

**Figure 5.11** NH3 production in nutrient broth using Nessler's indicator reagent. The gradient used to determine the amount of $NH_3$ produced by the isolates. A-none, B-low, C-moderate, D-high.

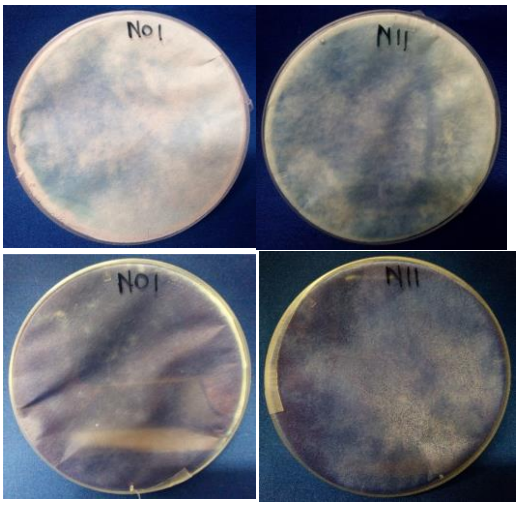


**Figure 5.12** HCN production on nutrient agar with and without glycine. Colour indicator paper changes from aqua green to blue in reaction to HCN. Top row- without glycine (N01 and N11), Bottom- with glycine (N01 and N11).

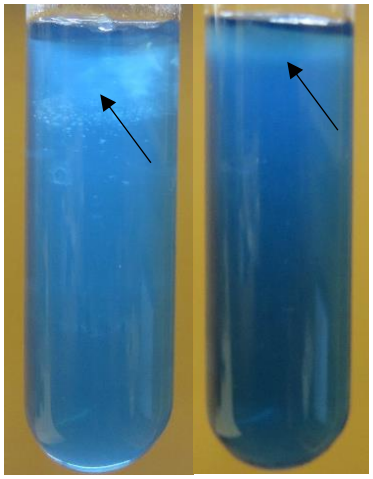

**Figure 5.13** Results of test for N-fixation. Example of bacterial growth (shown by the arrows) in JNFb medium testing for growth in N-free medium indicating the ability to fix nitrogen.

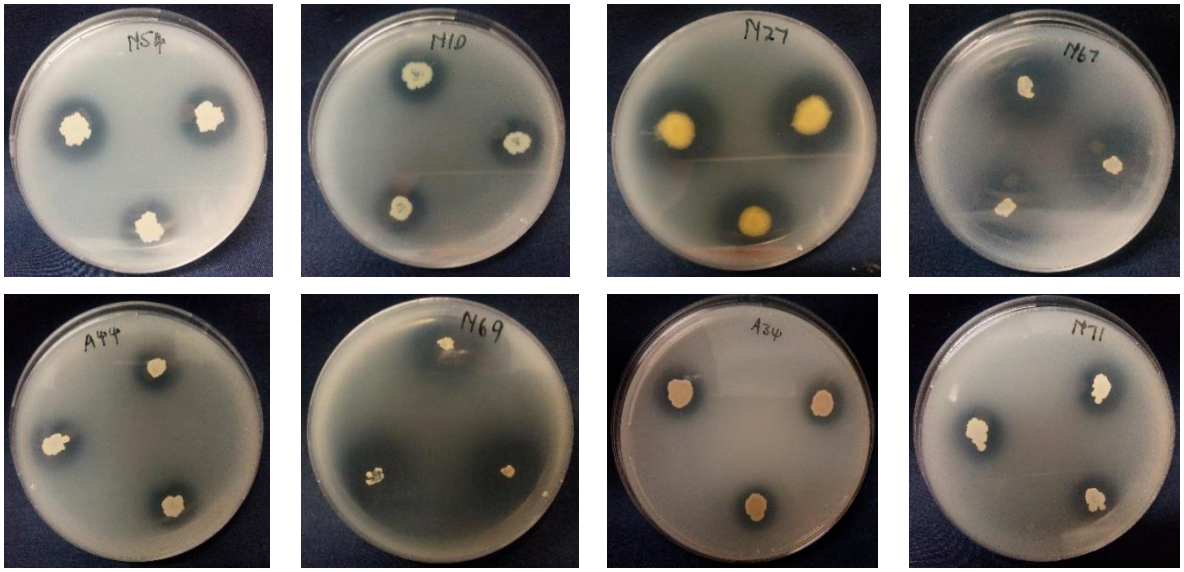


**Figure 5.14** P-solubilization on NBRIY (top row) and PVK (bottom row). Clear zones around the bacterial culture are indicative of P-solubilization by the isolates. Isolate codes from left to right N54, N10, N27, N67, A44, N69, A24 and N71.

**Figure 5.15** IAA production using Salkowski's reagent as an indicator solution by some of the rhizobacterial isolates. Colour change of the medium from colourless to pink is indicative of IAA production.

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# CHAPTER 6

## Selection of best-performing rhizobacterial isolates

**Abstract**

Based on the tests conducted in Chapters 3, 4 and 5 combined, this chapter is aimed at selecting the best-performing isolates to be used to conduct a final greenhouse trial (Chapter 7). A scoring system was developed, and the isolates were awarded points based on the results obtained during the *in vitro* and *in vivo* tests. However, great importance was placed on the performance of the isolates *in vivo*. Based on the results obtained during the *in vitro* tests and *in vivo* trials combined, isolates N54, A20, N28, N59A, N44, N04AC, N02, A17, A09AC and A45 were selected as the best-performers for biocontrol of *Fusarium pseudograminearum* while A45, N69, N67, N76, N34, KBS1F3, N29, A10AC, A33 and A43 were selected as the best wheat growth promoters. Spider diagrams were constructed for all the selected isolates to visualise their traits and points were awarded. These isolates showed potential to be applied as biocontrol agents and biofertilisers on wheat.

### 6.1 Introduction

The question addressed in this chapter is: based on all the *in vitro* and *in vivo* evaluations conducted, which of the isolates performed best overall? Answering this question is important as previous research has shown that there is poor correlation between the performance of rhizobacterial isolates *in vitro* and *in vivo* (Ran et al. 2005). Isolates that may show potential *in vitro* may not necessarily perform well under greenhouse/field conditions and *vice versa*. It is therefore important to give careful consideration to the selection process.

There is not a “one size fits all” method for selection of rhizobacterial isolates in the abovementioned context - selection largely depends on the specific aim of each study. Some studies place more importance on the selection of isolates that can inhibit the highest percentage of pathogen growth (Dinesh et al. 2015; Kurabachew and Wydra, 2013; Liu 2015) while others place more importance on plant growth promotion traits (Lucas et al. 2014; Son et al. 2014) whereas others selected isolates that can inhibit pathogen growth and also possess plant growth promotion-related traits (Thokchom et al. 2014).

Liu (2015) conducted a screening study for the selection of PGPR strains with broad-spectrum biocontrol activity and plant growth promotion in the presence of plant pathogens. The first

level of selection was based on the performance of the strains in *in vitro* antibiosis assays against nine pathogens after which the selected strains were assessed for plant growth promotion-related traits and also tested for plant growth promotion efficacy in the growth chamber. In many cases however, several such PGPR studies are based on the screening of rhizobacterial isolates *in vitro* followed by selection of those that possess the most traits associated with biocontrol activity and plant growth promotion to be used in subsequent studies either in the greenhouse or in the field (Ambrosini et al. 2012; Arruda et al. 2013; Dinesh et al. 2015; Kurabachew and Wydra 2013; Lucas et al. 2014; Son et al. 2014).

In the previous chapters (chapter 3-5), a collection of 113 rhizobacterial isolates was screened for biocontrol activity against *Fusarium pseudograminearum* on wheat and plant growth promotion of wheat using seedling assays in the greenhouse. In addition to this, the isolates were also screened for traits associated with biocontrol activity and plant growth promotion *in vitro*. Based on the tests conducted in chapters 3, 4 and 5 combined, the main aim of this chapter was aimed at selecting the best-performing isolates to be used to conduct a final greenhouse trial.

## 6.2 Materials and methods

The selection of best-performing isolates was done following the procedure outlined by Berg et al. (2001). The isolates were awarded points based on their performance *in vitro* and *in vivo* (Table 6.1 and 6.2). One point was awarded for each *in vitro* test performed and three points for each greenhouse trial test performed. The selection of the best-performing biocontrol and plant growth promoters was done separately. The points were summed to determine the total score for each isolate (Table 6.3 and 6.4). The top 10 best-performing isolates were selected for biocontrol and the same number of isolates were selected for plant growth enhancement. Spider diagrams were also constructed for the selected isolates using Microsoft Office Excel to visualize the points awarded.

## 6.3 Results

The developed scoring system was used to award all isolates points and the top 10 isolates with the highest point for biocontrol activity against *F. pseudograminearum* were selected. These isolates were *Bacillus* sp. (A09AC, A17, A20, N02, N28, N54), *Pseudomonas* sp. (N04AC, N44, N59A) and *Stenotrophomonas* sp. (A45). The top 10 isolates with the highest point were

also selected as the best wheat growth promoters. These isolates were *Bacillus* sp. (A10AC), *Paenibacillus* sp. (KBS1F3), *Pseudomonas sp.* (N29, N69, N67, N76), *Pantoea* (N34) and *Stenotrophomonas* sp. (A33, A43, A45). Spider web diagrams (Figure 6.1 and 6.2) were constructed for each isolate selected to give a visual illustration of traits of the isolates linked to points awarded for each trait.

## 6.4 Discussion

In studies that involve the selection of effective PGPR isolates, *in vitro* screening gives an indication of the possible mode of action of rhizobacteria. However, many screening methods adopt an elimination approach in between the screening stages. This results in the elimination of potentially good plant growth promoters or biocontrol agents. For instance, due to the difference in conditions, isolates that do not perform well *in vitro* can eliminated before proceeding to *in vivo* trials where they may have performed best. In the current study, the selection of best-performing isolates was based on the performance of the isolates *in vitro* and seedling assays performed in the greenhouse.

All selected rhizobacterial isolates increased the shoot and root weight of wheat even in the presence of *F. pseudograminearum*, thus indicating their biocontrol activity. In the *in vitro* tests that involved the screening the isolates, three isolates (N54, N28 and N44) inhibited mycelial growth of *F. pseudograminearum* while the other seven did not. These seven isolates, however, showed inhibition of the growth of the other pathogens tested. The lack of *in vitro* inhibition of pathogen growth, whilst increasing plant growth in the presence of the pathogen (compared to the pathogen only control treatment) in the greenhouse can be indicative of another mechanism of action such as induction of systemic resistance being present. It was also observed that all the isolates selected as the best-performers for biocontrol activity produced at least one antibiotic volatile compound, i.e. either HCN or $NH_3$ or both. These volatile compounds have been shown to have antifungal properties (Ghyselinck et al. 2013; Kumar et al. 2014; Wei et al. 1991).

Another trait that confers to rhizobacteria the ability to control soilborne pathogens is the production of siderophores. Siderophores are iron chelators that bind to iron in the soil. The siderophore-iron complex cannot be assimilated by pathogens thus starving them of iron (Ribeiro and Cardoso 2012). However, plants have developed mechanisms of taking up and metabolizing the complex (Grobelak et al. 2015). In the current study, all isolates selected for

biocontrol activity except N02 and A17, produced siderophores. The selected best-performing isolates for biocontrol were also found to produce at least one of the cellulytic enzymes (chitin, cellulase, protease and β-1,3-glucanase). These enzymes degrade fungal cell walls thus inhibiting the growth of pathogens (Bach et al. 2016; Choubane et al. 2016; Kavamura et al. 2013). Another biocontrol mechanism of action of PGPR is the induction of systemic resistance. Often, all these attributes together result in effective biocontrol activity.

Hydrogen cyanide has been found to be toxic to soil pathogens through the inhibition of the electron transport system (Szentes et al. 2013). HCN is formed from glycine through the action of HCN synthase enzyme which is associated with the plasma membrane of certain rhizobacteria (Shameer and Prasad 2018). For this reason, the production of HCN was enhanced when the growth medium was supplemented with glycine. The production of HCN is a common trait amongst the gram-negative Pseudomonas group from the rhizosphere (Fernando et al. 2006; Shameer and Prasad 2018). In the current study, of the selected isolates, only N59A, N04AC, A45 and N44 were found to produce HCN and according to the MALDI-TOF results these isolates are *Pseudomonads*. Choudhary and Varma (2016) found that *Pseudomonas flourescens* suppress disease caused by *Fusarium oxysporum* f. sp. *radices-lycopersici* in tomato with the help of the release on HCN. *Pseudomonas solanacearum* has been found to also hydrolyse fusaric acid which is a compound that damages plant tissue during infection.

Apart from isolates N29, A10AC and A43, all isolates were able to significantly increase both the dry shoot and root weight of wheat in the absence of the pathogen, indicating direct plant growth-promoting activity. Isolate N29 only increased root weight while A10AC and A43 increased only the shoot weight. All these isolates tested positive for the production of IAA which is directly involved in growth promotion of plants (Goswami et al. 2016; Kavamura et al. 2013; Khan et al. 2016). All selected isolates were also found to fix atmospheric nitrogen and solubilize phosphate. These are important plant growth-promoting traits. In addition to this, A45, N67, KBS1F3 and N29 were found to produce ACC deaminase which is involved in the alleviation of salt and drought stress in plants (Parray et al. 2016). ACC deaminase has also been found to increase the plant's ability to absorb N, P, K and Ca from the soil (Parray et al. 2016).

In line with inconsistencies found in previous studies, some of the isolates that produced three or four of the biocontrol traits (antibiotic enzymes, siderophore and volatile compounds) *in vitro* were not amongst the best-performing isolates during the screening in the greenhouse on

seedlings. Examples of such isolates include N70 and A19. In studies conducted by Mavrodi et al. (2012), some isolates that showed pathogen inhibition *in vitro* were unable to suppress the pathogen *in planta* and some of those that showed no inhibition *in vitro* could suppress the pathogen *in planta*. This could be due to the difference in conditions between the two environments. Some isolates can induce systemic resistance against pathogens in plants which cannot be detected *in* vitro. For this reason, using seedling assays provides a rapid method to assess the performance of isolates *in planta*. The same phenomenon was observed with regards to the isolates that were able to fix nitrogen, solubilize phosphate, produce IAA and ACC deaminase *in vitro*; *in planta,* these isolates did not significantly increase the weight of the wheat seedlings compared to the uninoculated control. These isolates include A08, A09AC, N04AC, N10, N12 and N14. Isolate N04AC was, however, selected as one of the best-performing isolates for plant growth promotion in the presence of *F. pseudograminearum.*

There are several methods that can be employed to screen and select for effective isolates with high potential for promotion of plant growth and biocontrol of plant diseases. As mentioned before, each method is dependent on the objectives of the study. The current study employed the method of screening all isolates in both seedling assays in the greenhouse and again *in vitro* before any selection or elimination of isolates was done. Furthermore, the selection of the best-performing biocontrol and plant growth promoters was done separately. It was observed that some of the isolates that performed the best as plant growth promoters did not perform well as biocontrol agents and *vice versa*. The selected isolates showed good potential as biocontrol agents and wheat growth promoters and should be further screened under field conditions in future studies.

### 6.5 Tables and Figures

**Table 6.1** Scoring system for the selection of effective biocontrol isolates.

| Type of experiment | | Points awarded | Total possible points in category |
|---|---|---|---|
| **Seedling assay in the greenhouse** | **Increase in dry wheat weight*** | | 6 |
| | Increase in shoot weight | 3 | |
| | Increase in root weight | 3 | |
| ***In vitro* screening** | | | |
| | **Production of volatiles** | | 2 |
| | HCN | 1 | |
| | $NH_3$ | 1 | |
| | **Production of antibiotic enzymes** | | 4 |
| | Chitin | 1 | |
| | Cellulase | 1 | |
| | B-1,3-glucanase | 1 | |
| | Protease | 1 | |
| | **Production of siderophores** | | 1 |
| | Chrome azurol S (CAS) agar | 1 | |
| | **Dual culture (antibiosis)** | | 4 |
| | *F. pseudograminearum* | 1 | |
| | *R. solani* | 1 | |
| | *P.capsici* | 1 | |
| | *M. phaseolina* | 1 | |

* Increase in dry wheat weight in the presence of *F. pseudograminearum*.

**Table 6.2** Scoring system for the selection of effective plant growth promoting isolates.

| Type of experiment | | Points awarded | Total possible points in category |
|---|---|---|---|
| **Seedling assay in the greenhouse** | **Increase in dry wheat weight*** | | 6 |
| | Increase in shoot weight | 3 | |
| | Increase in root weight | 3 | |
| ***In vitro* screening** | | | |
| | **Nitrogen fixation** | | 2 |
| | Nitrogen-free semisolid media (JNFb) | 1 | |
| | Glucose nitrogen-free mineral medium (GNFMM) | 1 | |
| | **Phosphate solubilisation** | | 2 |
| | Pikovskaya (PVK) growth medium | 1 | |
| | National Botanical Research Institute's Phosphate (NBRIY) growth medium | 1 | |
| | **IAA production** | | 1 |
| | Salkowski's indicator solution | 1 | |
| | **ACC deaminase production** | | 1 |
| | Dworkin and Foster (DF) minimal medium | 1 | |

* Increase in dry wheat weight in the absence of *F. pseudograminearum*.

**Table 6.3 S**election of the best-performing biocontrol isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry weight* | | Dual culture assays | | | | Production of volatiles | | Siderophore production | Antibiotic enzyme production | | | | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | *F. pseudogra-minearum* | *R. solani* | *P. capsici* | *M. phaseolina* | HCN | $NH_3$ | | Chitin | Cellulase | B-1,3-glucanase | Protease | |
| A05A | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 6 |
| A05B | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 6 |
| A05C | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 6 |
| A06 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 7 |
| A07 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 7 |
| A07AC | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 8 |
| A08 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 9 |
| A09 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 12 |
| A1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 5 |
| A10 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 7 |
| A10AC | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 6 |
| A12 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 6 |
| A14W | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 10 |
| A14Y | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 11 |
| A16 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 10 |
| A17 | 3 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 13 |
| A19 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 9 |
| A20 | 3 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 14 |
| A21 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 11 |
| A22 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| A24 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 1 | 7 |
| A25 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 10 |
| A26 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 11 |
| A27 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 6 |

*Increase in wheat dry weight in the presence of *F. pseudograminearum*.

**Table 6.3 continued** Selection of the best-performing biocontrol isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry weight* | | Dual culture assays | | | | Production of volatiles | | Siderophore production | Antibiotic enzyme production | | | | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | *F. pseudogra-minearum* | *R. solani* | *P. capsici* | *M. phaseolina* | HCN | $NH_3$ | | Chitin | Cellulase | B-1,3-glucanase | Protease | |
| A28 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 9 |
| A29 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 7 |
| A32 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 5 |
| A33 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 8 |
| A34 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 11 |
| A35 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| A36 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 10 |
| A37 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 4 |
| A38 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| A39 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| A40 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 4 |
| A41 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 9 |
| A43 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 3 |
| A44 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 7 |
| A45 | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 12 |
| A46 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 5 |
| A47 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 11 |
| A48 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| KBS IF3 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 10 |
| N01 | 0 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 1 | 8 |
| N02 | 3 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 13 |
| N03 | 0 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 7 |
| N04 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 9 |

*Increase in wheat dry weight in the presence of *F. pseudograminearum*.

**Table 6.3 continued S**election of the best-performing biocontrol isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry weight* | | Dual culture assays | | | | Production of volatiles | | Siderophore production | Antibiotic enzyme production | | | | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | *F. pseudogra-minearum* | *R. solani* | *P. capsici* | *M. phaseolina* | HCN | $NH_3$ | | Chitin | Cellulase | B-1,3-glucanase | Protease | |
| N04AC | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 13 |
| N05 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 4 |
| N07 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 7 |
| N08 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 5 |
| N10 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 7 |
| N11 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 7 |
| N12 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| N13 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 4 |
| N14 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 0 | 0 | 6 |
| N15ii | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| N16 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 5 |
| N16W | 0 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 9 |
| N17 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 11 |
| N19 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 7 |
| N20 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 7 |
| N21 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 6 |
| N26 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 5 |
| N27 | 3 | 3 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 12 |
| N28 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 14 |
| N29 | 3 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 10 |
| N30 | 3 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 9 |
| N31 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| N32 | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 10 |
| N33 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 5 |

*Increase in wheat dry weight in the presence of *F. pseudograminearum*.

**Table 6.3 continued** Selection of the best-performing biocontrol isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry weight* | | Dual culture assays | | | | Production of volatiles | | Siderophore production | Antibiotic enzyme production | | | | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | *F. pseudogra-minearum* | *R. solani* | *P. capsici* | *M. phaseolina* | HCN | $NH_3$ | | Chitin | Cellulase | B-1,3-glucanase | Protease | |
| N34 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 6 |
| N37 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| N38 | 3 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 7 |
| N39 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 7 |
| N41 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 8 |
| N42 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| N43 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 7 |
| N44 | 3 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 13 |
| N45 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| N51 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 12 |
| N53 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 10 |
| N54 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 15 |
| N55A | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 4 |
| N55B | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 9 |
| N56 | 0 | 3 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| N58 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 6 |
| N59 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| N59A | 3 | 3 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 13 |
| N59B | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 9 |
| N59C | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 6 |
| N60 | 0 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 11 |
| N62 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| N63 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 0 | 1 | 1 | 12 |
| N64 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 5 |

*Increase in wheat dry weight in the presence of *F. pseudograminearum*.

**Table 6.3 continued S**election of the best-performing biocontrol isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry weight* | | Dual culture assays | | | | Production of volatiles | | Siderophore production | Antibiotic enzyme production | | | | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | *F. pseudogra-minearum* | *R. solani* | *P. capsici* | *M. phaseolina* | HCN | $NH_3$ | | Chitin | Cellulase | B-1,3-glucanase | Protease | |
| N65 | 3 | 3 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 10 |
| N66 | 3 | 3 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 10 |
| N67 | 3 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 11 |
| N68 | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 7 |
| N69 | 0 | 3 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 10 |
| N70 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 9 |
| N71 | 0 | 3 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 10 |
| N72 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 7 |
| N74 | 0 | 3 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 0 | 1 | 0 | 10 |
| N76 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 7 |
| N77W | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 5 |
| N77Y | 3 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 12 |
| N78 | 0 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 7 |
| NAS696 | 3 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 10 |
| T11 | 3 | 0 | 1 | 1 | 1 | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 12 |
| T19AC | 3 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 8 |
| T22 | 3 | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 10 |
| T29AC | 3 | 0 | 1 | 1 | 0 | 1 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 9 |

*Increase in wheat dry weight in the presence of *F. pseudograminearum*.

**Table 6.4 S**election of the best-performing plant growth promoting isolates: points awarded to each isolate for each respective trait.

| Isolate | Increase in wheat dry seedling weight | | Nitrogen fixation | | Phosphate solubilisation | | IAA production | ACC deaminase production | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | JNFB | GNFMM | PVK | NBRIY | | | |
| A05A | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| A05B | 3 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| A05C | 3 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| A06 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 5 |
| A07 | 0 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | 7 |
| A07AC | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| A08 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A09 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 3 |
| A10 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| A10AC | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| A12 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A14W | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 1 | 5 |
| A14Y | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| A16 | 0 | 3 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| A17 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 3 |
| A19 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| A20 | 0 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | 7 |
| A21 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| A22 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| A24 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 5 |
| A25 | 0 | 3 | 1 | 1 | 0 | 0 | 1 | 0 | 6 |
| A26 | 0 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | 7 |
| A27 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| A28 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A29 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A32 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 5 |

**Table 6.4 continued** Selection of the best-performing plant growth promoting isolates: points awarded to each isolate for each of the respective traits.

| Isolate | Increase in wheat dry seedling weight | | Nitrogen fixation | | Phosphate solubilisation | | IAA production | ACC deaminase production | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | JNFB | GNFMM | PVK | NBRIY | | | |
| A33 | 3 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 8 |
| A34 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 0 | 7 |
| A35 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A36 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| A37 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 3 |
| A38 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| A39 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A40 | 0 | 0 | 1 | 0 | 0 | 1 | 1 | 0 | 3 |
| A41 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 1 | 5 |
| A43 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| A44 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| A45 | 3 | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 11 |
| A46 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| A47 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| A48 | 0 | 0 | 0 | 1 | 1 | 1 | 1 | 0 | 4 |
| KBS IF3 | 3 | 3 | 1 | 1 | 0 | 0 | 1 | 0 | 9 |
| N01 | 0 | 3 | 1 | 0 | 1 | 1 | 1 | 0 | 7 |
| N02 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N03 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N04 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 5 |
| N04AC | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N05 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N07 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N08 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N10 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N11 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |

**Table 6.4 continued** Selection of the best-performing plant growth promoting isolates: points awarded to each isolate for each of the respective traits.

| Isolate | Increase in wheat dry seedling weight | | Nitrogen fixation | | Phosphate solubilisation | | IAA production | ACC deaminase production | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | JNFB | GNFMM | PVK | NBRIY | | | |
| N12 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N13 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N14 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N15ii | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N16 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N16W | 0 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 4 |
| N17 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N19 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 3 |
| N20 | 0 | 3 | 1 | 0 | 0 | 0 | 1 | 0 | 5 |
| N21 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N26 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| N27 | 3 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N28 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N29 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 1 | 9 |
| N30 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N31 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N32 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| N33 | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| N34 | 3 | 3 | 1 | 1 | 0 | 1 | 1 | 0 | 10 |
| N37 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| N38 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N39 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N41 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N42 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N43 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N44 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 1 | 5 |

**Table 6.4 continued** Selection of the best-performing plant growth promoting isolates: points awarded to each isolate for each of the respective traits.

| Isolate | Increase in wheat dry seedling weight | | Nitrogen fixation | | Phosphate solubilisation | | IAA production | ACC deaminase production | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | JNFB | GNFMM | PVK | NBRIY | | | |
| N45 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 1 | 3 |
| N51 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N53 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N54 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N55A | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| N55B | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| N56 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N58 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N59 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N59A | 0 | 0 | 1 | 1 | 0 | 1 | 1 | 0 | 4 |
| N59B | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| N59C | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N60 | 0 | 0 | 1 | 0 | 1 | 0 | 1 | 0 | 3 |
| N62 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N63 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N64 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N65 | 0 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 8 |
| N66 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 7 |
| N67 | 3 | 3 | 1 | 0 | 1 | 1 | 1 | 1 | 11 |
| N68 | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| N69 | 3 | 3 | 1 | 1 | 1 | 1 | 1 | 0 | 11 |
| N70 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N71 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| N72 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N74 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 6 |
| N76 | 3 | 3 | 1 | 0 | 1 | 1 | 1 | 0 | 10 |
| N77W | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |

**Table 6.4** continued. Selection of the best-performing plant growth promoting isolates: points awarded to each isolate for each of the respective traits.

| Isolate | Increase in wheat dry seedling weight | | Nitrogen fixation | | Phosphate solubilisation | | IAA production | ACC deaminase production | Total points awarded |
|---|---|---|---|---|---|---|---|---|---|
| | Shoot | Root | JNFB | GNFMM | PVK | NBRIY | | | |
| A05A | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 1 | 4 |
| N77Y | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| N78 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| NAS696 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 2 |
| T11 | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |
| T19AC | 0 | 0 | 1 | 1 | 0 | 0 | 1 | 0 | 3 |
| T22 | 0 | 0 | 1 | 0 | 1 | 1 | 1 | 0 | 4 |
| T29AC | 0 | 0 | 1 | 1 | 1 | 1 | 1 | 0 | 5 |

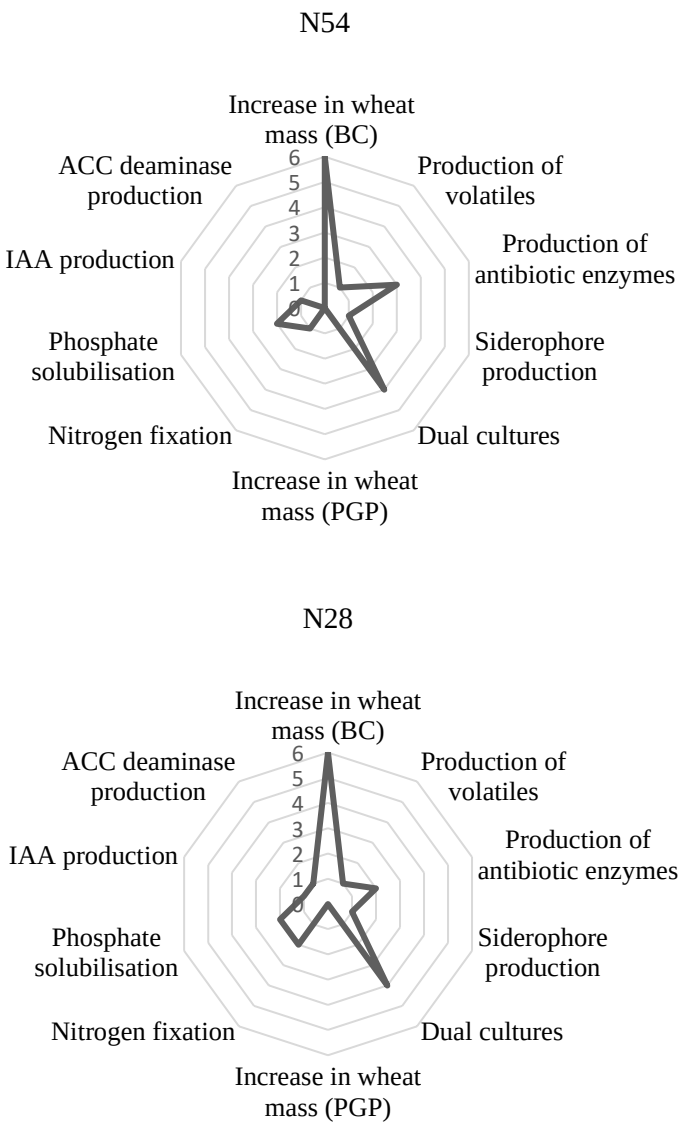
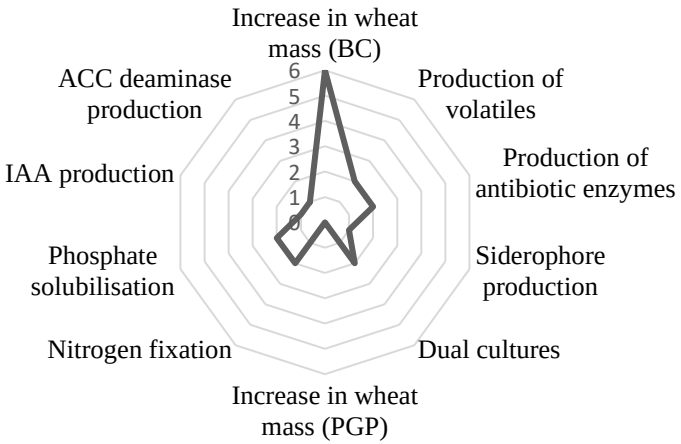
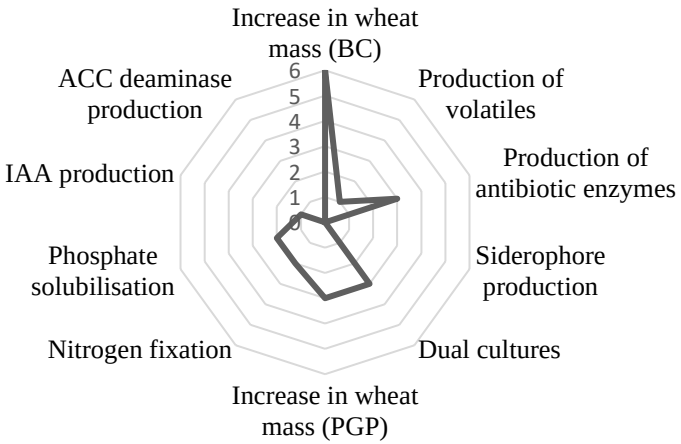



**Figure 6.1** Spider diagrams for isolates that performed the best as biocontrol agents against *F. pseudograminearum* on wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP)= plant growth promotion in absence of pathogen i.e. direct plant growth promotion.

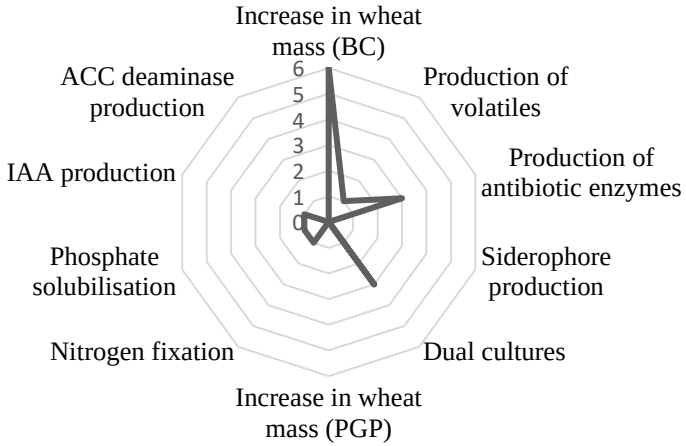
N04AC




N02




A17




A09AC

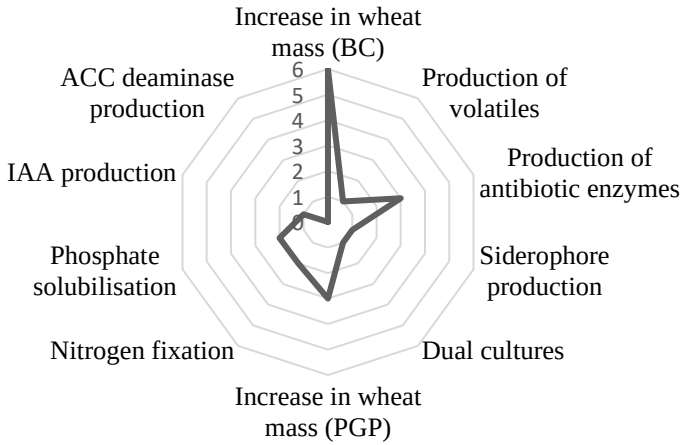
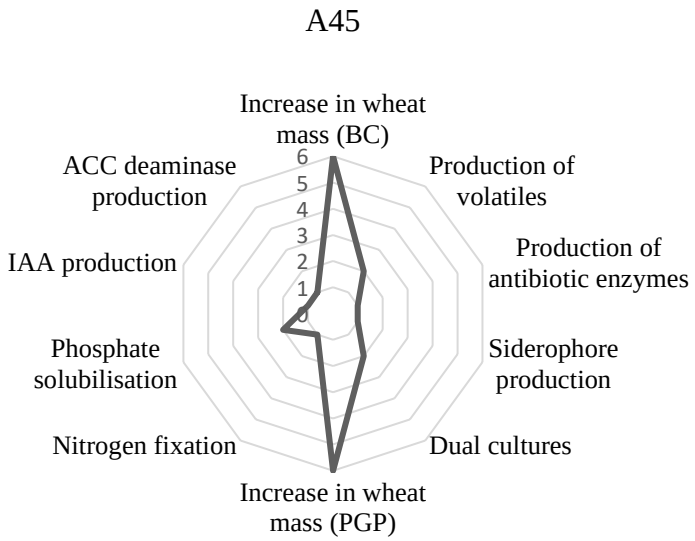



**Figure 6.1 cont** Spider diagrams for isolates that performed the best as biocontrol agents against *F. pseudograminearum* on wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP)= plant growth promotion in absence of pathogen i.e. direct plant growth promotion.



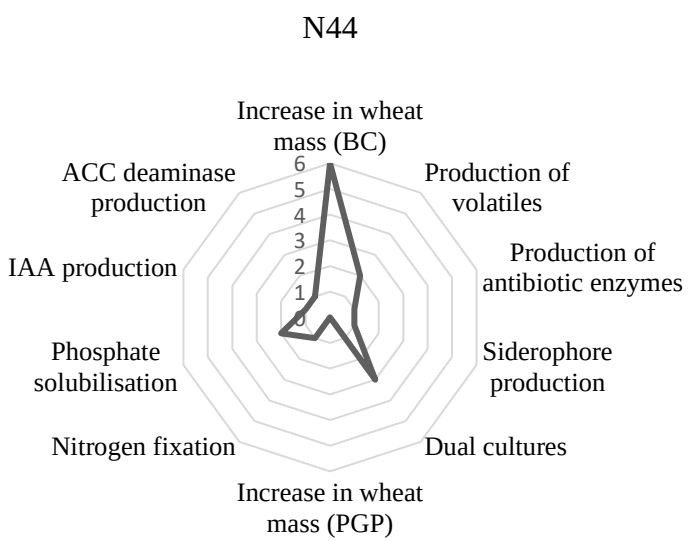




**Figure 6.1 cont** Spider diagrams for isolates that performed the best as biocontrol agents against *F. pseudograminearum* on wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP)= plant growth promotion in absence of pathogen i.e. direct plant growth promotion.

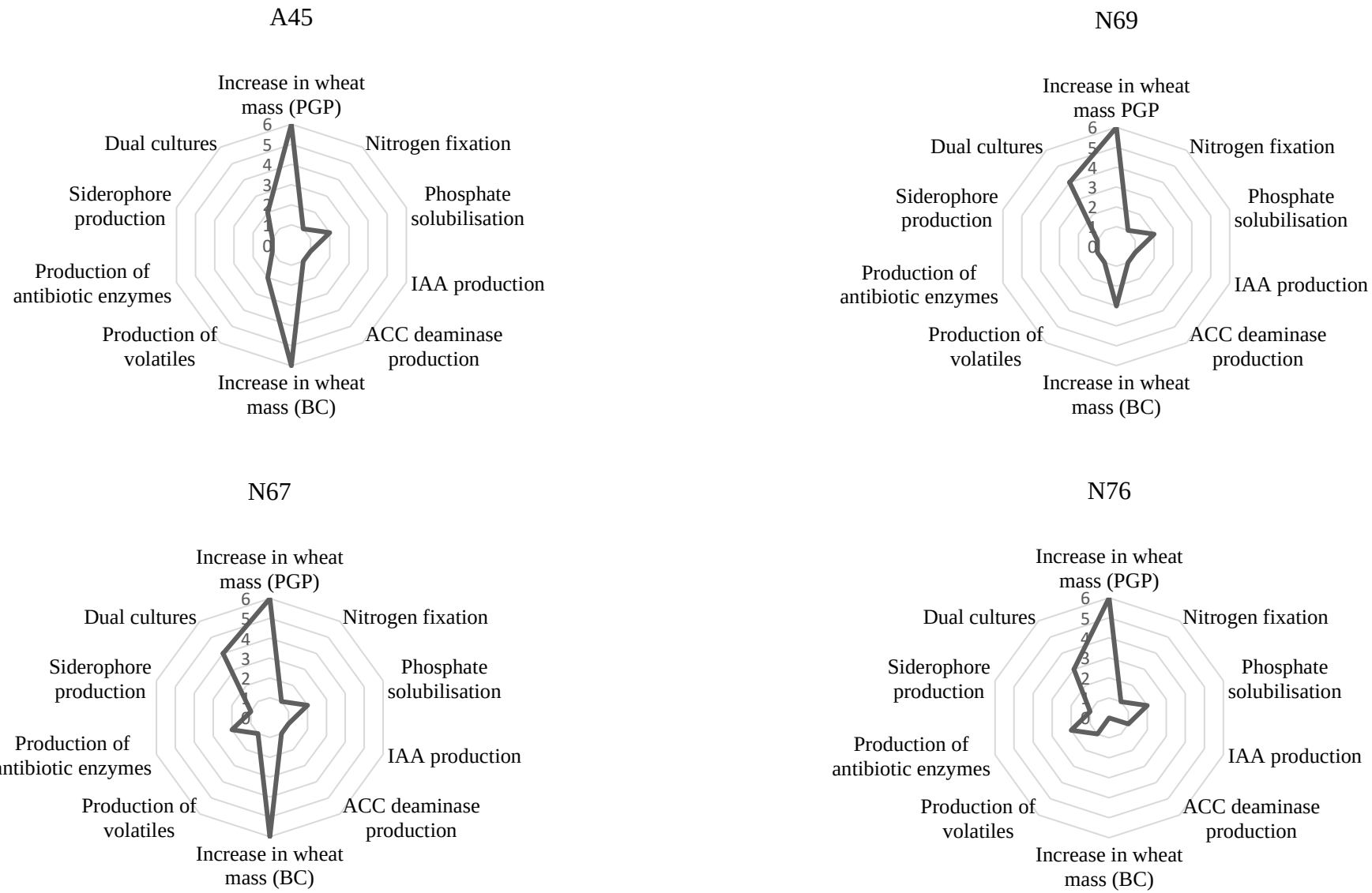
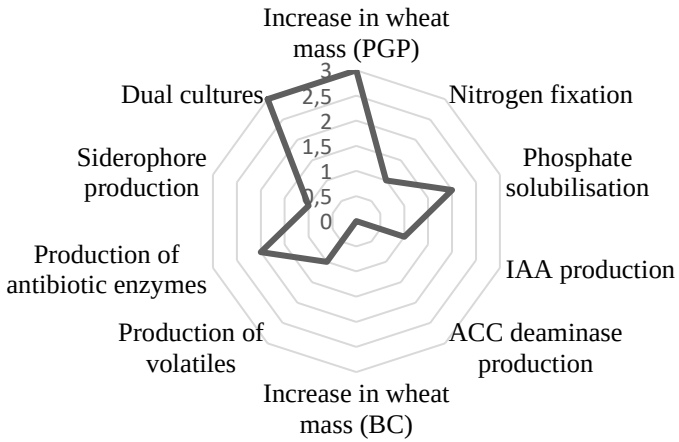
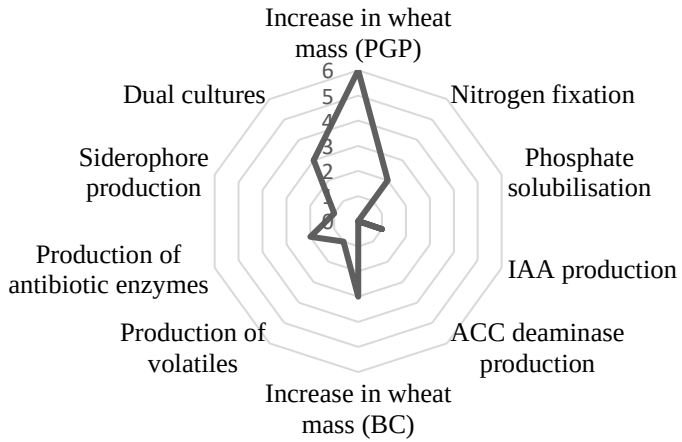



**Figure 6.2** Spider diagrams for isolates that performed the best for growth enhancement of wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP=) plant growth promotion in absence of pathogen i.e. direct plant growth promotion.

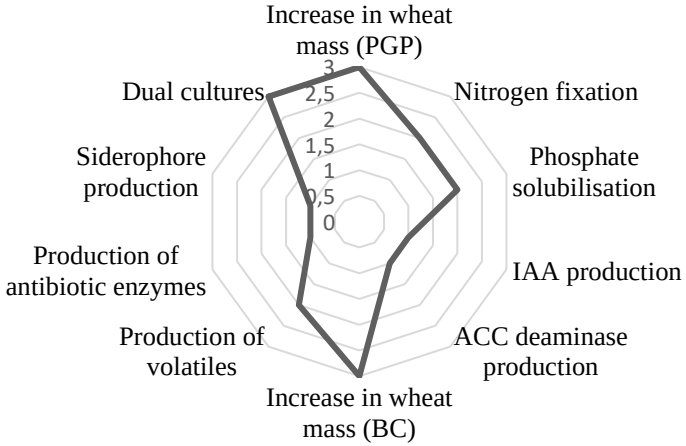
N34




KBS1F3




N29




A10AC

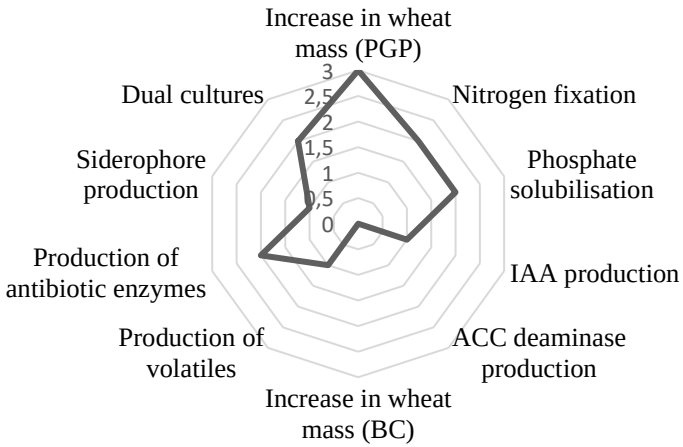
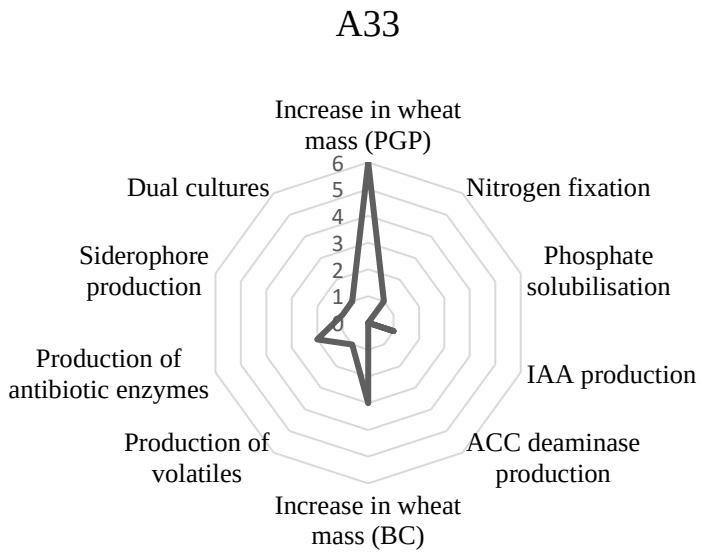



**Figure 6.2 continued** Spider diagrams for isolates that performed the best for growth enhancement of wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP)= plant growth promotion in absence of pathogen i.e. direct plant growth promotion.



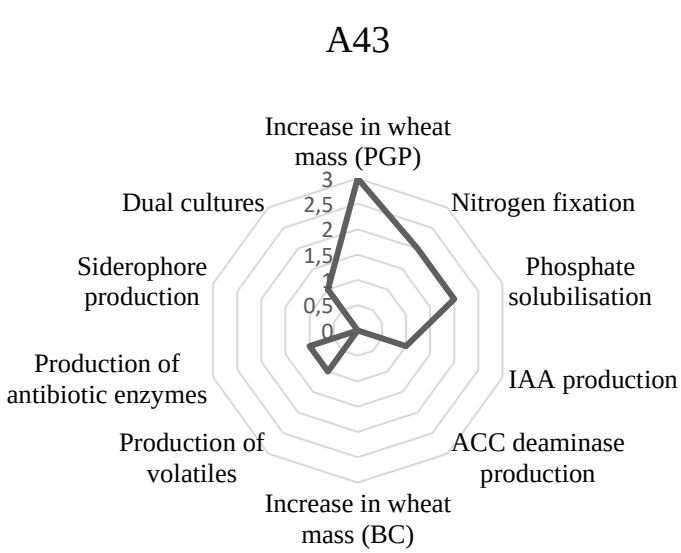




**Figure 6.2 continued** Spider diagrams for isolates that performed the best for growth enhancement of wheat. Increase in wheat weight (BC)= in presence of the pathogen, indicating biocontrol. Increase in wheat weight (PGP)= plant growth promotion in absence of pathogen i.e. direct plant growth promotion.

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# CHAPTER 7

## Disease suppression and plant growth promotion of selected rhizobacteria against *Fusarium pseudograminearum* on wheat in the greenhouse


### Abstract

The aim of the current chapter was to further assess the 10 selected best-performing isolates (from chapters 3, 4 and 5) for biocontrol activity and plant growth promotion in greenhouse pot experiments. The effect of temperature, pH, NaCl and different carbon sources on the growth of the isolates were also assessed *in vitro*. Eight (A17, N44, N54, A09AC, N04AC, N28, A20 and A45) isolates showed biocontrol activity by increasing the total dry weight of *F. pseudograminearum* inoculated wheat seedlings by 16.1 to 29.8%, with 5.79 to 18.26% of this increase being due to plant growth promotion effect of the isolates. Nine isolates (KBS1F3, A45, A33, N34, N76, N67, N53, N69, A10AC) showed direct plant growth promotion activity by increasing the total dry weight of uninoculated wheat seedlings by 16.9 to 29.7%. Isolate A17 (*Bacillus cereus/thuringiensis*) and KBS1F3 (*Paenibacillus alvei*) resulted in the highest increase in total dry weight of the seedlings. The optimum growth temperature of all isolates was between 26ºC and 35ºC whilst an increase in NaCl concentration decreased the growth of the isolates. The optimum pH for all the isolates was found to be pH 7. The isolates showed the ability to utilise a variety of carbon sources (starch, d+glucose, glycerol, d+galactose, lactose, d-mannitol and sucrose). Isolates A17 and KBS1F3 have the potential for development as sustainable and environmentally friendly biocontrol agents and biofertilisers. Their ability to grow under different conditions, some extreme, indicates that they may survive and aid plant growth under stress conditions.


### 7.1 Introduction

The soil is populated with a variety of organisms, amongst these are rhizobacteria which have been found beneficial for the growth of plants. There are many reports on the potential of rhizobacteria as biocontrol agents and biofertilizers. *Pseudomonas* isolates have shown great success against *Rhizoctonia solani* and *Pythium ultimum* root rot on wheat and also increase the shoot length and root weight of the crop (Mavrodi et al. 2012). The application of *Bacillus amyloliquefaciens* FLN13 and *Lactobacillus plantarum* SLG17 showed potential against Fusarium head blight of wheat under field conditions (Baffoni et al. 2015). *Trichoderma* (TrichC70) has been found to increase the emergence of wheat seedlings, increase the dry yield

as well as root rot severity caused by *Fusarium graminearum* (Xue et al. 2017). Other isolates such as *Azospirillum*, *Azoarcus* and *Azorhizobium* have been found to increase wheat root length in the field which could aid in the absorption of water and nutrients (Cortivo et al. 2017).

Even with all the success that is obtained with various isolates *in vitro* or in the greenhouse, the application of such isolates in the field does not always yield the same results (Mavrodi et al. 2012). As such, there is continuous research aimed at better understanding the mode of action of the isolates and ensuring their best performance for commercial use. The ability of PGPR to promote plant growth and control pathogens is limited by a number of factors in the environment such as the availability of nutrients, pH of the soil, temperature and humidity.

PGPR require a continuous supply of nutrients to grow and optimum pH and temperature to carry out metabolic processes. Some of these processes are beneficial for plant growth promotion such as nitrogen fixation, ACC deaminase, siderophore and IAA production, phosphate solubilisation (Delshadi et al., 2017; Parada et al., 2016); while others are beneficial for the control of pathogens such as the production of antibiotic compound, directly competing for nutrients and space with the pathogens and the induction of systemic resistance in plants (Baffoni et al., 2015; Mavrodi et al., 2012; Singh and Jha, 2017). *Strenotrophomonas maltophilia* SBP-9 was also reported to increase wheat yield even under salt stress (Singh and Jha 2017).

In chapters 3 and 4 of this thesis, 113 bacterial isolates were screened in the greenhouse by means of a seedling assay for plant growth promotion and biocontrol activity against *F. pseudograminearum* on wheat. The isolates were further assessed for traits associated with direct plant growth promotion and biocontrol activity *in vitro* (chapter 5). The top 10 best-performing isolates for biocontrol and plant growth promotion were selected (chapter 6) and assessed in a final greenhouse experiment (current chapter). In order to better understand the behaviour of the isolates, the effect of different carbon sources, temperature, pH and NaCl on the growth of the isolates will also be studied.

## 7.2 Materials and methods

Evaluation of selected rhizobacterial isolates for plant growth promotion and biocontrol activity against *F. pseudograminearum* on wheat in the greenhouse.

### 7.2.1.1 Bacterial culture preparation

All bacterial cultures were obtained from the UP-PGPR culture collection. A total of 10 isolates were used to assess biocontrol activity and another 10 to assess plant growth promotion. The isolates were maintained and prepared as described in chapter 3 paragraph 3.2.1.

### 7.2.1.2 Inoculum preparation

The fungal pathogen, *Fusarium pseudograminearum* M7816N, was supplied by Dr S. Lamprecht (Agriculture Research Council, Stellenbosch, South Africa). The inoculum was prepared and stored as described in chapter 3 paragraph 3.2.2.

### 7.2.1.3 Seed preparation

Wheat seeds of the cultivar Duzi were obtained from Klein Karoo Seed (P.O. Box 159, Oudtshoorn, 6620, South Africa). The seeds were surface disinfected with 70% ethanol for one minute and then washed five times with sterile $dH_2O$. They were subsequently submerged in 1% sodium hypochlorite solution for 30 seconds and rinsed five times in sterile $H_2O$. The seeds were then pre-germinated in sterile petridishes containing moist filter paper for three days.

### 7.2.1.4 Assessment of rhizobacterial isolates for biocontrol activity against *F. pseudograminearum*

The wheat seeds were prepared as described above. The isolates were streaked onto NA plates from working cultures. After 3 days of incubation, a loop full of the bacterial cells were transferred to 150ml of autoclaved nutrient broth in 250ml flasks. These were incubated in a shaking incubator (150rpm) for 48 hours at 25$^{o}$C. The pregerminated seeds were transferred into 9cm diameter pots containing steam-pasteurised soil. Five millilitres of the bacterial suspension was pipetted on top of the pre-germinated seeds at planting. Fourteen days following bacterial inoculation, the seedlings were inoculated with 5ml of $1\times10^{6}$ spores/ml of *F. pseudograminearum* by pipetting it at the crown of the seedling. The negative control was

treated with uninoculated NB while the positive control was treated with 5ml of $1\times10^6$ spores/ml spore suspension only. Each treatment comprised 5 replicates with 4 seedlings/replicate. The plants were grown for a further 8 weeks at an average temperature of 25$^o$C in the greenhouse. The seedlings were watered every second day with municipal tap water. Upon harvesting, soil was washed from the roots, disease symptoms were recorded and disease incidence determined (Disease incidence= number of diseased plants/total number of plants×100). The roots were excised and the fresh root and shoot weight of the seedlings were determined by weighing. The number of plants showing disease symptoms from each treatment was also recorded. Roots and shoots were placed in brown paper bags and dried in an oven for 72 hours at 50$^o$C. Dry root and shoot weight were determined by weighing. This experiment was done twice.

7.2.1.5 Screening of rhizobacterial isolates for plant growth promotion of wheat

The wheat seeds were prepared as described above. The isolates were streaked onto NA plates from working cultures. After 3 days of incubation, a loop full of the bacterial cells were transferred to 150ml of autoclaved nutrient broth in 250ml flasks. These were incubated in a shaking incubator (150rpm) for 48 hours at 25$^o$C. the pregerminated seeds were then transferred into 9cm diameter pots containing soil. Five millilitres of the bacterial suspension was pipetted at the top of the pre-germinated seeds at planting. The negative control was inoculated with uninoculated NB. Each treatment composed of 5 replicates with 4 seedlings per replicate. These were incubated for a further 8 weeks at an average temperature of 25$^o$C. The seedlings were watered daily with municipal tap water. Upon harvesting, excess soil was rinsed from the roots with tap water. The shoots were excised, and fresh root and shoot weight of the seedlings were recorded. The roots and shoots were placed in brown paper bags and dried in an oven at 50$^o$C for 72 hours. Following this, the dry root and shoot weight were determined by weighing. This experiment was done twice.

7.2.1.6 Experimental design and statistical analysis

Four seeds planted in one pot represented a single experimental unit. Each treatment was replicated five times. The experiment was set up according to a randomized block design. Analysis of variance (ANOVA) was performed using SAS 9.2 software (SAS Institute, Cary,

NC, USA). Fischer's Protected Least significant difference (LSD) was used to separate the means at a significance level of 5% (p=0.05).

### 7.2.2 Characterisation of the selected isolates

#### 7.2.2.1 Sequencing

Pure cultures of the best-performing isolates from the pot trials were sent to Inqaba Biotechnical Industries (Hatfield, South Africa) for sequencing of the 16sRNA gene region. At Inqaba the DNA was extracted with Zymo Fungal/Bacterial DNA extraction kit (Zymo Research Corp.), the PCR performed using DreamTaq (Fermentas Life Sciences, DreamTacTM Green PCR Master Mix) and the primers 27-F and 1492-R. The sequencing reaction was performed with ABI Bid Dye v3.1 and the clean-up performed with the Zymo Sequencing Clean-up kit (ZR-96, DNA Sequencing Clean-up KitTM).

#### 7.2.2.2 Gram-staining

Gram-staining was performed according to Somasegaran and Hoben (2012).

#### 7.2.2.3 Temperature response

The growth of each isolate was tested over a range of 15 temperatures ranging from ±14°C to ±56°C, in a temperature gradient shaking incubator (Scientific Industries Inc., Model TGI). Nutrient broth (NB) was prepared,10ml was dispensed into the apparatus glass tubes and autoclaved. The autoclaved NB was then inoculated with the respective rhizobacterial isolates and placed in the incubator for 24 h. Following incubation, 270µl of the culture was dispensed into 96-well microplates (Greiner Bio-One, Flat-Bottom) to quantify bacterial growth by measuring the optical density (OD) spectrophotometrically (600nm) (Mikkili et al. 2014).

#### 7.2.2.4 NaCl response

Nutrient broth medium with different NaCl concentrations (0%, 0.1%, 0.5%, 2%, 4% and 6% m/v). Each flask was inoculated with loopful of culture and incubated in a 150rpm shaking incubator at 25ºC for 24h. After incubation, 270µl of the culture was dispensed into 96-well microplates (Greiner Bio-One, Flat-Bottom) to quantify bacterial growth by measuring the optical density (OD) spectrophotometrically (600nm) (Mikkili et al. 2014).

7.2.2.5 pH response

To test for the effect of different pH (4-9) on the growth of the isolates. Nutrient broth was prepared and pH adjusted to the desired pH using either NaOH or $H_2SO_4$. The prepared NB was then dispensed in 250ml flasks and autoclaved. The autoclaved NB was inoculated with the isolates and incubated in a shaking incubator at 150rpm at 25$^oC$ for 24 h. Following incubation, 270µl of the culture was dispensed into 96-well microplates (Greiner Bio-One, Flat-Bottom) to quantify bacterial growth by measuring the optical density (OD) spectrophotometrically (600nm) (Mikkili et al. 2014).

7.2.2.6 Carbon source utilization

Carbon source utilization of the isolates was done according to the procedure described by Schofield et al. (1979). The basal medium contained 0.2g peptone, 0.2g casein hydrolysate, 0.2g $KH_2PO_4$, 0.6g $K_2HPO_4$, 0.1g $MgSO_4 \cdot 7H_2O$ and 15g agar in 1L $dH_2O$. An aqueous solution of individual carbon sources (starch, d+glucose, glycerol, d+galactose, lactose, d-mannitol and sucrose) was prepared by dissolving 5g of the carbon source in 30ml $dH_2O$. The two solutions were autoclaved and mixed before pouring into sterile petri dishes to make up a final concentration of 0.5% (w/v) of carbon source in the media). Control medium was prepared without a carbon source. The isolates were streaked onto plates containing different carbon sources and incubated at 30$^oC$ for 7 days. Following incubation, the plates were inspected for bacterial growth, which was indicative of utilization of the particular carbon source. In the case of starch, the plates were flooded with iodine and clear zones around the bacterial growth was an indication of starch hydrolysis.

7.2.2.7 Citrate utilization

Citrate utilization was done using Simmons's Citrate Agar (Holt et al. 1994). The medium was composed of 0.2g $NH_4H_2PO_4$, 0.08g bromothymol blue, 0.8g $Na_2(NH_4)PO_4$, 0.2g $MgSO_4 \cdot 7H_2O$, 5g NaCl and 2g sodium citrate in 1L $dH_2O$. The medium was dissolves and dispensed (10ml) into test tubes. The test tubes with the medium were autoclaved and allowed to cool down in a slanted position. The isolates were streaked onto the slants and incubated at 25$^oC$ for 5 days. Colour change of the medium from green to blue was indicative of citrate utilization.

7.2.2.8 Gelatine hydrolysis

Gelatine hydrolysis was done on medium composed of 16g NB supplemented with 120 g gelatine (Sheridans, Libstar Operations (Pty) Ltd) in 1L $dH_2O$ (Holt et al. 1994). This was dissolved and distributed (10ml) into test tubes. The test tubes with the medium were dissolved and allowed to cool. The test tubes were inoculated with bacteria by stabbing into the semi-solid medium with an inoculating loop five times. The test tubes were incubated at 25$^{o}$C for 5 days. Following incubation, the test tubes were placed in the refrigerator for an hour to allow the medium to solidify. Test tubes that did not solidify indicated gelatine hydrolysis while those that solidified indicated negative reaction for gelatine hydrolysis.

## 7.3 Results

7.3.1 Evaluation of selected rhizobacterial isolates for plant growth promotion and biocontrol activity against *F. pseudograminearum* on wheat in pot trials in the greenhouse

7.3.1.1 Screening of rhizobacterial isolates for biocontrol activity against *F. pseudograminearum*

Eight weeks after the start of the experiment the negative (untreated) control showed no symptoms associated with *F. pseudograminearum* infection whilst the positive (pathogen inoculated) control showed clear symptoms of stunting and browning of the crowns as well as the stems (Figure 7.1 and Figure 7.2). In addition, most of the diseased seedlings did not flower or produce any spikes. Significant differences between treatments were observed in terms of the fresh weight of the seedlings (Table 7.1). The shoot dry weight data also showed significant differences between the treatments ($p=0.012$). Compared to the pathogen-inoculated control, only N44 was able to increase the shoot dry weight of diseased wheat seedlings significantly by 26.5% (Figure 7.3). The root dry weight data also showed significant differences between the treatments ($p<0.0001$). All the isolates, except N02, significantly increased the root dry weight of diseased wheat seedlings; with A17 significantly different from all the other treatments with a 58.5% compared to the uninoculated control. The total dry seedling weight data also showed significant differences between the treatments ($p=0.0002$). Isolates A17 (29.8%), N44 (26.4%), N54 (23%), A09AC (21.2%), N04AC (22%), N28 (16.9%), A20 (16.5%) and A45 (16.1%) significantly increased the total dry weight of seedlings inoculated with *F. pseudograminearum*, with 5.79 to 18.26% of this increase being due to plant growth

promotion effect of the isolates. At least 80% disease incidence was also observed with all treatments.

7.3.1.2 Screening of rhizobacterial isolates for plant growth promotion of wheat

Some isolates clearly showed an increase of the wheat seedlings at the time of harvest. Others increased the shoot length while others increased the density of the shoot system and others did not show any clear effect on the aboveground growth of the seedlings. Significant differences were observed from the fresh shoot, root and total weight of the seedlings (Table 7.2 and Figure 7.4). There were also significant differences observed in the shoot dry weight of the seedlings ($p=0.0045$). All isolates, except N29, significantly increased the shoot dry weight of the seedlings (A10AC- 23.7%, KBS1F3- 35.3%, N34- 34.5%, A45- 34%, N76- 32.6%, A33- 29.5%, N67- 26.1%, N53- 24.7%, N69- 22.8%,). Isolate KBS1F3 significantly increased the root dry weight of the seedlings by 29.79%. The total seedling weight resulted in a significant difference between the treatments ($p=0.0147$). All isolates, except N29, significantly increased the total dry weight of the seedlings (KBS1F3- 29.7%, A45- 27.8%, A33- 26.2%, N34- 22.6%, N76- 22.4%, N67- 21.3%, N53- 20.3%, N69- 18.3%, A10AC- 16.9%).

7.3.2. Characterisation of the selected isolates

7.3.2.1. Sequencing

Based on the sequencing of the 16srRNA region the isolate with the best biocontrol activity, A7, was identified as *Bacillus cereus/thuringiensis* whereas the best-performing plant growth promoter, KBS1F3, was identified as *Paenibacillus alvei*.

7.3.2.2. Gram reaction

Isolates A09AC, A10AC, A17, A20, KBS1F3, N02, N28, N53 and N54 tested gram-positive whereas isolates A33, A45, N04AC, N29, N34, N44, N59A, N67, N69 and N76 were gram-negative (Table 7.3).

7.3.2.3 Temperature response

The optimal growth temperatures of the isolates were between ±29$^{o}$C and ±35$^{o}$C (Figure 7.5). Most of the isolates did not grow at temperatures above 50$^{o}$C except for N29, N34 and N44 that could still grow at about 56$^{o}$C.

7.3.2.4 NaCl response

Generally, the rhizobacterial isolates grew better with no NaCl added to the nutrient broth and increase in NaCl concentration decreased growth of the isolates (Figure 7.6). This was followed by 0.5% NaCl concentration for most of the isolates (A09AC, A17, A33, N02, N04AC, N28, N29, N34, N54, N59A, N67 and N76). Isolate A10AC grew better at 0.1% NaCl concentration. With the 6% NaCl concentration A09AC, A10AC, A17, KBS1F3, N04AC and N44 did not grow.

7.3.2.5 pH response

A pH value of 7 was optimal for all the isolates except A10AC, N28 and N54. None of the isolates showed growth at pH 4 (Figure 7.7). Isolate A10AC, N28 and N54 showed the highest growth at pH 8. Isolate KBS1F3 did not grow at pH 5 while A10AC showed very little growth.

7.3.2.6 Carbon source utilization

As shown in Table 7.3 , all isolates were able to grow on NA medium supplemented with starch but only A09AC, A20, KBS1F3, N02, N54 and N76 hydrolysed the starch. All isolates also utilised D+glucose and glycerol. All isolates except N04AC and N76 utilised sucrose. N76, KBS1F3, N04AC, N67 and N69 could not grow on medium with lactose as a carbon source, while all isolates except KBS1F3 utilised D+galactose and D-mannitol.

7.3.2.7 Citrate utilization

Isolates N54, N28, N34, N76, N02, N44, N29, N53, N67, N59A, N69 and N04AC tested positive for utilization of citrate as a sole carbon source whereas A45, A33, A17, A09AC, A20, KBS1F3 and A10AC tested negative (Figure 7.8).

7.3.2.8 Gelatine hydrolysis

Isolates N02, N76, A33, N34, A45, N28, N44, A17, N29, KBS1F3, N53, N67, N59A, N69, N04AC tested positive for gelatine hydrolysis whereas N54, A09AC, A20 and A10AC tested negative for gelatine hydrolysis (Figure 7.9).

## 7.4 Discussion

In the current chapter, the selected rhizobacterial isolates were screened again for biocontrol activity and plant growth promotion in pots in the greenhouse. The effect of temperature, pH, NaCl and different carbon sources on the growth of the isolates was also assessed. Of the 10 isolates tested in the final greenhouse experiment, eight (A17, N44, N54, A09AC, N04AC, N28, A20 and A45) showed biocontrol activity by increasing the total dry weight of *F. pseudograminearum* inoculated wheat seedlings by 16.1 to 29.8%, with 5.79 to 18.26% of this increase being due to plant growth promotion effect of the isolates; whereas nine isolates (KBS1F3, A45, A33, N34, N76, N67, N53, N69, A10AC) showed direct plant growth promotion activity by increasing the total weight of uninoculated wheat seedlings by 16.9 to 29.7%.

The results are similar to those of Mavrodi et al. (2012) who found that the application of PGPR could increase shoot length and root weight of wheat in the greenhouse. When wheat plants are infected with *F. pseudograminearum* more resource and energy are invested into controlling the disease which compromises growth and yield; thus, plant growth promotion is correlated with disease suppression (Mavrodi et al. 2012). The application of *Trichoderma* (TrichC70) increased the emergence of wheat by 10.9%, dry weight by 51.7%, yield by 11.0% and root rot severity by 51.7% which did not differ significantly from those obtained from the registered fungicide Vitaflo-280 which is a combination of carbanthiin and thiram (Xue et al. 2017). The ability of PGRP to perform on the same level as chemical fungicides further highlights what a more attractive alternative they are as a means of plant disease control.

In the current study, the selected rhizobacterial isolates displayed multiple traits associated with biocontrol activity and plant growth promotion. These include the production of antibiotics, siderophores, IAA, ACC deaminase and the action of solubilising phosphate and fixing nitrogen as reported in chapter 5. These traits may have played a role in their biocontrol and plant growth promotion efficacy in the greenhouse. PGPR produce siderophores such as pyochelin and pyoverdin which bind Fe(III) thus making it unavailable to pathogens but available for plant use, aiding in plant growth. Visca et al. (1993) found increased production of salicylic acid by *Pseudomonas aeruginosa* in response to Fe(III) limiting conditions thus suggesting its involvement in the uptake of Fe(III) from the soil. The production of antibiotic compounds and volatiles may have played a role in the control of *F. pseudograminearum* on wheat in the greenhouse.

Rijavec and Lapanje (2016) proposed that the production of the volatile compound HCN is not related to biocontrol activity but rather to plant growth promotion through the regulation of the availability of phosphate. They argue that the amount of HCN produced by rhizobacteria is not high enough to cause any toxicity to pathogens or other rhizobacteria. They further suggested that HCN is involved in the induction of systemic resistance and can act as a siderophore and bind to Fe(III) (Rijavec and Lapanje 2016). Regardless of the role of HCN, be it in biocontrol of pathogens or improving plant growth, one cannot ignore the importance of this trait in PGPR.

One other favourable characteristic of some PGPR is their ability to penetrate the plant cells, migrate towards the vascular bundles and can be translocated towards the stems leaves and seeds (Batista et al., 2018; Díaz Herrera et al., 2016). This also allows for the control of pathogens which may be carried in seeds. Upon sowing these PGPR can also establish themselves in the rhizosphere. *Acinebacter*, *Bacillus*, *Micrococcus*, *Paenebacillus*, *Pantoea*, *Pseudomonas* and *Staphylococcus* spp. for example, have been reported as endophytes (Díaz-Herrera et al. 2016). *Bacillus subtilis* has also been isolated from the stem of a healthy ginger plant (Yang et al. 2012).

To increase the efficacy of PGPR when applied in the field, a number of researchers (Cortivo et al. 2017; Delshadi et al. 2017; Saleemi et al. 2017) suggest the use of PGPR mixtures for enhanced plant growth promotion compared to individual applications. Zalila-kolsi et al. (2016) found *Bacillus* sp. to perform the best in controlling of *F. pseudograminearum* and suggested the use of a mixture of isolates with different traits to achieve the maximum protection against the pathogen.

There are a number of factors one needs to consider when choosing PGPR to enhance plant growth and control plant pathogens, from the plant to the environment and soil they are applied to (Salvo et al. 2018). The ability of PGPR to thrive at extreme environmental conditions ensures that they can still promote plant growth and control plant diseases. Generally, the optimum temperature of all isolates in the current study was between 26$^{o}$C and 35$^{o}$C. Isolate KBS1F3, which was identified as *Paenibacillus alvei*, still grew well at 47$^{o}$C while A17 which was identified as *Bacillus cereus/thuringiensis* could still grow at 50$^{o}$C. To withstand such high temperatures, the PGPR alter their phospholipid composition which allows them to maintain structural and functional integrity (Paulucci et al. 2015). *Bacillus* and *Paenibacillus* species form endospores which allow them to survive extreme conditions including temperatures as high as 50$^{o}$C.

Another factor that limits the growth of PGPR in the soil is the salt concentration. High salt concentrations disrupt cell membranes and affect cell water potential. The growth of KBS1F3 decreased with increase in NaCl concentration while A17 still grew well at 4% NaCl concentration. Woyessa and Assefa (2011) found that *Pseudomonas flourescens* to grow up to 5% NaCl concentration while *Bacillus cepacia* grew between 1% and 2%, *Bacillus subtilis* and *Bacillus coagulans* grew up to the 5% concentration. Barnawal et al. (2017) found that the application of *Arthrobacter protophormiae, Dietzia natronolimnaea* can encourage wheat salt stress tolerance while *Bacillus subtilis* can provide drought stress tolerance. To allow them to withstand high salinity levels, *Pseudomonas* strains have been found to produce osmolytes such as Ala, Gly, Ser, Glu, Thr and Asp in their cytosol (Egamberdieva 2012).

The pH of the soil is can influence the growth of PGPR in the soil. KBS1F3 (*P. alvei*) still grew well at pH 8 while A17 (*B. cereus/thuringiensis*) showed good growth at all pH except pH 4. This agrees with Woyessa and Assefa (2011) who reported *P. flourescens, B. cepacia* and *B. coagulans* to grow at pH 5.5-8.5 and 9.0-10.0; however, *B. subtilis* did not grow at pH 9.0-10.0. The ability of PGPR to grow at a variety of pH allows it to survive in the rhizosphere which is characterised by acidic conditions due to $CO_2$ and organic acids released from the plants.

Plants may release different root exudates depending on the growth stage they are in. (Salvo et al. 2018). Thus, the ability of PGPR to utilise different carbon and nitrogen sources is favourable to ensure their survival and growth. In the current study, the isolates utilised a variety of carbon sources. KBS1F3 hydrolysed starch and gelatine, make use of D+glucose, glycerol and sucrose as the sole carbon source while A17 utilised all carbon sources used and hydrolysed gelatine, but could not hydrolyse starch and citrate. The health and longevity of PGPR is dependent on the availability of continuous supply of nutrients and the ability of PGPR to utilise a variety of carbon sources ensures their survival in the soil and affords them the ability compete with other microorganisms.

The selected isolates showed great potential as agricultural additives in the production of wheat. However, the potential of the isolates still needs to be studied further under field conditions. The potential of these isolates could also be assessed on different crops and pathogens. The isolates can be used in conjunction with other control strategies to reduce pathogen inoculum load in the soil during and before the next growing season. In addition, the efficacy of the isolates could be assessed in soil from different backgrounds to see how previous cropping practices affect the performance of the different isolates.

### 7.5. Tables and Figures

**Table 7.1** Effect of selected rhizobacterial isolates on *F. pseudograminearum* inoculated wheat seedlings in pot trials in the greenhouse.

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight A[3] | | | | | | Percentage change in weight B[4] | | | Disease incidence[5] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight | | | Mean dry weight | | | Mean fresh weight | | | Mean dry weight | | | Mean dry weight | | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | |
| [1]NC | 0.788$^{bcde}$ | 0.802$^{ab}$ | 1.124$^{cd}$ | 0.417$^{bcd}$ | 0.358$^{bc}$ | 0.553$^{bcde}$ | 8.391 | 21.515 | 14.112 | 0.482 | 29.242 | 9.722 | 0 | 0 | 0 | 0.000$^{c}$ |
| [1]PC | 0.727$^{de}$ | 0.660$^{d}$ | 0.985$^{e}$ | 0.415$^{bcd}$ | 0.277$^{d}$ | 0.504$^{e}$ | 0 | 0 | 0 | 0 | 0 | 0 | -0.479 | -22.626 | -8.861 | 100.000$^{a}$ |
| A09AC | 0.881$^{ab}$ | 0.822$^{ab}$ | 1.207$^{abc}$ | 0.485$^{abc}$ | 0.362$^{bc}$ | 0.611$^{ab}$ | 21.183 | 24.545 | 22.538 | 16.867 | 30.686 | 21.230 | 16.307 | 1.117 | 10.488 | 100.000$^{a}$ |
| A17 | 0.884$^{ab}$ | 0.875$^{a}$ | 1.245$^{ab}$ | 0.482$^{abc}$ | 0.439$^{a}$ | 0.654$^{a}$ | 21.596 | 32.576 | 26.396 | 16.145 | 58.483 | 29.762 | 15.586 | 22.626 | 18.264 | 95.000$^{a}$ |
| A20 | 0.794$^{bcd}$ | 0.790$^{ab}$ | 1.125$^{bcd}$ | 0.483$^{abc}$ | 0.331$^{c}$ | 0.587$^{abc}$ | 9.216 | 19.697 | 14.213 | 16.386 | 19.495 | 16.468 | 15.827 | -7.542 | 6.148 | 96.667$^{a}$ |
| A45 | 0.759$^{cde}$ | 0.749$^{bcd}$ | 1.069$^{de}$ | 0.477$^{abc}$ | 0.335$^{c}$ | 0.585$^{abcd}$ | 4.402 | 13.485 | 8.528 | 14.939 | 20.939 | 16.071 | 14.388 | -6.425 | 5.787 | 80.000$^{b}$ |
| N02 | 0.703$^{de}$ | 0.673$^{cd}$ | 0.977$^{e}$ | 0.413$^{cd}$ | 0.316$^{cd}$ | 0.522$^{cde}$ | -3.301 | 1.969 | -0.812 | -0.482 | 14.079 | 3.571 | -0.959 | -11.732 | -5.606 | 100.000$^{a}$ |
| N04AC | 0.867$^{ab}$ | 0.843$^{ab}$ | 1.211$^{abc}$ | 0.492$^{ab}$ | 0.362$^{bc}$ | 0.615$^{ab}$ | 19.257 | 27.727 | 22.944 | 18.554 | 30.686 | 22.024 | 17.986 | 1.117 | 11.212 | 93.333$^{a}$ |
| N28 | 0.829$^{bc}$ | 0.761$^{bc}$ | 1.128$^{bcd}$ | 0.463$^{abc}$ | 0.356$^{bc}$ | 0.589$^{abc}$ | 14.030 | 15.303 | 14.518 | 11.566 | 28.519 | 16.865 | 11.031 | -0.559 | 6.501 | 100.000$^{a}$ |
| N44 | 0.939$^{a}$ | 0.873$^{a}$ | 1.287$^{a}$ | 0.525$^{a}$ | 0.357$^{bc}$ | 0.637$^{a}$ | 29.161 | 32.273 | 30.659 | 26.506 | 28.881 | 26.389 | 25.899 | -0.279 | 15.189 | 95.000$^{a}$ |
| N54 | 0.858$^{abc}$ | 0.838$^{ab}$ | 1.202$^{abc}$ | 0.478$^{abc}$ | 0.389$^{b}$ | 0.620$^{ab}$ | 18.019 | 26.969 | 22.03 | 15.181 | 40.433 | 23.016 | 14.628 | 8.659 | 12.116 | 100.000$^{a}$ |
| N59A | 0.689$^{e}$ | 0.689$^{cd}$ | 0.976$^{e}$ | 0.376$^{d}$ | 0.341$^{bc}$ | 0.514$^{de}$ | -5.227 | 4.394 | -0.914 | -11.566 | 23.105 | 1.984 | -9.832 | -4.749 | -7.052 | 100.000$^{a}$ |
| [2]LSD | 0.099 | 0.097 | 0.121 | 0.079 | 0.051 | 0.073 | | | | | | | | | | 9.829 |
| [2]CV (%) | 13.87 | 14.06 | 12.10 | 19.49 | 16.24 | 14.05 | | | | | | | | | | 12.55 |

[1] NC = Negative control (uninoculated), PC = Positive control (pathogen inoculated)

[2] LSD = least significant difference. CV = coefficient of variance expressed in percentages.

In a column, means followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[3] The percentage change in weight A = [(treatment-positive control)/positive control×100]. Negative values are treatments that are less than the positive (pathogen inoculated) control and positive values are treatments with a higher weight than the positive control. Data was square-root transformed.

[4] Percentage change in weight A = [(treatment- negative control)/negative control×100] negative values are treatments that are less than the negative control and positive values are treatments with a higher weight than the negative control.

[Percentage change in weight B gives an indication of direct growth promotion in the absence of the pathogen whereas percentage change in weight A reflects a combination of direct growth promotion and biocontrol activity. Subtracting B values from A values gives an indication of biocontrol effect].

[5] Disease incidence = diseased plants/total number of plants×100.

Highlighted figures indicate those for which a significant increase in dry weight was recorded in the bacteria treated plants compared to the control plants.

**Table 7.2** Effect of selected rhizobacterial isolates on the growth of wheat seedlings in pot trials in the greenhouse.

| Isolate | Seedling weight (g) | | | | | | Percentage change in weight[3] | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean fresh weight | | | Mean dry weight | | | Mean fresh weight | | | Mean dry weight | | |
| | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total | Shoot | Root | Total |
| [1]NC | 0.794$^{e}$ | 0.801$^{de}$ | 1.128$^{f}$ | 0.417$^{c}$ | 0.358$^{b}$ | 0.553$^{c}$ | 0 | 0 | 0 | 0 | 0 | 0 |
| A10AC | 0.953$^{bcd}$ | 0.789$^{e}$ | 1.239$^{e}$ | 0.516$^{ab}$ | 0.387$^{ab}$ | 0.647$^{ab}$ | 20.025 | -1.498 | 9.840 | 23.741 | 8.100 | 16.998 |
| A33 | 1.018$^{ab}$ | 0.915$^{abc}$ | 1.369$^{abc}$ | 0.540$^{ab}$ | 0.424$^{ab}$ | 0.689$^{ab}$ | 28.212 | 14.232 | 21.365 | 29.496 | 18.436 | 26.221 |
| A45 | 1.009$^{ab}$ | 0.916$^{abc}$ | 1.365$^{abc}$ | 0.559$^{a}$ | 0.426$^{ab}$ | 0.707$^{a}$ | 27.078 | 14.357 | 21.010 | 34.053 | 18.994 | 27.848 |
| KBS1F3 | 1.051$^{a}$ | 0.996$^{a}$ | 1.449$^{a}$ | 0.564$^{a}$ | 0.436$^{a}$ | 0.717$^{a}$ | 32.368 | 24.345 | 28.457 | 35.252 | 21.788 | 29.656 |
| N29 | 0.883$^{d}$ | 0.882$^{bcd}$ | 1.249$^{de}$ | 0.474$^{bc}$ | 0.372$^{ab}$ | 0.605$^{bc}$ | 11.209 | 10.112 | 10.727 | 13.669 | 3.911 | 9.403 |
| N34 | 1.041$^{ab}$ | 0.924$^{ab}$ | 1.393$^{ab}$ | 0.561$^{a}$ | 0.376$^{ab}$ | 0.678$^{ab}$ | 31.108 | 15.356 | 23.493 | 34.532 | 5.028 | 22.604 |
| N53 | 0.919$^{cd}$ | 0.976$^{a}$ | 1.346$^{abcd}$ | 0.520$^{ab}$ | 0.410$^{ab}$ | 0.665$^{ab}$ | 15.743 | 21.848 | 19.326 | 24.700 | 14.525 | 20.253 |
| N67 | 0.918$^{cd}$ | 0.917$^{abc}$ | 1.299$^{bcde}$ | 0.526$^{ab}$ | 0.412$^{ab}$ | 0.671$^{ab}$ | 15.617 | 14.482 | 15.159 | 26.139 | 15.084 | 21.338 |
| N69 | 0.953$^{bcd}$ | 0.855$^{bcde}$ | 1.281$^{cde}$ | 0.512$^{ab}$ | 0.403$^{ab}$ | 0.654$^{ab}$ | 20.025 | 6.742 | 13.564 | 22.782 | 12.569 | 18.264 |
| N76 | 0.982$^{abc}$ | 0.833$^{cde}$ | 1.289$^{cde}$ | 0.553$^{a}$ | 0.386$^{ab}$ | 0.677$^{ab}$ | 23.678 | 3.995 | 14.273 | 32.614 | 7.821 | 22.423 |
| [2]LSD | 0.088 | 0.086 | 0.104 | 0.074 | 0.069 | 0.085 | | | | | | |
| [2]CV (%) | 10.36 | 10.89 | 8.95 | 16.03 | 19.64 | 14.49 | | | | | | |

[1] NC - Negative control (uninoculated)

[2] LSD- least significant difference. CV- coefficient of variance expressed in percentages.

In a column, means followed by the same letter are not significantly different according to Fisher's LSD test at p=0.05.

[3] The percentage change in weight= [(treatment-negative control)/negative control×100] negative values are treatments that are less than the disease control and positive values are treatments with a higher weight than the disease control. Square-root transformed data.

Highlighted figures indicate those that are significantly different from the applicable control.

**Table 7.3** Characterisation of selected PGPR isolates

| Test | | Isolates | | | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | A09AC | A10AC | A17 | A20 | A33 | A45 | KBS1F3 | N02 | N04AC | N28 | N29 | N34 | N44 | N53 | N54 | N59A | N67 | N69 | N76 |
| **Gram-staining** | Gram-positive | ✓ | ✓ | ✓ | ✓ | | | ✓ | ✓ | | ✓ | | | | ✓ | ✓ | | | | |
| | Gram-negative | | | | | ✓ | ✓ | | | ✓ | | ✓ | ✓ | ✓ | | | ✓ | ✓ | ✓ | ✓ |
| **Carbon source utilization** | Starch | ✓ | × | × | ✓ | × | × | ✓ | ✓ | × | × | × | × | × | × | ✓ | × | × | × | ✓ |
| | D+glucose | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Glycerol | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | D+galactose | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Lactose | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | × | × |
| | D-mannitol | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| | Sucrose | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × |
| **Citrate utilization** | | × | × | × | × | × | × | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ |
| **Gelatine hydrolysis** | | × | × | ✓ | × | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | ✓ | × | ✓ | ✓ | ✓ | ✓ |

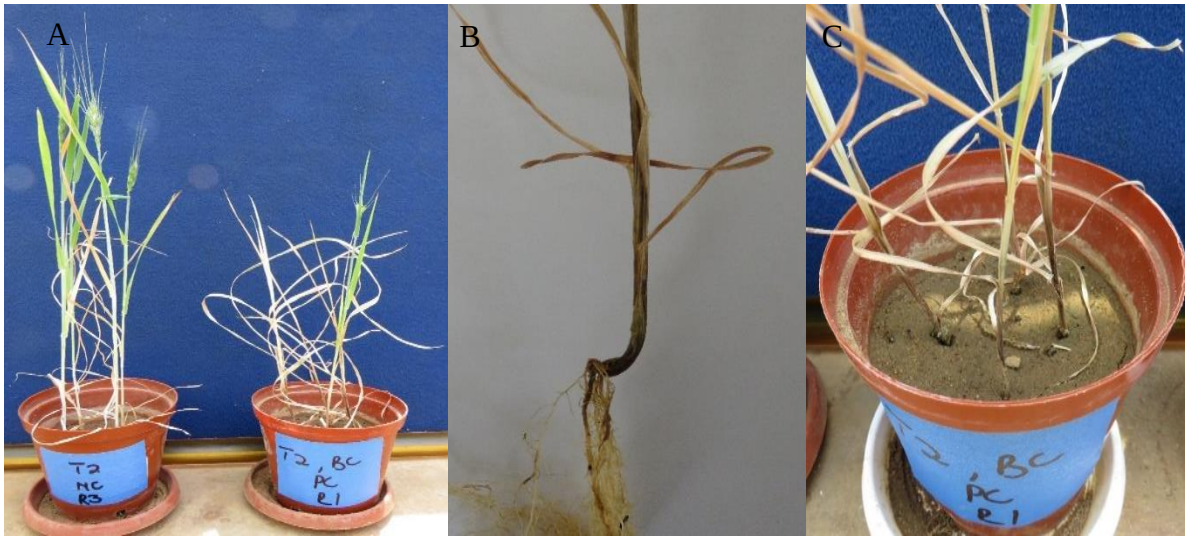


**Figure 7.1** Effect of *F. pseudograminearum* inoculation on wheat plants in the greenhouse. A: left= uninoculated (negative) control; right= pathogen (*F. pseudograminearum*) inoculated control. B and C: Symptoms on wheat seedlings inoculated with *F. pseudograminearum* in the greenhouse pot trial.

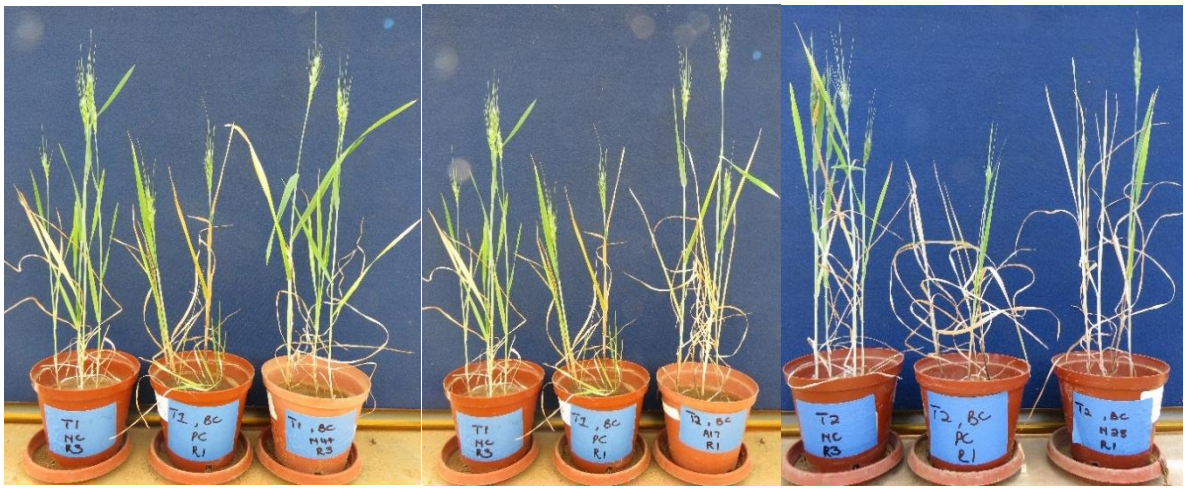

**Figure 7.2** Effect of different rhizobacterial isolates (N44, A17, N28 with each picture containing a negative and a positive control) on the growth of wheat seedlings inoculated with *F. pseudograminearum* in the greenhouse.

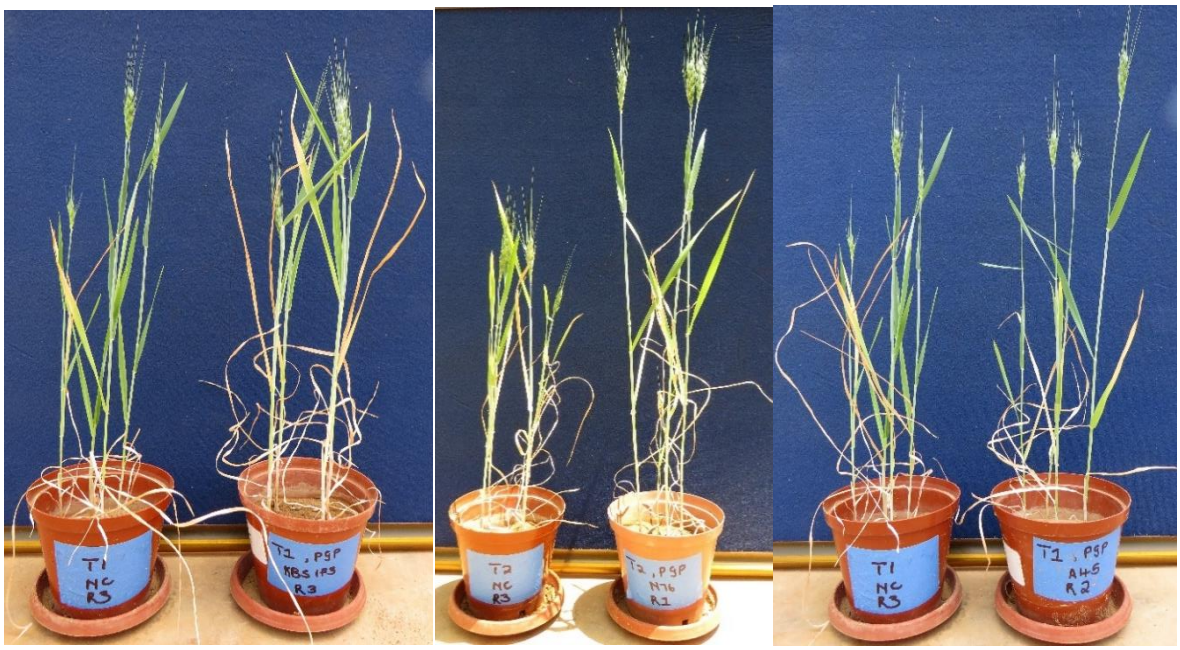


**Figure 7.3** The effect of different rhizobacterial isolates (KBS1F3, N76 and A45 with the negative control on the left of each picture) on the growth of wheat seedlings compared to the untreated control in greenhouse pot trials.

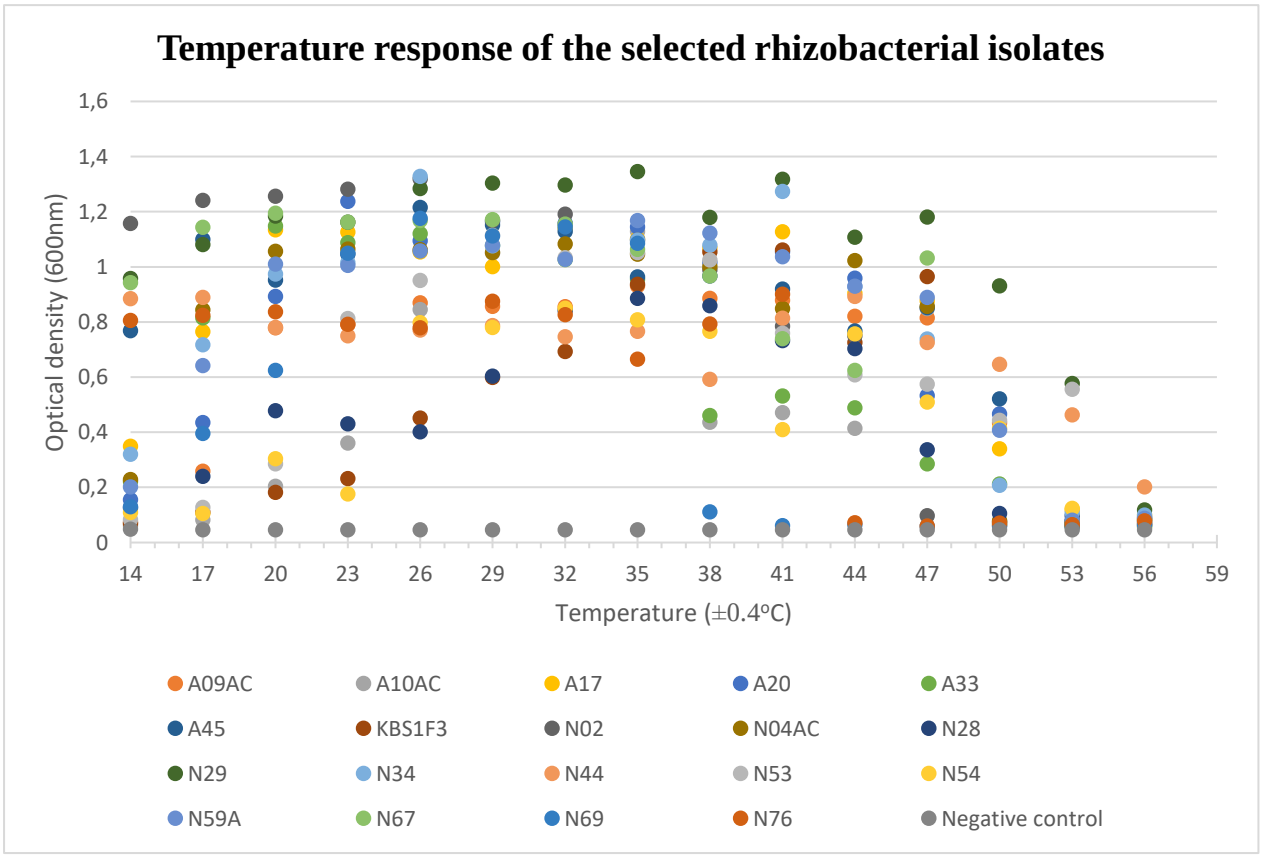


**Figure 7.4** Effect of different temperatures (14±4ºC to 56±4ºC) on the growth of the respective rhizobacterial isolates in nutrient broth.

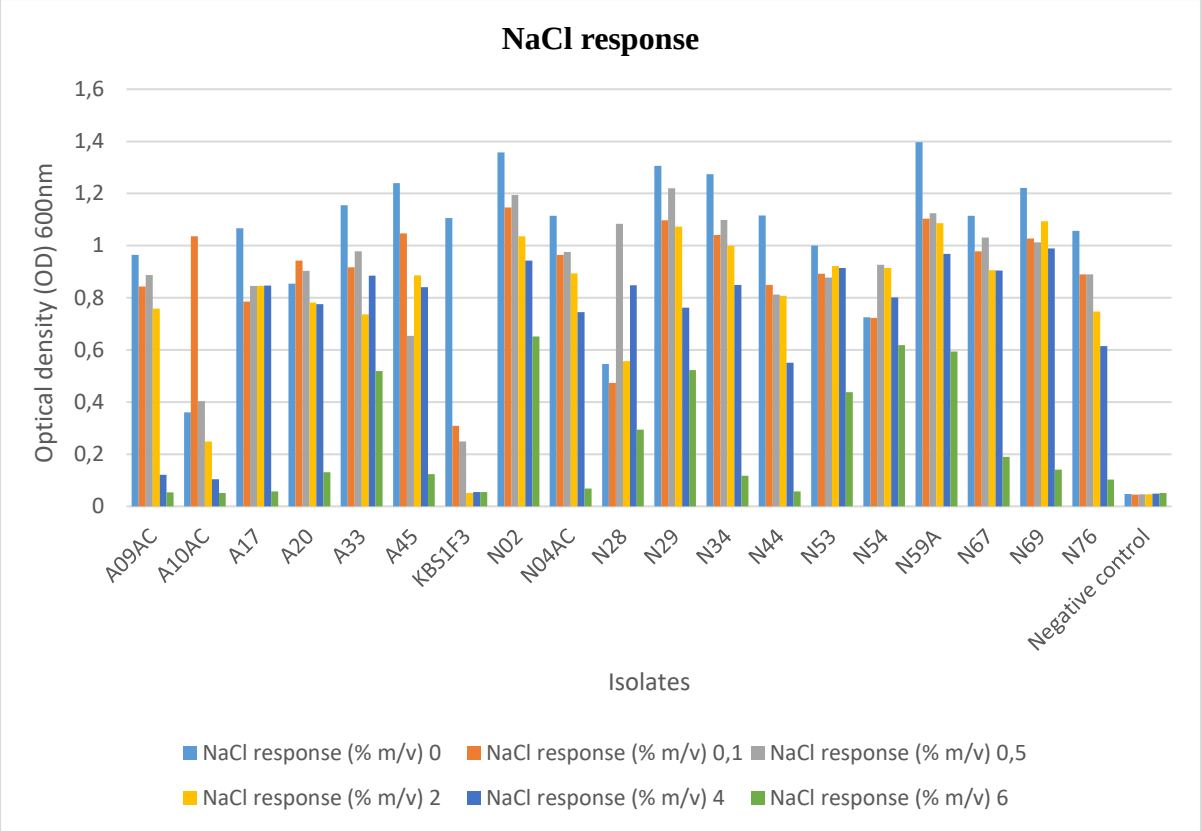


**Figure 7.5** Effect of different NaCl concentrations (0-6% m/v) in nutrient broth on the growth of the respective rhizobacterial isolates.

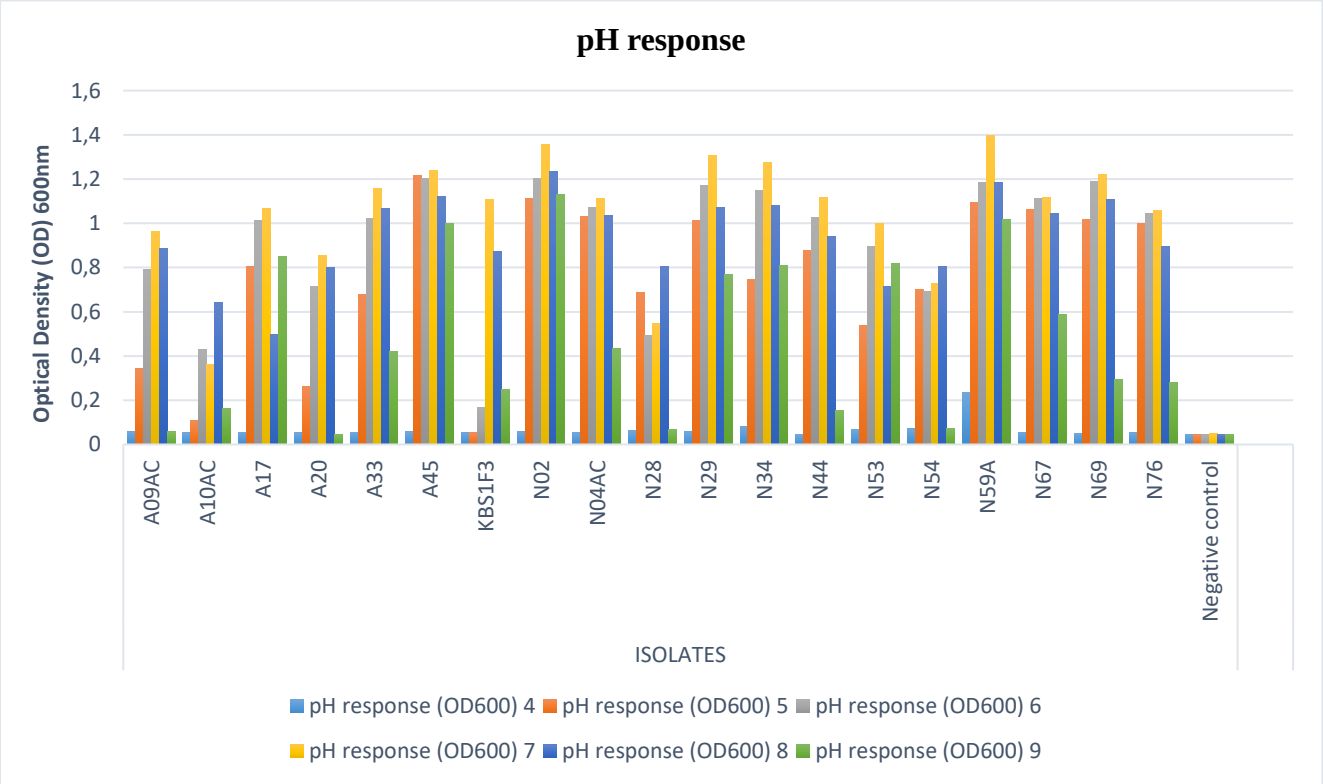


**Figure 7.6** Effect of different pH (4-9) levels in nutrient broth on the growth of the respective rhizobacterial isolates.

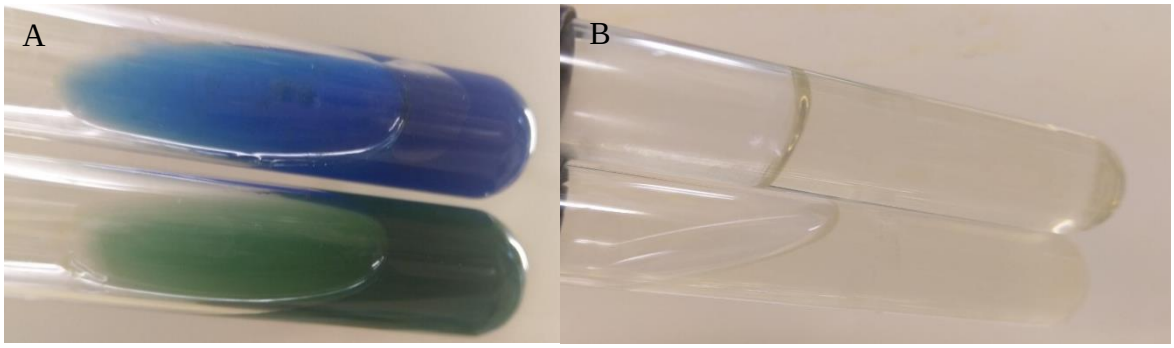


**Figure 7.7** A: Colour change of citrate utilization medium from green to blue upon citrate hydrolysis. B: Gelatine medium consistency change from solid to liquid upon gelatine hydrolysis.

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# CHAPTER 8

## General discussion

The search for PGPR with novel modes of action is ongoing. The use of PGPR as agricultural inoculants offers many advantages over the use of synthetic chemicals and can contribute towards sustainable and environmentally friendly agriculture. Many successes have been obtained with the use of PGPR to promote plant growth and control diseases of various crops and numerous strains have been commercialised as biofertilisers and biocontrol products. To be effective under a diverse range of field conditions strains with multiple modes of action or a novel mode of action are desirable (Thokchom et al. 2014).

In chapter 3, a seedling bioassay was developed and used to screen a collection of 113 rhizobacterial isolates for biocontrol against *Fusarium pseudograminearum* on wheat. The bacterial suspension was applied to pre-germinated seeds at planting and the pathogen spore suspension applied two weeks after planting. Fifty-two percent of the isolates significantly increased the shoot dry weight of the seedlings inoculated with *F pseudograminearum*, 41% increased the root dry weight and 32% increased the total dry weight of the seedlings. Total dry weight of *F.pseudograminearum* inoculated seedlings increased between 39.13% and 123.61%. When compared to the increase in dry weight of pathogen free seedlings it has been calculated that 14.58% to 106.41% of this was due to direct plant growth promotion effect due to the bacterial treatments.

In chapter 4, the developed seedling bioassay was used to screen the rhizobacterial isolate collection (113) for the promotion of wheat seedling growth in the absence of the pathogen. Of the 113 isolates screened, 12% increased the shoot dry weight of the seedlings, 22% increased the root dry weight whereas the total weight of the seedlings was increased by 32% of the isolates. Increase in total seedling weight ranged from 32.35% to 87.95%. This growth may be due to nitrogen fixation, phosphate solubilisation or phytohormone production of the isolates (Goswami et al. 2016; Lwin et al. 2017; Vejan et al. 2016). This small-scale, rapid method of screening rhizobacterial isolates showed that the isolates have direct plant growth promotion on wheat. However, this type of method has its drawbacks. The seedlings were grown for a short period of time which can result in limited information on the ultimate effect of the treatments on the crop when grown to maturity.

*In vitro* screening was performed (chapter 5) on the 113 isolates to assess their biocontrol activity and plant growth promotion traits. The isolates were screened for antibiosis activity using the dual-culture assay on different media (PDA, NA and MEA) against *Rhizoctonia solani*, *Fusarium pseudograminearum*, *Phytophthora capsici* and *Macrophomina phaseolina*. Biocontrol traits such as the production of siderophores, cellulolytic enzymes (cellulase, chitinase, β-1,3-glucanase and protease) and volatile compounds ($NH_3$ and HCN) were assessed. The isolates were also screened for direct plant growth promotion traits such as N-fixation, P-solubilisation, IAA and ACC-deaminase production. Eleven of the isolates inhibited mycelial growth of all four pathogens while 41 isolates inhibited 3 of the pathogens on either of the media used. These bacterial isolates thus exhibit a broad-spectrum of activity. Of the 113 isolates screened for pathogen inhibition, 13.27% showed 50% or more inhibition of mycelial growth of *R. solani*, 16.81% of *M. phaseolina* and 3.54% of *P. capsici in vitro.* All isolates also displayed multiple traits associated with biocontrol activity and plant growth promotion.

In chapter 6, the 10 best-performing isolates for biocontrol and plant growth promotion were selected based on the tests conducted in chapters 3, 4 and 5 combined. A scoring system was developed and used to award points to each isolate. *Bacillus* sp. strain A09AC, A17, A20, N02, N28, N54 *Stenotrophomonas* sp. strain A45, *Pseudomonas* sp. strain N04AC, N44 and N59A were selected as the best-performers for biocontrol of *F. pseudograminearum* whereas *Bacillus* sp. strain A10AC, *Stenotrophomonas* sp. strain A33, A43, A45, *Paenibacillus* sp. strain KBS1F3, *Pseudomonas* sp. strain N29, N69, N67, N76 and *Pantoea* sp. strain N34 were selected as the best-performers for direct growth promotion of wheat in the absence of a pathogen. The selected isolates increased the root and shoot dry weight of wheat in the greenhouse and exhibited multiple traits associated with either biocontrol activity or plant growth promotion. Any or all of the traits assessed may have contributed towards disease suppression and growth of the wheat plants (Kamei et al. 2014; Khare & Arora 2010; Niranjan et al. 2005; Satyanarayana et al. 2017). There are different methods that can be employed to select for effective isolates. Each of these has their own advantages and disadvantages (Agaras et al. 2015; Liu 2015; Zhang et al. 2015; Zheng et al. 2011). One most important advantage of the selection method used in the current study is that all isolates were screened *in vivo* and *in vitro* before any isolates were eliminated.

In chapter 7, the efficacy of the selected isolates from chapter 3, 4 and 5 was further assessed for biocontrol and plant growth promotion in pot trials in the greenhouse. The selected isolates showed biocontrol activity by increasing the total dry weight of *F. pseudograminearum*

*inoculated* wheat seedlings by 16.1 to 29.8%, with 5.79 to 18.26% of this increase being due to direct plant growth promotion effect of the isolates; whereas the isolates selected for plant growth promotion in absence of the fungal pathogen showed plant growth promotion activity by increasing the total dry weight of pathogen free seedlings by 16.9 to 29.7%. Isolate A17 which was very closely related in its nucleotide sequence to both *Bacillus cereus* and *Bacillus thuringiensis* and isolate KBS1F3 (*Paenibacillus alvei*) resulted in the greatest increase in total dry weight of the seedlings in the crown rot biocontrol experiments and direct growth promotion experiments respectively.

The optimum growth temperature for all bacterial isolates was observed to be between 26ºC and 35ºC while bacterial growth decreased with an increase of NaCl concentration in the growth medium. The optimum pH for growth of most isolates (A09AC, A17, A20, A33, A45, KBS1F3, N02, N04AC, N29, N34, N44, N53, N59A, N69, N67) was 7 whereas isolates A10AC and N54 grew optimally at a pH of 8 and isolate N67 grew equally well at a pH of 6 and 7. The isolates showed the ability to utilise a variety of carbon sources. Based on the performance in pot trials, it is concluded that rhizobacterial isolates A17 and KBS1F3 have the potential to be developed as sustainable and environmentally friendly biocontrol agents and biofertilisers.

Isolate A17 (*Bacillus cereus/thuringiensis*) showed potential as a biocontrol agent of crown rot caused by *F. pseudograminearum* on wheat while isolate KBS1F3 (*Paenibacillus alvei*) showed potential as a wheat plant growth promoter (biofertiliser). The results obtained confirm that the isolates possess multiple traits that are desirable in PGPR. The isolates have great potential for inclusion in integrated biocontrol and plant growth promotion strategies for wheat and their ability to grow under different conditions, some extreme, indicates that they may survive and aid plant growth under stressful conditions. Future development and use of these strains as inoculants in crop production could reduce the dependence on chemical fungicides and fertilizers and lead to the adoption of more sustainable and environmentally friendly farming methods.

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# Appendix A: Inhibition of mycelial growth

**Table A1** Inhibition of *R. solani*, *M. phaseolina*, *P. capsici* and *F. pseudograminearum* mycelial growth by bacterial isolates on PDA, NA and MEA

| Isolates | % inhibition of mycelial growth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R. solani* | | | *M. phaseolina* | | | *P. capsici* | | | *F. pseudograminearum* | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| A05A | 39 | 0 | 0 | 0 | 34 | 0 | 0 | 19 | 0 | 0 | 0 | 0 |
| A05B | 46 | 0 | 0 | 0 | 55 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| A05C | 43 | 0 | 0 | 0 | 36 | 0 | 0 | 19 | 0 | 0 | 0 | 0 |
| A06 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 |
| A07 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A07AC | 25 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| A08 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| A09 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A1 | 55 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A10 | 56 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 |
| A10AC | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| A12 | 44 | 0 | 0 | 0 | 22 | 0 | 0 | 3 | 30 | 0 | 0 | 0 |
| A14W | 48 | 0 | 0 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 |
| A14Y | 65 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 0 | 0 | 0 |
| A16 | 49 | 0 | 0 | 0 | 47 | 0 | 31 | 7 | 0 | 0 | 0 | 0 |
| A17 | 42 | 0 | 0 | 0 | 30 | 0 | 32 | -2 | 0 | 0 | 0 | 0 |
| A19 | 45 | 0 | 0 | 0 | 21 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| A20 | 41 | 0 | 0 | 0 | 30 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| A21 | 41 | 0 | 0 | 0 | 38 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| A22 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 |
| A24 | 43 | 0 | 0 | 0 | 0 | 0 | 0 | -6 | 0 | 0 | 0 | 0 |
| A25 | 41 | 0 | 0 | 0 | 0 | 40 | 0 | 5 | 0 | 0 | 0 | 0 |

**Table A1 continued** Inhibition of *R. solani*, *M. phaseolina*, *P. capsici* and *F. pseudograminearum* mycelial growth by bacterial isolates on PDA, NA and MEA.

| Isolates | % inhibition of mycelial growth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| A26 | 47 | 0 | 0 | 0 | 21 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| A27 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | -1 | 0 | 0 | 0 | 0 |
| A28 | 37 | 0 | 0 | 0 | 31 | 0 | 0 | 1 | 51 | 0 | 0 | 0 |
| A29 | 40 | 0 | 0 | 0 | 28 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A32 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| A33 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A34 | 46 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A35 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A36 | 42 | 0 | 0 | 0 | 18 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| A37 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A38 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| A39 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A40 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| A41 | 40 | 0 | 50 | 0 | 52 | 0 | 0 | 31 | 0 | 0 | 38 | 0 |
| A43 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A44 | 40 | 0 | 40 | 0 | 41 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| A45 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| A46 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A47 | 41 | 0 | 0 | 0 | 41 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| A48 | 50 | 0 | 45 | 0 | 0 | 0 | 38 | 17 | 0 | 0 | 0 | 19 |
| KBS IF3 | 34 | 0 | 0 | 0 | 33 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N01 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| N02 | 51 | 0 | 0 | 0 | 40 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N03 | 46 | 0 | 0 | 0 | 48 | 0 | 0 | -1 | 0 | 0 | 0 | 0 |
| N04 | 41 | 0 | 0 | 0 | 54 | 0 | 0 | 2 | 0 | 5 | 0 | 0 |

**Table A1 continued** Inhibition of *R. solani*, *M. phaseolina*, *P. capsici* and *F. pseudograminearum* mycelial growth by bacterial isolates on PDA, NA and MEA.

| Isolates | % inhibition of mycelial growth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N04AC | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N05 | 42 | 0 | 0 | 0 | 0 | 0 | 43 | 8 | 0 | 0 | 0 | 0 |
| N07 | 39 | 0 | 0 | 0 | 24 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N08 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N10 | 40 | 0 | 0 | 0 | 34 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N11 | 41 | 0 | 0 | 0 | 44 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N12 | 38 | 0 | 50 | 0 | 46 | 43 | 0 | -1 | 0 | 0 | 0 | 0 |
| N13 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N14 | 42 | 0 | 0 | 0 | 52 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| N15ii | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -19 | 0 | 0 | 0 | 0 |
| N16 | 32 | 0 | 0 | 0 | 0 | 0 | 32 | -2 | 0 | 0 | 0 | 0 |
| N16W | 36 | 0 | 40 | 0 | 61 | 0 | 0 | 5 | 0 | 0 | 0 | 0 |
| N17 | 38 | 0 | 0 | 0 | 59 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N19 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N20 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 |
| N21 | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 20 | 0 | 0 | 0 | 0 |
| N26 | 40 | 0 | 0 | 35 | 49 | 0 | 0 | 14 | 0 | 0 | 0 | 0 |
| N27 | 36 | 0 | 37 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N28 | 42 | 0 | 55 | 0 | 47 | 39 | 0 | 7 | 25 | 0 | 0 | 25 |
| N29 | 41 | 0 | 0 | 0 | 71 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| N30 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N31 | 44 | 0 | 0 | 0 | 44 | 0 | 0 | 4 | 0 | 0 | 0 | 9 |
| N32 | 44 | 0 | 41 | 0 | 49 | 0 | 0 | -1 | 0 | 0 | 0 | 3 |
| N33 | 36 | 0 | 0 | 0 | 0 | 0 | 36 | 0 | 0 | 0 | 0 | 0 |
| N34 | 44 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table A1 continued** Inhibition of *R. solani*, *M. phaseolina*, *P. capsici* and *F. pseudograminearum* mycelial growth by bacterial isolates on PDA, NA and MEA.

| Isolates | % inhibition of mycelial growth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N37 | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N38 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N39 | 38 | 0 | 41 | 0 | 54 | 53 | 36 | 2 | 0 | 0 | 0 | 0 |
| N41 | 36 | 0 | 39 | 0 | 31 | 0 | 36 | 1 | 0 | 0 | 0 | 0 |
| N42 | 40 | 0 | 43 | 0 | 46 | 0 | 0 | 48 | 0 | 0 | 0 | 0 |
| N43 | 42 | 0 | 0 | 0 | 0 | 0 | 45 | 10 | 0 | 0 | 0 | 0 |
| N44 | 46 | 0 | 37 | 0 | 0 | 0 | 0 | 3 | 0 | 11 | 0 | 0 |
| N45 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N51 | 42 | 0 | 0 | 0 | 0 | 0 | 0 | -7 | 0 | 0 | 0 | 0 |
| N53 | 47 | 0 | 38 | 54 | 30 | 39 | 48 | 1 | 32 | 0 | 0 | 0 |
| N54 | 68 | 53 | 0 | 63 | 16 | 47 | 30 | 33 | 28 | 7 | 0 | 36 |
| N55A | 36 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N55B | 41 | 0 | 0 | 0 | 0 | 0 | 0 | 17 | 0 | 0 | 0 | 0 |
| N56 | 37 | 0 | 0 | 0 | 0 | 0 | 59 | 9 | 0 | 0 | 0 | 0 |
| N58 | 40 | 0 | 0 | 0 | 0 | 0 | 25 | 0 | 0 | 0 | 0 | 0 |
| N59 | 41 | 0 | 0 | 49 | 44 | 0 | 68 | 2 | 0 | 0 | 0 | 26 |
| N59A | 41 | 0 | 0 | 0 | 0 | 0 | 34 | 1 | 0 | 0 | 0 | 0 |
| N59B | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N59C | 31 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N60 | 42 | 0 | 0 | 0 | 59 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N62 | 59 | 0 | 0 | 0 | 50 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N63 | 38 | 0 | 0 | 50 | 0 | 0 | 31 | 57 | 0 | 0 | 32 | 0 |
| N64 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N65 | 40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N66 | 39 | 0 | 0 | 0 | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table A1 continued** Inhibition of *R. solani*, *M. phaseolina*, *P. capsici* and *F. pseudograminearum* mycelial growth by bacterial isolates on PDA, NA and MEA.

| Isolates | % inhibition of mycelial growth | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N67 | 70 | 0 | 0 | 70 | 54 | 48 | 37 | 0 | 0 | 0 | 0 | 7 |
| N68 | 33 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N69 | 14 | 0 | 59 | 64 | 67 | 76 | 38 | 1 | 0 | 7 | 0 | 18 |
| N70 | 32 | 0 | 0 | 0 | 59 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N71 | 29 | 0 | 0 | 0 | 59 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| N72 | 47 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| N74 | 37 | 0 | 0 | 0 | 0 | 0 | 0 | 12 | 0 | 9 | 0 | 0 |
| N76 | 22 | 0 | 0 | 0 | 56 | 39 | 30 | 8 | 0 | 0 | 0 | 0 |
| N77W | 39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N77Y | 68 | 0 | 0 | 0 | 27 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N78 | 21 | 0 | 0 | 0 | 38 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| NAS696 | 35 | 0 | 0 | 0 | 71 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T11 | 60 | 0 | 40 | 0 | 27 | 0 | 0 | 3 | 0 | 0 | 0 | 32 |
| T19AC | 35 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| T22 | 33 | 0 | 0 | 0 | 38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T29AC | 39 | 59 | 48 | 57 | 12 | 41 | 0 | 40 | 34 | 0 | 0 | 21 |

# Appendix B: Inhibition zones

**Table A2** Diameter of inhibition zones (mm) in dual culture plates between the pathogen and rhizobacterial colonies on PDA, NA and MEA.

| Isolates | Diameter of inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| A05A | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A05B | 5 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A05C | 4 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A06 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A07 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A07AC | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A09 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A1 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A10 | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| A10AC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A12 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 |
| A14W | 5 | 0 | 0 | 0 | 8 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A14Y | 4 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| A16 | 5 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| A17 | 2 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A19 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A20 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A21 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A22 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A24 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| A25 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A26 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A27 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A28 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table A2 continued** Diameter of inhibition zones (mm) in dual culture plates between the pathogen- and rhizobacterial colonies on PDA, NA and MEA.

| Isolates | Diameter of inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *R. solani* | | | *M. phaseolina* | | | *P. capsici* | | | *F. pseudograminearum* | | |
| | PDA | NA | MEA | PDA | NA | MEA | PDA | NA | MEA | PDA | NA | MEA |
| A29 | 0 | 0 | 0 | 0 | 5 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| A32 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A34 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A35 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A36 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A37 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A39 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A40 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A41 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| A42 | 6 | 0 | 2 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 1 | 0 |
| A43 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A44 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 |
| A46 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A47 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| A48 | 2 | 0 | 3 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| KBS IF3 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 1 | 0 | 0 | 0 | 0 |
| N01 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N02 | 4 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N03 | 3 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N04 | 0 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N04AC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N05 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| N07 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |

**Table A2 continued** Diameter of inhibition zones (mm) in dual culture plates between the pathogen- and rhizobacterial colonies on PDA, NA and MEA.

| Isolates | Diameter of inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N08 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N10 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N11 | 0 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N12 | 0 | 0 | 8 | 0 | 8 | 4 | 0 | 0 | 0 | 0 | 0 | 0 |
| N13 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 7 | 0 | 0 | 0 | 0 |
| N14 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N15ii | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N16 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 |
| N16W | 0 | 0 | 1 | 0 | 1 | 0 | 0 | 4 | 0 | 0 | 0 | 0 |
| N17 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N19 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N20 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N21 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| N26 | 0 | 0 | 0 | 1 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N27 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 15 | 0 | 0 | 0 | 0 |
| N28 | 1 | 0 | 5 | 0 | 7 | 2 | 0 | 8 | 1 | 0 | 0 | 0 |
| N29 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N30 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N31 | 1 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N32 | 1 | 0 | 2 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N33 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N34 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N37 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N38 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N39 | 0 | 0 | 4 | 0 | 5 | 2 | 4 | 0 | 0 | 0 | 0 | 0 |

**Table A2 continued** Diameter of inhibition zones (mm) in dual culture plates between the pathogen- and rhizobacterial colonies on PDA, NA and MEA.

| Isolates | Diameter of inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N41 | 0 | 0 | 2 | 0 | 5 | 0 | 4 | 9 | 0 | 0 | 0 | 0 |
| N42 | 0 | 0 | 1 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N43 | 0 | 0 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 |
| N44 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N45 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0 | 0 | 0 | 0 |
| N51 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N53 | 5 | 0 | 1 | 7 | 2 | 1 | 6 | 14 | 4 | 0 | 0 | 0 |
| N54 | 4 | 5 | 0 | 4 | 13 | 4 | 3 | 0 | 3 | 0 | 0 | 0 |
| N55A | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N55B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N56 | 0 | 0 | 0 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 |
| N58 | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| N59 | 1 | 0 | 0 | 6 | 2 | 0 | 11 | 0 | 0 | 0 | 0 | 0 |
| N59A | 1 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 0 | 0 | 0 | 0 |
| N59B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N59C | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N60 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N62 | 8 | 0 | 0 | 0 | 2 | 0 | 0 | 12 | 0 | 0 | 0 | 0 |
| N63 | 5 | 0 | 0 | 7 | 0 | 0 | 3 | 3 | 0 | 0 | 6 | 0 |
| N64 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N65 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N66 | 3 | 0 | 0 | 0 | 6 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N67 | 13 | 0 | 0 | 12 | 7 | 5 | 4 | 0 | 0 | 0 | 0 | 0 |
| N68 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N69 | 7 | 0 | 8 | 10 | 11 | 13 | 8 | 0 | 0 | 0 | 0 | 0 |

**Table A2 continued** Diameter of inhibition zones (mm) in dual culture plates between the pathogen- and rhizobacterial colonies on PDA, NA and MEA.

| Isolates | Diameter of inhibition zone (mm) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***R. solani*** | | | ***M. phaseolina*** | | | ***P. capsici*** | | | ***F. pseudograminearum*** | | |
| | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** | **PDA** | **NA** | **MEA** |
| N70 | 9 | 0 | 0 | 0 | 5 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N71 | 6 | 0 | 0 | 0 | 4 | 0 | 0 | 2 | 0 | 0 | 0 | 0 |
| N72 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| N74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N76 | 0 | 0 | 0 | 0 | 8 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| N77W | 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N77Y | 7 | 0 | 0 | 0 | 9 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| N78 | 0 | 0 | 0 | 0 | 4 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| NAS696 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| T11 | 5 | 0 | 1 | 0 | 1 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| T19AC | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 |
| T22 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 11 | 0 | 0 | 0 | 0 |
| T29AC | 0 | 9 | 5 | 8 | 12 | 2 | 0 | 0 | 5 | 0 | 0 | 0 |