CHAPTER- 5

Growth promotion in sorghum (*Sorghum bicolor* (L.) Moench) by rhizobacterial isolates from the rhizosphere of sorghum and grasses in Ethiopia and South Africa

Abstract

Mineral fertilizers have long been used as the quickest way of improving crop productivity. However, due to their cost and associated environmental problems, continues use of fertilizers has resulted in a search for alternative approaches such as the use of plant growth promoting rhizobacteria. In most developing countries including Ethiopia and South Africa, the application of this approach in agriculture is scanty. This paper presents the isolation and screening of rhizobacteria for growth promotion of sorghum under greenhouse conditions. A total of 78 bacteria isolated from the rhizosphere of sorghum in Ethiopia and 86 isolates from the rhizosphere and rhizoplane of grasses in South Africa were screened for their growth promoting ability in sorghum. Statistically significant growth promotion was achieved with three isolates from Ethiopia and ten isolates from South Africa.. The effective isolates colonized the sorghum roots at a concentration of $\geq 10^{5}$ cfu g^{-1} root and were further tested (*in-vitro*) for the major/direct modes of action for plant growth promotion. Six isolates changed the colour of CAS-agar medium from blue to yellow/orange due to their ability to produce siderophores. The phytohormone indoleacetic acid was detected in the culture filtrate of 11 isolates with concentrations ranging between 4.2 µg – 22.8µg in the presence of trypthophan. Thirteen effective isolates solubilized tricalcium phosphate on Pikovskaya (PVK) agar medium which was evident from the formation of clear zones of varying diameter. Of the effective isolates identified by means of the API and / or sequencing of the bacterial 16S rDNA genes, 44 % were *Bacillus cereus*, 19% *Chrysoemonas luteola*, 13% *Serratia marcescens,* 13% *Sphingomonas paucimobilis* and 6% each of *Stenotrophomonas maltophila* and *Brevibacterium laterosporus*

5. 1. Introduction

Sorghum (*Sorghum bicolour* (L.) Moench) ranks fifth among the World's major cereals following wheat, maize, rice and barley (House, 1995; FAO, 1999). It is one of the dry land summer cereals and is a staple crop in arid and semi-arid areas in Ethiopia. It is also a staple food for more than 500

million people in more than 30 countries, although maize has to some extent replaced its use in Southern Africa. In countries like Ethiopia where traditional agriculture predominates, the average yield is very low, ranging between 200 to 1500 kg/ha compared to developed countries such as the USA where commercial production yielded between 3775-4400 kg/ha in the 1980's (House, 1995). Diseases caused by fungal pathogens (Huluka and Esele, 1992; Davis and Bockus 2001) and unavailability of essential nutrients such as phosphorous and iron (Rodriguez and Fraga, 1999; Igual *et al.,* 2001) are among some of the major causes for the low sorghum yield in these developing countries.

Bacteria are constantly involved in interactions with plant roots. By benefiting from the nutrients secreted by plant roots within the rhizosphere, the bacteria influence the plants in a direct or indirect way and result in stimulation of plant growth (Bloemberg and Lugtenberg, 2001). Bacteria inhabiting the rhizosphere and positively influencing plant growth are referred to as Plant Growth Promoting Rhizobacteria (PGPR) (Kloepper *et al.,* 1986). Significant yield increases have been achieved in crops such as maize, rice, potato, wheat and canola after inoculation with PGPR (Khalid *et al.,* 1997; Zahir *et al.,*. 1998; Bertrand *et al.,* 2001; Thakuria *et al*.2004) which resulted in increased interest in this group of organisms (Asghar *et al.,*. 2004; Thakuria *et al.,*. 2004).

Data on the application of PGPR as growth promoting agents ultimately resulting in yield increases in sorghum is lacking in Ethiopia and South Africa. Although there is a growing interest in replacing chemical fertilizers and pesticides with bacterial inoculants (Mayak *et al.,* 2001), little effort has been made in terms of laboratory and field studies concerning the potential role of PGPR as plant growth promoting agents. This study is therefore aimed at isolating bacteria from the rhizosphere of sorghum and other grasses in Ethiopia and South Africa and evaluating them for growth promotion of sorghum under greenhouse conditions. It is anticipated that the study will provide important information toward application of PGPR as inoculants in agriculture.

5. 2. Materials and Methods

5. 2. 1. Soil sample collection and Isolation of bacteria

Soil sample collection and isolation of bacteria followed the same procedure as described in Chapters 2 and 3.

5. 2. 2. Bacterial inoculum preparation

Bacterial isolates were grown in nutrient broth (Biolab, South Africa) on a rotary shaker (Labotech) at 28 ^{o}C for 24 hrs. The suspension was centrifuged in 50 ml capacity sterile plastic tubes at 3000 x *g* for 10 min using an Avanti TM J-25 centrifuge (Beckman, Ireland). The pellets were re-suspended in quarter strength sterile Ringer's (Merck, South Africa) solution and the suspension was adjusted to give a final concentration of 10^{8}-10^{9}cfu/ml (OD = 0.1- 0.5) at 550 nm using the viable plate count method and optical density measurement.

5. 2. 3. Evaluation of bacterial isolates for growth promotion

Prior to the greenhouse pot experiment, a preliminary screening of 78 bacterial isolates from Ethiopia and 86 isolates from South Africa was conducted for early root and shoot development in sorghum in 96 cell styrofoam seedling trays. The trays were filled with steam-pasteurized sandy loam topsoil. Sorghum seeds (Ethiopian variety, Meko) were surface sterilized with 70% ethanol for 5 min, 1% sodium hypochlorite for 1 min and rinsed five times in sterile water. Three cells in each tray received four sorghum seeds and constituted one replicate and three replications were used in a randomized block design in the tray. The bacterial inoculum (10^{8} – 10^{9} cfu/ml) was applied in the form of soil drenching twice a week apart. The temperature of the greenhouse was maintained at 28 ^{o}C and watering was done twice daily. The plants were harvested three weeks after the first inoculation. Shoot and root length as well as fresh and dry weight measurements were compared with the un-inoculated control (data not shown).

Plate 2. Experiment on 96 Styrofoam seedling trays for the preliminary screening of bacterial isolates for growth promotion of sorghum in the greenhouse.

Based on the results from the screening experiment in the seedling tray, a total of 20 Ethiopian and 39 South African isolates were selected for the greenhouse pot trial. For the pot trial, eight surface sterilized seeds were sown in a 12cm x 10 cm diameter pot containing steam-pasteurized topsoil. The seeds were watered regularly until the emergence of the first shoot. Five days later, 30ml of the bacterial inoculum (10^8-10^9 cfu/ml) was applied to the pots as a soil drench and a second application was made one week later. The pots were watered twice daily with an automatic watering system. The experiment was arranged in a randomized block design with three replications and was repeated twice. Plants were harvested 35 days after planting and 30 days after inoculation. Growth promoting ability of the bacterial isolates was determined based on the data collected on shoot height, shoot dry and fresh weight, root length and root dry weight, leaf width and leaf chlorophyll content as measured with a Minolta SPAD 502 chlorophyll meter (Minolta, Japan) and expressed in spad units.

5. 2. 4. Root colonization

Root colonization by the bacterial isolates was assessed according to the procedure described by Han *et al.* (2000). Briefly, surface sterilized roots of two plants per pot in each replication were macerated in 3 ml 0.1M Phosphate buffer (pH = 7) using a homogenizer. The suspension was serially diluted in quarter strength Ringer's solution (Merck, South Africa). Aliquots of 0.1ml were plated on Nutrient agar (Biolab, South Africa) amended with 50 µg rifampicin per ml. The plates were incubated at 28 ± 2 ^{o}C for 24h. The resulting colonies were counted and root colonization by each bacterial isolate was expressed as CFU/g root.

5. 2. 5. Modes of action for growth promotion

5. 2. 5. 1. Siderophore production

Siderophore production was detected by the universal chemical assay using chrome-azurol S (CAS) agar (Schwyn and Neilands, 1987). Cultures of selected isolates that resulted in significant growth promotion in the greenhouse pot experiment were grown in a modified minimal medium (under iron restricted and high iron condition) at 27 ± 1 ^{o}C for 48 hr and at 180 rpm on a rotary shaker. Each culture was centrifuged at 12,000 x *g* for 10 min and the supernatant was collected. Three wells were made equidistantly on the CAS agar plate using # 1 cork borer after which 30 µl of the culture supernatant was deposited into the wells. Control plates received sterile broth media without bacteria

under both low and high iron conditions. The plates were incubated at room temperature for 8 hrs after which any colour change in the medium was recorded.

5. 2. 5. 2. Indole acetic acid production

The production of the hormone indoleacetic acid (IAA) was detected from the culture supernatants of the bacterial isolates selected based on their growth promoting efficiency following the procedure described by Thakuria *et al.* (2004). Briefly, pure colonies from a 24 hr. culture were inoculated into nutrient broth with 2 % trypthophan and in the absence of trypthophan, and were incubated at 28 ± 2 ^{o}C for 48 hrs. Five ml culture was removed from each tube and centrifuged at 12,000 x *g* for 15 min. Two millilitre aliquot of the supernatant was transferred to a fresh tube and washed with ethyl acetate to extract free IAA like substance. This was then treated with 4 ml salkowsky reagent (1ml 0.5 M FeCl in 50ml $HClO_4$) and incubated at room temperature for 25 min. The absorbance of the solution (pink colour developed) was read at 530 nm. For the control experiment sterile nutrient broth was used. The concentration of IAA in the culture supernatants was determined using a calibration curve of pure IAA as a standard.

5. 2. 5. 3. Phosphate solubilization

Phosphate solubilization activity of the selected rhizobacterial isolates was detected by means of a plate assay using Pikovskaya (PVK) agar (Pikovskaya, 1948) which results in a clear halo formation. To compare the reproducibility of the halo formation, isolates were also tested on PVK agar supplemented with 0.1 % bromophenol blue (Gupta *et al.,* 1994) and a modified PVK medium devoid of yeast extract i.e. NBRIY medium (Nautiyal, 1999). A pure colony from a fresh culture of each isolate was stab inoculated in duplicate into each of the three-agar media using a sterile needle. The diameter of the resulting clear halo was measured after 14 days of incubation of plates at 28 ^{o}C. Control plates were inoculated with sterile nutrient broth.

5. 2. 6. Identification of bacterial isolates

Identification of the bacterial isolates effective in the growth promotion of sorghum in the current study was conducted by means of the API system and sequencing of the bacterial 16S rDNA genes as described in chapter 2 section 2. 2. 7.

5. 2. 7. Statistical analysis

The data were subjected to analysis of variance (ANOVA) using SAS-9.1 software (SAS Institute, 2003). Data on root colonization of the bacterial isolates were log transformed before subjecting to ANOVA. Mean values in each treatment were compared by the least significant difference (LSD) and Duncan's Multiple Range tests at 5 % ($P = 0.05$) level of significance. Pearson's linear correlation coefficient was used to evaluate the relationship between phosphate solubilization and root/ shoot dry weight as well as the relationship between IAA concentration and root/shoot length.

5. 3. Results

5. 3. 1. Greenhouse pot trials

5. 3. 1. 1. Ethiopian isolates

Of all the isolates obtained from the rhizosphere of sorghum, three isolates resulted in a significant increase ($P = 0.05$) in at least two growth parameters. Two isolates (KBE5-1 and KBE7-8) resulted in a significant increase in at least five parameters (Table 5. 1). Isolate KBE5-1 displayed increases in all parameters tested except chlorophyll content and root length. This isolate increased shoot and root length by 53 and 93 % respectively, increased shoot and root biomass by more than 90 %, increased leaf width by 68 %. Isolate KBE7-8, on the other hand resulted in a significant increase in all seven parameters (Table 5. 1; Fig. 5. 1). Likewise, the increase in the shoot and root biomass by this isolate was > 90 % while the increase in shoot and root length was 51 % and 77 % respectively. Another isolate, KBE9-1 resulted in a 95 % and 64 % increase in the length and dry weight of the roots respectively (Table 5.1). This isolate however rendered no significant increase in shoot length, shoot biomass, chlorophyll content as well as shoot width of the leaves over the control by this isolate (Table 5. 1).

5. 3. 1. 2. South African isolates

Isolates KBS9-B and NAS4-3 resulted in significant increases in all seven parameters measured (Table 5. 2; Fig.5. 2). The percentage increases in all parameters were very similar for the two isolates. The increase in both shoot fresh weight and root dry weight was 92 % by isolate KBS9-B and 93 % by isolate NAS4-3 (Table 5. 2) whereas both isolates resulted in ca. 64 and 73 % increase

in shoot and root length respectively. Another isolate (KBS5-H) also rendered significant increases in shoot and root length (Table 5. 2; Fig.5. 2). This isolate increased shoot and root length by 66 and 76 % respectively but it had less effect on shoot and root dry weights than isolates KBS9-B and NAS4-3. Other isolates which stimulated growth in sorghum were isolates KBS6-H, KBS5-F, KBS2-12 and KFP9-E all of which resulted in significant increases in five parameters. The rest of the isolates resulted in significant increases in at least three parameters except for isolates KBS1-T and KBS10E which resulted in increases in only two parameters. Most of the isolates that resulted in significant growth promotion mainly affected plant biomass (fresh and dry weights of shoots and roots), root length and chlorophyll content. Compared to the control, these isolates had no effect on shoot length and leaf width by.

5. 3. 2. Root colonization

Both Ethiopian and South African isolates, which resulted in significant growth promotion in sorghum, were able to colonize the roots successfully. Six isolates from the rhizosphere of grasses in South Africa and five isolates from sorghum rhizosphere in Ethiopia colonized sorghum roots with a concentration of $\geq 10^5$ cfu /g root. The count of bacterial colonies similar to the inoculated ones for each of the isolates KBS9-B, KFP9-K, KBS9-H, KBS6-H, KBS5-H and NAS4-3 was 8 log units compared to the un-inoculated control which rendered a count of only 4 log units (Table 5. 3). Similarly Ethiopian isolates KBE7-8, KBE5-1, KBE9-1, KBE5-7 and KBE5-8 were detected at levels $\geq 10^8$ cfu /g root. Most of the other isolates were however detected at a lower level than the initial inoculum level of $10^8 – 10^9$ cfu /ml and the decrease from the initial inoculum level ranged between 1 – 4 log units.

5. 3. 3. Modes of action for growth promotion

5. 3. 3. 1. Siderophore production

Six bacterial isolates, two from sorghum rhizosphere in Ethiopia and four from the rhizosphere of grasses in South Africa, were able to produce siderophores on CAS agar plates (Table 5. 4). This was confirmed by a change in the colour of the CAS agar plates from orange/yellow to blue as a result of the siderophores sequestering and binding iron from the medium. Only one of the two isolates from the rhizosphere of sorghum in Ethiopia that produced siderophores in culture enhanced growth in sorghum (KBE9-1) reflected in a significant increase in root length and root dry weight.

On the other hand, of all the isolates from the rhizosphere of grasses obtained within South Africa and only isolates KBS6-H, KBS5-F and KBS6-17 tested positive for the production of siderophores on CAS agar plates (Table 5. 4). The production of siderophores in culture by isolates NAE5-7 from Ethiopia and isolates KBS9-R and KBS6-17 from South Africa did not result in any significant growth promoting effect in sorghum (Tables 5. 1 and 5. 2).

5. 3. 3. 2. Indole acetic acid (IAA) production

Of the 15 isolates tested for the production of the hormone IAA, 11 (73 %) were capable of producing the hormone in liquid culture with concentrations ranging between 4.2 - 22.8 µg/ml in the presence of 0.2 % trypthophan (Table 5. 4). This concentration however decreased significantly in the absence of trypthophan ranging between 1.82 - 5.43 µg/ml indoleacetic acid. The highest amount of IAA was produced by isolate KBS5-H (22.8 µg/ml) followed by isolates KBS9-H (22.6 µg/ml), KBS6-H (21.4 µg/ml), KBE9-1 (20.8 µg/ml) and NAS4-3 (20.5 µg/m) in the presence of 0.2 % trypthophan. In the absence of trypthophan the amount produced by these isolates decreased to 2.24, 5.21, 5.43, 2.17, 3.4 µg/ml respectively. Isolates KBS5-F, KFP9-K and KBS1-T all of which affected some aspect of sorghum growth under greenhouse condition (Table 5. 2) were unable to produce IAA in culture (Table 5. 4).

5. 3. 3. 3. Phosphate solubilization

Thirteen isolates (86 %) were able to solubilize tri-calcium phosphate on Pikovskaya (PVK) agar medium. Nine of these isolates were also capable of solubilizing phosphate on a modified PVK medium devoid of yeast extract (NBRIY medium). Isolates KBS5-F, KBE9-1 and KBS6-H resulted in the greatest level of phosphate solubilization, rendering a 10 mm diameter clear zone followed by isolate KBS5-H that rendered a clear zone of 7mm in diameter (Table 5. 4). Eight other isolates showed some ability to solubilize phosphate on PVK medium rendering a clear zone ranging between 0.5 - 5 mm in diameter. The same isolates, which effectively solubilized phosphate on PVK medium, displayed improved phosphate solubilization ability on NBRIY medium with clear zone diameters of 12 mm (KBS6-H, KBS9-1) and 14.5 mm (KBS5-F). Similarly isolates KBS9-R and KBE7-8, both of which tested positive for phosphate solubilization on PVK medium (clear zone of 5mm in diameter each) solubilized phosphate better on NBRIY medium (clear zone of 8.5 and 10 mm in diameter respectively) (Table 5. 4).

5. 3. 4. Identification of bacterial isolates

The three isolates obtained from the sorghum rhizosphere in Ethiopia and which resulted in significant growth promotion in sorghum in the present study have been identified as *Bacillus cereus*. Identification of effective isolates obtained from the rhizosphere of grasses and rhizoplane of roots (South African isolates) resulted in 30 % *B. cereus*, 23 % *Chryseomonas luteola*, 15% each of *Serratia marcescens* and *Sphingomonas paucimobilis* and 8 % each of *Stenotrophomonas maltophilia* and *Brevibacterium laterosporus* (Table 5.5).

5. 4. Discussion

The present study demonstrated that rhizobacteria isolated from the rhizosphere and rhizoplane of grasses in South Africa and from the rhizosphere and of sorghum in Ethiopia have the ability to promote growth of sorghum under greenhouse conditions.

According to our knowledge, this is the first report of PGPR associated with enhancement of growth in sorghum. Other similar studies have been done on the role of plant growth promoting bacteria in increasing the growth and yield of wheat (Khalid *et al.,* 2004), rice (Thakuria *et al.,* 2004), maize (Berg *et al.,* 1991) and several other crops. However, there are no reports on the occurrence of PGPR in the sorghum rhizosphere, and their beneficial effect on the growth of this crop.

Three isolates from the rhizosphere of sorghum in Ethiopia and 16 from the rhizosphere of grasses within the Nylsvlei Nature Reserve in South Africa resulted in significant growth promotion of sorghum under greenhouse conditions. The most frequently isolated species with growth promoting abilities in the current study were *B. cereus*, *C. luteola* followed by *S. marcescens, Sphmon. paucimobilis, S. maltophilia, and B. laterosporus.*

Three isolates from sorghum rhizosphere in Ethiopia viz. isolates KBE7-8, KBE5-1 and KBE9-1 and four isolates from the rhizosphere and rhizoplane of grasses in South Africa viz. NAS4-3, KBS5-H, KBS9-H and KFP9-K, all of which resulted in significant growth promotion, were identified as *B. cereus*. Several reports on the growth promoting activity of *B. cereus* support the results obtained in the current study. Chen *et al.* (1994), reported that inoculation with *B. cereus* increased grain yield in rapeseed. In a similar study, Xia *et al.* (1990), reported that *B. cereus* strain 83 -10 promoted growth and increased grain yield of rapeseed in a repeated field trial. In the current study the *B. cereus*

isolates significantly promoted early growth of sorghum by mechanisms involving the production of the auxin IAA, siderophores and by their ability to solubilize phosphate.

All seven strains of *B. cereus* were highly efficient in colonizing sorghum roots and significantly increased root length. The stimulation of root growth may be attributed to the production of IAA in culture at a concentration ranging between 4.2 - 22.8 µg/ml in the presence of 0.2% trypthophan. Patten and Glick (2002), reported the production of IAA by wild type *Pseudomonas putida* from as little as 0.5 µg/ml in the absence of trypthophan to as much as 32.7 µg/ml in the presence of 500 µg/ml trypthophan which resulted in the development of the host plant root system. Bacterial secreted indoleacetic acid may promote root growth directly by stimulating plant cell elongation or cell division or indirectly by influencing bacterial 1-aminocyclopropane-1-carboxylic acid deaminase (ACC-deaminase) activity (Patten and Glick, 2002). ACC- deaminase produced by plant growth promoting bacteria is involved in the stimulation of root elongation in seedlings (Li *et al.*, 2000). This enzyme hydrolyses plant ACC, the immediate precursor of the plant growth inhibiting phytohormone ethylene (Penrose *et al.,* 2001). In our study however, no attempt was made to determine the ACC deaminase activity of the rhizobacterial isolates. Suzuki *et al.* (2003), observed that IAA producing strains of *Psedomonas fluorescens* stimulated root development in black currant.

Many root-associated bacteria have been reported to produce IAA in culture media (Patten and Glick, 1996; Patten and Glick, 2002). In the present study we have observed a positive linear relationship with significant r value (r = 0.57, P = 0.05) between the *in-vitro* IAA production and increase in root length. Asghar *et al.* (2002) and Khalid *et al.* (2004) have previously reported a positive correlation between *in-vitro* auxin production and growth promoting activities of PGPR. The ability to produce IAA by the most effective isolates in our study might have contributed to the successful root colonization by these isolates (Suzuki *et al.,* 2003) and resulted in significant increase in root growth.

Glick (1995) and Rodriguez and Fraga (1999) indicated that many PGPR promote plant growth by increasing the availability of nutrients in the rhizosphere by means of solubilization of unavailable forms of nutrients and /or siderophore production. The solubilization of phosphate in the rhizosphere is the most common mode of action implicated in PGPR by increasing nutrient availability to host plants (Vessey, 2003). Of the *B. cereus* strains isolated in this study, *B. cereus* strains KBE7-8 and KBE9-1 solubilized phosphate on Pikovskaya (PVK) medium with a clear zone formation of 5 and

10 mm in diameter respectively while clear zone formation by *B. cereus* strains KBS9-H and KBS5-H was 5 and 7mm respectively.

Although we did not conduct a comparative study of the influence of soil type and plant species on the microbial flora in the rhizosphere, the occurrence of only a specific group of bacteria *(Bacillus* spp.) from the rhizosphere of the sorghum fields from two separate sites in Ethiopia is noticeable and could be related to the nature of the soil and plant type. In this regard, Lemanceau *et al.* (1995), reported that non-leguminous crops select specific groups of bacteria in the rhizosphere. The colonization of the maize rhizosphere, for example, by specific groups of bacteria was consistently found at two distinct geographical locations (Tilak *et al.*, 2005). Earlier, Gryston *et al.* (1998), indicated that the abundance and activities of soil microorganisms are influenced by, among other factors, the types of plant species.

Unlike the rhizosphere of sorghum where the majority of isolates were found to be *Bacillus* spp., the rhizosphere of grasses from the Nylsvlei Nature Reserve was found to be colonized by Gram negative isolates such as *S. marcescens, C. luteola, S. maltophila* and *Sphingom. paucimobilis* which exhibited one or more of the properties for growth promotion. *S. maltophilia* and *Serratia* spp. have been reported as members of the naturally occurring rhizosphere community (Lottman *et al.,* 1999).

The Gram-negative isolates that resulted in significant growth promotion of sorghum in this study showed the ability to solubilize phosphate on PVK medium by producing clear zones ranging from 3 - 10 mm in diameter. Clear zone formation was improved by *C. luteola* KBS5-F and *S. marcescens* KBS6-H when these isolates were inoculated on a modified PVK medium devoid of yeast extract (NBRIY) medium. The diameter of the clear zone was increased by 4mm and 2mm for the two isolates respectively. This was also true for the Gram-positives *B. cereus* KBE7-8 and KBE9-1. These results are in agreement with the findings of Nautiyal (1999), who demonstrated that by omitting yeast extract from PVK medium, higher phosphate solubilization was achieved by *Pseudomonas* sp. For some isolates such as *S. maltophila* KBS9-B, phosphate solubilization was similar with and without yeast extract. For other isolates like *B. cereus* KBS5-H and *Sphmon. paucimobilis* KBS1-T, the extent of solubilization (observed from the clear zone formation) decreased or was not observed when yeast extract was removed from the medium. The main effect of phosphate solubilization on plant growth is an increase in biomass and P content (Bashan and de-Bashan, 2005) and in this study a positive linear correlation (significant r-value) was obtained

between phosphate solubilization and root dry weight (r = 0.92, P = 0.005) with the most effective bacterial isolates.

Serratia marcescens strain KBS6-H resulted in growth promotion of sorghum by increasing shoot fresh and dry weights, chlorophyll content as well as root length. The growth promoting effect of some *Serratia* strains in soybean was previously studied under controlled condition where growth promotion was achieved through the production of plant growth regulating compounds (Zhang *et al.*, 1996). Ryu (2005), evaluated the efficacy of *S. marcescens* 90-166 for of *in-vitro* and *in-vivo* growth promotion in Arabidopsis. The results indicated that the bacteria increased foliar fresh weight of Arabidopsis by means of signal transduction of IAA, salicylic acid, and gibberellins. In another study in soybean Dashti *et al.* (1997), observed an increase in grain and protein yield as a result of co-inoculation with *Bradirhizobium japonicum* and *Serratia* strains. In the current study, *S. marcescens* (KBS6-H) colonized the roots at a higher level (8. 16 log cfu/g) than the control and other non effective isolates and tested positive for the production of siderophores, indoleacetic acid and phosphate solubilization. Interestingly, the other *Serratia marcescens* strain (KBS9-R) did not stimulate plant growth (i.e. root and shoot weight, leaf diameter and chlorophyll content) although it tested positive for the production of siderophores, indoleacetic acid and phosphate solubilization. This probably indicates that the ability to manifest these modes of actions by a given bacterium may not necessarily mean that the bacterium is a PGPR (Vessey, 2003). Cattelan *et al.* (1999), for instance found that out of five rhizosphere isolates which tested positive for P solubilization, only two had a positive effect on soybean seedling growth. In chapters two and three of this study however, *S. marcescens* (KBS9-R) has been proven to be very effective in the suppression of *F. oxysporum* and *P. ultimum* associated with soilborne diseases of sorghum.

Previous reports on *S. maltophila* focussed mainly on their antagonistic activity due to traits associated with biocontrol mechanisms including production of antibiotics, production of the enzyme chitinase and rhizosphere colonization (Kobayashi *et al.,* 1995; Kobayashi *et al.,* 2002; Zhang *et al.,* 2000). Sturz *et al.* (2001), recovered plant growth promoting strains of *S. maltophila* from the root zones of quack grass (*Agropyron repens* (L.) Beauv. that significantly increased the biomass of shoots and roots in an *in-vitro* bacterization study. In the current study, *S. maltophilia* strain KBS9-B resulted in a significant increase in all the seven parameters used to evaluate growth promotion in sorghum. The growth promoting ability of this strain was probably due to its high level of root colonization (8.3 Log cfu/g), phosphate solubilization and production of indoleacetic acid. Our result partly concurs with that of Donnate-Correa *et al.* (2004), who previously isolated IAA

producing strains of *S. maltophilia, S. paucimobilis* and *C. luteola* from the rhizosphere of the perennial legume tagasate. However, *C. luteola* KBS5-F and *S. paucimobilis* KBS1-T did not show any signs of indoleacetic acid production in this study and their growth promoting ability is probably due to their ability to solubilize phosphate and produce siderophores.

Enhanced iron nutrition resulting in increased plant growth can also be achieved due to the ability of some plants to bind and release iron from bacterial iron-siderophore complexes and utilizing the iron for growth (Bashan and de-Bashan, 2005). Of all the isolates that resulted in significant growth promotion in the current study, only *S. marcescens* KBS6-H, *C. luteola* KBS5-F and *B. cereus* KBE9-1 produced siderophores.

The present study offers a significant impetus to the application of PGPR for use in agriculture in Ethiopia and South Africa. However, in order to develop the best performing PGPR strains for commercial applications, further selection and screening in field trials will be required and additional studies on modes of action need to be conducted.

5. 5. References

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Table 5. 1. Effect of rhizobacterial isolates from sorghum rhizosphere in Ethiopia on shoot and root growth enhancement of sorghum under greenhouse conditions

Bacterial Isolates	Shoot height(cm)*	Shoot fresh weight (g)*	Shoot dry Weight (g)*	Chlorophyll* (spad units)	Leaf* Width(cm)	Root* Length(cm)	Root dry mass (g)*
KBE4-3	19.50^{cd}	1.50^{d}	0.16^{b}	21.40^{abc}	0.39^{bc}	12.80^{de}	0.07^{abc}
KBE5-3	22.93^{bcd}	2.17^{cd}	0.27^{b}	21.56^{abc}	0.44^{bc}	16.00^{de}	0.08^{abc}
KBE5-7	23.53^{bcd}	2.25^{cd}	0.33^{b}	21.76^{abc}	0.42^{bc}	14.33^{de}	0.01^{abc}
NAE4-1	21.56^{cd}	1.73^{d}	0.23^{b}	21.80^{abc}	0.44^{bc}	19.33^{bcd}	0.06^{abc}
NAE7-1	21.00^{cd}	2.08^{d}	0.30^{b}	22.46^{abc}	0.42^{bc}	17.33^{cde}	0.06^{abc}
KBE7-6	21.06^{cd}	1.97^{d}	0.22^{b}	22.73^{abc}	0.42^{bc}	16.93^{cde}	0.05^{abc}
KBE5-2	19.46^{cd}	1.17^{d}	0.17^{b}	22.93^{abc}	0.48^{bc}	11.93^{de}	0.04^{bc}
KBE9-4	28.80^{abcd}	4.52^{cd}	0.66^{b}	23.83^{abc}	0.82^{abc}	13.80^{de}	0.10^{abc}
KBE9-1	35.26^{ab}	4.09^{cd}	0.50^{b}	29.43^{a}	0.70^{abc}	26.26^{ab}	0.15^{a}
NAE2-8	23.20^{bcd}	1.71^{d}	0.21^{b}	24.36^{abc}	0.65^{bc}	14.86^{de}	0.03^{bc}
KBE8-2	21.66^{cd}	1.33^{d}	0.23^{b}	24.40^{abc}	0.52^{bc}	17.40^{cde}	0.06^{abc}
KBE6-3	30.60^{abc}	1.55^{d}	0.13^{b}	25.30^{abc}	0.59^{bc}	13.40^{cde}	0.05^{bc}
NAE9-5	23.20^{bcd}	1.65^{d}	0.26^{b}	25.33^{abc}	0.54^{bc}	18.73^{bcd}	0.04^{bc}
KBE5-1	38.40^{a}	12.40^{ab}	1.33^{a}	26.16^{abc}	0.93^{ab}	24.40^{abc}	0.11^{ab}
KBE5-8	25.00^{abcd}	7.70^{bc}	0.59^{b}	28.36^{ab}	0.92^{ab}	12.80^{de}	0.08^{abc}
KBE7-8	36.86^{ab}	14.84^{a}	1.41^{a}	29.83^{a}	1.28^{a}	29.56^{a}	0.13^{ab}
NAE5-7	17.73^{cd}	1.78^{d}	0.22^{b}	19.40^{bc}	0.39^{bc}	14.53^{de}	0.07^{abc}
KBE1-7	20.26^{cd}	1.39^{d}	0.19^{b}	18.53^{c}	0.40^{bc}	14.73^{de}	0.06^{abc}
KBE8-3	15.43^{d}	1.43^{d}	0.17^{b}	18.23^{c}	0.40^{bc}	17.93^{cd}	0.04^{bc}
KBE6-1	22.33^{cd}	2.08^{d}	0.27^{b}	20.36^{abc}	0.42^{bc}	19.33^{bcd}	0.09^{abc}
Control	18.13^{cd}	0.63^{d}	0.10^{b}	19.93^{bc}	0.30^{c}	9.43^{e}	0.008^{c}
Pr > F	< 0.0001	< 0.0001	< 0.001	0.0021	< 0.0001	< 0.0001	0.0008
$LSD_{0.05}$	14.14	5.54	0.62	9.58	0.59	7.98	0.1024

* Means within each column followed by the same letter/s are not significantly different according to the least significant difference (LSD) test at *P = 0.05* using the GLM procedure

Table 5. 2. Effect of rhizobacterial isolates from the rhizosphere and rhizoplane of grasses at Nylsvlei Nature Reserve in South Africa on shoot and root growth enhancement of sorghum under greenhouse conditions

Bacterial Isolates	Shoot height(m)	Shoot fresh weight (g)	Shoot dry Weight (g)	Chlorophyll (spad units)	Leaf Width(cm)	Root Length(c)	Root dry Weight(g)
KBS9-B	41.53^{a*}	7.36^{ab}	1.79^{a}	29.96^{ab}	1.27^{a}	21.56^{b}	0.25^{ab}
NAS4-3	40.58^{a}	9.37^{a}	1.36b	28.26^{ab}	1.14^{ab}	20.93^{b}	0.28^{ab}
KBS1-F	30.50^{b}	3.93^{def}	0.68^{cd}	20.23^{fgh}	0.66^{def}	5.76^{g}	0.16^{a-d}
KBS5-F	30.47^{b}	6.80^{a-d}	0.99^{c}	24.76^{cd}	0.65^{def}	19.80^{bc}	0.20^{abcd}
KBS2-12	30.27^{b}	3.90^{ef}	0.74^{cd}	26.80^{bc}	0.72^{d}	21.23^{b}	0.25^{ab}
KBS9-H	29.67^{b}	4.27^{c-f}	0.49^{de}	29.16^{ab}	0.73^{d}	21.50^{b}	0.10^{cde}
KFP9-K	28.90^{b}	2.93^{efg}	0.56^{de}	28.36^{ab}	0.75^{d}	21.00^{b}	0.10^{cde}
KBS5-H	43.50^{a}	11.85^{a}	0.58^{de}	30.33^{a}	0.78^{cd}	24.13^{a}	0.08de
KFP9-E	28.53^{b}	5.50^{b-e}	0.99^{c}	24.30^{cd}	0.45^{f}	11.50^{e}	0.21^{abc}
KBS6-1	28.27^{b}	2.97^{efg}	0.77^{de}	23.40^{c-f}	0.61^{def}	15.46^{d}	0.10^{cde}
KBS6-H	28.17^{b}	6.97^{abc}	1.72^{a}	30.46^{a}	1.00^{bc}	20.63^{bc}	0.13^{b-e}
NAS2-B	26.93^{b}	4.07^{def}	0.48^{de}	24.50^{cd}	0.60^{def}	8.26^{f}	0.08^{de}
NAS1-6	25.77^{b}	3.90^{ef}	0.72^{cd}	20.16^{fgh}	0.70^{de}	8.26^{f}	0.09^{cde}
NAS6-N	25.60^{b}	4.23^{c-f}	0.56^{de}	19.70^{gh}	0.63^{def}	19.76^{bc}	0.10^{cde}
KBS1-J	25.20^{b}	3.70^{ef}	0.51^{de}	23.36^{def}	0.63^{def}	7.53^{fg}	0.09^{cde}
KBS10-E	24.83^{b}	1.53^{g}	0.35^{ef}	23.30^{def}	0.47^{ef}	8.66^{f}	0.10^{cde}
KBS6-11	23.80^{b}	2.83^{efg}	0.60^{de}	20.90^{efg}	0.63^{def}	18.30^{c}	0.13^{b-e}
KBS1-T	23.70^{b}	3.23^{efg}	0.45^{de}	18.63^{gh}	0.63^{def}	19.96^{bc}	0.09^{cde}
KBS9-R	23.50^{b}	3.10^{efg}	0.38^{f}	19.80^{gh}	0.70^{de}	7.65^{fg}	0.08^{de}
Control	14.70^{b}	0.62^{g}	0.46^{f}	16.83^{h}	0.56^{def}	5.73^{g}	0.02^{e}
Pr > F	0.0001	< 0.0001	<0.0001	< 0.0001	<0.0001	< 0.0001	0.0111
$LSD_{0.05}$	8.07	2.89	0.32	3.42	0.24	2.41	0.128

* Means within each column followed by the same letter/s are not significantly different according to the least significant difference (LSD) test at *P = 0.05* using the GLM procedure.

Table 5.3 Origin of rhizobacterial isolates from Ethiopia and South Africa and their ability to colonize sorghum roots under greenhouse conditions

South African isolates				*Ethiopian isolates(sorghum rhizosphere)*		
Isolate	*Log cfu g $^{-1}$ ± SE*†	*Origin*	*Host grass*	*Isolate*	*Log cfu g $^{-1}$ ± SE*†	*Origin*
KBS9-B	8.30 ± 0.17^{a}	rhizoplane	*Cyprus esculentus* L.	KBE7-8	8.19 ± 0.11^{a}	rhizosphere
KFP9-K	8.19 ± 0.57^{ab}	rhizoplane	*Cyprus esculentus* L.	KBE5-1	8.09 ± 0.11^{a}	,, ,, ,, ,,
KBS9-H	8.17 ± 0.16^{ab}	rhizoplane	*Cyprus esculentus* L.	KBE9-1	8.08 ± 0.15^{a}	,, ,, ,, ,,
KBS6-H	8.16 ± 0.17^{ab}	rhizoplane	*Arstidia canescens* subsp. canescens	KBE5-7	8.07 ± 0.21^{a}	,, ,, ,, ,,
KBS5-H	8.08 ± 0.20^{ab}	rhizoplane	*Eragrostis biflora*	KBE5-8	8.06 ± 0.12^{a}	,, ,, ,, ,,
NAS4-3	8.03 ± 0.12^{b}	rhizosphere	*Themeda triandra*	KBE7-6	7.03 ± 0.13^{b}	,, ,, ,, ,,
KBS6-11	7.22 ± 0.22^{c}	rhizosphere	*Arstidia canescens* subsp. canescens	KBE4-3	6.56 ± 0.64^{bc}	,, ,, ,, ,,
KBS2-12	7.06 ± 0.09^{cd}	rhizosphere	*Stipagrotis zeyheri* subsp. sericans	NAE7-1	6.06 ± 0.14^{cd}	,, ,, ,, ,,
KBS6-1	6.98 ± 0.08^{d}	rhizosphere	*Arstidia canescens* subsp. canescens	KBE6-1	5.99 ± 0.06^{d}	,, ,, ,, ,,
KBS1-T	6.61 ± 0.42^{e}	rhizoplane	*Sporobolus fimbriatus*	KBE6-3	5.97 ± 0.10^{d}	,, ,, ,, ,,
NAS6-N	5.94 ± 0.13^{f}	rhizoplane	*Arstidia canescens* subsp. canescens	NAE4-1	5.93 ± 0.22^{d}	,, ,, ,, ,,
KFP9-E	5.83 ± 0.20^{f}	rhizoplane	*Cyprus esculentus* L.	KBE5-2	5.90 ± 0.03^{d}	,, ,, ,, ,,
NAS2-B	5.74 ± 0.21^{f}	rhizoplane	*Stipagrotis zeyheri* subsp. sericans	KBE8-2	5.84 ± 0.15^{d}	,, ,, ,, ,,
KBS2-3	4.97 ± 0.73^{g}	rhizosphere	*Stipagrotis zeyheri* subsp. sericans	NAE2-8	4.88 ± 0.08^{e}	,, ,, ,, ,,
NAS1-6	4.92 ± 0.07^{g}	rhizosphere	*Sporobolus fimbriatus*	KBE9-4	4.88 ± 0.03^{e}	,, ,, ,, ,,
KBS1-F	4.88 ± 0.32^{g}	rhizoplane	*Sporobolus fimbriatus*	Control	4.32 ± 0.26^{f}	
KBS1-J	4.84 ± 0.02^{g}	rhizoplane	*Sporobolus fimbriatus*	*Pr > F*	*< 0.0001*	
KBS10-E	4.77 ± 0.09^{g}	rhizoplane	*Cyprus esculentus* L.	*LSD$_{(0.05)}$*	*0.53*	
Control	4.44 ± 0.15 ^{h}					
Pr > F	*< 0.0001*					
LSD $_{(0.05)}$	*0.24*					

† Values represent means of three replications plus or minus standard errors of the means and means with the same letter are not significantly different (*P = 0.05*) according to the least significant difference (LSD)t test using the GLM procedure.

Table 5. 4. Modes of action of rhizobacterial isolates in the growth promotion of sorghum under greenhouse conditions

Bacterial isolate	Siderophore production†		Phosphate solubilization		Indoleacetic acid (µg/ml)*	
	High iron	Low iron	Pikovskaya agar	NBRIY medium	0.2 % trypthophan	no trypthophan
KBS6-11	-	-	-	-	10.4^{cd}	2.62^{abcd}
KBS6-H	+	+ +	++++	+++++	21.4^{a}	5.43^{a}
KFP9-K	-	-	+	+	0.0^{f}	0.00^{d}
KBS5-H	-	-	+++	+	22.8^{a}	2.24^{abcd}
KBE5-1	-	-	-	-	10.6^{cd}	2.71^{abcd}
NAS4-3	-	-	+	-	20.5^{a}	3.42^{abc}
KBS9-H	-	-	++	-	22.6^{a}	5.21^{ab}
KBS9-R	++	+ + +	++	+++	4.2^{e}	2.13^{bcd}
KBE7-8	-	-	++	++++	12.4^{bc}	2.10^{bcd}
KBS9-B	-	-	+	+	15.5^{b}	2.30^{abcd}
KBS1-T	-	-	++	-	0.0^{f}	0.00^{d}
KBE9-1	++	+ + +	++++	+++++	20.8^{a}	2.17^{abcd}
KBS5-F	+	+ +	++++	+++++	0.0^{f}	0.00^{d}
NAE5-7	+	+ +	-	-	7.5^{de}	1.82^{cd}
Control	-	-	-	-	0.0^{f}	0.00^{d}

† Siderophore production was compared among the bacterial isolates by measuring the diameter of yellow/orange halo on CAS agar plates: + = halo diameter ≤ 3mm, ++ = 4 - 5mm, +++ = > 5 mm.

ƒ Diameter of clear zone formed (mm) around the colony as a result of solubilization of tri-calcium phosphate on Pikovskaya and NBRIYmedium was measured: + = ≤ 3mm clear zone, ++ = 3 – 5mm clear zone, +++ = 5 – 8 mm clear zone, ++++ = 8 – 10mm clear zone, +++++ = > 10mm clear zone formation.

* Values are means of three separate experiments. Means followed by the same letter/s are not significantly different according to Duncan's Multiple Range test ($P = 0.05$).

Table 5. 5. Identification of the most effective South African and Ethiopian rhizobacterial isolates based on the API system and 16 S rDNA sequencing

Bacterial Isolates	Gram reaction	Endo-spore#	Catalase test	Cytochrome oxidase	Motility test	O/F reaction*	Bacterial species	Type of identification†
KBE7-8	+	+	+	+	Motile	Nd	*Bacillus cereus*	*16S rDNA sequencing*
KBE5-1	+	+	+	+	Motile	Nd	*B. cereus*	*16S rDNA sequencing*
KBS9-H	+	+	+	-	Motile	Nd	*B. cereus*	*16S rDNA sequencing*
NAS4-3	+	+	+	+	Motile	Nd	*B. cereus*	*API-50CHB*
KFP9-K	+	+	+	+	Motile	Nd	*B. cereus*	*16S rDNA sequencing*
KBE9-1	+	+	+	+	Motile	Nd	*B. cereus*	*16S rDNA sequencing*
KBS5-H	+	-	+	-	Motile	Nd	*B. cereus*	*16S rDNA sequencing*
KBS6-H	-	-	+	+	Motile	Oxidative	*Serratia marcescens*	*16S rDNA sequencing*
KBS9-R	-	-	+	-	Motile	Oxidative	*S. marcescens*	*16S rDNA sequencing*
KBS5-F	-	-	-	+	Motile	,, ,, ,, ,,	*Chryseomonas luteola*	*API 20 NE*
NAS1-6	-	-	+	+	Motile	,, ,, ,, ,,	*C. luteola*	*API 20 NE*
KBS6-11	-	-	+	+	N. motile	Oxidative	*C.luteola*	*API 20 NE*
NAS2-B	-	-	+	+	N. motile	,, ,, ,,	*Sphingomonas puacimobilis*	*API 20 NE*
KBS1-T	-	-	+	+	N. motile	,, ,, ,,	*S. puacimobilis*	*API 20 NE*
KBS2-12	+	-	+	-	N. motile	Nd	*Brevibacterium.laterospors*	*API 20 NE*
KBS9-B	-	-	+	-	Motile	Oxidative	*Stenotrophomonas maltophila*	*API 20 NE*

* Nd = oxidation fermentation test not conducted, # += endospore present, - endospore absent.

† Sequencing of the bacterial 16S r DNA was performed for those isolates identified by the API system with less than 80 % identity with the isolates on the data base.

Figure 5. 1. Growth promotion in five-weeks- old sorghum plants in the greenhouse by *Bacillus cereus* KBE5-1, *B. cereus* KBE7-8 and *B. cereus* KBE9-1 isolated from sorghum rhizosphere in Ethiopia. Note that the un-inoculated control resulted in retarded growth as compared to the treatments inoculated with bacterial isolates.

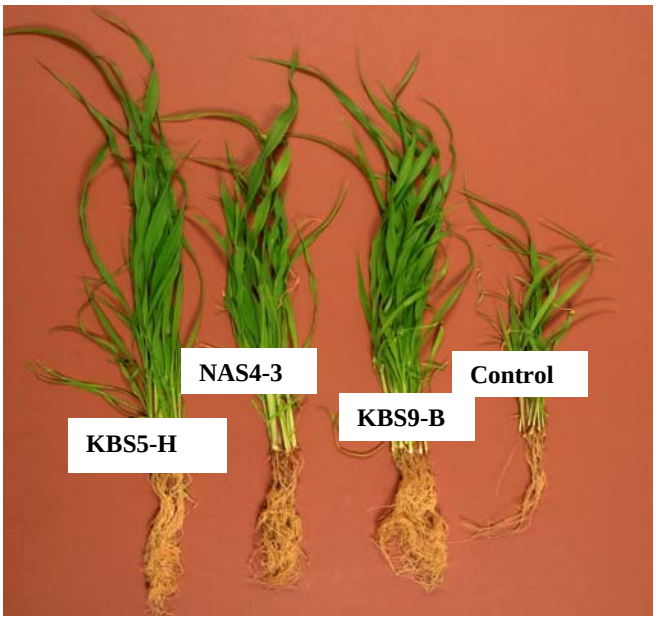

Figure 5. 2. Shoot and root growth enhancement in 4-weeks-old sorghum plants by bacterial isolates *Bacillus cereus* KBS5-H, *B. cereus* NAS4-3 and *Stenotrophomonas maltophilia* KBS9-H all isolated from the rhizosphere of grasses within the Nylsvlei Nature Reserve in South Africa in comparison with the un-inoculated control.

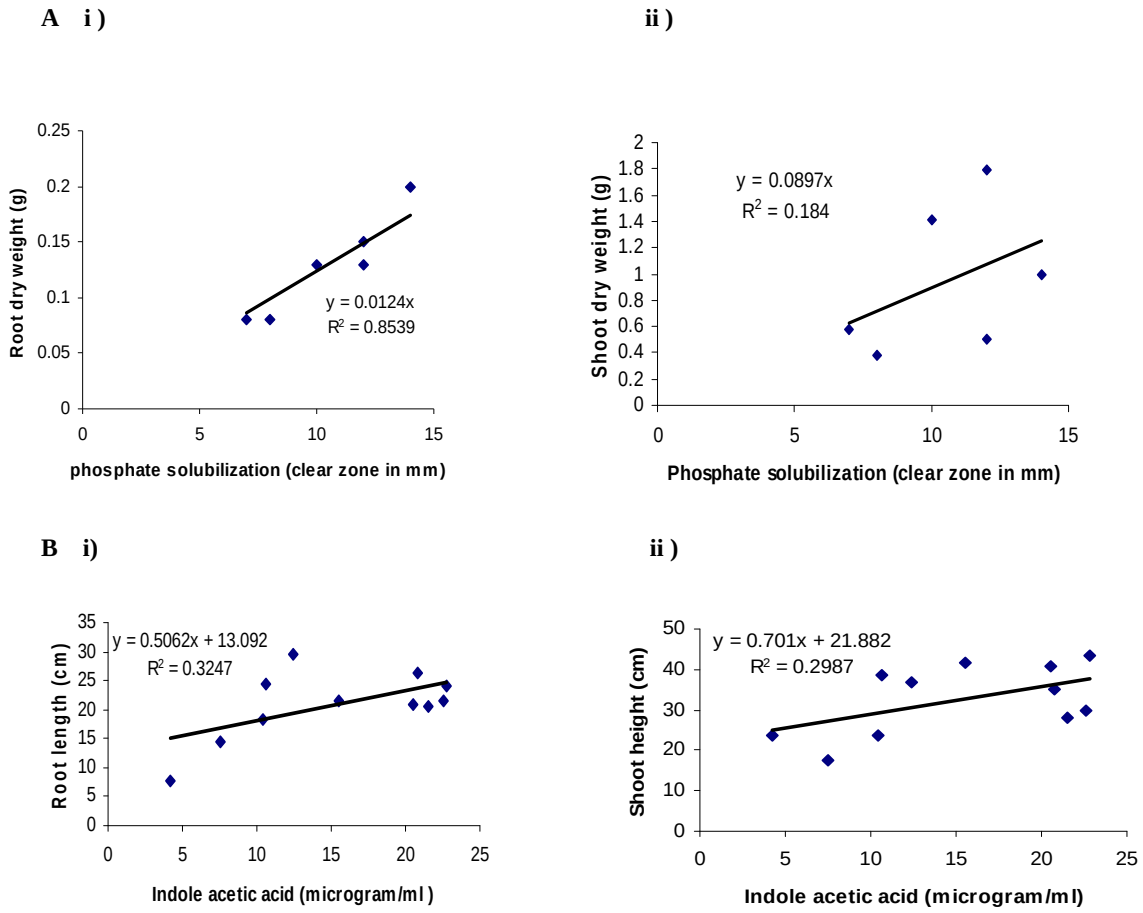

Figure 5. 3. Relationship between P- solubilization and root/shoot dry weight: i) r is significant (*P = 0.005*) for root dry weight and P- solubilization. ii) r is not significant (*P = 0.05*) for shoot dry weight and P- solubilization **(A)**. Relationship between concentration of IAA and root/shoot length: i) r is significant (*P = 0.05*) for root length and IAA concentration. ii) r is significant (*P = 0.05*) for shoot growth and IAA concentration according to Pearson's linear correlation coefficient test **(B).**

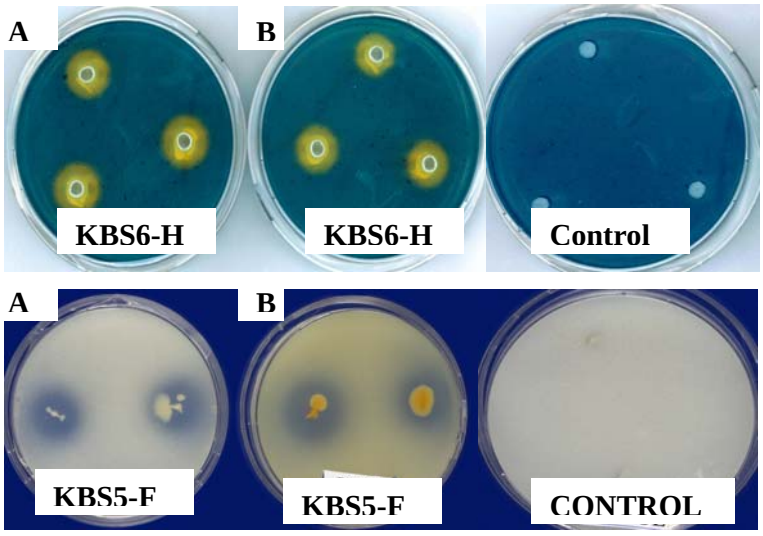

Figure. 5. 4. Siderophores production by *Serratia marcescens* (KBS6-H) as shown by the yellow halo on CAS agar plates under low iron (A) and high iron (B) conditions with the controls inoculated with sterile broth only (Top). Phosphate solubilization by *Chrysoemonas luteola* KBS5-F stab inoculated on NBRIY medium (A) and PVK medium containing bromophenol blue (B) (bottom)