Efficacy of rhizobacteria for growth promotion and biocontrol of *Pythium ultimum* and *Fusarium oxysporum* on sorghum in Ethiopia and South Africa

By

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DECLARATION

I, the undersigned, declare that the PhD thesis entitled: ‘’Efficacy of rhizobacteria for growth promotion and biological control of *Pythium ultimum* and *Fusarium oxysporum* on sorghum in Ethiopia and South Africa’’ submitted to the University of Pretoria is my own original work and it has not formed previously the basis for the award of any degree.

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CONTENTS

LIST OF ABBREVIATIONS

PGPR = Plant growth promoting rhizobacteria
cfu = colony forming units
RBGU = Rose-bengal-glycerol-urea medium
API = Analytical profile index
ANOVA = Analysis of variance
DMR = Duncan's Multiple Range
ISR = Induced systemic resistance
IAA = Indole 3- acetic acid
ACC deaminase = 1-amino cyclopropane 1-carboxylic acid deaminase
KB medium = Kings B medium
PDA = Potato dextrose agar
PVK = Pikovskaya agar
CAS = Chrome-azurol-sulphur
NBRIY = National botanical research institute (modified PVK) medium.
2, 4- DAPG = 2-4-diacetyl phloroglucinol.
PCR = Polymerase chain reaction
DNA = Deoxyrhibonucliec acid
dNTP = deoxy nucleotide triphosphate.
et al. = and others
sp = species (single)
spp. = species (plural)
µg = microgram
µl = microlitre

SUMMARY

In-vitro and greenhouse screening of 78 bacterial isolates from sorghum rhizosphere in Ethiopia and 86 isolates from the rhizosphere of grasses at Nylsvlei Nature Reserve in South Africa was conducted in terms of inhibition of *Fusarium oxysporum* that causes root rot in sorghum. Among the Ethiopian isolates KBE5-7, KBE5-1, KBE2-5 and NAE5-5 resulted in 100% disease suppression while disease suppressions ranging from 85.6% - 95.8% were rendered by South African isolates KBS9-H, KBS9-B, KFP9-A, NAS6-B and KBS5-F. According to identification by means of API and 16S rDNA sequencing, the majority of the effective isolates belong to members of the genus *Bacillus*. Other Gram negative isolates effective in this study have been identified as *Serratia marcescens*, *Chryseomonas luteola*, *Stenotrophomonas maltophilia* and *Enterobacter sakazaki*.

Screening of rhizobacterial isolates was also conducted in terms of *in-vitro* and *in-vivo* antagonistic activity against *Pythium ultimum* Trow, a common soilborne pathogen causing yield reductions in a wide variety of crops including sorghum. Statistically significant disease suppression was achieved by a number of isolates both from Ethiopia and South Africa. Most of the effective isolates maintained themselves in the rhizosphere at a level of $\geq 10^{5}$ cfu/g four weeks after inoculation. While *Bacillus cereus* was the predominant isolates from both sites, *Brevilbacterium laterosporus*, *Serratia marcescens* and *Pseudomonas fluorescens* were among the most effective isolates with the potential to suppress *Pythium ultimum in-vitro* and *in-vivo*.

Modes of action studies assessing production of antibiotics, siderophores, chitinolytic activity and induction of systemic resistance in sorghum were conducted for rhizobacterial isolates effective against *F. oxysporum* and *P. ultimum*. The antibiotic substances produced in the culture filtrates of many of these effective bacteria resulted in strong antifungal activity against both pathogens. The antibiotics from *Bacillus cereus* (KBS5-H) and *Bacillus subtilis* (KBS6-3) resulted in an efficient antagonistic activity against *F. oxysporum* and *Pythium ultimum* respectively. Siderophore production was evident in the Gram-negative strains *Serratia marcescens* (KBS9-R), *C. violaceum* (KBE9-1) and *E. sakazaki* (NAS6-B) with prominent yellow/orange halo development on CAS-agar plates demonstrating the potential by these isolates to produce siderophores under iron stressed conditions. Chitinolytic activity on chitin-agar plates was shown by isolates which mostly (83 %) belonged to strains of *B. cereus* The split root system has also demonstrated that *B. cereus* (KBS5-H), *C. violaceum* (KBE9-1) and *S. marcescens* (KBS9-R) were capable of rendering significant induction of systemic resistance against *F. oxysporum* in sorghum. The successful *in-vitro* and *in-vivo* suppression of *F. oxysporum* and *P. ultimum* by the effective rhizobacterial isolates and the

presence of various modes of action provide useful information on the potential of these isolates as biocontrol agents against soilborne fungal pathogens.

The isolation and screening of rhizobacteria for growth promotion of sorghum has also been conducted under greenhouse condition in pathogen free soils. Three isolates from Ethiopia and 10 isolates from South Africa have been identified as the most effective growth promoting isolates in these studies. The isolates also tested positive for the production of siderophores, production of indoleacetic acid and phosphate solubilization, the direct modes of actions through which bacteria promote plant growth in the rhizosphere of several plants. Of the most effective isolates 44 % were identified as *Bacillus cereus,* 19 % as *Chryseomonas luteola,* 13 % as *Serratia marcescens*, 13 % as *Sphingomonas paucimobilis*, and 6% each as *Stenotrophomonas maltophilia* and *Brevibacterium laterosporus* respectively.

The best biocontrol agents were selected out of a total of 24 isolates both from Ethiopia and South Africa. The selection procedure was conducted by using criteria such as the *in-vitro* and *in-vivo* suppression of *Fusarium oxysporum* and *Pythium ultimum,* the root colonization ability of the bacterial isolates and selected modes of action including production of antibiotic substances and siderophores, chitinolytic activity and induction of systemic resistance in sorghum. According to this procedure five isolates from Ethiopia (KBE5-7, KBE5-1, KBE9-1, NAE1-7 and NAE5-7) and six isolates from South Africa (KBS5-F, KBS9-R, KBS6-H, KBS5-H, KFP9-K and KBE6-17) have been selected as the most efficient biocontrol isolates. The selection of the best performing growth promoting isolates was conducted out of 12 efficient isolates using the following criteria: root colonization, siderophores and indoleacetic acid (IAA) production, phosphate solubilization and bacterial growth profiles in liquid cultures. Two isolates from Ethiopia (KBE7-8 and KBE9-1) and five isolates from South Africa (KBS5-H, KBS5-F, KBS6-H, KBS9-B and NAS4-3) have been selected as the best growth promoting isolates. As the screening and selection of this study are based on laboratory and greenhouse studies, further evaluation of the best isolates under field conditions and additional modes of action studies are warranted to ascertain their full potential as biocontrol and growth promoting agents.