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Screening of plant growth promoting rhizobacteria for antifungal activity against *Fusarium oxysporum*

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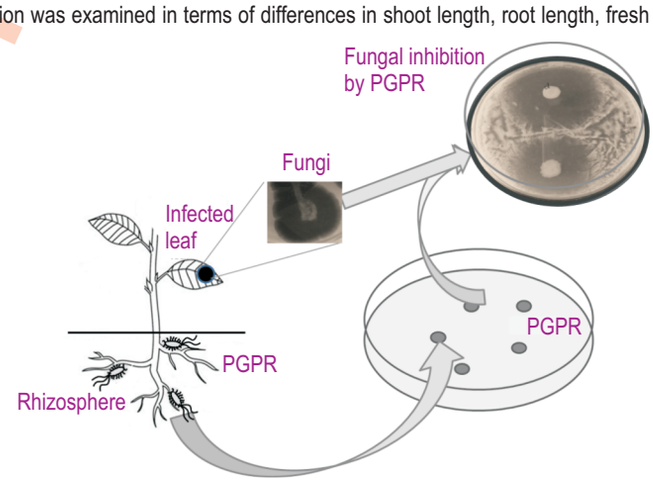
Abstract

Aim: New species of Plant Growth Promoting Rhizobacteria (PGPR), with varying growth promoting and biocontrol ability are often being discovered. They facilitate plant growth either directly by secreting nutrients and hormones or indirectly by providing defence mechanism to the plant. The present study was undertaken to isolate PGPR from the rhizosphere of *Solanum lycopersicum* and *Arachis hypogaea,* and test their growth promoting ability and antifungal activity against *Fusarium oxysporum.*

Methodology: PGPRs were isolated from the rhizosphere of *S. lycopersicum* and *A. hypogaea* by serial dilution of the rhizospheric soil and identified by 16s rDNA sequencing. The isolates were analysed for antifungal activity against *F. oxysporum*, indole 3-acetic acid (IAA) production and phosphate solubilisation. For the growth promotion assay, aseptically grown *Vigna radiata* seedlings were dipped separately in isolated bacterial suspension of PGPR (10^9 CFU ml^{-1}) and planted in autoclaved soil. Plants were irrigated with 50% Hoagland solution for every 48 hr and maintained at 25 ± 2 °C with 16/8 hr of light and dark photoperiod. Growth promotion was examined in terms of differences in shoot length, root length, fresh weight and dry weight after 12 days of treatment.



Results: Six isolates were found to have antifungal activity towards plant pathogen, *F. oxysporum*. Five isolates showed similarity to *Pseudomonas aeruginosa* (B7-1, B11-5, B3-1, Rh-1, Rh-2) and one to *Pseudomonas putida* (B53). All six strains were able to produce IAA, where B53 and B13-1 showed the highest production compared to other strains. *P. putida* B53 demonstrated the highest plant growth promotion activity by significantly ($p<0.05$) increasing the growth of *V. radiata* plants as evidenced by increase in shoot length, root length, fresh and dry weight.

Interpretation: The results obtained from the present study supports that PGPRs like *Pseudomonas* sp. could serve as potential eco-friendly bio-fertilizer and bio- fungicide.

Key words: Biofungicide, *Fusarium oxysporum,* Indole 3-acetic acid, Phosphate solubilization, Plant growth promoting rhizobacteria

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Introduction

Plants are prone to infection by many fungal species. The food and agriculture organization states that pests and diseases are responsible for most of the crop loss worldwide (Savary *et al*., 2012). It has been stated that plant diseases are responsible for 10% and 20% yield loss every year in the more developed and less developed areas. Among these, most virulent diseases are caused by fungal species. Most of the pathogenic fungi belong to the class Ascomycetes (e.g. Fusarium wilt disease by *Fusarium* sp.). *Fusarium* sp. mostly infects banana, tomato and rice plants, which are the most predominantly used food crops all over the world (Ploetz *et al*., 2006). Fungi reproduce both sexually and asexually *via* spores. Spores are widely distributed in soil and associated with many plants. Fungal diseases are controlled by use of chemical fungicides; however, the fungi develop resistance to various fungicides with time and the usage of fungicide leads to environmental pollution (Al-Assiuty *et al*., 2014). Therefore, bio-control of fungal pathogens developed from antagonistic rhizobacteria can be an effective eco-friendly alternative to chemical fungicide. Most of the PGPR are able to control pathogenic bacteria and fungi through microbial antagonism. Combination of PGPR and fungi such as *Trichoderma* have shown enhanced control of plant diseases (Mishra *et al*., 2013). PGPR helps in plant growth and defense by either direct or indirect mechanisms. In direct mechanism, rhizobacteria promote plant growth by synthesizing phytohormones, fixing nitrogen and solubilizing organic phosphates (Shahab *et al*., 2009). In the indirect mechanism, rhizobacteria impart defense in plants by producing various antibiotics and lytic enzymes that inhibit the growth of other plant pathogens. Besides antagonism, certain plant-microbe interactions can induce mechanisms in which the plant can defend themselves against pathogenic bacteria, fungi and other microorganisms. This kind of resistance is called Induced Systemic Resistance (ISR) (Pieterse *et al*., 2014) where the bacterial components like lipopolysaccharides, homoserine lactone, acetoin and 2, 3-butanediol stimulate the plant defense mechanism by inducing the jasmonate and ethylene signaling (Pangesti *et al*., 2016).

In the present study, PGPRs were screened from the rhizosphere of *A. hypogea* and *S. lycopersicum* and analysed for antifungual activity against *F. oxysporum* and their growth promotion activity using *V. radiata*.

Materials and Methods

Isolation and identification of fungi: For fungal isolation, leaves were surface sterilized with 95% ethanol; lesions were cut from the infected leaves of *S. lycopersicum,* placed on potato dextrose agar (PDA) and incubated at 28°C for 3-5 days (Bayona *et al*., 2011). Molecular identification was done by amplifying Internal Transcribed Spacer (ITS) region using the primers ITS3-KYO1 (5' AHCGATGAAGAACRYAG 3') and ITS4-KYO2 (5' RBTTTCTTTTCCTCCGCT 3'). The PCR condition for the designed primers was 95°C for 10 min as initial denaturation, followed by 35 cycles of denaturation at 95°C for 30 sec, annealing at 38°C for 30 sec, extension at 72°C for 20 sec and final extension at 72°C for 7 min (Toju *et al*., 2012). The amplified PCR product was sequenced and the sequences were blasted against NCBI database to identify the isolated strains.

Isolation and identification of PGPR: Plants such as *S. lycopersicum* and *A. hypogea* were collected from the agricultural fields. The roots of the plants were washed in autoclaved distilled water, cut into small pieces and incubated for 1-2 hrs in the conical flasks containing distilled water at 37 °C. From this, 1ml was taken for serial dilution, spread plated on Luria Bertani (LB) agar medium and the plates were incubated at 37°C overnight. The bacteria with unique and distinct morphology were isolated and identified by ribotyping using 16S rDNA universal primers fD1 5' AGAGTTTGATCCTGGCTCAG 3' and rP1- 5' ACGGCTACCTTGTTACGATT 3' (Weisburg *et al*., 1991).

Antifungal activity by PGPR: The antifungal activity of the isolated PGPRs was screened by well diffusion method. One hundred microliters of fungal culture (0.4 OD_{600}) was spread plated on potato dextrose agar plates. An overnight grown exponential phase bacterial cultures were adjusted to 0.4 OD at 580 nm and 5 µl of the culture was added to the wells of PDA plates. The plates were incubated at 28°C for 2-3 days (Perez *et al*., 1990) to check for antifungal activity.

Production of Indole 3-Acetic Acid by PGPR: The isolates were screened for IAA production using microplate method (Sarwar *et al*., 1995). The qualitative and quantitative determination of IAA production was performed with isolates showing antifungal activity. These isolates were grown in LB broth amended with 0.1 mg ml^{-1} tryptophan and incubated at 30°C for 3 days. Cultures were centrifuged, 100µl of the supernatant was transferred to 96 microwell plate followed by addition of 150µl of Salkowski reagent (0.01 M of $FeCl_3$ in 35% $HClO_4$). IAA was used as standard. The samples were incubated at room temperature for 25 min in dark. Change in colour of the mixture to pink or deep red was noted and their absorbance was measured at 540nm.

Phosphate solubilisation by PGPR: The qualitative estimation of phosphate solubilization was performed by well diffusion method using Pikovskaya agar (Pikovskaya *et al*., 1948). An overnight grown bacterial culture was adjusted to 0.4 OD at 580 nm and 5 µl of the sample was loaded onto the wells punctured on Pikovskaya agar plate. The isolates with the ability to solubilise inorganic phosphate showed halo zone of clearance and were taken as phosphate solubilising PGPRs.

Plant growth promotion by PGPR: The bacterial isolates B7-1, B11-5, B13-1, Rh-1, Rh-2 and B53 were grown in LB broth and adjusted to 0.4 OD at 580nm. A 10 ml of culture was taken,

centrifuged and the pellet dissolved in saline water was used for growth promotion assay. The seeds of *V. radiata* were surface sterilized with sodium hypochlorite followed by Tween 20 and kept overnight for germination. Germinated seeds of equal size were selected, dipped in bacterial suspension (10^9 CFU ml^{-1}) and planted in 1.5 kg of autoclaved potting soil. The experiment was carried out at 25 ± 2°C with 16/8 hr of light and dark photoperiod. Plants were irrigated with 50% Hoagland's solution (300 ml per pot) for every 48 hrs. The plants were uprooted after 12 days of plantation and the shoot length, root length, fresh and dry weights were measured (Naqqash *et al*., 2016). Untreated seeds were taken as control.

Statistical analysis : The values were expressed as mean ±SD for each group. Statistical analyses were performed by one-way ANOVA followed by Tukey's multiple comparison tests. Statistical analyses were performed using Graphpad prism 7 and the level of significance was set at p value < 0.05.

Results and Discussion

For molecular identification of isolated fungus, 400 bp of ITS region was amplified and sequenced (Fig.1 A). The sequence was subjected to BLAST analysis against NCBI database which showed that the isolated fungus was *F. oxysporum* with 99% similarity. For molecular identification of bacteria, 1.5 kb of 16S rDNA gene was amplified for all the six isolated bacterial strains (Fig.1 B) and sequenced. Among six bacterial isolates, five (B7-1, B11-5, B13-1, Rh-1 and Rh-2) showed 99% similarity to *P. aeruginosa* and one (B53) showed 99% similarity to *P. putida*. *Pseudomonas* sp. has already been reported as plant growth promoting bacteria with biocontrol activity (Vacheronl *et al*., 2016).

The bacterial strains were tested for their antifungal activity against *F. oxysporum*. All six bacterial isolates were able to inhibit the growth of *F. oxyporum* species. Among these B7-1, B11-5 and B13-1 strains showed maximum zone of clearance compared to other bacterial strains. These bacteria were able to inhibit the growth of fungi under normal room temperature. Similarly, PGPR mediated suppression of plant fungi has been previously reported by many bacterial strains. Liu *et.al*., (2010) reported that *Pseudomonas* sp., isolated from recycled soilless crops showed antifungal activity against *F. oxysporum*. *Pseudomonas* isolates from rice phylloplane also showed biocontrol ability against fungi, *Rhizoctonia solani* (Akter *et al*., 2016) and PGPR isolated from cucumber showed antifungal activity against *Phytophthora capisci* (Islam *et al.,* 2016).

IAA is one of the important plant growth hormones which helps in cell proliferation and elongation. The bacterial isolates have the ability to produce IAA with different pathways at different concentration. These bacterial strains produce IAA in the presence of tryptophan at concentrations of 1µg- 5µg ml^{-1}. Similar results have been previously reported by PGPR isolated from the roots of wheat plants, where it produced IAA in the range of 0.27 - 77.98 µg ml^{-1} (Majeed *et al*., 2015). Among six bacterial isolates, B53 showed statistitically increased production of IAA compared with other strains (Fig. 2). Similarly, Bharucha *et al*. (2013) also reported highest production of IAA by *P. putida* UB1 among the isolated PGPR strains.

The qualitative estimation of phosphate solubilization was done in Pikovskaya agar which is rich in tricalcium phosphate. After three days of incubation, the zone of clearance was observed in the plates. The isolated PGPRs were able to reduce tricalcium phosphate to mono calcium phosphate, which

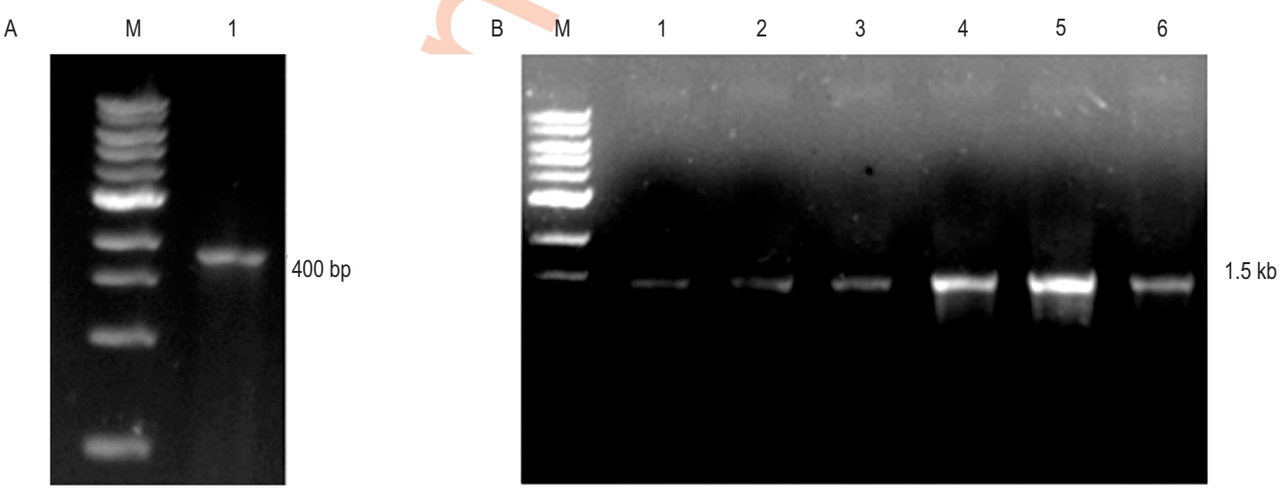

Fig.1 : (A) PCR amplification of ITS region of isolated fungi. Lane M, 100bp marker, Lane 1, amplification of ITS region of fungi and (B) PCR Amplification of 16S rDNA. Lane M, 1Kb marker, Lane 1-6, amplification of 16 S rDNA from six PGPR isolates.

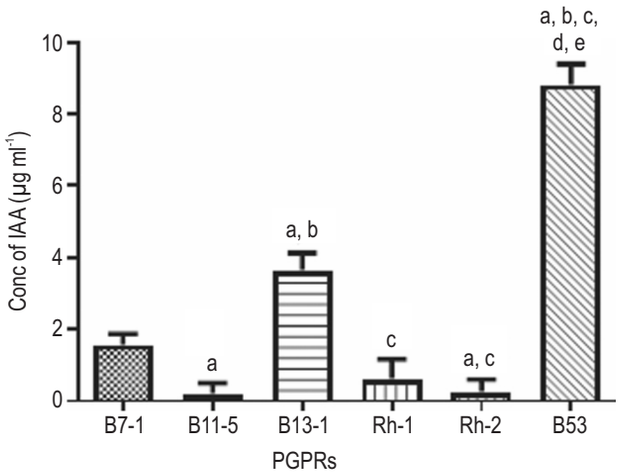

Fig. 2 : Production of IAA by rhizobacterial isolates. Data represents means ± SD. Different letters indicate a significant difference between groups (a: vs B7-1 group, b: vs B11-5 group, c: vs B13-1 group, d: vs Rh-1 group, e: vs Rh-2 group : $p < 0.05$; one-way ANOVA and then Tukey's multiple comparison tests).

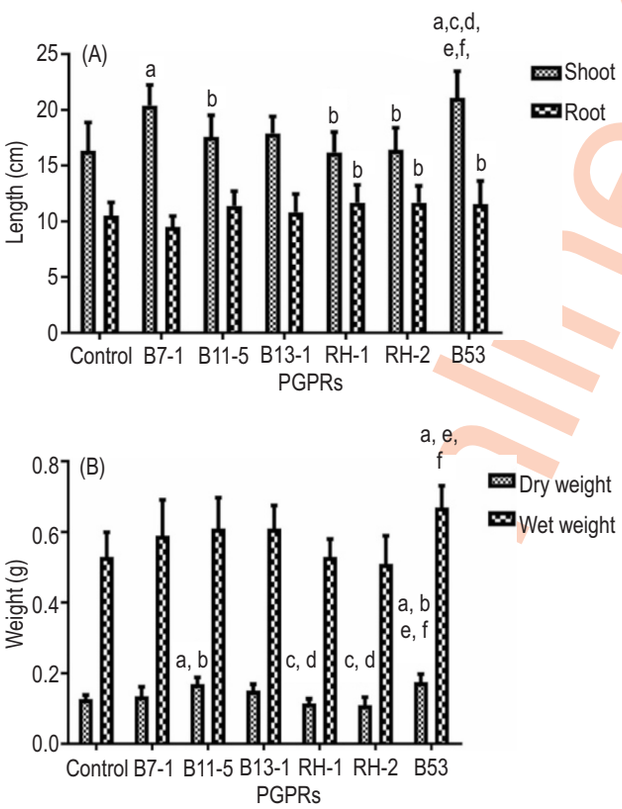

Fig. 3 : (A) Effect of PGPR isolates on shoot and root length of *Vigna radiate* plant; (B) Effect of PGPR isolates on wet and dry weight of *Vigna radiate* plant. Data represents means ± SD. Different letters indicate a significant difference between groups. (a: vs control, b: vs B7-1, c vs B11-5 group, d: vs B13-1 group, e: vs Rh-1 group, f: vs Rh-2 group : $p < 0.05$; one-way ANOVA and then Tukey's multiple comparison tests).

is readily absorbed by the plants. All isolates, except B53, showed greater zone of clearance. This indicated the ability of the isolates to reduce phosphate and its potential use as plant growth promoter in agriculture. This result was similar to the phosphate solubilising ability of *Pseudomonas* sp. reported by Park *et al*. (2009) and Akter *et al*. (2016). Gluconic acid (GA) producing endophytic bacteria was also reported to solubilize insoluble phosphate and stimulate growth of *Pisum sativum* plants (Oteino *et al*., 2015).

Plant growth promotion assay was performed by growing *V. radiata* in the presence and absence of isolated bacterial strains. Plants which were inoculated with PGPR showed an increase in root and shoot length compared to control. Among the six bacterial isolates, B7-1 showed maximum (26% - 30%) shoot length followed by B53 in comparison to other isolates. While comparing root length, B53 and Rh-2 showed significant increase of about 15% - 20% (Fig. 3A). Bacterial isolate B53 showed 27% higher biomass followed by B11-5 and B13-1 as compared to untreated control plants (Fig. 3B). Similarly, fluorescent Pseudomonads were also reported to posses the ability to promote plant growth in normal and saline conditions (Anitha *et al*., 2015). Oteino *et al.* (2015) reported the ability of *P. flourescens*, *P. putida* and *Bacillus subtilis* to promote the growth of *P. sativum* (Oteino *et al*., 2015). Rhizobacteria such as *Lysinibacillus sphaericus*, *Paenibacillus alvei*, *Bacillus safensis*, *Bacillus pumilus* and *Brevundimonas vesicularis* facilitated 24 - 34% increase in the yield when the seeds were treated with these bacteria (Breedt *et al*., 2017).

This is a preliminary study for screening the plant growth promoting rhizobacteria from *S. lycopersicum* and *A. hypogaea* rhizosphere. It is concluded from the present study that the PGPR *isolates*, B7-1, B11-5, B13-1 Rh-1, Rh-2 and B53 identified as *Pseudomonas* sp. have antifungal activity against *F. oxysporum* and also have the potential to produce IAA and solubilize inorganic phosphate to organic form. Among these, *P. putida* B53 demonstrated greater plant growth promotion activity by increasing the growth of *V. radiata* plants. Hence, these bacterial species can be used as potential bio-fertilizer and fungicide for plants infected by *F. oxysporum* sp.

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References

Akter, S., J. Kadir, A.S. Juraimi and H.M. Saud: *In vitro* evaluation of *Pseudomonas* bacterial isolates from rice phylloplane for biocontrol of *Rhizoctonia solani* and plant growth promoting traits. *J. Environ. Biol*., **37**, 597-602 (2016).

Al-Assiuty, A.N.I.M., M.A. Khalil, A.W. Ismail, N.M. van Straalen and M.F.

Ageba: Effects of fungicides and biofungicides on population density and community structure of soil oribatid mites. *Sci. Total. Environ*., **466–467**, 412–420 (2014).

Anitha, G. and B.S. Kumudini: Isolation and characterization of fluorescent pseudomonads and their effect on plant growth promotion. *J. Environ. Biol.*, **35**, 627-634 (2014).

Bharucha, U., K. Patel and U.B. Trivedi: Optimization of indole acetic acid production by *Pseudomonas putida* UB1 and its effect as plant growth- promoting rhizobacteria on mustard (*Brassica nigra*). *Agric. Res*., **2**, 215–221 (2013).

Breedt, G., N. Labuschagne and T.A. Coutinho: Seed treatment with selected plant growth-promoting rhizobacteria increases maize yield in the field. *Ann. Appl. Biol*., **17**, 229-236 (2017).

Bayona, L.G., A. Grajales, M.E. Cárdenas, R. Sierra, G. Lozano, M.F. Garavito, M.C. Cepero de García, A. Bernal, P. Jiménez and S. Restrepo: Isolation and characterization of two strains of *Fusarium oxysporum* causing potato dry rot in *Solanum tuberosum* in Colombia. *Rev. Iberoam. Micol*., **28**, 166–172 (2011).

Liu, J., G. Gilardi., M. Sanna., M.L. Gullino and A. Garibaldi: Biocontrol of *Fusarium* crown and root rot of tomato and growth-promoting effect of bacteria isolated from recycled substrates of soilless crops. *Phytopathol. Mediterr.,* **49**, 163–171 (2010).

Islam, S., A.M. Akanda, A. Prova, M.T. Islam and M.M. Hossain: Isolation and identification of plant growth promoting rhizobacteria from cucumber rhizosphere and their effect on plant growth promotion and disease suppression. *Front. Micro. Biol*., **6**, Article 1360 (2016).

Majeed, A., M.K. Abbasi, S. Hameed, A. Imran and N. Rahim: Isolation and characterization of plant growth-promoting rhizobacteria from wheat rhizosphere and their effect on plant growth promotion. *Front. Microbiol*., **6**, Article 198 (2015).

Mishra, D.S., A. Kumar, C.R. Prajapati, A.K. Singh and S.D. Sharma: Identification of compatible bacterial and fungal isolate and their effectiveness against plant disease. *J. Environ. Biol.*, **34**, 183-189 (2013).

Naqqash, T., S. Hameed, A. Imran, M.K. Hanif, A. Majeed and J.D. van Elsas: Differential response of potato toward inoculation with taxonomically diverse plant growth promoting rhizobacteria. *Front. Plant. Sci*., **7**, 144 (2016).

Oteino. N., R.D. Lally, S. Kiwanuka, A. Lloyd, D. Ryan, K.J. Germaine and D N. Dowling: Plant growth promotion induced by phosphate solubilizing endophytic *Pseudomonas* isolates. *Front. Microbiol*., **6**, Article 745 (2015).

Pangesti, N., M. Reichelt, J.E. Van De Mortel, E. Kapsomenou, J. Gershenzon, J.J.A., VanLoon, M. Dicke and A. Pineda: Jasmonic acid and ethylene signaling pathways regulate glucosinolate levels in plants during rhizobacteria-induced systemic resistance against a leaf-chewing herbivore. *J. Chem. Ecol.*, **42**, 1212–1225 (2016).

Park, K.H., C.Y. Lee and H.J. Son: Mechanism of insoluble phosphate solubilization by *Pseudomonas fluorescens* RAF15 isolated from ginseng rhizosphere and its plant growth-promoting activities. *Lett. Appl. Microbiol.*, **49**, 222-228 (2009).

Perez, C., M. Pauli and P. Bazerque: An antibiotic assay by the well agar diffusion method. *Acta Biol. et Med. Exper.*, **15**, 113–115 (1990).

Pieterse, C. M. J., C. Zamioudis, R.L. Berendsen, D.M. Weller, S.C.M. Van Wees and P.A.H.M. Bakker: Induced systemic resistance by beneficial microbes. *Annu. Rev. Phytopathol.*, **52**, 347–375 (2014).

Pikovskaya, R.I.: Mobilization of phosphorus in soil connection with the vital activity of some microbial species. *Microbiology*, **17**, 362-370 (1948).

Ploetz, R.C.: Fusarium wilt of banana is caused by several pathogens referred to as *Fusarium oxysporum f.* sp. cubense. *Phytopathology*, **96**, 653–656 (2006).

Sarwar, M. and R.J. Kremer: Determination of bacterially derived auxins using a microplate method. *Lett. Appl. Microbiol*., **20**, 282–285 (1995).

Savary, S., A. Ficke, J.N. Aubertot and C.A. Hollier: Crop losses due to diseases and their implications for global food production losses and food security. *Food Security*, **4**, 519–537 (2012).

Shahab, S., N. Ahmed and N.S. Khan: Indole acetic acid production and enhanced plant growth promotion by indigenous PSBs. *Afr. J. Agric. Res*., **4**, 1312–1316 (2009).

Toju, H., A.S. Tanabe, S. Yamamoto and H. Sato: High-coverage its primers for the DNA-based identification of ascomycetes and basidiomycetes in environmental samples. *Plos One*, **7** (2012).

Vacheronl, J., Y.M. Loccoz, A. Dubost, M.G. Martins, D. Muller and C.P. Combaret: Fluorescent *Pseudomonas* strains with only few plant-beneficial properties are favored in the maize rhizosphere. *Front. Plant Sci*., **7**, Article 1212 (2016).

Weisburg, W.G., S.M. Barns, D.A. Pelletier and D.J. Lane: 16S ribosomal DNA amplification for phylogenetic study. *J. Bacteriol*., **173**, 697-703 (1991).