Curr Microbiol (2015) 71:566–571
DOI 10.1007/s00284-015-0886-7

Isolation and Screening of Rhizosphere Bacteria from Grasses in East Kavango Region of Namibia for Plant Growth Promoting Characteristics

D. H. Haiyambo[1] · P. M. Chimwamurombe[1] · B. Reinhold-Hurek[2]

Received: 11 March 2015 / Accepted: 4 July 2015 / Published online: 9 August 2015

Abstract A diverse group of soil bacteria known as plant growth promoting rhizobacteria (PGPR) is able to inhabit the area close to plant roots and exert beneficial effects on plant growth. Beneficial interactions between rhizospheric bacteria and plants provide prospects for isolating culturable PGPR that can be used as bio-fertilizers for sustainable crop production in communities that cannot easily afford chemical fertilizers. This study was conducted with the aim of isolating rhizospheric bacteria from grasses along the Kavango River and screening the bacterial isolates for plant growth promoting characteristics. The bacteria were isolated from rhizospheres of *Phragmites australis*, *Sporobolus* sp., *Vetiveria nigritana*, *Pennisetum glaucum* and *Sorghum bicolor*. The isolates were screened for inorganic phosphate solubilization, siderophore production and indole-3-acetic acid (IAA) production. The nitrogen-fixing capability of the bacteria was determined by screening for the presence of the *nifH* gene. Up to 21 isolates were obtained from *P. australis*, *Sporobolus* sp., *S. bicolor*, *P. glaucum* and *V. nigritana*. The genera *Bacillus*, *Enterobacter*, *Kocuria*, *Pseudomonas* and *Stenotrophomonas*, identified via 16S rDNA were represented in the 13 PGPR strains isolated. The isolates exhibited more than one plant growth promoting trait and they were profiled as follows: three phosphate solubilizers, four siderophore producers, eight IAA producing isolates and five nitrogen-fixers. These bacteria can be used to develop bio-fertilizer inoculants for improved soil fertility management and sustainable production of local cereals.

✉ P. M. Chimwamurombe
pchimwa@unam.na

[1] Department of Biological Sciences, University of Namibia, Private Bag 13301, Windhoek, Namibia

[2] Department of Microbe-Plant Interactions, University of Bremen, P.O. Box 330440, 28334 Bremen, Germany

Introduction

Soil-dwelling bacteria are important components of soil health status as they play crucial roles in recycling and transforming nutrients so that they are available to plants [15]. A diverse group of soil bacteria known as plant growth promoting rhizobacteria (PGPR) isable to inhabit the area close to plant roots and provide beneficial effects on the growth of the plant [16]. Plant growth promotion by PGPR involves direct mechanisms which improve nutrient uptake and regulation of phytohormones [6, 10]. PGPR facilitate nitrogen, phosphorus and iron uptake via N_2-fixation, inorganic phosphate solubilization and production of iron chelators known as siderophores, respectively [30]. PGPR are able to modulate phytohormones by synthesizing the auxin, indole-3-acetic acid (IAA) and producing 1-aminocyclopropane-1-carboxylate (ACC) deaminase which decreases ethylene levels [1]. Indirect mechanisms of plant growth promotion mainly involve biocontrol and reducing inhibitory effects on plant growth [10]. Biocontrol by PGPR is achieved by competition for nutrients and sites, reduction of virulence factors, production of antifungal compounds and induced systemic resistance [18]. The beneficial features that bacteria exert on plants, while the two are in association can be harnessed by isolating the relevant bacteria and re/introducing them into crop agricultural environments at favourable conditions, i.e., at increased population numbers or in a conducive substrate.

The majority of Namibians based in the North and North Eastern regions depend on subsistence agriculture for their livelihood [36]. Land degradation and environmental

threats that are linked to the long-term impacts of climate change can lead to disastrous situations [38]. Therefore, it is important to identify adapted solutions in order to realise sustainable land management practices for the benefit of using natural biological resources for crop farming [38]. The sustainable agriculture system which comes to mind is one in which, instead of synthetic inputs, natural resource management is used to maximise crop production [34]. Agricultural soils used by small scale subsistence farmers lack sufficient amounts of nutrients to support ideal crop growth [10]. By incorporating native plant-associated bacteria into agricultural farming systems, it is possible to improve sustainability and productivity of crop farming in Namibia. This study was done to isolate rhizospheric bacteria from grasses along the Kavango River and screen the isolates for the plant growth promoting characteristics.

Materials and Methods

Sampling

The samples were taken from a total of 8 sites located in the Kavango River in the East Kavango region of Namibia. Half of the sites were situated in pristine lands (17°53′38.49″S; 20°09′08.97″E, 17°52′40.49″S; 20°10′31.03″E, 17°53′33.62″S; 20°14′56.13″E and 17°52′30.82″S; 20°15′21.88″E) and the other half were located in small subsistence farmers' crop fields (17°55′00.13″S; 20°06′16.14″ E, 17°53′49.75″S; 20°09′07.07″E, 17°53′43.80″S; 20°14′05.26″E and 17°54′04.40″S 20°14′14.34″E). Convenience sampling coupled with a plant health scale that was based on the physical appearance of the plants was used in subsistence farming fields. Plants were ranked on three levels: 0 = wilted/pale yellow leaves, 1 = wilted/green leaves and 2 = not wilted/green leaves. Only those with a healthy appearance level 2 were selected. A systematic sampling method, where samples were selected along a 30–50 m transect line at 10-m intervals, was used to select plants from pristine areas. Samples were collected by digging up a whole plant, removing the surrounding soil followed by aseptically detaching the root system from the plant and then stored at 4 °C.

Isolation of Bacteria

For each sample, one gram of root material was rinsed with 2 ml sterile distilled water and transferred into 9 ml phosphate buffer saline (Oxoid Ltd.) solution, followed by vortexing. Thereafter, 1 ml of the mixture was transferred into 9 ml of enrichment media i.e., combined carbon medium [3] and synthetic malate (SM) [25], and incubated at 30 °C for 14 days. Serial dilutions up to dilution factor of 10^{-6} were prepared from the incubated tubes followed by spread plating on VM-ethanol agar [26]. The inoculated plates were incubated at 30 °C for 5–7 days. After incubation, single colonies were subcultured onto fresh VM-ethanol agar plates and incubated at 30 °C for 5–7 days.

Inorganic Phosphate Solubilization

The ability to solubilize inorganic phosphate was tested by growing the bacterial isolates on Pikovskaya agar (Oxoid Ltd) dyed with bromophenol blue (Sigma-Aldrich Co.) [14] for 10 days at 30 °C. The formation of more transparent zones around the bacterial colonies was indicative of inorganic phosphate solubilization on Pikovskaya agar. The diameter of the zones and the diameter of the colonies were measured and recorded. This test was done in triplicate. The solubilization index was calculated using the published formula [7].

Siderophore Production

Chromeazurol-*S* (Chromeazurol *S*; Sigma-Aldrich Co.) [33] agar was used to determine if the bacteria were able to produce iron chelating siderophore complexes. After growing the bacteria at 30 °C for 5 days on CAS agar [33], plates were observed for the formation of orange halos around the bacterial colonies indicating siderophore activity. This test was done in triplicates.

Quantitative Valuation of Indole-3 Acetic Acid Production

The quantitative valuation of indole-3 acetic acid (IAA) was done according to [14], with slight modifications. Bacterial isolates were grown in VM-ethanol broth and adjusted to a concentration of $OD_{600} = 1$. The broth cultures were used to prepare 10 ml suspensions (10 % v/v) in VM-ethanol broth supplemented with 5 mM L-tryptophan (Sigma-Aldrich Co.) and placed in a shaking incubator under dark conditions at 30 °C for 2 days. Cultures were centrifuged at 4,400 rpm for 15 min followed by removing and mixing the supernatant with Salkowski's reagent in the ratio of 1:2. The mixture was incubated at room temperature for 30 min. Absorbance values of the supernatant and Salkowski reagent (Sigma-Aldrich Co) mixtures were measured at 530 nm. Non-inoculated broth was kept as a control. This test was done in triplicates. The quantity of IAA produced by the bacterial isolates was determined by comparing absorbance values with those from a standard curve. Pure IAA (Sigma-Aldrich Co.) was used to prepare standard concentrations of 0, 5, 10, 15, 20, 25, 30, 35, 40 and 45 mg l^{-1} [39].

Amplification of Nitrogenase nifH Gene Amplicons

The gene that codes for the Fe protein polypeptide of the nitrogenase enzyme is the *nifH* gene. Thus, the detection of the *nifH* gene in the genomic DNA sample of a bacterial isolate would label that particular isolate as a nitrogen fixer. In order to amplify the *nifH* gene, a nested PCR using outer primers FGPH19 (5′-TACGGCAARGGTGG NATHG-3′), PolR (5′-ATSGCCATCATYNTCRCCGGA-3′) and inner primers PolF (5′TGC GAYCCSAARGCB GACTC-3′) and AQER (5′-GACGATGTAGATYTCCTG-3′) [31] was performed in a Esco Swift™ MaxPro Thermal Cycler. In the first step, a 25 µl PCR mixture consisted of 12.5 µl DreamTaq Green PCR Master Mix 2× (ThermoScientific), 2 µl Genomic DNA prepared with Zymo Research Fungal/Bacterial DNA MiniPrep™, 0.5 µM of each primer and 10 µl nuclease-free water (ThermoScientific). The reaction was carried out as follows: initial denaturation at 94 °C for 4 min, 30 cycles at 94 °C for 1 min, 55 °C for 1 min and 72 °C for 2 min, and final extension at 72 °C for 5 min. In the second step, a 25 µl PCR mixture consisted of 12.5 µl DreamTaq Green PCR Master Mix 2 × (ThermoScientific), 2 µl template from the first step, 0.5 µM of each primer and 10 µl Nuclease-free water. The reaction was carried out as in the first step except the annealing temperature was set to 56 °C instead of 55 °C. A strain of *Enterobacter* sp. MOP 1-1 [4] was used as a positive control. The PCR products were separated by electrophoresis in 1.2 % agarose gels stained with ethidium bromide (Sigma-Aldrich) and visualized under UV light.

PCR Amplification of 16S rRNA and Sequence Analysis

Genomic DNA from each isolate was used as a template for amplifying the 16S RNA gene fragment by PCR using forward primer 27F (5′-GAGTTTGATCMTGGCTCAG-3′) and reverse primer 1492r (5′-GGTTACCTTGTTAC GACTT-3′). The 50 µl PCR mixture consisted of 25 µl DreamTaq Green PCR Master Mix 2 × (ThermoScientific), 2 µl Genomic DNA, 1 µM of each primer and 21 µl nuclease-free water. The reaction was carried out according to Grönemeyer et al. [13] in a Esco Swift™ MaxPro Thermal Cycler. More specifically, PCR cycle steps were as follows: initial denaturation at 95 °C for 4 min, 35 cycles at 95 °C for 1 min, 50 °C for 30 s and 72 °C for 1 min, and final extension at 72 °C for 10 min and holding at 4 °C. The products were separated by electrophoresis in 1 % agarose gels stained with ethidium bromide and visualized under UV light. Purification and sequencing of PCR fragments were carried out at Inqaba Biotec (Pretoria, South Africa). The Sequences were analysed using the Basic Local Alignment Search Tool (BLAST) to determine which organisms the sequences are most similar to. The BLAST programme is available in the National Center for Biotechnology Information website (NCBI-http://www.ncbi.nlm.nih.gov/gene bank).

Results

Bacteria Isolated from Grasses

A total of 21 bacterial isolates were isolated from the rhizospheres of *Pennisetum glaucum* (12) and *Sorghum* bicolor (5) of subsistence farmers; and from rhizospheres of *Sporobolus* sp. (1) and *Phragmites australis* (1). One of the bacteria was isolated from both *Vetiveria nigritana* and *Sporobolus* sp. and the last was a common isolate from *P. glaucum* and *Sorghum bicolor* plants, see Table 1. The majority of the isolates were isolated from the fields of subsistence farmers.

Plant Growth Promoting Characteristics

Bacterial isolates LCM1-14 (*Pseudomonas veronii*), ACM2-32 (*Pseudomonas stutzeri*) and WSS2-47 (*Bacillus megaterium*) were the only phosphate solubilizing isolates as tested via Pikovskaya agar plate assay. The solubilisation index was used to assess the relative phosphate solubilisation of the bacteria, see Table 2. Isolate ACM2-32 (index = 3.5) had a desirable index value of greater than 3, while WSS2-47 and LCM1-14 had indexes of 1.4 and 2.8, respectively.

The qualitative measure of siderophore production depends on an iron mobilization reaction which causes a change in pH thus bringing about a colour change in the medium. Four of the isolates, LCS2-11, LCM1-14, WSS2-47 and LSM1-65, were able to produce iron siderophore complexes in CAS agar. As with phosphate solubilization, only bacteria isolated from *P. glaucum* and *S. bicolor* crops had the ability to chelate iron.

The bars in Fig. 1 show the amount of IAA produced by isolates with the capability to produce indole in broth supplemented with L-tryptophan. Isolate FCM2-50 (*E. cloacae*) produced the highest value of 8.98 ± 0.46 mg IAA L^{-1}, whilst the isolate LCS2-11 (*S. maltophilia*) produced the lowest value of 3.63 ± 0.54 mg IAA L^{-1} amongst the selected isolates. The other isolates that produced IAA were LCM1-14 (*P. veronii*), LSS1-21 (*P. validus*), ASM1-59 (*B. subtilis*), FCM1-66 (*B. licheniformis*), LSE-68 (Bacillus sp.) and LSM1-61 (*B. amyloliquefaciens*).

Table 1 Molecular identification of bacterial isolates based on 16S rDNA sequences

Isolate designation	Name of isolate with closest similarity	Similarity (%)	NCBI genbank accession numbers of isolates of this study	Plant from which isolated
^{S7}GRDB-1^{cc}	–	–	–	*P. australis*
^{S6}GXLB-6$^{cc/sm}$	–	–	–	*Sporobolus* sp.
^{S1}LCS2-11$^{cc\ P}$	*Stenotrophomonas maltophilia* JN256	100	KP019209	*S. bicolor*
^{S1}LCM1-14$^{cc\ P}$	*Pseudomonas veronii* NK7	100	KP019210	*P. glaucum*
^{S1}LSS1-21$^{sm\ P}$	*Paenibacillus validus* SB 3263	99	KP019211	*S. bicolor*
^{S1}LSM1-23^{sm}	–	–	–	*P. glaucum*
^{S1}LSM1-26^{sm}	–	–	–	*P. glaucum*
^{S2}ACM2-32$^{cc\ P}$	*Pseudomonas stutzeri* strain *1*	100	KP019212	*P. glaucum*
^{S2}ACM1-35^{cc}	–	–	–	*P. glaucum*
$^{S1\text{-}7}$A-40$^{cc/sm}$	–	–	–	*P. glaucum*/*S. bicolor*
^{S3}WCM1-41$^{cc\ P}$	*Bacillus cereus* JN244	100	KP019213	*P. glaucum*
^{S3}WCM2-45^{cc}	–	–	–	*P. glaucum*
^{S3}WSS2-47$^{sm\ P}$	*Bacillus megaterium* SRRNINew52	100	KP019214	*S. bicolor*
^{S3}WSS2-48^{sm}	–	–	–	*S. bicolor*
^{S3}WSS2-49$^{sm\ P}$	*Bacillus cereus* DkBoA3-1	98	KP019215	*S. bicolor*
^{S4}FCM2-50$^{cc\ P}$	*Enterobacter cloacae* GL7	99	KP019216	*P. glaucum*
^{S2}ASM1-59$^{sm\ P}$	*Bacillus subtilis* Y38	100	KP019217	*P. glaucum*
^{S5}LSM1-61$^{sm\ P}$	*Bacillus amyloliquefaciens* DMKUB24	99	KP019218	*P. glaucum*
^{S5}LSM1-65$^{sm\ P}$	*Kocuria* sp. MI-46a	99	KP019219	*P. glaucum*
^{S4}FCM1-66$^{cc\ P}$	*Bacillus licheniformis* 55N2-3	99	KP019220	*P. glaucum*
^{S6}LSE-68$^{cc/sm\ P}$	*Bacillus sp*. b29 (2010)	100	KP019221	*V. nigritana*/ *Sporobolus* sp.

^{P} Isolates that had at least one plant growth promoting characteristic were identified via 16S rDNA sequence

Enrichment medium used to isolate bacteria: *cc* combined carbon medium, *sm* synthetic malate medium. *S1* site 1 (17°53′43.80″S 20°14′05.26″E), *S5* site 5 (17°53′43.80″S 20°14′05.26″E), *S2* site 2 (17°53′49.75″S 20°09′07.07″E), *S6* site 6 (17°53′33.62″S 20°14′56.13″E), *S3* site 3 (17°55′00.13″S 20°06′16.14″E), *S7* site 7 (17°52′30.82″S 20°15′21.88″E), *S4* site 4 (17°54′04.40″S 20°14′14.34″E)

Table 2 Relative quantification of inorganic phosphate solubilized zone

Isolate	Colony diameter (mm)	Zone + colony diameter (mm)	Index value
LCM1-14	5.5	15.5	2.8
ACM2-32	4	14	3.5
WSS2-47	4.5	6.5	1.4

Detection of nifH Gene from Genomic DNA

The *nifH* gene fragment was amplified from the genomic DNA of 5 isolates (Fig. 2). The amplified fragments were as expected ~320 bp long. The *nifH* was detected in DNA from isolates LCS2-11(*S. maltophilia*), WCM1-41 (*B. cereus*), WSS2-49 (*B. cereus*), FCM2-50 (*E. cloacae*) and LSE-68 (*Bacillus* sp.). The presence of the *nifH* gene is an indication of N_2-fixation capability by the isolates.

Discussion

The resilient spore producing *Bacilli* and the versatile *Pseudomonas* spp., is predominant genera in the rhizosphere [23, 24]. The ubiquitously occurring *Stenotrophomonas* spp. are mainly found in soils and are known to be in association with plants. Beneficial relationships between *Stenotrophomonas* spp. and plants have been reported by Ryan et al. [27]. The actinomycete isolate *Kocuria* sp. LSM1-65 had demonstrated

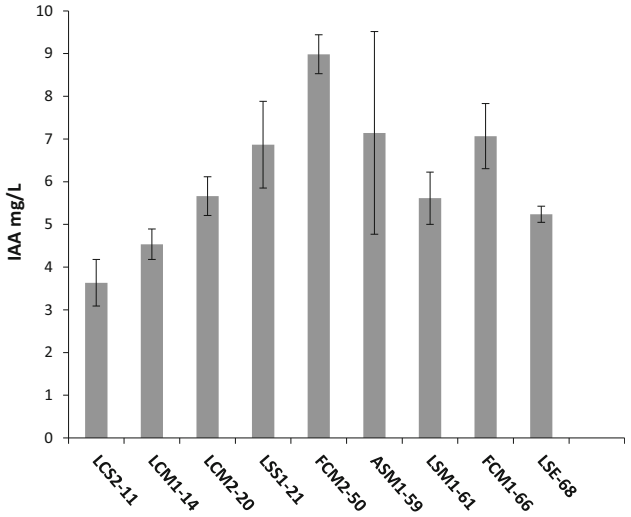

Fig. 1 Mean average indole-3-acetic acid (IAA) produced (milligrams per litre) by eight selected bacterial isolates. *Error bars* denote one standard error around the mean

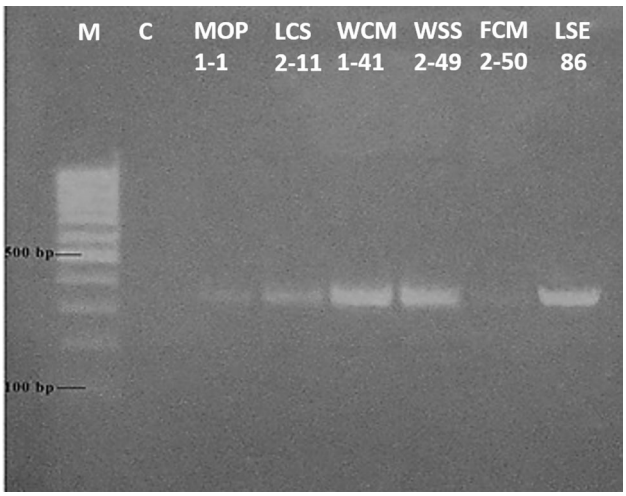

Fig. 2 Agarose gel of nifH amplicons from the DNA of five isolates and Enterobacter sp. MOP 1-1, which was used as a positive control

siderophore producing capability. *Kocuria turfanensis* strain 2M4 isolated from the rhizospheric soil was found to be an IAA producer, siderophore producer and phosphate solubilizer [11]. *Enterobacter* bacteria are prevalent in the rhizosphere as well as the endorhizosphere; they have been isolated from the rhizosphere of various plants [17, 21, 32]. *Enterobacter* spp. belong to a group (Enterobacteriaceae) which have rhizosphere colonization advantage [2]. Most of the isolates considered as plant growth promoting bacteria are known human pathogens. Reassuringly, we are aware that the pathogenic strains come into being via induced stress factors and selective pressures [5, 35]. Avoiding the risk to human health requires the use of a multidisciplinary approach to better understand interactions that take place between the microbes plant associates, the host plant, and the environment to accomplish proper screening so that the clinical strains are distinguished from the agricultural ones [8].

Phosphate solubilizing bacteria can play an essential role in maintaining soil phosphorus balance. *Pseudomonas* spp. are amongst the most effective phosphate solubilizing bacteria [17, 19, 37]. Siderophore activity by isolate *P. veronii* LCM1-14 may be attributed to pyoverdine, pyochelins and/or quinolobactin [20, 29], whilst *S. maltophilia* LCS2-11 is believed to secrete siderophores of the catechol-type [9]. *Bacillus* spp. are known to produce hydroxamate-type siderophores [22]. Tryptophan introduced as an external source initiates IAA production by the bacteria. Bacteria that reside in the rhizosphere produce plant hormones that can positively influence plant growth [12], particularly the root system development [28]. Fixing of atmospheric nitrogen is a valuable feature of diazotrophic PGPR. These bacteria should be regarded as vital components in inoculation formulations for the reason that other than chemical fertilizers, nitrogen-fixing bacteria offer the only practical alternative to solving the problem of nitrogen deficiency in agricultural soils.

Thirteen bacterial isolates with various plant growth promoting characteristics were isolated from the rhizospheres of grasses along the Kavango River. Efforts aimed at discovering native microorganisms that can improve crop growth are encouraged. A diverse collection of plant-beneficial bacteria is accommodated in the rhizospheres of a wide variety of plants. Native PGPR bacteria inoculants have the potential to alleviate challenges of local chemical fertilizer production and low cereal grain production. Using bacteria as a bio-fertilizer for sustainable soil health improvement and increased crop production can be achieved in a moderately cheap and ecologically safe manner.

Acknowledgments This study was funded by The Future Okavango (TFO) Project through the BMBF (Federal Ministry of Education and Research, Germany) Research Framework Programme, Research for Sustainable Development (FONA).

Compliance with Ethical Standard

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ahemad M, Kibret M (2014) Mechanisms and applications of plant growth promoting rhizobacteria: current perspective. J King Saud Univ Sci 26(1):1–20
2. Barraquio WL, Segubre EM, Gonzalez MAS, Verma SC, James EK, Ladha JK, Tripathi AK (2000) Diazotrophic enterobacteria: what is their role in the rhizosphere of rice. In: Ladha JK, Reddy PM (eds) The quest for nitrogen fixation in rice. Kluwer Academic Publishers, Philippines, pp 93–118

3. Bashan Y, Holguin G, Lifshitz R (1993) Isolation and characterization of plant growth-promoting rhizobacteria. In: Glick BR, Thompson JE (eds) Methods in plant molecular biology and biotechnology. CRC Press, Florida, pp 331–345
4. Burbano Roa CS (2011) Molecular analysis of root-associated diazotrophs in important plants from Southern Africa and South America. Dissertation, University of Bremen
5. Carvalho-Assef APDA, Gomes MZR, Silva ARA, Werneck L, Rodrigues CAS, Souza MJ, Asensi MD (2010) IMP-16 in *Pseudomonas putida* and *Pseudomonas stutzeri*: potential reservoirs of multidrug resistance. J Med Microbiol 59(9):1130–1131
6. Chaiharn M, Chunhaleuchanon S, Kozo A, Lumyong S (2008) Screening of rhizobacteria for their plant growth promoting activities. KMITL Sci Technol J 8(1):18–23
7. Edi-Premono M, Moawad MA, Vleck PLG (1996) Effect of phosphate solubilizing *Pseudmonas putida* on the growth of maize and its survival in the rhizosphere. Indones. J Crop Sci 11:13–23
8. Fletcher J, Leach JE, Eversole K, Tauxe R (2013) Human pathogens on plants: designing a multidisciplinary strategy for research. Phytopathology 103(4):306–315
9. Garcia CA, De Rossi BP, Alcaraz E, Vay C, Franco M (2012) Siderophores of *Stenotrophomonas maltophilia*: detection and determination of their chemical nature. Rev Argent Microbiol 44(3):150–154
10. Glick BR (2012) Plant growth-promoting bacteria: mechanisms and applications. Scientifica 2012
11. Goswami D, Pithwa S, Dhandhukia P, Thakker JN (2014) Delineating *Kocuria turfanensis* 2M4 as a credible PGPR: a novel IAA producing bacteria isolated from saline desert. J Plant Interact 9(1):566–576
12. Govindasamy V, Senthilkumar M, Magheshwaran V, Kumar U, Bose P, Sharma V, Annapurna K (2011) *Bacillus* and *Paenibacillus* spp.: potential PGPR for sustainable agriculture. In: Maheshwari DK (ed) Plant growth and health promoting bacteria. Springer, Germany, pp 333–364
13. Grönemeyer J, Berkelmann D, Mubyana-John T, Haiyambo D, Chimwamurombe P, Kasaona B, Hurek T, Reinhold-Hurek B (2014) A survey for plant-growth-promoting rhizobacteria and symbionts associated with crop plants in the Okavango region of Southern Africa. Biodivers Ecol 5:287–294
14. Grönemeyer JL, Burbano Roa CS, Hurek T, Reinhold-Hurek B (2012) Isolation and characterization of root-associated bacteria from agricultural crops in the Kavango region of Namibia. Plant Soil 356(1–2):67–82
15. Hopkins DW, Dungait JA (2010) Soil microbiology and nutrient cycling. In: Dixon GR, Tilston EL (eds) Soil microbiology and sustainable crop production. Springer, The Netherlands, pp 59–80
16. Kloepper JW, Schroth MN (1978) Plant growth-promoting rhizobacteria on radishes. In: Station de pathologie vegetale et phyto-bacteriologie (Ed.), Proceedings of the 4th International Conference on Plant Pathogenic Bacteria 2: 879–882
17. Lodewyckx C, Vangronsveld J, Porteous F, Moore ER, Taghavi S, Mezgeay M, der Lelie DV (2002) Endophytic bacteria and their potential applications. Crit Rev Plant Sci 21(6):583–606
18. Lugtenberg B, Kamilova F (2009) Plant-growth-promoting rhizobacteria. Ann Rev Microbiol 63:541–556
19. Mander C, Wakelin S, Young S, Condron L, O'Callaghan M (2012) Incidence and diversity of phosphate-solubilising bacteria are linked to phosphorus status in grassland soils. Soil Biol Biochem 44(1):93–101
20. Matthijs S, Baysse C, Koedam N, Tehrani KA, Verheyden L, Budzikiewicz H, Cornelis P (2004) The Pseudomonas siderophore quinolobactin is synthesized from xanthurenic acid, an intermediate of the kynurenine pathway. Mol Microbiol 52(2):371–384
21. Mehnaz S, Mirza MS, Haurat J, Bally R, Normand P, Bano A, Malik KA (2001) Isolation and 16S rRNA sequence analysis of the beneficial bacteria from the rhizosphere of rice. Can J Microbiol 47(2):110–117
22. Ollinger J, Song KB, Antelmann H, Hecker M, Helmann JD (2006) Role of the Fur regulon in iron transport in *Bacillus subtilis*. J Bacteriol 188(10):3664–3673
23. Piggot PJ (2009) *Bacillus Subtilis*. In: Schaechter M (ed) Desk encyclopedia of microbiology, 2nd edn. Academic Press, California, pp 154–165
24. Podile AR, Kishore GK (2006) Plant growth-promoting rhizobacteria. In: Gnanamanickam SS (ed) Plant-associated bacteria. Springer, The Netherlands, pp 195–230
25. Reinhold B, HurekT Niemann EG, Fendrik I (1986) Close association of *Azospirillum* and diazotrophic rods with different root zones of Kallar grass. App Environ Microb 52(Suppl 3):520
26. Reinhold-Hurek B, Hurek T (2000) Reassessment of the taxonomic structure of the diazotrophic genus *Azoarcus* sensu lato and description of three new genera and new species, *Azovibrio restrictus* gen. nov., sp. nov., *Azospira oryzae* gen. nov., sp. nov. and *Azonexus fungiphilus* gen. nov., sp. nov. Int J Syst Evol Microbiol 50(2):649–659
27. Ryan RP, Monchy S, Cardinale M, Taghavi S, Crossman L, Avison MB, Dow JM (2009) The versatility and adaptation of bacteria from the genus *Stenotrophomonas*. Nat Rev Microbiol 7(7):514–525
28. Ryu RJ, Patten CL (2008) Aromatic amino acid-dependent expression of indole-3-pyruvate decarboxylase is regulated by TyrR in *Enterobacter cloacae* UW5. J Bacteriol 190(21):7200–7208
29. Saha R, Saha N, Donofrio RS, Bestervelt LL (2012) Microbial siderophores: a mini review. J Basic Microb 52:1–15
30. Saharan BS, Nehra V (2011) Plant growth promoting rhizobacteria: a critical review. Life Sci Med Res 21:1–30
31. Sato A, Watanabe T, Unno Y, Purnomo E, Shinano T, Osaki M (2009) Analysis of diversity of diazotrophic bacteria associated with the rhizosphere of a tropical Arbor, *Melastoma malabathricum* L. Microb Environ 24(8):81–87
32. Schütz A, Golbik R, Tittmann K, Svergun DI, Koch MHJ, Hübner G, König S (2003) Studies on structure–function relationships of indolepyruvate decarboxylase from *Enterobacter cloacae*, a key enzyme of the indole acetic acid pathway. Eur J Biochem 270:2322–2331
33. Schwyn B, Neilands JB (1987) Universal chemical assay for the detection and determination of siderophores. Anal Biochem 160(1):47–56
34. Tilston EL, Sizmur T, Dixon GR, Otten W, Harris JA (2010) The Impact of Land-Use Practices on Soil Microbes. In: Dixon GR, Tilson EL (eds) Soil microbiology and sustainable crop production. Springer, The Netherlands, pp 273–295
35. Turrientes MC, Baquero MR, Sánchez MB, Valdezate S, Escudero E, Berg G, Martínez JL (2010) Polymorphic mutation frequencies of clinical and environmental *Stenotrophomonas maltophilia* populations. Appl Environ Microbiol 76(6):1746–1758
36. United Nations Development Programme (UNDP) (2010) Country pilot partnership: adapting to climate change through the improvement of traditional crops and livestock farming in Namibia. www.thegef.org/gef/sites/thegef.org/files/gef_prj_docs/GEFProjectDocuments/M&E/TE/FY2012/UNDP/G002915/2915_3598_Namibia_CCA_TE.pdf
37. Vassilev N, Vassileva M, Nikolaeva I (2006) Simultaneous P-solubilizing and biocontrol activity of microorganisms: potentials and future trends. Appl Microbiol Biotechnol 71(2):137–144
38. World Bank (2009) Namibia: country brief. Open knowledge repository. https://openknowledge.worldbank.org/handle/10986/2630
39. Yasmin F, Othman R, Sijam K, Saad MS (2010) Characterization of beneficial properties of plant growth-promoting rhizobacteria isolated from sweet potato rhizosphere. Afr J Microbiol Res 3(11):815–821