APPLICATION AND IMPORTANCE OF BENEFICIAL MICROBES ON CROP PRODUCTION

BY

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Abstract

The performance of designed specific bacteria isolate comprising to bacillus pumllus pseudomonas aeruginosa and Brevibacterium spp in terms of their reduction capability and degrading activities of the bacteria were studied.The aim of this study was to isolate some authoctothonous bacteria from niche area soil and investigate their capability to degrade the components in the soil and as well tested for different plant growth promotion activities of the bacterial.Out of 38 isolates collected from different sites from niche area three efficient strains were selected for further studies, and analyzed for plant promoting activities. The media used in this research were nutrient broth and agar. Bacteria strains were identified by their morphological and biochemical characteristics. The reduction of sewage wastewater weight and degrading properties of pseudomona aeuraginosa. Bacillus Pumillus and brevibacterium spp were examined. The identified Bacteria strain included Brevibacterium spp (86.6%), pseudomona aeuroginosa (9.6%), and B.pumilus (3.6%). Evaluation of morphological, physiological and Biochemical activity of the designed consortia was carried out. Acid production tests indicated that this bacterium capable to utilize the citrate and hydrolyze casein catalase, and oxidase positive. This study indicated that some *autochthonous Bacteria* have the potential to Fix Nitrogen which can be used to enriched soil fertility. The potentiality of PGPR in agriculture is steadily increased as it offers an attractive way to replace the use of chemical fertilizers, pesticides and other supplements. Growth promoting substances are likely to be produced in large quantities by these rhizosphere microorganisms that influence indirectly on the overall morphology of the plants. Use of PGPR as inoculants increased nutrient availability to plants. The progress, to date in using the rhizosphere bacteria in a variety of applications related to agricultural improvement along with their mechanism of action with special reference to plant growth-promoting traits.

Keywords: Rhizosphere, Autochtonous, Morphology, Bactria, Nitrogen.

Introduction

Soil fertility and nutrient cycling are influenced by the soil microbial population. An understanding of the interrelationships between functional microbial communities and cycling of C, N, P and S as an indicator of soil health is essential for soil fertility management. The major shortcomings associated with the use of chemical fertilizers include high cost, limited supply and environmental polluting effects (especially from N and P fertilizers). Thus, there is a need for alternative and sustainable sources of nutrients for crops. The adoption of an integrated nutrient management (INM) approach others_an alternative strategy against the adverse effects of using conventional N and P fertilizers alone. An INM system involves minimal chemical fertilizer application in combination with both natural and man-made sources of crop nutrients to improve nutrient-use efficiency and crop productivity in an environmental-friendly manner . In addition, advances in the understanding of the relationships between crops and microorganisms colonizing rhizospheric soils have increased screening efforts for microbial strains showing plant growth-promoting (PGP) ability. This ability is through direct and/or indirect enhancement of plant nutrient uptake by use as microbial inoculants or in microbial-based fertilizers termed as bio-fertilizers (Amelia et al., 2020).

Microorganisms play an important role in\decomposition of organic matter as they take part in the production of humus as well as soil's quality and structure are greatly influenced by microbes. They participate in the preservation

of biological equilibrium, recycling of nutrients between the soil and roots and in the process of transforming nutrients. They aid in the surface blooming lessens erosion losses. While maintaining soil pH, microorganisms also maintain mineral and nutrient balance and increase the fertility of the soil. Some microbes like Rhizobium, create root nodules and symbiotic relationships with plants. These organisms help in fixing the atmospheric nitrogen to soil which is further available to plants (Keithellakpam et al., 2022) microbes in sustainable agriculture is due to the genetic dependency of plants on the beneficial functions provided by symbiotic co-habitants. Therefore, microbial biotechnology and its applications in sustainable development of agriculture and environmental health are getting better attention. Interest in the microbial biodegradation of pollutants has intensified in recent years as mankind strives to find sustainable ways to clean up contaminated environments. These bioremediation and biotransformation methods use the naturally occurring, microbial catabolic diversity to degrade, transform or accumulate a huge range of compounds including hydrocarbons, polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), pharmaceutical substances, radionuclide's and heavy metals (Narendra et al., 2020) 2012). When the factors and conditions that control a particular beneficial microbiological function are known, that function can potentially be stimulated through targeted management measures.

Alternatively, beneficial microorganisms can be applied to the cropping system, either as consortia in, e.g., organic fertilizers or soil conditioners, or as isolated and propagated single microbial strains. The latter approach is used most widely for the microbial control of agricultural pests (the word 'pests' is used in a broad sense, including invertebrate pests, plant pathogens, and weeds) and microbial stimulation of plant growth or health by mechanisms other than pest control, i.e., 'biostimulation' (Ingvar et al, 2021). Biofertilizers are the rhizobacteria that under particular conditions mainly enhance plant growth via providing required nutrition. The bacteria can accelerate certain microbial processes in the soil that augment the availability of nutrients in a form easy to assimilate by plants. They can be grouped, based on their nature and function, as N2 fixing, phosphate solubilizing, phosphate mobilizing, or biofertilizers for micronutrients. Biocontrol agents (BCA) or biopesticides main function is suppressing or controlling plant disease. They can be categorized as bacteria per se or as compounds derived from bacteria acting as biocontrol agents. Most likely plants use different strategies for growth promotion at different times of plant growth and development. All these mechanisms of plant growth promotion act dynamically, and only by monitoring the various parameters over the entire growth period, we can estimate the role of each of the different factors (Salme et al., 2017).

A fundamental knowledge of the genetics, molecular biology ecology, and evolution of symbiotic interactions could enable the development of microbe-based sustainable agriculture. Undoubtedly, getting biased rhizosphere opens new opportunities for future agricultural developments based on exploiting the beneficial microbial services to reduce the inputs of agrochemicals, thereby reaching sustainable environmental and economic goals (Narendra et al., 2020). Soil fertility and nutrient cycling are essential factors in sustainable agriculture, heavily influenced by soil microbial communities. Beneficial microbes, including plant growth-promoting rhizobacteria (PGPR), play a crucial role in soil health, improving nutrient availability and supporting plant growth through nitrogen fixation, phosphate solubilization, and disease suppression (Glick, 2020). The over-reliance on chemical fertilizers poses significant environmental risks, such as soil degradation, water pollution, and greenhouse gas emissions. Recent research suggests that integrating beneficial microbes as biofertilizers can mitigate these issues, enhancing soil microbial diversity and improving crop productivity (Liu et al., 2023). Microbial inoculants, including Bacillus, Pseudomonas, and Rhizobium species, promote plant growth through mechanisms such as phytohormone production, induction of systemic resistance, and organic matter decomposition (Kumar et al., 2022). Advancements in microbiome-based agricultural solutions have shown that microbial consortia can be tailored to specific crops, enhancing their resilience to environmental stressors such as drought and soil salinity (Singh & Sharma, 2023). In addition, the use of metagenomics and synthetic biology approaches is enabling the identification and development of novel microbial strains with improved plant growth-promoting properties (Chen et al., 2023). Given the increasing global demand for sustainable agricultural practices, integrating beneficial microbes into crop management strategies offers a promising alternative to chemical fertilizers. This approach not only enhances soil fertility and crop yields but also aligns with the principles of regenerative agriculture and climate-smart farming (Patel & Meena, 2024).

Literature Review

Soil fertility and nutrient cycling are significantly influenced by microbial populations, which play a crucial role in nutrient dynamics and soil health (Amelia et al., 2020). Beneficial microbes, particularly plant growth-promoting rhizobacteria (PGPR), have been widely studied for their role in improving plant growth and productivity. These microorganisms enhance soil fertility by fixing nitrogen, solubilizing phosphorus, and producing phytohormones that promote root growth (Keithellakpam et al., 2022). Microorganisms such as Rhizobium, Bacillus, and Pseudomonas species contribute significantly to soil health by decomposing organic matter, enhancing nutrient uptake, and protecting plants from pathogens (Narendra et al., 2020). The ability of PGPR to suppress plant diseases through biological control mechanisms makes them an essential component of sustainable agriculture (Ingvar et al., 2021). PGPRs produce siderophores, hydrolytic enzymes, and antibiotics, which help in controlling plant pathogens and improving soil microbial diversity (Salme et al., 2017).Beneficial microbes enhance soil fertility through several mechanisms, including:Nitrogen Fixation: Certain bacteria such as Rhizobium and Azospirillum fix atmospheric nitrogen into a form available to plants (Narendra et al., 2020).Phosphate Solubilization: Microorganisms like Bacillus and Pseudomonas solubilize insoluble phosphates, making them available for plant uptake (Whitelaw, 2000).

Biodegradation of Organic Matter: Microbial enzymes break down organic matter, releasing essential nutrients into the soil (Pradhan & Sukla, 2006). PGPRs have been increasingly used as biofertilizers to reduce the dependency on chemical fertilizers. They enhance nutrient uptake, stimulate root development, and improve plant resistance to stress conditions (Saleem et al., 2007). The application of PGPR as microbial inoculants has been shown to increase crop yield and quality while maintaining soil fertility (Bashan et al., 2004). Microbial biotechnology has also been employed in environmental conservation. Microbes capable of degrading pollutants such as heavy metals, hydrocarbons, and pharmaceutical residues contribute to soil and water purification (Narendra et al., 2020). Bioremediation techniques using microbial consortia have proven effective in restoring contaminated soils, thus supporting agricultural sustainability (Amann et al., 1995). Despite their benefits, the large-scale application of beneficial microbes faces challenges such as inconsistent field performance and limited shelf life of microbial inoculants. Advances in molecular biology and biotechnology can help in developing more resilient microbial strains with enhanced growth-promoting abilities (Compant et al., 2008). Future research should focus on understanding microbial interactions in the rhizosphere to optimize their application in sustainable agriculture.

Materials and Methods

Collection of *Samples*

Soil samples were collected using some clean sterile polythene bags and sterile spatula in a niche area near sewage dump sites at Oyun river within Kwara State.

Isolation of Bacteria

Isolation of bacteria was done by Streaking plate method. For bacterial isolation, soil extract and enrichment media were prepared. The soil extract was prepared by incubating, 0.1 mL of the supernatant of each tube containing suspension of soil and enrichment media was inoculated in nutrient agar plates by streaking at 30°C for 24 hours. The plates were examined and the suspected colonies stained by crystal violet for 1-2min. grams iodine use to flood the slide (Bergey's manual, 1986). Stain were decolourized by acetone for 2-3secs counterstain with safranin for 2min after incubation, a part of the culture medium was directly mixed with ethyl acetate (50:50) and then stirred using a magnetic stirrer for six hours. Isolates were later biochemically characterized using the commercial kit Analytical profile index system. Confirmed colonies were kept as consortium.

Screening of Different Consortium for Bioremediation

A consortium is a group of specific bacterial isolates which possesses the capability to degrade the components present in the Soil. For preparation of one consortium, we have used three to four different isolates. Different consortia were formulated randomly primarily on the basis of their morphology, color, size, shape, and so forth. Different cultures were inoculated in 25mL of NB and incubated overnight at 32–37°C and 180–200 rpm. These primary cultures were checked by streaking on nutrient agar plates which were then incubated at 32–37°C. These primary cultures were used for subculturing. 100µL of culture was inoculated into 100mL of NB and incubated at 32–37°C under shaking conditions for a period of 16–18h. The culture was harvested by centrifugation at 4°C and 7000rpm followed by washing twice with sodium phosphate buffer (pH 6.8–7.0). The supernatant was discarded and pellets were stored for the further experiments. At the time of experiment, different pellets were re-suspended according to the 20 consortia designed and inoculated in the sample and sample flasks were kept in shaking incubator at 180–200 rpm and 32–37°C for 32–36 h. After incubation (Bergey Manual,1986).

Isolation of phosphate Solubilizing Bacteria

10.0g of soil samples was suspended in 90ml of sterilized distilled water and 10^1 dilution was obtained. Serial dilutions were prepared by mixing 1ml of the suspension made into 9ml sterilized water blanks, until the 10^7 dilution was obtained. From these dilutions 100.0µl was spread plated on Pikovskayas Agar plates. These plates were the incubated at 30^oC and were observed for11 2-7 days. The total bacterial types were counted after 48 hours of incubation. The PSB showing halo zones of clearance were streaked again on PVK agar plate to check for purity and ''P'' solubilizing ability. The pure strains forming zone of clearance were maintained by streaking on nutrient agar slants and stored at 4^0C. (Bergey's Manual, 1986)

PCR amplification of 16S rRNA gene

The 16S rRNA gene of the target bacterial isolates was amplified by using universal eubacterial primers as reported by Heddi *et al*. (1998). The base sequences of the primers used are presented in Table 3.1. These were custom synthesized (Sigma, Pvt. Ltd.).

Sequencing and Data Analysis

PCR products of 16S rRNA gene of six efficient bacterial isolates obtained through amplification with specific primer were freeze dried in a lyophilizer (CHRIST *ALPHA I-2LD*) and sent for custom sequencing using same upstream and downstream primers used for the amplification of 16S rRNA gene (Ocimum Biosolutions, Pvt. Ltd.).

Evaluation of efficient strains under field conditions

A field experiment was conducted at the River bank via Asa river at harmony estate along Kulende area , Ilorin to check the effect of selected efficient strains on plants growth and soil fertility. Groundnut was selected for the field study. The isolates used in this field study were selected based on their efficiency of P-solubilization and/or production of growth promoting substances under *in vitro* conditions. Bio inoculants were applied in the soil as seed inoculations.

Seed Inoculation

Seed inoculation of PGPR is done by mixing the PGPR culture i.e inoculum with slurry to whish treated seeds are added with the result a uniform coat of PGPR culture around is formed the seed. The inoculated seeds were dried in shade and then sown in the field. Field experiment was conducted in Asa river (harmony river) along harmony estate Ilorin on Groundnut crop in which selected isolates were amended in soil as seed inoculation. Six field plots

measuring 2x2 m^2 each were Prepared. The plots with different treatments were arranged in a randomized complete block design with three replicates per treatment. Size of inoculum on each seed was 2×10^5 to 2.5×10^5. During the field study irrigation was done once before Maize was sown to have adequate soil water storage for seedling establishment. The plants were irrigated regularly. No fertilizer was added except to the control soil sample. The crop was harvested in first week of November and studied for various parameters such as growth parameters and P content in the plant parts. From each plot, ten randomly selected plants were uprooted and checked for shoot height. The plants were oven dried at 65 ^{0}C for 24 hours and measured for shoot weight and root weight and total P (Kitson and Mellon 1944). Total P (Kitson and Mellon, 1944), available P (Olsen *et al*., 1954), Total N content and Phosphatase (Tabatabai and Bremner, 1969) and Phytase activity (Kim and Lie, 2005). One week after before harvesting, the viability of inoculated bacteria in the soil was determined by isolating PSB bacteria using serial dilution technique.

Physiochemical properties of soil

For the isolation of PGPRs soil samples were collected from soil of different crops planted along the river bank in Asa river (harmony estate). Prior to the processing for PGPR isolation, soil samples were tested for their physiochemical properties like pH, TDS, EC, Organic carbon, total phosphorus, available phosphorus, total nitrogen were determined

Statistical Analysis

Three replicates were used for each experiment. The data were analyzed by analysis of variance (ANOVA) and the means were compared with Tukey's test at $P < 0.05$. Data was analyzed by ANOVA and the means were compared using Tukey's test at $P < 0.05$. All the analysis was performed by using Graph Pad Prism 5.0 software

Results

Table 1: Base sequences of 16Sr RNA gene primers

Name of the Primer		Sequence (5' to 3')
16S rRNA gene	F	AGA GTT TGA TCA TGG CTC AG
	R	TAC CTT GTT ACG ACT TCA CC

Table 2: Different Field Treatments

S/N	**Treatment**
Treatment 1	Soil
Treatment 2	Soil + GAL-1
Treatment 3	Soil + GAL-2
Treatment 4	Soil + GAL-3
Treatment 5	Soil + GAL-4
Treatment 6	Soil + GAL-5
Treatment 7	Soil + GAL-6
Treatment 8	Soil + GAL-7
Treatment 9	Soil + GAL-8**Table 3**

Morphological Characteristics of the Identified Strains

	Colony Morphology		
Test	**Bacillus pumilus**	**Brevibacterium sp**	**Pseudomonas aureginosa**
Margin	Irregular	Irregular	Irregular
Elevation	Concave	Convex	Flat
Surface	Dull	Glistening	Dull
Opacity	Opaque	Opaque	Translucent
Gram's reaction	+ve	-ve	-ve
Cell shape	Rods	Cocci/rods	Short rods
Endospore	+	-	-
Shape	Oval	+	+
Motility	+	+	-
Fluorescence	(UV)	(UV)	

Table 4.: Physicochemical Properties of Soil Samples

PH	7.85±0.85
TDS	0.45±0.06
EC	0.35±0.07
Organic Carbon (%)	0.55±0.02
Available P($mgkg^{-1}$)	3.28±0.45
Total P ($mgkg^{-1}$)	120±3.16
Total Nitrogen ($mgkg^{-1}$)	0.46±0.12
	99.44±8.21

The results in table 4a showed that COD concentration versus the different doses of bacteria is from 0.0 to 4.0 gm/l after retention time (14) days were much closed. The COD in reference sample nearly closed to the samples with bacteria. After three weeks the volume of sludge and the concentration of COD in sludge and sludge mass were calculated. Morphological characteristics (margin, elevation, surface, opacity, gram's reaction, cell shape, endospore, position, shape, motility, and fluorescence) of the identified strains is shown in Table 4b. Physiological tests and various biochemical tests were also carried out, and the results showed that Bacillus pumilus,

is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C, capable to starch hydrolysis, and catalase positive. Brevibacterium sp. (is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C catalase and oxidase positive. Pseudomonas aeruginosa is aerobic in nature, gram negative, motile, shows its growth from 25 to 42°C, this bacterium capable to utilize the citrate and hydrolyze casein, catalase, and oxidase positive (Tables 4c and 4d). Different temperatures were also studied for better COD reduction. The results reveal that better COD (chemical oxygen demand) reduction could be achieved in the flask incubated at 35°C as compared to the other flasks incubated at 25°C, 40°C, and 45°C

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Discussion

Strains were identified on the basis of physiological, morphological, biochemical, and 16rRNA techniques performed at MTCC (Chandigarh). Strains of the selected consortium 13 were identified as Bacillus pumilus, Brevibacterium sp, and Pseudomonas aeruginosa. Morphological characteristics (margin, elevation, surface, opacity, gram's reaction, cell shape, endospore, position, shape, motility, and fluorescence) of the identified strains is depicted in Table 4b. Physiological tests and various biochemical tests were also performed, and the results showed that Bacillus pumilus, MTCC (5305) is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C, capable to starch hydrolysis, and catalase positive. Brevibacterium sp. (MTCC 5306) is aerobic in nature, gram positive, motile, shows its growth from 25 to 45°C catalase and oxidase positive. Pseudomonas aeruginosa (MTCC 5307) is aerobic in nature, gram negative, motile, shows its growth from 25 to 42°C, this bacterium capable to utilize the citrate and hydrolyze casein, catalase, and oxidase positive (Tables 4c and 4d).

Effect of inoculum size and Agitation: the effect of biomass on the COD reduction ability of consortium was studied. Different effluent: biomass ratios of 1:1, 1:2, 1:5, and 1:10 were tried. Agitation was tested simultaneously; flasks were incubated at different rpm (80rpm, 150rpm, 180rpm, 200rpm, and 220rpm). Results suggested that consortium 13 produced the best results when used in the 1:2 effluent: biomass ratio and incubated at 200rpm. The reduction in COD was observed up to 79% after 36h of incubation period. (a) Comparison of percentage degradation of sewage sample after 8h at different shaking speed and by using different inoculum size of consortium 13. (b) Comparison of percentage degradation of sewage sample after 4h at different incubation temperature.

Different temperatures were also studied for better COD reduction. The results reveal that better COD reduction could be achieved in the flask incubated at 35°C as compared to the other flasks incubated at 25°C, 40°C, and 45°C. The selected consortium comprises 3 bacterial isolates found to be capable of reducing COD, BOD, and TSS of sewage wastewater. In order to reduce the time of degradation, the consortium was acclimatized. Table 5 shows the reduction in COD, BOD, and TSS by using selected consortium 13 while reducing time of incubation from 36h to 4h. The reduction achieved after 36h in case of COD, BOD, MLSS, and TSS was from 196–48mg/L, 78–15mg/L, 425–170mg/L, and 546–200mg/L. After 30min of settling, 80% of wastewater was removed and fresh 80% was added, and the sample was incubated for 24h. The results of this cycle show the reduction in COD from 172 to 40mg/L, BOD from 68 to 10mg/L, MLSS 600 to 225mg/L, and TSS from 624 to 198mg/L. In the same manner, settling was done for 30min and incubation time was further reduced to 20hr. Estimation revealed the reduction 152–32mg/L, 62.8–11mg/L, 525–191mg/L, and 569–190mg/L in COD, BOD, MLSS, and TSS, respectively. This reduction in incubation time was carried on further from 12h to 8 and then finally to 4h. The final results show that the percentage degradation was 79% (155–32mg/L), 85.5% (55–8mg/L), 67.6% (500–162mg/L), and 67.1% (578–190mg/L1) for COD, BOD, MLSS, and TSS, respectively, after 4h of incubation.

Phosphorus solubilizing bacteria are reported to dissolve insoluble phosphates by production of inorganic or organic acids and/or by the decrease of the pH (Whitelaw, 2000). Most of the previous reports stated that calcium phosphates are dissolved by acidification. Therefore, any microorganism that acidifies its external medium will show some level of Phosphate Solubilizing Activity (Pradhan and Sukla, 2006). It is well known that Phosphate Solubilizing Bacteria in soil solubilize insoluble phosphates mainly by secreating acids into the medium (Dave and Patel, 2003; Chung *et al*., 2005). Isolates showed maximum P solubilization activity might have used the same mechanism to solubilize the insoluble form of phosphate into soluble form. Similar method was also used by Achal *et al.,* (2007) to analyze the soluble content of phosphate in culture filtrate of *Aspergillus tubingensis* and by Himani and Reddy, (2011) to analyze the soluble content of phosphate in culture supernatant of *Bacillus sp*.

In the present study no relationship could be ascertained with the quantity of P-solubilized and value of pH. These results are in concurrence with those of various other workers who also could not establish any correlation between the quantity of phosphate solubilized and decrease in pH (Dave and Patel 1999; Narsian *et al.* 2000; Sujatha *et al.* 2004). Thus the pH does not seem to be the sole factor responsible for P-solubilization. Phosphatases are enzymes of wide specificity which cleave phosphate ester bonds and this plays an important role in hydrolysis of insoluble polyphosphates and organic phosphates (Yadav *et. al*., 2004). In all the treatments, the phytase activity was observed to be more than phosphatase activity. This may be due to the higher extracellular phytase enzyme activity of selected bacterial isolates as compared to extracellular phosphatase enzyme activity similar to which was reported by Aseri *et al*. (2009) that microbes executes extracellular phytase activity many times more than extracellular phosphatase activity.

The exact mechanism by which PGPR stimulate plant growth is not clearly known, although several mechanisms such as production of phytohormones, suppression of deleterious organisms, activation of phosphate solubilization and promotion of the mineral nutrient uptake are usually believed to be involved in plant growth promotion (Lalande, *et al*., 1989; Glick, 1995). IAA is one of the most important phytohormone and function as important signal molecule in the regulation of plant development. It has been reported that IAA production by PGPR can vary among different species and strains, and also influenced by culture conditions, growth stage and substrate availability (Mirza *et al.,* 2001). Higher level of IAA production by Pseudomonas was recorded by other research workers (Xie *et al*.,1996). Joseph *et al*., (2007) reported that PGPR associated with *Cicer arietinum Pseudomonas* and *Bacillus* species had a high potential to produce siderophores (Joseph *et al*., 2007). Regarding the results of production of IAA, siderophore, protease and HCN, very distinct behaviors were observed among different isolates. Extracellular protease, lipase and HCN can contribute to the ability of bacteria to suppress fungal diseases. Inadequate supply of iron and other metals due to chelation by siderophores may act as a biocontrol agent of some plant disease in their niche (Joseph *et al*., 2007; Loper and Henkels, 1997). This finding showed that PGPR with ability of IAA and siderophore production could improve plant growth.

Soluble bacteria in the nitrogen biosolids by growth hormones, natural enzymes, antibiotics and different compounds such as volatile gasses and sidrophore are capable to develop the aerial parts of plant (Astaraei, and Koocheki, 1997).There are number of sources of ammonia secretion by microorganisms. Ammonia and extracellular proteins are the nitrogenous secretions of nitrogen fixers in nitrogen free or deficient medium (Saribay 2003; Behl *et al*. 2006). Amidases catalyze the hydrolysis of the carboxylic amide bonds to liberate carboxylic acid and ammonia (Asano and Lubbehusen 2000). One of the major mechanisms utilized by PGPR to facilitate plant growth and development is lowering of the ethylene levels by hydrolysis of 1-aminocyclopropane-1- carboxylic acid (ACC), the immediate precursor of ethylene in plants. The enzyme catalyzing this reaction (ACC deaminase) hydrolyzes ACC to α-ketobutyrate and ammonia (Zahir *et al*. 2008).

Some authors consider the production of ammonia to be involved in antagonistic interactions that result in disease control (Saraf *et al*. 2008), however, meticulous experimentation is required to exactly pin point the role of ammonia in influencing the growth of plant and suppressing the diseases. Some of the indigenous microorganisms obtained in the present study possessing high nitrogen fixing and P-solubilizing capacities coupled with high IAA production,

siderophore production and ammonia production might provide them better tools to survive under the local conditions and thus can become good candidate for biofertilizers production.

An attempt was made to characterize the efficient bacteria isolated from the authosphere of plants using 16S rRNA gene sequencing to identify and decipher their phylogenetic affilation of these bacteria. The 16S rRNA gene sequence is about 1,500 bp long and is composed of both variable and conserved regions. Universal primers are usually chosen as complementary to the conserved regions at the beginning of the gene or at either the 540-bp region or at the end of the whole sequence (about the 1,500-bp region), and the in between sequence of the variable region is used for the comparative taxonomy (Chen *et al*. 1989; Relman 1999). The gene is large enough, with adequate interspecific polymorphisms, to allow distinguishing and statistically valid measurements. The 16S rRNA gene serve as molecular chronometer, since it is the most conserved part during evolution (Clarridge 2004). Therefore, 16S rRNA gene sequencing is used and accepted worldwide for identification and phylogenetic analysis of the bacterium. Sequence data of 16S rRNA gene of eight efficient strains obtained through automated sequencer using eubacterial universal primers revealed 1366 bp partial sequence in isolates.

The test bacterial isolates clustered with members of the genera *Stenotrophomonas, Bacillus, Brevibacterium,Micrococcus, Azospirillum, Acinetobacter Pseudomonas* and *Burkholderia*, thus differentiating the bacterial isolates on the genetic basis. Researchers have also reported the isolation of these genera from the authosphere of various crop plants (Chan *et al*. 1994; Estrada-de Los Santos *et al*. 2001; Minkwitz and Berg 2001; Vessey 2003; Bashan *et al*. 2004).Various other workers also used this technique for identification and phylogenetic analysis of the isolates (Catara *et al*. 2002; Khan and Doty 2009; Islam *et al*. 2010). Presence of various genera in the agricultural soils shows the extent of microbial diversity existing in these specialized niches of different plants. Similar observations were made by Louw (1970) and Thakkar *et al.* (1993). Results of this study showed that in the agricultural soils, most of the bacterial isolates exhibited various traits like phosphate solubilization, IAA production, ammonia production and siderophore production, and the predominant genus was of *Pseudomonas*. Dominance of this genus in the root zones of various crops has also been reported by other workers (Parmar and Dadarwal 1997; Vazquez *et al.* 2000; Saxena and Sharma 2003).

Therefore, in the present study the observance of *Pseudomonas and Bacillus* as the dominant native flora reflects that these organisms are probably adapted to the agroclimatic conditions of Ilorin in a better way and thus need to be exploited for the preparation of bioinoculants. Although *Stenotrophomonas* and *Burkholderia* spp. occurs ubiquitously in the environment, soil and plants are their main environmental reservoirs and several studies subsequently demonstrated that these two genus are capable of great metabolic versatility (Tabacchioni *et al*. 2002; Ryan *et al.,* 2009). *Burkholderia* spp. were for many years included in the genus *Pseudomonas* owing to its broad and vague phenotypic definition. However, rRNA–DNA hybridization analyses during the early 1970s indicated considerable genetic diversity among members of this genus (Compant *et al*., 2008). Therefore, to get reliable and accurate identification of bacterial isolates, molecular characterization (16S rRNA gene sequencing) is an important tool.

The present study clearly confirmed that selected isolates effectively enhance the plant growth total P uptake and soil fertility in all the treatments as compared to conrol soil treatment. Many studies in relation to crop improvement by PGPR were carried out either in pot cultures or field conditions (Whitelaw *et al*. 1997; Saber *et al*. 2009 and Omar, 1998). Microbial populations are key components of soil plant entity where they are associated in a framework of interactions affecting plant development (Vassilev *et al*., 2006). In the present study significant increase in plant growth was recorded with the inoculation of selected PGPR strains. The microorganisms involved in plant growth promotion can enhance plant growth by increasing the availability of plant growth promoting substances and other trace elements. There was significant increase in the height of groundnut crops in all the treatments compared to control. Similarly increase in shoot and root dry weight was significantly increased in all the treatments in comparison to that of control. PGPR may effectively increase the surface area of roots (Bashan *et al*., 2004) and the root weight (Bertrand *et al*., 2001). Similar findings were reported by Puente *et al*. (2004). This shows

higher nutrient uptake by inoculated roots significantly improved seedling growth. Kundu and Gaur (1984) also reported, increased P uptake and plant growth in various crops inoculated with PSMs. PSB are also reported to produce metabolites such as phytoharmones which aid in plant growth (Kloepper *et al*., 1989).

Thus, the inoculation of these bacterial isolates considerably increased P uptake and enhanced plant growth as compared to uninoculated soil. These results could be attributed to the ability of these microorganisms to solubilize phosphorus already present in the soil. Son *et al*., (2003) reported that, phosphate solubilisation was mainly due to the acidification of the culture by bacteria; however high level of phosphate solubilisation may not be achievable in soil because most soils have a great pH buffering capacity. Moreover, the final pH did not reduce to strongly acidic levels. It is known that the production of organic acids by soil microorganisms and commensurate pH decrease is the major mechanism of phosphate solubilisation (Whitelaw *et al*., 1999). The available P increased in the soil having bacterial inoculums as compared to control. P solubilising microorganisms can solubilise and mineralize P from organic and inorganic pools of total soil P and may be used as inoculants to increase P availability to plants (Kucey *et al*., 1989; Richardson, 2001; Illmer *et al*., 1995; Whitelaw *et al*., 1999). Phosphate solubilising bacteria are slowly emerging as important organisms used to improve soil health as *in vitro* studies have demonstrated that they reduce P deficiency in soil (Yosef *et al.,* 1999). Thus, the P content was more in bio inoculated treatments. Previous studies involving plants inoculated with PSMs showed growth enhancements and increased P contents but large variations were found (Kucey *et al*., 1989).

The organic carbon level was significantly increased in all the treatments in comparison to the initial values. Our results are in agreement with Himani and Reddy (2011) who reported an increase in soil organic carbon level in bio-inoculated and RP fertilized soil as compared to control soil. Little decrease in soil pH was observed in all the treatments compared to control soil. Inorganic P is solubilized by the action of organic and inorganic acids secreted by P-solubilizing bacteria in which hydroxyl and carboxyl groups of acids chelate cations and decreases the pH in basic soils and increases the concentration of phosphorous in soil (Stevenson 2005). Soil enzymes have been suggested as potential indicators of soil quality because of their relationship to soil biology, ease of measurement, and rapid response to changes in soil management (Dick *et al.,* 1996). Inorganic P is released from organic matter by hydrolysis of C-O-P ester bonds by phosphatases, which are therefore important in the P nutrition. Activities of enzymes such acid phosphatase and phytase in all the treatment soils were higher than the control soil. In all the treatments, the phytase activity was observed to be more than phosphatase activity. This may be due to the higher extracellular phytase enzyme activity of selected bacterial isolates as compared to extracellular phosphatase enzyme activity similar to which was reported by Aseri *et al*. (2009) that microbes executes extracellular phytase activity many times more than extracellular phosphatase activity.

This statement is in agreement with our findings which showed that in laboratory conditions extracellular phytase activity was more pronounced than extracellular phosphatase activity by selected isolates. Higher enzyme activities in soils indicated the potential of soil to affect the biochemical transformations necessary for the maintenance of soil fertility (Rao et al. 1990).All the results together suggested that the treatments of maize crops with PGPR improved the crop growth, yield and soil field experimental conditions. After harvesting the viability of inoculated P solubilizing bacteria were tested and it was found that there was significant increase in the viability of bacteria which was 3.7×10^7 to 2.4×107 in all the inoculation treatments compared to control soil. In control soils where inoculums was not added, it was found no halozones were present around the grown colonies which suggested that phosphate solubilizing bacteria were absent in the control soils.

Conclusion

There are many species of bacteria which are found in soil reported to promote plant growth by producing growth regulators, inducing root exudation and enhancing the availability of nutrients to plant, besides controlling soil borne plant pathogen. The means by which PGPR enhance the nutrient status of host plants can be categorized into four area. biological nitrogen fixation, inducing root surface area, enhancing other beneficial symbiosis of the host, and

combination of modes of action. The roots of leguminous plants are colonized by numerous microorganisms and which cause definite influence on the survival and nodulation ability of seed inoculated . Authocthonous microorganisms may not only influence the inoculated rhizobia adversely through saprophytic competition, but also help them in survival through synergism resulting in an increase in their nodulation ability and N2 fixing efficiency.

In the present study we have isolates the PGPER from agricultural soil. A total of 8 bacterial isolates were selected based on their growth on Pikovskayas agar plates. Isolates were quantitatively analysed for their P solubilization activity in different soluble form of P (rock phosphate, aluminium phosphate and ferrous phosphate). Four selected isolates significantly released the soluble P in the PKV broth with decreased in pH of the medium. All the isolates were positive for acid, alkaline phosphatses and phytase enzyme production. Bacterial isolates were further tested for their plant growth promotion activities. All the selected isolates were positive for siderophore and HCN production. Selected isolates were tested for their indole acetic acid production with and without tryptohane and were showed positive results. It was found that these phosphate solubilizing strains along can substitute the chemical fertilizer, might be used to reduce the alkalinity of soil by neutralization phenomenon through organic acid exudation and can survive in the soil system to retain the phosphate solubilizing potential for long time.

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