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METABOLIC FINGERPRINTING OF BACTERIAL STRAINS ISOLATED FROM NORTHERN AREAS OF PAKISTAN

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Abstract

The diversity of Plant Growth Promoting Rhizobacteria (PGPR) in the rhizosphere plays a key role in the maintenance of sustainable agricultural system. In this study, samples were obtained from northern areas of Pakistan. Thirty bacterial strains were isolated, purified, characterized biochemically and subjected to the metabolic fingerprinting by performing nitrogen fixation, phosphate solubilization, protease, indole acetic acid (IAA) production, antibiotic susceptibility and heavy metal resistance test, lead acetate assay for the H_2S production. Strains showing distinct characteristics were further characterized by 16S rDNA sequencing and characterized as *Bacillus pumilus* (KT273321), *Acinetobacter baumanii* (KT273323), *Acinetobacter junii (*KT273324), *Pseudomonas aeruginosa* (KT273325), *Bacillus circulans* (KT273326) *and Bacillus* cereus (KT273327). As most of the strains show positive results for resistance against heavy metals, phosphate solubilization, nitrogen fixation, IAA production, and so these strains might be utilized for the removal of heavy metals from the ecosystem as well as biofertilizer in agriculture lands of northern areas

Key words: Biofertilizer; Lead acetate; Nitrogen fixation; *Pseudomonas*; Phosphate solubilization.

Introduction

Microorganisms are ubiquitous in nature. They are found everywhere and live almost in every kind of habitat including terrestrial, aquatic, and atmospheric. It is because of the diversity of microorganism that enables them to survive in extremely cold or hot environment. Microorganisms also exert beneficial effect into the environment. Temperature has a strong effect on microbial diversity. The diversity of microorganisms in tropical areas is different from temperate areas. The abundance and types of microorganisms present in cold areas are different from plain and forest areas. Cold environment are main habitat for psychrophiles including bacteria, yeasts and fungi (Petrova *et al.*, 2009).

In cold environment, microorganisms have to face specific challenges that include limited water supply and nutrients, reduced activation of enzymes, and extremes in pH. To survive successfully in extreme environment, microorganisms have naturally evolved a structural and functional adaptations including changes in their metabolic activities (Christner, 2010). Microorganisms abundant in cold environment demonstrated the dominance of gram positive bacteria, especially *Actinobacteria* and *Firmicutes* (Willerslev *et al*., 2004).

The rhizosphere is the area around plant roots that includes the variety of microbes which are influenced by great majority of stresses (Johri *et al.*, 2003). Rhizobacteria are usually present adjacent to the roots or on its outer plane and play a critical role in various soil biochemical processes such as atmospheric nitrogen fixation, phosphorus solubilization, siderophores production that chelate iron and synthesis of plant growth regulators (Gothwal *et al.*, 2008). Substantial amount of nitrogen fixing and phosphate solubilizing bacteria present in soil and plant rhizosphere which enhance the plant yield and replace the chemical fertilizers, can be used as biofertilizer to enhance growth of plant (Zaidi *et al.*, 2014). The application of the fertilizers can cause serious harmful environmental pollution. One way to overcome the potential harmful effect of fertilizers is to replace them with biofertilizer i.e. plant growth promoting rhizobacteria (PGPR). Different genera are commercialized that are used in the improvement of the plant growth in modern agriculture system (Adesemoye *et al.*, 2009).

One of most important mechanism of PGPRs is to synthesize IAA which enhance the growth of plant (Hsu, 2010). IAA is a secondary metabolite produced by microorganism by the conversion of tryptophan into (IAA) by IAM-hydrolase in tryptophan dependent pathway.

Generally, heavy metals are not removed biologically and remained in the environment for an indefinite period. However, these metals become toxic to human and also effect the microbial communities and their metabolic activities at high concentration (Hookoom & Puchooa, 2013). In addition, the greater amount of metals in the soil is harmful for the growth of plants and subsequently reduces the yields of crop. The remediation of metal-polluted soils thus becomes essential for the maintenance of sustainable agricultural system (Eckford *et al.*, 2002). Bioremediation based on microorganisms offer a cost effective and environmental friendly method for cleaning of metals (Eckford *et al.*, 2002). Microorganisms exhibit different type of systems to cope with the elevated concentration of these heavy metals that are usually specific to a particular metal (Chien *et al.*, 2008). Microorganisms have adopted different mechanisms in order to tolerate high concentration of heavy metals either by removing them through efflux systems, or by reduction in metal ions concentration by using these metals as an electron acceptor in different respiration reactions (Haferburg & Kothe, 2010).

Biofertilizer refers to the microorganisms which enhance the yield of plant by supplying adequate nutrient to the plants. According to the climate and agricultural conditions, it is necessary to select appropriate strains as biofertilizer. For a particular area, climatic conditions and soil characteristics vary widely, so a great variety of strains as bio-fertilizers needs to be isolated from the specific area. The diversity and metabolic profiling of microorganisms in a particular area is different from the diversity of

microorganisms in other areas. The climate condition of northern areas of Pakistan is different from the climate condition of plain area of Punjab and Sindh. The bacterial strains isolated from the northern areas have different genetic and metabolic characteristics. The northern areas were selected for this research work because no such type of work has been reported yet from these areas. The isolated strains can be used as a biofertilizer in the same area for sustainable agriculture as well as for bioremediation of heavy metals such as mercury to reduce the environmental pollution.

Material and Methods

Isolation and purification of bacterial strains: Twelve samples including soil and water were collected from different regions of northern areas of Pakistan such as Khanaspur, Nathia gali, Ayubia, Siri Lake, Murree, Kali Matti, Namli mari, Barkha gali and Naran. For the isolation of bacterial species from different samples, nutrient agar medium (Cappuccino & Sherman, 2004) was prepared. Soil samples were serially diluted in the test tubes and 50µl of each dilution was spread on agar plate with the help of spreader under aseptic conditions and 50µl of water samples were spread directly as a control on agar plate. The plates were incubated for 24 h at 37ºC. Distinct colonies were purified by sub-plating on the same medium. 30% glycerol stocks of isolated colonies were prepared and preserved at -20ºC for further use.

Biochemical characterization of bacterial strains: Bacterial strains were identified by subjecting to different morphological and biochemical tests, i.e., gram staining, catalase, oxidase, starch hydrolysis, citrate utilization and mannitol test (Cappuccino & Sherman, 2004).

Metabolic fingerprinting of selected bacterial strains

Screening of nitrogen fixing bacteria: A selective nitrogen free mannitol (NFM) agar medium (Okon *et al.*, 1977; Rafique *et al.*, 2015) was prepared for the screening of nitrogen fixing bacteria. Overnight grown culture of bacterial strains in nutrient broth containing 0.5% peptone and 0.3% beef extract were streaked on NFM plates in the form of grid and incubated at 37ºC for 48-72 h (Ahmad *et al.*, 2008).

Phosphate solubilization test: The potential of isolated bacterial strains for phosphate solubilization was determined on the basis of halo zones formation on National botanical research institute phosphate (NBRIP) agar plates (Linu *et al.*, 2009). Overnight grown cultures in LB broth were spotted on NBRIP agar plates and incubated at 37ºC up to five days. The isolates were examined for the presence of a halo zone of phosphate solubilization around the colony (Nautiyal, 1999).

Protease test: The activity of isolated bacterial strains for the production of protease enzymes was checked by spotting onto skim milk agar (Kazanas, 1968). After incubation at 37ºC for 48 h, plates were examined for the development of clear zones around each bacterial colony (Kumar *et al.*, 2004).

Hydrogen cyanide (HCN) production test: Test for the production of HCN by isolated bacterial strains was performed by following the method of Castric and Castric (1983). Isolates were streaked on nutrient agar plates containing 4.4g glycine l^{-1} (Kremer & Souissi, 2001). A Whatmann filter paper No. 1 immersed in 2% sodium carbonate in 0.5% picric acid solution was placed inside the lid of petri plate. All inoculated plates were sealed carefully with parafilm in order to prevent the release of gaseous metabolite (HCN). A nutrient agar plate with bacterial inoculation was used as a control. All plates were incubated at 37ºC for 5 days and observed for color change in the filter paper padding.

Colorimetric method for the determination of IAA: In order to estimate the IAA production by selected bacterial strains, a colorimetric method using Salkowski's reagent containing 50 ml of 35% per chloric acid and 1ml of 0.5M ferric chloride was used (Ehmann, 1977). Four days old, 1.5 ml of each bacterial culture grown in yeast extract mannitol (YEM) (Sahasrabudhe, 2011) was centrifuged at maximum speed. The supernatant was shifted to a clean test tube and 2 ml of Salkowski's reagent was added in it and kept in dark for 20-30 minutes for color development. After incubation in dark, optical density was measured at 535nm and IAA concentration in all the samples were determined by using the standard curve generated from serial dilution of IAA stock solution (Ahmad *et al*., 2016)

Heavy metal resistance test: Metal resistance test of selected bacterial strains on nutrient agar plates against mercury, chromium, cadmium, and lead was carried out by following the method of Tariq & Latif (2014). The solution of each metal (50 and 100µg ml^{-1}) was poured (20 µl) into each well marked on the plate. After incubation at 37ºC for 24 h, metal resistance or susceptibility was assessed by measuring the inhibition zone diameter in mm.

Qualitative detection of hydrogen sulfide (H_2S) production: H_2S production was done by spotting bacterial strains in a grid pattern in replica on lead acetate medium Amin & Latif (2011). A plate without lead acetate was used as a control and spotted in the similar pattern as in experimental plates. The plates were incubated at 37ºC for 48-72h. After incubation, blackening of colonies were observed indicating the presence of H_2S production (Amin & Latif, 2013).

Antibiotic susceptibility test on bacterial isolates: Antibiotic susceptibility pattern of bacterial isolates was performed with Ceftriaxone and Cephalosporin (Sarwar & Latif, 2015). The solution of both antibiotics (50 and 100 µg ml^{-1}) was prepared as recommended by Roche manufacturer. The bacterial culture was spread on Muller Hinton agar plates containing 38g Muller Hinton l^{-1} (Varghese, 2015) and wells (5mm in diameter) were made on each plate with the help of sterile borer. Each antibiotic solution was poured (20 ul) into the well under aseptic conditions and plates were incubated at 37ºC for 24 h. Antibiotic susceptibility pattern was assesses by measuring the inhibition zone diameter in mm.

Molecular characterization of selected bacterial strains by 16S rDNA sequencing: Seven bacterial strains (AZ-1, AZ-2, AZ-3, AZ-4, AZ-5, AZ-6, AZ-7) were selected for molecular characterization through 16s rDNA sequencing. DNA from 24 h old cultures of selected bacterial strains was isolated by using the Thermo Scientific Genejet Genomic Purification Kit (catalogue #K0721). By using universal primers F (5′AGAGTTTGATCCTGGCTCAG′) and R (5′AAGGAGGTGATCCAGCCGCA3′) (Normand, 1995), amplification of 16S rRNA gene was performed and amplified products were forwarded to Macrogen sequencing services at Korea. The resulted sequences were matched with nucleotide BLAST on NCBI for identification and were also submitted to GenBank for accession numbers.

Phylogenetic analysis of selected bacterial strains: Phylogenetic analysis of selected isolates was carried out using MEGA 6 software and phylogenetic tree was constructed by aligning the sequences using multiple sequence alignment tool through neighbor joining method with Bootstrap value 1000 (No. of data sets).

Statistical analysis: Results of antibiotic susceptibility and IAA production test were done in triplicate and data were subjected to mean, standard error and analysis of variance (ANOVA). Data was calculated in mean ± SE of each replicate and then comparison was performed by using Duncan's Multiple range test (DMRT).

Results

Isolation and biochemical characterization of purified bacterial strains: Thirty bacterial strains were isolated from different soil and water samples as shown in Table 1. Purified bacterial strains were observed for colony morphology and other biochemical test for their preliminary characterization.

For metabolic fingerprinting, bacterial strains were subjected to other specific tests including nitrogen fixation, phosphate solubilization, protease, hydrogen cyanide, H_2S and IAA production, antibiotic susceptibility and heavy metal resistant tests. Bacterial strains were screened out for nitrogen fixing ability. Only one bacterial strain (AZ-14) could not grow on NFM while the remaining bacterial strains showed their growth on NFM under optimum conditions (Fig. 1).

In phosphate solubilization test, formation of halo zone in NBRIP medium surrounding growth of bacterial colonies was indication of positive results. Eighteen bacterial strains showed holo zones in NBRIP medium while 12 strains showed negative results for phosphate solubilization. Protease production test identifies the ability of bacterial strain to hydrolyze casein (protein) with the help of enzyme protease. For this test clear zone were observed around the bacterial growth on skim milk agar plates. All bacterial strains were positive for this test as clear zone were observed around the bacterial growth (Fig. 2).

Isolated bacteria strains were examined for hydrogen cyanide (HCN) production that functions as antifungal agent in order to enhance the plant growth. The production of HCN is determined normally by change in color of filter paper from cream to dark brown. All the bacterial strains showed negative results for HCN production, as the color of filter paper remained the same (cream) after incubation.

All bacterial strains were found to be positive for IAA production ranging in concentration from 0.5 to 8 µg ml^{-1} as determined by the development of red color after reaction with Salkowski reagent. Only ten bacterial strains were producing more than 5µg ml^{-1} IAA (Fig. 3b) whereas sixteen were producing less than 2.5µg ml^{-1} IAA (Fig. 3a) and remaining intermediate level (Fig. 3c).

Heavy metal resistance and H_2S production test: Resistance of heavy metal in bacteria reflects the degree of environmental contaminations. For this purpose, bacteria were subjected to three heavy metals (Chromium, Lead, and Mercury in the form of mercuric chloride) (Fig. 5). Only four bacterial strains (AZ-3, AZ-5, AZ-13, and AZ-27) were resistant to both concentration of $HgCl_2$ (50 µg and 100 µg ml^{-1}) and almost all bacterial strains were resistant to both concentration (50 µg and 100 µg ml^{-1}) of lead and chromium. It is already reported that H_2S aids in the volatilization of methyl mercury and reduces its toxicity (Amin & Latif, 2011). Results indicate that all those bacterial strains which showed resistance to $HgCl_2$ are also H_2S producers (Fig. 4).

Fifteen bacterial strains displayed blackening in their colonies on LA medium (Fig. 5). Bacterial strains that are H_2S producers exhibited black (dark brown) colored colonies due to the formation of PbS (lead sulfide) while strains that are non H_2S producers showed white colored colonies.

Table 1. Isolated and purified bacterial strains from different sources (Northern areas of Pakistan).

Serial No.	Sampling source	Number of bacterial isolates	Labels of isolates
1.	Siri Lake 3 water	3	AZ-9,AZ-10,AZ-11
2.	Siri Lake 5 water	2	AZ-12.AZ-13
3.	Khanspur water	2	AZ-14 , AZ-15
4.	Barkha gali soil	3	AZ-3 , AZ-16, AZ-17
5.	Nathia gali soil	2	AZ-1, AZ-18
6.	Nathia gali water	2	AZ-6, AZ-19
7.	Ayubia soil	2	AZ-20,AZ-21
8.	Murree soil	5	AZ-2, AZ-7,AZ-22,AZ-23,AZ-24
9.	Namli mari water	1	AZ-8
10.	Kali matti soil	3	AZ-4,AZ-25,AZ-26
11.	Naran soil (Muree highway)	2	AZ-5,AZ-27
12.	Naran water	3	AZ-28,AZ-29,AZ-30

Fig. 1. Growth of bacterial strains on NFM medium indicate their ability to fix nitrogen.

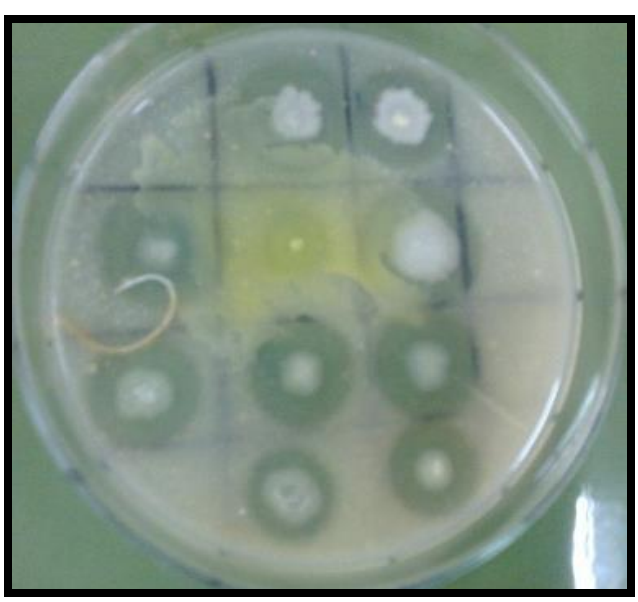

Fig. 2. Protease test result of bacterial strains. Holo zones formation by the hydrolysis of casein protein indicate the production of protease.

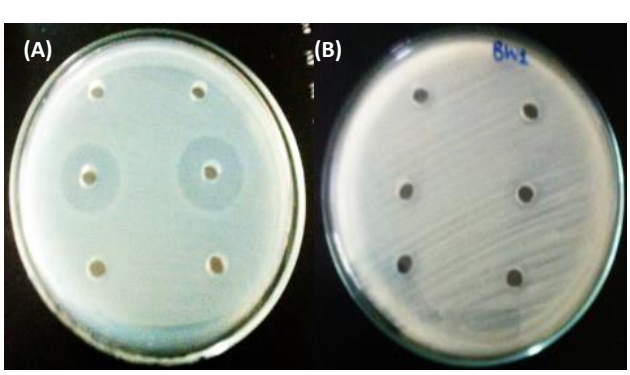

Fig. 4. Bacterial resistance against heavy metal in well plate method. A, C, E= 50µg ml^{-1} whereas B, D, F = 100µg ml^{-1}, $K_2Cr_2O_7$= Potassium dichromate, $HgCl_2$=mercuric chloride, $PbCl_2$=lead chloride respectively. Clear zones around C and D (5A) indicate the sensitivity of bacterium to $HgCl_2$.

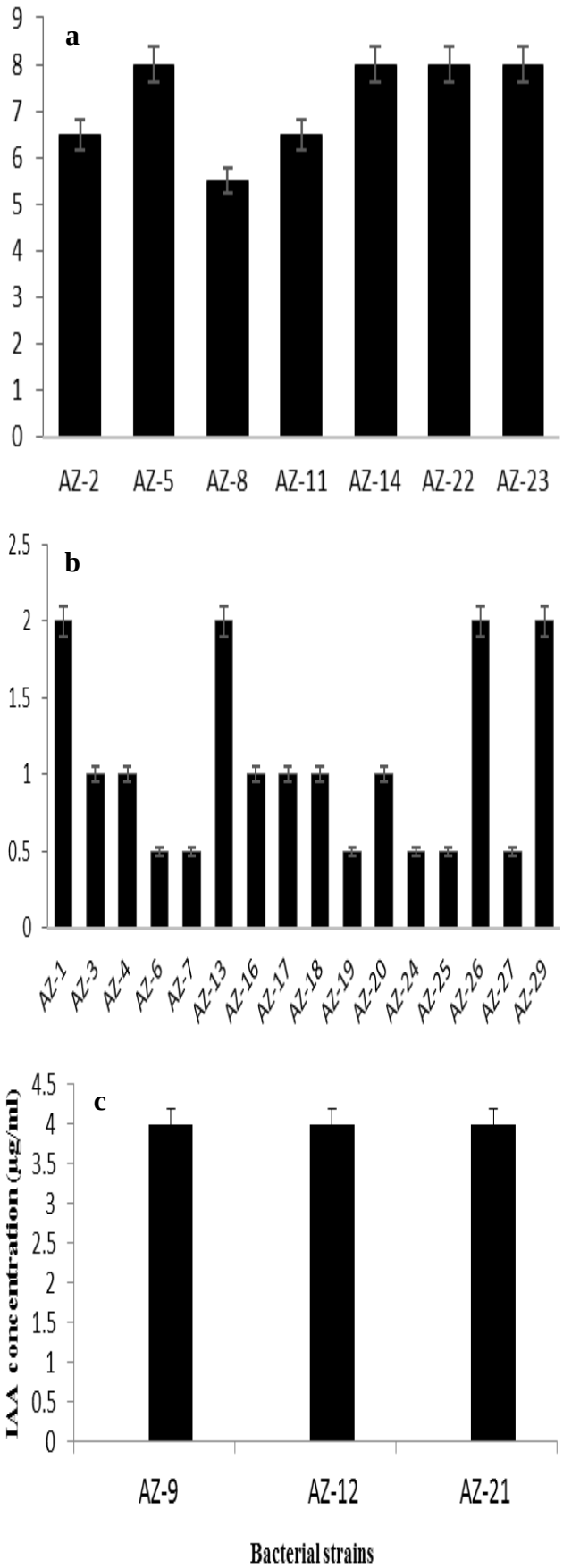

Fig. 3. Quantification of IAA production by different bacterial strains. (3a) Bacterial strains producing more than 5µg ml-1 IAA (3b) bacterial strains producing less than 2.5µg ml-1 and (3c) bacterial strains producing intermediate level of IAA.

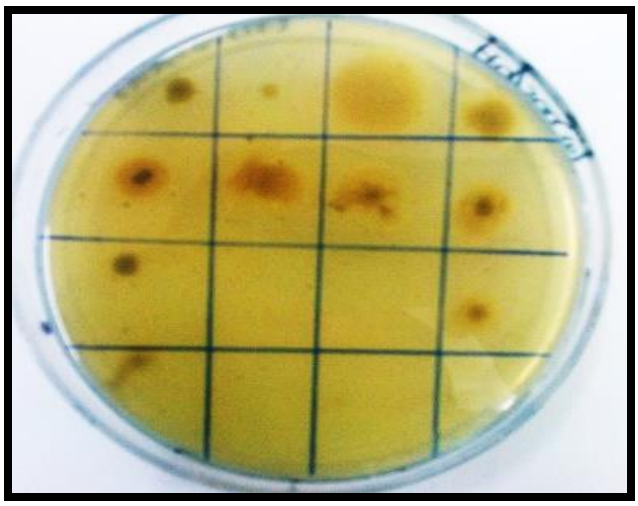

Fig. 5. H_2S production test on lead acetate medium. Production of H_2S was indicated by blackening around the bacterial colonies which is due to the formation of lead sulphide (PbS).

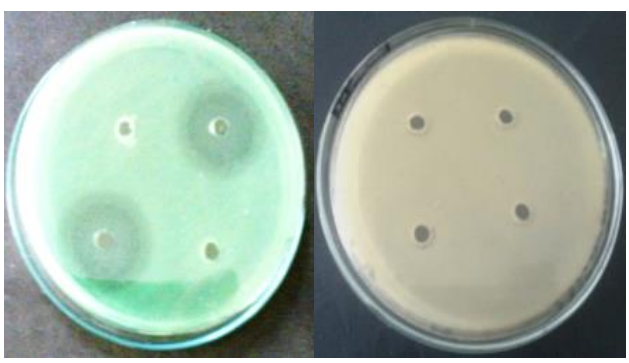

Fig. 6. Response of bacterial strains to both concentrations of antibiotics (Ciprofloxacin and Ceftriaxone) (A) AZ-4 (*Acienetobacter junii*) All resistant; (B) AZ-6 (*Pseudomonas aeruginosa*) sensitive to 100 µg ml^{-1} of both antibiotics.

Antibiotic susceptibility testing against cephalosporin and ceftriaxone: Antibiotic susceptibility test with two antibiotics Ceftriaxone and Cephalosporin was performed against different bacterial strains effective in stopping the growth on MH agar plates. Most of bacterial strains were resistant to 50 µg ml^{-1} drug except for three strains which were sensitive to only Ciprofloxacin (50 µg ml^{-1}) and produce the zone of inhibition measuring 19 mm for AZ-15, AZ-17 and AZ-30 but most of the strains were susceptible for 100 µg ml^{-1} drug producing variable range (16 to 30 mm) of inhibition zone (Fig. 6). Only three bacterial strains (AZ-4, AZ-7, and AZ-8) were completely resistant for both concentrations of drugs (Figs. 7 and 8).

Phylogenetic analysis of selected isolates on the basis of 16S rDNA sequencing method: Seven bacterial strains (AZ-1, AZ-2, AZ-3, AZ-4, AZ-5, AZ-6, and AZ-7) were subjected for molecular characterization on the basis of their ability to fix nitrogen, production of IAA, phosphate solubilization, H_2S production and mercury resistance. Blast query revealed that bacterial strains AZ-1 and AZ-2 *Bacillus pumilus* (KT273321 and KT273322) respectively were 76% homologous to each other and 65% homologous to *Bacillus pumilus* (EU5945521 and KU844052.1) which are 99% homologous to each other. But in combined form clade 1 showed 41% similarity with clade 2 which included *Bacillus cereus* (KJ812465.1 and KT600324.1) which were 100% homologous to each other but 81% homologous to AZ-7 *Bacillus cereus* (KT273327). In clade 3, strain AZ-5 *Bacillus circulans* (KT273325) was found to be 77% homologous to *Bacillus circulans* (NR 112632.1 and NR 104566.1) which were 76% homologous to each other and showed 99% homology to clade 1. In clade 4 strain AZ-6 *Pseudomonas aeruginosa* (KT273326) was found to be 99% homologous to *Pseudomonas aeruginosa* (NR 117678.1). This clade 4 had 100% similarity with other four clades. In clade 5, strain AZ-3 *Acinetobacter baumanii* (KT273323) showed 61% similarity with *Acinetobacter baumanii* (NR 074737.1 and NR 117677.1) but are 73% homologous to each other. This clade showed 61% similarity with clade 6 which include AZ-4 *Acinetobacter junii (*KT273324) and 73% homologous *A.junii (*NR 117623.1). Both clades collectively showed 100% homology to clade 4 and other clades in the tree (Fig. 9).

Discussion

A great diversity of microorganisms present in the soil enhance the growth of plants by variety of mechanism. The beneficial effect of these microorganisms can be derived from their metabolic activities in the environment, their association with plants and provide benefit to them by enhancing their growth and their use as biofertilizer to replace the artificial fertilizer for sustainable agricultural system. It is reported that these microorganisms as plant growth promoting rhizobacteria (PGPRs) are commonly used as inoculants for improving the growth and yield of agricultural crops and replace the need of chemical fertilizers (Sivasakthi *et al.*, 2014).

PGPRs can also be used to promote the plant growth by observing different methods like phosphate solublization, IAA production, nitrogen fixation and HCN production. PGPRs are reported to be used as an alternative of chemical fertilizers to provide protection against the pathogens (Bhardwaj *et al.*, 2014).

In addition to be used as PGPRs, wide variety of microorganisms are being used for the purpose of bioremediation. This process can be applied to remediate specific pollutants such as heavy metals (mercury) which are neurotoxin and potent damage for human life. It is already reported that majority of heavy metals can lead to poisoning at higher concentration (Kavamura and Esposito, 2010). Bioremediation by microorganisms is a natural process and cost effective as compared to traditional methods. It is reported that *Ochrobactrum* and *Pseudomonas* species have been used for the bioremediation of environmental pollutants (Cheng *et al.*, 2010; Pandey *et al.*, 2013). These observation support the current study to use these strains for the purpose of bioremediation in contaminated soil.

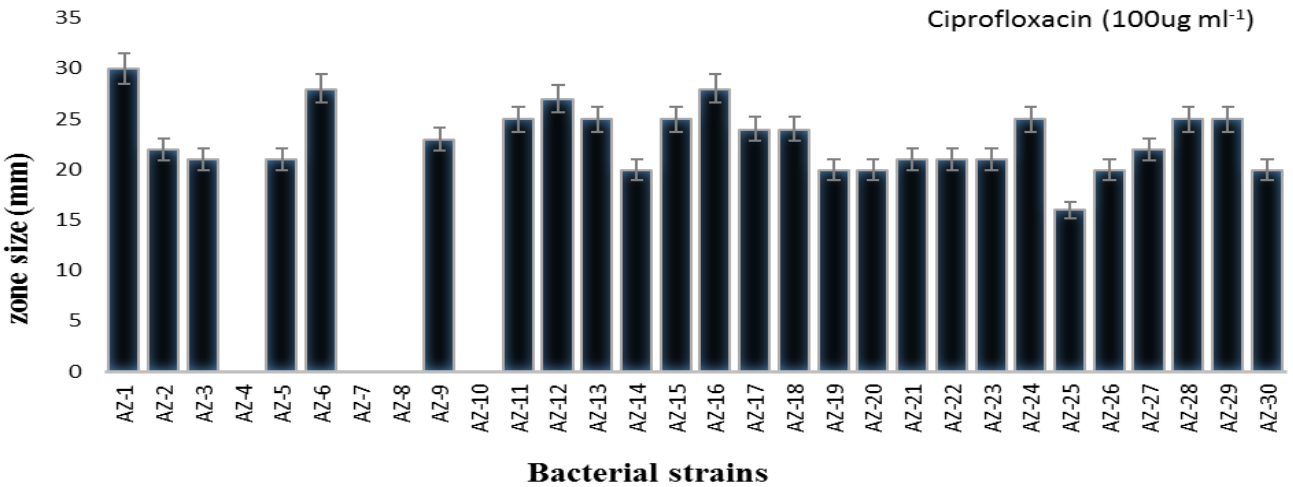

Fig. 7. Measurement of zone of inhibition in diameter (mm) at a final concentration (100µg ml-1) of ciprofloxacin. Bars represent means ± standard deviation (SD). Each was replicated three times (p<0.05)

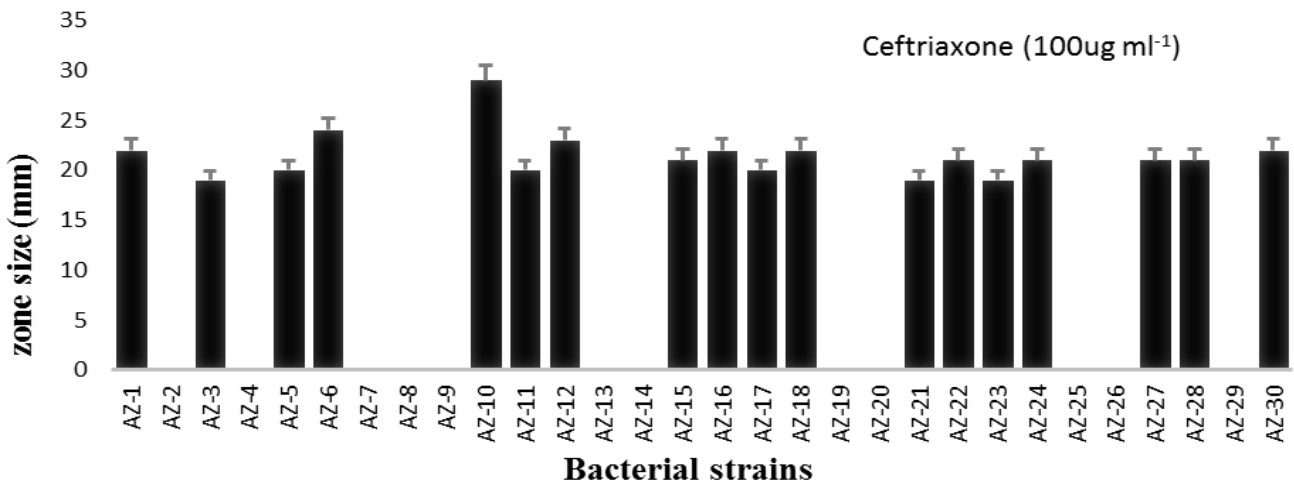

Fig. 8. Measurement of zone of inhibition in diameter (mm) at a final concentration (100µg/ml) of ceftriaxone. Bars represent means ± standard deviation (SD). Each was replicated three times (p<0.05).

In the current study, different soil and water samples were taken from different sources and used to purify bacterial cultures by growing on simple N-agar medium. Then the culture were screened for their ability to fix nitrogen. Selected isolated cultures were further screened by investigating their potential to remediate mercury by using heavy metal resistance test against different concentration of $HgCl_2$. Biochemical characterization was performed by different tests including catalase, oxidase, starch hydrolysis, citrate utilization and mannitol salt agar. Bacterial strains were further subjected to additional tests like HCN and H_2S production test.

Majority of the isolates produce positive result for H_2S production test and gave black colonies on lead acetate plates. It was found that almost all the strains which were resistant to mercury produced H_2S (Linderholm *et al.*, 2008). It is already mentioned that H_2S aids in the reduction of methyl mercury and convert it to a toxic state to a nontoxic state with the help of mercuric reductase enzyme. Selected bacterial isolates exhibited black (dark brown) color colonies on lead acetate plates due to the formation of lead sulphide (PbS).

Nitrogen fixation and phosphate solubilization tests of selected bacterial strains were carried out to determine the ability of isolates to fix nitrogen and solubilize phosphate respectively. Both are important feature of PGPRs and therefore can be used to enhance the crop yield for sustainable agricultural system. Almost all bacterial strains showed positive result for both test; they are agronomically important because such microbes fix nitrogen and utilize insoluble phosphate from the soil and make it available to plant by converting it into soluble form. Most of the genera that showed positive results for these tests included *Pseudomonas* and *Bacillus* species. Mohammadi (2012) has reported that bacterial strains from genera *Pseudomonas* and *Bacillus* play an efficient role in phosphate solubilization that support the current study for the maintenance of sustainable agriculture system. In addition to *Pseudomonas* and *Bacillus*, *Rhizobium* is also reported as a more potent phosphate solubilizers (Han & Lee, 2006). All the strains showed positive result for protease test also. In this way these bacteria can be used as protein degrader.

The ability of selected bacterial strains to produce IAA was measured by colorimetric method. Most of the strains showed promising results for IAA production. It is

mentioned in literature that inoculation of plant with IAA producing bacteria increases the yield of plant by proliferation of lateral roots and root hairs (Mohite, 2013). Fatima *et al.* (2009) also showed that germination rate, roots, shoot growth of plant were increased by IAA producer PGPRs. This study is similar to our results as most of the strains are good producer of IAA and can be used in agriculture system for enhancing the crop yield.

Bacterial strains were also subjected to heavy metal resistance test. Four metals (chromium, lead, mercury and cadmium) were used to check the ability of bacteria for their resistance. Majority of the strains in this study are resistant to these heavy metals, so they can be used as a potent source of bioremediation in contaminated soil as it is already reported that bacteria resistant to these metals exhibited a critical role in the biogeochemical cycling of heavy metals and thus play an important role in bioremediation (Duruibe *et al.*, 2007). In additon to heavy metals, bacterial strains were also subjected to antibiotic susceptibility test against two concentration (50 and 100 µg ml^{-1}) of Ciprofloxacin and Ceftriaxone. Majority of the strains showed resistance to these antibiotics.

Different bacterial strains isolated in this study are beneficial with respect to environmental perspective as they have the ability to resist heavy metals in their surrounding environment. If use in combination, these strains can not only resist mercury but can also have the potential to synthesize high concentration of IAA in order to enhance the plant yield. So in conclusion, these strains can be used as an agent for bioremediation because of their ability to resist mercury in one way and as PGPRs in the other way as their ability to enhance the plant growth. These selected bacterial strains can be used in agricultural areas contaminated with mercury. In this way these strains can not only remediate mercury from the environment but can also allow plants to grow well due to their capability of nitrogen fixation, phosphate solublization and most importantly to synthesize greater amount of IAA which is plant growth hormone.

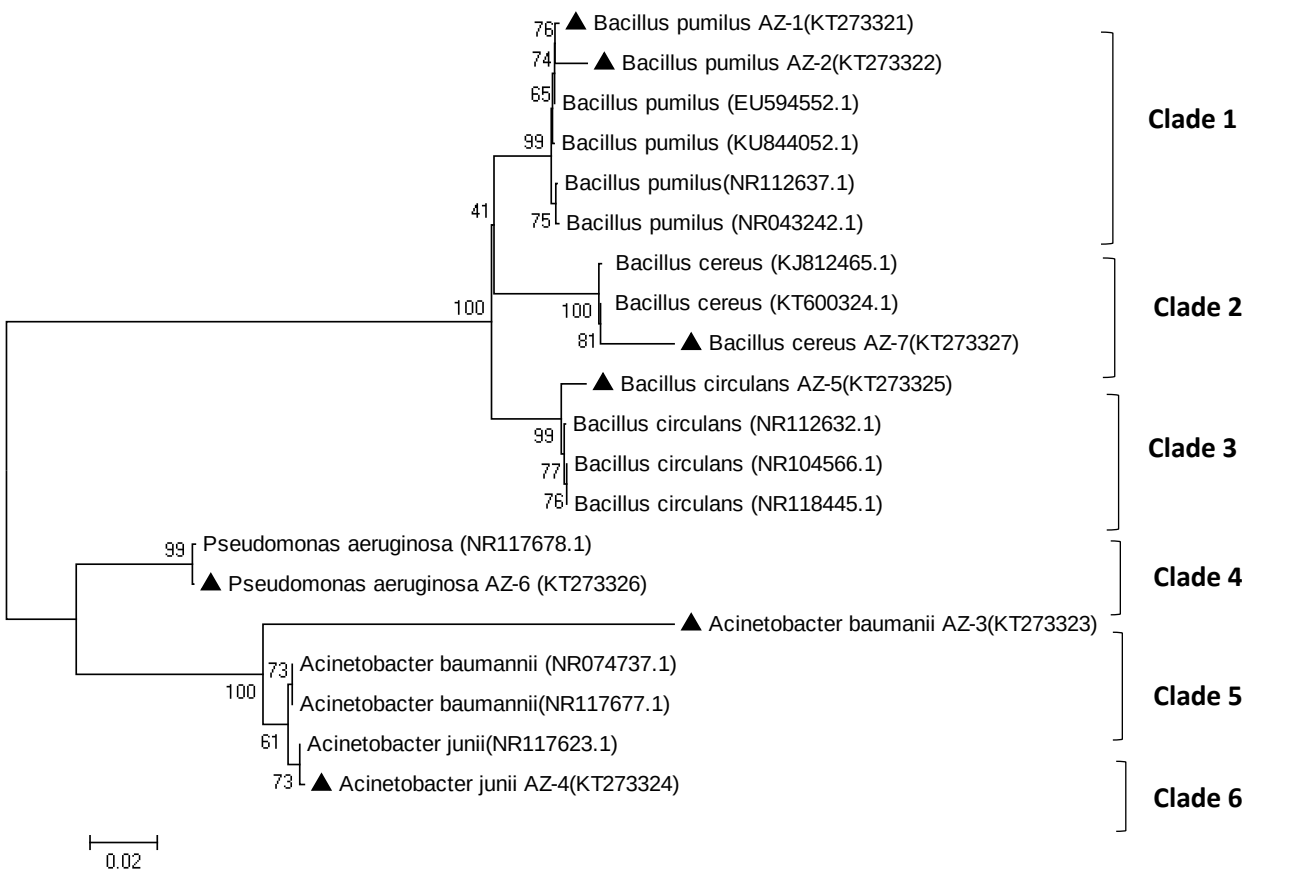

Fig. 9. Phylogenetic tree of selected bacterial strains with NCBI reported closely related BLAST sequences using neighbor-joining method. Scale bar represents 0.02 changes per nucleotide position.

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