Isolation and partial characterization of phosphate solubilizing bacteria isolated from soil and marine samples

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Abstract: In the present study the potential of indigenous bacterial isolates from soil rhizosphere and marine environment to promote plant growth was determined. Eight bacterial strains isolated from Soil and marine samples were characterized for the Phosphate solubilizing activity. Qualitative and quantitative estimation of phosphate solubilization is done. MIC of antibiotic and heavy metals were checked for these strains. Strains show a diverse pattern of antibiotic and heavy metals resistance.

Keywords: Phosphate solubilizing activity, qualitative, quantitative, plant growth.

INTRODUCTION

Soil bacteria are very important in biogeochemical cycles and have been used for crop production for decades. Plant bacterial interactions in the rhizosphere are the determinants of plant health and soil fertility. Free-living soil bacteria beneficial to plant growth, usually referred to as plant growth promoting rhizobacteria (PGPR), are capable of promoting plant growth by colonizing the plant root. PGPR are also termed plant health promoting rhizobacteria (PHPR) or nodule promoting rhizobacteria (NPR). These are associated with the rhizosphere, which is an important soil ecological environment for plant microbe interactions. Generally, PGPR functions in three different ways: synthesizing particular compounds for the plants, facilitating the uptake of certain nutrients from the soil, and lessening or preventing the plants from diseases (Hayat *et al*, 2010). Among the bacterial genera with this capacity are *Pseudomonas, Bacillus, Rhizobium, Burkholderia, Achromobacter, Agrobacterium, Microccocus, Aereobacter, Flavobacterium* and *Erwinia.* The use of phosphate solubilizing bacteria as inoculants simultaneously increases Phosphate uptake by the plant and crop yield. Strains from the genera *Pseudomonas, Bacillus* and *Rhizobium* are among the most powerful phosphate solubilizers. The principal mechanism for mineral phosphate solubilization is the production of organic acids, and acid phosphatases play a major role in the mineralization of organic phosphorus in soil (Rodríguez *et al*., 2004). Inadequate soil management by the use of chemical fertilizers has caused a global problem of nutrition depletion in soil and has made the pH of the soil acidic. Such adverse effects have caused reductions in crop production (Hungria and Vargas, 2000). There is an immediate need to replace the use of chemical fertilizers by alternative biological fertilizers. The use of microorganisms with the aim of improving soil fertility by maintaining biogeochemical cycles for nutrition management in the soil is necessary for agriculture (Freitas *et al*., 2007). However, during the past couple of decades, the use of PGPR for sustainable agriculture has increased tremendously worldwide (Figueiredo *et al*., 2008).

The aim of this study is to isolate bacterial strains from Soil and marine samples and study their Phosphate solubilizing activity both qualitatively and quantitatively to check their efficiency *in vitro* as plant growth promoting bacteria. MIC of antibiotic and heavy metals were also checked for these strains.

MATERIALS AND METHODS

Isolation of phosphate solubilizing bacteria

Seven soil samples were collected from agricultural fields of Malir and Karachi University and three Water samples were collected from the Arabian Sea, in sterilized glass bottles. One gram of collected soil sample was transferred to 9 ml sterile distilled water and shaken well to get a homogenous mixture. Serial dilutions were made up to 10^{-10} from the homogenous mixture. 100 µl of serially diluted sample from 10^{-8}-10^{-10} dilutions were streaked on nutrient agar plates and incubate at 37°C for 24 hrs. Next day morphologically distinct bacterial colonies were selected and streaked on new nutrient agar plates to get purified colonies. Plates were incubated at 37°C for 24 hrs. Free living bacteria in sea were isolated from surface water of the sea. 1 ml water sample was transferred to 9 ml peptone water of 1% concentration and shaken well to get a homogenous mixture. Serial dilutions were made up to 10^{-10} from the homogenous mixture. 100 µl of serially diluted sample from 10^{-8}-10^{-10} were streaked on nutrient agar plates supplemented with 1% NaCl and incubate at 30±2°C for 24-48 hrs (Uzair and Ahmed, 2007). Next day

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morphologically distinct bacterial colonies were selected and streaked on new nutrient agar plates supplemented with 1% NaCl to get purified colonies. Plates were incubated at 37°C for 24 hrs. Purified bacterial colonies were observed for their colonial and cellular morphology. Colonial morphology was observed on nutrient agar. Cellular morphology was studied through Gram staining.

Initial screening of Phosphate Solubilization activity

Isolated bacterial isolates were checked for phosphate solubilization activity by placing a drop of culture on Tris minimal broth for 24 hrs, on Tris minimal medium agar and Pikovskaya medium agar amended with inorganic, insoluble phosphate salts i.e. Zinc Phosphate ($Zn_3(PO_4)_2$) and Tri-calcium phosphate [$Ca_3(PO_4)_2$]. Plates were incubated at 28±2°C for 24-48 hrs. Bacterial strains producing large halo zones were selected for further studies. Eight best solubilizers, namely CMG4, CMG5, CMG7, CMG13, CMG15, CMG22, CMG24 and CMG26 were selected for further studies.

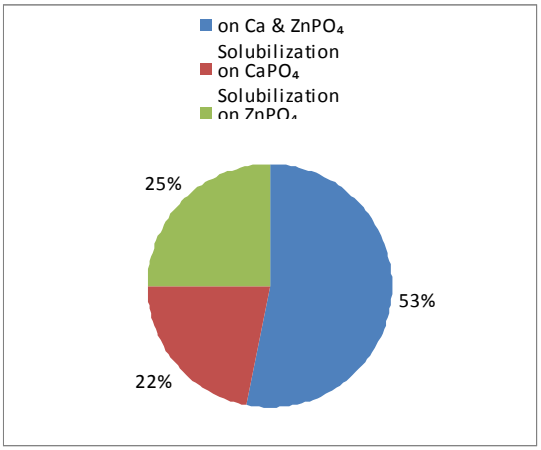

Fig. 1: Phosphate Solubilization (Halozone formation using $ZnPO_4$ & $Ca_3(PO_4)_2$)

MIC of antibiotics

Antibiotic resistance profile of bacterial isolates was performed by incorporating different antibiotics separately in nutrient agar by replica plate technique. Concentrations used were 100-600 mg/ml of Chloramphenicol and 50-500 mg/ml of Rifampicin.

MIC of heavy metals

MIC of heavy metal was determined by using replica plate technique for $ZnCl_2$, $CdCl_2$, $NiCl_2$ and $CuSO_4$. Working solutions were prepared in D/W to give the concentrations 0.5-2.0mM (millimole) and filtered through 0.2µm membrane filter. Heavy metal solutions were incorporated separately in Tris minimal agar and incubated for 24-48 hours at 37°C and MIC was determined by observing the growth.

16S rRNA identification

Selected bacterial isolates, which showed best phosphate solubilizing efficiency, i.e. CMG4, CMG7, CMG13, CMG15 and CMG22 were identified by 16S rRNA gene amplification and sequencing. Genomic DNA of the bacterial isolates was extracted by using DNA extraction kit (Promega, USA).

Qualitative Estimation of phosphate Solubilization activity

For qualitative estimation of phosphate solubilization activity, selected bacterial strains i.e. CMG4, CMG5, CMG7, CMG13, CMG15, CMG22, CMG24 & CMG26 were inoculated in Tris minimal broth and incubated at 37 °C for 24hrs in a shaker at 100 rpm. After 24 hrs, 10µl of broth cultures were transferred to four different types of agar plates with the following amendments:

1-Tris minimal agar amended with 5mM ($Zn_3(PO_4)_2$)

2-Tris minimal agar amended with 5mM $Ca_3(PO_4)_2$

Agar plates were incubated at 28±2°C for 5 days. Agar plates were observed for halo zone formation, and increase in the size of halo zone was recorded every day. Efficiency of the solubilization was calculated by the following formula (Nguyen *et al*., 1992; Vazquez *et al*., 2000):

$$\text{Solubilization Efficiency} = \frac{\text{Diameter of the halo}}{\text{Diameter of colony}} \times 100$$

Eight best solubilizers that were CMG4, CMG5, CMG 7, CMG13, CMG15, CMG22, CMG24 and CMG26 were selected for further studies.

Quantitative estimation of Phosphate solubilization activity:

Quantitative estimation of phosphate solubilization activity was performed by slight modification in single solution method of Murphy and Riley (1958). Single colony of selected strains were inoculated in Pikovskaya broth for 24 hrs, 10 µl of overnight grown (O/N) cultures was inoculated in 100 ml Pikovskaya medium supplemented with 5 mM Ca_3 $(PO_4)_2$ and 5 mM ($Zn_3(PO_4)_2$) and un-supplemented control. Flasks were incubated at 28±2°C in shakubator at 300 rpm for 15 days in the dark. The amount of phosphate released was estimated on 3rd, 5th, 7th, 9th, 11th, 13th and 15thday by aseptically taking 1ml samples from the bulk and centrifuging at 10,000rpm for 10 minutes. After centrifugation the supernatant was collected and the pellet was discarded. Supernatant was passed through 0.45 µm filter to remove cell and insoluble contents. This filtrate was then added with 1ml of reagent and was used to estimate the released phosphate at 880 nm wavelength on UV spectrometer. Working standards were prepared by making 100ppm stock solution of KH_2PO_4 to 1ppm. Solubilization of PO_4 was calculated by comparing with the absorption of standard KH_2PO_4.

Identification by 16SrRNA

Five selected strains were further identified by 16S rRNA using universal primers 16S rRNA was done commercially by Macrogen Inc. Korea.

Table 1: Gram reaction and cellular morphology of Bacterial isolates

Strain code	Gram Reaction	Arrangement
CMG 1	Gram +ve	cocci bunches and duploids
CMG 2	Gram -ve	cocobacilli .scattered
CMG 3	Gram -ve	cocobacilli .scattered
CMG4	Gram -ve	short rods scattered
CMG 5	Gram +ve	bacilli
CMG 6	Gram -ve	coccobacilli chain
CMG 7	Gram -ve	short rods scattered
CMG 8	Gram -ve	bacilli in chain
CMG 9	Gram -ve	bacilli scattered
CMG 10	Gram -ve	bacilli in chain
CMG 11	Gram -ve	cocci in chain
CMG 12	Gram +ve	cocci in bunches
CMG 13	Gram -ve	short rods scattered
CMG 14	Gram -ve	short rods scattered
CMG 15	Gram -ve	short rods scattered
CMG 16	Gram +ve	short in chains
CMG 17	Gram -ve	short rods scattered
CMG 18	Gram +ve	cocci in bunches
CMG 19	Gram -ve	rods in chain
CMG 20	Gram -ve	rods scattered
CMG 21	Gram -ve	short rods scattered
CMG22.	Gram -ve	short rods scattered
CMG 23	Gram -ve	rods in chain
CMG 24	Gram +ve	bacilli in chain
CMG 25	Gram -ve	bacilli scattered
CMG 26	Gram +ve	short rods
CMG 27	Gram -ve	rods in chain
CMG 28	Gram -ve	rods scattered
CMG 29	Gram +ve	cocci tetrads
CMG 30	Gram -ve	rods in chain
CMG 31	Gram -ve	rods in chain
CMG 32	Gram -ve	rods scattered

RESULTS

Morphological characterization

Out of 32 isolated bacterial strains only 8 strains were gram positive whereas the remaining ones were gram negative. All gram-negative strains were rod in shape and arranged either in chain or scattered. Out of 8gram positive bacterial strains, 5 were cocci in bunches and 3 were bacilli (table 1).

Phosphate solubilization (halozone formation on tris minimal medium + ($Zn_3(PO_4)_2$) and on tris minimal medium and $Ca_3(PO_4)_2$

Halozone formation was observed on tris minimal medium amended with ($Zn_3(PO_4)_2$) and $Ca_3(PO_4)_2$ and all the 32 straines exhibited the phosphate solubilization to some extend either on the medium amended with (Zn3(PO4)2) or $Ca_3(PO_4)_2$ or on both the medium (table 2 and fig. 1).

Table 2: Phosphate Solubilization (Halozone formation using $ZnPO_4$ & $Ca_2(PO_4)_2$)

Strain code	Halozone in $ZnPO_4$	Halozone in $CaPO_4$
CMG 1	+	+
CMG 2	+	+
CMG 3	+	-
CMG4	+	+
CMG 5	+	+
CMG 6	+	+
CMG 7	-	+
CMG 8	+	-
CMG 9	+	-
CMG 10	-	+
CMG 11	+	+
CMG 12	+	-
CMG 13	+	+
CMG 14	+	+
CMG 15	+	+
CMG 16	-	+
CMG 17	+	+
CMG 18	-	+
CMG 19	-	+
CMG 20	+	-
CMG 21	+	+
CMG 22.	+	+
CMG 23	+	-
CMG 24	+	+
CMG 25	-	+
CMG 26	+	+
CMG 27	+	-
CMG 28	+	-
CMG 29	+	+
CMG 30	-	+
CMG 31	+	+
CMG 32	+	+

Selection of promising strains

Bacterial strains which showed 10 mm zone in both salts ($Zn_3(PO_4)_2$) or $Ca_3(PO_4)_2$ within 48 hours and more than 200% efficiency (calculated by formula) for PO_4 solubilization on Tris minimal agar CMG4, 5, 7, 13, 15, 22, 24 and 26 were selected as promising strains (table 3).

Study of Genetic markers

Antibiotic Resistance

Chloramphenicol

4% strains showed resistance up to 400µg/ml of chloramphenicol, where as 50 % strains showed resistance up to 300µg/ml. Remaining 46% showed sensitivity to 100µg/ml of Cm (table 4).

Table 3: Selection of promising strains

Strain code	Halo zone size m.m	Colony size m.m	Diameter of Halozone Diameter of colony	Efficiency in %
CMG 4	12.5	6.0	12.5	208
			6.0	
CMG 5	9.8	5.2	9.8	188
			5.2	
CMG 7	20.0	6.0	20.0	333
			6.0	
CMG 13	21.0	7.0	21.0	300
			7.0	
CMG 15	22.0	8.0	22.0	275
			8.0	
CMG 22	15	6.0	15.0	250
			6.0	
CMG 24	9.3	5.0	9.3	186
			5	
CMG 26	14.0	7.0	14.0	200
			7.0	

Table 4: Minimal inhibition Concentration of Chloramphanicol against Indigenous bacterial Isolates (µg/ml)

Strain code	C 1	100 µg/ml	200µg/ml	300µg/ml	400µg/ml	500 µg/ml	600 µg/ml	C2
CMG 4	+	+	+	+	-	-	-	+
CMG 5	+	+	+	+	+	+	+	+
CMG 7	+	+	+	+	+	+	+	+
CMG 13	+	+	+	+	+	+	+	+
CMG 15	+	+	+	+	+	+	+	+
CMG 22	+	+	+	+	+	+	+	+
CMG 24	+	-		+	+	+	+	+
CMG 26	+	-	-	-	-	-	-	+

Table 5: Minimal inhibition Concentration of Rifimpicilin against Indigenous bacterial Isolates (µg/ml)

Strain code	C1	50 µg/ml	100 µg/ml	200µg/ml	300µg/ml	400µg/ml	500 µg/ml	C 2
CMG 4	+	+	-	-	-	-	-	+
CMG 5	+	+	+	-	-	-	-	+
CMG 7	+	+	+	+	-	-	-	+
CMG 13	+	+	-	-	-	-	-	+
CMG 15	+	-	-	-	-	-	-	+
CMG 22	+	+	-	-	-	-	-	+
CMG 24	+	+	-	-	-	-	-	+
CMG 26	+	+	-	-	-	-	-	+

Table 6: Minimal inhibition Concentration of $CuSO_4$ against Indigenous bacterial Isolates(mM/ml)

Strain code	C1	0.5	1.0	1.5	2.0	C2
CMG 4	+	+	+	+	-	+
CMG 5	+	+	+	+	+	+
CMG 7	+	+	+	-	-	+
CMG 13	+	+	+	+	+	+
CMG 15	+	+	+	+	+	+
CMG 22	+	+	+	-	-	+
CMG 24	+	+	+	+	+	+
CMG 26	+	+	+	+	+	+

Rifampicin

All the strains were sensitive from 300-500µg/ml of rifampicin. 96.7% of the strains showed resistance to 50 µg/ml of Rifampicin while nearly 42% of the strains grew at 100µg/ml and only 6.4% show resistance up to 200 µg/ml (table 5).

Metal Resistance

All the strains showed resistance up to 2mm concentration of $ZnCl_2$, $CdCl_2$ & $NiCl_2$. For $CuSO_4$ 25% of the strains showed resistance up to 1mM $CuSO_4$, 12.5% showed resistance up to 1.5 mM of $CuSO_4$ and only 62% of the strains showed resistance up to 2mM $CuSO_4$ (tables 6-8).

Table 7: Minimal inhibition Concentration of $ZnCL_2$ against Indigenous bacterial Isolates (mM/ml)

Strain code	C1	0.5	1.0	1.5	2	C2
CMG 4	+	+	+	+	-	+
CMG 5	+	+	+	+	+	+
CMG 7	+	+	+	+	+	+
CMG 13	+	+	+	+	+	+
CMG 15	+	+	+	+	+	+
CMG 22	+	+	+	+	+	+
CMG 24	+	+	+	+	+	+
CMG 26	+	+	+	+	+	+

Table 8: Minimal inhibition Concentration of $NiCl_2$ against Indigenous bacterial isolates (mM/ml)

Strain code	C1	0.5	1	1.5	2	C2
CMG 4	+	+	+	+	+	+
CMG 5	+	+	+	+	+	+
CMG 7	+	+	+	+	+	+
CMG 13	+	+	+	+	+	+
CMG 15	+	+	+	+	+	+
CMG 22	+	+	+	+	+	+
CMG 24	+	+	+	+	+	+
CMG 26	+	+	+	+	+	+

Qualitative estimation of phosphate solubilization activity

Total 32 bacterial strains were tested for phosphate solubilization on Tris minimal agar amended with 5 mM $(Zn_3(PO_4)_2)$ and Tris minimal agar amended with 5mM $Ca_3(PO_4)_2$. Out of 32 strains 7 strains exhibited halozone formation on $Ca_3(PO_4)_2$ and 8 strains exhibited halozone formation on $(Zn_3(PO_4)_2)$. 17 strains gave phosphate solubilization activity both on $(Zn_3(PO_4)_2)$ and $Ca_3(PO_4)_2$ (tables 11-12).

Quantitative analysis

Phosphate estimation by UV-Visible spectrometer

CMG4 and CMG22 gave the best results for liquid phosphate solubilization of Tri Calcium PO_4 and $(Zn_3(PO_4)2)$, while CMG7 gave good results in $TriCaPO_4$ and CMG13 gave better solubilization in $(Zn_3(PO_4)_2)$. All the strains gave maximum solubilization in 15 days in case of $TriCaPO_4$, while in case of $(Zn_3(PO_4)_2)$ some of the strains gave maximum solubilization in 13 days. Slight decrease in solubilized PO_4 was observed in some strains CMG22 gave best results by producing maximum amount of soluble phosphate in both the sources, that is 1785 mg/L in $Ca_3(PO_4)_2$ and 1326 mg/L in $(Zn_3(PO_4)_2)$ in 15 days. CMG4 gave 1185 mg/L soluble phosphate in $Ca_3(PO_4)_2$ and 1229 mg/L soluble phosphate in $(Zn_3(PO_4)_2)$ within 15days of incubation (tables 9-10).

Identification by 16SrRNA

Five strains which were identified by QTS 24 (tables 6.3) were further identified by 16S rRNA using universal primers 16S rRNA was done commercially by Macrogen Inc. Korea. CMG4 and 22 were identified as *Pseudomonas aerogenosa* while CMG7, CMG13 and CMG15 were identified as *Serretia marcesens*.

DISCUSSION

In the present study the potential of indigenous bacterial isolates from soil rhizosphere and marine environment to promote plant growth was determined. Thirty-two bacterial strains (CMG1-CMG32) were isolated from agricultural soil and marine environment. Bacterial isolates were characterized by cellular and colonial morphology, antibiotic resistance and heavy metal resistance. 25% of total bacterial strains were gram positive and 75% were gram negative. The majority of the organisms were antibiotic resistant and all the bacterial isolates showed resistance to the metal salts. All isolated

Table 9: Quantitative Analysis of Phosphate solubilization in Liquid medium (Tri calcium phosphate)

Strain code	Days						
	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
CMG 4	959	962	1027	1119	1180	1178	1185
CMG 5	987	1083	1213	1219	1414	1581	1631
CMG 7	153	465	660	795	791	811	824
CMG 13	198	280	326	413	641	773	831
CMG 15	927	972	1685	1685	1750	1743	1785
CMG 22	215	300	317	321	615	728	820
CMG 24	103	178	188	239	337	408	403

Table 10: Quantitative Analysis of Phosphate solubilization in Liquid medium (Zinc phosphate)

Strain code	Days						
	3rd Day	5th Day	7th Day	9th Day	11th Day	13th Day	15th Day
CMG 4	833	878	1051	1175	1233	1235	1229
CMG 5	195	188	276	305	371	403	40
CMG 7	788	889	995	986	1010	1032	1030
CMG 13	35	72	76	82	89	91	101
CMG 15	687	987	1038	1186	1181	1207	1326
CMG 22	231	275	289	333	389	550	623
CMG 24	254	356	386	389	437	482	681

Table 11: Solubilization of Ca Tri Phosphate by selected indigenous bacterial strains

Strain code	Zone of solubilization in m.m				
	Day 2	Day 4	Day 6	Day 8	Day 10
CMG 1	-	5.4	8.0	9.3	10
CMG 2	5.0	6.2	7.0	10.0	11.0
CMG 4	10.0	10.6	12.00	12.4	14.0
CMG 5	8.8	10.0	14.0	14.2	14.2
CMG 7	15.0	15.7	18.0	19.3	20.0
CMG 11	8.5	12.4	14	14.5	15.5
CMG 13	20.0	22.0	24.5	26.0	26.5
CMG 14	9.0	11.3	13.0	14.5	16.0
CMG 15	22.0	24.0	25.5	26.1	27.0
CMG 17	6.5	6.5	7.0	7.0	7.0
CMG 21	5.9	7.0	7.5	8.0	8.5
CMG 22	12.0	15.2	18.0	20.0	22.0
CMG 24	9.3	9.6	10.0	10.5	10.5
CMG 26	12.0	14.0	18.0	19.5	21.0
CMG 29	7.0	13.0	13.6	13.6	13.8
CMG 31	4.6	5.0	5.8	6.0	6.4
CMG 32	5.7	8.0	8.4	8.4	8.4

strains were checked for phosphate solubilization activity by halo zone formation on tris minimal medium agar amended with tri calcium phosphate and zinc phosphate separately. Those bacterial strains exhibiting higher phosphate solubilization activity (i.e. halo zone measuring >5mm within approximately 48 hours) on phosphate compound supplemented Tris minimal medium were selected for further studies. Out of thirty two bacterial isolates there were eight isolates (three gram positive and five gram negative) which exhibited more than 5mm zone. Those five bacterial strains were identified by 16S rRNA gene sequencing using universal primers. Results of BLAST via NCBI showed that CMG4 and CMG22 had 99% homology with *Pseudomonas aeroginosa,* CMG7 and CMG13 showed 99% homology with *Serratia marcescens*, serratia uncultured clone and with *serrtia nematophilia* while CMG15 showed 100% homology with *serratia marcescens*. CMG 4, CMG7, CMG13, CMG15 and CMG22 were analyzed by quantification of soluble phosphate in broth by UV spectrometer.

CMG4, CMG5, CMG7, CMG13, CMG15, CMG24, and CMG26 were further checked for antibiotic and metal resistance. When selected isolates were exposed to the different concentrations of protein synthesis inhibiting broad spectrum antibiotic Chloramphenicol CMG4, CMG5, CMG13, CMG15and CMG22 show resistance up to 300ug/ml of Chloramphenicol but CMG24 and CMG26 did not show resistance even to 100ug/ml of the same it shows that Bacillus species isolated from the soil are sensitive to Cm even in a low concentration of 100ug/ml where as all gram negative bacteria can resist up to certain level of Cm**.** Same isolates when exposed to the 50-500ug/ml of Rifampicin and RNA synthesis inhibiting antibiotics by replica plate CMG4, CMG13, CMG22, CMG24 and CMG26 showed resistance up to 50ug/ml only while CMG5 and CMG 7 showed their growth up to 100ug/ml where as CMG15 did not show their growth even to 50ug/ml of Rifampicin. Walsh, 2000 reported that usually it has been seen that bacterial isolates showed resistance to a range of antibiotics as compared to a single resistance of isolates to antibiotics, which may lead to a suggestion that the bacterial isolates may have been already exposed to the antibiotic in their native environment. This suggestion may strongly be supported by the facts reported by many authors

Table 12: Solubilization of $ZnPO_4$ by selected indigenous bacterial strains

	Zone of solubilization in m.m				
Strain code	Day 2	Day 4	Day 6	Day 8	Day 10
CMG 1	6.0	8.0	8.0	8.0	8.0
CMG 2	9.0	11.5	13.0	15.0	16.0
CMG 4	18.0	22.0	26.0	28.0	29.5
CMG 5	10.0	13.0	15.0	18.0	22.0
CMG 7	20.0	24.0	28.0	32.0	32.0
CMG 11	8.0	9.5	13.0	15.0	16.2
CMG 13	22.0	26.0	28.0	33.0	34.0
CMG 14	7.0	9.5	10.5	10.5	12.0
CMG 15	25.0	26.5	28.0	30.0	32.0
CMG 17	9.0	9.4	10.0	12.0	12.0
CMG 21	5.5	6.0	8.0	10.0	12.0
CMG 22	15.0	18.0	19.0	22.0	24.0
CMG 24	12.0	14.0	18.0	18.0	18.0
CMG 26	13.0	14.5	16.0	18.0	22.0
CMG 29	8.8	10.0	10.5	12.6	14.0
CMG 31	5.0	6.8	7.5	9.0	9.5
CMG 32	8.8	10.0	14.0	14.8	16.0

(Compant *et al*., 2005; Mazurier *et al*., 2009; Raaijmaker *et al*., 2002).

When selected isolates were exposed to 0.5-2 mM metals solution of $CuSO_4$, $ZnCl_2$, $CdCl_2$ and $NiCl_2$, all the strains show resistance up to 2mM metal solutions but CMG4 and CMG7 showed sensitivity to 1.5mM $CuSO_4$. Day by day remarkably increasing industrialization and population causes the increase in environmental contamination with the variety of toxic metal and organic compounds (Glick, 2010). It might be suspected that the multiple resistance gene may be present on same genetic element or the same gene performs broad range resistance i.e to antibiotics and metals. The toxicity of heavy metals in the soil can be reduced by PGPR mycorrhizal fungi as they help to decrease the bioavailability of toxic heavy metal; on the other hand this increases the bioavailability of non toxic heavy metal by changing the oxidation states of metals (Denton, 2000).

CONCLUSION

The results of qualitative and quantitative estimation of phosphate solubilization prove that the isolated strains can be used as biofertilizer to increase the phosphate uptake by the plants. Bacterial consortium can be developed that can be used as biofertilizer. Biofertilizers are environment friendly, free from hazardous chemicals, posses no detrimental health effects and are cost effective.

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