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Plant Growth Promotion Potential of Heavy Metal Resistant Bacteria From The Sewage Contaminated Coovum River, Chennai, India

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Rivers contaminated with sewage are the most disturbed ecosystems due to anthropogenic activities such as release of heavy metals, organic pollutants and pharmaceuticals as well as faecal and pathogenic microorganisms, which coexist with the indigenous microbial population. Coovum is such a much polluted river in Chennai city, India. There have been several programs in operation and plan to remediate the Coovum river. In this context, the present study has aimed to explore some of the beneficial bacteria from this polluted river to be used for environmental and horticultural applications. A total of 43 bacteria were isolated from two different water samples collected from Coovum river. Physicochemical parameters were analysed and found the water is not suitable for either drinking purposes or irrigation purposes. As such, the Coovum water also inhibited the germination and reduced growth of tested seeds and seedlings, respectively thus double confirms its unfitness for irrigation purposes. All these 43 bacteria were screened for different plant growth promotion potentials such as production of phytohormones (Indole Acetic Acid-IAA) and phosphate solubilization. They were also characterized for the production of hydrolytic enzymes, heavy metal resistance and antibiotic resistance to characterize their bioremediation potential. While screening for IAA production, 7 strains were found to produce this plant growth promoting hormone. Similarly, 10 strains were able to solubilize phosphate. All the 17 strains were also characterized for their production of lytic enzymes and found only nine strains were able to show amylolytic activity and nine strains showed proteolytic activity. While screening their antagonistic activity against plant pathogenic fungi, one strain has inhibited the mycelia growth of *Macrophomina phaseolina* and five strains were able to inhibit *Alternaria alternata*. Three efficient strains, AMET3006, AMET3028 and AMET3030 were able to grow even at 5 μm concentrations of Hg, Cu, Fe, Mn and Cr. Among these, the best performing strain AMET3006 was identified as *Pseudomonas* sp. Thus, the present study concludes that even in much polluted ecosystem like Coovum river, bacteria were having plant growth promotion activities, bioremediation potential, heavy metal and antibiotic resistance and added advantage of antifungal activity. These bacteria can be employed to bioremediate the contaminated water which can be used for the purpose of irrigating ornamental plants due to their plant growth promotion activities. Further studies on the practical applications of the selected strains will produce more encouraging results which are underway.

Keywords: Plant growth promotion, heavy metal resistance, *Pseudomonas*, Coovum river, bioremediation.

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Aquatic pollution has become a global concern, but even so, most developing nations are still producing huge pollution loads and the trends are expected to increase[1]. The twenty-first century has witnessed rapid urbanization in Asia, particularly for the large population giants like China and India. Such unprecedented urbanization in India has thrown up several issues and challenges[2]. One such important issue is river pollution. The Chennai City is the fourth largest metropolis in India, and the coastal region of this city is a typical example for uncontrolled disposal of wastewater and serious pollution level[3]. The city has been subject to a multitude of anthropogenic impacts attributable to accelerated population growth and development of small-scale and large-scale industries, expansion of harbours and tourism related activities in the coastal zone, disposal of municipal wastes, industrial wastes and numerous recreational and commercial activities that not only degrade the quality of coastal water but also pose a serious health hazard to marine biotas and human[4, 5, 6, 7, 8]. Coovum or Kuvam River almost bisects the city of Chennai, India. It is also considered to be the shortest classified river draining into the Bay of Bengal and is only about 65 km long and collects surpluses of 75 small tanks of a minor basin. The Coovum River which was once navigable and recreational has now become a sink of garbage dumping and a sewage outlet. Coovum is presently a river spoiled by filth and pollution and the water quality is considered to be highly toxic and completely non-potable [9].

The physiochemical properties of Coovum river water has been studied often. Invariably all the studies concludes that the lower parts of the river closer to estuary always indicates heavy pollution which is strongly affected by anthropogenic activities, rock-water interaction, and saline water intrusion. The major contaminant sources identified in the areas were the samples were polluted were liquid and solid wastes, discharges from factories and refineries, drainage of sewage, and brine water in estuaries. Coovum river water from certain locations, were even found to be not fit for irrigation as they had high acidity, low dissolved oxygen and high amount of dissolved solids[10, 11, 9]. Moreover, when groundwater quality in Chennai city along the Coovum river was studied, the water quality parameters such as pH, EC, TDS, BOD, COD, Na and Pb have not complied with the WHO standards, and the water is not fit for drinking and domestic purposes [12].

Bioremediation is the remarkable capacity of microorganisms to degrade or to transform pollutants into secondary compounds that are compatible with the equilibrium of the local ecosystem[13]. Bioremediation of polluted sites is increasingly considered as a potent tool to clean and to detoxify soils and waters contaminated by unwanted residues mainly generated by human activities[14, 15]. Bacteria isolated from such polluted environments have well proven to have very good bioremediation potentials because of their diverse adaptable characteristics. The Coovum river water has been the source of microbiological studies for various purposes such as analysis of pathogenic microorganisms, antiobiotic resistant organisms etc[16, 9]. However, to our knowledge, the Coovum river water has not been comprehensively studied for the exploration of beneficial microorganisms. In the present study, we have made an attempt to explore beneficial bacteria from the Coovum River Water and characterized them for the use of bioremediation and reuse of the same water for the purpose of irrigation of ornamental plants. The number of organisms present varies considerably between different water types, and it is generally accepted that sewage-polluted surface waters contain greater numbers of bacteria than do unpolluted waters. Because of highly polluted environment these organisms have adopted themselves to produce a variety of metabolites for their growth and developments. The present study aimed on the isolation and selection of efficient bacteria from Coovum River and screening for their plant growth promotion potential traits along with characterization for bioremediation properties. This work has significance at the current scenario while Chennai Corporation is developing Parks all over the city. If such a successful technology developed, the Coovum water can be remediated with efficient bacteria and reused for irrigation purpose to the ornamental plants of public parks since those bacteria would also improve plant growth.

MATERIALS AND METHODS

Sample collection

The Coovum river water samples were collected using sterile containers from two different locations such as estuary mouth and at 1 km upstream in the river during 2010. The collected water samples were tested for various Physical and chemical Properties. Ten ml of water sample were taken and added to 90 ml of sterile distilled water in a conical flask. The suspension was serially diluted and spread plated on Nutrient and King's B agar (KBA) medium[17]. After 48 h of incubation distinct colonies with different morphologies were subcultured to purity.

Effect of Coovum estuary water samples on seed germination of crop plants

Eight different seeds such as Green gram, Wheat, Groundnut, Green pea, Yellow pea, Lobia, Kidney bean and Chick pea were soaked for six hours in normal water and in the raw water samples collected from the Coovum estuary. Thus treated seeds were kept in blotting papers in the moist chamber set up using plastic trays and moistened with sterile distilled water whenever needed and covered with a layer of blotting paper to avoid moisture loss. The trays were kept in darkness at room temperature and after 3 days the germinated seeds were counted. Percentage of seed germination was calculated using the following formula

$$\text{Percent seed germination} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds}} \times 100$$

1.3. Screening for IAA Production

The bacterial strains were tested for IAA production by the method, described by Gupta et al. (2002). The bacterial strains were inoculated in sterile Nutrient broth supplemented with L-tryptophan and incubated at 37°C for 3 days in shaking conditions. After incubation period, the cultures were centrifuged at 10,000 rpm for 10 minutes. To the supernatant (2 ml), two drops of ortho phosphoric acid was added and incubated at room temperature for 10 minutes, followed by addition of 4 ml of Salkowski reagent (50 ml of 35% sulphuric acid, 1 ml of 0.5 M $FeCl_3$). Development of pink colour indicates the positive result for IAA production and no color change indicates the negative result for IAA production[18].

Screening for Phosphate Solubilization

All the isolates were screened for phosphate solubilization by streaking in Pikovskaya's agar medium. All the bacterial strains were spot inoculated in Pikovskaya's medium. The plates were incubated for five days at room temperature. After the incubation period, the plates were observed for the solubilization of phosphates. Halo zone around the colonies indicates the positive result for solubilization of phosphates by bacteria[19].

Screening for Production of extracellular enzymes

Nutrient agar was prepared supplemented with substrates for enzymes such as Gelatin, Skimmed Milk, Casein, Starch and Tween 80 for the assay of extracellular enzymes *viz.*, protease, amylase and lipase at a concentration of 1% (w/v)., Pectin and cellulose at 0.3%(w/v). The selected bacterial strains were streaked on substrate-amended media and kept incubation of four days after which subjected to visualize enzyme activity. Pectinase and CM cellulose activity was visualized by a translucent zone of lysis around the bacterial patch after staining with 0.3% Congo Red and destained with 1 N NaCl for 15 min each. Amylase activity was visualized by a zone of clearance on flooding the plates with iodine solution. Protease activity was visualized as a clear zone around colony when the plate was flooded with saturated ammonium sulfate prepared in 0.1N HCl. Lipase activity was visualized as opaque zone around the bacterial growth.

Screening for Antifungal activity

The antifungal activity of the isolated bacterial strains was tested *in vitro* by dual plate assay against various plant pathogenic fungi such as *Alternaria alternata, Fusarium oxysporum* and *Macrophomina phaseolina*. The fungal discs measuring 8 mm were cut out from 4 days old culture and inoculated at the center of a fresh PDA plate. The selected bacterial strain were streaked on the periphery of each plate and incubated at room temperature. The growth of *Alternaria alternata, Fusarium oxysporum, Macrophomina phaseolina* and bacteria were observed periodically and the inhibition zone was measured. Efficient antagonistic bacteria were identified by their ability to produce a zone of inhibition.

Screening for Heavy metal tolerance

The selected bacterial strains were streaked on NA plates amended with various concentrations (1 µm , 3 µm and 5 µm) of different heavy metals *viz.*, mercury (Hg) , Copper (Cu) , Iron (Fe) , Manganese (Mn) and Chromium (Cr). The NA plates without heavy metals served as controls. The plates were incubated at room temperature for 24 h and observed for bacterial growth. The heavy metal tolerant strains were further selected and screened quantitatively by inoculating them on tubes containing 1 µm , 3 µm , 5 µm, of the heavy metals and observing the O.D at 600 nm after 48 hrs of incubation .

Screening for antibiotic resistance

The Hexadiscs (Himedia Mumbai) containing different antibiotics at different concentrations was placed on the surface of lawn cultures of selected strains prepared in Nutrient Agar Medium. The discs were containing the antibiotics such as ceftazidime(Ca), ciprofloxacin (CF) , Amikacin (AK) , Netillin (Nt) , Nitrofurantoin (Nf). The NA plates without antibiotics served as controls. The plates were incubated at room temperature for 24 h and observed for zone of inhibition if any. The sensiticity/resistance of the particular strain to the respective antibiotics was recorded with reference to CLSI standards mentioned the manufacturers information.

Identification of selected Strain

The selected potential strain AMET3006 was subjected to various biochemical and staining techniques as described by Cappuccino and Sherman (2004)[20] and the results were interpreted with the key provided in the Bergy's Manual of Determinative Bacteriology.

Analyses of Results

All the experiments were conducted with two replicates. Data presented were average of two replicates with standard deviation.

RESULTS AND DISCUSSION

In the Era of urbanization coupled with rapid economic development, the river pollution occurred continuously, which seriously damages the river ecosystems. Vast quantities of domestic and industrial wastewater flowed into the river, which leads to the water system become severely polluted. Consequently, the function of river as resources was lost and the urban ecology and water environment are seriously deteriorated. Scientists have also speculated that sooner in future there will not be almost a complete natural river in the World[21, 22]. Polluted aquatic environment contains various types of toxic chemicals. The major categories of these pollutants are heavy metal ions, toxic aromatic hydrocarbons, and pesticides. Increasing environmental pollution by heavy metals results from their increasing utilization in industrial processes. The micro-organism present in the polluted environment has many beneficial activities and they are well proven to have very good bioremediation potentials because of their diverse adaptable characteristics. The quality of water is of great importance also for human lives[23]. Water Quality Index can be evaluated on the basis of various physical, chemical and bacteriological parameters. The water quality in different reservoirs, bays and rivers was evaluated using dissolved oxygen (DO) and the parameters affecting DO[24].

In the present study, two different water samples were collected from Coovum River Estuary *i.e.*, one from the mouth of estuary and other from 1 km upstream in the river. Several physical and chemical parameters such as Appearance, Odour,

Table 1. Physical properties of water samples collected from Coovum River Estuary, Chennai

Parameters	Water from Coovum estuary mouth	Water from Coovum River (2 km upward)
Appearance	Turbid	Turbid
Odour	Offensive smell	Offensive smell
Turbidity	0.5	15.7
Total dissolved solids (mg/L)	38800	18984
Total suspended solids (mg/L)	5	6
Total solids (mg/L)	38805	18990
Electrical conductivity(micro mho/cm)	55329	27066

Turbidity, Total solids (suspended and dissolved), pH, alkalinity, salts, metals, BOD and COD level were tested in TWAD Board (Tamil Nadu water Supply and Drainage Board) and values are presented in tables 1 & 2. The BOD is a value that indicates how fast biological organisms utilize oxygen in a water body and is usually taken as a measure the quality of the water. In general, the unpolluted natural aquatic environments shall have very low BOD so as to have more DO. Clean rivers usually have a BOD of below 6 mg/L where as BOD levels exceeding 6 mg/L are considered as polluted rivers and the rivers which has BOD more than 30 mg/L are considered as heavily polluted rivers (CPCB, 2014). In the present study, the biological oxygen demand (BOD) estimated at the mouth of estuary was 50 mg/L while it was 96 mg/L in water collected from 1 km upstream in the river. The relatively low BOD at estuary mouth may be due to the diluting effects of salt water being mixed at the estuary mouth. The BOD value tested in the present study clearly shows Coovum River is more polluted than treated sewage.

The water at both the locations appeared turbid with offensive smell. Both total suspended solids and total dissolved solids found very high along with high electrical conductivity. The water was slightly acidic, high in hardness (values ranging 2900 to 4400 (as $CaCo_3$) mg/mL). The tested minerals such as Calcium, Magnesium, Sodium, Potassium, Iron and Manganese were at different levels. The ionic forms such as ammonia, Nitrite, Nitrate, Chloride, Fluoride, Sulphate and Phosphate are also analyzed and values are presented in table 2. Invariably the values of all the parameters tested were strongly indicating that the River Coovum is heavily polluted which cannot be used for domestic, agricultural and industrial uses[25, 26].

Table 2. Chemical properties of water samples collected from Coovum River Estuary, Chennai

Parameters	Water from Coovum estuary mouth	Water from Coovum River (2km upward)
Ph	6.61	6.81
Alkalinity pH (as $CaCo_3$)mg/mL	0	0
Alkalinity Total (as $CaCo_3$)mg/mL	312	488
Total Hardness (as $CaCo_3$)mg/mL	4400	2900
Calcium(as Ca) mg/mL	1120	840
Magnesium (as Mg) mg/mL	384	192
Sodium(as Na)mg/mL	9100	4400
Potassium (as K) mg/L	600	400
Iron (as Fe) mg/L	1.12	0.98
Manganese (as mn) mg/L	Nil	Nil
Free ammonia (as NH_3) mg/L	32.48	28
Nitrite (as NO_2) mg/L	0.10	0.11
Nitrate (as NO_3) mg/L	7	11
Chloride (as Cl) mg/L	12227	7425
Fluoride (as F) mg/L	0.46	0.28
Sulphate (as SO_4) mg/L	8978	1419
Phosphate (as PO_4) mg/L	0.21	0.24
Tidy's test (as O) mg/L	4.6	1.7
Silica (as SiO_2)	42.15	43.77
COD mg/L	148	181
BOD mg/L	50	96
Copper mg/L	0.00875	0.00921
Zinc mg/L	0.139	0.153
Chromium mg/L	0.00560	0.00709
Total kjeldhal Nitrogen mg/L	42.56	39.20
Oil and Grease mg/L	0.0039	0.0067

Besides, the other physicochemical parameters such as pH, alkalinity, total hardness and metal ions and salts were also analyzed in both the water samples and presented in table 2. These interdependent parameters are found valuable in assessing the water quality of rivers. Both pH, acidity and alkalinity are the important parameters in many studies on river pollution[27, 28]. A recent review has concluded the limits for water quality parameters for drinking water based on the recommendations of various regulatory bodies of national and international importance[29] This report has been taken for comparison of tested values and standards. pH of the Coovum river water samples found to be around 6 and it is in permissible limits. Total hardness in both the locations was around 2900-4400 mg/L (as $CaCo_3$) which is almost 10 times more than the permissible limits of any of the standards. The range of total alkalinity (312-488 mg/ml) found to be within the permissible limits only.

Table 3. Effect of Coovum estuary water on the germination of crop plant seeds

Water sample	Treatment period	Green gram	Kidney beam	Wheat	Ground nut	Green pea	Yellow pea	Lobia	Chick pea
Coovum water	3 hrs	72%	-	-	-	-	-	-	-
Estuary water	3 hrs	75.9%	-	-	-	-	-	-	-
Distilled water	3 hrs	80.6%	85%	83.3%	89%	79.9%	82.9%	80.6%	84.8%

Table 4. Screening of estuarine bacteria for the production of hydrolytic enzymes

S. No.	Isolate code	Amylase (Starch)	Protease (Skim milk)	Protease (Gelatin)	Protease (Casein)	Cellulase (CM Cellulose)
1	AMET 3001	1.4 ±0.1	1.3 ± 0.18	1.8±0.2	-	-
2	AMET 3002	2.2±0.1	3 ± 0.2	2.3±0.1	-	-
3	AMET 3006	-	-	-	-	-
4	AMET 3011	2±0.2	-	1.4±0.2	-	1.5±0.1
5	AMET 3017	-	-	2±0.1	-	-
6	AMET 3018	-	2.9 ± 0.05	2.6±0.08	1.3±0.1	-
7	AMET 3024	-	-	-	-	-
8	AMET 3028	2.4	2.1 ± 0.45	2.3±0.1	-	-
9	AMET 3029	2.1±0.2	2.5 ± 0.1	2.8±0.1	-	-
10	AMET 3030	1.4±0.2	-	-	-	-
11	AMET 3031	1.5±0.2	-	-	1.4±0.1	-
12	AMET 3032	-	1.7 ± 0.2	-	-	-
13	AMET 3034	-	1.6 ± 0.2	-	-	-
14	AMET 3035	-	-	-	1.6±0.2	-
15	AMET 3036	2.2±0.1	2.5 ± 0.1	-	-	1.5±0.08
16	AMET 3038	2.8±0.2	2.6 ± 0.1	-	-	-
17	AMET 3043	-	-	-	-	-

+ indicates the production of respective enzyme while – indicates no such production

In case of metal ions, all the metal ions tested were found high in the estuaries and low in upstream samples. Metal ions such as Calcium (840-1120 mg/L), Magnesium (192-384 mg/L), Sodium (4400-9100 mg/L), Potassium (400-600 mg/L), Iron (0.98-1.12 mg/L) were all found to be at higher level than the permissible limits. Manganese was not found in the samples. However, similar trend was not observed in the in the analyses of different salts. Different salts were found at different concentrations in both the locations through which no common trend could be judged. Free ammonia was in the range of 28-32.5 mg/L. Salts like Nitrite (0.10-0.11 mg/L), Nitrate (7-11 mg/L), Chloride (7425-12227 mg/L), Fluoride (0.28 -0.46 mg/L), Sulphate

(1419-8978 mg/L), Phosphate (0.21-0.24 mg/L). However, all the detected levels of salts are extremely higher than that of the permissible limits. Heavy metals such as Copper (0.00875-0.00921 mg/L), Zinc (0.139-0.153 mg/L) and Chromium (0.00560 -0.00709 mg/L) were also detected at alarmingly higher levels. The total nitrogen content (Kjeldhal Nitrogen 39.20-42.56 mg/L) was also found higher. The Oil and Grease also found high (0.0039-0.0067 mg/L). The above analyses made in the present study concludes that the water collected from Coovum was ineligible for both drinking and irrigation purposes.

The germination percentage of crop plants seeds such as such as Green gram, Wheat, Groundnut, Green pea, Yellow pea, Lobia, Kidney bean and Chick pea was observed after treating them with the water samples collected from the Coovum river in comparison with distilled water. All the selected crop plants seeds were able to germinate well in distilled water. However, a very mild treatment of seeds for 3 h with the Coovum river water samples has completely inhibited the germination of all the seeds tested excepting green gram (Table 3). This study again proves that the Coovum river water at present is not eligible for irrigation purposes. Undiluted or untreated domestic sewage was found to have phytotoxicity and reduced germination and seedling parameters of crop plants[30].

Mostly, polluted river water samples were subjected to Microbiological studies to find the load and prevalence of human pathogenic bacteria. However, in the present study, we have used the Coovum River water to isolate and characterize few beneficial bacteria from this polluted aquatic environment. A total of 43 bacterial strains were isolated obtained as pure cultures from two Coovum samples and named as AMET3001 to AMET3043. IAA is one of the most important phytohormone and function as main indicator particle in the regulation of plant growth. It has been evident that IAA production by plant growth promoting bacteria can vary among different species and strains. It is also influenced by culture conditions, growth stage and substrate availability[31]. In the present study, all the 43 strains were tested for their ability to produce IAA and Phosphate solubilization. In the qualitative assay for IAA screening, seven strains have formed brown colour after adding Salkowski reagent which clearly indicated that they are IAA positive. However, the intensity of brown has varied between the isolates. All these seven IAA positive strains were quantified for the production of IAA. This study has revealed that out of seven strains AMET 3028 have produced higher amounts of IAA than the other isolates (Table 4). When screening for the Phosphate solubilization, 13 strains were able to produce halo zones which indicates that these strains have the ability to solubilize tri calcium phosphate. Production of phytohormones such as IAA and solubilization of complex nutrients such as phosphate were always considered as important traits for bacteria to be used in plant growth promotion. Bacteria isolated from diverse ecosystems and characterized for different environmental applications were also subjected to screen for IAA production and phosphate solubilization[32, 33, 34]. Hence, this part of the study has proved that the bacteria isolated from Coovum river water has the potential to be used for environmental and plant growth promotion applications and hence further characterized on other aspects for safe usage.

In recent years, the enzymes have been played an important role in manufacturing of chemicals, textiles, pharmaceuticals, paper and food agricultural chemicals. Enzyme based industrial bioprocess now directly competes with established chemical based process[35]. Hence, all the 17 strains shortlisted in the present study were also characterized for the production of different enzymes. Out of 17 strains, nine strains were able to show amylolytic activity, nine strains showed proteolytic activity using skimmed milk as substrate, 7 strains showed proteolytic activity using gelatin as substrate and 3 strains showed proteolytic activity using casein as substrate (Table 4). None of the strains were able to degrade lipid or pectin and thus confirming that they could not produce lipase and pectinase. Production of extracellular lytic enzymes has been regarded as one of the most desired characteristics for both plant growth promotion and environmental applications such as bioremediation[36, 33, 37].

The concept behind this study is to find a useful microorganism from Coovum river water which has both plant growth promotion and bioremediation potential. Antagonistic potential is

considered as one of the desirable character for plant growth promoting bacteria, as it is a indirect mechanism for plan growth promotion. Generally, plant growth promoting rhizobacteria facilitate the plant growth directly by either assisting in resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents[38]. In the present investigation, out of 17 strains, AMET 3018 was able to inhibit the growth of *Macrophomina phaseolina*, which is a serious soil borne plant pathogen causing root rot in many crop plants. Five strains namely AMET 3006, AMET 3028, AMET 3029, AMET 3030 and AMET 3032 were able to inhibit *Alternaria alternate.* None of the strains were able to inhibit *Fusarium oxysporum*. AMET 3006 and AMET 3029 showed a maximum zone of 1.9 cm against *Alternaria alternata* (Table 2).

The waste coming from industrial sources in environment is a reservoir of microorganisms that can develop resistance to heavy metals. The presence of small amount of heavy metals in the solid waste can induce the emergence of heavy metal resistant microorganisms[39]. Selected 3 strains namely AMET 3006, AMET 3028, AMET 3030 which showed positive in both IAA and Phosphate solubilization were screened for the heavy metal tolerance and Antibiotic resistance. All the three strains were able to grow even at 5 µm concentrations of Hg, Cu, Fe, Mn and Cr (Table 1). All the three strains showed resistance to all the six antibiotics tested. Heavy metal resistant bacteria were previously reported suitable for environmental application. Samanta *et al.* (2012)[40] have isolated a *Bacillus* sp. from municipal waste, which was found to grow in presence of a wide range of metals namely nickel, cadmium, chromium and cobalt in the order Cd2+> Cr6+> Ni2+> Co2+. Heavy metal tolerance test showed maximum microbial tolerance to cadmium and minimum tolerance to cobalt and also it was observed that the isolate was even resistant to a wide range of antibiotics namely Kanamycin (30µg/disc), Ampicillin (25µg/disc) and Methicillin (5µg/disc). The above studies have indicated that three strains, AMET3006, AMET3028 and AMET3030 which were having the ability to grow even at 5 µm concentrations of Hg, Cu, Fe, Mn and Cr were found efficient. Among these, the best performing strain AMET3006 was subjected to various staining and biochemical tests. It is a gram negative rod shaped bacterium which does not produce endospores. It is catalase and oxidase positive. It exhibited motility in hanging drop method. Moreover, it was found to grow in Cetrimide agar which indicates that the strain belongs to the genus *Pseudomonas*. From the above results, the strain AMET3006 was identified as *Pseudomonas* sp. AMET3006.

CONCLUSION

Bacteria in this environment are metabolically active which leads to the production of various enzymes and bioactive compounds and sticky banned for untreated waste water. Therefore, it is important to waste derived bacteria in biological terms and also as a resource for biotechnology. The present study concludes that isolation of beneficial bacteria having plant growth promotion activities, bioremediation potential, heavy metal and antibiotic resistance and added advantage of antifungal activity is possible even from much polluted ecosystem like Coovum river. These bacteria can be employed for the bioremediation of sewage contaminated Coovum river water which further can be used for the purpose of irrigating ornamental plants.

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