RESEARCH PAPER

Isolation, Morphological and Biochemical Characterization of Phosphate-Solubilizing Bacteria from Agricultural and Garden Soils, and Evaluation of Their Efficiency in the Bioremediation of Detergent Waste water

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Abstract

Phosphate-rich effluent originating from household detergents is a major contributor to eutrophication and to the deterioration of receiving-water quality, and the conventional physicochemical methods used to remove phosphate remain costly and often generate secondary waste. In the present study, phosphate-solubilizing bacteria (PSB) were isolated from four soil environments – rice-field, corn-field and tuberose-field soil (grouped as agricultural soil) and garden soil – and their efficiency in reducing the turbidity of synthetic detergent waste water was evaluated. Soil suspensions were plated onto Pikovskaya's agar by the spread-plate method, and colonies that produced a clear halo zone were purified by quadrant streaking. Four representative isolates (AG-1 and AG-2 from agricultural soils; GD-1 and GD-2 from garden soil) were characterized by Gram staining and by catalase, oxidase and in doletests, and were then inoculated separately into detergent waste water to evaluate turbidity reduction over five days of incubation by spectrophotometric measurement of optical density at 600 nm. The agricultural isolates were Gram-negative rods that were catalase- and oxidase-positive but indole-negative, resembling Pseudomonas-type organisms, where as the garden soil isolates were Gram-positive rods that were catalase-positive, oxidase-negative and indole-positive, resembling Bacillus-type organisms. All four isolates produced a measurable halo zone on Pikovskaya's agar and reduced the turbidity of detergent wastewater relative to the un inoculated control, with reductions ranging from 47.5% to 57.6%. Isolate AG-2 produced the greatest turbidity reduction (57.6%), followed by AG-1 (53.4%), GD-2 (50.8%) and GD-1 (47.5%). These findings indicate that phosphate-solubilizing bacteria native to agricultural soil possess a superior phosphate-solubilizing and wastewater-clarifying capacity relative to garden-soil isolates, and support the use of indigenous PSB as an eco-friendly biological alternative to chemical treatment of phosphate-laden detergent wastewater.

Keywords: Phosphate-solubilizing bacteria; Pikovskaya's agar; detergent wastewater; turbidity reduction; bioremediation; halo zone

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INTRODUCTION

Rapid urbanization, industrialization and population growth have greatly increased the volume of domestic and industrial waste water produced globally. Among the many pollutants contained in these effluents, detergent-based waste water represents a significant environmental pollutant owing to its high phosphate and surfactant loads. Household detergents are widely used for the washing and sanitation purposes, and a phosphate-rich effluent generated there by is often discharged into sewers and natural water bodies with little or no treatment at all.

Detergent formulation includes surfactants, builders, bleaching agents and fillers, where phosphate builders, such as sodium tripolyphosphate, are usually included to soften water and improve cleaning performance. When discharging these effluents into the aquatic system, phosphate compounds act as limiting nutrients, stimulating the overgrowth of algae and macrophytes, resulting in eutrophication. The latter leads to reduction of oxygen content, mortality of fish and decrease in water quality. Since phosphorus represents the most common limiting nutrient in fresh water systems, even slight increase in phosphate loading causes disproportional ecosystem disturbance (Aliyat et al., 2022).

Conventional physicochemical methods of phosphate removal, including chemical precipitation, ion exchange, adsorption and membrane filtration, are capable of achieving high removal efficiencies but they are frequently expensive, energetically-intensive and generate chemical sludge or other secondary waste streams having to be disposed of too. Such drawbacks have led to the growing interest towards biological methods of phosphate removal utilizing the natural metabolic potential of microorganisms in a more sustainable way (Kim et al., 2005).

Soil bacteria, fungi and algae are capable of metabolizing a variety of organic and inorganic pollutants, and a number of soil bacterial genera are able to convert insoluble or complex compounds into simpler, soluble forms as a result of the organic acids and extracellular enzymes secretion. Among them, phosphate-solubilizing bacteria (PSB) are of particular interest owing to their capability of converting insoluble mineral phosphate – present in soils principally as calcium, iron or aluminium phosphate – into soluble orthophosphate (Wang et al., 2023).

MATERIALS AND METHODS

Chemicals, media and equipment

Standard laboratory glassware (conical flasks, test tubes, measuring cylinders, Petri plates, glass slides and cover slips) and equipment (autoclave, incubator maintained at 28-30°C, laminar air flow chamber, compound microscope and UV-visible spectrophotometer) were employed throughout the study. Pikovskaya's agar was prepared from glucose, tricalcium phosphate, ammonium sulphate, potassium chloride, magnesium sulphate, yeast extract, manganese sulphate, ferrous sulphate and agar, and nutrient agar (peptone, beef extract, sodium chloride and agar) was used for sub culturing before biochemical testing. Analytical-grade hydrochloric acid, sodium hydroxide, 3% hydrogen peroxide, oxidase reagent (tetra methyl-p-phenylene di amine dihydrochloride) and Kovac's reagent were used for biochemical characterization, and commercially available household detergent powder was used for preparation of synthetic detergent wastewater.

Soil Sample Collection

Soil samples were collected from four sites chosen on the basis of their expected phosphate content and microbial activity: rice-field soil, corn-field soil and tuberose-field soil, representative of agricultural land that had been regularly fertilized and manured with phosphate and organics, and garden soil, representative of non-agricultural environment. At each site, the surface litter layer was removed and approximately 100-200 g of soil was collected aseptically from a depth of 5-10 cm, on one chosen to exclude superficial contamination while ensuring a metabolically.

Preparation of detergent waste water and bacterial inoculation:

A synthetic waste water was prepared by dissolving a fixed amount of commercially available household detergent powder in distilled water to obtain an even solution with reproducible phosphate and surfactant content; the use of the synthetic effluent of standardized composition allowed to attribute any difference in treatment effect between flasks solely to bacterial action. Coarse undissolved particles were allowed to settle briefly and the supernatant was used as working wastewater. Aliquots of 100 mL of this wastewater were aseptically dispensed into sterile 250 mL conical flasks marked AG-1,AG-2, GD-1andGD-2depending on the isolate to be tested, and an equal volume of freshly grown culture of the appropriate isolate was inoculated into each flask; additionally, one flask with no bacterial inoculum was kept as uninoculated control. Flasks were statically incubated at 28–30°C, with periodic gentle agitation to provide adequate aeration and even suspension, for five days of treatment.

Determination of Turbidity and Calculation of Turbidity Reduction Efficiency

Turbidity was measured spectrophotometrically as optical density (OD) at 600 nm, wavelength commonly used for turbidity estimation in microbiological and wastewater researches, with higher absorbance corresponding to greater turbidity. OD of each wastewater sample was determined immediately prior incubation (final turbidity,OD_t).The percentage reduction in turbidity attributable tobacterial treatment was calculated using the following expression:

$$Percentageturbidityreduction = [(OD_0 - OD_t)/OD_0] \times 100$$

where OD_0 is the optical densityofthe detergent wastewater before PSBtreatment and OD_t is the optical density of the same sample after treatment. A higher percentage value indicates a greater removal of suspended particles and more effective clarification of the wastewater by the inoculated isolate. All measurements represent

single-flask determinations for each isolate; the absence of biological replication should be borne in mind in interpreting the magnitude of the differences reported below, and replicated trials are recommended for future confirmatorywork.

RESULTS

Isolation and screening of phosphate-solubilizing bacteria

Isolation of soil suspension onto Pikovskaya's agar resulted in the formation of bacteria colonies in all four soil samples, and some of these bacterial colonies had a clear halo zone of tricalcium phosphate solubilization. Bacteria colonies from agricultural soils (rice field and corn field) tended to have bigger halo zones compared to bacteria colonies from garden soil, which was a preliminary observation that there was a greater level of phosphate-solubilizing bacteria in the agricultural soil samples. Two isolates from each agricultural soil sample (AG-1 and AG-2) and from garden soil sample (GD-1 and GD-2 from two different garden soil samples) were isolated by quadrants streaking method.

Morphological characterization

The gram stain results for the two agriculturally derived bacterial cultures, AG-1 and AG-2, revealed that they were gram-negative rod-shaped bacteria existing either singly, which is in line with Pseudomonas-type soil PSB. On the other hand, the garden soil-derived cultures, GD-1 and GD .The results of Gram staining for all four isolates are summarized in Table 1.

Table1.1-Morphological (Gramstaining) characteristics of the phosphate-solubilizing bacterial isolates

Isolate code	Soil source	Gram reaction	Cell shape	Probable group
AG-1	Agricultural(ricefield)	Negative	Rod	Pseudomonas-like
AG-2	Agricultural(cornfield)	Negative	Rod	Gram-negative PSB
GD-1	Gardensoil	Positive	Rod	Bacillus-like
GD-2	Gardensoil	Positive	Rod	Bacillus-like

Biochemical characterization

All four isolates were catalase-positive, evident from immediate and vigorous effervescence on addition of hydrogen peroxide, indicating an aerobe mode of metabolism. The agricultural isolates AG-1 and AG-2 were oxidase-

positive,developing a dark purple color within 10 seconds of reaction with oxidase, while Garden isolates GD-1 and GD-2 showed no difference in color and were marked as oxidase-negative. In the indole test, AG-1 and AG-2 did not produce a red ring and were marked as indole-negative while GD-1 produced a faint redring and GD-2 a pred ring and were marked as indole-positive. The total biochemistry profile is catalase-positive, oxidase-positive and indole-negative for the agricultural isolates while catalase-positive, oxidase-negative and indole-positive for the garden isolates. This biochemistry profile is indicative of Pseudomonas type biochemistry profile. The complete biochemical profile of all isolates is presented in Table 2.

Table 2. Biochemical characteristics of the phosphate-solubilizing bacterial isolates

Isolate code	Soil source	Catalase	Oxidase	Indole	Probable genus
AG-1	Agricultural(ricefield)	+	+	−	Pseudomonas-like
AG-2	Agricultural(cornfield)	+	+	−	Gram-negativePSB
GD-1	Gardensoil	+	−	+	Bacillus-like
GD-2	Gardensoil	+	−	+	Bacillus-like

Turbidity reduction of detergent waste water

When each ofthe four PSB isolates was inoculated in detergent wastewater, clarification was observed along with measurable decrease in optical density at 600nm compared to the initial reading while the un inoculated controls showed negligible difference in turbidity within the same five days period. Out of the isolates tested, the agricultural isolates AG-2 (57.6%) and AG-1 (53.4%) exhibited the highest percentage reduction in turbidity compared to the garden soil isolates which had slightly lower percentages (GD-2 = 50.8% and GD-1=47.5%).

Table 3. Turbidity reduction of detergent waste water by PSB isolate safter 5days of incubation

S.No.	Isolatesource	Isolatecode	OD_{600} (before treatment)	OD_{600} (after treatment,day5)	Turbidity reduction (%)
1	Agricultural soil (rice field)	AG-1	1.18	0.55	53.4
2	Agricultural soil (corn field)	AG-2	1.18	0.50	57.6
3	Gardensoil	GD-1	1.18	0.62	47.5
4	Gardensoil	GD-2	1.18	0.58	50.8

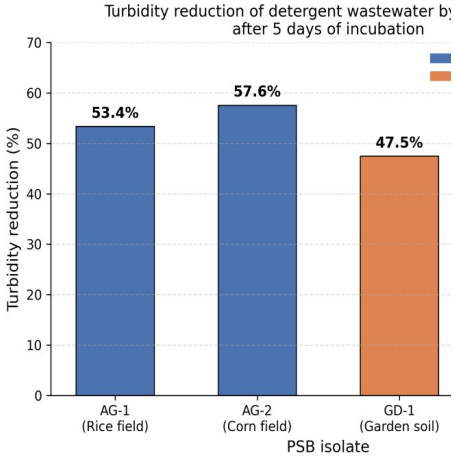


Figure1. Percentage reduction in turbidity of detergent wastewater by agricultural (AG-1, AG-2) and garden-soil (GD-1, GD-2) PSB isolates after 5 days of incubation.

Overall, all four isolates demonstrated the ability to solubilize phosphate on selective medium and to clarify detergent wastewater under the conditions tested, with the two agricultural isolates consistently out performing the two garden-soil isolates in turbidity-reduction efficiency.

DISCUSSION

This study attempted to isolate and characterize phosphate-solubilizing bacteria from agricultural and garden soil sand to evaluate and compare their performance inturbinating the wastewater of a detergent. The findings indicated that the all the four isolates showed phosphate- solubilizing ability as indicated by their halo-zone formation on Pikovskaya's agar and ability to reduce the turbidity of wastewater, with the agricultural isolates showing higher reduction than the garden-soil isolates.

Soil is known to be a habitat rich in phosphate-solubilizing microorganisms, and agricultural soils that are constantly exposed to phosphate fertilizers and root exudates from cultivated plants are generally expected to harbour more efficient PSB populations compared to the uncultivated or non-agricultural soils. In agreement with this, the isolates obtained from agricultural soils here exhibited larger halo zones compared to the garden soil isolates, just as reported previously that rhizosphere and cultivated soils are inhabited by more efficient phosphate-solubilizing strains. Halo zone formation in itself indicates the dissolution of insoluble tricalcium phosphate by organic acids secreted by the growing colony, and hence larger halo diameters generally indicate more efficient solubilization which would be expected to translate into better performance in phosphate-containing effluents such as detergent wastewater.

The isolates were characterized to belong to two broad phenotypic groups. The agriculturl isolates AG-1 and AG-2 were Gram-negative rods that were catalase- and oxidase-positive.

CONCLUSION

Phosphate solubilizing bacteria have been successfully isolated from agricultural (rice field and corn field) and garden soils through halo-zone formation in Pikovskaya's agar medium, and four representative isolates have been purified and characterized. Based on morphological and biochemical properties, the agricultural isolates were found to be Gram-negative, catalase and oxidase positive, indole negative rods, representing a Pseudomonas-type phenotype, while the garden soil isolates were found to be Gram-positive, catalase positive, oxidase negative, indole positive rods, representing a Bacillus-type phenotype. This suggests that PSB recovered from different soil environments can show significant differences in their phenotypic characteristics.

Upon inoculation into synthetic detergent wastewater, all four isolates showed a significant reduction in turbidity over five days compared to uninoculated control samples, where the reduction achieved by agricultural isolates, AG-1 and AG-2 were 53.4% and 57.6%, respectively, while garden isolates GD-1 and GD-2 showed 47.5% and 50.8%, respectively. This study provides evidence for the fact that native phosphate solubilizing bacteria, which are recovered from ordinary soil sources without further optimization, have the actual capability of biological clarification of phosphate-laden detergent effluent, and agricultural soils appear to be a relatively better source for highly efficient strains compared to non-agricultural soils, though both have provided.

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