A high throughput method and culture medium for rapid screening of phosphate accumulating microorganisms

Vasvi Chaudhry, Chandra Shekhar Nautiyal *

Division of Plant Microbe Interactions, National Botanical Research Institute, Rana Pratap Marg, Lucknow 226 001, India

ARTICLE INFO

Article history:
Received 1 April 2011
Received in revised form 17 May 2011
Accepted 18 May 2011
Available online 26 May 2011

Keywords:
Bacteria
Medium
High-throughput screening
Phosphate accumulation
Serratia marcescens

ABSTRACT

A novel PA Medium [PAM] for efficient screening of phosphate-accumulating organisms (PAOs) was developed taking Serratia marcescens NBRI1213 as model organism. The defined National Botanical Research Institute’s growth medium (NBRI) supplemented with 0.1% maltose, designed for quantitative estimation of phosphate accumulation was designated as PAM. Our work suggested usage of PAM for efficient qualitative screening and as a microbiological medium for preferential selection of PAOs on Petri-plates. For qualitative screening of PAOs, Toluidine blue-O dye (TBO) was supplemented in PAM, designated as PAM-TBO. Qualitative analysis of phosphate accumulated by various groups correlated well with grouping based upon quantitative analysis of PAOs, effect of carbon, nitrogen, salts, and phosphate accumulation-defective transposon mutants. For significantly increasing sample throughput, efficiency of screening PAOs was further enhanced by adaptation of PAM-TBO assay to microtiter plate based method. It is envisaged that usage of this medium will be salutary for quick screening of PAOs from environment.

1. Introduction

Phosphate is an essential nutrient for all organisms, used in the biosynthesis of diverse cellular components, including nucleic acids, proteins, lipids, and sugars (Rao et al., 1985). In bacterial cells part of biomolecules (e.g. DNA, polyphosphates, phospholipids, and ATP) consists of phosphorus (Juhna et al., 2007). Microorganisms play a key role in phosphorus circulation in the biosphere (Oehmen et al., 2007). Their ability to accumulate inorganic polyphosphates (polyP) and orthophosphate (Pi) act as phosphate storage reservoirs (one of the constituents of nucleic acids) and as energy storage reservoirs (for ATP formation) is one of the mechanisms of adaptation to changing environmental conditions (Smirnov et al., 2005; Karl-Kroppa, 1956; Schink, 2005). Eutrophication of aquatic environments caused by excessive release of phosphorus (P) through wastewaters is a worldwide pollution problem results in negative environmental effects in recent years, due to rapid industrialization, urbanization, and population growth (Hrenovic et al., 2009). Removal of phosphorus from domestic and industrial wastewater is thus central in curbing the water pollution problem of eutrophication (Ahn et al., 2007). Biological phosphorus removal processes therefore have been attracting attention in recent years because of low investment and operational costs compared with chemical precipitation processes (van Loosdrecht et al., 1997; Hirota et al., 2010; Pijuan et al., 2005; Omelon and Grynpas 2007; Ohtake et al., 1985; Ryazanova et al., 2007). Isolation of phosphate-accumulating microorganisms for understanding of their role in the phosphorus turnover in nature and technology has been conducted for more than 30 years. Due to insufficient understanding of the bacteria involved in these processes, especially phosphorus-accumulating bacteria, the mechanism of phosphorus metabolism is not fully understood. Therefore search for new microorganisms capable of effective phosphate accumulation and a fast, simple, sensitive, and accurate method for their isolation and screening will be salutary. Although various media have been used in microbial P uptake research (Serafim et al., 2002; Morohoshi et al., 2003; Nautiyal et al., 2008; Ryazanova et al., 2007; Trépanier et al., 2002; Kulaev et al., 2004; Reddy and Bux 2002), but to the best of our knowledge there is no rapid method for the isolation and screening of phosphate-accumulating organisms (PAOs).

Accordingly, aim of the study was to design a new medium and a method for the isolation of PAOs from the environment. In the present investigation, we have developed a new method based on novel medium for isolation and screening of PAOs. In addition, we describe a high-throughput method based on 96 well microtiter plate model that enables a large number PAOs to be screened based on color assay, which is readily adaptable to automation.

2. Methods

2.1. Bacterial strain, culture condition, sample collection and isolation

As an experimental organism Serratia marcescens NBRI1213 (NBRI1213) was used throughout the optimization studies. Starter
cultures of NBR1213 were inoculated from single colony isolates grown from frozen glycerol stock cultures into glass test tubes containing 5 ml of Nutrient broth medium and grown overnight at 28 °C incubated in a rotary shaker (New Brunswick Scientific Co., Inc., Edison, NJ) and can be used as a pre-inoculum for further studies. Water for all media was deionized and double distilled prior to use.

For transposon mutagenesis NBR1213, an efficient phosphate-accumulating strain resistant to ampicillin (100 mg/ml), was used as a parent strain. Transposon5 (Tn5) was introduced into NBR1213 cells by conjugation with Escherichia coli WA803/pPGS (Salvaraj and Iyer 1983), using a modification of the procedure by Rostas et al. (1984). Transconjugants were obtained by membrane filter mating on NA medium for 48 h at 28 °C and were selected on NA containing ampicillin (100 mg/ml) and kanamycin (100 mg/ml) as described earlier (Nautiyal et al., 1989).

Samples were collected from three different ecosystems. Soil sample was collected from Tangdhar (34°23′52″N 73°51′49″E), water sample from Dinapuri (25°38′24″N 85°2′24″E) and sludge sample was collected from Refinery of Reliance Industries, Jamnagar (22°28′0″N, 70°4′0″E). All samples collected in sterile plastic bags or bottles were transported to the laboratory and stored at 4 °C immediately for further studies. Spread plate technique was employed to enumerate total heterogeneous bacterial population from soil, water and sludge using PAM Agar, after incubation at 28 °C for 48 h morphologically distinct colonies present on the plates were selected at random and subjected to screening for phosphate accumulators using PAM-TBO medium. The percentage of PAOs were determined by the formula = [No. of PAOs obtained/No. of total bacteria screened] × 100.

2.2. Pi accumulation experiments

For Pi accumulation experiments the cells of NBR1213 were inoculated in 50 ml of liquid media at 28 °C under shaking (180 rpm) for 48 h. After incubation, the cells were harvested by centrifugation at 10,000g for 10 min at 4 °C and used for extraction of accumulated Pi. A weighed amount of fresh wet cells obtained after centrifugation was used as biomass.

2.3. Extraction and assay of total polyP

Total polyP were extracted by a Rao et al. (1985) based on the insolubility of polyP in alkaline hypochlorite. NaF (1 mM) was added, and the pH of the reagent was adjusted to 9.8. The cells (about 109 cells per ml) were collected by centrifuging 50 ml of culture at 10,000g for 10 min at 4 °C. The cells were resuspended in 0.145 M NaCl containing 1 mM NaF, and the centrifugation was repeated. Washed pellet was suspended in 1 ml of alkaline hypochlorite reagent and incubated at 25 °C for 60 min. The insoluble fraction was collected by centrifugation at 27,000g for 15 min. The fraction was suspended in a pool of a wash solution containing 1.5 M NaCl, 5 mM EDTA, and 1 mM NaF and adjusted to pH 4.6 with HCl. This suspension was centrifuged (27,000g for 15 min), and the supernatant was discarded. The residue was solubilized in 1 ml of 0.154 M NaCl solution adjusted to pH 7.0 and centrifuged at 10,000g for 10 min, and the supernatant fluid was collected. This polyP extract was stored at 0–4 °C. The content of P was estimated by Mo-Blue method (Muyima and Cloete, 1995), 100 μl of polyP solution was mixed with an equal volume of 2 N HCl and heated at 95 °C for 30 min. Then the solution was diluted to 300 μl with distilled water, mixed with 700 μl of the Mo-blue assay solution (6 parts of 0.42% (NH4)6Mo7O24·4H2O in 1 N H2SO4 and 1 part of 10% ascorbate), and the solution was made 5 ml with distilled water and incubated at 45 °C for 20 min. Pi concentration was determined based on absorbance at 820 nm with reference to the standard curve of 0–10 mM phosphate. Wet biomass value was used for calculations.

2.4. Medium composition and preparation

Preliminary work to optimize the medium was started with Minimal medium-9 (M9) salts and glucose was subsequently replaced with sucrose, sorbitol, mannitol, maltose, arabinose, lactose, sodium benzoate, sodium acetate and sodium citrate. Ammonium chloride as nitrogen source was replaced with magnesium nitrate, ammonium acetate, ammonium nitrate, sodium nitrate, potassium nitrate, aluminum ammonium sulfate and ammonium sulfate and combination of salts of magnesium and calcium were optimized to prepare complete defined medium for growth and screening of PAOs. Water for all media was deionized and double distilled prior to use. For quantitative analysis, composition of the complete defined medium (PAM) was (g/L) Sodium citrate, 4; NaCl, 0.5; (NH4)2SO4, 2.5; CaCl2, 0.25; MgSO4, 0.25; Na2HPO4, 12.8; KH2PO4 3; Maltose, 0.01; and Agar, 20 (for initial screening of PAOs on Petri-plates). For qualitative screening of PAOs Toluidine blue-o dye (0.025 g/L) was added and the medium was designated as PAM-TBO. Stock solutions of Maltose, CaCl2 and MgSO4 were autoclaved for 20 min at 10 psi or filter sterilized individually with a 0.2-mm-poresize filter and aseptically added and mixed thoroughly prior to inoculation of culture. For comparative studies PAM was tested against M9, NM (Nakamura et al., 1995) and synthetic waste water (SWW) (Ohtake et al., 1996). Compositions of M9 was (g/L) NaH2PO4,12.8; KH2PO4, 3.0; NaCl, 0.5; NH4Cl, 1.0; glucose, 2.0; MgSO4·7H2O, 0.4940; CaCl2·2H2O, 0.0152; NM was (g/L) glucose, 0.5; peptone, 0.5; monosodium glutamate, 0.5; yeast extract, 0.5; KH2PO4, 0.44; (NH4)2SO4, 0.1; MgSO4·7H2O and SWW (g/L) was peptone, 0.2; citric acid, 0.2; yeast extract, 0.02; NaHCO3, 0.075; MgSO4·7H2O, 0.15; CaCl2·2H2O, 0.05; NaCl, 0.1; KH2PO4, 0.053.

2.5. Optimization of color based method for qualitative screening of PAOs

Qualitative (Toluidine blue-O color based qualitative estimation of phosphate accumulation) and quantitative analyses (quantitative estimation of phosphate accumulation as Pi) in broth were carried out by using 50 ml of PAM in a 150 ml test tube inoculated in triplicate with the bacterial strain (500 μl inoculum with approximately 1–2 × 106 cfu/ml). Autoclaved, uninoculated medium served as controls. For qualitative estimation of TBO in PAM-TBO, the optical density of the culture supernatant was measured at 625 nm. For the quantitative analysis, phosphate accumulated in the culture biomass was extracted by using Rao et al. (1985) and estimated by the Mo-Blue method (Muyima and Cloete, 1995) was measured at 820 nm, by using Milton Roy Spectronic 20D1, USA.

2.6. Development of high throughput microtiter plate based method

Bacterial cultures were individually cultivated in the microtiter plates with 4 replicates entire row of the microtiter plate. Upper 4 wells contained 200 μl PAM-TBO, while lower 4 wells contained 200 μl PAM medium. Initial 20 μl inoculum prepared in NB was used into selected wells of the microtiter plate and served to blank the plate reader. Typically, wells in column 12 on each plate remained unseeded, as these 8 wells acted as negative background controls during subsequent analyses. When all the selected wells were seeded, the microtiter plates were covered with its original lid, sealed with parafilm, placed inside an incubator and incubate statically for 24 h at 28 °C. To remove the interference with O.D. of bacterial cells, after incubation microtiter plates were centrifuged for 15 min at ~1800g (Sigma 3-16K). After centrifugation,
the media removed from the wells was then transferred to fresh microtiter plates. The resulting color production was quantitated at the absorbance (the optical density at 625 nm [OD_{625}]) in a microtiter plate reader (Biotek). To reduce the effects of plate-to-plate variations and day-to-day variations, NBRI-28 with low phosphate accumulation and NBRI1213 with high phosphate accumulation ability properties as controls were incorporated on each assay plate and two designated control plates were included in each screening session. Plate-based controls were incorporated; and assay wide controls were included to determine background levels, signal-to-noise ratios, and standards for normalization. Using the protocol described as above no statistically significant differences were noted when comparing all pairs of rows to each other (P > 0.05), under our experimental conditions.

2.7. Statistical analysis

The experiments were conducted with three independent replications. Results of all analysis were judged for its significant at 5% level. Statistical analysis was performed with Sigma Plot 7.1 (SPSS Science, USA) and MS-Excel (Microsoft Corp., USA). Sigma Plot 7.1 software was used for graphical data presentation.

3. Results and discussion

3.1. Effect of various carbon, nitrogen and salt on phosphate accumulation in NBRI1213

In our endeavor to develop an efficient defined medium for quantitative estimation of phosphate accumulation using strain NBRI1213 grown in M9 liquid medium for 2 days by replacing glucose present in M9 with a range of carbon sources and it was found that the type and amount of carbon source played an important role in phosphate accumulation. Among a range of carbon source, in the presence of sodium citrate NBRI1213 accumulated maximum phosphate with 90.23 mM per gm biomass (Table 1). The ability of nitrogen source to influence the phosphate accumulation ability was checked by replacing NH4Cl with a range of nitrogen sources as indicated above. NBRI1213 accumulated phosphate efficiently in presence of (NH4)2SO4. Phosphate accumulation ability was further improved by increasing the concentration of (NH4)2SO4 by 0.25% instead of 0.1% (Table 2). Various salts were used to improve the phosphate accumulation ability of NBRI1213 and it was observed that MgCl2 at 0.25% had a synergistic effect on phosphate accumulation activity, in the presence of CaCl2 at 0.25% as compared to other salts of magnesium and calcium (data not shown). This National Botanical Research Institute’s phosphate growth medium designed for quantitative estimation of phosphate accumulation was designated as NBRI. To enhance the efficiency of the NBRI, the medium was individually supplemented further with additional sugars glucose, sucrose, arabinose, glycerol, mannitol, sorbitol and maltose at a concentration of 0.1 g/L. Maximum phosphate accumulation of Pi (141.49 ± 1.95 mM gm biomass\(^{-1}\)) occurred in the presence of maltose. The NBRI medium supplemented with 0.1% maltose thus formulated for an efficient quantitative estimation of phosphate accumulation was designated as PAM.

An efficient screening strategy for isolating effective PAOs in environments with diverse microbial populations and limited organic resources will be salutary, where it is possible that PAOs are often out- competed by organisms capable of utilizing available nutrients more rapidly (Appeldoorn et al., 1992). The only avenues currently available for the study of uncultured bacteria are cultivation-independent molecular ecological techniques that have proved to be very powerful for the study of bacteria in their natural settings (Bashan and Bashan, 2004). Parallel study of laboratory cultures would however, strongly complement molecular ecological investigations and enhance research into the roles of PAOs and their biotechnological potentials due to lack of suitable media that would support the growth of all viable nutritional types.

3.2. Comparison analysis of PAM with previously known media

For quantification of phosphate accumulation ability of PAOs by PAM, an efficient phosphate accumulating bacterial strain NBRI1213 was used a reference strain and compared with known media, using M9, NM and SWW broth for up to 10 days (Fig. 1). The result suggested that new medium PAM was about 2.5–7-fold more efficient as compared to NM medium, M9 medium and SWW, respectively. As compared with other media, the ability of the strain to accumulate phosphate in PAM was also maintained at a higher level throughout the duration of 10 days.

Efficacy of PAM was evaluated to screen PAOs among heterogeneous population on Petri-plates from different ecosystems. Five hundred heterogeneous bacteria from different environmental sources, viz. soil, water, and sludge at random and calculated the percentage of PAOs by qualitative screening of 500 heterogeneous bacteria taken. Percentage of PAOs from soil, water, and sludge were 20%, 35% and 64%, respectively by using PAM agar medium (Table 3).

A new defined medium PAM was formulated which was more efficient to accumulate phosphate and maintain it at a higher level.

<table>
<thead>
<tr>
<th>N sources</th>
<th>Pi content (mM gm biomass(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>M9C</td>
<td>90.23 ± 8.76</td>
</tr>
<tr>
<td>M9C + NH4Cl (0.1%)</td>
<td>90.23 ± 8.76</td>
</tr>
<tr>
<td>M9C + MgNO3 (0.1%)</td>
<td>2.34 ± 0.43</td>
</tr>
<tr>
<td>M9C + C6H2(NO3)2 (0.1%)</td>
<td>24.15 ± 3.55</td>
</tr>
<tr>
<td>M9C + NH4NO3 (0.1%)</td>
<td>15.55 ± 1.20</td>
</tr>
<tr>
<td>M9C + NaNO3 (0.1%)</td>
<td>0.955 ± 0.51</td>
</tr>
<tr>
<td>M9C + KNO3 (0.1%)</td>
<td>4.4 ± 0.14</td>
</tr>
<tr>
<td>M9C + AlNH4(SO4)2 (0.1%)</td>
<td>5.18 ± 0.47</td>
</tr>
<tr>
<td>M9C + NH4Cl (0.1%)</td>
<td>90.23 ± 8.76</td>
</tr>
<tr>
<td>M9C + (NH4)2SO4 (0.025%)</td>
<td>6.74 ± 0.23</td>
</tr>
<tr>
<td>M9C + (NH4)2SO4 (0.1%)</td>
<td>99.3 ± 1.80</td>
</tr>
<tr>
<td>M9C + (NH4)2SO4 (0.25%)(M9CN)</td>
<td>103.65 ± 5.05</td>
</tr>
<tr>
<td>M9C + (NH4)2SO4 (0.5%)</td>
<td>97.4 ± 1.86</td>
</tr>
<tr>
<td>M9C + (NH4)2SO4 (1%)</td>
<td>48.36 ± 0.84</td>
</tr>
</tbody>
</table>

Control M9 medium contained g/L: glucose, 4; NaCl, 0.5; NH4Cl, 1; CaCl2, 0.014; MgSO4, 0.25; Na2HPO4, 12.8; KH2PO4, 3.

Changes in concentration of M9 medium components are indicated within brackets.

The data are the means of three independent experiments ± SE.
longer as compared to NM, M9 and SWW, respectively. Our work suggest the use of PAM as an efficient phosphate accumulation medium over other media and also that PAM should serve as a synergistic composition for qualitative screening of PAOs and preferential isolation of potential phosphate accumulators on Petri-plates from different ecosystems. The results suggest that PAM should serve as a synergistic microbiological growth medium composition for efficient screening of PAOs that grow on the culture medium, in the presence of the other microorganism. This further augments well for the use of PAM as an efficient phosphate accumulation medium over other media for isolation of PAOs from environment.

3.3. Qualitative and quantitative performance of dye based formulation PAM-TBO

In our attempt to reduce the time required to perform a quantitative assay, PAM medium was further modified by using Toluidine blue-O (TBO) as an indicator dye for visual observation, to quickly evaluate the level of phosphate accumulation. We compared the influence of TBO on qualitative and quantitative analysis using NBRI1213, grown in PAM TBO containing 0.01, 0.025, 0.05, and 0.1 g/L TBO for 2 days (Fig. 2). The TBO dye concentration 0.025 g/L correlated well with quantitative assay and was used to formulate the new medium. The PAM growth medium thus formulated containing 0.025 g/L TBO was designated as PAM-TBO.

The potential of PAM-TBO to evaluate a large number of PAOs was tested by initial screening of 2500 bacterial strains. Quantitative and quantitative analysis was carried out with 10 bacterial strains (Fig. 3) NBRI-21 (A), NBRI-3 (B) NBRI-4 (C), NBRI-RHP88 (D), NBRI1213 (E), NBRI-28 (F), NBRI-36 (G), NBRI-44 (H), NBRI-37 (I), and NBRI-49 (J) grown on PAM-TBO medium for 2 days. The strains selected could be placed into two distinct groups based upon the level of phosphate accumulation. The first group of five strains – (Fig. 3) NBRI-21 (A), NBRI-3 (B), NBRI-4 (C), NBRI-RHP88 (D), NBRI1213 (E) accumulated phosphate and was at least 5-fold more efficient than the second group of five strains – NBRI-28 (F), NBRI-36 (G), NBRI-44 (H), NBRI-37 (I), and NBRI-49 (J). Phosphate accumulation ability of NBRI1213 was monitored in the presence of various carbon, nitrogen and salts. NBRI1213 as compared with control PAM-TBO –Fig. 4(A), demonstrated diverse level of phosphate accumulation activity in the presence of various carbon sources – glucose (B), sorbitol (C), glycerol (D) and sucrose (E). Nitrogen sources – C2H7NO2 (F), KNO3 (G), NH4NO3 (H), NH4Cl (I) and salts – Na2CO3 (J), KCI (K), Na2SO4 (L) and MnSO4 (M). The pattern of phosphate accumulation by NBRI1213 in qualitative assay with PAM-TBO correlated well with the quantitative assay.

An experiment was conducted to screen 500 phosphate accumulation-defective Tn5 mutants of NBRI1213. Based upon visual observation, owing to their incapability to decolorize TBO efficiently, as compared with NBRI1213 (Fig. 4), two mutants – NBRI1213-4052 (N) and NBRI1213-4053 (O) were easily distinguishable by day 2. Quantitative analysis further confirmed the diverse levels of phosphate accumulation ability of the mutants (Fig. 4). These findings further demonstrate that there is a correlation between the pattern of phosphate accumulation by the mutants in qualitative assay and quantitative assay.

3.4. Development of high throughput method for the screening of PAOs

To effectively evaluate the potential application of PAM-TBO assay in screening possible PAOs in environmental samples, efficiency of the screening was further enhanced by using 96 well plates. The screening media used in the study was PAM-TBO using a range of Na2HPO4 and KH2PO4 (47, 94, 200, 300, and 500 mM) as phosphate sources in PAM-TBO medium. It was observed that high

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Sample</th>
<th>Total heterogenous CFU</th>
<th>% Phosphate accumulators</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Soil</td>
<td>5.42 ± 0.029</td>
<td>20 ± 0.57</td>
</tr>
<tr>
<td>2.</td>
<td>Water</td>
<td>5.19 ± 0.020</td>
<td>35 ± 2.30</td>
</tr>
<tr>
<td>3.</td>
<td>Sludge</td>
<td>7.40 ± 0.060</td>
<td>64 ± 0.57</td>
</tr>
</tbody>
</table>

The data are the means of three independent experiments ± SE.
and low phosphate accumulators can be easily distinguished using phosphate concentration in the range of 94–300 mM by visual observations after 24 h of growth. We opted for 94 mM for further work as it was optimally suited for screening wider range of phosphate accumulators. PAOs were isolated from soil, water and activated sludge representing fungi, actinomycetes and bacteria and PAM-TBO assay proved to be effective for the microorganisms tested (data not provided). To validate the screening method, 940 PAOs were screened. A collection of 10 PAOs with non-phosphate accumulation and phosphate accumulation abilities properties each were successfully identified using this assay (data not provided). Fig. 5 clearly demonstrates potential of the adaptability of PAM-TBO assay to microtiter plate based method to enhance sample throughput, as highest limit of decolorization of TBO in PAM was maximum in NBRI1213 followed by NBRI28, a low phosphate accumulator (Fig. 5A). Introduction of the centrifugation step further enhanced the sensitivity of the assay, especially for low phosphate accumulator NBRI28 (Fig. 5B).

In the present investigation an attempt has been made to develop a new protocol based upon visual observation, for a quick and reliable detection of PAOs. Therefore, it was envisaged to modify the PAM medium by using TBO, a blue colored dye that we serendipitously discovered that it decolorizes owing to a drop in the concentration of phosphate of the medium, as an indicator to quickly evaluate the level of phosphate accumulation based upon visual observations. The metachromatic activity of polyP allows for staining with TBO both within cellular vacuoles (Lin et al., 2003). TBO has been used to identify the nonradioactive polyP species obtained from biological systems after separation by PAGE. The polyP hydrolytic degradation–visualization strategy was first employed in paper chromatography by Karl-Kroupa (1956). Omelon and Grynpas (2007) used a similar methodology that is applicable for polyP species resolved by PAGE that visualizes linear polyP species less than 5 Pi units long, as well as Pi and the 3 m ion. This method involves hydrolysis of separated polyP species to Pi, followed by complexation of the product Pi with molybdate and the cationic dye methyl green. However to the best of our knowledge this is the first report of directly using TBO in a microbiological growth media as an indicator dye for visual observations, to quickly evaluate the level of PAOs.
Large scale screening of PAOs deploying both qualitative and quantitative assay gave similar results, indicating that there is a correlation between a qualitative and a quantitative assay. However, in a qualitative assay with PAM-TBO, it was possible to quickly distinguish the two groups of low and high phosphate accumulating bacteria without any need for time consuming biochemical methods usually involved in the quantitative assay of PAOs. The suitability of the assay for different microorganisms was further confirmed by screening PAOs isolated from soil, water and activated sludge representing fungi, actinomycetes and bacteria. The results suggest that PAM-TBO should serve as an excellent formulation for the initial screening of a large number of PAOs and an early detection of the effect of various physiological parameters and screening efficiency of a large number of phosphate-accumulation defective mutants, based upon visual observation. Pure culture evaluation by using PAM-TBO may be a useful tool in the search for PAOs better suited for environments where physiological factors may constitute a limitation for phosphate accumulation. Fast decolorization of the dye is an indicator of a superior phosphate accumulating microbe. Therefore colorimetric reaction using PAM-TBO of the present work was further applied in a high-throughput manner to detect efficient PAOs, growing on PAM Petri-plates. Adaptation of PAM-TBO assay in screening PAOs in environmental samples for microtiter plate assay enhanced the effectiveness of the assay system efficiently and effectively. Therefore, it is envisaged that use of this protocol will be salutary for the quick screening of PAOs.

4. Conclusions

Our work has demonstrated that PAM can be used as an efficient medium for enumeration, screening and growth of PAOs. The results suggest that, by using PAM-TBO based upon visual observations, it is indeed possible to classify the PAOs on a qualitative basis. Qualitative analysis of the phosphate accumulation by various groups also correlated well with our grouping based upon quantitative analysis of bacteria isolated from environment. Further high-throughput screening of PAOs using PAM-TBO medium on microtiter plate based model offers a simple and flexible method to screen large number of PAOs and to examine multiple parameters and factors influencing phosphate accumulation.

Conflict of Interest

The authors have declared no conflict of interest.

Acknowledgements

Financial support of the work from Task Force Council of Scientific and Industrial Research (CSIR) Grant NWP-019 is duly acknowledged. V.C. would like to thank CSIR for providing her Senior Research Fellowship.

References


