Biocatalysis and Agricultural Biotechnology 5 (2016) 168–172

Contents lists available at ScienceDirect

Biocatalysis and Agricultural Biotechnology

journal homepage: www.elsevier.com/locate/bab

Assessment of phosphate solubilization activity of Rhizobacteria in mangrove forest

Manouchehr Teymouri [a], Javad Akhtari [b], Maryam Karkhane [c], Abdolrazagh Marzban [a,*]

[a] Biotechnology Research Center, Mashhad University of Medical Sciences, Mashhad, Iran
[b] Department of Physiology and Pharmacology, Immunogenetics Research Centre, Faculty of Medicine, Mazandaran University of Medical Sciences, Sari, Iran
[c] Gastroenterology and Liver Diseases Research Center, Research Institute for Gastroenterology and Liver Diseases, Shahid Beheshti University of Medical Sciences, Tehran, Iran

ARTICLE INFO

Article history:
Received 28 September 2015
Received in revised form
25 January 2016
Accepted 28 January 2016
Available online 29 January 2016

Keywords:
Phosphate solubilizing bacteria
Mangrove
Biofertilizer
Rhizosphere

ABSTRACT

The current study was performed to assess the phosphate (P) solubilization potential of inhabiting bacteria from rhizosphere of *Avicennia marina* plants. Hence, sampling was carried out from soils near the mangrove roots in the intertidal zone. Among 13 isolates screened, four high potential solubilizing bacteria (PSB) were selected based on the formation and dimension of clear halo around the colonies. Phylogenetic analysis of 16 S rDNA of PSBs showed their close similarity to *Bacillus*, *Pseudomonas* and *Acinetobacter* species. Regarding phosphate solubilization index (PSI), the dimension of the clearance zone divided by that of bacterium-inoculated spot, PSB1 and PSB3 were found as the most potent isolates with 3.5 and 2.6-PSI values, respectively. However, maximum P solubilization was found in broth media for PSB10 (357 mg/l) and PSB3 (282 mg/l), respectively. The consortium experiment showed a positive effect on total P solubilized by these isolates as solubilizing efficiency increased by 373 mg/l. P solubilizing efficiency also achieved in a high percentage range for consortium culture (74% ± 8%) and PSB10 (71 ± 7%). The experiments showed that P solubilization was promoted along with acidification in BSB10 and consortium culture during incubation course, while such trend was not seen for other isolates. Our investigation proposes that the isolates can be applied as biofertilizer in marine ecosystems via bioaugmentation to sustain and even restore mangrove forests.

1. Introduction

Phosphate (P) compounds act as one of the most important fertilizers in soil and promote growth of plants and algae. Amongst different P compounds, only orthophosphate can be assimilated by plants and algae (Hameeda et al., 2008). Moreover, variability in pH, humidity and cation contents of soil influences P solubilization. Considering that the use of chemical fertilizers in agriculture has created potential health hazards, the role of rhizospheric microorganisms to improve soil structure has been highly regarded (Sharma et al., 2013).

Phosphate solubilizing microorganisms (PSM) play a major role in supplementing plants with P and protect plant roots against parasites (El-Hadad et al., 2011; Tallapragada and Gudimi, 2011). In addition, PSMs have been widely reported to possess many potentials, including nitrogen fixation, metal chelating, organic acid and phytohormone production, beneficial to plant health and growth (Kim et al., 2005; Pandey et al., 2006; Jiang et al., 2008; Hariprasad and Niranjana, 2009; Matilla et al., 2011; Kaplan et al., 2013). Up to now, a variety of microorganisms involved in P solubilization has been discovered from ectorhizospheric zone and endosymbiotic rhizobia. The most important groups belong to fungi and bacteria (known as phosphate solubilizing bacteria or PSB) such as *Penicillium*, *Aspergillus*, *Pseudomonas*, *Azospirillum*, *Burkholderia*, *Bacillus*, *Enterobacter*, *Rhizobium*, *Erwinia*, *Serratia*, *Alcaligenes*, *Arthrobacter*, *Flavobacterium* and *Acinetobacter* species (Rodriguez and Fraga, 1999; Kuklinsky-Sobral et al., 2004; Ayyadurai et al., 2006; Morales et al., 2007; Kundu et al., 2009).

There are some reports concerning the isolation of several marine bacterial genera such as *Pseudomonas*, *Bacillus*, *Vibrio*, *Alcaligenes*, *Micrococcus*, *Corynebacterium* and *Flavobacterium* having potential for solubilizing P in seawater and marine sediments. Although high concentrations of cations in sea water cause P to precipitate in sediments, bacteria inhabited marine environments have shown the greater capability to redissolve inorganic P compounds (Mudryk, 2004). Moreover, they are capable of assimilating organic P compounds and metabolizing them to inorganic soluble compounds. The aim of this study was to assess P solubilizing potential of microorganisms inhabited sediments of

* Corresponding author.
E-mail address: marzban86@gmail.com (A. Marzban).

http://dx.doi.org/10.1016/j.bcab.2016.01.012
1878-8181/

mangrove forests and rhizosphere of *Avecinia marina* at Qeshm Island, located in the Persian Gulf, south of Iran.

2. Materials and methods

2.1. Soil sample collection

Soil samples were collected from rhizosphere and sediments of mangrove forest in the intertidal coast of Qeshm Island, south of Iran. The season at the time of sampling was mid-autumn with temperature and pH variation of 30–35 °C and 7–7.7, respectively. In addition, as the location was an intertidal zone, it was difficult to assign a given amount to P and chemical oxygen demand (COD) concentrations. However, the COD varied from four to 20 mg/l and 3.5 to 10, respectively, among the samples. The samples were transferred into containers by a sterile spoon taken from the top surface of soils near the plant roots at the depth of 15 cm. To maintain aerobic microorganisms, especially bacteria, the container caps were loosed, and remained away from heat and sunlight. In the laboratory, they were kept at 4 °C in refrigerator until the start of the experiments.

2.2. Isolation of P solubilizing bacteria

One gram of each collected soil sample was diluted fivefold by autoclaved sea water. One-tenth microliter of each dilution was inoculated on Pikovskaya (PVK) agar medium by an L-shape glass rod. The plates were incubated at 30 °C for one week. Then, PSBs were determined through appearing clear halo around their colonies. The isolates were purified for the study of P solubilization activity under different conditions like varying temperature, pH and salinity.

2.3. Characterization of P solubilizing bacteria

Preliminary identification was performed through biochemical experiments and morphological characteristics based on macroscopic and microscopic observations. All the studies were carried out according to the experiments described in Bergey's manual of determinative bacteriology (Holt et al., 1994). To extract genomic DNA from the bacterial colonies, they were cultured on nutrient agar and incubated overnight at 35 °C. Then, DNA was isolated using the phenol/chloroform/isoamyl alcohol method as described by Sambrook et al. (2001). Polymerase chain reaction (PCR) of 16 S rDNA gene fragment was done using universal primers of Fd1 (5′-AGAGTTTGATCCTGGCTCAG-3′) and Rd1 (5′-AGGAGGTGATCCAGCC-3′). PCR mixture contained reaction buffer (20 mM, Tris pH 8.4, KCl 50 mM), $MgCl_2$ (1.2 mM), Taq polymerase (1.25 units), dNTP (10 mM) and each of primers (0.1 mM). DNA amplification was performed by a DNA thermal cycler Primus 25 with the following cycling program: initial denaturation at 94 °C for 5 min, and 30 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 45 s, extension at 72 °C for 1 min, and final extension at 72 °C for 7 min.

Phylogenetic analysis was conducted using 16 S rDNA sequencing followed by alignment with NCBI nucleotide database, from which the closest species-related sequences were retrieved and analyzed by MEGA version 4.0 software. Neighbor joining method was employed with bootstrap values generated from 1000 replicates.

3. Assessment of P solubilization efficiency

3.1. Evaluation of P solubilization activity on agar medium

To evaluate the P solubilization activity, pure colonies were spot-inoculated on PVK agar medium by a metal loop and incubated for 3 days at 30 °C. P solubilization potency was measured based on Phosphate Solubilization Index (PSI) as the following equation (Morales et al., 2011):

$$PSI = \frac{\text{Total diameter of halo zone}}{\text{Colony diameter}}$$

PSIs were calculated in triplicate for the various colonies. The most potent bacteria were selected for further studies.

3.2. Evaluation of P solubilization activity in broth medium

The selected bacterial strains were evaluated for P solubilization activity in 100 ml broth medium containing 1 g glucose, 0.5 g $MgCl_2 \cdot 6H_2O$, 0.025 g $MgSO_4 \cdot 7H_2O$, 0.02 g KCl, 0.01 g $(NH_4)_2SO_4$, 2.0 g NaCl as well as 0.5 g tricalcium phosphate amended as insoluble P source. All experiments were carried out in 250 ml Erlenmeyer flasks containing 100 ml liquid medium. The flasks were autoclaved at 121 °C under 15-atmosphere pressure for 15 min prior to adding tricalcium phosphate. Culture conditions for the isolates were initial pH 7.0 and temperature 30 °C with rotary shaking at 100 rpm for seven days. P solubilization activity was assayed as pure isolates and a consortium of all isolates. Growth rate and P solubilization were measured at daily intervals. Two milliliters of media was centrifuged at 6000 rpm for 15 min, and then the supernatant was taken to measure the P concentration using ascorbic acid method as described by Murphy and Riley (1962).

Briefly, 1 ml of the supernatant was added to 5 ml ammonium heptamolybdate solution (2.5 g/ml) in 0.2 M H_2SO_4. Then, 1 ml fresh-made ascorbic acid solution (0.4% w/v) was added and mixed. The solution was incubated in dark for half an hour and the absorbance was measured via spectrophotometry at 587 nm wavelength. Using KH_2PO_4 standard calibration line, the P content of the supernatants was determined. Control flasks without bacterial inoculation were also incubated at the same condition used for samples. This control showed non-bacterial P solubilization at the end of the incubation period and their values were subtracted from experimental values.

3.3. Statistical data analysis

Statistical analyses were performed using GraphPad Prism version 5 (GraphPad Software, San Diego, CA). All experiments were conducted in triplicate and the values expressed as mean along with standard deviation. One-way ANOVA and Tukey's multiple comparison were employed at the significance level of 5%.

4. Results and discussion

From the total soil samples, 86 colonies were isolated in the primary screening, of which 13 colonies exhibited P solubilizing activity. Among these colonies, four colonies (PSB1, PSB3, PSB10 and PSB12) showed maximum P solubilizing potential on agar medium (Fig. 1). As seen in Table 1, maximum PSIs were determined for PSB1 and PSB3 strains, which were 3.5 and 2.6, respectively. PSB1 was the main prominent P solubilizer on agar media, while the highest P solubilization occurred in the broth