Biotechnology Research and Innovation (2017) xxx, xxx–xxx

Biotechnology Research & Innovation

http://www.journals.elsevier.com/biotechnology-research-and-innovation/

RESEARCH PAPER

Alkaline phosphatase activity of a phosphate solubilizing *Alcaligenes faecalis*, isolated from Mangrove soil

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Received 26 September 2016; accepted 13 January 2017

KEYWORDS
Mangrove ecosystem;
Alcaligenes faecalis;
Phosphate solubilization;
Alkaline phosphatase

Abstract Microorganisms are capable of converting insoluble phosphate into a bioavailable form through solubilization and mineralization processes. Hence in the present study a phosphate solubilizing bacterium, PSB-26, was isolated from mangrove of the Mahanadi delta using NBRIP-agar and NBRIP-BPB broth containing tricalcium phosphate as the phosphate source. Based on phenotypic and molecular characterization, the strain was identified as *Alcaligenes faecalis*. The maximum phosphate solubilizing activity of the strain was found to be 48 μg/ml with decrease in pH of the growth medium from 7.0 to 3.2. During phosphate solubilization, various organic acids, such as oxalic acid (289 mg/L), citric acid (0.2 mg/L), malic acid (0.3 mg/L), succinic acid (0.5 mg/L) and acetic acid (0.4 mg/L) produced in the broth culture were detected through HPLC analysis. Crude alkaline phosphatase activity of the strain was determined by *p*-nitrophenyl phosphate assay and optimized with different growth parameters to obtain maximum enzyme production. Under optimized sets of conditions, maximum alkaline phosphatase activity of 93.7 U/ml was observed. Partially purified alkaline phosphatase exhibited three protein bands of sizes approximately 45 kDa, 25 kDa and 17 kDa. Partially purified alkaline phosphatase during characterization showed maximum activity at pH 9.0 (96.53 U/ml), temperature of 45 °C (97.99 U/ml) and substrate concentration of 1.75 mg/ml (96.51 U/ml). The effect of the bacterium on growth of *Arabidopsis thaliana* plant showed that inoculation of bacterial culture exhibited better growth in comparison to the control. Hence the phosphate solubilizing and alkaline phosphatase production activity of the bacterium may have probable use for future biotechnological application.

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http://dx.doi.org/10.1016/j.biori.2017.01.003

Please cite this article in press as: Behera, B. C., et al. Alkaline phosphatase activity of a phosphate solubilizing *Alcaligenes faecalis*, isolated from Mangrove soil. *Biotechnology Research and Innovation* (2017), http://dx.doi.org/10.1016/j.biori.2017.01.003

Introduction

Mangrove forests inhabit the tropical and subtropical regions of the world, thriving in the transitional regions between the land and the sea and offering a unique environment for diverse groups of organisms (Flores-Mireles, Winans, & Holguin, 2007). These ecosystems are characterized by periodic tidal flooding, which results in a highly saline soil profile, with variable levels of nutrients. Muddy mangrove soil has a strong capacity to absorb nitrates and insoluble phosphates carried out by the tides (Vazquez, Holguin, Puente, Lopez Cortes, & Bashan, 2000). Phosphorus usually precipitates in mangrove sediment due to its binding with various cations available in the interstitial water (De, Sarkar, Maity, Mukherjee, & Das, 2011). As a result, phosphorus becomes largely unavailable to plants which is detrimental as phosphorous is vital to plant growth, especially in nutrient-limited mangrove environments. The developments of stalk, stem strength, root, flower, seed formation, crop maturity and resistance to plant diseases are factors associated with phosphorus nutrition (Khan, Jilani, Akhtar, Naqvi, & Rasheed, 2009). Deficiency of soil P is one of the most important chemical factors restricting plant growth in soils. Phosphate solubilizing bacteria as potential suppliers of soluble phosphorus should confer a great advantage for plants through solubilization and mineralization (Hong, Geun, Mi, & Moon, 2006; Rodriguez & Fraga, 1999). Solubilization of mineral phosphate by phosphate solubilizing bacteria is generally associated with the release of low molecular weight organic acids (Goldstein, 1995). Their hydroxyl and carboxyl groups are able to form complexes with the iron and aluminium of corresponding phosphate compound in soil, thereby releasing bioavailable phosphate into the soil which can be utilized by plants (Gyaneshwar, Kumar, Pareka, & Podle, 2002). Solubilization of phosphate-rich compounds is also carried out by the action of a phosphatase enzyme called alkaline phosphatase (AlPase). In all bacteria, this enzyme catalyzes the hydrolysis of a wide variety of phosphomonoesters and catalyzes a transphosphorylation reaction by transferring the phosphoryl group to alcohol in the presence of certain phosphate acceptors (Coleman, 1992). Hence, application of phosphate solubilizing bacteria (PSB) with triple super phosphate can increase plant height, growth, yield, number of tillers and mineral nutrient content in tissues (Chen, 2006; Panhwar et al., 2013; Sarkar et al., 2012).

In recent years, different screening programs have been performed in saline habitats in order to isolate and characterize novel enzymatic activities with different properties to those of conventional enzymes. Besides being intrinsically stable and active at high salt concentrations, halophilic enzymes offer important opportunities in biotechnological applications, such as food processing, environmental bioremediation and biosynthetic processes. In this sense, the finding of novel enzymes showing optimal activities at various ranges of salt concentrations, temperatures and pH values is of great importance (Gomez & Steiner, 2004). With the above notions in consideration, this study focuses on phosphate-solubilizing bacterium which have been identified and characterized from saline mangrove soils of the Mahanadi river delta, Odisha, India. Further attempt has been made to purify and characterize the alkaline phosphatase enzyme produced by the bacterial isolates which may have potential biotechnological application. In addition, the efficiency of the isolated and identified bacterium was also evaluated in relation to its plant growth promotion.

Materials and methods

Isolation of PSB

Phosphate solubilizing bacteria were isolated from different locations of mangrove forest soil of the Mahanadi river delta by culturing strains on plates containing National Botanical Research Institute's phosphate agar, (NBRIP) contained L^{-1}: glucose, 10 g; $Ca_3(PO_4)_2$, 5 g; $MgCl_2{\cdot}6H_2O$, 5 g; $MgSO_4{\cdot}7H_2O$, 0.25 g; KCl, 0.2 g and $(NH_4)_2SO_4$, 0.1 g; pH 7.0 (Nautiyal, 1999). Colonies of phosphate-solubilizing bacteria were recognized by the formation of clear halos around them. They were also screened for their ability to change the blue colour of NBRIP–bromophenol blue broth medium (pH 7.0) to colourless state by the formation of organic acid and lowering of pH (Mehta & Nautiyal, 2001).

Morphological and biochemical characterization of bacteria

Culture characteristics such as colony appearance, spore formation and motility of each strain were determined according to standard methods. Cell shape and size were determined by scanning electron microscopy (SEM) (Zeiss, Sigma). Several biochemical characteristics such as production of catalase, urease, indole, nitrate reduction, citrate utilization, acid-gas production from sugar, Voges–Proskauer (V–P) reaction, hydrolysis of tributyrin, tween-80, cholesterol, gelatine, casein, pectin and chitin etc. were also determined. The results were compared with Bergey's Manual of Determinative Bacteriology (Buchanan & Gibbons, 1974).

Molecular identification of bacteria

The 16S rRNA gene of the bacterial isolate was amplified using universal 27F forward primer (5′-AGGCCTAACACATGCAAGTC-3′) and 1492R reverse primer (5′-GGGCGGWGTGTACAAGGGC-3′) described by Das et al. (2014). PCR product of 16S rRNA gene was purified using QIA quick gel extraction kit (Qiagen GmbH, Germany) and nucleotide sequences were determined using the Big dye terminator v 3.1 cycle sequencing kit in an automated 3130xl genetic analyzer system (Applied Biosystems, Hitachi, USA) and submitted to gene bank. The sequences were finally aligned in the alignment explorer tool of the Molecular Evolutionary genetics analysis software (MEGA5.0; Tamura et al., 2011) using Clustal-W. The phylogenetic tree was prepared with the help of Neighbour Joining method and Kimura-2 as the model taking boot strap value as 1000.

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