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Isolation and Characterization of a Phosphate-solubilizing Bacterium *Pseudochrobactrum* sp. PSB13 from Petroleum-Contaminated Drill Cuttings

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Abstract. Phosphate-solubilizing bacteria were extensively studied in many environment. However, little is known about them in drill cuttings, as wastes from drilling process. A phosphate-solubilizing bacterium strain PSB13 was isolated from petroleum-contaminated drill cuttings. This strain was identified as *Pseudochrobactrum* sp. based on its 16S rDNA sequence and phenotypic characteristics. This strain could solubilize 97.6 μg/ml phosphates in 6 days when grown in NBRIP liquid medium. The increase in solubilization of phosphate coincided with the drop in pH, which indicates organic acid was responsible for the phosphate-solubilization. Phosphate-solubilizing bacterium was reported in drill cuttings for the first time, which suggests its potential in the bioremediation of petroleum-contaminated drill cuttings.

Introduction

Bacteria play a key role in the natural remediation of petroleum-contaminated drill cuttings [1, 2], which are wastes from drilling process. Phosphorus, one of the most essential elements for bacteria growth, usually remains in insoluble form in drill cuttings. Therefore, phosphates in drill cuttings are deficient and mostly unavailable for most bacteria, which hider the degradation of petroleum in drill cuttings. A large portion of soluble inorganic phosphate can be applied to drill cuttings as phosphatic fertilizers. However, it is costly and inefficient. Therefore, much attention should be attached to the phosphorus-solubilizing bacteria which can transform the insoluble phosphorus to available form in drill cuttings. A number of phosphate-solubilizing bacteria were isolated in soil and rhizosphere, which attracted increasing attention[3,4]. However, little is known about these bacteria in drill cuttings.

In this study, phosphate-solubilizing bacterium was isolated from petroleum-contaminated drill cuttings. Moreover, the change of concentration of phosphate and pH value was measured in the incubation of phosphate-solubilizing bacterium, which may be helpful for the understanding of phosphate biocycle in drill cuttings.

Materials and Methods

Isolation of phosphate-solubilizing bacteria. The petroleum-contaminated drill cuttings sample was collected from Tianjin in China. Sample was added into 50 ml of Pikovskaya's broth media containing tricalcium phosphate (TCP) as insoluble phosphate source at pH 7 and shaken for 7 days.

The serially diluted enrichment samples were plated on Pikovskaya's agar [5]. Distinct colonies with clear halo zones were selected for further analysis.

16S rDNA sequencing and phylogenetic analysis. The genomic DNA from the isolated bacterial strain was extracted and amplified for 16S rDNA sequence, which was sequenced with an ABI 3730 XL DNA analyzer according to previous methods [6]. The sequence (KJ569102) was blast against Genbank database and highy related sequences were selected manually. Sequences were aligned using ClustalW, and phylogenetic analyses were performed with the MEGA v4.0 using neighbor-joining method based on Jukes–Cantor corrected distances with bootstrap value of 1000.

Qualification of available phosphate in liquid medium. Qualification of phosphate-solubilization was carried out by inoculating 1 ml of bacterial suspension (3×10^7 cells/ml) in 50 ml of National Botanical Research Institute Phosphate (NBRIP) broth [7] in flasks. The flasks were incubated at 30 °C with 150 rpm for 9 days. Sterile water-inoculated media was treated as control. Samples were taken with the intervals of 0, 3, 6, 9 days. The quantification of soluble phosphate was carried out with the Phosphomolybdenum blue method [8]. pH value of the medium was recorded on a pH meter equipped with glass electrode.

Result and Discussion

Isolation of Phosphate-Solubilizing Bacteria and Phenotypic Analysis. Strain PSB13 showed a visible halo zone on Pikovskaya's agar, which was pale-white, circular and convex. Phylogenetic analysis showed strain PSB13 was closely related to *Pseudochrobactrum lubricantis* KSS7.8^T (100%) as shown in Fig. 1. However, strain PSB13 was also highy related to *Pseudochrobactrum saccharolyticum* CCUG33852^T (99.9%), *Pseudochrobactrum asaccharolyticum* CCUG46016^T (99.8%) and *Pseudochrobactrum kiredjianiae* CCUG49584^T (99.3%). Therefore, the identification of strain PSB13 to the species level need further study. Moreover, physiological and biochemical characterization of strain PSB13 was analyzed as shown in Table 1. Strain PSB13 was able to grow in the present of rhamnose, melezitose, cellobiose, arab sugar, glucose, mannitol, sorbitol and gelatin. The NaCl concentration range for growth was between 0 and 9%, which indicates its halo-tolerance.

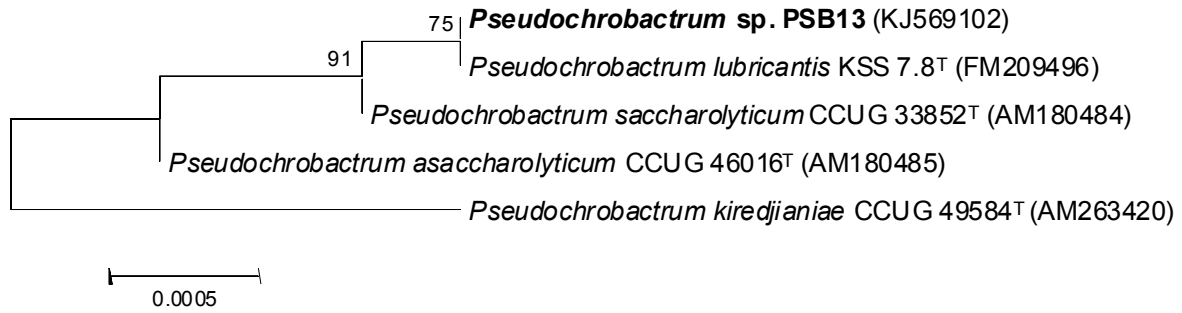

Fig. 1 Phylogenetic tree of *Pseudochrobactrum* sp. strain PSB13 and related species constructed on the basis of 16S rDNA sequences using the neighbor-joining method. Bootstrap values below 70% were not presented. The scale bar represents 0.05% nucleotide sequence difference.

Table 1 Physiological and biochemical characteristics of strain PSB13

Characteristic	Strain PSB13
NaCl tolerance (0–9 %)	+
Xylose	−
Lactose	−
Maltose	−
Rhamnose	+
Melezitose	+
Cellobiose	+
Arab sugar	+
Glucose	+
Mannitol	+
Sorbitol	+
Urease	+
Malonate	−
Gelatin	+
Aesculin	−

P solubilization and pH. Concentraion of soluble P increased in the period of incubation with maximum P solubilization of 97.59 μg/ml at the sixth day after inoculation (Fig. 2a), which was similar with the report of S.G. Dastager and S. Damare [9]. In addition, the pH value decreased with the increase of concentraion of soluble P (Fig. 2b) with minimum pH value of 5.02 at the end of incubation. The decrease in pH clearly indicates the production of organic acid, which is considered to be responsible for the phosphate-solubilization. E. Perez et al. [10] stated that the acidity of a liquid culture is the main mechanism of phosphate dissolution. Lower molecular weight organic acids, such as citric and oxalic acid were reported to be the main contributors to phosphate solubilization and decrease in pH [11]. The organic acid in volved in the P solubilization of strain PSB13 need further study, which is helpful for the understanding of mechanism of phosphate dissolution in drill cuttings.

Collectively, this is the first and the only report about the capacity of phosphate-solubilizing of *Pseudochrobactrum*, which was isolated from drill cuttings. The present study suggests the potential for application of phosphate-solubilizing bacteria in the bioremediation of petroleum-contaminated drill cuttings.

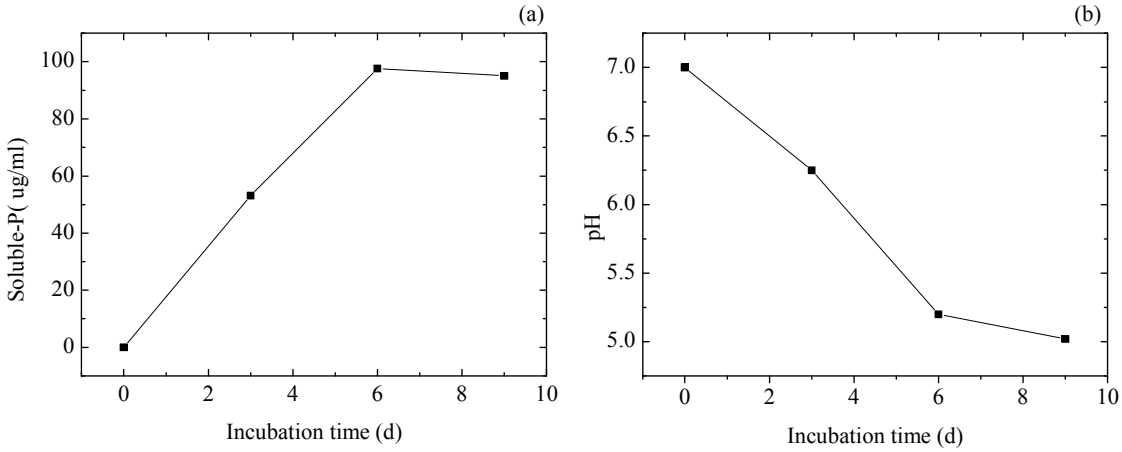

Fig. 2 Capacity of strain PSB13 to solubilize TCP (a) and changes in the pH (b) in NBRIP liquid medium. The initial pH was 7.0.

Conclusions

A phosphate-solubilizing bacterium *Pseudochrobactrum* sp. PSB13 was isolated from petroleum-contaminated drill cuttings, which could release inorganic phosphate from TCP by decreasing pH of the NBRIP liquid media. Phosphate-solubilizing bacterium was reported in drill cuttings for the first time, which suggest its potential in the bioremediation of petroleum-contaminated drill cuttings.

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