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# Extraction of potassium from K-feldspar through potassium solubilization in the halophilic *Acinetobacter soli* (MTCC 5918) isolated from the experimental salt farm

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ABSTRACT

Out of 81 isolated bacteria from salt pan of CSIR-CSMCRI's experimental salt farm with salinity range from 10 to 24 Be′, one of the promising bacterial strain identified as *Acinetobacter soli* showed potassium solubilizing potential of 66-68% (w/w) w.r.t. cell dry weight. It was further optimized with cost effective carbon source for K release in the broth itself which was 10.67% of K (with respect to the K-feldspar present in the broth) utilizing sugarcane baggase hydrolysate with production age of 120 h.



## 1. Introduction

N, P, Ca, Mg and K are important macronutrients for plants (Prajapati and Modi, 2012). Out of these macronutrients, K is limited due to lesser availability in the soluble form in the soil (Prajapati and Modi, 2012, Maathuis, 2009). The soluble K in the soil which is accessible by the plant for growth, is limited although, there are many potassium rich mineral ores in pedosphere are available (Hayashi et al., 2014). There are many growing and established technologies for making the chemical fertilizers to suffice the need of potash for the crops.

Currently, available chemical processes for making potassic fertilizers are not eco- friendly as well as their long term use affects the physical and chemical quality of the soil (Horikoshi, 1999, Leaungvutiviroj et al., 2010). In order to overcome these problems, it is an emergent need for the development of microbial potassic fertilizers. Some of the fungi and very few bacteria are known for the potassium solubilization (Epstein, 2003; Maurya et al., 2014). Higher yields can be obtained in bacterial leaching as compared to the chemical leaching (Bhatti et al., 2012). Mineralogy of pedosphere of India is very rich in insoluble potassium. Therefore, to capitalize the existing low value potassic minerals, green microbial processes may be developed for meeting the potassic fertilizer demands (Epstein, 2003).

The present study deals with isolation of promising potassium solubilizing halophilic bacteria and further optimizing the upstream process for increasing potassium solubilization by the bacteria.

## 2. Materials and methods

Saline nutrient broth, sucrose, dextrose and yeast extract was procured from Himedia. Wheat bran and sugarcane baggase were procured from Bhavnagar local market.

### *2.1. Isolation of Acinetobacter soli from brine of CSMCRI's experimental salt farm*

Isolation of bacteria from salt pans of CSIR-CSMCRI's experimental salt farm having different salinity ranging from 10 to 24 Be′ was done. Saline water samples were collected from experimental salt farm, CSIR-CSMCRI Bhavnagar from 0 to 10 cm distance from the surface having salinity in range from 10 Be′ to 24 Be′ and a pH of 7.0–7.5.

The saline water samples were serially diluted, plated on five different media and kept for 5 days incubation at 30 °C. The media composition consists of (g/l): (A) NaCl 16.2, $MgSO_4 \cdot 7H_2O$ 9.7, $MgCl_2 \cdot H_2O$ 7.0, $CaCl_2$ 3.6, KCl 2.0, $NaHCO_3$ 0.06, NaBr 0.026 for moderately halophilic bacteria (Ventosa et al., 1998) and 20% for extreme halophilic bacteria with supplementation of 5 g yeast extract (Rohban et al., 2009); (B) Horikoshi-I supplemented with 10% NaCl for haloalkaliphilic bacteria (Prajapati and Modi, 2012) (C), Horikoshi-II supplemented with 20%

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**Table 1**
Optimum upstream process parameters for maximizing K solubilization.

| Strain | | | | |
|---|---|---|---|---|
| *Acinetobacter soli* (MTCC 5918) | | | | |
| | Production media composition | Experimental conditions | Production age | % K (biomass) |
| Temperature | | | | |
| Activity at 30 °C | 0.05% yeast extract, 0.5% potassium feldspar and 1% glucose | 30 °C temp., 120 rpm | 144 h | 66.1 |
| Activity at 37 °C | 0.05% yeast extract, 0.5% potassium feldspar and 1% glucose | 37 °C temp., 120 rpm | 144 h | 66.1 |
| Activity at 40 °C | 0.05% yeast extract, 0.5% potassium feldspar and 1% glucose | | 144 h | No growth was observed. |
| Production age of *Acinetobacter soli* | | | | |
| Cheap carbon sources | 0.05% yeast extract, 0.5% potassium feldspar and 1% glucose | 37 °C temp., 120 rpm | 120 h | 68.3 |
| | Wheat bran hydrolysate having 0.006784 g. | 37 °C temp., 120 rpm | 120 h | 5.17 |
| | Sugarcane baggase hydrolysate having 0.28 g. total sugar | 37 °C temp., 120 rpm | 144 h | 10.67 |

NaCl for extreme haloalkaliphilic bacteria (D), nutrient medium at pH 10 with 35 g/l sodium chloride for haloalkaliphilic bacteria (F). Well isolated and differentiated colonies from these media were transferred on the respective medium slants and cultures were maintained as glycerol stocks.

The halophilic bacteria (CSMCRI's *Acinetobacter soli* — MTCC 5918) was isolated from brine present in salt pans of CSIR-CSMCRI's experimental salt farm (21°47.74 N, 072°07.63 E) and was inoculated in nutrient broth (consists 0.3% beef extract, 0.5% peptone and 0.5% NaCl) and maintained at pH 7.0 ± 0.2 and kept at 30 °C incubation period at an agitation 120 rpm for obtaining the seed culture having 100 ml working volume.

The seed culture inoculated (equivalent to 1% inoculum size of 24 h seed culture having 1.24 OD at 540 nm) under aseptic conditions (laminar flow) in the production medium which contained 0.05% yeast extract, 0.5% potassium feldspar and 1% glucose. Potassium content present in the biomass was estimated at regular intervals by using flame photometer (Systronics flame photometer 128). Potassium feldspar containing 12% potassium was procured from WMA India.

### 2.2. Activity at different temp.; production age

Further, an independent experiment with optimum upstream parameters was carried out to study the effect of varying production age on the potassium solubilizing potential of organism. Potassium solubilization (KS) activity was measured at different temperatures (30 °, 37 ° and 40 °C).

### 2.3. Substitution of glucose with cheap carbon sources

#### 2.3.1. Biomass pre-treatment using acid hydrolysis

Hydrolysis of the wheat bran and sugarcane baggase biomass was done using 1 M HCl at 110 °C, 120 rpm for 12 h. After reaction is complete i.e. after complete hydrolysis, the material was dried completely at 120 °C for 4 h. to remove the HCl present in it. Finally the hydrolysates were extracted in distilled water.

#### 2.3.2. Microorganism and culture media

100 ml seed culture of *Acinetobacter soli* was prepared in medium containing (g/l) peptic digest of animal tissue 5.000, Sodium chloride 5.000, Beef extract 1.500, Yeast extract 1.500. pH of the medium was maintained at 7.4 ± 0.2 and thereafter autoclaved at 121 °C.

#### 2.3.3. Fermentation

1% of the above raised seed culture of *Acinetobacter soli* inoculated into the production medium A & B; Production medium A containing 0.5% K-feldspar, 0.05% yeast extract and wheat bran hydrolysate having 0.006784 g. Total sugar in 100 ml distilled water; production medium B containing 0.5% K-feldspar, 0.05% yeast extract and sugarcane baggase hydrolysate having 0.28 g. Total sugar in 100 ml distilled water.

## 3. Results and discussions

### 3.1. Isolation of Acinetobacter soli from brine of CSIR-CSMCRI's experimental salt farm

Total 81 bacteria were isolated from the brine samples collected from CSIR-CSMCRI's experimental salt farm. From medium A 16, medium B 24, medium C 15, medium D Nil and medium F 25 bacteria were isolated. The supplementation of yeast extract in medium B and yeast extract with other carbon sources in medium F might be the reason for getting maximum bacterial isolates on these media. Since, medium A contains inorganic salts and medium C contains 10% NaCl with pH 10, therefore, less bacteria were isolated on these two media.

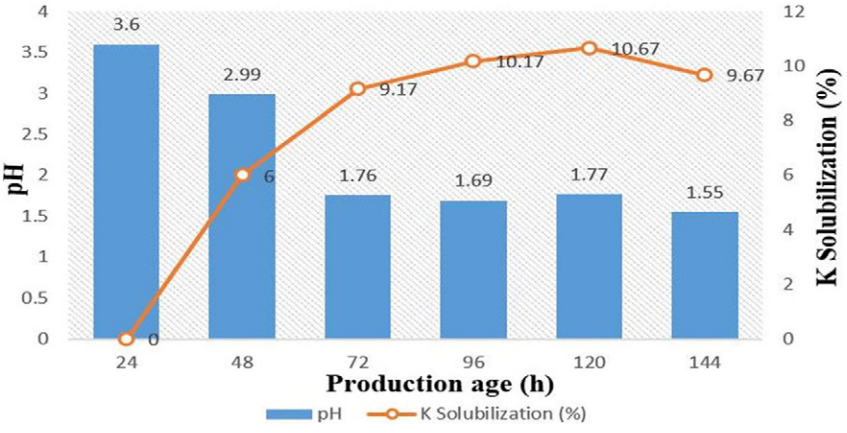


**Fig. 1.** Trend of pH and potassium solubilization percentage at various production age utilizing sugarcane baggase hydrolysate as carbon source.