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A N I N T E R N A T I O N A L J O U R N A L

Release of Potassium from Rock Powder by the Yeast *Torulaspora globosa*

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ABSTRACT

The alteration of minerals in rocks and the availability of elements for plant nutrition require long periods of time, and microorganisms are thought to induce the release of potassium and phosphate from rocks. In this context, this work evaluates the role of the yeast Torulaspora globosa*, isolated from the sugar cane rhizosphere, in the solubilization of potassium from alkaline ultramafic rock powder. The experiments were performed in liquid medium, with or without agitation, at 30°C with the following treatments: culture medium + alkaline ultramafic; culture medium + yeast suspension; and culture medium + yeast suspension + alkaline ultramafic. The results showed that as much as 38% of the total potassium in the rock was released in the medium with the yeast during a 15-day period of incubation. Acid production may be the mechanism by which the yeast solubilizes potassium because the total acidity increased during the sampling period. Agitation (which increased oxygen availability) resulted in approximately 20% more biosolubilization of the alkaline ultramafic rock than with the static culture. These data indicate the potential for this yeast in biosolubilization processes and biofertilizer production.*

Key words: *biosolubilization, insoluble rocks, alkaline ultramafic*

INTRODUCTION

Potassium (K) is an essential nutrient for plant growth and is extremely important for the productive farming of major agricultural crops. This mineral is required in high quantities by plant cells and possesses essential physiological and biochemical functions involving cell osmotic regulation and enzyme activation (Mengel and Kirkby 1987; Marschner 1995; Valmorbida and Boaro 2007). Potassium is highly utilized in Brazilian agriculture; however, only 10% of the total potassium needed is produced domestically (Moraes 2005).

Some alternatives have been tried regarding the use of rocks incorporated into soils in an effort to reduce the use of chemicals and improve environmental and agricultural sustainability. Nevertheless, the long period of time required for the release of the minerals from the rocks to increase the availability of important elements for plant nutrition make this alternative unfeasible.

Many igneous, metamorphic and sedimentary rocks may be used as fertilizers or natural correctives since the management is adequate. Moreover, studies on the kinetics of mineral dissolution from the rocks indicate their agronomic potential as natural nutrient sources (Song and Huang 1988; Kalinowski and Schweda

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1996). The possibility of using microorganisms as an alternative biological process to induce the release of potassium from potassium-containing rocks also exists.

Many groups of microorganisms, such as actinomycetes, other bacteria and molds, are capable of solubilizing the potassium contained in silicate minerals through decomposition (Weed et al. 1969). The release of potassium from these minerals is primarily caused by the acids produced by the microorganism during biological activity and by its absorption of soluble potassium, which causes an increase in the concentration gradient during mineral hydrolysis.

Reports have indicated satisfactory results for potassium and phosphate solubilization by molds such as *Aspergillus*, *Penicillium* and *Fusarium* and bacteria such as *Bacillus*, *Pseudomonas* and *Micrococcus* (Gaur, 1990). The mold *Aspergillus niger* is known for its ability to solubilize phosphate rocks, which is caused by the production of organic acid, especially citric acid (Nahas et al. 1990; Vassileva et al. 1998). Lopes-Assad et al. (2006) have verified that medium acidification by a strain of *A. niger* (CCT4355) caused the solubilization of potassium from two rocks (alkaline ultramafic and phlogopite) after 21 days of incubation.

Phosphate- and potassium-solubilizing bacteria such as *Bacillus megaterium* and *Bacillus mucilaginosus* are also organic acid producers (Han and Lee 2005). However, a few reports describe the use of yeasts in the solubilization of rocks. Vassileva et al. (2000) have studied the yeast *Yarrowia lipolytica* for the solubilization of phosphate rocks and obtained significant results for the release of soluble phosphate.

The species *Torulaspora globosa* is found in the composition of some Indian commercial biofertilizers (*Agriland Biotech Limited* and *Sundaram Overseas Operation*), which shows the potential of rock phosphate solubilization and plant growth promotion (Rosa 2009). Vora and Shelat (1998) have observed that this yeast is able to solubilize tricalcium phosphate through the production of acetic acid. The inoculation of this species in different cultures in the field has resulted in a significant increase in the productivity of the inoculated cultures when compared to that of the non-inoculated ones.

In this context, the aim of this work was to evaluate the biosolubilization of the alkaline ultramafic rock for potassium release by a strain of the yeast *Torulaspora globosa*, isolated from the rhizosphere of sugar cane, that possesses the potential to be a biological control agent (Rosa et al. 2010; Rosa-Magri et al. 2011).

MATERIALS AND METHODS

Rock powder

The rock powder utilized in the experiments was alkaline ultramafic from Lages, Santa Catarina, Brazil. According to Sobral et al. (2006), the potassium concentration in this rock is 2.73%, presenting a grain size of between 0.002-0.05 mm.

Microorganism and inoculum production

A strain of the yeast *Torulaspora globosa* (1S112), isolated from the sugar cane rhizosphere (variety 867515) in a cultivated area belonging to UFSCar – Araras, SP, Brazil, was utilized. The yeast was identified by the sequencing of the ITS region from ribosomal DNA, according to Rosa (2009). For the inoculum production, a loop of yeast cells was inoculated in liquid GYMP medium (2% glucose; 0.5% yeast extract; 1% malt extract; 0.2% potassium dihydrogen phosphate) and incubated at 30°C and 160 rpm for 48 hours. A suspension of 10^7 cells/mL was used for the inoculum.

In vitro experiments

The following treatments were used: 1) alkaline ultramafic + medium; 2) yeast + medium; and 3) alkaline ultramafic + yeast + medium. The assays were carried out in triplicate, using 500-mL Erlenmeyer flasks containing 200 mL of the culture medium as proposed by Vassileva et al. (2000): 80 g/L glucose; 0.7 g/L magnesium sulfate; and 0.1 g/L yeast extract. Before autoclaving at 120°C and 1 atm for 20 minutes, 0.8 g (0.4% m/v) of the rock powder was added to the culture medium in the flasks for treatments 1 and 3, and the pH was adjusted to 5.3. A volume of 2 mL of the yeast suspension was also added to the flasks for treatments 2 and 3. First, the flasks were sampled (10 mL) at 12-hour intervals during a 72-hour incubation period. In a second experiment, the flasks were sampled (10 mL) every 3 days during 15 days of incubation. The flasks were maintained at 30°C, either at a constant speed of 160 rpm or without agitation.

Analysis

The quantity of soluble potassium was determined by flame emission photometry; the pH was determined by a digital pH-meter; and the acidity was determined by titration of the samples until pH 7 with 0.05 M NaOH solution. Statistical analysis using analysis of variance and Tukey´s test at 5% of significance was performed with STATISTICA 6 software. The solubilization rate was calculated as follows: average of soluble potassium concentration (in $mmol_c/dm^3$) X 100 / total potassium concentration in the rock powder (2.921 $mmol_c/dm^3$).

RESULTS AND DISCUSSION

The results showed that the yeast *T. globosa* is very effective for the solubilization of the alkaline ultramafic rock, releasing higher concentrations of potassium in treatment 3 (yeast + alkaline ultramafic + medium) compared to treatment 1 (only alkaline ultramafic), as shown in Figure 1.

A decrease of 20% in the potassium concentration was obtained with the static culture, showing that the availability of oxygen promotes the solubilization process (Fig. 1).

During the 15-day incubation period, the concentration of soluble potassium in the liquid medium increased slightly after 3 days with a slower rate of solubilization (Fig. 1).

There was an increase in the titratable acidity in the treatments where the yeast was inoculated, which indicates that this yeast produces acids (Fig. 2). Vora and Shelat (1998) isolated a strain of *T. globosa* in India and observed good results with this species for phosphate solubilization (tricalcium phosphate) due to acetic acid production.

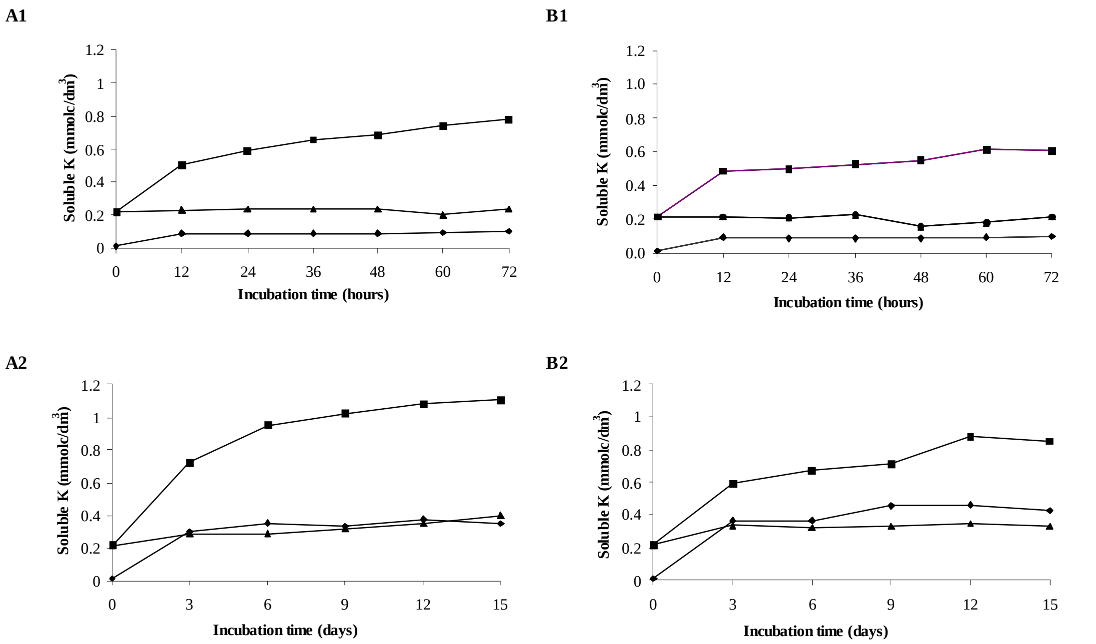

Figure 1 - Concentration of soluble potassium ($mmol_c/dm^3$) in culture medium that was cultivated with the yeast *T. globosa* and the alkaline ultramafic rock powder at 30ºC with agitation at 160 rpm (A) and without agitation (B) for 72 hours (1) and 15 days of cultivation (2). Legend: (♦) *T. globosa* + medium; (■) *T. globosa* + rock powder + medium; (▲) rock powder + medium.

The medium pH continued to fall during the incubation period in the shaken flasks when compared to the static culture, which showed stabilization in pH values during 3 days of cultivation. However, the pH did not decrease below 6 in the treatment of yeast + alkaline ultramafic + medium (Fig. 3).

Therefore, in the biosolubilization assays with *T. globosa*, the acids produced may attack the minerals from the rock powder and release ions with an alkalinizing effect, which contributes to the elevation in pH values along with solubilization of K. Similar results were obtained by Lopes-Assad et al. (2010) with the same rock but using *A. niger*. Indeed, the ultramafic rock presents a complex mineralogy with approximately 12% CaO and 22% MgO (data not shown).

Lian et al. (2007) studied a strain of the thermophilic fungus *Aspergillus fumigatus* that was cultured with K-bearing minerals to determine if the microbe–mineral combination enhances the release of mineralic potassium. They observed that the potassium solubilization rate showed a positive dependence upon pH when the fungi and minerals were mixed directly and exhibited no correlations with solution acidity if contact between the cells and rock was restrained.

Here, the acid production was higher when the flasks were shaken and in the presence of the yeast, although it was slightly lower when the rock powder was added (Fig. 2).

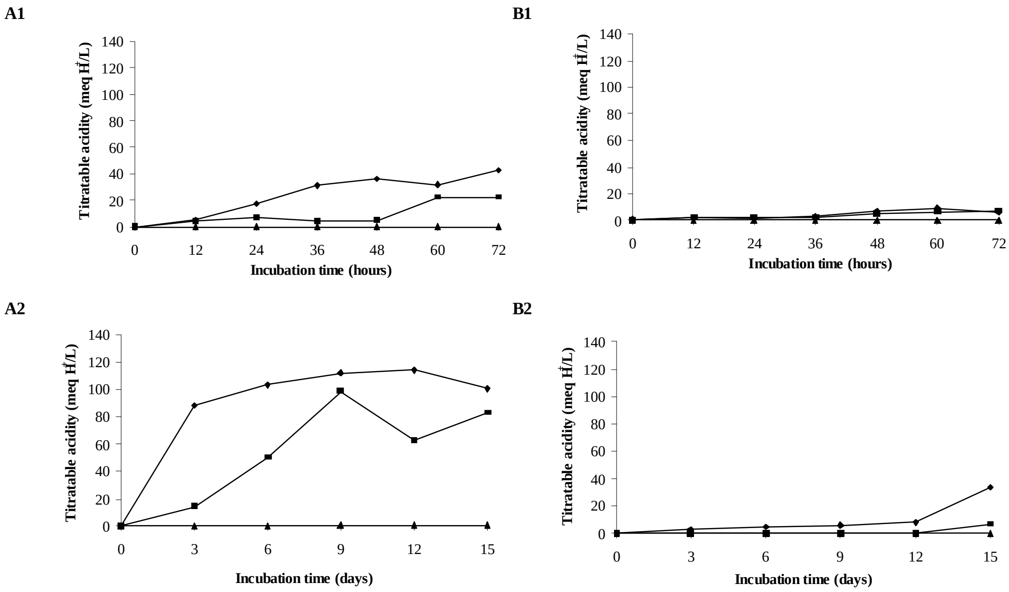

Figure 2 - Titratable acidity (mEq H^+/L) of culture medium that was cultivated with the yeast *T. globosa* and the alkaline ultramafic rock powder at 30ºC with agitation at 160 rpm (A) and without agitation (B), for 72 hours (1) and 15 days of cultivation (2). Legend: (♦) *T. globosa* + medium; (■) *T. globosa* + rock powder + medium; (▲) rock powder + medium.

The ability to produce acids is related to oxygen availability. Freer (2002) reported that acetic acid production by yeasts occurs through the oxidation of acetaldehyde by the enzyme acetaldehyde dehydrogenase, which requires oxygen as an electron acceptor. This author also observed that acetic acid production results from a combination of high concentrations of glucose and aeration of the culture.

In this work, concentrations as high as 80 g/L of glucose were utilized in the process, which undoubtedly led to higher acid production and subsequent potassium release from the rock powder.

For *Saccharomyces cerevisiae,* Gutierrez (1991) concluded that an increase in the medium pH resulted in higher production of acetic acid. The biochemical mechanism of acetic acid production in *T. globosa* is unknown, but if it is the same as in *S. cerevisiae,* it will have an interesting effect on the biosolubilization process after basic ions are released from the rocks during cultivation. This mechanism would contribute to the medium alkalinity and the synthesis of acids by the yeast.

The culture medium may be optimized to promote more efficient potassium release from the alkaline ultramafic with less energy cost, for instance, in a fed-batch system or using agricultural waste.

In any event, to guarantee the potassium solubilization, conditions that benefit acid production must be employed.

When considering the use of rock powder in agricultural systems, soil pH is of particular importance; phosphates (and potassium) will be released more quickly in moderately acidic soils than in neutral or alkaline soils.

Experiments using the same rock powder under the same cultivation conditions and when scaled-up (200 mL culture medium into 500-mL Erlenmeyer flasks) but using the mold *A. niger* (Lopes-Assad et al., 2010), have shown similar results for soluble potassium concentration but over a longer period of time. Using yeast, approximately 37% of the solubilization rate (more than 1 $mmol_c$ K/dm^3) was reached after 12 days of incubation (Table 1), while a 35-day period was needed for similar results for the mold (39.7% of solubilization and 1.16 $mmol_c$ K/dm^3).

Supanjani et al. (2006) studied the direct applications of phosphate rock and potassium rock in conjunction with phosphate-solubilizing bacteria and potassium-solubilizing bacteria for cultivation of the hot pepper *Capsicum annuun* and verified a variety of benefits (improved nutrient uptake, higher biomass harvest and fruit yield), which promises a sustainable alternative to the use of classical fertilizers.

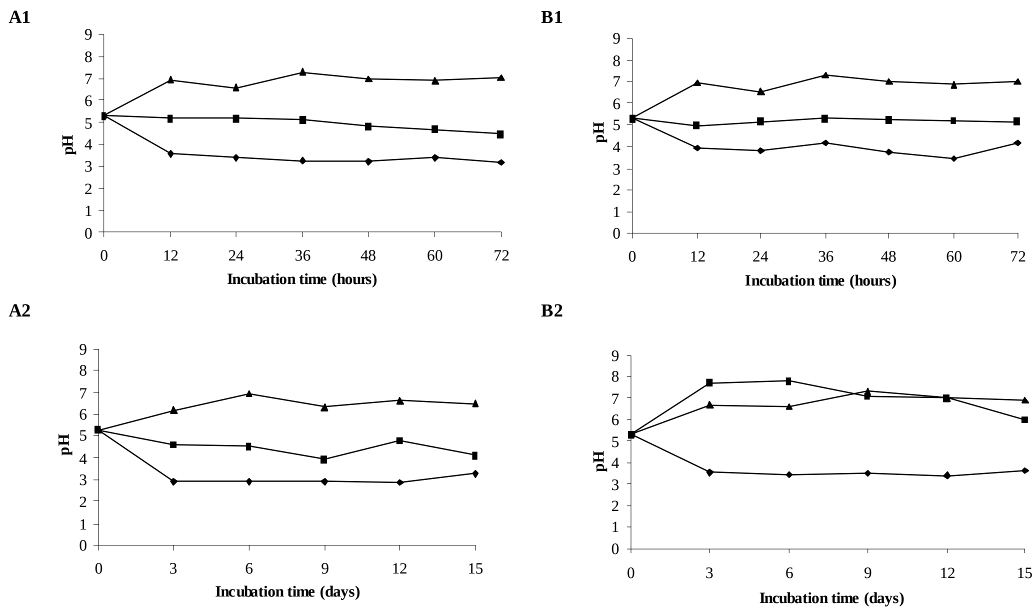

Figure 3 - pH of culture medium that was cultivated with the yeast *T. globosa* and the alkaline ultramafic rock powder at 30ºC with agitation at 160 rpm (A) and without agitation (B), for 72 hours (1) and 15 days of cultivation (2). Legend: (♦) *T. globosa* + medium; (■) *T. globosa* + rock powder + medium; (▲) rock powder + medium.

Table 1 - Soluble potassium ($mmol_c/dm^3$) and solubilization rate (%) of the alkaline ultramafic rock powder by *T. globosa* at 30ºC and 160 rpm.

Incubation time (days)	***T. globosa* + rock powder + medium**[1]	**Solubilization rate (%)**
0	0.22[a]	7.5
3	0.72[b]	24.6
6	0.95[c]	32.5
9	1.02[d]	34.9
12	1.08[e]	37.0
15	1.10[e]	37.7

[1] Different letters in the column indicate a significant difference by Tukey´s test (p<0.05).

This yeast offers great potential for biotechnological applications, especially in the production of organic acids and solubilization of rocks for biofertilizers.
The fact that the yeast *T. globosa* is found in the sugar cane rhizosphere is a positive factor because the application of rock powder in agricultural soils depends greatly on the presence of these microorganisms to convert insoluble nutrients into available elements for plant uptake, thus promoting nutrition and development.

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