Isolation of Bacterial Cultures from *Helianthus* Annuus L. Rhizosphere and Assessment of Bioweathering of Verdete (Glauconitic Sandstone)

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Abstract— **Brazil imports more than 90% of potassium fertilizer needs for food production in order to meet an annual consumption of about 200 million dollars. This demand tends to increase 3% per year, only to be maintained at current agricultural production. It is therefore imperative for the nation to develop strategies to reduce import dependence. One possibility for implementation in agricultural productivity can be harnessing of alternative sources of potassium, for example, rocks containing K_2O in the composition. In this context, various microbial species have highlighted the role of biossolubilization capacity of chemical elements from these rocks, releasing them as chemical forms assimilable by plants. In this study, two separate cultures from sunflower rhizosphere, identified as *Paenibacillus* sp and sp *Bhurkholderia* were evaluated for the ability to release K, Si, and Mg a partir de rock powder (verdete 9,5% K_2O) in Aleksandrov's medium. *Paenibacillus* sp. was the most effective to solubilize K (circa 32 $mg.L^{-1}$), while the solubilizing performance of *Bhurkholderia* sp was worse than the abiotic control (uninloculated sterile medium). Changes in particle size distribution of rock powder (20-28#; 28-35#; <35#) greatly affected the solubilization of Si and Mg in biotic assays, but potassium solubilization did not statistically differ for both biological treatments and control. Was not identified change in the crystalline rock structure by XRD analysis in any of the grain size conditions. In EPS, only produced by *Burkholderia* sp were found amounts of Si, K and Mg twice larger than those released into the aqueous phase. The results indicate that the strategy adopted for the isolation of high mineral efficient solubilizing bacteria was satisfactory, which may be an alternative in the future to biofertilizer production.**

***Index Term*— mineral weathering; mineral solubilizing microrganisms; rhizosphere bacteria; EPS trapped elements.**

I. Introduction

The Brazilian economy is strongly related to agricultural commodities [1]. The country is one of the world's largest producer of sugarcane, used for both food (sugar) and fuel (ethanol), and ranks among the world six largest agricultural producer and exporter [2]. However, although Brazil comprises a huge arable land extension, with more than 20% of its territory cultivated for crops, most of Brazilian soils present low fertility [3]. Moreover, many of the agricultural soils also have low fertility as a result of continued crops associated with the tropical climate, causing increased soil acidity. As soils become more acid, particularly when the pH drops below 4.5, the supply of most plant nutrients decreases while a few elements, such as aluminum, become more soluble and toxic to plants [4]. Therefore, chemical fertilizers must be added to soil to provide nutritional support for sustainable crop production. This places Brazil both as one of the biggest exporters of agricultural products and importer of fertilizers [5,6].

Not only Brazil, but most agricultural producing countries depend on external sources of nitrogen (N), phosphorus (P), and potassium (K) as they are essential macronutrients for plant growth [7]. Besides N and P, K has several metabolic and structural functions in plants. Due to the high solubility of potassium ion in water, this element is highly mobile in the plant system, acting as carriers of electrical charge and as catalysts for many of the enzymatic processes in the plant cells [8; 9]. Likewise, this element is related to plants tolerance to stress, temperature variation, drought, diseases, pest episodes, as well as to osmoregulation of water in plants. So, in order to enable sustainable crops productivity, it is necessary to supplement the soil with potassium for the cultivation of the majority of high carbohydrate plants such as bananas, sugarcane, and potatoes [8-10]. Particularly in tropical region soils, where the potassium content is usually low (below 1.5 $mmol.L^{-1}$).

In Brazil, the most important source of commercial potassium fertilizer is of geological origin – sylvite – composed of KCl and NaCl, with an average grade of 9.7% of equivalent K_2O [11]. The potash production in Brazil, restricted to the mine Taquari - Vassouras, in Sergipe, is performed by underground mining of sylvite ore, that is distributed to the market among standard (0.2 to 1.7 mm) and granular (0.8 to 3.4 mm) grain sizes. Even though the unit produces potassium on the top limit of its nominal capacity (500,000 t / year of KCl), it is still too far from achieving the domestic demand for the product, producing less than 10% of what is needed [5;6;12]. The small domestic production of potassium fertilizer and the large internal demand places

Brazil as one of the biggest importers of potassium. The import of potassium for use as agricultural fertilizer accounts for about 90% of the total domestic consumption [5;6;12].

Industrial minerals and rocks can be used as alternative sources for producing potassium salts or can be directly applied to soils in the form of rock powder to act as a slow release fertilizer [13;14]. One possibility is the use of verdete (glauconitic sandstone), a rock rich in potassium present in Minas Gerais, in Brazil, which stands out for the K_2O content ranging from 6 to 14% [15]. The disadvantage of verdete processing is the difficulty in the release of the potassium which is imprisoned in the chemical structure of the rock. As a consequence, the use of inorganic acids or thermal treatment is needed; this can increase the cost of the recovery of this chemical element and does not always result in a satisfactory extraction [13-16]. Therefore, there is a growing interest in finding alternative routes that provide a cost-effective technology for potassium extraction from alternative sources [13-17].

Microorganisms may be used to obtain different elements by biosolubilization of natural rock. Some studies have already showed the ability of soil bacterial and fungal species in solubilizing mineral phosphates and silicates [18-28]. However, as pointed out by Certini et al. [19], later corroborated by McNamara et al. [20], there are significant differences between the microbial communities of the rhizosphere and the bulk soil. Comparatively, rhizosphere microorganisms have a higher capability to biosolubilize chemical elements from rocks than the others. The roots may influence the colonization and development of some specific microbial communities, whose metabolism can increase the availability of nutrients in the soil, thus stimulating plant growth [21-24].

Becerra-Castro et al. [21] studying the action of two bacteria (*Arthrobacter* strains) *in vitro* in weathering of ultramafic rock, concluded that microorganisms act on different phases of minerals and that the mechanism adopted by each strain to release the elements is different. According to these authors, it is necessary to make the isolation and selection of promising strains to be used at different stages of solubilization of components from the rock matrix. In fact, the ecological relationships between soil microorganisms and rocks, mostly those associated with the plant rhizosphere, are complex and still need further investigation [18].

This scenario suggests that rhizosphere soil supplemented with rock powder is suitable for the isolation of rock solubilizing microorganisms, noting that each microbial activity can cause different mineral structure weathering. The biosolubilization of chemical elements from rock material can occur by different mechanisms including metabolic by-products action, such as protons, organic acids, chelator, and EPS (Extracellular Polymeric Substances) or functional molecules present in outer membrane [18;26].

Therefore, the main objective of this study was to investigate the ability of culture isolates to solubilize different chemical elements, particularly potassium, from rock matrix. The isolation strategy was to sample rhizosphere soil in which sunflower plant (*Helianthus annus* L.) had been growing. A soil of low potassium content was used for the plant cultivation and it was supplemented with rock powder containing potassium (K_2O) as the only source of this element.

II. MATERIALS AND METHODS

A. Rock powder (verdete)

The rock used was verdete (glauconitic sandstone) from Cedro do Abaeté region, Serra da Saudade, Minas Gerais, Brazil. For the experimental procedure, the rock was firstly crushed and sieved to obtain particles less than or equal to 1.68 mm in diameter. Then, subsamples were obtained by further sieving: (1) between 20-28# (0.841 mm and 0.595 mm); (2) 28-35# (0.595 mm and 0.420 mm) and (3) below 35# (0.420 mm).

The elemental composition of the mineral was determined by X-ray fluorescence (FRX), atomic absorption spectrometry and gravimetric analysis, which were carried out at the Chemical Analysis Laboratory of Center for Mineral (LQA-COAM/CETEM), revealing (in %w/w): K_2O (9.5); SiO_2 (63.7); Al_2O_3 (13.9); Fe_2O_3 (3.8); and MgO (1.2).

B. Isolation of bacteria from sunflower rhizosphere

For bacteria isolation, a sunflower cultivar (*Helianthus annuus* L. 122 V-2000) given by EMBRAPA (Brazilian Agricultural Research Corporation) was cultivated in a soil with a low potassium content (0.02 cmol/l), collected at the city of Recife, Pernambuco state, located in the northeast of Brazil. The soil's texture and elemental composition are presented as follows: sand 75%; silt 14%; clay 1%; particle density 2 g/mL; bulk density 1.3 g/mL; porosity 43%; pH (H_2O) 6.8; water holding capacity 34%; organic matter 17 g/l; and the elements (in cmol/l): Na (0.066); Ca (24); Mg (7.7); K (0.02); H+Al (9.2); Al (0); S (31.8).

Sunflower sowing was carried out in pots containing 1.8 kg of moist soil previously supplemented with N-P-K. Rock powder, grain size ≤1.68 mm, was added to the soil as the sole source of potassium, while NH_4NO_3 and H_3PO_4 were used as nitrogen and phosphorus sources, respectively. The N-P-K nutrient ratio based on the soil analysis was 40:70:40.

Figure 1 shows a general scheme of the steps employed from the sunflower planting to the isolation of potassium-solubilizing bacteria. Five sunflower achenes were directly sown into the soil in each of three pots, in X equidistant spatial arrangement. The soil moisture was maintained with the addition of 100 mL of water every 2-3 days, to prevent the plants from drying out. The total plant growth time was about 90 days (time of its inflorescence). Thereupon, sampling procedure consisted of collecting randomly the rhizospheric soil at the sunflower roots surroundings, per plant in each pot, totaling fifteen samples. After thoroughly homogenized, 20 g of soil were placed in a Erlenmeyer flask of 500 mL capacity that contained 200 mL of glucose yeast extract (GYE) medium [32] plus 20 g of rock powder (verdete, grain size below 1.68 mm), as the sole source of potassium. Medium and rock

powder were autoclaved separately at 121°C for 20 min and mixed together under aseptic conditions before being used.

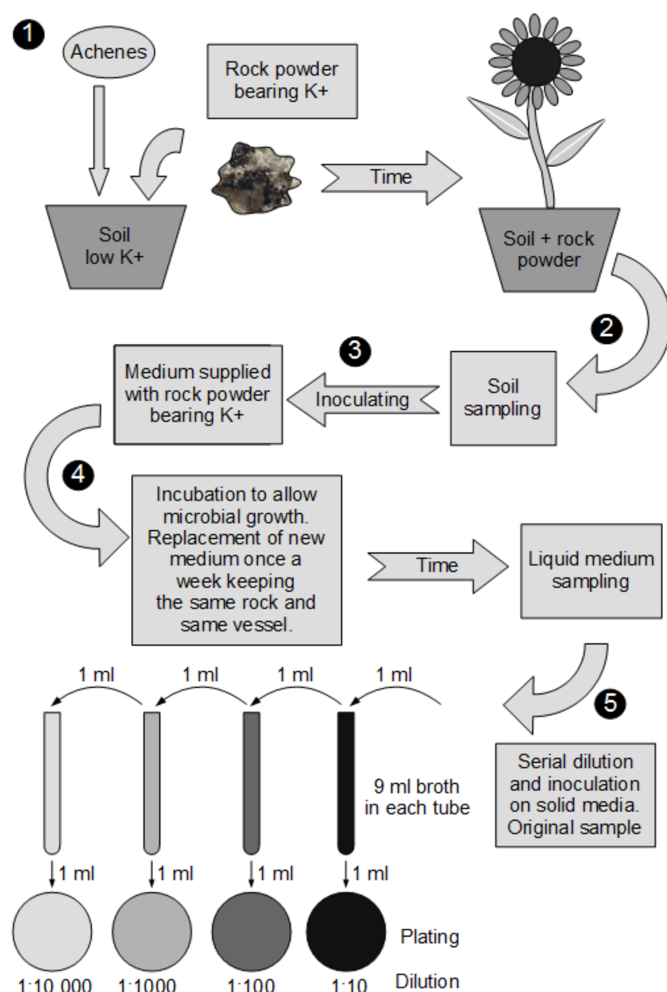

Fig. 1. General scheme used for seeding *Helianthus annuss* 122 V 2000 (1), rhizosphere soil sampling (2) and isolation of bacterial strains with the ability to solubilize chemical elements from the rock matrix (3, 4 and 5). Adapted from Melamed et al. [33].

The GYE medium used for bacterial growth and isolation was composed of (g/L): Glucose (10.0); yeast extract (1.0); NaCl (0.01); Na_2HPO_4 (4.0); NH_4NO_3 (0.005); 2 mL of $MgSO_4.7H_2O$ (10%); 2 mL of $CaCl_2$ (1%); 4 mL of Fe-EDTA (0.045%), and 2 mL of a micronutrient solution. The micronutrient solution consisting of (g/L): $NaMoO_4.2H_2O$ (1.0); $MnSO_4.2H_2O$ (1.175); H_3BO_3 (1.4); $CuSO_4.5H_2O$ (0.04); $ZnSO_4.7H_2O$ (0.12), was prepared separately and sterilized by filtration through a 0.22 µm diameter membrane filter (Millipore).

The culture was incubated at 30°C and at 150 rpm for 28 days in an orbital shaker to stimulate the growth of bacterial populations. After 7 days of incubation, about 180 mL of the culture medium was replaced by fresh GYE medium supplemented with the same amount of rock powder used before. This procedure was repeated every week for the four consecutive weeks to promote growth of potassium solubilizing bacteria.

At the end of the incubation period, serial dilutions were performed in phosphate buffered saline (PBS), till 10-10, and plated in GYE agar, containing rock powder as the sole source of potassium. The Agar plate preparation consisted of spreading 0.02 g of sterile rock powder, with the aid of a Drigalsky spatula, over a solidified GYE medium, followed by covering it with 4 mL of the agar medium in liquid state. Uninoculated Petri dishes were incubated at 30°C for 48 h before being used in order to check for contamination.

The different morphotypes obtained were isolated and later purified by streaking onto the same solid medium. The purified isolates were maintained by monthly transfers, stored in refrigerator at 4°C.

C. Biological Weathering of Glauconite

This test was performed to determine the influence of contact time of the selected isolates with the rock in the release of its constituents, particularly potassium, as a direct result of biological activity. Erlenmeyer flasks (250 ml capacity) containing 10 g of sieved verdete (≤1.68 mm) and 100 mL of Aleksandrov modified medium were used and individually inoculated with the isolated cultures Nigla 05 or Ig 04 (biotics assays). For comparative purposes, an assay without inoculation (abiotic control) was also conducted. Triplicate samples were sacrificed at each sampling event at 7, 14, and 21 days.

The Aleksandrov modified medium used was constituted of (g/L): glucose (5.0); Na_2HPO_4 (2.0); $(NH_4)_2SO_4$ (0.5); $CaCO_3$ (0.1); K_2HPO_4 (0.12); $MgSO_4$ (0.5). There was no addition of the iron source ($FeCl_3$) from the original medium [34] because preliminary tests (data not shown) showed that neither bacterial growth nor elements solubilization was influenced by the presence of this element. The pH of the medium was adjusted to between 6.5 and 6.7 before autoclaving for 20 min at 121°C.

The standard inoculum (10^4 $CFU.mL^{-1}$) was obtained from the growth of a loopful of a 48 hour old culture of the isolated bacteria aerobically grown on Aleksandrov modified broth, under agitation condition, at 28±2°C.

For the quantitative estimation of the dissolution of the chemical elements (K, Si, Mg, and Al) from the rock matrix, culture samples were centrifuged at 4000 x g, at 20°C for 20 minutes. Then, the supernatant was filtered through a cellulose ester membrane with a pore size of 0.22 µm, for cells removal, and acidified at pH 2 with HCl P.A. to avoid further contamination. Chemical analyses in cultures filtrates were determined by atomic absorption at the Laboratory of Chemical Analyses (LQA-COAM/CETEM).

D. Effect of the distribution of the rock powder particle-size on the solubilization activity

Experiments were carried out in 500 mL Erlenmeyer flasks containing 100 mL of Aleksandrov modified medium, using three different particle-size distributions of rock powder: (1) between 20-28# (0.841 mm and 0.595 mm); (2) 28-35# (0.595 mm and 0.420 mm) and (3) below 35# (< 0.420 mm).

The inoculum was prepared as previously described. After 14 days of incubation at 28±2°C, 150 rpm, the amounts of K, Si, and Mg released in culture broths were determined by atomic absorption spectrometry. An abiotic control (uninoculated sterile medium) was done for each tested rock powder granulometry.

E. Crystalline structure changes by microbial action

In order to assess changes on the surface-structure of rock particles, solids from a 14 days old culture of a previous study were characterized by X-ray diffraction (XRD) analysis. The solids from the culture of the most effective solubilizing isolate were chosen for this XRD analysis. Prior to being analyzed, the solids were filtered through a 0.22 µm cellulose ester membrane filter, washed three times with distilled water, dried at 60°C overnight, grounded using an agate mortar, and sieved below 200#.

F. Determination of EPS production and trapped elements

The selected isolates were grown in 100 mL of modified Aleksandrov medium containing rock powder (below 35#, 0.420 mm). After 14 day in incubator shaker at 150 rpm and 28±2°C, the contents of six flasks of each culture were individually homogenized, and centrifuged for 20 min at 4000 x g, at 20°C for cells removal. Samples of three flasks were removed, filtered through a cellulose ester membrane of 0.22 µm and acidified to pH 2.0 with HCl P.A. for subsequent chemical analysis. Samples of the three other vials (containing the solution and the EPS) were sterilized at 121° C for 20 minutes with the addition of 4% v/v of H_2O_2 to decompose the polysaccharides [35] The recovered supernatant was filtered through a cellulose ester membrane of 0.22 microns and acidified to pH 2.0 for subsequent chemical analysis.

G. Microbial identification of selected strains by molecular biology

Bacteria strains were isolated in pure cultures from sunflower rhizosphere at 90-day cultivation on soil supplemented with rock powder as the unique potassium source, in GYE medium. The isolated strains were observed under the microscope for the determination of morphotinctorial characteristics and macroscopically characterized from colonies grown on agar. The strains were preserved by freezing in triplicate in DMSO at -80° C.

The phylogenetic identification was performed by molecular biology techniques. DNA from the pure strains was extracted with the help of the commercial kit Ultra Clean Soil Isolation (MO BIO Laboratories) according to instructions provided by the seller.

The amplification of the 16S rRNA gene by Polymerase Chain Reaction (PCR) was performed using the kit Top Taq Master Mix (Qiagen) in a total volume of 50 µL, containing 0.5 µM of each primer and 5µL of the extracted DNA. The primers used were SAdir (5’-AGAGTTTGATCATGGCTCAGA-3’, forward) and S17 Rev (5’-GTTACCTTGTTACGACTT-3’, reverse). PCR reactions were performed in a PCR thermocycler System 9700 (Applied Biosystems). A first denaturation was performed at 94 ºC for 4 minutes followed by 30 cycles in the following conditions: 94 °C for 30 seconds, 50ºC for 30 seconds and 72ºC for 30 seconds. The PCR ended at 72 ºC for 10 minutes. The amplicons were purified using the PCR Clean-Up System (Promega) before sequencing, as previously described by [36].

The PCR products were further purified using a PCR cleaning kit and sent to the University of São Paulo (USP) for sequence analysis.

The products were purified using the BigDye XTerminator Purification Kit and electronically injected in the sequencer. The sequence results were analysed using Chromas Lite software, version 2.01 and Bioedit Sequence Alignment, as previously described by [36]. The sequences validated by the programs were aligned to the sequences from the Genbank using BLAST (Basic Alignment Search Tool). Just fragments with similarity levels over 98% were considered.

H. Statistical analysis

All experiments were carried out in at least triplicate. All data are reported as means ± SD (standard deviation). The Independent-Samples t-test was used to compare means and the variance homogeneity determination (ANOVA) was conducted with the General Linear Model using type II sum of squares and Tukey’s Honestly Significant Difference (P = 0.05) using statistical analysis system software (SAS Institute, 2002).

III. RESULTS AND DISCUSSION

A. Bacterial isolation and Identification

Nine bacterial cultures were successfully isolated from sunflower rhizosphere at 90-day cultivation on soil supplemented with rock powder as the unique potassium source, in GYE medium. Two isolates were selected because of their capacity of growing in modified Aleksandrov medium. These two strains were referred as Nigla 05 and Ig 04. This medium is commonly used to investigate the ability of microbial strains to convert insoluble forms of potassium and other chemical elements from different rock minerals, including the silicate one, which is the subject of the present research [29-33]. The isolation of biosolubilizing microorganisms considered that soils with low nutrient levels may have a rhizospheric region with a greater diversity of microorganisms capable of solubilizing chemical elements from rock and/ or minerals in the soil, which happen due to selective pressure and to better use the oligotrophic niche [34,35].

The identification of the bacterial strains was performed by the Laboratory of Biocorrosion and Biodegradation at the National Institute of Technology INT / MCTI (Ministry of Science, Technology and Innovation). The 16S ribosomal RNA gene amplicons obtained for strains Nigla 05 and Ig 04 showed homology to *Burkholderia* sp. (Acession number

KC241903 99% identity) and *Paenibacillus* sp. (Accession number JQ691537 98% identity), respectively.

These two genres are widely recognized in the scientific literature as rhizosphere inhabitants of different plants, which contribute positively for plants growth, and are also recognized as solubilizing agents into rock matrix, providing macro and micronutrients to plants in soil lacking those elements [37-40]. Observations from both, laboratory and field conditions, have shown that the change of mineral and nutritional composition of the soil can influence the diversity of microbiota in the rhizosphere [41-44]. According to some authors, the effect of minerals on the microbial diversity is due to the ability of some species to use this oligotrophic niche more efficiently than others [18;26;44]. Gleeson and collegues [29] demonstrated through the profile of the bacterial communities that colonize granite that the communities varied according to the type of mineral inclusion (muscovite, plagioclase, quartz, and K-feldspart). Uroz et al. [18;26] suggests a new term to define the region adjacent to the minerals in the soil, "mineralosphera" where microorganisms are selected for their ability to preferentially use inorganic nutrients released by minerals of the soil.

B. Biological weathering of verdete

The biotic assays with Nigla 05 and Ig 04 cultures showed similar behavior to the abiotic control. Under the conditions tested, the Ig 04 strain showed the best performance in 7 days, resulting in increased potassium solubilization in the shortest time tested. There was no significant difference for both strains in the days 14 and 21 days and therefore, 14 days was chosen for the subsequent biotic assays.

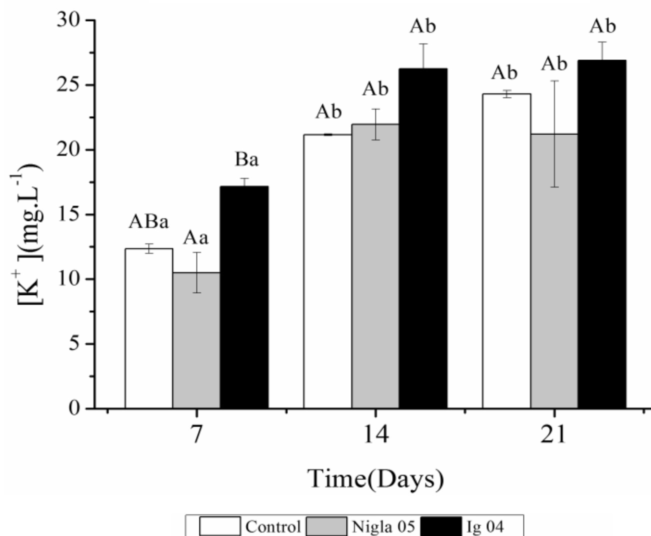

Fig. 2. Biological leaching of potassium (K^+) from rock powder for Nigla 05, Ig 04, and abiotic control (modified Aleksandrov medium, sterile and non-inoculated). Means with the same letter do not differ in Tukey's test ($p < 0.05$). Equal capital letters do not differ within the same time (7, 14 or 21 days) and equal lowercase letters do not differ within the same treatment. Error bars indicate standard error of mean.

One explanation for the poor performance of Nigla 05 strain in relation to the abiotic test may be due to EPS synthesis which results in potassium imprisonment on the cell surface [29], which makes it difficult to evaluate its biosolubilization. The biological impact on weathering process is difficult to separate from purely abiotic process and this can corroborate the observations of biotic and abiotic assays [18,38].

C. Effect of rock powder particle-size distribution on solubilization activity

The solubilization capacity of Si, Mg, and K by the two isolates as a function of rock particle size distribution (20-28#, 28-35#, <35#) are presented in Figure 3. Data of iron and aluminum release are not shown because their concentrations were below the analytical detection limits (circa 1 mg.L^{-1}), for both biotic and abiotic assays.

In general, the greatest amounts of different elements were released from the rock by Ig 04 strain activity, independently of its particle size. However, the granulometry of the rock powder greatly affected the solubilization of each chemical element for both abiotic and biotics assays. This isolate was able to solubilize the maximum amounts of Si (59.3 mg.L^{-1}) and Mg (60 mg.L^{-1}) using rock particle sizes between 28 and 35# and between 20 and 28#, respectively.

In the particle size between 20 and 28# (Figure 3A), the amounts of Si solubilized did not differ statistically (28 mg.L^{-1}), while the larger amount of Mg (statistically significant, $p < 0.05$) was 60 mg l^{-1}, two times higher than that obtained in tests with 05 Nigla and the control test (approximately 30 mg L^{-1}). For the intermediate particle size (between 28 and 35#), Figure 3B, the concentration of solubilized Si (59 mg.L^{-1}) was statistically significant and greater then the control assay (25 mg.L^{-1}), while for Mg, there was no statistically significant difference between Ig 04 and the control, and the values obtained by Nigla 05 were lower than the control and Ig 04. For the smaller particle size (<35#), Figure 3C, the solubilization of Si was statistically similar in the biotic tests (Ig 04 and Nigla 05), while the Mg solubilization decreased in the following order: Ig 04> Control> Nigla 05. Nevertheless, the amounts of potassium released in the biotic and abiotic tests were statistically similar.

The elements solubilization by one of the isolates ratifies that the strategy adopted to supplement a naturally poor nutrient soil with rock powder, as the sole source of potassium, is suitable for obtaining solubilizing agents. Beauregard et al.[42] suggest that plants can compensate for the low nutrient availability in soils with a more efficient ecological relationship with microorganisms in the rhizosphere that have the ability to influence the elements cycles. Furthermore, Koreem et al. [41] observed that a decrease in the K content of the soil can be correlated with the increase of indigenous microbial population, including bacteria, saprotrophic and ectomycorrhizal fungi.

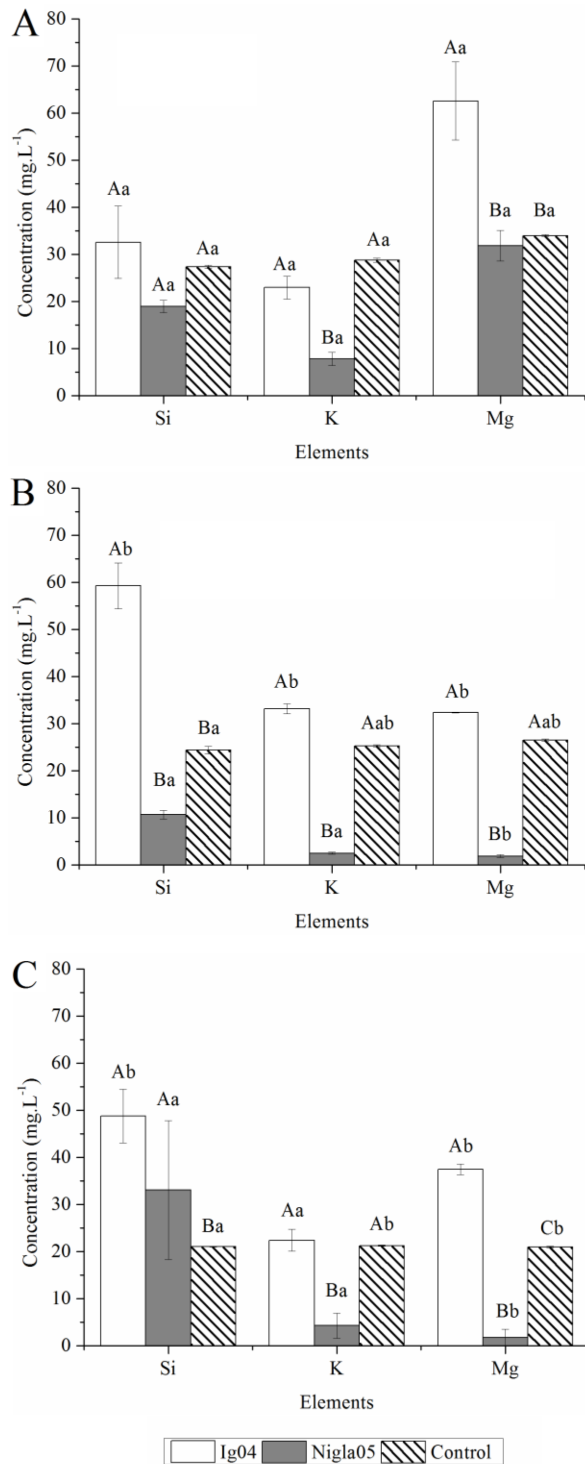

Fig. 3. Effect of particle-size distribution of the powdered potassium rock on the solubilization of chemical elements in biotic (Ig 04 and Nigla 05) and abiotic (uninoculated steril medium) assays, during 14 days under the same environmental conditions [A: 20-28#; B: 28-35#; C: <35#]. Values are means ± SE of three replicates. Different letters indicate significant differences among the means of different treatments (p<0.05).

C. Rock structure changes related to bacterial activity

The XRD diffractograms of the three particle sizes tested (Figure 4) were not able to show differences in the peaks. It was also not possible to determine which crystal structures have undergone weathering and released the elements observed in the analyzed solution; as in Figure 3, in which it was seen the release of Si and Mg when Ig 04 was used as inoculum. The formation of new peaks was also not observed, which does not suggest changes in the crystalline structure of the rock.

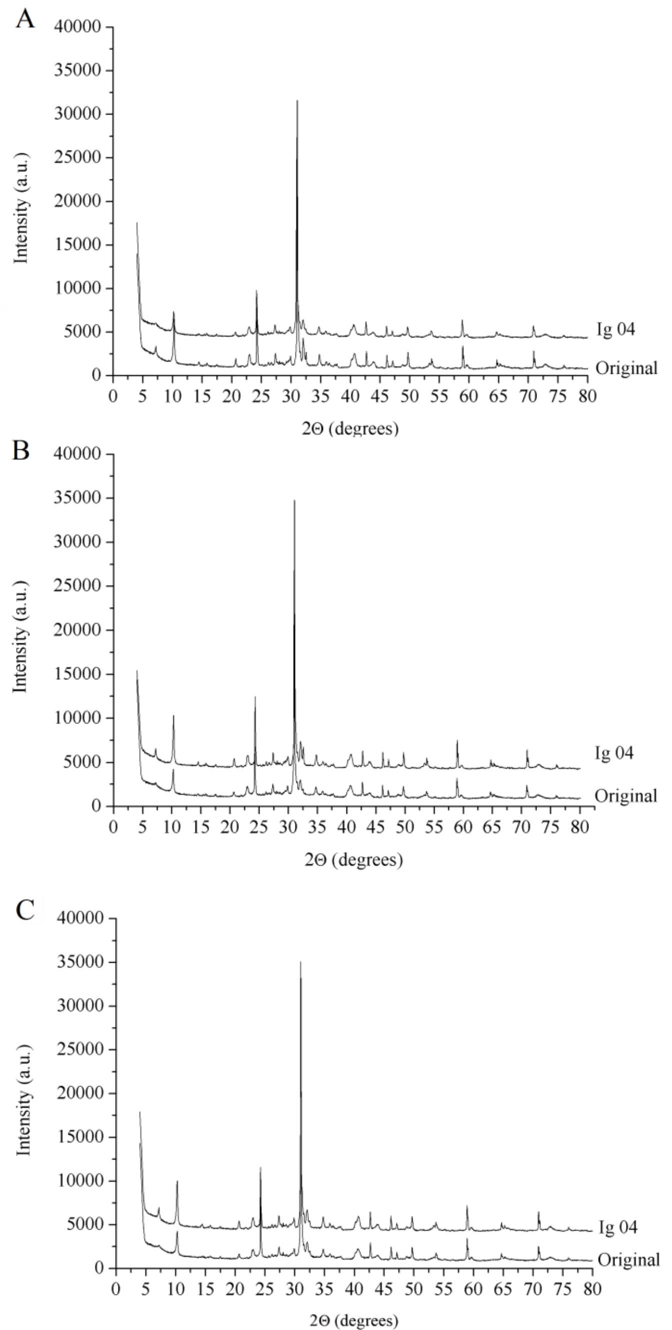

Fig. 4. XRD diffractograms of the verdete before (original) and after 14 days of growth of Ig 04 as a function of particle size distribution [A: 20-28#; B: 28 - 35#; C: <35#].

D. EPS production and trapped elements

Among the two selected isolates, only Nigla 05 strain was identified as an EPS producer (data not shown). So, this

strain was further tested to evaluate microbial rock weathering accomplished by mineral bound-EPS and mineral solubilization (Figure 5). The EPS was able to retain K, Si, and Mg, by the action of Nigla 05 strain on the rock powder during 14 days. However, there was no solubilization of Al and Fe, and no adsorption of these elements to the EPS.

According to Liermann et al. [45], the EPS shifts the equilibrium reactions between the mineral structure of the rock and the liquid solution, favoring the dissolution of the constituent minerals and the subsequent release of more elements to the solution. However, since the EPS and mineral interaction is dependent of electric charges, the removal will be different for the mineral species involved. Liu et al. [35] observed approximately 65% of Si adsorption and only 1% of K to the EPS produced by *Bacillus mucilaginosus* employing K^+ content of 100 to 300 $mg.mL^{-1}$ and from 200 to 500 SiO_2 $mg.mL^{-1}$.

Another important action of the EPS in the release of minerals is the increase in fracture of the crystalline structure through the EPS deposition and subsequent cycles of swelling and shrinking, acting as mechanical force and causing stress in the structure of the stone [18;38]. Therefore, more studies are needed to enhance EPS production by Nigla 05, and to define when the saturation by adsorption of cations in relation to the amount in solution occurs.

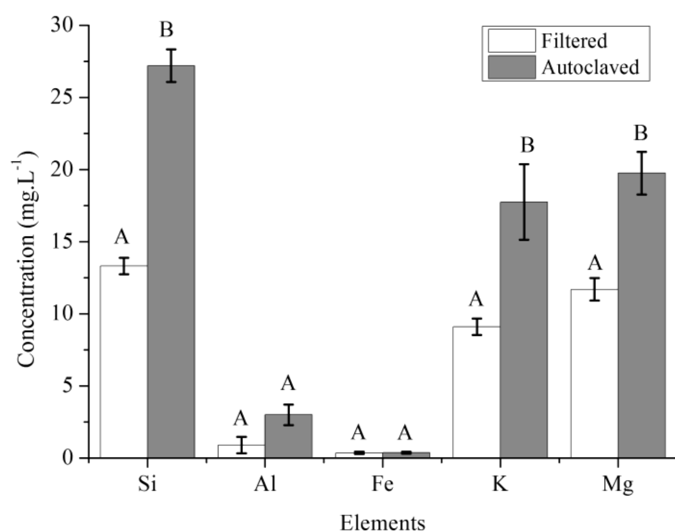

Fig. 5. Mineral amounts in solution and bounded to EPS after 14-day cultivation of Nigla strain in rock powder (below 35#, 0.420 mm). Means with the same letter do not differ in Tukey's test 5%.

IV. CONCLUSIONS

The strategy adopted to isolate microorganism from sunflower rhizosphere containing rock powder as the sole source of nutrient (potassium) resulted in the isolation of nine bacterial cultures and two isolates were selected due to their capacity of growing in modified Aleksandrov medium. The selected strains, referred as Nigla 05 and Ig 04, were identified as *Burkholderia* sp. and *Paenibacillus* sp., repectively.

Biotic assays with Nigla 05 and Ig 04 cultures showed similar behavior to the abiotic control. Under these experimental conditions, the Ig 04 strain (*Paenibacillus* sp.) showed a little better performance in terms of potassium solubilization, indicating the potential use of this microorganism in further studies for the development of agrominerals biosolubilization processes.

Nigla 05 (*Burkholderia* sp.) strain was identified as an EPS producer, and it was also verified that this EPS is able to trap the solubilized elements (Si, K, and Mg). Therefore, more studies are needed to enhance EPS production by Nigla 05, to identify the influence of EPS in the process of solubilization/retention of K and to define when saturation by adsorption of cations in relation to the amount of EPS in solution occurs.

The granulometry of the rock powder greatly affected the solubilization of each chemical element for both abiotic and biotics assays. The decrease of the particle size increased the elements dissolution, but without significant action below 0.420 mm. The XRD diffractograms were not able to identify which crystal structure released cations or to determine if there was the formation of new structures.

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