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Mobilization of potassium from waste mica by potassium-solubilizing bacteria (*Bacillus mucilaginosus*) as influenced by temperature and incubation period under *in vitro* laboratory conditions

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SUMMARY. – An attempt was made to mobilize potassium (K) from a K-bearing mineral (waste mica) inoculated with K-solubilizing bacteria (*Bacillus mucilaginosus*) as a function of temperature and incubation period under *in vitro* laboratory conditions. A series of incubation experiments were carried out to study the mobilization of K from waste mica. Destructive samplings were drawn at different periods of incubation and analyzed for pH, total sugar content and NH_4OAc-K. The results showed that irrespective of temperature and period of incubation, a significantly higher mobilization of K was recorded in all the treatments when incubation was carried out in the presence of the bacterial strain compared to control. The pH and total sugar content were found to decrease in the presence of *Bacillus mucilaginosus* as compared to control, while in the presence of the bacterial strain the NH_4OAc-K content increased from 12.3 to 46.0%, irrespective of treatments. Thus, it may be concluded that waste mica inoculated with a K-solubilizing strain could be a promising technology to utilize waste mica, and could be used effectively as an alternative source of K-fertilizer for crop production.

INTRODUCTION. – Potassium (K) is the third major essential macronutrient required for plant growth and development, and it is commonly applied as fertilizer for crop production. The development of intensively managed agriculture resulted in an increased K removal from soil, and nowadays many areas of India have soils showing K-deficiency, thus becoming one of the constrains in agricultural production. In India, the whole amount of K-fertilizers is imported, costing a huge amount of foreign exchange, as there is no K-reserve in India for commercial manufacturing of K-fertilizers. Thus, the use of alternative indigenous resources

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of low-grade K-bearing minerals such as feldspar and waste mica is getting importance to reduce the dependence of imported or commercial K fertilizers (Badr *et al.*, 2006; Nishanth and Biswas, 2008; Basak and Biswas, 2009, 2010). The use of plant growth promoting rhizobacteria, particularly phosphate- and K-solubilizing microorganism as biofertilizers, was reported as an alternative solution to improve plant growth and nutrition (Vessey, 2003). There are some bacteria in the soil which are capable of solubilizing K-bearing minerals to bring K into an available form. These bacteria are specially known as K-solubilizing bacteria (KSB) or K-dissolving bacteria (KDB) or silicate-dissolving bacteria (SDB). Some attempts have been made on the use of K-solubilizing bacteria, also known as biological potassium biofertilizers (BPF), to investigate the activation of minerals inoculated with bacteria (Lin *et al.*, 2002; Sheng, 2005; Han and Lee, 2005; Han *et al.*, 2006; Sheng and He, 2006; Supanjani *et al.*, 2006; Liu *et al.*, 2006; Basak and Biswas, 2009, 2010). Increased bioavailability of P and K in soils – leading to higher P and K uptake and plant growth – by inoculation of phosphate-solubilizing bacteria (PSB) and KSB, or by co-inoculation of bacteria with naturally occurring rocks and materials was reported by few studies (Lin *et al.*, 2002; Girgis, 2006). Some of the studies showed that K-bearing mineral inoculated with KSB improves the available K content in the culture medium. Therefore, the application of K-solubilizing bacteria holds a promising approach for increasing K availability in soils.

Different hypotheses have been postulated to explain the mechanisms of K solubilization from K-bearing minerals by microorganisms. The main mechanism of mineral-K solubilization involves the production of low molecular organic acids by soil microorganisms (Huang and Keller, 1972; Leyval and Berthelin, 1989). Many researchers opined that production of carboxylic acids and capsular polysaccharides or extracellular polysaccharides (EPS) and enzymes play an important role in the dissolution of silicate minerals by application of K-solubilizing microorganism such as *Bacillus mucilaginosus* (Lin *et al.*, 2002). Thus, it is very important to investigate the microbial solubilization of K-bearing minerals and their potentiality as K-fertilizers.

In many soils there are considerable amounts of insoluble K-reserves, most of which present in alumino-silicate minerals from which K cannot be absorbed directly by plants. Mica, specially waste mica, is an ideal example of such mineral. India has the world's largest deposit of mica distributed over a total area of about 3,888 km^2 in Munger district of

Bihar, and Koderma and Giridih districts of Jharkhand. This material is not used in agriculture, though it contains a significant amount of K (8-12% K_2O) and is dumped near mica mines (NISHANTH and BISWAS, 2008; BASAK and BISWAS, 2009). Mica can effectively be used as a source of K, if modified or altered by some suitable chemical or biological means. Thus, by treating these indigenous sources of waste micas with suitable microbial cultures the mobilization of K can be increased. Therefore, dissolution of K-bearing minerals like waste mica by K-solubilizing bacteria holds a good proposition to evaluate the effect of bacteria for releasing K, which then could be utilized by plants as source of K. However, information regarding K release from minerals like mica treated with K-solubilizing bacteria is meager. Therefore, the aim of this study was to evaluate the potential of the K-solubilizing bacterial strain *Bacillus mucilaginosus* to mobilize K from waste mica as a function of temperature and incubation period under *in vitro* laboratory conditions.

MATERIALS AND METHODS. – *Waste mica.* – Waste mica, a K-bearing mineral, was collected from the nearby area of mica mines located at the Koderma district of Jharkhand, India. This is a byproduct of mica mines and is produced during the cleaning of fresh mica, which is mainly used in electrical industry. This byproduct material is usually dumped in surrounding area of mica mines and has no use in agriculture as such. Waste mica, a 2:1 clay mineral, comes under muscovite mica according to clay mineral classification. The structure of mica is flake-like with a theoretical composition of $(OH)_4K_2(Si_6Al_2)Al_4O_{20}$. Before use, it was ground thoroughly in a Wiley mill and then passed through a 2-mm sieve. The finely ground waste mica contained 20.0 mg kg^{-1} of available K and had 10.0% total K. Mica was sterilized by dry autoclaving at 20 psi for 120 min before using in the incubation study.

Bacterial strain. – Potassium-solubilizing bacteria (*Bacillus mucilaginosus*), a carrier-based biological K fertilizer (BPF), was obtained from the Hebei Research Institute of Microbiology (P.R. China) with due permission from the Government of India, Ministry of Agriculture. The active bacterial strain was isolated from the BPF material using a nutrient agar medium and subsequently multiplied for further use. The composition of the nutrient agar medium was: beef extract 3.0 g, peptone 5.0 g, agar 15.0 g, distilled water 1000 mL, pH 6.6 to 7.0. The isolated *Bacillus mucilaginosus* strain was maintained on potato-dextrose agar slants in a refrigerator at 4ºC. The strain was cultured in a special medium suitable for K-solubilizing bacteria (SHENG and HE, 2006). The special broth culture medium had the following compositions: sucrose 5.0 g, sodium hydrogen phosphate (Na_2HPO_4) 2.0 g, magnesium sulphate ($MgSO_4 \cdot 7H_2O$) 0.5 g, ferric chloride ($FeCl_3$) 0.005 g, calcium carbonate ($CaCO_3$) 0.1 g, sterilized waste mica (K source) 1.0 g, distilled water 1000 mL and pH was adjusted to 7.

Incubation study. – A series of incubation experiments were carried out under *in vitro* laboratory conditions. The first experiment was carried out to investigate the mobilization of K from waste mica inoculated with bacterial culture (broth culture) and incubated at different temperatures for different periods of incubation. The K-solubilizing bacterial strain, isolated from biological K fertilizer and multiplied according to the standard method mentioned above, was used for mobilization of K from waste mica. About 1000 mL of medium containing 20 g waste mica was prepared. From this medium, 100 mL aliquots were transfererd in separate conical flask having 250 mL capacity. In one set, 1 mL of *Bacillus mucilaginosus* culture was added (1.98×10^7 cells). In another set, no bacterial strain was added (control). Both sets were incubated at four different temperatures (15, 28, 37 and 50ºC) in four separate incubators for different periods of incubation (7, 14, 21 and 28 days). Destructive samplings were drawn at different periods of incubation and analyzed for pH, available K (NH_4OAc-K) and total sugar content.

Changes of pH in the medium were determined at different periods of incubation. The pH was measured directly by a pH-meter after having filtered the medium through a 0.2 µm Whatman membrane filter (RICHARD, 1954). Separation of capsular polysaccharide from the culture fluid was carried out using the extracellular polysaccharides (EPS) quantification method (GANCEL and NOVEL, 1994). The culture fluid was treated with 6% (v/v) H_2O_2 and sterilized at 121°C for 20 min to decompose EPS, thereafter centrifuged at 10,000 rpm for 20 min. The total sugar content of the EPS was determined by the modified phenol-sulfuric acid method (DRAPRON and GUILBOT, 1962), using glucose as a standard. Potassium content in the medium (broth culture) was determined by extracting sample with 10 mL of 1 N NH_4OAc extractant followed by determination of K content using a flame photometer (HANWAY and HEIDEL, 1952).

Release kinetics. – To compute the release kinetics of K from waste mica treated with the bacterial strain *Bacillus mucilaginosus*, the release of K was measured at different incubation periods. To work out the release rate of K from waste mica, data of NH_4OAc-K generated in incubation experiments were fitted into the following simple first-order kinetic equation (JARDINE and SPARKS, 1984):

$$\ln (a - p) = \ln a - kt$$

where, a is the initial amount of NH_4OAc-K (mg L^{-1}), p the amount of NH_4OAc-K (mg L^{-1}) released at a particular time t (days), $(a - p)$ the final amount of NH_4OAc-K (mg L^{-1}), and k the rate constant.

Statistical analysis. – The experiment was carried out in three replicates. Data presented are the means of three independent experiments. Standard error was calculated and put in the graphical representation.

RESULTS AND DISCUSSION. – *Changes of pH.* – Data obtained from the first incubation experiment showed that in presence of BPF broth culture containing *Bacillus mucilaginosus* the pH of the medium decreased at all temperatures compared to control (Fig. 1). In the presence of the bacterial culture, the lowest pH (5.47) was observed at 28°C after 21 days of incubation, while the highest pH (7.33) was observed at 50°C after 7 days of incubation. A relatively higher pH was observed at 50°C, which might be due to a slower growth of microorganism at that temperature. Decrease in pH in the presence of the bacterial strain might be due to the release of some organic acid substances from microorganisms. It was reported that the bacterial intervention on silicate minerals is closely associate with the formation of mucilaginous compounds consisting of EPS as well as with the production of different low molecular organic acids (GROUDEVA and GROUDEV, 1987). It is also reported that mineral-K solubilization is due to the production of low molecular acids by soil microorganisms (HUANG and KELLER, 1972; LEYVAL and BERTHELIN, 1989). Protons associated with organic acid molecules decrease the pH of the solution and, therefore, induce the releasing capacity of cations such as iron, potassium and magnesium.

Mobilization of K from waste mica. – Mobilization of K from waste mica estimated by measuring the release of NH_4OAc-K is reported in Fig. 2. The results show that addition of the bacterial strain had a significant effect in enhancing the mobilization of K throughout the periods of incubation. The overall mean release of NH_4OAc-K in the presence of the bacterial culture was 29.8% higher over its counterpart without the bacterial culture. Data also showed that temperature and period of

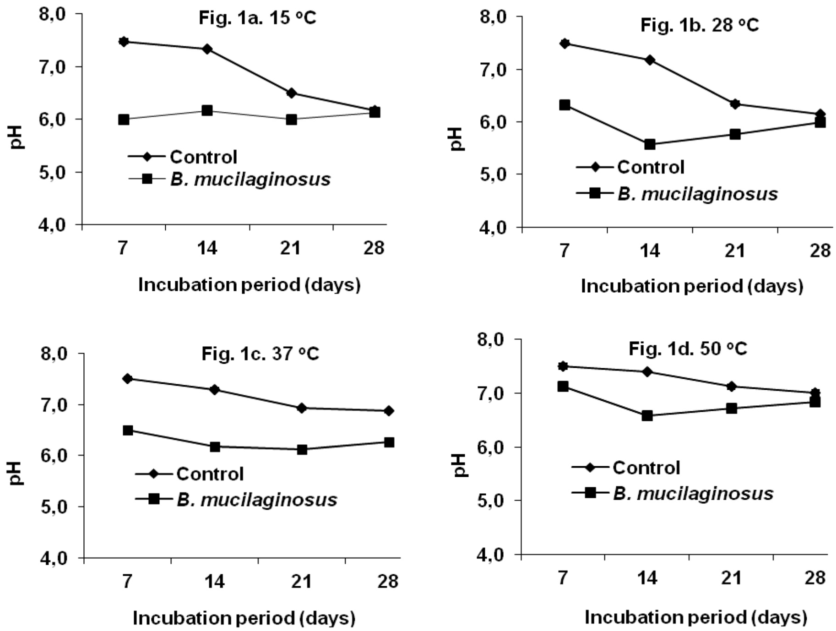

Fig. 1. – Changes in pH of the medium containing waste mica treated with the bacterial strain *Bacillus mucilaginosus* under different temperatures and incubation periods. Bars indicate standard error (n = 3).

incubation had a significant impact on NH_4OAc-K. In the presence of the bacterial culture, the NH_4OAc-K content increased from 12.3 to 46.0%, irrespective of treatments over their respective controls (Fig. 2). The highest increment of NH_4OAc-K was observed 28 days after incubation at 37°C (Fig. 2c). The NH_4OAc-K content increased gradually up to 14 days, then increased slowly up to 21 days when the culture was incubated at 37 and 50°C; thereafter, it increased considerably up to 28 days. The results showed that mobilization of K from waste mica could be enhanced significantly by introducing a bio-inoculum such as *Bacillus mucilaginosus*.

Many workers observed that species of *Bacillus* could solubilize K from K-bearing minerals, which may be attributed to different mechanisms of dissolution of K from these minerals. It was proposed (Vandevivere *et al.*, 1994) that *Bacillus mucilaginosus* increases the dissolution rate of silicate and alumino-silicate minerals and releases K^+ and SiO_2 from the crystal lattice primarily due to production of organic

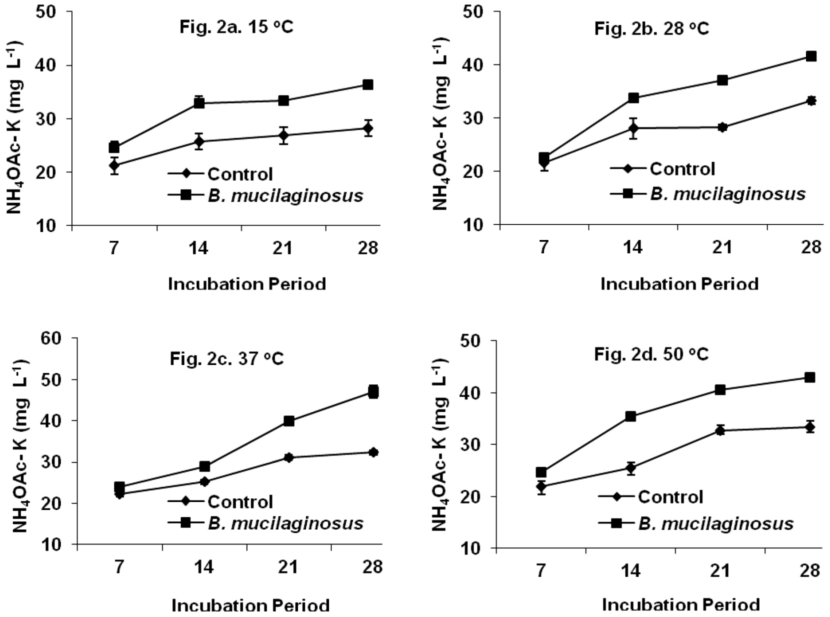

FIG. 2. – Changes in NH_4OAc-K in the medium containing waste mica treated with the bacterial strain *Bacillus mucilaginosus* under different temperatures and incubation periods. Bars indicate standard error ($n = 3$).

acids. It is suggested that the activity of silicate-dissolving bacteria played a crucial role in the release of Si^{+4}, Fe^{+3} and K^+ from feldspar and Fe-oxyhydroxides (SONG and HUANG, 1988; FRIEDRICH *et al.*, 1991; BENNETT *et al.*, 1998; STYRIAKOVA *et al.*, 2004). The association of silicate minerals and bacterial strain can be explained as an outer sphere complex formation as it occurs through electrostatic interactions. The present study indicates that release of K from waste mica by *Bacillus mucilaginosus* strain may occur as a result of the action of both EPS and organic acids produced, which can form bi-dentate complexes with metals ions and tend to be more effective in enhancing dissolution than mono-dentate ligands formed by acetate or propionate (WELCH and ULLMAN, 1993).

Total sugar content. – The total sugar content (Fig. 3) was found to decrease considerably in the presence of bacterial culture as compared to control (medium only). The decrease in total sugar content in the medium due to introduction of the bacterial culture proves that the bac-

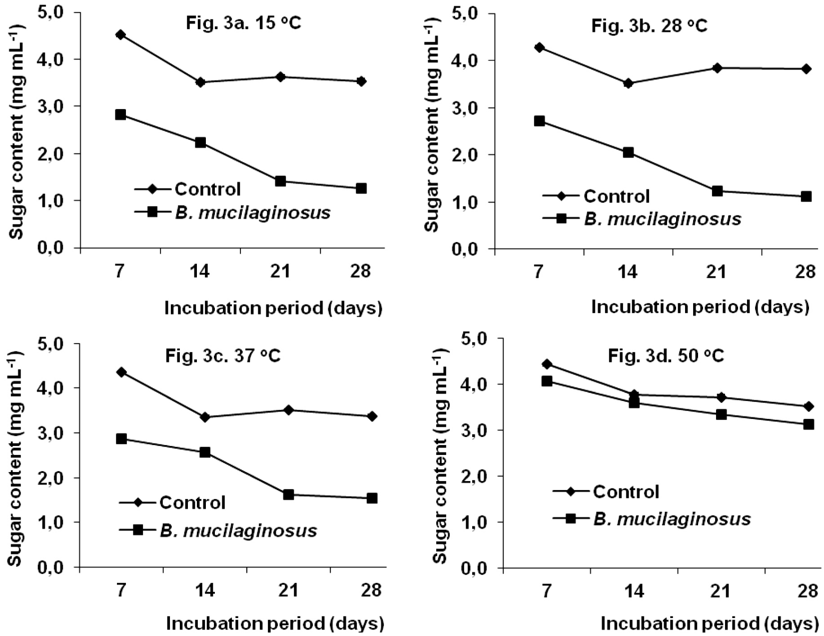

Fig. 3. – Changes in total sugar content (mg mL^{-1}) in the medium containing waste mica inoculated with the bacterial strain *Bacillus mucilaginosus* under different temperatures and incubation periods. Bars indicate standard error (n = 3).

terial strain depleted sugar content in the medium for their own growth and nutrition, and shows that they are very active in mobilizing K from K-bearing mineral like waste mica. With the lengthening of the period of incubation, total sugar content decreased. In the presence of bacterial culture, the highest total sugar content (4.08 mg mL^{-1}) was observed at 50°C, while the lowest value (1.11 mg mL^{-1}) was observed after 28 days at 28°C, which was 71.0% lower compared to control (medium only). The higher amount of total sugar content at 50°C was an indication of a slower growth of the microorganisms under higher temperatures, while the lowest amount indicates the highest utilization of sugar by the bacterial strain found after 28 days of incubation at 28°C. This indicates that growth of microorganisms is higher during 28 days of incubation at 28°C. The bacterial culture showed a variable degree of metabolic effectiveness under different temperatures and incubation periods leading to different degree of utilization of total sugar content in the medium. The lowest sugar content found at 28°C after 28 days of incubation also

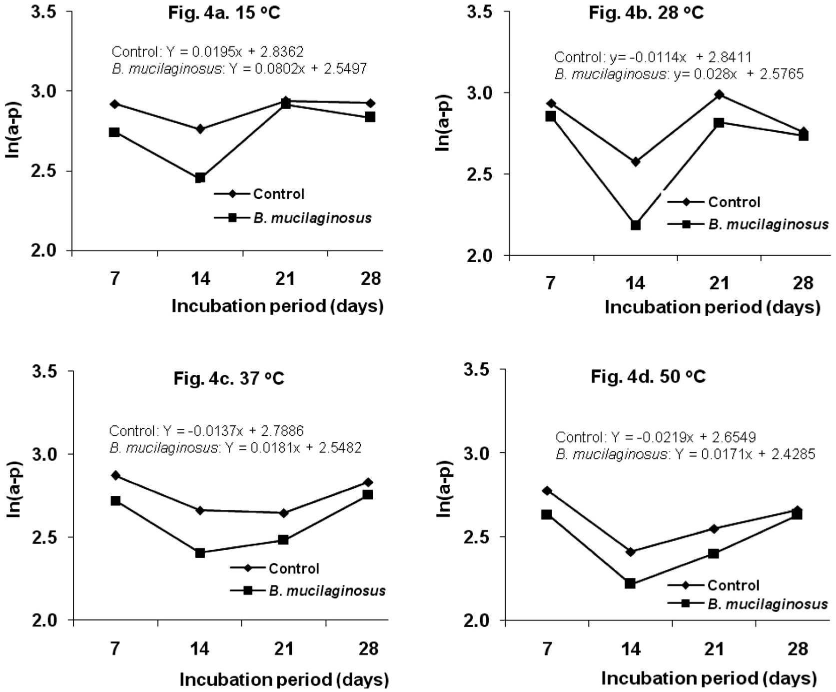

FIG. 4. – Effect of the bacterial strain *Bacillus mucilaginosus* on potassium release rate from waste mica under different temperatures and incubation periods. Bars indicate standard error (n = 3).

reflects the highest metabolic activity of the bacterial culture under this condition. It was found that the metabolic activity of silicate-dissolving bacteria played an important role in the release of Si, Fe and K from silicate minerals by production of different low molecular organic acids (STYRIAKOVA *et al.*, 2004).

Potassium release kinetics. – Data obtained from the incubation experiment were fitted into a first-order kinetics equation and K release kinetics from waste mica treated with bacterial culture was measured. According to the best-fitted first-order equation, a release curve of ln (a - p) versus time was plotted for different treatments. It can be observed that a significantly higher release rate (k value) was found in the case of mica inoculated with the bacterial culture as compared to un-inoculated mica, irrespective of temperature and duration of incubation period (Fig. 4). The rate of release was quite higher at 14 days of incubation then decreased gradually at 21 and 28 days of incubation, irrespective of temperature. The higher K release rate from waste mica treated with bacterial culture might be due to the microbial dissolution of waste mica, which resulted in more K released from waste mica. It is reported that organic acids secreted by microbes may increase dissolution of minerals (Grinsted *et al.*, 1982). Moreover, organic acid have been shown to dissolve feldspar and micas (Huang and Kiang, 1972; Huang and Keller, 1972; Boyle and Voigt, 1973). Thus, release of K might be due to the production of organic acids mediated by microbes during the incubation that leads to dissolution of K from mica.

Conclusions. – The results demonstrated that irrespective of temperature and period of incubation, compared to control a significantly higher mobilization of K was recorded in all treatments when incubation was carried out in the presence of the bacterial strain *Bacillus mucilaginosus*. The pH and total sugar content were found to decrease in the presence of the bacterial culture as compared to control. Application of waste mica along with bacterial culture increased NH_4OAc-K content in the medium indicating notable amount of K release from waste mica. From the release kinetics equation of K, it was found that substantial amount of K derived from waste mica when treated with the bacterial culture. Thus, it may be concluded that bio-intervention of waste mica with the bacterial strain could be an alternative and viable technology to utilize waste mica, and could be used effectively as an alternative source of K-fertilizer for crop production.

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