



Bio-Weathering of Feldspar and Talc by Silicate- Solubilizing Bacteria Isolate from Green Fodder Cultivated Sandy Soil

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ABSTRACT

Silicon and potassium are important nutrients to plant growth and development. However, their mineral resources in soil matrix are poorly soluble. The current study aims to increase the concentration of these nutrients in rhizosphere soil to meet the plant need. To achieve this goal, two strains of silicate solubilizing bacteria (SSB) were isolated from rhizosphere of green fodder grown on sandy soil and identified as *Bacillus cereus* AUMC-B 477 with accession number SUB12524150 L1 OQ220474 and *Enterobacter kobei* AUMC-B 478 with accession number SUB12524150 L2 OQ220475. Their efficiency to release silicon and potassium from silicate, rich minerals viz, feldspar and talc were evaluated. The SEM images indicated degradation of feldspar and talc surfaces by both strains. Release of silicon and potassium was obviously enhanced in the inoculated culture media compared to the non-inoculated ones. This was correlated with the decrease in culture pH due to secretion of organic acids. Such acidification process may figure out that acidolysis process might be one of the possible breaking down of feldspar and talc by either *Bacillus cereus* AUMC-B 477 or *Enterobacter kobei* AUMC-B 478. The findings revealed that *Enterobacter kobei* AUMC-B 478 applied with K-feldspar was more efficient for releasing more silicon and potassium into the culture media. Further study in pot experiments is recommended using *Enterobacter kobei* AUMC-B 478 to insist its capability to overcome soil deficiency in silicon and potassium.

Keywords: *Bacillus cereus*, *Enterobacter Kobei*, silicate solubilizing bacteria, organic acids, rhizosphere of green fodder, sandy soil

1. Introduction

Silicon and potassium are important nutrients for plant growth. They are the second and fourth most abundant elements in the earth crust, respectively (Heckman, 2012). As for silicon, attention has been brought lately. The Association of American Plant Food Control Officials classified Si as a beneficial element (Heckman, 2013). Silicon has been reported to improve plant growth and yield as well as protect plant from various biotic and abiotic stresses (Epstein, 2009). As for potassium, it is an essential element for plant growth and reproduction (Read *et al.*, 2006). The plant ability to uptake these elements is limited due to the low solubilization of their attached minerals. Si is absorbed by the plant in form of mono-silicic acid, which has maximum solubility in solution of 2 mM. In soil solutions, its concentration varies between 0.1 and 0.6 mM (Klotzbucher *et al.*, 2014). As for potassium, the total K content in soils ranges between 0.04 and 3%, while only 1 to 2% of the total are easily available to plants (Bahadur *et al.*, 2019). In order to allow plants to have more access to these elements, a method is needed to facilitate transformation of Si and K in soil from unavailable to available forms. One of the possible techniques is bio-weathering which is a typical geochemical process that involves the disintegration of rocks and minerals by living organisms (Gadd, 2007; Abdulla, 2009). In this case,

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microorganisms known as silicate solubilizing bacteria (SSB) are the bio-weathering agents that disintegrate silicate-bearing minerals viz: mica, quartz, pyroxene, talc, and feldspar, which assigned as a potential source of macro- and micronutrients (Ahmed and Holmström, 2015; Samuels *et al.*, 2020). Silicate solubilizing bacteria (SSB) are distributed in soil, water, aquatic sediments and in silicate minerals but their population is low compared to other bacteria in rhizosphere. The transformation of polymerized silica to monomeric form by bacteria is important in the biogeochemical cycles of silicates in nature (Lauwers, 1974). Dissolution of silicates by bacteria in soil gained greater attention not only because of their involvement in the silica cycle but also because of its role in the release of some plant nutrients like silicon, potassium, calcium, and magnesium from silicate minerals. Thus, silicon solubilizing bacteria could be applied as biofertilizer to meet the plant need of Si and K and enhance crop production either alone or in conjunction with silicate minerals.

Some of the SSB strains belong to the genera *Bacillus*, *Pseudomonas*, *Proteus*, *Rhizobia*, *Burkholderia*, and *Enterobacter*. They are known to release silicon from silicate minerals and promote plant growth (Lee *et al.*, 2019). Several mechanisms of silicate disintegration by bacteria have been proposed, including solubilization by ligands exchange, acids formation (organic and inorganic), nucleophilic attack and production of extracellular polysaccharides. Nevertheless, acidolysis is acknowledged as the most commonly occurring mechanism by which silicate minerals are weathered. Silicate solubilizing microbe-based biofertilizers seems to be a better option, which can help to maintain the concentration of orthosilicic acid in soil and regulate the biogeochemical cycle of Si (Meena *et al.*, 2014).

This study was undertaken due to shortage of available silicon in our Delta Valley (95% of cultivated lands) which attributes to the construction of Aswan High Dam and Ethiopian renaissance Dam which led to the sequestration of silt rich in silica minerals deposits. Meanwhile, the feldspar mine production in Egypt is up to about 400,000 metric ton and considered the largest reserves worldwide (Smith and Brown, 1988).

Therefore in this study, silicate mineral-solubilizing bacteria were isolated from the rhizosphere soil of green fodder and experimenting their solubilizing activities on two silicate minerals: feldspar (64.59 % SiO₂) and talc (54.97 % SiO₂). The possible mechanisms of potassium and silicon release in the presence of the silicate mineral-solubilizing bacteria were also examined.

2. Materials and Methods

Soil samples were collected from rhizosphere of green fodder, cultivated sandy soil at El-Amal Village, El-Ismailia Governorate, Egypt. Soil physical and chemical properties were determined including pH, EC and soluble anions and cations as described by Jackson (1973), while soil texture was evaluated according to Piper (1950).

Silicate solubilizing bacteria (SSB) were isolated from the rhizosphere soil. Aleksandrov agar medium was used for isolation of the bacteria on plates, containing 0.5% glucose, 0.05% MgSO₄·7H₂O, 0.0005% FeCl₃, 0.01% CaCO₃, 0.2% calcium phosphate and 0.2% feldspar (Hu *et al.*, 2006)

After incubating the inoculated plates for 72 h in dark at 28–30 °C, they were observed for the appearance of clear zones around bacterial colonies, as an indication of silicate solubilization. The bacterial colonies displaying the largest solubilization zone were selected and purified.

The selected bacterial isolates were characterized for their ability to solubilize and release soluble silica and potassium from two insoluble silicate sources i.e. feldspar (KAlSi₃O₈) and talc (Mg₃Si₄O₁₀(OH)₂) in Aleksandrov broth medium. Erlenmeyer flasks (250 ml) with 100 ml of the broth medium containing (0.2%) of either insoluble silicate sources were inoculated individually with each bacterial isolate at a concentration of 1 % (OD = 0.778 at 600 nm), while uninoculated flasks served as control, then incubated on a shaker incubator (120 rpm) for 14 days at 30–35°C.

Triplicate individual flasks sampling were taken periodically every 3 days to determine bacterial counts (CFU) on the silicate agar medium, while their supernatants (centrifuged at 7000 rpm for 10 min) were used to determine soluble Si by inductivity coupled plasma mass spectrometry Ultima Expert (Horiba scientific) and soluble K by flame photometer (Jenway, PFP-7). While the pH of the supernatant was determined using pH meter (Jenway 3510).

After two weeks, the liquid cultures were centrifuged for 20 min at 2000 rpm. Supernatants were filtrated through nonsterile 0.45 µm, 4 mm sized micro filter syringes for organic acids determination by HPLC (Agilent 1200). The column temperature of HPLC (model Agilent 1100 DAD) at 30 °C. The

mobile phase consisted of basic ammonium and methanol with the ratio of 99:1. The phosphoric acid was applied to adjust the pH to 2.6 at a flow rate of 0.5 ml/min. Detection was performed with a UV detector set at 214 nm. Organic acids quantitation was achieved by the absorbance recorded in the chromatograms relative to external standards.

2.1. Molecular identification of bacterial isolates

Bacterial isolates were cultured in sterile test tubes containing 10 ml of nutrient broth medium (Zimbro *et al.*, 2015). Cultures were incubated at 28°C for 48 hours. Cultures were sent to the Molecular Biology Research Unit, Assiut University for DNA extraction using Patho-gene-spin DNA/RNA extraction kit provided by Intron Biotechnology Company, Korea. DNA samples were sent to SolGent Company, Daejeon South Korea for polymerase chain reaction (PCR) and gene sequencing. PCR was performed using the two universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACG ACTT-3'). The purified PCR products (amplicons) were reconfirmed using a size nucleotide marker (100 base pairs) by electrophoreses on 1% agarose gel. The amplicons were sequenced with the incorporation of dideoxynucleotides (dd NTPs) in the reaction mixture. Bacterial amplicons were sequenced in the sense and antisense directions using 27F and 1492R primers (White *et al.*, 1990). Sequences were further analyzed using Basic Local Alignment Search Tool (BLAST) from the National Center of Biotechnology Information (NCBI) website. Phylogenetic analysis of sequences was done with the help of MegAlign (DNA Star) software version 5.05.

2.2. Scanning electron microscopy (SEM) examination

After two weeks of the experiment, bacterial colonization of the selected isolates on silicate minerals was morphologically investigated using scanning electron microscopy (SEM). Live specimens were concentrated on pallet affixed to stubs and spitter coated with gold- palladium, while microscopic examination was performed using JEOL GM 5200 microscope (Shamseldean and Platzer, 1989).

2.3. Statistical analysis

Data accomplished in triplicates were statistically evaluated by least significant differences (LSD) in one-way completely randomized analysis of variance (ANOVA) at 5% significance calculated using CoHort software under windows (Costat, model 6.311). Graphic presentations were done using Excel software (Microsoft Co. 2010). Standard error (+_S.E) of means were also calculated for comparison with bar diagram.

3. Results

3.1. Physical and chemical properties of selected soil

The selected soil was analyzed as shown in Table (1). The soil is sandy in texture, neither saline nor alkaline with pH 7.2

Table 1: Soil physical and chemical analyses

Soil texture	SP (%)	EC (dS m-1)	pH	CaCO ₃ (%)	Anions (meq. L ⁻¹)			Cations (meq. L ⁻¹)				SAR
					HCO ₃ ⁻	Cl ⁻	SO ₄ ⁻	Ca ⁺⁺	Mg ⁺⁺	Na ⁺	K ⁺	
Sandy	23.5	1.68	7.2	1.4	2.0	12.0	2.23	6.0	7.0	2.3	0.93	0.9

SP=Saturation percent, SAR= Sodium adsorption ratio.

3.2. Isolation and identification of promising silicate solubilizing bacteria

The two bacterial isolates which exhibited the widest zones of solubilization have shown higher silicon and potassium concentrations as compared to control. On the other hand, both bacterial isolates were identified after BLAST analysis as *Bacillus cereus* AUMC-B 477 (Figure 1) and *Enterobacter kobei* AUMC-B 478 (Figure 2).

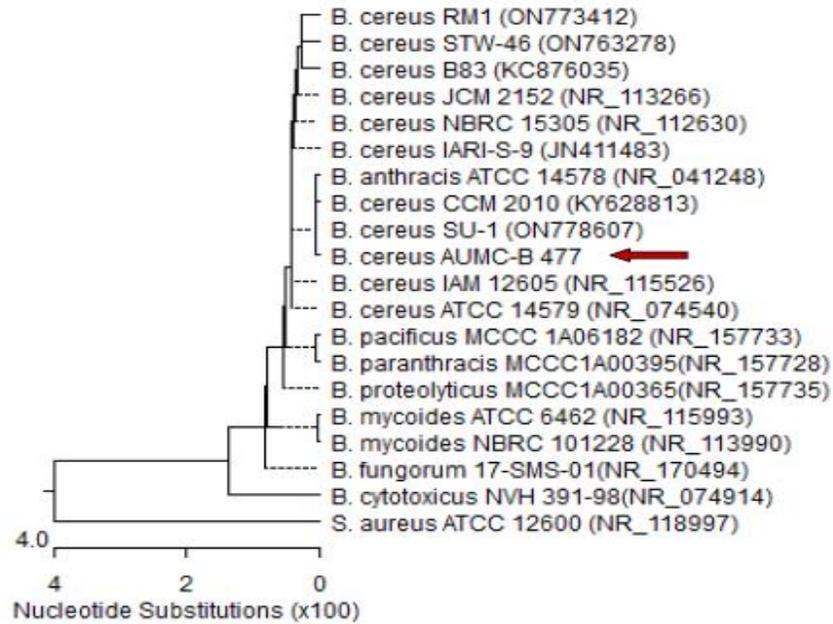


Fig. 1: Phylogenetic tree based on 16S sequences of rDNA of the bacterial strain isolated in the present study (*Bacillus cereus* AUMC-B477, arrowed) aligned with closely related sequences accessed from the GenBank. (B. = Bacillus, S.= Staphylococcus).

Bacillus cereus AUMC-B477 showed 99.90 - 100% identity and 100% coverage with several strains of *Bacillus cereus* including the type strain JCM 2152 with GenBank accession No. NR_113266. *Staphylococcus aureus* is included in the tree as an outgroup strain.

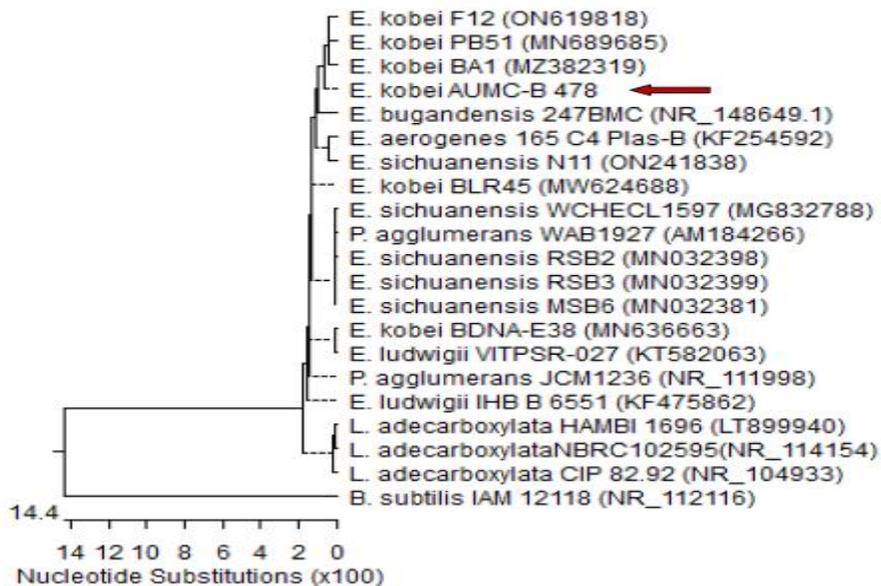


Fig. 2: Phylogenetic tree based on 16S sequences of rDNA of the bacterial strain isolated in the present study (*Enterobacter kobei* AUMCB- 478, arrowed) aligned with closely related sequences accessed from the GenBank. *Bacillus subtilis* is included in the tree as an outgroup strain.

Sample *E. kobei* AUMC-B 478 showed 99.01-100% identity and 99-100% coverage with several strains of *Enterobacter kobei*.

E. = *Enterobacter*, *L.* = *Leclercia*, *P.* = *Pantoa*.

3.3. Silicon release from feldspar and talc in broth medium

As seen in Figure (3,) the amounts of silicon released from the insoluble silicate sources varied according to the source type and microbial treatment. In the control test, the silicon dissolution from feldspar after two weeks was 12.5 mg/L, while recorded 31.1 and 17 mg/L when treated with *Enterobacter kobei* AUMC-B 478 and *Bacillus cereus* AUMC-B 477, respectively. On the other hand, silicon dissolved from the sole talc control after two weeks was relatively lower and only accounted 3.4 mg/L but increased up to 25.5 and 8.2 mg/L after inoculation with *Enterobacter kobei* AUMC-B 478 and *Bacillus cereus* AUMC-B 477, respectively. It obvious that *Enterobacter kobei* AUMC-B 478 was more successful in releasing silicon from both silicate sources, than *Bacillus cereus* AUMC-B 477. Meantime, the release of Si from either feldspar or talc was more efficient by *Enterobacter kobei* AUMC-B 478 with increasing incubation period.

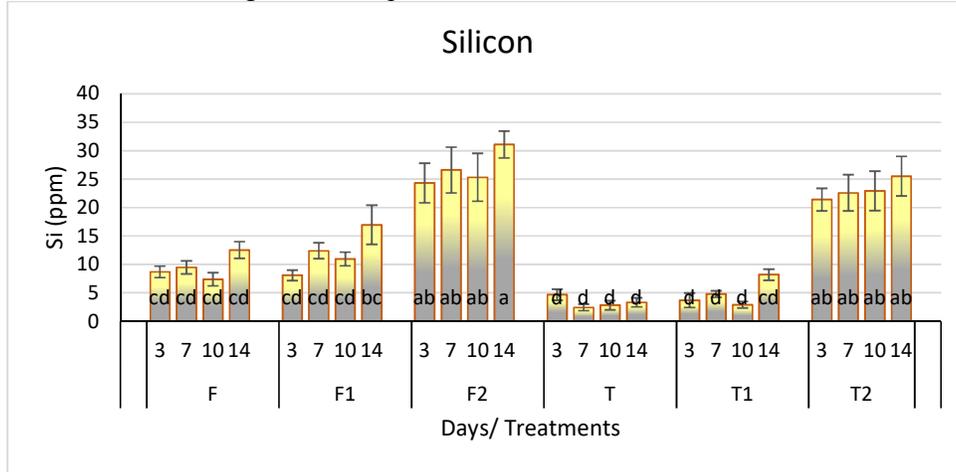


Fig. 3: Release of Si from feldspar (F) and talc (T) by *Bacillus cereus* AUMC-B 477 (F1, T1) and *Enterobacter kobei* AUMC-B 478 (F2, T2) in accordance to incubation period in days. Ranks shown at the base of each column.

3.4. Potassium release from feldspar in broth medium

The amount of potassium released from the non-inoculated feldspar (control) after two weeks was 8.9 mg/L. The ability of *Enterobacter kobei* AUMC-B 478 to release potassium from feldspar mineral was superior compared to that of *Bacillus cereus* AUMC-B 477 as the former recorded 13.8 mg/L, while the latter recorded 10.6 mg/L. (Fig 4).

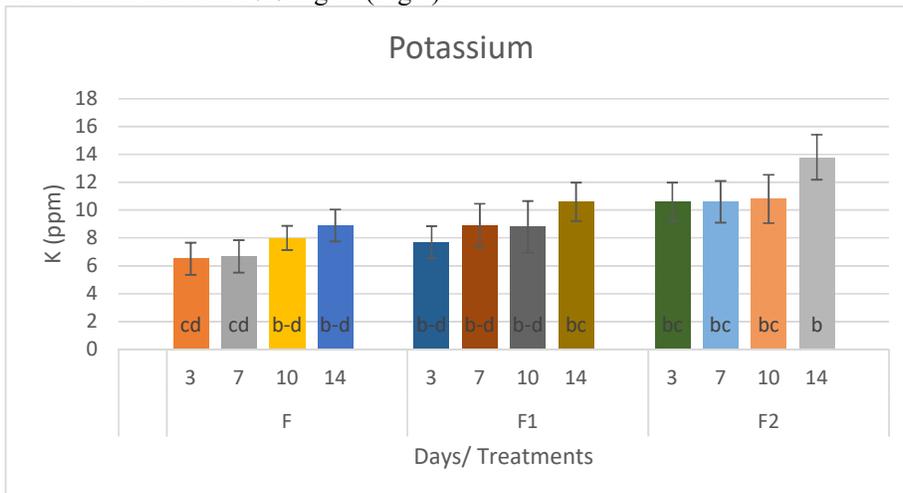


Fig. 4: Release of K from feldspar (F) by *Bacillus cereus* AUMC-B 477 (F1) and *Enterobacter kobei* AUMC-B 478 (F2) in accordance to incubation period in days. Ranks shown at the base of each column.

Representing an efficacy of 30 % higher than that of *Bacillus cereus* AUMC-B 477. Meanwhile, the amounts of K released by either bacterial strains progressively increased with increasing incubation time up to 14 days. The variability of potassium release from feldspar by tested microbiota may due to differences in their ability to release organic acids.

3.5. The pH changes in the liquid medium

Figure (5) shows the pH changes within 3-14 days in the media of feldspar and talc incubated with and without *Bacillus cereus* AUMC-B 477 or *Enterobacter kobei* AUMC-B 478. Results revealed significant reductions in pH of feldspar culture media from 5.57 to 3.9 and from 5.9 to 4.5 when inoculated with *Enterobacter kobei* AUMC-B 478 and *Bacillus cereus* AUMC-B 477, respectively. These drops in pH values were higher than those of talc inoculated by *Enterobacter kobei* AUMC-B 478 (from 5.9 to 4.0) or with *Bacillus cereus* AUMC-B 477 (from 6.1 to 4.9).

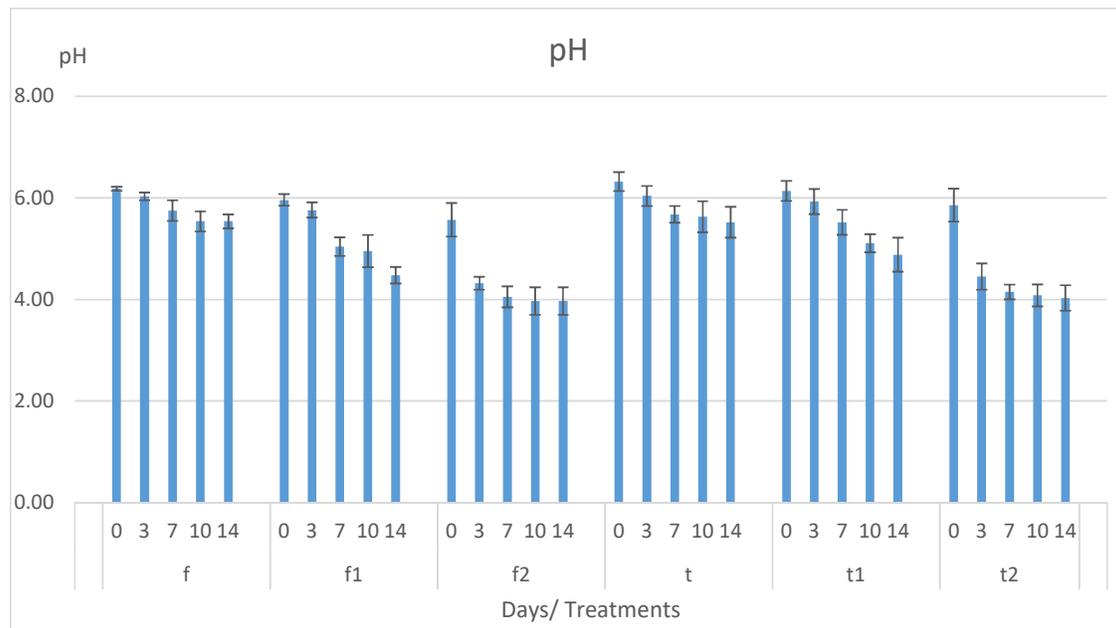


Fig. 5: The pH of *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 with the pH values measured during the whole incubation period.

3.6. Biomass production of *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 in culture media containing feldspar or talc

As seen in Figure (6), the log no of *Bacillus cereus* AUMC-B 477 with feldspar at zero time accounted 8.78 decreased to 8.2 in the presence of talc. Meantime, *Enterobacter kobei* AUMC-B 478 accounted 6.18 and 6.4 with feldspar and talc, respectively. However, when incubation time increased to 10 days, the log cfu/ ml of *Bacillus cereus* AUMC-B 477 declined with both talc (from 8.2 to 5.5) and feldspar (8.78 to 5.73). In contrast, the log no. of *Enterobacter kobei* AUMC-B 478 recorded an increase with feldspar (from 6.18 to 6.4), and decreased with talc (from 6.4 to 5). This pattern was positively correlated to pH changes in the surrounding environment.

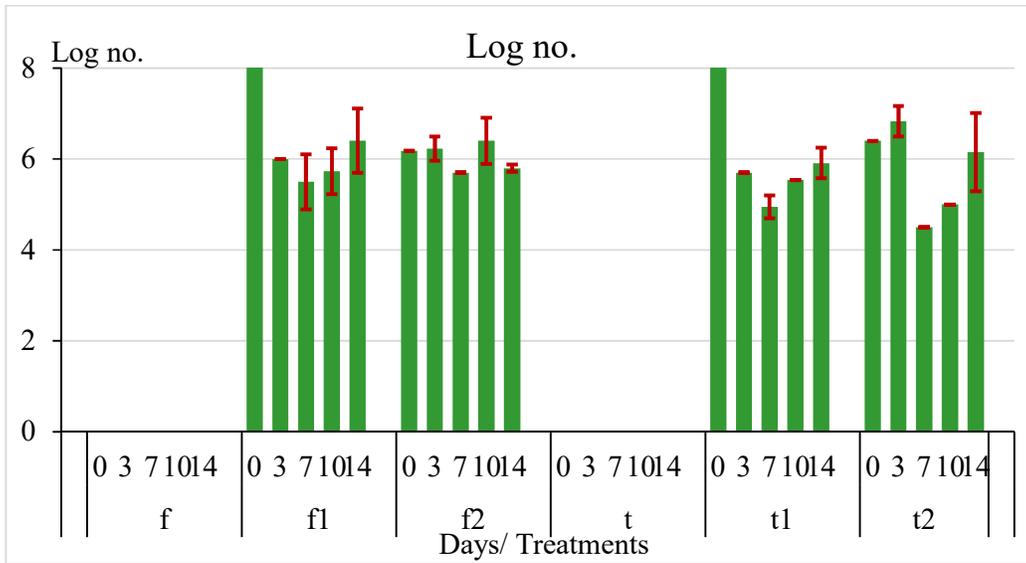


Fig. 6: The Log no. of *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 with the Log no. values measured during the whole incubation period.

3.7. Organic acids produced by *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478

Both *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 formed varied and copious amounts of organic acids in culture media with feldspar or talc (Table 2).

In media containing feldspar, the most pronounced organic acids produced by *Bacillus cereus* AUMC-B 477 were oxalic followed by maleic and formic acids. While *Enterobacter kobei* AUMC-B 478 produced higher amounts of lactic acid followed by citric and formic acids.

The production of formic and salicylic acids depended on variation between strain types. Never the less, *Enterobacter kobei* production of citric, lactic and ascorbic acids on feldspar were much bigger than that produced on talc and by *Bacillus cereus* regardless of the substrate. While on the other hand, the production of Oxalic and maleic as dicarboxylic acids by both strains were nearly similar on talc.

Table 2: Organic acids released during 14 day incubation of feldspar and talc with silicate solubilizing bacteria.

Org. acid (µg/ml)	<i>Bacillus cereus</i>						<i>Enterobacter kobei</i>						LSD _{0.05}
	Feldspar			Talc			Feldspar			Talc			
	Mean ± SE	Rank		Mean ± SE	Rank		Mean ± SE	Rank		Mean ± SE	Rank		
Oxalic	62.20 ± 1.79	a		56.28 ± 0.53	a		34.00 ± 2.31	b		56.87 ± 2.02	a		5.854
Citric	5.41 ± 0.38	b		3.33 ± 0.20	b		43.55 ± 1.93	a		6.45 ± 0.35	b		3.277
Lactic	10.42 ± 0.66	b		12.43 ± 0.19	b		83.62 ± 1.52	a		11.69 ± 0.22	b		2.739
Ascorbic	3.30 ± 0.25	c		5.40 ± 0.20	b		14.16 ± 0.15	a		3.20 ± 0.18	c		0.645
Maleic	47.71 ± 0.54	b		50.98 ± 1.15	a		2.52 ± 0.22	c		47.43 ± 1.34	b		3.027
Formic	32.86 ± 0.89	c		150.53 ± 1.02	a		32.86 ± 1.47	c		122.61 ± 7.29	b		12.305
Salicylic	6.55 ± 0.29	c		8.33 ± 0.12	b		3.91 ± 0.10	d		9.86 ± 0.12	a		0.577

3.8. Scanning electron microscopy (SEM) studies

Scanning electron microscopy (SEM) was used to investigate the morphological changes of feldspar or talc in absence or presence of *Enterobacter kobei* AUMC-B 478. The culture media of this strain was selected on basis of its great efficacy in solubilizing silicon and potassium from the selected silicate minerals. Figure (7a, c) show that the surface of blank control feldspar was smooth with sharp edges. After two weeks of inoculation, the upper surface of the mineral was found to accommodate small irregular etched particles with evidence of bacterial colonization near the pits Figure (7b, d). On the other hand, blank control talc was platy particles 1-100 µm Figure (8a, c). Similarly, when talc was

treated with *Enterobacter kobei* AUMC-B 478, the platy particles of blank control talc was found to contain pits and smashed particles attached with the strain Figure (8b, d).

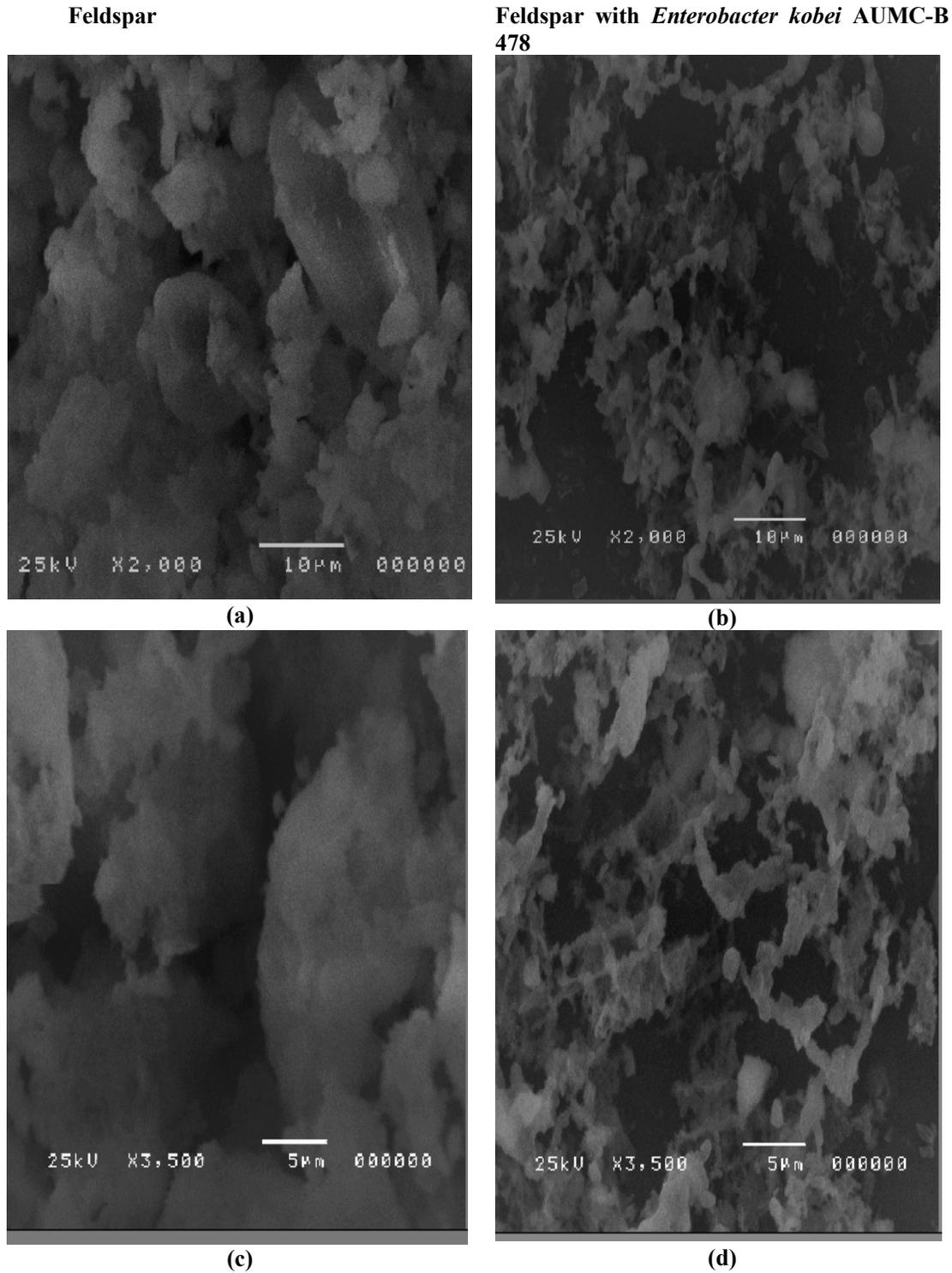


Fig. 7: (a) blank feldspar (SEMx2000) (b) treated feldspar with *Enterobacter kobei* AUMC-B 478 (SEMx2000) (c) blank feldspar (SEMx3500) (d) treated feldspar with *Enterobacter kobei* AUMC-B 478 (SEMx3500)

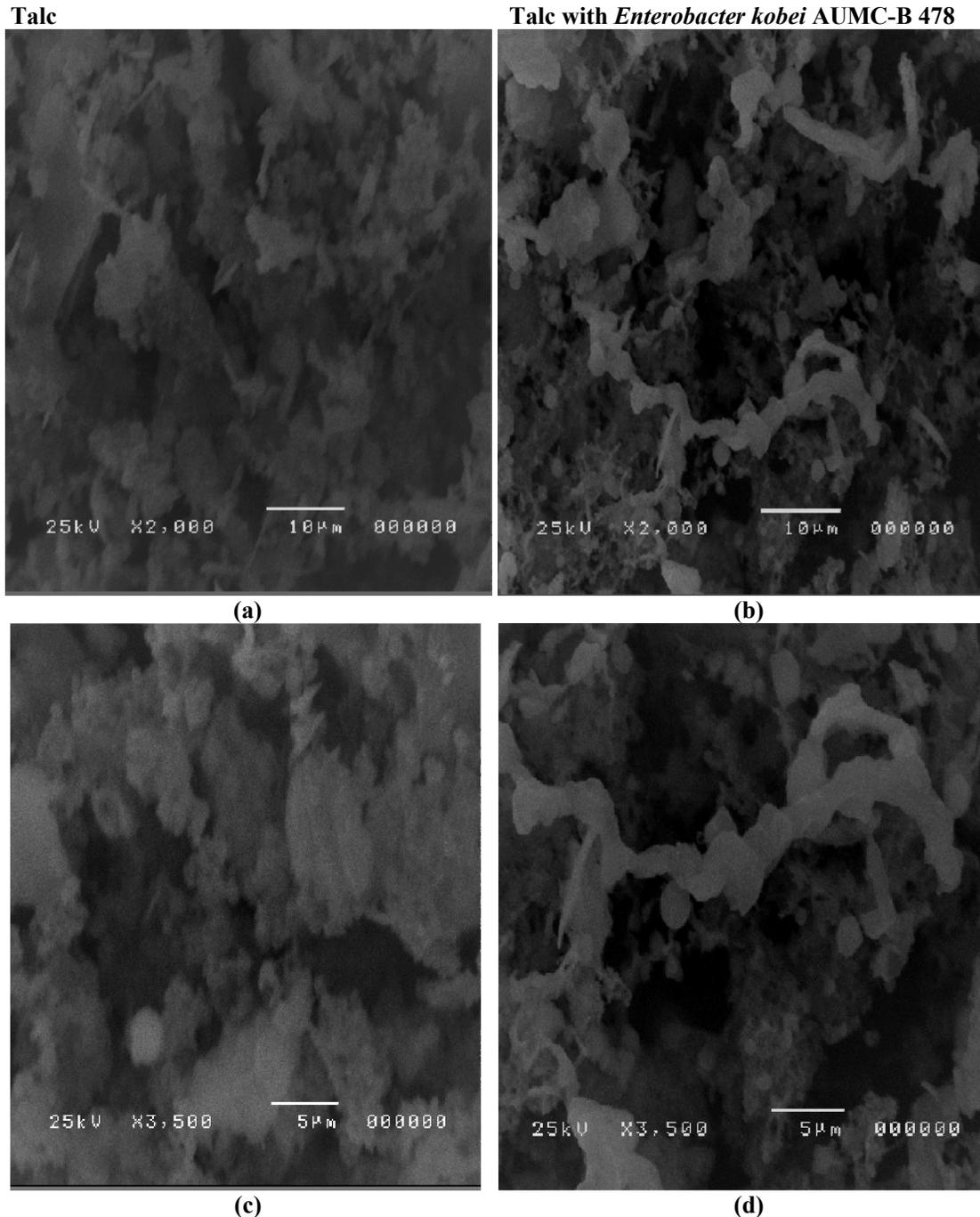


Fig. 8: (a) blank talc (SEMx2000) (b) treated talc with *Enterobacter kobei* AUMC-B 478 (SEMx2000) (c) blank talc (SEMx3500) (d) treated talc with *Enterobacter kobei* AUMC-B 478 (SEMx3500)

4. Discussion

On the basis of the foregoing results, both *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 strains secreted various organic acids as secondary metabolites in culture media supplemented with K- feldspar or talc which lowered the pH of the broth media to 4.0 at 14 days of inoculation. The study of Liu (2006) showed that *B. mucilaginosus* with cfu 1.85×10^8 and pH 5.96 caused dissolution of feldspar in liquid culture after 5 days and released K at about 13.4 mg/l which is coincide with our results upon using *Enterobacter kobei* AUMC-B 478. After two weeks of incubation.

Bacillus cereus is a potassium solubilizing bacterium (KSB) and has been used in several studies to increase the solubility of K. In addition, *B. cereus* enhances plant growth via increasing nutrient uptake and production of plant hormones (Singh, et.al, 2017).

It was reported that (*Enterobacter* sp.) isolate UPMSSB7 isolated from rhizosphere soil of rubber plantation showed the highest solubilization of silicate, phosphate and potassium (solubilizing indexes of 4.67, 2.52 and 2.61, respectively). It also showed highest silicate solubilization at 5 and 10 days (9.76 and 11.55 mg L⁻¹, respectively) when assayed in Calcium silicate medium (Imran *et al.*, 2020).

Under low pH, acidolysis is expected to occur causing degradation of silicate minerals and release Si and K in the culture media. In this respect, the efficacy of *Enterobacter kobei* AUMC-B 478 surpass that of *Bacillus cereus* AUMC-B 477 under two types of silicate minerals. This drop in pH could be an argument supporting the role of SSB in decreasing pH for enhancing solubilization of silicate minerals. In this respect, the efficacy of *Enterobacter kobei* AUMC-B 478 in dissolution of Si or K from both silicates surpass that of *Bacillus cereus* AUMC-B 477.

It seems that *Enterobacter kobei* AUMC-B 478 was highly capable in lowering the pH of the surrounding mineral media to a limit that causes acidolysis which ultimately enhance chelating of cations bound to silicon and potassium. These results are generally agreed with those reported by Buragohain *et al.*, (2018) and Yifan (2020) who recorded decreases in pH of the culture fluid media up to 4.3 within the first day and further drop to 3.90 after 7 days of *Bacillus aryabhatai* SK1-7 incubation. These results are consistent with the previous findings of many authors. According to Sheng and He (2006) the solubilization of illite and feldspar by microorganisms is due to the production of organic acids like oxalic and tartaric acids besides formation of capsular polysaccharides which would help in dissolution of mineral silicates. Similar finding was also reported by Prajapati and Modi (2012) who showed the action of different organic acids like citric, oxalic, malic, succinic and tartaric acid on dissolution of potassium from potassium aluminum silicate. The potential of silicate-solubilizing bacteria has often been reported to extract K and P from insoluble silicates and secrete some of important metabolites such as organic acids and polysaccharides (Lee *et al.*, 2019). It seems that the potential of SSB in releasing silicon from soil is largely dependent on bacterial species and nature of silicate minerals. *Enterobacter ludwigii* GAK2 could efficiently solubilize silicate and phosphate, in glucose NBRIP media and able to produce organic acids (citric, acetic, and lactic acids), indole-3-acetic acid, and gibberellic acid (GA1, GA3) in Luria-Bertani media (Arjun *et al.*, 2020)

On the other hand, vasanthi *et al.*, (2018) indicated that the strains of silicate bacteria are capable to produce thick and sticky biofilms of polysaccharides as a hydrated gel that wrapped microorganisms and adheres them to rock surfaces causing erosion of the rock minerals (Kemmling *et al.*, 2004). The biofilm formation on aluminosilicates was shown to increase the time of water contact to mineral surface which ultimately promote corrosion of the silicate minerals and greater release of potassium, silicon, and aluminum in the bacteria – mineral contact model.

Here, *Enterobacter kobei* AUMC-B 478 strain should be further tested for use in amelioration of silicon and potassium deficiency in saline soils of north Delta Valley which represent 25% of the total irrigated lands.

5. Conclusion

Two types of bacteria were isolated from rhizosphere of green fodder, i.e. *Bacillus cereus* AUMC-B 477 and *Enterobacter kobei* AUMC-B 478 Treatment of K-feldspar and talc indicated that the former strain surpass the latter type in terms of increasing soluble Si and K in the culture media. The mechanism of Si and K release is not yet clear, though some evidence points to the force effect of pH drop due to secretion of some organic acids causing acidolysis of the surrounding area.

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