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ISOLATION AND EVALUATION OF SILICATE SOLUBILIZING BACTERIA FOR PLANT GROWTH PROMOTING TRAITS

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Abstract – Silicate solubilizing bacteria are known for their ability to dissolve silicates to monosilicic acid, a form of silica absorbed by plant roots. In the present study, 40 bacterial isolates (SSB1-SSB40) were retrieved from rhizospheric soil of paddy and garden soil on Bunt and Rovira medium. Silicate solubilization indices of the forty silicate solubilizing bacterial isolates varied from 1.09 to 2.66, six promising silicate solubilizer (SSB1-SSB6) were further evaluated for solubilization of phosphate, zinc and potassium; indole acetic acid and hydrocyanic acid production and ammonia excretion. Isolate SSB2 exhibited maximum solublization, indices for silicate (2.66) and potassium (3.33), while isolate SSB3 was efficient zinc and phosphate solubilizer. All six isolates were producing indole acetic acid (IAA) ranging from 5.72-12.40 µg/ml. Highest IAA production (12.40 µg/ml) and ammonia excretion (4.60 µg/ml) was observed in isolate SSB2. Promising silicate solubilizing bacterial isolates with growth promoting traits could be further assessed as silicon biofertilizer in different crops.

INTRODUCTION

Weathering of rocks from earth crust by action of microbes is an essential part of nutrient cycle, required for sustainability of biosphere (De Tombeur *et al*. 2020). Chemolithotrophy is well-studied phenomenon for microbial metabolism, which is an important factor in chemical weathering. Chemical weathering provides key nutrients to microbes and plants; transform organic and inorganic minerals present in rocks and soil (Uroz *et al*., 2009; Napieralski *et al*., 2019). Silicon is second most abundant element on earth crust and an important micronutrient for plant growth. Wollastonite, feldspar, silicon dioxide, mica and quartz are common silicate sources present in soil (Tubana *et al*.2016).

Silicon in monocotyledonous plants has fascinating role in stress relief, delay wilting and enhancement of plant's ability to resist metal toxicity (Emamverdian *et al*. 2018). Reduced lipid peroxidation and enhanced enzyme activity of chitinase, peroxidase, superoxide dismutase and catalase is reported upon silicon application (Liang *et al*., 2003). Silicon reduces salt toxicity in the silicon accumulating monocot plants, wheat (*Triticum aestivum*) and rice (*Oryza sativa*) by obstructing flow of sodium ions in saline conditions (Ahmad *et al*. 1992). Usage of calcium silicate slag, potassium silicate, sodium silicate and fly ash as silicon source in agricultural crops is reported and disclosed as beneficial for nutrient acquisition and disease control (Datnoff *et al*., 1997; Ishiguro2001; Parab *et al*., 2015; Peera *et al*., 2016).

Microorganisms (bacteria, fungi and actinomycetes) that provide iron, nitrogen, sulphur, phosphorus and many other key elements to plants have been well reported in literature (Billah *et al*. 2019; Estrada-Bonilla *et al*. 2021). Nowadays, microorganism based biofertilizers are well known for growth enhancement and provide key nutrients to plants. Diazotrophs like *Azotobacter*, *Rhizobium* and *Frankia* efficiently deliver nitrogen to the plants (Nosrati *et al*., 2014; Jnawali *et al*., 2015; Marappa *et al*., 2020), whereas fungi like mycorrhiza and *Trichoderma* are well recognized and documented for their beneficial effect on many crops via supply of phosphorus, water and pathogenesis control (Barman *et al*., 2016; Poveda *et al*., 2020).

Silicate present in soil can be efficiently dissolved upon augmentation of silicate solubilizing bacteria hence firmly absorbed by plant roots. Santi and

Goenadi (2017), used quartz mineral to reveal the silicate solubilization potential of different bacteria. Microorganisms like *Burkholderiaeburnea, Bacillus globisporous, Bacillus mucilaginosus*, and *Enterobacter ludwigii* are some examples of silicate solubilizing bacteria (SSB) reported in literature (Sheng *et al.*, 2008; Kang *et al.*, 2017; Lee *et al.*, 2019). *Rigidoporos microporus*, causative agent of root rot disease of rubber can be controlled by using silicate solubilizing bacteria (Shabbir *et al.*, 2020). *Magnaporthe grisea* causative agent of rice blast, one of most pernicious disease in rice crop can be effectively controlled by foliar spray of silicon and soil amendment (Abed-Ashtiani *et al.*, 2012).

Silicon plays a vital role in stress tolerance and disease control in many plants like rice, wheat, barley and sugarcane as it naturally accumulate in these plants. The present investigation was carried out to isolate and characterize silicate solubilizing bacteria for plant growth promoting attributes.

MATERIALS AND METHODS

Isolation and screening of silicate solubilizing bacteria

Different soil samples were collected from Chaudhary Charan Singh Haryana Agricultural University Hisar farms for isolation of silicate solubilizing bacteria on Bunt and Rovira agar medium (1955). Selected isolates were screened for silicate solubilizing activity by spotting 3 µl of 5 days old culture on Bunt and Rovira agar plates. The plates were incubated for 72h at 30±2 °C and solubilization index (SI) was calculated by using the formula:

$$SI = \frac{\text{Diameter of colony+ Diameter of halozone}}{\text{Diameter of colony}}$$

Phosphate, zinc and potassium solubilization

Phosphate solubilization activity of bacterial isolates was detected by using Pikovaskaya medium containing calcium triphosphate as insoluble phosphate (Pikovaskaya, 1948). A spot of about 3 µl from 5 day old bacterial culture was spotted on plate and clear zone formation around the colony was inferred as phosphate solubilization by culture. Potential of bacterial isolates for zinc solubilization was investigated by spot test method on plates having minimal medium amended with zinc oxide. For measurement of potassium solubilizing activity, bacterial cultures were spotted on modified Aleksandrov medium having mica as insoluble potassium source. Clear zone formation around the colony was observed and solubilization index for phosphate (PSI), zinc (ZSI) and potassium (KSI) was calculated by using the formula described earlier for silicate solubilization index.

Plant growth promoting traits

Indole acetic acid production

Silicates solubilizing bacterial isolates were tested for their potential for production of indole acetic acid (IAA). Microbial cultures were grown in nutrient broth having 100 µg/ml DL- tryptophan for 72h at 30±2 °C. Two ml of Salkowaski reagent was added in test tube containing 2 ml of 72h incubated culture broth supernatant and allowed to develop pink color (Glickmann and Dessaux, 1995) and the intensity of colour developed was measured at 530 nm using Spectrophotometer. IAA produced was calculated by reference standard curve (10-100 ìg IAA/ml).

Hydrocyanic acid (HCN) production

HCN production was assessed by method proposed by Alstrom and Burns (1989). Selected SSB isolates were grown in test tube containing King's B medium which comprised of 4.4 g/l glycine. Filter paper strips soaked in solution (0.5% picric acid in 2% sodium carbonate solution) were hanged in test tubes containing medium inoculated by bacterial cultures andincubated for 5 days at 30±2°C. The change in colour of filter paper from yellow to brown or light red indicated HCN production.

Ammonia excretion

Test tubes having 5 ml peptone broth were inoculated with 5 days old culture and incubated for 4 days at 30±2°C. After incubation, 0.5 ml of Nessler's reagent was added to each test tube and shaken vigorously. Two ml aliquot was taken in eppendorf and centrifuged at 10,000 rpm for 15 minutes and ammonia excreted was calculated by referring to standard curve (1-10 µg/ml ammonia). The absorbance of supernatant was read at 450 nm with of UV-Vis spectrophotometer (Chaney and Marbach, 1962).

RESULTS AND DISCUSSION

Isolation and screening of silicate solubilizing bacteria

Total 40 morphologically distinct bacterial isolates

(SSB1-SSB40) with zone of silicate solubilization were obtained from different soil samples by spreading different dilutions on Bunt and Rovira agar medium plates. Silicate solubilization indices (SSI) of bacterial isolates were ranging from 1.09-2.66 (Plate 1). Highest SSI was exhibited by SSB2 (2.66) followed by SSB4 (2.45), SSB5 (2.36), SSB3 (2.33), SSB6 (1.83) and SSB1 (1.58) (Fig. 1). Silicate solubilizing bacteria with varying SSI have been reported in literature. Sulizah *et al.* (2018) isolated five silicate solubilizing bacteria from rice rhizospheric soil, with maximum SSI of 1.10. Six promising silicate solubilizing bacterial isolates (SSB1, SSB2, SSB3, SSB4, SSB5 and SSB6) were evaluated further for mineral solubilization and other plant growth promoting traits.

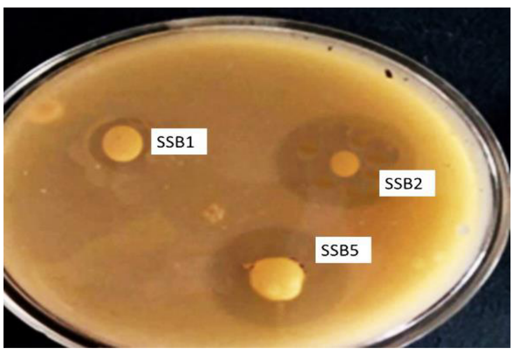

Plate 1. Silicate solubilization on Bunt and Rovira medium

Fig. 1. Silicate solubilization potential of bacterial isolates

Phosphate, zinc and potassium solubilization by silicate solubilizing bacteria

The ability of six silicate solubilizing bacterial isolates for phosphate solubilization varied from 1.42 to 2.33 (Table 1). Maximum phosphate solubilization was exhibited by isolate SSB3 (2.33) while other isolates have lower value of PSI (Plate 2 (A)). Different bacteria have been reported in literature as phosphate solubilizers along with silicate solubilizing activity. Shabbir *et al.* (2020) screened 26 bacterial isolates for solubilization of silicate and phosphate, isolate UPMSSB7 exhibited maximum solubilization of silicate and phosphate (solubilization index 4.67 and 2.52 respectively).

Minimal medium having 0.1% zinc oxide had shown zone of clearance when inoculated with different SSB isolates and incubated for 72h. Three out of six silicate solubilizing bacterial isolates (SSB3, SSB4 and SSB5) had shown ZSI of 1.5, 1.33 and 1.33 respectively (Table 1, Plate 2(B)). Saravanan *et al.* (2004) screened three bacterial isolates for zinc solubilization by using two different insoluble zinc sources (zinc carbonate and zinc oxide) and reported that two out of three bacterial isolates have dissolved more zinc oxide in comparison of zinc carbonate. The results of present study also confirm that zinc oxide is best insoluble zinc source to investigate zinc solubilization potential of bacterial isolates.

Table 1. Silicate, potassium, phosphorus and zinc solubilizing indices of bacterial isolates

Bacterial Isolates	SSI	PSI	ZSI	KSI
SSB1	1.58	-	-	1.28
SSB2	2.66	1.85	-	3.33
SSB3	2.33	2.33	1.50	2.57
SSB4	2.45	1.71	1.33	2.83
SSB5	2.36	1.42	1.50	3.00
SSB6	1.83	1.80	1.33	2.50

In present investigation, potassium solubilization indices of bacterial isolates varied from 1.28 to 3.33 after 48 h incubation at 30±2 °C (Table 1). Highest solubilization of potassium was shown by SSB2 (3.33) followed by SSB5 (3.00), SSB4 (2.83) and SSB3(2.57) (Plate 2(C)). Similarly, silicate solubilizing bacteria have been reported as potassium solubilizers suggesting that some common mechanism may be involved in solubilization of minerals (Priyadharsini and Muthukumar, 2016; Etesami *et al.*, 2017; Shabbir *et al.*, 2020).

Plant growth promoting traits of bacterial isolates

Plant growth is affected by the presence of microorganisms. Plant growth promoting traits are characteristics of microorganisms that determine the effect of their presence on the plant. Many plant growth promoting traits have been revealed in literature; production of IAA, HCN production, ammonia excretion, mineral solubilization (K, Zn

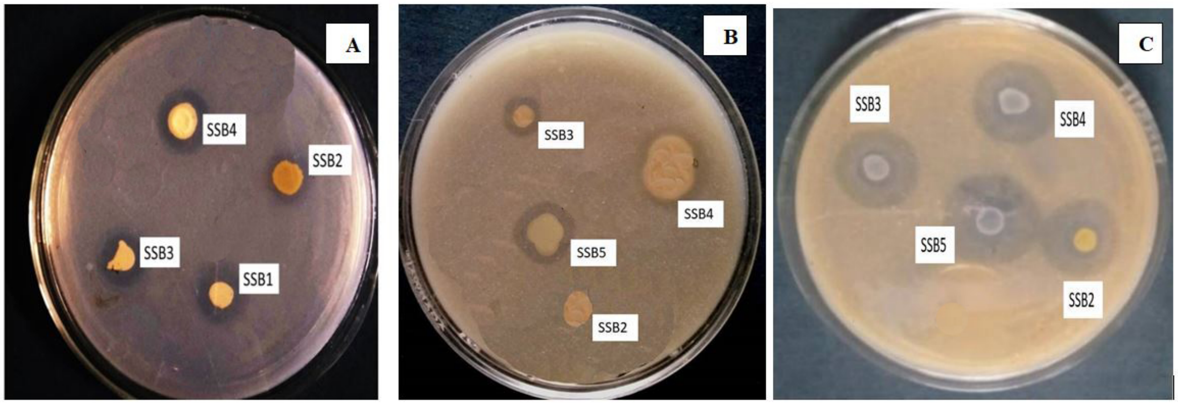

Plate 2. Mineral solubilization by Silicate solubilizing bacterial isolate: A) Phosphate solubilization B) Zinc solubilization C) Potassium solubilization

and P) and nitrogen fixation are some of them.

Indole acetic acid production

Auxins are naturally occurring plant hormones and IAA is anatural auxin, which is involved in different processes related to plant physiology like cell differentiation, geotropic and phototropic responses (Aloni *et al.* 2006). The IAA production potential of promising SSB was checked and it was found that isolate SSB2 produced maximum IAA (12.40 µg/ml) among all six isolates (Plate 3, Table 2). Bacterial isolates capable of producing different range of IAA have been reported in literature. Singh *et al.* (2015) evaluated bacterial isolates for IAA production and found all isolates able to produce IAA ranging from 3.17-13.29 µg/ml. Mike-Anosike *et al.* (2018) reported 4-10 µg/ml IAA production by rhizobacteria and similar study reported the IAA production of different bacterial isolates in range of 0.38-2.37 µg/ml (Restu *et al*. 2019).

HCN production

Biocontrol agents are becoming more popular in integrated pest management and sustainable agriculture because of their specificity and eco-friendly nature. Different mechanisms of biocontrol have been discovered over the time and production of volatile organic compounds like HCN is one of them. In present study, four out of six (66.67%) SSB isolates changed the colour of filter paper strips confirming HCN production (Plate 4). Similar results were also obtained by Rasool *et al.* (2021), they retrieved thirteen bacterial isolates from Saffron (*Crocus sativus* L.) rhizospheric soil and 61% bacterial isolates were reported as HCN producer. Similarly, Singh *et al.* 2015 reported that five out of ten (50%) bacterial isolates clearly showed HCN production.

Ammonia excretion

Chemical fertilizers for nitrogen are subjected to about 50% leaching and application of biological nitrogen fixer to soil can be finest replacement of these pollution (water and soil) causing compounds (Zahran. 1999). Diazotrophs are the prokaryotic organisms having potential to convert atmospheric nitrogen to ammonia, nitrate and organic compounds (proteins and nucleic acid) (Hartono *et al.* 2016). In present investigation, ammonia excretion by silicate solubilizing bacterial isolates varied from 1.75-4.60 µg/ml of ammonia (Table 2). The isolate SSB2 had produced maximum colour change of solution after Nesslerization reaction (Plate 5). Ammonia excreted by microorganisms in soil can be utilized as nitrogen source by plants.

CONCLUSION

In present investigation isolation and screening of silicate solubilizing bacteria having plant growth

Table 2. Multiple plant growth promoting traits of silicate solubilizing bacterial isolates

Bacterial Isolate	IAA production (µg/ml)	HCN Production	Ammonia excretion (µg/ml)
SSB1	6.74	+	2.72
SSB2	12.40	+	4.60
SSB3	11.03	+	2.34
SSB4	7.69	-	3.60
SSB5	8.67	+	1.82
SSB6	5.72	-	1.75

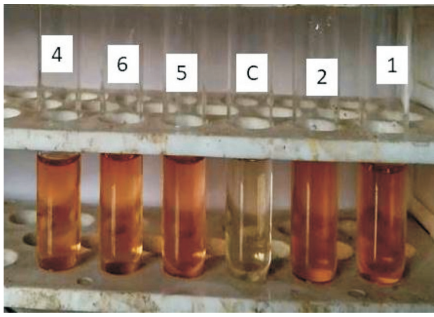

Plate 3. IAA production by SSB isolates

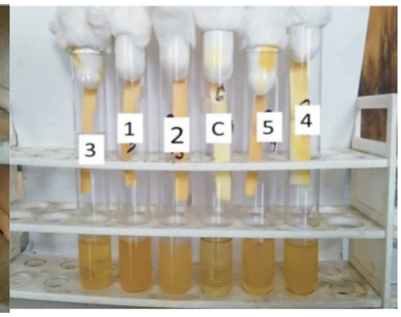

Plate 4. HCN production by SSB isolates

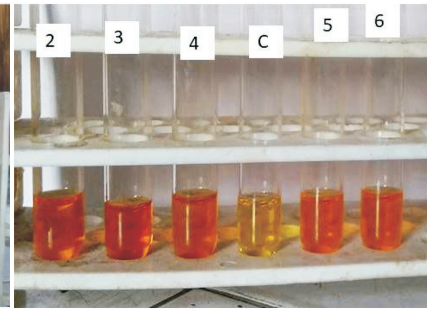

Plate 5. Ammonia excretion by SSB isolates

promoting attributes such as IAA production, ammonia excretion, mineral solubilization (phosphate, potassium and zinc), and biocontrol properties like HCN production was carried out. Among different bacterial isolates screened for various plant growth promoting attributes the isolate SSB2 exhibited maximum silicate and potassium solubilization and exhibited IAA and HCN production, ammonia excretion and phosphate solubilization potential which are advantageous for plant growth promotion. The promising isolate can be explored as biofertilizer after investigating its plant growth promoting effects on different crops.

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