Studies on Potassium Solubilization by Bacterial isolate RSML S2 from Saline and Alkaline Lonar Crater Lake of Maharashtra

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***Abstract*—For plants to flourish, potassium is a necessary nutrient, and it is crucial for agricultural production. It is needed in large amounts and has two main functions as it activates enzymes that are vital to metabolic activities and required mainly for the production of proteins and starch.**

Plants can only use 1-2 percent of the potassium that is found naturally in soil or that is provided by synthetic fertilizer; the remainder is bound with other minerals and is therefore inaccessible to plants. One of the most significant microorganisms in saline soil that influences soil health is bacteria. Effective soil microbes that mobilize potassium have a significant impact on soil fertility, potassium ion cycling, and mineral availability. Meteoric impact crater Lonar Lake located Maharashtra is known for its great treasure of alkaline extremophilic bacteria. The present study is carried out to study the potassium solubilizing traits of bacterial isolates from Lonar Lake. Eight bacterial isolates are screened. Out of which the RSML S2 isolate exhibited maximum potassium solubilisation ability at varying pH and salinity conditions. The isolate may be exploited for development of biofertilizer after exploring its other plant growth promoting traits.

Key words—Crater Lake, effect of pH, effect of salinity, potassium solubilizing bacteria

I. INTRODUCTION

In general a mineral soil ranges between 0.04 and 3 % of potassium (K). The upper and subsurface layers of the soil profile contain between 3 and 100 ton ha $^{-1}$ of total K. Of this total K, about 98 % is bound in the mineral form, whereas only 2 % is in soil solution and is in exchangeable phases (Schroeder, 1979; Bertsch and Thomas et al. 1985)

The amount of K in a soil depends upon nature of parent material, degree of weathering, addition of manures and fertilizers, and losses due to leaching, erosion, and crop removal. In India approximately 72 % agricultural soils need instant K fertilization for good crop production as these soils are categorized under low (130 kg K2O ha^{-1}) and medium (130–335 kg K2O ha^{-1}) K status. (Hasan, 2002)

K is an essential plant nutrient for its growth and is extremely important for the productive farming in agriculture. It is required in high quantities and plays two major roles in the activation of enzymes which are essential to metabolic processes, mainly for the production of proteins and starch.

The K is a “plant-preferred” ion for the maintenance of turgor (rigidity) of each plant cell. The turgid cells sustain the leaf’s vigour which supports efficiently the process of photosynthesis. (Almeida et al, 2017)

The K controls the size of the stomatal openings in response to ecological and internal plant conditions and influences the transpiration rates. Deficiencies of K in crops were evident generally in high crop yields areas, continued nitrogen fertilization, with or without phosphorus, depletes the soils of their K. Being mobile nutrient, plants, under deficient conditions, are not able to transport the K to younger leaves. Typical K deficiency symptoms in plants include brown scorching and curling of leaf tips as well as chlorosis (yellowing) between the leaf veins in older or lower leaves. Sometimes purple spots may also appear on the leaf undersides. Under insufficient supply of K, the plants have poorly developed root system, grow slowly, produce lesser seeds, and have lower yields. (Yadav and Sidhu, 2016; Olaniyan et al., 2022). Sugars produced in photosynthesis require an adequate supply of potassium for their transport through the phloem to other parts of the plant for utilization and storage. The adequate supply of potassium is important for the transport of the water as well as nutrients through xylem and phloem (Cochrane and Cochrane, 2009).

Only 1–2 % of naturally occurring soil K or supplied through synthetic fertilizer is available to plants, and the rest is bound with other minerals and therefore unavailable to plants. The efficient potassium mobilizing soil microorganisms influence the availability of minerals in soil and play a major role in K ion cycling and soil fertility. (Bin L, Bin W, Mu P, Liu C, Teng HH. 2010)

The balance of the K+/Na+ ratio in soil plays an important role in maintaining crop growth Soil salinization is a significant threat to soil health, especially to the agricultural ecosystem; it reduces vegetation biomass, destroys ecosystem diversity, and limits land use efficiency. This area of investigation has garnered extensive attention in China, especially in the arid and semi-arid areas, High levels of Na+ in the soil can depolarize plant membranes, making it harder for K+ to be absorbed. High levels of Na+ can activate potassium outward-rectifying channels (KORs), which causes K+ to leak out of the cell.(Abdul Wakeel, 2013; Rao et al., 2023)

Bacteria are one of the most important micro-organisms in saline soil, which affects soil health. The reclamation of saline–alkali soil using micro-organisms has been confirmed as a useful method (Chookietwattana et al., 2019; Yin and Zhang, 2022)

With intensive agriculture, availability of K in soils dropped due to crop removal, leaching, runoff, and erosion. Thus, it becomes urgent to explore the bio-activation of soil K reserves to improve the potash fertilizer shortage.

Soil microorganisms affect soil fertility by influencing several soil processes via many mechanisms such as decomposition, immobilization, and mineralization. Various groups of soil microflora were reported to be involved in solubilisation /mobilization of unavailable forms of K into available forms of K which is easily absorbed by plants' roots. These KSMs are economically viable and eco-friendly and are capable to dissolve K-bearing minerals and rocks, enhance plant growth, and improve the crop yield. (Pandey et al. 2020; Jain et al.2022)

Meteoric impact crater Lonar Lake is located in Buldhana district of Maharashtra, formed around 30,000-50,000 years ago. The lake is a closed system without any outlet and the lake is unique due to its salinity, alkalinity and biodiversity. The pH values of the lake water are generally higher than 10and occasionally reaching 12 (Singh and Singh, 2018) and the salinity range from 8460 mg/L (0.08%) to 10250 mg/L (1.02%) (Borul S. B. 2012) 0.08%

The present study deals with efforts to explore alkaline saline environment loving potassium mobilizing bacteria from the Lonar Crater Lake of Maharashtra, India.

II. MATERIALS AND METHODS

Sample Collection: The sediments from eight different sites of Meteoric impact crater Lonar Lake of Maharashtra were collected in sterile polythene bags and brought to the laboratory.

Enrichment: The sediment samples were mixed with Mica and kept at room temperature for one week. 1gm of sample inoculate in 100 ml liquid media containing 0.95% glucose, 0.045% yeast extract and 0.45% Mica make and incubated at room temperature at 120 rpm for 7 days.

Screening and Isolation of Potassium Solubilizing bacteria

Samples were inoculated after serial dilution from 10^{-2} up to 10^{-6} on Aleksandrov's agar medium composed of 1% glucose, 0.05% MgSo .7H O, 0.0005% FeCl , 0.01% CaCO3, 0.2% CaPo and 0.5% potassium aluminium silicate, agar 2.5 % and pH-7.5 and incubated at 28 0 + 2 C for 1 week. Colonies exhibiting clear zone of potassium solubilization were selected. (Anukriti, et al., 2016)

Potassium solubilizing colonies were selected from 10^{-5}, and 10^{-6} dilution containing plates. Isolation of colonies was done on the basis of zone formed around the colonies only those isolates were selected whose diameter showed more D/d ratio according to Khandeparkar's selection ratio.

Ratio = D/d = Diameter of zone of clearance/ Diameter of growth.

The isolate showing highest D/d ratio was maintained on nutrient agar slants and used for further studies. (Anukriti, et al., 2016)

Identification of bacterial isolate:

The colony characters such as size, shape, colour, opacity, consistency were noted for the isolate from Aleksandrov agar showing highest D/d ratio of potassium solubilisation zone. Gram staining was done for morphological characterization of the isolate.

Indole test, Methyl red test, Simmons Citrate test, Voges-Proskauer test, Starch hydrolysis test, Glucose, Lactose, Maltose, Catalase Oxidase Urease Gelatin Hydrolysis Casein hydrolysis test, H2S production were determined for biochemical characterization.

Study of effect of pH on the potassium solubilisation:

The Aleksandrov agar plates of varying pH from 6,7,8,9 and 10 were prepared and spot inoculated and incubated at room temperature for 72 hours. The diameter of zone of solubilisation appeared around colony in each plate was determined and the D/d ratio is calculated.

Study of effect of salinity on the potassium solubilisation:

The Aleksandrov agar plates of varying NaCl concentration from 0.07,0. 08, 0.10, 0.5, 1.0, and 1.5 % were prepared and spot inoculated and incubated at room temperature for 72 hours. The diameter of zone of solubilisation appeared around colony in each plate was determined and the D/d ratio is calculated.

III. RESULTS AND DISCUSSION

Screening and Isolation of Potassium Solubilizing bacteria

The samples obtained from different sites of Meteoric impact crater Lonar Lake of Maharashtra in the month of December 2024. The samples were enriched and screened for potassium solubilisation activity on Aleksandrov's agar medium. The colonies exhibiting clear yellow zone of potassium solubilization were observed. Eight bacterial isolates were obtained from the colonies whose diameter of zonebshowed more D/d ratio. The bacterial isolate RSML S2 showing highest D/d ratio is selected and isolated. (Table: 1) The isolate was maintained on Aleksandrov's agar slant in refrigerator for further studies.

Table: 1 Zone of Potassium solubilization by bacterial isolates on Aleksandrov's agar medium

Bacterial isolates	Clear zone diameter (D) mm	Growth diameter(d) mm	D/d (ratio)
RSML S2	34	6	5.66
RSML S14	26	5	5.20
RSML S8	24	5	4.80
RSML S6	28	6	4.66
RSML S11	29	7	4.10
RSML W6	23	6	3.83
RSML W 3	18	5	3.60
RSML W9	22	7	3.14

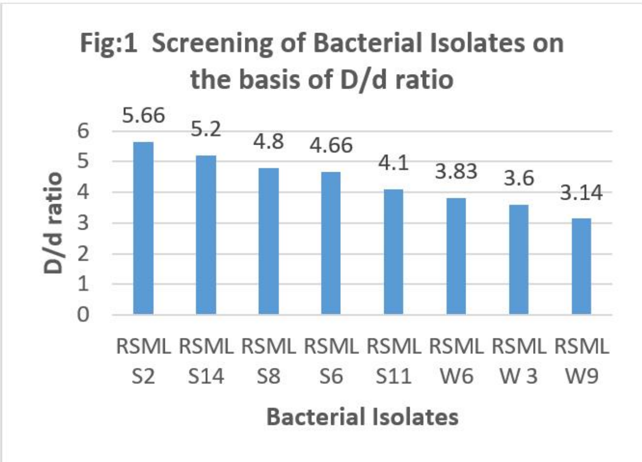
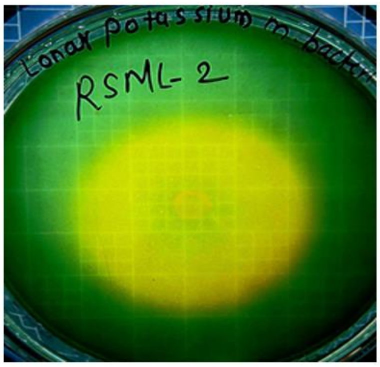


Photo: 1 Yellow around the colony RSML S2 indicating strong potassium solubilization



Identification of bacterial isolate:

The colony of the bacterial isolate RSML S2 from Aleksandrov agar was circular, 4mm in size, cream coloured, opaque and butter like in consistency. The Gram staining revealed gram positive rod shaped bacteria. (Photo:1). The observation of biochemical tests are as given in Table: 2. The cultural, morphological and biochemical tests are in accordance with the Bergey's, Manual of Systematics of Archaea and Bacteria identifying the bacterial genera as *Bacillus* species. Molicular identification may provide more concrete identification. (Logan and Vos, 2015).

Table: 2 Biochemical characterization of KSB RSML S2

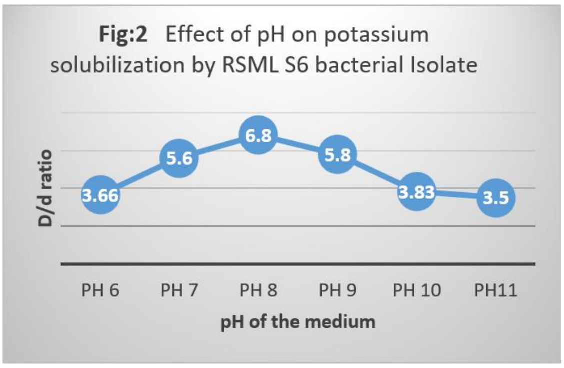
Test	Observation
Indole test,	Negative (-ve)
Methyl red test,	Negative (-ve)
Simmons Citrate test,	Positive (+ve)
Voges-Proskauer test,	Positive (+ve)
Starch hydrolysis test,	Positive (+ve)
Glucose	Positive (+ve)
Lactose	Negative(-ve)
Maltose	Positive (+ve)
Catalase	Positive (+ve)
Oxidase	Negative(-ve)
Urease	Negative (-ve)
Gelatin Hydrolysis	Positive (+ve)
Casein hydrolysis test,	Positive (+ve)
H2S production	Negative (-ve)
Gram's nature	Gram positive rods

Effect of pH on the potassium solubilisation:

In order to determine the effect of different pH on the growth of isolated bacteria and zone of potassium solubilization the isolate was grown at pH range from 5, 6, 7, 8, and 9. It was found that maximum potassium solubilization was achieved at pH 8.0 with sharp decline potassium solubilization from pH 9 onwards. (Table: 3 and Fig: 2). The growth remained same at pH 7 and pH9 while it is increased at pH10 and then decreased. The one to one relation with growth and potassium solubilisation was not evident. The increase in growth at pH 10 indicates adaptability of the bacterial isolate to its natural habitat. Anukriti Verma et al, (2016) reported a positive correlation between growth and potassium solubilization.

Table: 3 Effect of pH on potassium solubilisation by Bacterial isolate RSML S2

Bacterial isolate RSML S2	Clear zone diameter (D) mm	Growth diamete r(d) mm	D/d (ratio)
pH 6	11	3	3.66
pH 7	28	5	5.60
pH 8	34	5	6.80
pH 9	29	5	5.80
pH 10	23	6	3.83
pH11	14	4	3.50

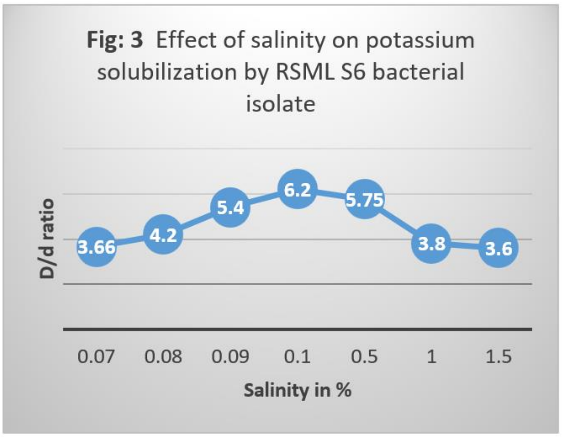


Effect of salinity on the potassium solubilisation:

The effect of salinity on potassium solubilizing ability of RSML S2 was tested on the Aleksandrov's agar plates amended with varying NaCl concentration from 0.07,0. 08, 0.10, 0.5, 1.0, and 1.5 % with salinity values of 700, 800, 900, 1000, 5000, 10,000 and 10,500 mg/ L. The salinity values for study were selected on the basis of recorded average salinity of Lonar lake water throughout a year. (Borul, 2012). Maximum potassium solubilization was observed at 1000mg/L salinity and then the potassium solubilisation was decreased. (Table: 4 and Fig: 3). The growth remained constant at all salinity values. Effect of salinity on potassium solubilisation by plant growth promoting *Bacillus* species and its role in plant growth promotion ius also reported. Salt tolerant Potassium Solubilizing Bacteria (KSB) reduced the impact of salinity on plant growth and improved productivity by solubilizing unavailable potassium in the soil. (Suman, et al. 2019 : Priyanka, and Sindhu, 2013)

Table: 4 Effect of salinity on potassium solubilisation by Bacterial isolate RSML S2

Percent Salinity (mg/ L)	Clear zone diameter (D) mm	Growth diameter(d) mm	D/d (ratio)
0.07 (700)	11	4	3.66
0.08 (800)	21	5	4.20
0.09 (900)	27	5	5.40
0.10 (1000)	31	5	6.20
0.50 (5000)	23	4	5.75
1.00 (10000)	19	5	3.80
1.05 (10500)	18	5	3.60



IV. CONCLUSION

The bacterial isolate RSML S2 has a high efficiency of potassium solubilization, which could be used to generate bio inoculants, in elevated pH and saline soils that can help plants with potassium deficiencies. More study may be done on the additional characteristics that promote plant growth.

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