**Annals of Plant and Soil Research 25 (2): 285-296 (2023)**
https://doi.org/10.47815/apsr.2023.10268

# Zinc solubilization and potash mobilization by potent plant growth promoting bacteria isolated from Odisha

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Received, February, 2023; Revised accepted, May, 2023

## ABSTRACT

*Potassium and Zinc are essential macro and micro nutrients required for growth, development and immunity of plants. In order to improve the soil Zn & K content, microorganisms with ability to solubilise insoluble Zn (ZSB) & K (KMB) in the soil can make it available to plants increasing nutrition availability and help in overall growth of the crops plants. Twenty soil bacteria were isolated from rice rhizosphere of OUAT fields, Bhubaneswar, Odisha, out of which 4 isolates showed both KMB and ZSB traits. They were identified as species of Acenitobacter through VITEK-2 system and their plant growth promoting traits were studied. Out of all the isolates, BZ-2 reported maximum PGPR traits. Effect of the isolates on the rice seed germination was also studied and BZ-4 and BZ-5 treated seeds showed maximum germination, root length, shoot length, fresh weight and dry weight of the biomass. The isolates also produced acid that lowered the pH of the liquid broth over 0-10 days interval. The quantitative estimation for the solubilisation of K & Zn was done using ICP-OES and all the isolates reported solubilisation of these elements and produced more available K &Zn as compared to control. BZ-2 and BZ-5 reported maximum solubilisation of K & Zn. The organism depicting higher PGP traits along with the potassium & zinc solubilization have a greater agricultural and environmental significance as they can be a good replacement for chemical fertilizers.*

***Keywords***: Zinc solubilising bacteria, Potassium mobilizing bacteria, Plant growth promoting rhizobacteria, Soil bacteria

## INTRODUCTION

Agriculture plays a vital role in India being source of livelihood for around 58% of the population (Chand, 2008). Crops requires a number of macro and micro nutrients like nitrogen, potassium, calcium, phosphorous, sulphur, boron, zinc, manganese, iron, copper, molybdenum etc for its growth. Potassium (K) is a major plant macronutrient that influences plant growth & development, grain quality and also plays a key role in the synthesis of cells, enzymes, proteins, starch, cellulose, vitamins (Verma *et al.,* 2017) etc. Moreover, K participates in nutrient uptake, transportation and provides resistance to abiotic & biotic stresses, thus playing an important role in increased crop production and providing resistance to plant pathogens (Maqsood et al. 2013). Zinc is a vital micro-nutrient involved in auxin metabolism and carbohydrate metabolism (Alloway, 2008) and acts as a significant anti-oxidant in plants. Zn also plays an important role in the normal development of floral tissues, flowering, fertilization and fruiting in plants. Plants absorb Zn and other elements from soil where as the source for animals including human is from plants through the food chain. Soil contains 2-25 ppm of zinc, whereas ideally it should contain 1-200 ppm and 50ppm of potassium, where the requirement is 70-100ppm. In humans Zn deficiency affects approximately 2 billion people in the world (Zhang et al. 2011).

Nutrient deficiency in plants occurs due to the availability of zinc and potassium in insoluble form in the soil. Deficiency of these essential elements leads to retarded shoot growth, chlorosis & reduced leaf size, increased susceptibility to heat, light and fungal infections, as well as it affects grain yield, pollen formation, root development, water uptake and transport. To overcome deficiency of these elements, various chemical fertilizers in the form of DAP, NPK, Zinc sulphate or Zn-EDTA (Karak *et al.,* 2005) are being used over time which is economically not viable and environmental hazards. Moreover, these elements are transformed into insoluble complex forms within

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7 days of application. Even though application of agrochemicals such as chemical pesticides & fertilizers may enhance productivity, nevertheless the residual agrochemicals in the crop fields drastically affect the soil health; with respect to nutrient availability, microbial diversity and also increased in pathogen attack to the photo-insensitive crops. As a result of this, recycling of several elements like nitrogen, zinc, potassium, phosphorous etc through microbial intervention is evident to reduce deficiency in the soil.

An environmentally friendly alternative to all these approaches is the use of plant growth promoting rhizobacteria who have the capability to solubilise these complex Zn and K compounds. Plant Growth Promoting Rhizobacteria (PGPRs) are group of microbes that inhabit the rhizospheric region of the root and help to increase crop productivity for which they are also considered as bio fertilizer. PGPRs promote growth of plants through various direct & indirect mechanisms. In direct mechanism it facilitates nutrient uptake, nitrogen fixation, Phosphorus solubilization, siderophore production, IAA and other growth hormone production (Bhardwaj *et al.,* 2014). Indirect mechanisms are control of phytopathogenic microorganisms which are a major & constant threat to sustainable agriculture and environment stability through production of antibiotics, siderophore, lytic enzymes.These zinc solubilising bacteria (ZSB) and potash mobilizing bacteria (KMB) have the ability to solubilize complex zinc and potassium respectively in the soil into simpler forms, thus increasing their availability to plants. Mechanisms used by these microbes are; production of organic acids, ions, chelators and siderophores (Saravanan *et al.,* 2011). Some potential bacterial species perform processes like mineralization and solubilization of organic and inorganic phosphorus. In some cases, KMBs produce exo-polymeric substances (Bhattacharyya and Jha, 2012) as a mechanism to solubilize the complex elements. The potential of these bacteria can be better utilized in nutrient recycling and nutrient bio fortification in crop field.

Fortification of essential nutrients through bacteria is an effective measure to surmount malnutrition caused due to macro & micro-nutrient deficiency by growing cereal crops with enhanced levels of bioavailable nutrient concentration (Raghavendra et al. 2016; Zahedi 2016). The use of PGPR having the capability to solubilize complex elements into simpler bioavailable form can be an environmentally friendly and economical approach of improving micronutrients availability to plants and subsequently by eliminating nutrient deficiency in plants and animals (Saravanan et al. 2011). Inoculation of crops with such microbial strains for biofortification may not only deal with the problem of malnutrition through increased nutrient concentration but also improve crop yield and soil fertility through their PGPR traits. Cereals are the staple food in almost all parts of the world. Rice is known to be deficit of zinc and potassium. Keeping that in mind, here we aim to isolate PGPRs showing ZSB & KMB properties in particular and study their effect on rice var. Swarna.

## MATERIAL AND METHODS

### 2.1: Isolation and Screening of KMB and ZSB

Soil samples were collected aseptically from the rhizospheric region of Rice field OUAT farms. Microorganisms were isolated by in vitro cultivation method of bacterial isolation in basal medium (Donadio *et al.,* 2002). One gram of soil sample was suspended in 10ml sterile distilled water, logarithmic dilutions were made up to $10^{-4}$ level, and 100 µl suspension was spread on Nutrient agar plate (NA). Pure isolates were obtained pure isolates by streak plate method on different media will be screened for potassium solubilization by plate assay. Screening of rhizobacterial isolates for formation of K solubilization zone. The solubilization of potassium by rhizobacteria was studied on the modified Feldspar medium plates (GYEA) and zinc solubilization was studied on the synthetic ZSB media containing 0.1% $ZnCO_3$ by the spot inoculation method. A loopful of 48 h old culture was spotted on the prepared plates and were incubated at 28˚ C for 3 days.

### 2.2: Characterization and identification of the potent isolates

Shape, colour, margin, elevation of the bacterial colonies was observed with the 24hrs incubated cultures and their gram’s variability was studied (Tripathi and Sapra, 2022). The biochemical characterization and identification was done using VITEK-2 system (Ligozzi *et al.,* 2002)

**2.3: Plant growth promoting activities exhibited by the isolates**

Plant growth promoting activities of potassium mobilizing bacteria were tested *in vitro*. The PGP activities included IAA production, Phosphate solubilisation, Hydrogen cyanide production and Ammonia production, which was determined using following standard methods (Pahari and Mishra, 2017; Panda *et al.,* 2021).

**2.4: Seed germination and growth variables through roll towel method**

This experiment was carried out with rice var. Swarna to determine the effect on seed germination of the isolates on by the standard roll towel method in germination paper. Seeds of rice were surface sterilized with 0.2% $HgCl_2$ solution for 2-3 min. the seeds were then soaked in 10ml of the bacterial suspension. The brown germination paper was soaked in distilled water. Seeds were placed on the paper and another pre-soaked paper towel was placed on the first one so that the seeds were held in position. After 14 days, the towels were removed and the number of germinated seeds and were taken for determination of root length and shoot length. Germination percentage (%) = Seeds germinated/Total seeds germinated x 100 seeds

**2.5: Acid production by the isolates**

The isolates were inoculated in the basal media supplemented with Feldspar and $ZnCO_3$. The pH of the media was measured using pH meter in the interval of 2,4,6,8 and 10 days. The decrease in pH records the production of acid by the isolates.

**Quantitative estimation of potash solubilisation by isolates through liquid assay**

Erlenmeyer flasks (250 ml) with 100 ml of sterilized GyEA media and ZSB media were taken as mineral potassium and zinc source resp. Post autoclaving the media was inoculated with 1 ml of bacterial suspension and incubated at 30±1°C for 10 days. The flasks were incubated on rotary shaker (100 rpm) at 30±1˚C. In the interval of 2, 4, 6, 8 and 10 days, the suspension was centrifuged at 6,300 rpm for 10 min and supernatant was retained (Saiyad *et al.,* 2015). Dilution was done by 1:10 ratio, 1ml of suspension was taken and 10 ml of deionised water was added and mixed thoroughly. Further the solution was sent for analysis in inductively coupled plasma-optical emission spectroscopy (ICP-OES) for estimating K & Zn.

**RESULT AND DISCUSSION:**

**3.1 Isolation and screening of potash and zinc solubilizing bacteria from collected soil sample**

A total of 20 morphologically distinct colonies were isolated from different rhizospheric soil sample. The isolates were streaked and stabbed into slants and butts resp. for storage. The pure cultures of the bacteria were maintained. The isolates were screened for potassium mobilizing bacteria on synthetic medium (GYEA with Feldspar) and zinc solubilising bacteria using synthetic media with $ZnCO_3$ as the source of Zn. By observing clear zone in agar plates, a total of 4 potential bacteria portraying both ZSB & KMB characteristics were found (Fig.1 & 2; Table 1). Bhattacharya et al (2016) isolated around 10 potash mobilizing bacteria from tea soil using a serial dilution plate method on modified GYCaA media. Gontia-Mishra *et al.,* (2017) isolated ZSB using $ZnCO_3$ as the source of Zn.

Table 1: KMB +ve clear zone through plate assay

| Isolate name | KMB + zone (after 72 h) | ZSB + zone (after 24h) |
|---|---|---|
| BZ-2 | 24mm | 20mm |
| BZ-3 | 22mm | 24mm |
| BZ-4 | 22mm | 22mm |
| BZ-5 | 16mm | 19mm |

**3.2: Colony morphology and gram's variability of the isolates**

The colony morphology of the isolates were studied based on their shape, size, colour and texture etc. Colonies were Circular or oval, white, off white or light yellow; elevation was raised or flat; margins were entire or undulate.

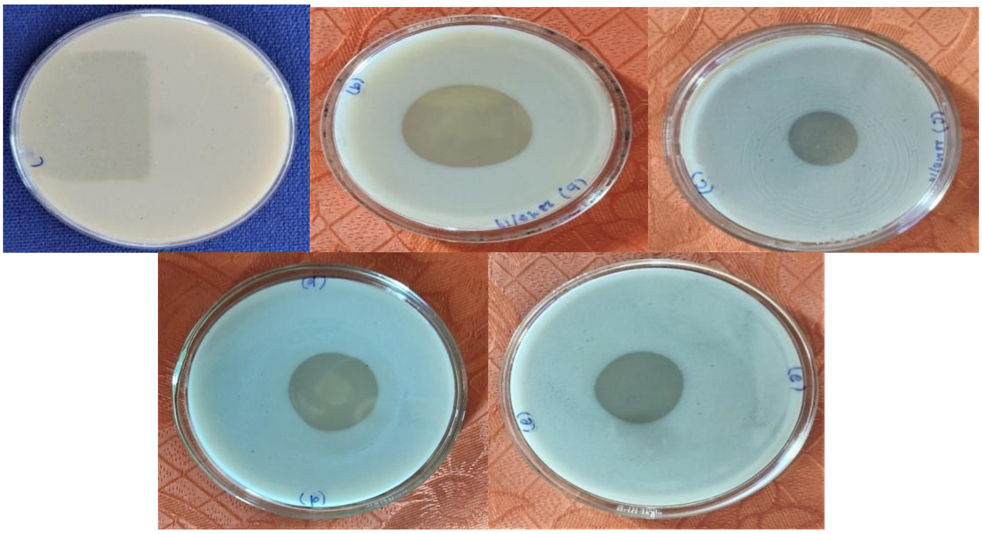

Figure1: KMB control plate and KMB positive plates

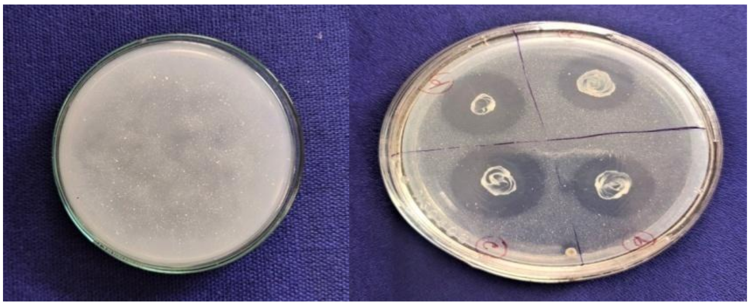

Fig.2: ZSB control plate and ZSB positive plate

Further the single bacterial colonies were taken to study their gram's reaction through staining procedure. Results of Gram's reaction showed the organisms to be species of Gram negative *Cocco-bacilli* (Fig.3) which corroborates with the findings of Khan et al (2018) who isolated gram negative bacteria from soil samples. The organisms were further characterized and identified based on their biochemical attributes using VITEK-2 system (Table 2).

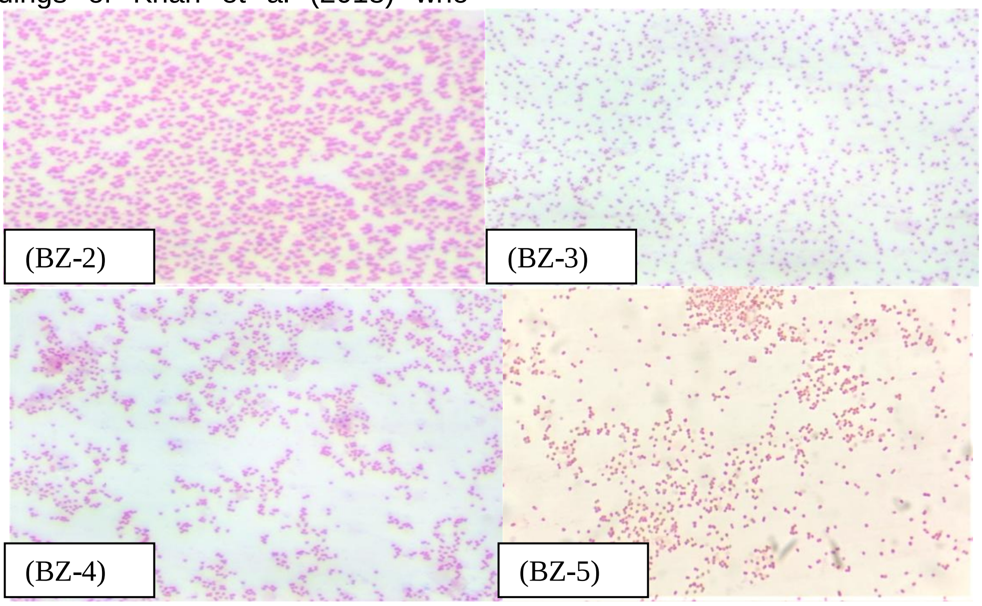


Figure 3: Gram's reaction of the isolates

Table 2: Biochemical characteristics and sugar utilization by the isolates

| Biochemical Test | BZ-2 | BZ-3 | BZ-4 | BZ-5 |
|---|---|---|---|---|
| Ala-Phe-Pro-ARYLAMIDASE | - | - | - | - |
| ADONITOL | - | - | - | + |
| L-Pyrrolydonyl-ARYLAMIDASE | - | - | - | - |
| L-ARABITOL | - | - | - | - |
| D-CELLOBIOSE | + | + | + | + |
| BETA-GALACTOSIDASE | - | - | - | - |
| $H_2S$ PRODUCTION | - | - | - | - |
| BETA-N-ACETYL-GLUCOSAMINIDASE | - | - | - | - |
| Glutamyl ArylamidasepNA | - | - | - | - |
| D-GLUCOSE | + | + | + | + |
| GAMMA-GLUTAMYL-TRANSFERASE | - | - | - | - |
| ERMENTATION/GLUCOSE | - | - | - | - |
| BETA-GLUCOSIDASE | - | - | - | - |
| D-MALTOSE | - | - | - | - |
| D-MANNITOL | - | - | - | - |
| D-MANNOSE | + | + | + | + |
| BETA-XYLOSIDASE | - | - | - | - |
| BETA-Alanine Arylamidase | - | - | - | - |
| L-Proline ARYLAMIDASE | - | - | - | - |
| LIPASE | - | - | - | - |
| PALATINOSE | - | - | - | - |
| Tyrosine ARYLAMIDASE | + | + | + | + |
| UREASE | + | + | + | + |
| D-SORBITOL | - | - | - | - |
| SACCHAROSE/SUCROSE | - | - | - | - |
| D-TAGATOSE | - | - | - | - |
| D-TREHALOSE | - | - | - | - |
| CITRATE(SODIUM) | + | + | + | + |
| MALONATE | - | - | - | - |
| 5-KETO-D-GLUCONATE | - | - | - | - |
| L-LACTATE alkalinisation | + | + | + | + |
| ALPHA-GALACTOSIDASE | - | - | - | - |
| SUCCINATE alkalinisation | + | + | + | + |
| Beta-N-ACETYL-GALETOSAMINIDASE | - | - | - | - |
| ALPHA-GALACTOSIDASE | - | - | - | - |
| PHOSPHATASE | - | - | - | - |
| Glycine ARYLAMIDASE | - | - | - | - |
| ORNITHINE DECARBOXYLASE | - | - | - | - |
| LYSINE DECARBOXYLASE | - | - | - | - |
| L-HISTIDINE assilimilation | - | - | - | - |
| COUMARATE | + | + | + | + |
| BETA- GLUCORONIDASE | - | - | - | - |
| 0/129 RESISTANCE (com. Vibrio) | + | + | + | + |
| G;lu-Gly-Arg-ARYLAMIDASE | - | - | - | - |
| L- MALATE assimilation | - | - | - | - |
| ELLMAN | - | - | - | - |
| L-LACTATE assimilation | - | - | - | - |

The biochemical tests were confirmed with VITEK-2 system and the organisms were found to be of *Acenitobacter baumanii* complex belonging to *A. nosocomilis, A. pitti, A. calcoaceticus, A. baumanii*. (Table-3). Joyanes et al (2010) confirmed isolation and identification of *Acenitobacter baumanii* from clinical samples using VITEK-2 system. Bhattacharya *et al.,* (2016) isolated potassium solubilising *Acenitobacter soli* from experimental salt farm that was found to release 10.67% of soluble potassium in broth assay. Upadhyay *et al.,* (2021) isolated *Acenitobacter baumanii* a potent ZSB that also portrayed plant growth promoting traits.

Table 3: Organisms identified in VITEK-2 system

| Organism name | Identified name |
|---|---|
| BZ-2 | *Acenitobacter nosocomilis* |
| BZ-3 | *Acenitobacter pitti* |
| BZ-4 | *Acenitobacter calcoaceticus* |
| BZ-5 | *Acenitobacter baumanii* |

### 3.3 Plant growth promoting characteristics of the isolates

The 4 potential ZSB & KMB isolates were tested for other Plant Growth Promoting traits like phosphate solubilisation, IAA production, nitrification, ammonification and HCN production for further screening and trial application (Table 4; Fig. 4). All the four isolates tested positive for ammonification and phosphate solubilisation. Out of all the isolates BZ-3 and BZ-5 tested positive for Nitrate reduction and BZ-2 tested positive for HCN production. BZ-2 and BZ-3 only tested positive for IAA production. Hence, BZ-3 and BZ-2 depicted maximum plant growth promoting traits. Lakra and Mishra (2018) isolated metal tolerant bacterial isolates from industrial effluents and studied their plant growth promoting traits such as IAA production, Ammonification, siderophore production, Phosphate solubilization and HCN production. Dinesh et al (2018) reported that bacteria isolated from different soil conditions showed various PGP traits as reported in the present investigation.

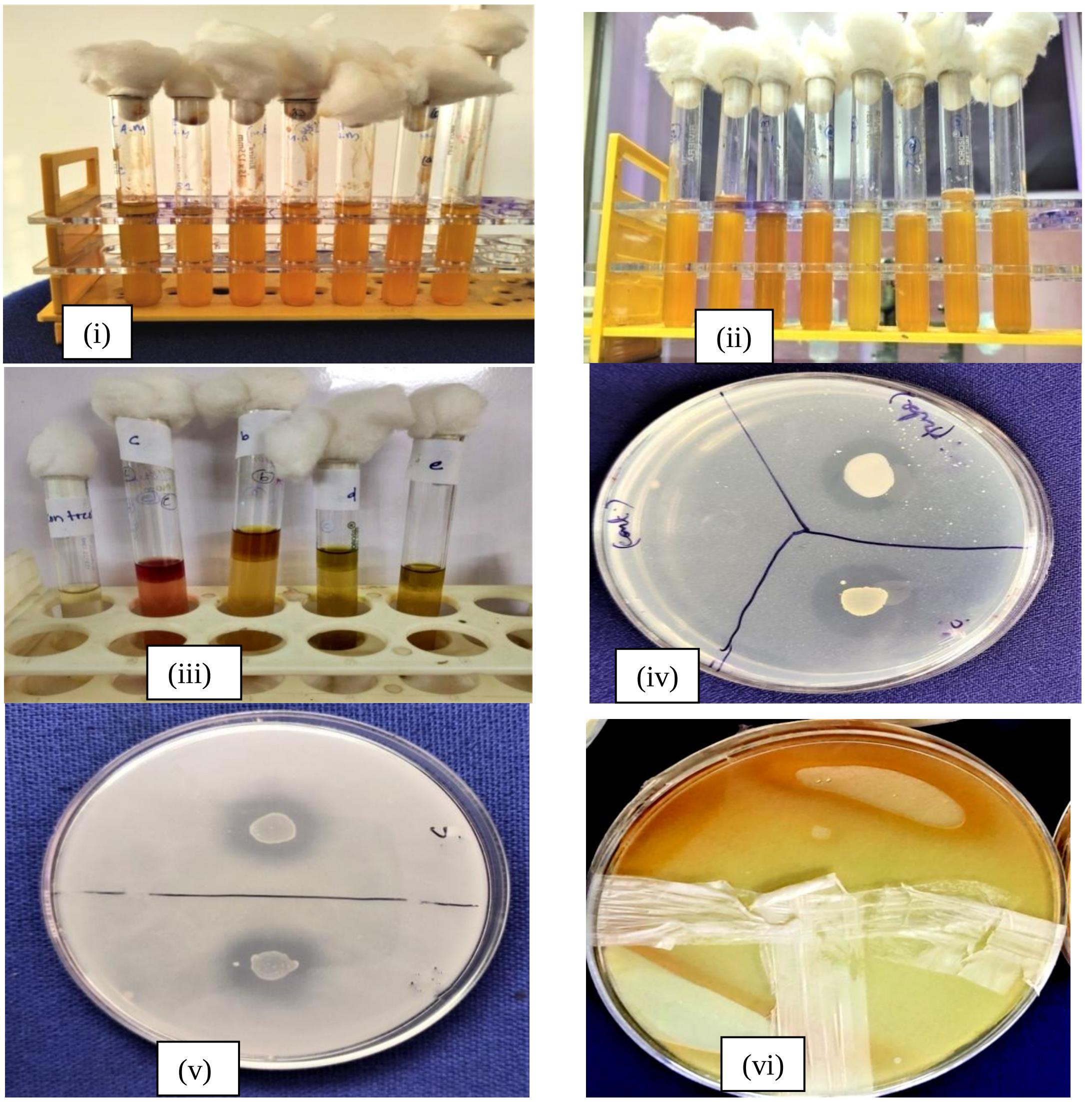


Figure 4: PGP traits exhibited by the KMB isolates ((i) Ammonification (ii) Nitrate reduction (iii) IAA production (iv) & (v) Phosphate solubilisation (vi) HCN production)

Table 4: Plant growth promoting characteristics exhibited by the isolates

| Isolate name | Ammonification | Nitrate Reduction | IAA | PSB (zone) | HCN Production |
|---|---|---|---|---|---|
| BZ-2 | + | - | + | + (13mm) | + |
| BZ-3 | + | + | + | + (14mm) | - |
| BZ-4 | + | - | - | + (12mm) | - |
| BZ-5 | + | + | - | + (11mm) | - |

**3.4: Effect of the isolates on the rice seed germination and growth variables**

All the isolates were tried with Rice seeds (Swarna sub-1 variety) to study effect on germination percentage, root length and shoot length. Hundred rice seeds were taken in triplicate in each roll towel germination paper. After 14 days, changes in germination percentage, root length and shoot length was observed in bacteria treated seeds and compared with control. Maximum germination percentage 95% was observed in case of BZ-3 treated seeds followed by BZ-4, whereas the germination percentage in control was 87%. Root length and shoot length was also increased when the seeds were treated with bacterial inoculants. In control, 10.27 cm root length and 7.64 cm shoot length observed whereas 13.565 (+32.08%) cm root length and 8.13 cm (+6.49%) shoot length was observed in case of BZ-4 and BZ-5 respectively. Similarly fresh biomass of the seedlings also increased. Highest root fresh weight was observed in case of BZ-4 (+112.2%) and for shoot fresh weight in case of BZ-5 (+11.26). Maximum root dry weight was observed in case of BZ-4 (+301.9%) and for shoot fresh weight in case of BZ-4 (+359.7%) (Table 5 and Fig. 5).

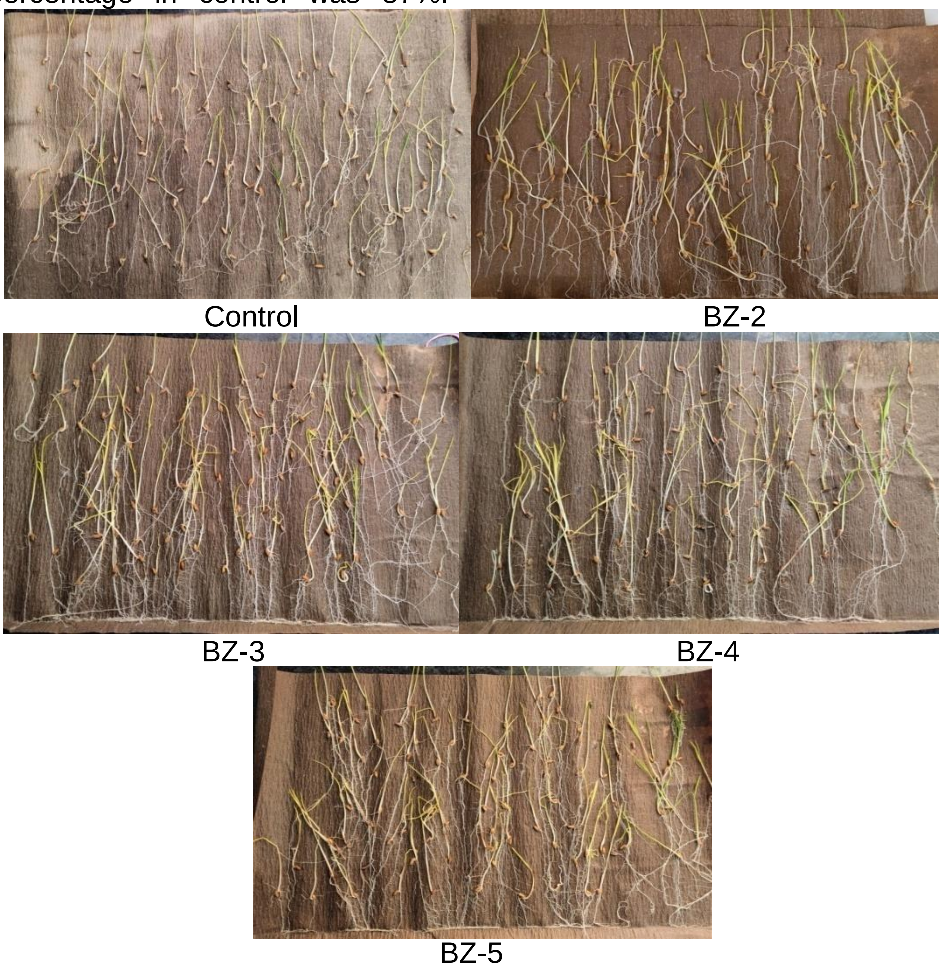


Figure 5: Effect of KMB isolates on rice seed germination and growth variables

All the values were statistically significant at ($p<0.05$) by one way ANOVA analysis. Pradhan and Mishra, (2015) also experimented the effect of plant growth promoters on rice seed germination and reported an increase in plant growth by seed bacterization. They also opined that PGPR enhance the growth, seed emergence and yield of the crops.

**3.5: Acid production by the isolates**

To study the acid production by the isolates the organisms were inoculated in the basal media supplemented with feldspar and $ZnCO_3$ and the pH of the media was measured using pH meter in the interval of 2,4,6,8 and 10 days. The decrease in pH records the production of acid by the isolates. The pH declined in the interval of two days till 6$^{th}$ day, then a sudden increase was observed (Fig-6) Ability of potash mobilizing bacteria to produce organic or inorganic acid is one of the major mechanisms of zinc and potassium solubilisation. A decrease in pH was observed in case of all the organisms till the 6$^{th}$ day which indicated acid production by the isolates. Sunithakumari *et al.,* (2016) reported decrease in pH by ZSB *Pseudomonas aeruginosa*. Jha (2017) isolated potash mobilizing bacteria to improve the potassium nutrition in paddy through several mechanisms that also induced acidification.

Table 5: Effect of isolates on rice seed germination and growth variable

| Isolate no. | Germination % | Root length (in cm) | Shoot length (in cm) | Root Fresh weight (g) | Shoot fresh weight (g) | Root Dry weight (g) | Shoot Dry weight (g) |
|---|---|---|---|---|---|---|---|
| BZ-2 | 91% | 11.147± 0.33 (+8.53%) | 7.866 ± 0.82 (+2.95%) | 1.203± 0.49 (+5.99%) | 2.795± 0.53 (-21.2%) | 0.295 ± 0.71 (+44.6%) | 0.263± 0.39 (+82.6%) |
| BZ-3 | 95% | 13.062± 0.85 (+27.18) | 7.935 ± 0.55 (+3.86%) | 1.22± 0.93 (+7.48%) | 3.907± 0.77 (+10.05%) | 0.342 ± 0.38 (+67.6%) | 0.155± 1.33 (+7.63%) |
| BZ-4 | 94% | 12.512± 0.52 (+21.8%) | 8.136± 0.69 (+6.49%) | 2.409± 0.32 (+112.2%) | 3.92 ± 0.94 (+10.42%) | 0.820 ± 0.45 (+301.9%) | 0.662± 0.28 (+359.7%) |
| BZ-5 | 90% | 13.565± 0.51 (+32.08%) | 7.953± 0.95 (+4.09%) | 2.129 ± 0.66 (+87.96%) | 3.95± 0.72 (+11.26%) | 0.346± 0.63 (+69.6%) | 0.180 ± 0.84 (+25%) |
| Control | 87% | 10.27± 0.74 | 7.64 ± 0.93 | 1.135 ± 0.62 | 3.55 ± 1.06 | 0.204 ± 0.38 | 0.144 ± 0.74 |

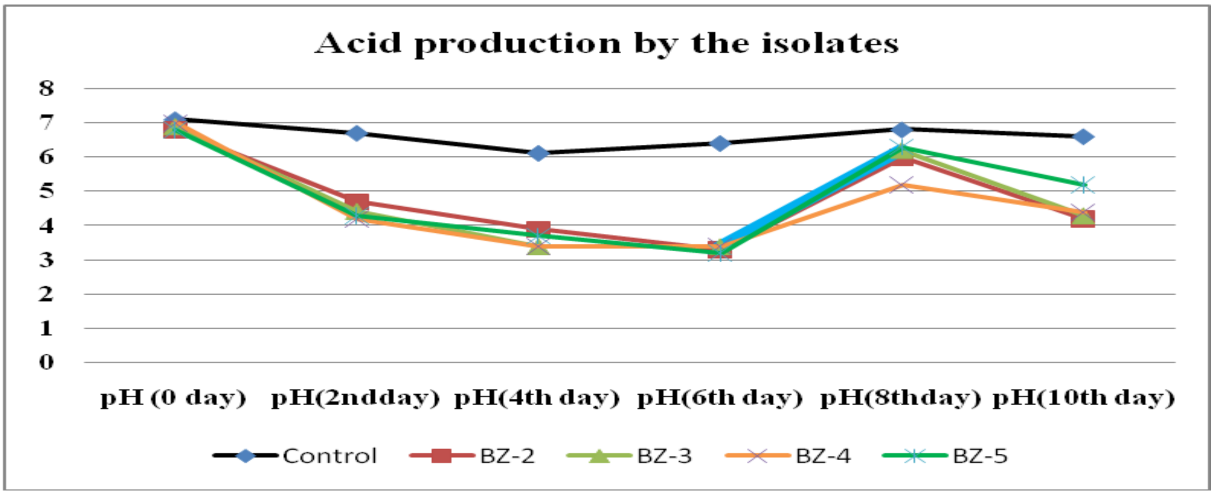


Figure 6: Change in pH by the isolates

**3.5: Quantitative estimation of potash and zinc solubilisation by isolates through liquid assa**

The amount of available potassium and zinc through broth assay showed increased amount of available K and Zn with increase in time within the period of 0 day, 2$^{nd}$ day, 4$^{th}$ day, 6$^{th}$ day, 8$^{th}$ day and 10$^{th}$ day (Fig.7). Maximum soluble potassium was produced by BZ-2 i.e., 3.809 mg/L (+280.9%) on 2$^{nd}$ day. In case of BZ-4 (+205.6%) and BZ-5 (217.5%) a constant

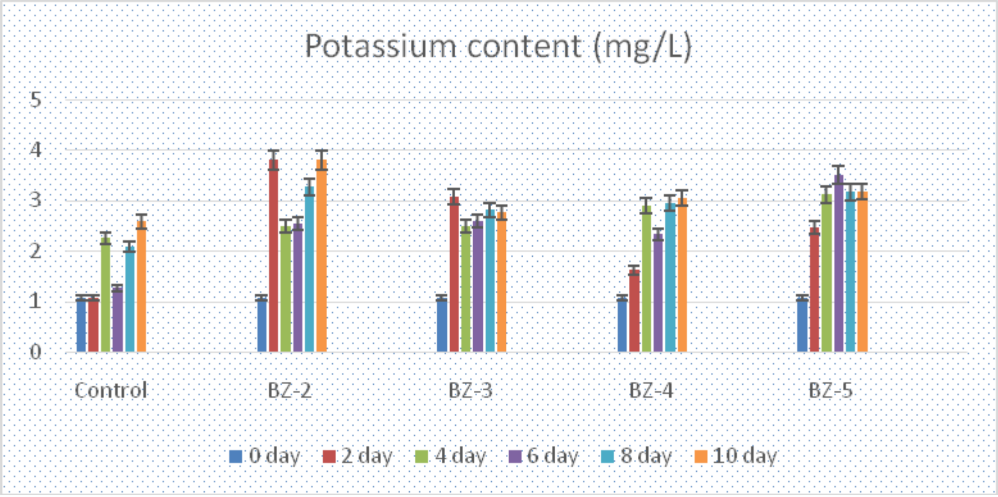


Figure 7: Increase in potassium content by the organisms

increase in potash content was observed. With respect to control, higher percentage of soluble K was found in all the isolates, BZ-2 and BZ-5 reported maximum amount of available potassium. The amount of available zinc increased in all the isolates as compared to control over the period of 0 day to 10 day. Maximum increase in zinc with respect to control was found in case of BZ-2 (+91.76%) (Fig. 8).BZ-2 & BZ-5 reported maximum zinc solubilisation. The increase in soil potassium and zinc content with time was statistically significant ($p \leq 0.05$). Quantitative estimation of potassium solubilisation by the isolates performed by Saiyad *et al.,* (2015) and available zinc quantitative estimation using AAS by Rehman *et al.,* (2021) corroborates with the present findings i.e., with increase in number of days there is increase in potassium and zinc content in the liquid media. Hence this report shows that KMBs and ZSBs are capable of solubilising complex potassium and zinc and converting it into soluble forms.

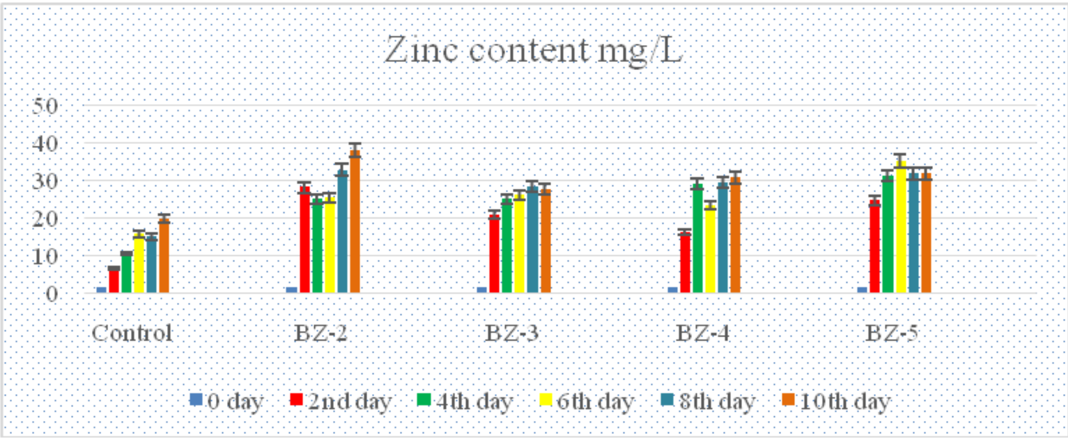


Figure 8: Increase in zinc content by the organisms

**CONCLUSION**

Four of the 20 bacterial isolates showed different PGP traits with special reference to potassium and zinc solubilizing ability. The isolates were identified based on their biochemical attributes through VITEK-2 system as *Acenitobacter* sp. Maximum available zinc was reported in case of BZ-2 (+91.76%). With respect to control, higher percentage of soluble K and Zn was found in all the isolates, BZ-2 and BZ-5 reported maximum amount of available potassium and zinc. The organism showing higher PGP traits along with the potassium and zinc solubilizing capacity have a greater agricultural and environmental significance as they can be a good replacement for chemical fertilizers. Increase in crop production leads to supply crops with good nutritional benefits to the increasing population.

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## ACKNOWLEDGEMENT

Financial assistance by Department of Science and Technology (DST), Government of India through DST-INSPIRE Fellowship is duly acknowledged.


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