Biological Forum – An International Journal **16(7): 112-116(2024)**

ISSN No. (Print): 0975-1130
ISSN No. (Online): 2249-3239

Characterization of Zinc Solubilizing Bacteria

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(Received: 21 April 2024; Revised: 18 May 2024; Accepted: 07 June 2024; Published: 15 July 2024)
(Published by Research Trend)

ABSTRACT: Onion is one of the most important commercial bulbous crops that grown for regular consumption. Zinc is an essential and important minor key element which makes significant contribution in various physiological and metabolic processes in plants. Inoculation of zinc solubilizing bacteria is recommended to overcome the zinc deficiency in plants and humans. Zinc solubilizing bacteria alone has been found to increase the bioavailability of native and applied zinc to plants. In the present investigations, out of twenty-three soil samples, six isolates of zinc solubilizing bacteria were obtained from different villages of Kolhapur District. All the isolates were identified on the basis of morphological, cultural, microscopic features and biochemical analysis. Most of the isolates showed variability in cell morphology *i.e.,* rod and cocci. Colony colour and shapes also varied *i.e.,* circular, round and irregular. In the biochemical analysis all the isolates showed positive results for catalase test and H_2S production test except ZSB-3. All isolates showed positive results for starch hydrolysis, gelatine liquefaction and methyl red test.

Keywords: zinc solubilizing bacteria, morphological, cultural and biochemical analysis.

INTRODUCTION

Onion (*Allium cepa* L.) is one of the most important commercial bulbous crops cultivated extensively in world and belonging to *Alliceae* family (Hanelt, 1990). Onion is the queen of kitchen is one of the most important commercial crop not only in India but also in the world. Central Asia. It is primarily consumed for its unique flavour that enhance the flavour of other food (Randle, 2000). It is rich source of vitamin C and E and it has medicinal value also (Bosekeng, 2012). The edible portion of the bulb are enlarged leaf base and compact stem. Green onions also called scallions are eaten for their immature and green foliage. The chief constituent of the onion which is responsible for pungency due to allyl propyl disulphide (Baloch *et al.*, 2014). As per the final estimates of 2020-21 the production of onion is reported to be 26.64 Million tonnes and out of this Maharashtra produced 13.3 Million tonnes. Productivity of onion could be increased by the use of suitable varieties, balanced nutrition, water management and plant protection measures. Among the many constituents for the low productivity in onion imbalanced nutrition is the main factor. Plants require major (Nitrogen, Phosphorous and Potassium) and micronutrients *viz.*, zinc, sulphur, magnesium, manganese, boron, copper, ferrous and molybdenum are the essential elements. Among these, zinc has great role in the fertilization program to achieve higher and sustainable yield (Singh and Tiwari 1996). Zinc makes significant contribution in various physiological and metabolic processes in plants. Zinc is indispensable element for proper plant growth which can be reverted back to available form by zinc solubilizing bacteria (Saravanan *et al.,* 2003). The availability of zinc in soil and its concentration in plant tissues are generally affected by composition of microbial population present rhizosphere region of soil (Dotaniya and Meena 2015). Zinc solubilizing bacteria alone has been found to increase the bioavailability of native and applied zinc to plants. Thus, the management of biological fertilizers containing zinc solubilizing bacteria can be an effective alternative to chemical fertilizers. Application of zinc increased the growth and yield of onion (Phor *et al*., 1995). Zinc solubilizing biofertilizer conserves soil fertility and environment (Ilyas *et al*., 2018). It plays an important role in maintaining fertility and increasing productivity (Sindhu *et al*., 2017).

MATERIALS AND METHOD

Total 23 soil samples were collected from rhizosphere of onion crop from different regions of Kolhapur District. All 6 isolates were identified as zinc solubilizing bacteria on the basis of morphological

studies (cell morphology, Gram reaction and stain colour), cultural characteristics (colony colour and colony shape). Cultural studies were carried out on Kings B media containing ZnO and $ZnCO_3$, Rovira and Bunt media containing ZnO and $ZnCO_3$ and Nutrient Agar media containing ZnO and $ZnCO_3$. The individual colonies of zinc solubilizing bacteria were examined for colony morphology, cell shape and gram reaction as per standard procedure given by Barthalomew and Mittewar (1950). Biochemical characterization (catalase, starch hydrolysis, gelatin liquefaction, acid production from carbohydrate, methyl red and H_2S production test).

Isolation of zinc solubilizing bacteria from rhizosphere of onion. Soil was carefully removed from roots region and kept in fresh plastic bags after completion of labelling and tagging. Samples were preserved in refrigerator at 4°C temperature for further use. The isolation was carried out by using serial dilution and pour plate technique using Kings B media.

Serial dilution and pour plate technique One gram of soil sample was added in 9 ml of distilled water blank. After that tenfold serial dilutions were prepared up to 10^7 dilutions then one ml of aliquot was taken from 10^4 to 10^6 in sterilized petri plates with aliquot and poured with sterilized Kings B medium and mixed gently. After solidification of medium, plates were incubated at 28 ± 2°C for 4 to 5 days. Later on, morphological, cultural, microscopic features and biochemical analysis were carried out.

Morphological characteristics Morphological characteristics such as cell morphology, Gram reaction and stain colour were studied. Gram staining was carried out as per the standard procedure described by Cappuccino and Sherman (2001).

Biochemical analysis. For the identification of zinc solubilizing bacterial isolates, biochemical tests such as catalase, starch hydrolysis, gelatine liquefaction, acid production from carbohydrate, methyl red and H_2S production tests were carried out.

Catalase test. The sterilized glass slides were taken and using sterile loop a loopful of freshly incubated culture transfer on sterilized glass slide. After add drop of H_2O_2 (3% Hydrogen peroxide) on it. The occurrence of gas bubbles was shows positive result for catalase activity.

Starch hydrolysis test. Starch agar media were prepared and pour into sterilized petri plates under aseptic condition. After solidification of media plates were inoculated with efficient zinc solubilising bacterial isolate and incubate at 28 ± 2°C for 48 hr. The incubated plates were flooded with lugol's iodine solution.

Formation of clear zone around colony indicates starch positive test.

Gelatine liquefaction. The sterilized nutrient gelation deep tubes were inoculated with efficient zinc solubilizing bacterial isolate and tubes were incubated at 28±2°C for 48 hr. The tubes were kept in a refrigerator at 4°C for 30 minutes. The tubes with cultures that remained liquefied were taken as positive and those solidified on refrigeration were taken as negative for the test.

Methyl red test. Fresh isolates of ZSB were inoculated in the test tubes containing Kings B medium under aseptic conditions and were inoculated at 28°C for 48 hours. 5 ml of methyl red solution was added to each after the incubation period. The formation of red colour in the broth indicated the positive result and the formation of yellow colour indicated negative result for the test.

Acid production from carbohydrates Test tubes containing peptone, bromocresol purple along with growth factors and medium C of dye firstly sterilized and then

inoculated with ZSB and kept for 7 days at 28°C in incubator. The bromocresol purple is a pH indicator and turn yellow indicates the presence of acid.

H_2S production test. Bacterial cultures are grown in a suitable medium in a tube containing sulphur compounds like thiosulphate. Then the inoculated culture is incubated for 24-48 hours at room temperature. The presence of black precipitate indicates positive test and yellow colour indicates negative test.

RESULT AND DISCUSSION

During isolation, out of twenty-three rhizospheric region of soil samples collected from onion crop, six isolates of zinc solubilizing bacteria (ZSB) were obtained. Isolation was done on Kings B medium (Savaranan *et al.,* 2003) by adopting enrichment culture technique. Bacterial colonies were obtained after 5 to 7 days of incubation at 27±2°C. During isolation, out of 23 soil samples, 6 isolates were obtained. One each isolate of zinc solubilizing bacteria was obtained from different villages *viz.,* Murgud (Kagal teh.), Jakhale (Panhala teh), Bastawade (Kagal teh.), Rashiwade (Radhanagari teh), Kodoli (Panhala teh.) and Bhadagoan (Gadhinglaj teh.). Two isolates of zinc solubilizing bacteria were obtained from Kagal and Panhala and designated as ZSB-1, ZSB-3, ZSB-2 and ZSB-5 respectively. Moreover, one isolate each from Radhanagari, Gadhinglaj and designated as ZSB-4 and ZSB-6 respectively.

Out of total six obtained isolates four isolates were Gram negative whereas remaining two are Gram positive. The obtained isolates *viz.,* ZSB-1, ZSB-3, ZSB-4, ZSB-5 were Gram negative and ZSB-2, ZSB-6 were Gram positive. Most of the zinc solubilizing bacterial isolates were cocci shaped *viz.,* ZSB-3, ZSB-4, ZSB-5 and ZSB- 6 except ZSB-1 and ZSB-2 with rod shape. The results are in conformity with Cappuccino and Shermen (2001); Kumar *et al.* (2012).

Table 1: Collection of soil samples of onion from Kolhapur District.

Name of Tehsil	Name of village	No. of sample	Obtained isolates of ZSB	Latitude	Longitude	Altitude (m)
1. Kagal	1. Murgud	1	ZSB-1	16.3942°	74.1901°	556
	2. Chimgoan	1	—	16.7296°	74.5285°	547
	3. Bhadgoan	1	—	16.7154°	74.4996°	549
	4. Bastawade	1	ZSB-3	16.7240°	74. 5373°	564
	5. Hamidwada	1	—	16.8350°	74.4924°	558
2. Gadhinglaj	1. Aurnal	1	—	16.2110°	74.3368°	623
	2. Bhadagoan	1	ZSB-6	16.2552°	74.3734°	627
	3. Gijawane	1	—	16.9240°	74.0348°	625
	4. Gadhinglaj	1	—	16.8542°	74.2059°	635
3. Karveer	1. Pachagoan	1	—	16.6780°	74.2694°	554
	2.Bele	1	—	16.5571°	74.1328°	546
	3. Mudshingi	1	—	16.7084°	74.3528°	541
	4. Ispurli	1	—	16.5608°	74.1798°	559
4.Panhala	1. Jakhale	1	ZSB-2	16.8382°	74.1957°	674
	2. Panhala	1	—	16.8107°	74.1181°	689
	3. Kodoli	1	ZSB-5	16.8752°	74.1902°	695
5. Shirol	1.Yadrav	1	—	16.5202°	74.7260^0	543
	2. Jambhali	1	—	16.7195°	74.5317°	547
	3.Haroli	1	—	16.7240°	74. 5141°	539
	4. Bastawad	1	—	16.7787°	74.5544°	544
6. Radhanagari	1. Saravade	1	—	16.1419°	74.1034°	570
	2. Rashiwade	1	ZSB-4	16.5476°	74.1020°	564

Table 2: Morphological characteristics of Zinc solubilizing bacterial isolates.

Sr. No.	Obtained isolates	Cell morphology	Gram reaction	Stain colour
1.	ZSB-1	Rod	-Ve	Pink
2.	ZSB-2	Rod	+Ve	Purple
3.	ZSB-3	Cocci	-Ve	Pink
4.	ZSB-4	Cocci	-Ve	Pink
5.	ZSB-5	Cocci	-Ve	Pink
6.	ZSB-6	Cocci	+Ve	Purple

Table 3: Cultural characteristics of isolates of Zinc solubilizing bacteria.

Media	Obtained isolates	Colour	Shape
Kings B media containing ZnO	ZSB-1	Creamy white	Irregular
	ZSB-2	Transparent white	Round
	ZSB-3	Milky white	Circular
	ZSB-4	Opaque white	Circular
	ZSB-5	Milky white	Round
	ZSB-6	Milky white	Irregular
Rovira and Bunt media containing ZnO	ZSB-1	Opaque white	Irregular
	ZSB-2	Transparent white	Round
	ZSB-3	Creamy white	Round
	ZSB-4	Milky white	Round
	ZSB-5	White	Round
	ZSB-6	Transparent white	Irregular
Nutrient agar media containing ZnO	ZSB-1	Milky white	Round
	ZSB-2	Milky white	Round
	ZSB-3	White	Round
	ZSB-4	Milky white	Round
	ZSB-5	White	Round
	ZSB-6	Milky white	Round
Kings B media containing $ZnCO_3$	ZSB-1	White	Round
	ZSB-2	White	Round
	ZSB-3	—	—
	ZSB-4	White	Round
	ZSB-5	—	—
	ZSB-6	White	Round
Rovira and Bunt media containing $ZnCO_3$	ZSB-1	—	—
	ZSB-2	—	—
	ZSB-3	—	—

	ZSB-4	—	—
	ZSB-5	—	—
	ZSB-6	—	—
NA media containing $ZnCO_3$	ZSB-1	Transparent white	Circular
	ZSB-2	Transparent white	Circular
	ZSB-3	Milky white	Round
	ZSB-4	Milky white	Round
	ZSB-5	White	Round
	ZSB-6	Opaque white	Round

The cultural characteristics of zinc solubilizing bacteria (ZSB) in Table 4 revealed that, Kings B media containing ZnO showed, ZSB-1 isolate colony was creamy white whereas ZSB-3, ZSB-5 and ZSB-6 isolates colonies were milky white. The isolate ZSB-2 has transparent while colonies whereas ZSB-4 was opaque white. Rovira and Bunt media containing ZnO showed, ZSB-1 colony isolate was opaque white, ZSB-2 and ZSB-6 isolates colonies were transparent white, ZSB-3 isolate was creamy white, ZSB-4 isolate was milky white while ZSB-5 isolate colony was white. NA media containing ZnO showed ZSB-1, ZSB-2, ZSB-4 and ZSB-6 isolates colonies were milky white whereas ZSB-3 and ZSB-5 isolates colonies were white. Kings B media containing $ZnCO_3$ showed, ZSB-1, ZSB-22, ZSB-4 and ZSB-6 isolates colonies were white in colour. No growth was observed on Rovira and Bunt media containing $ZnCO_3$. Nutrient agar (NA) media containing $ZnCO_3$ showed ZSB-1, ZSB-2 isolate colonies were transparent white whereas ZSB-3, ZSB-4 isolates colonies were milky white, ZSB-5 isolate colony was white and ZSB-6 isolate colony was opaque white. The findings of present investigation are in confirmation with Chandra Shekhar *et al*. (2019); Tamboli (2019).

Based on morphological, cultural characteristics (colour and shape), microscopic observations, biochemical characterization and solubilization index, the efficient zinc solubilizing bacterial isolates were identified as *Bacillus* sp. and *Pseudomonas* sp. however designated as ZSB-1 and ZSB-2 respectively.

Table 4: Biochemical characterization of isolates of Zinc solubilizing bacteria.

Sr. No.	Biochemical test	ZSB-1	ZSB-2	ZSB-3	ZSB-4	ZSB-5	ZSB-6
1.	Catalase test	+	+	–	+	+	+
2.	Starch hydrolysis	+	+	+	+	+	+
3.	H_2S production test	+	+	–	+	+	+
4.	Gelatin liquefaction	+	+	+	+	+	+
5.	Acid production from carbohydrates	+	+	–	+	+	–
6.	Methyl-red test	+	+	+	+	+	+

All the obtained isolates showed positive results for catalase test except ZSB-3. All the obtained isolates showed positive result for starch hydrolysis test. Except ZSB-3, all other isolates showed positive result for H_2S production test.

Gelatine liquefaction test results showed positive results for all the obtained isolates. ZSB-1, ZSB-2 ZSB-4 and ZSB-5 gave positive results whereas ZSB-3 and ZSB-6 showed negative results for acid production from carbohydrates. In case of methyl red test all the obtained isolates showed positive results. The results of biochemical tests were noticed to be similar with Cappuccino and Shermen (2001); Kumar *et al*. (2012).

CONCLUSIONS

All ZSB isolates were studied and evaluated efficient isolates on the basis of morphological, cultural, microscopic features, biochemical characterization and solubilisation index. Out of six ZSB isolates, two showed highest solubilising index (5.23) and (4.33) on kings B medium respectively. Efficient isolates ZSB were identified as *Bacillus* sp. and *Pseudomonas* sp.

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How to cite this article: V.S. Patil, S.J. Waghmare, M.S. Kambale, R.A. Karande and T.S. Koli (2024). Characterization of Zinc Solubilizing Bacteria**.** *Biological Forum – An International Journal, 16*(7): 112-116.