Isolation and identification of potassium solubilizing bacteria and its effect on growth of *Vigna radiata*

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***Abstract*: Three macronutrient Nitrogen (N), Phosphorous (P) and Potassium (K) are essential for plant growth and development. Among them Potassium is having its role in transpiration, photosynthesis, respiration, transport of sugar. Potassium is required by all the plants for growth, metabolism and development. It is observed that, potassium is present in the soil in abundant amount in the form minerals (mica, feldspar) and rocks. Most of the potassium is in nonexchangeable form in nature and cannot be easily up taken by plants. The uptake of potassium by the plants can be increased by application of potassium solubilizing bacteria in the field. The potassium solubilizing bacteria converts insoluble mineral form of potash into soluble form by secretion of enzymes; acids etc. and make available for plants. Instead of using chemical fertilizers, use of potassium solubilizing bacteria is environmentally safe and eco-friendly method. The present study is based on the isolation, identification, characterization of potassium solubilizing bacteria from soil and its effect on the growth of plant (*Vigna radiata)*.**

***Keywords*: Mica, potassium solubilizing bacteria, *Vigna radiata*, pot study**

Introduction

Potassium (K) is the third most essential macronutrient among Nitrogen (N), phosphorous (P) and potassium (K) present in soil. Potassium is most abundantly absorbed cation which plays important role in the growth, metabolism and development of all crops (Archana, 2007). Potassium helps in nitrogen uptake, transpiration, protein synthesis, grain filling, increasing disease and insect resistance, sugar and starch transport, photosynthesis along with some physiological and biochemical processes. Without adequate potash, plants will grow slowly; have poorly developed roots, small seeds, weak stem and leaves etc. Potassium deficiency can be easily observed by the signs like chlorosis, yellow spots on leaves, falling of leaf prematurely etc. As potassium is a mobile nutrient its deficiency can be passed from older to the younger leaves. Soil contains abundant amount of potassium. Potassium constitutes about 2.5% of lithosphere but the actual soil concentration of this macronutrient varies widely ranging from 0.04 to 3.0% (Parmar and Sindhu, 2013). More than 98% potassium present in soil exists in the form of silicate minerals (microcline, feldspar, muscovite, mica etc. (Buchholz and Brown, 1993). The soil minerals contain 90 to 98% of soil potassium which is in unavailable form and only 2% is in available form to plants (Archana, 2007). For providing potassium, farmers knowingly or unknowingly applying chemical fertilizers like potassium chloride, potassium nitrate, potassium sulphate, monopotassium phosphate etc. The heavy application of chemical fertilizers can cause environmental pollution. All rest amount is bound with minerals and are unavailable for uptake by plants.

Soil microbes have reported to play a key role in the natural (K) cycle and therefore, Potassium solubilizing bacteria present in soil could provide an alternating technology to make potash available to the plants by converting insoluble minerals of potassium to soluble form which can be easily up taken by plants (Parmar and Sindhu,2013). Potassium solubilizing bacteria are able to solubilize rock potassium mineral powder such as mica, feldspar and orthoclases by weathering and solubilizing. They are readily weathered through organic acids and acidic polysaccharides secreted by microorganisms.Organic acids can directly enhance dissolution of potassium minerals (Sugumaran and Janarthanam, 2007). Though several laboratory techniques states that bacteria produces acids, alkalis, acidic polysaccharides etc. the actual mechanism of potassium released from the minerals still not known (Groudev, 1987). Growth and yield of plant can be enhanced by inoculating potassium solubilising bacteria which secrets enzymes, acids etc. and make available for uptake in plants. It will reduces use of chemical fertilizers and become eco-friendly method.

Materials and methods

Collection of soil sample for isolation of potassium solubilizing bacteria

The soil sample was collected from rhizosphere of sugarcane field, located in Solapur district. The latitude of Solapur, Maharashtra is 17.6599 and longitude is 75.90638.

Culture medium used for isolation of potassium solubilizing bacteria

The modified sterile Aleksandrove's media containing 1% glucose, 0.5% peptone, 0.05% $MgSO_4.7H_2O$, 0.01% $CaCO_3$, 0.2% $CaPO_4$, 0.3% mica, 0.0005% $FeCl_3$, 2% agar, pH 6.5 was used for isolation of potassium solubilizing bacteria from soil. (Sugumaran and Janartham, 2007)

Isolation of potassium solubilizing bacteria

One gram of soil was serially diluted from 10^{-1} to 10^{-5} by using sterile distilled water. 0.1ml of sample from each test tube was spread inoculated on the sterile Aleksandrove's media containing mica as a mineral source and incubated for one week at 37℃. The well isolated seven bacterial colonies were selected for screening of its potassium solubilizing activity.

Screening of potassium solubilizing bacteria

Screening of potassium solubilizing bacteria were carried out on the basis of zone activity by using Aleksandrove's media containing phenol red as a indicator as per Khandeparkar's selection ratio (Prajapati and Modi, 2012).
Ratio= Diameter of zone of clearance/diameter of growth
The seven isolated bacterial colonies were labelled as KSB1 to KSB7. Among them the colony labelled as KSB2 was showed maximum zone activity on Aleksandrove's medium. Hence KSB2 was subjected for its further study.

Morphological characteristics of KSB2

The potassium solubilizing bacteria were grown on Aleksandrove's media to study colony characteristics (Parmar and Sindhu, 2013).Gram staining was performed. Motility was studied by hanging drop technique.

Biochemical characteristics of KSB2

The biochemical characteristics of isolated potassium solubilizing bacteria (KSB 2) were studied. Indol, methyl red, Voges Proskaur, citrate utilization test, urease test, gelatin hydrolysis test, H_2S production test, nitrate reductase test, starch hydrolysis test,oxidase test, growth in KCN and phenylalanine deaminase test were performed.
The ability of bacteria to utilize sugars (glucose, dextrose, sucrose, fructose, maltose, mannitol, lactose, mannose) (Aneja, 2002; Deshmukh, 2007) was checked by performing sugar utilization test. The isolated bacterial culture was identified by using Vitek 2 software (www.biomerieux-diagnostics.com/vitekr-2compact-0).

Physiological characteristics of KSB2

For study of physiological characteristics of KSB2, cell density was adjusted to 10^6 to 10^8 CFU/ml on the basis when culture reaches 0.1 optical units at 600 nm with colorimeter (Schaad, 1992). Further optimization of pH, temperature, carbon and nitrogen sources for maximum growth of KSB2 was done. (Prasad, 2014)

Optimization of pH

50 ml of sterile modified Aleksandrove's broth was prepared in 7 different 250 ml capacity conical flask. pH of broth in each conical flask was adjusted to 4, 5, 6, 7, 8, 9, 10. Then 0.1 ml of KSB2 culture was inoculated into respective conical flask and incubated at 37º C for 4 days on rotary shaker at 120 rpm. The optical density was measured aseptically after each 24 hrs by using colorimeter at 600 nm. (Prasad, 2014)

Optimization of temperature

50ml of sterile modified Aleksandrove's broth was prepared in 5 different 250 ml capacity conical flask. Then 0.1 ml of KSB2 culture was inoculated into respective conical flask and incubated for 4 days at 10º C, 20º C, 30º C, 40º C and 50º C on incubator shaker at 120 rpm. The optical density was measured aseptically after each 24 hrs by using colorimeter at 600 nm. (Prasad, 2014).

Optimization of carbon source

Except the carbon source in Aleksandrove's broth, sterile 50 ml of modified Aleksandrove's broth was prepared with 0.5% respective carbon source viz. glucose, dextrose, sucrose, lactose, cellulose in 5 different 250 ml capacity conical flask. Then 0.1 ml of KSB2 culture was inoculated into respective conical flask and incubated at 37° C for 4 days on incubator shaker at 120 rpm. The optical density was measured aseptically after each 24 hrs by using colorimeter at 600 nm. (Prasad, 2014).

Optimization of nitrogen source

Except the nitrogen source in Aleksandrove's broth, sterile 50 ml of modified Aleksandrove's broth was prepared with 0.5% respective nitrogen source viz. peptone, Di-ammoniun hydrogen phosphate, sodium nitrate, urea and ammonium chloride in 5 different 250 ml capacity conical flask. Then 0.1 ml of KSB2 culture was inoculated into respective conical flask and incubated at 37° C for 4 days on incubator shaker at 120 rpm. The optical density was measured aseptically after each 24 hrs by using colorimeter at 600 nm. (Prasad, 2014).

Pot study

The pot experiment was carried to observe the effect of potassium solubilising bacteria (KSB2) on the growth of *Vigna radiata*. The soil was collected from non-fertilized field site in Solapur, Maharashtra, India. Available potassium was determined by flame photometer for control pot and test pots (Olsen *et al*, 1954).
Three earthen pots were filled with soil and twenty seeds of *Vigna radiata* were sowed into each pot. The germinated plants were maintained in each pot for further study. The isolated KSB2 was inoculated into all three pots. 50 ml of inoculum containing 10^6-10^8 CFU/ml cells were mixed into sterile water and added into each pot. The control pots were inoculated with water only. After 20 days of incubation, total number of leaves, root length, shoot length, total height, number of lateral roots, dry weight and wet weight of *Vigna radiata* were measured (Sugumaran and Janarthanam, 2007).

Determination of potassium content in soil

Soil analysis was carried out by using flame photometer to determine available potassium in control soil and inoculated soil with KSB2.The 5 gm of air dried each soil sample were mixed with 25 ml 0.5 M ammonium acetate solution separately and shaked for 30 minutes. Both the solutions were filtered through Whatmann No. 1 filter paper. The filtrate was subjected to determine potassium content in soil by using flamephotometer (Knudsen *et al*, 1982).

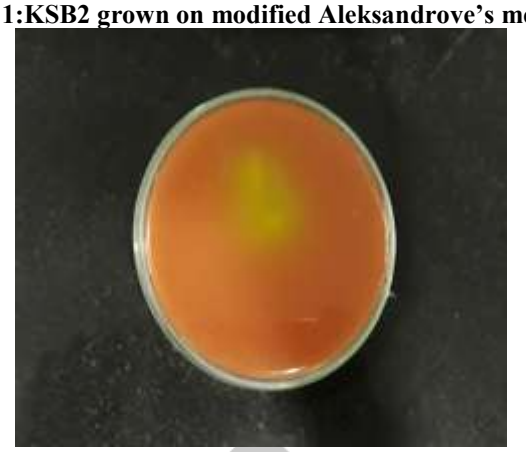
Results and Discussion

Isolation and screening of KSB

The well isolated seven bacterial colonies (labelled as KSB1, KSB2, KSB3, KSB4, KSB5, KSB6, KSB7) from modified Aleksandrove's media were checked for its capability to solubilize potassium mineral. It was determined on the basis of

Khandeparkar selection ratio. Out of seven KSB isolates, KSB2 showed maximum zone of clearance than 6 isolated KSB cultures. (Fig 1)

Fig. 1:KSB2 grown on modified Aleksandrove's media



Morphological characteristics of KSB2

The colony of KSB2 was 2mm in size, circular, moist, opaque and white with entire margin. The bacteria were observed as Gram negative, rod shaped, motile (Table 1).

Table 1: Morphological characteristics of KSB2

Size	Shape	Margin	Elevation	Consistency	Opacity	Colour	Cell shape	Gram nature
2 mm	circular	entire	elevated	moist	opaque	white	rod	Gram negative

Biochemical characteristics of KSB2

The KSB2 isolate showed positive results for Voges-Proskaur test, citrate utilization, urease test, nitrate reductase, starch hydrolysis, growth in KCN. The negative results were observed for Indol test, methyl red test, gelatin hydrolysis, H_2S production, phenylalanine deaminase. The KSB2 isolate can utilize glucose, dextrose, sucrose, maltose, mannose, lactose sugar and produces acid and gas while KSB2 unable to utilize fructose (Table2). Results of biochemical characters of KSB2 were compared with Bergeys Manual of Systematic Bacteriology.(Don J. Brenner etal,2005) The isolated bacterial culture (KSB2) was identified by using Vitek2 software and identified as *Enterobacter cloacae* (www.biomerieux-diagnostics.com/vitekr-2compact-0).

Table 2: Biochemical characteristics of KSB2

Sr. No.	Biochemical test	Result
1	Indol	-
2	Methyl red	-
3	Voges-Proskaur	+
4	Citrate	+
5	Urease test	+
6	Gelatin hydrolysis	-
7	H_2S production	-
8	Nitrate reductase	+
9	Starch hydrolysis	+
10	Phenylalanine deaminase	-
11	Oxidase	-
12	Growth on KCN	+
13	Glucose	+
14	Dextrose	+
15	Sucrose	+
16	Fructose	-
17	Maltose	-
18	Mannose	+
19	Lactose	+

+ : positive − : negative

Optimization of temperature and incubation time

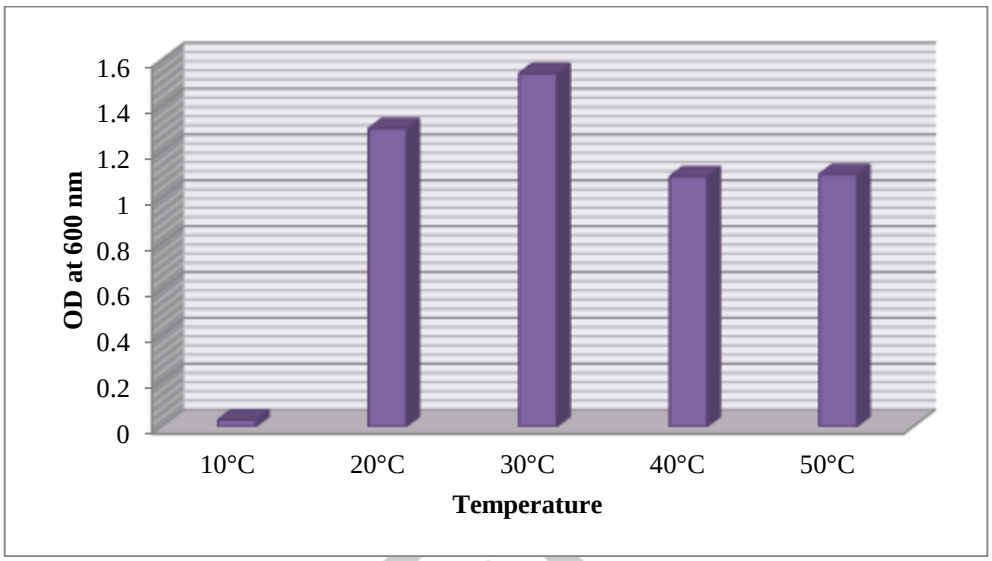


Fig 2: Effect of temperature on growth of KSB2 isolate

The maximum growth of KSB2 isolate was observed at 30 °C followed by 20 °C (Fig 2).

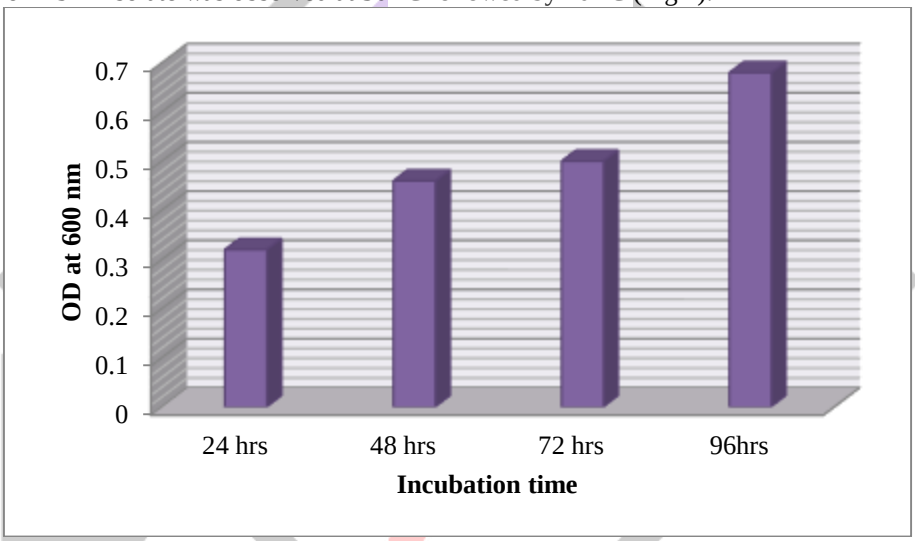


Fig 3: Effect of incubation time on growth of KSB2 isolate

The maximum growth of KSB2 isolate was observed after incubation of 96 hrs followed by 72 hrs (Fig 3).

Optimization of pH

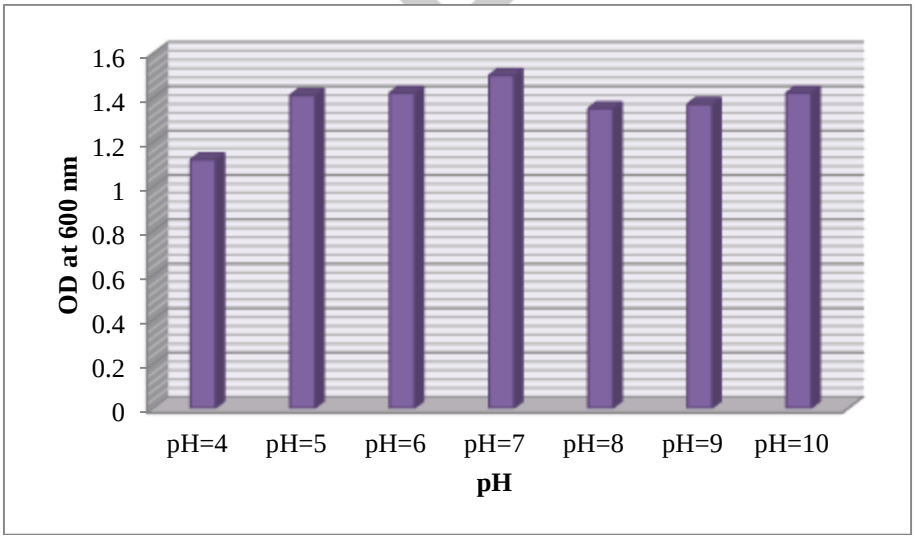


Fig 4: Effect of pH on growth of KSB2 isolate

The highest growth of KSB2 isolate was observed at pH 7 followed 5 and 6 (Fig 4).

Optimization of carbon source

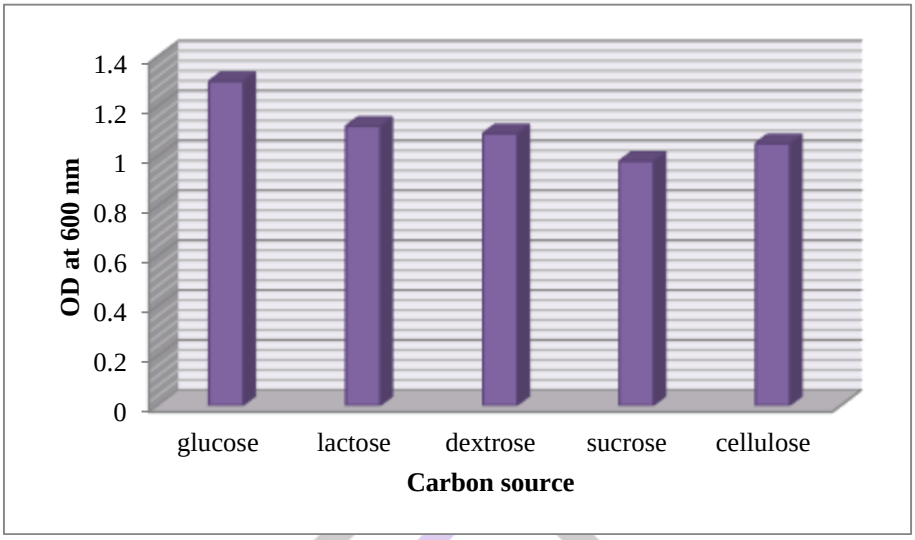


Fig 5: Effect of carbon source on growth of KSB2 isolate

Carbon is major source of energy and nutrition for the activities and growth of microorganisms. The KSB2 isolate having ability to utilize glucose followed by lactose and dextrose as a carbon source followed by cellulose and sucrose (Fig 5).

Optimization of nitrogen source

Ammonium chloride, Di-Ammonium hydrogen phosphate, sodium nitrate, peptone and urea were used for optimization of nitrogen source. The bacterial isolate (KSB2) showed maximum growth with peptone followed by Di-Ammonium hydrogen phosphate and sodium nitrate, moderate growth for ammonium chloride and low growth for urea (Fig 6).

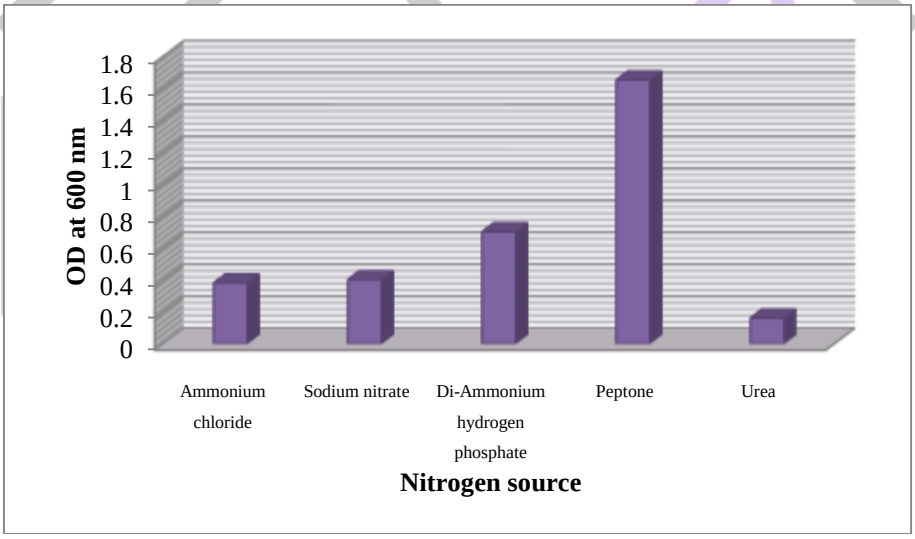


Fig 6: Effect of nitrogen source on KSB2 isolate

Pot experiment

The inoculation of KSB2 into soil sowed with plant *Vigna radiata* had significant influence on different growth characters. The total germinated seeds, average root length, average shoot length, average number of leaves, average lateral roots, average wet weight and dry weight of plants were considerably higher in plants treated with KSB2 and comparatively lower in uninoculated plants (Table 3).

Table 3: Effect of KSB2 isolate on germination and growth of *Vigna radiata*

Sr. No.	Particular	Control	Plant treated with KSB2
1	Total germinated seeds	12.00	16.00
2	Average root length (cm)	1.35	15.26
3	Average shoot length (cm)	11.09	18.84
4	Average of total number of leaves	16.00	25.00
5	Average of lateral roots	7.80	13.50
6	Average of wet weight (gm)	0.75	1.21
7	Average of dry weight (gm)	0.13	0.39

Determination of potassium content in soil

The control pot showed 38 ppm of potassium content in soil while KSB2 treated soil showed 56 ppm potassium content. It was determined by flame photometer. (Table 4)

Table 4: Determination of potassium content in soil

Soil sample	Potassium
Control (without KSB2)	38 ppm
KSB2 inoculated	56 ppm

Discussion

In present study KSB2 isolate grown on Aleksandrove's medium showed maximum zone of clearance around the colony. The KSB2 isolate was identified as *Enterobacter cloacae* on the basis of its morphological, cultural, physiological, biochemical characteristics and identified by using Vitek-2 software. The isolated KSB2 was grown at different temperature, pH, carbon and nitrogen sources for optimization of its growth parameters. It was found that KSB2 showed maximum growth at 30°C, pH 7, glucose as carbon source and peptone as nitrogen source.

As reported by various researchers, inoculation with potassium solubilizing bacteria has beneficial effects on growth of cotton and rape (Sheng, 2005), eggplant (Han and Lee, 2005), pepper and cucumber (Han and Lee, 2006; Sangeeth *et al.*, 2012), peanut (Youssef *et al.*, 2010), maize (Abou-el-Seoud and Abdel-Megeed, 2012; Leaungvutiviroj *et al.*,2010; Singh *et al.*, 2010), sorghum (Badr *et al.*, 2006), wheat (Sheng and He, 2006), Sudan grass (Basak and Biswas, 2012; Basak and Biswas, 2010), tea (Bagyalakshmi *et al.*, 2012), Okra (Prajapati *et al.*, 2013), potato (Abdel-Salam and Shams, 2012), and tomato (Lynn *et al.*, 2013). On the basis of previous findings it was concluded that KSB can used as bio-fertilizers for agriculture improvement which reduce the use of agrochemicals and support eco-friendly crop production (Archana *et al.*, 2013; Archana *et al.*, 2012; Prajapati *et al.*, 2013). Results of present study clearly indicated that the isolated *Enterobacter cloacae* have ability to solubilize potassium from its insoluble form and enhance the growth of *Vigna radiata*. Genetic modification can be possible to decrease pathogenicity of *Enterobacter cloacae* was reported by Andreote *et al.*, 2004. Therefore, it was concluded that *Enterobacter cloacae* isolate may be a potential candidate to be used as potassium solubilising bacteria by eco-friendly way.

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