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Exploration of chickpea rhizobia for solubilization potential of phosphorus, zinc and potassium

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Abstract

Plants require various nutrients for their unremitting growth and reproduction, among those phosphorus, zinc and potassium plays a crucial role. Like most micronutrients, these are not easily available to the plants due to their highly insoluble nature which is a costly practice for the farmer, associated with loss of production. Twenty four chickpea rhizobial isolates were characterized for their potential to solubilize insoluble sources of zinc, phosphorus and potassium. Out of 24 isolates, ten rhizobial isolates were found to solubilize phosphate (41.66%), with a solubilization index ranging from 2.15 to 3.33. Sixteen and ten rhizobial isolates were found as zinc and potassium solubilizers with an index varied from 2.18 to 5.14 and 2.33 to 4.16, respectively. So, transformation of unavailable form of nutrients to available form by microbes is going to be an important approach for sustainable agriculture.

Keywords: Chickpea, rhizobia, solubilization, zinc, phosphate, potassium

Introduction

Biofertilizers are one of the most important components of modern and sustainable agriculture as products containing living microorganisms which have the ability to solubilise or mobilize nutritionally important elements from plant non-usable to usable form through some biological processes. Biofertilizers include: nitrogen fixing biofertilizers (*Rhizobium, Bradyrhizobium, Azospirillum* and *Azotobacter, Frankia*), phosphate solubilizing biofertilizers (PSB) (*Bacillus, Pseudomonas, Aspergillus* etc.), plant growth promoting rhizobacteria (PGPR), potassium solubilizing bacteria and zinc solubilizing bacteria. It was reported by many reseacrhers that rhizobia nodulate legumes like beans, soybean, chickpea, pigeon pea can fix 50-500 kg atmospheric N/hectare under favourable environmental conditions [1]. Plants require various nutrients for their growth which are supplemented through inorganic or organic forms. Among these, zinc, phosphorus and potassium are essential micronutrients required for the normal healthy growth and reproduction of crop plants. However, availability of zinc from these sources depends on many factors which plays an important role in converting such unavailable sources into available ones [2]. Bacteria plays an important role in solubilising these unavailable nutrients required by the plants.

Beans are the most important grain legumes for direct human consumption in the world. In nutritional terms, alongwith great protein source, beans are also rich in minerals (especially iron and zinc) and vitamins. It is grown principally for its high protein seeds that are used as human food, which can be prepared by cooking, fermenting, milling or sprouting. Chickpea is one of the most important winter season legume crop of arid zones of India [3]. It is commonly known as by many names, among which chana is most popular one and highly prosperous in fiber, minerals (phosphorus, calcium, magnesium, iron and zinc), β-carotene and large amount of unsaturated fatty acids [4]. It is used as a vegetable, dry snacks and also as animal fodder. Soil mineral supplementation relies heavily on the use of chemical fertilizer, which has a considerable pessimistic impact on the environment. Use of highly energy consuming process in chemical production of nitrogen interferes with global nitrogen cycle that ultimately harms the environment [5]. Unwise application of chemical fertilizers in India has a substantial depressing impact on economy and environmental sustainability. So, there is a rising need to turn back to sustainable agents like microbes as biofertilizers that promote evergreen agriculture.

Materials and Methods

Detection of rhizobial isolates for phosphorus solubilization

All the selected chickpea rhizobial isolates were inoculated in 30 ml YEM broth in 100 ml conical flasks.

These flasks were incubated at 28±2ºC on a rotary shaker-cum-BOD incubator for 2-3 days. Five µl of each log phase grown rhizobial culture was taken and spotted on Pikovskaya's medium agar plates. These plates were incubated for 3-7 days at 28±2ºC in a B.O.D incubator and observed for P-solubilization zone. Formation of solubilization zone surrounding bacterial colony indicates the presence of P-solubilization ability of the rhizobial isolates. Zone of solubilization and colony size were measured and solubilization index was calculated using the following formula.

Solubilization index = $(H)^2 / (A)^2$
A = Colony diameter
H = Diameter (Colony + Halo zone)

Zinc solubilization by rhizobial isolates

Screening of rhizobial isolates for zinc solubilization was done on Minimal medium agar plates containing insoluble zinc oxide at a concentration of 0.1%. Degree of zinc solubilization for each isolate was determined by measuring zone of solubilization.

Screening of rhizobial isolates for potassium solubilization

Screening of potassium solubilization by rhizobial isolates was done on modified Aleksandrov medium plates containing insoluble mica powder as potassium source by the spotting method. Five µl of each log phase grown rhizobial culture was spotted on modified Aleksandrov's medium plates and plates were incubated at 28±2°C for 3 days. Potassium solubilizing activity was determined by formation of solubilization zone and solubilization index (K-SI) was calculated for each rhizobial isolate by the following formula:

Potassium solubilizing index (K-SI) = (Colony diameter + Clear zone diameter)/(Colony diameter)

Results

Phosphorus solubilization

Out of 24 chickpea rhizobial isolates, only ten isolates were found to solubilize phosphate (41.66%), although all the isolates were able to solubilized phosphate efficiently with remarkable halo zone formation (Figure 1). These rhizobial isolates solubilized P with a solubilization index ranging from 2.15 to 3.33 (Table 1). Maximum phosphate solubilization (3.33) was observed in the two rhizobial isolates namely CPR4C and CPR54A. Minimum capacity to solubilize phosphate was observed in the rhizobial isolate CPR27A as compared to the other phosphate solubilizer rhizobia. The solubilisation zone was observed upto seven days and afterthat no increase in zone was found. Fourteen isolates were not able to solubilise phosphate. It was also reported earlier by many researchers that not all the rhizobial isolates were able to soulbilize phosphate source [6].

Zinc solubilization

Sixteen rhizobial isolates were found to solubilize zinc by forming solubilisation zone on zinc minimal medium agar plates containing insoluble zinc oxide. These rhizobial isolates solubilized zinc with an index ranging from 2.18 to 5.14 (Table 1). All rhizobial isolates showed significant zinc solubilization with a zinc solubilisation index (Z-SI) more than 2.0. Among which, 4 rhizobial isolates CPR26C, CPR20A, CPR21F and CPR4C showed Z-SI index more than 3.0. Maximum zinc solubilization (5.14) was observed in the the rhizobial isolate CPR26C. The solubilization zone was observed upto seven days and no further increase in zone was observed after seven days.

Potassium solubilization

Potassium solubilisation capacity of all the twenty four chickpea rhizobial isolates was tested depending on the ability of rhizobia to form zone of solubilization on modified Aleksandrov medium plates. Only ten isolates were found to solubilize potassium with significant zone formation (Figure 2). These rhizobial isolates solubilized potassium with a solubilization index ranging from 2.33 to 4.16 (Table 1). Maximum phosphate solubilization (4.16) was observed in the the rhizobial isolate CPR46A. Five rhizobial isolates i.e., CPR21A, CPR22A, CPR30C, CPR46A and CPR65A showed potassium-solubilising index (K-SI) more than 3.0 and remaining 5 rhizobial isolates showed K-SI more than 2.0. The solubilisation zone was observed upto seven days.

Table 1: Phosphate, potassium and zinc solubilization capacity of chickpea rhizobial isolates

Sr. No.	Rhizobial isolates	Phosphate Solubilization Index (P-SI)	Potassium Solubilization Index (K-SI)	Zinc Solubilization Index (Z-SI)
1.	CPR65E	3.20		
2.	CPR43A			2.60
3.	CPR57C	-		2.22
4.	CPR2B	-		2.62
5.	CPR10B	-		-
6.	CPR26C	-		5.14
7.	CPR20A	3.00	2.66	3.33
8.	CPR24B	-	-	2.66
9.	CPR40E	-	-	-
10.	CPR21F	-	-	4.00
11.	CPR21A	2.72	3.11	-
12.	CPR65A	-	3.12	-
13.	CPR4C	3.33	2.33	3.81
14.	CPR22A	2.60	3.40	2.50
15.	CPR30C	-	3.33	2.33
16.	CPR59A	2.63	-	-
17.	CPR11E	-	2.77	-
18.	CPR41B	3.16	2.7	2.37
19.	CPR54A	3.33	-	2.55
20.	CPR51B	-	-	2.22
21.	CPR27A	2.15	-	2.18
22.	CPR1A	2.83	2.69	-
23.	CH25	-	-	2.92
24.	CPR46A	-	4.16	2.55

Fig 1: *Rhizobium* isolate CPR65E showing P-solubilization zone on Pikovskaya medium plate

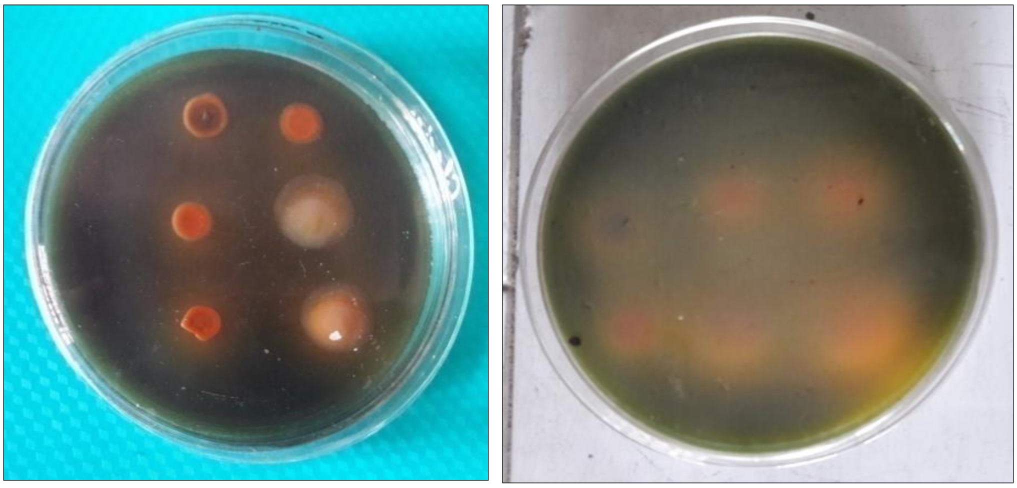

Fig 2: Rhizobial isolates showing K-solubilization zone on modified Aleksandrov's medium plates

Discussion

Most agricultural soils contain large reserves of phosphorus, a considerable part of which has accumulated as a consequence of regular applications of phosphate fertilizers. Individual or co-inoculation of PSB with other groups of microorganisms enhanced the plant growth by increasing the efficiency of biological nitrogen fixation [7]. Rhizobial isolates are differentially variable in their P-solubilizing ability with different sources of inorganic phosphate. For example, Alikhani *et al*. (2007) [8] assessed 446 bacteria belonging to the genera *Bradyrhizobium*, *Mesorhizobium*, *Sinorhizobium* and *Rhizobium* for phosphate solubilization potential and observed that 44% of the isolates solubilized tricalcium phosphate, while 76% solubilised phytate. *R. leguminosarum* bv. *viciae* was most efficient P-solubilizer followed by *M. ciceri*, *M. mediterraneum*, *S. meliloti* and *R. phaseoli* [8]. In other similar studies, Singh *et al*. (2014) reported that 70% of rhizobial strains showed phosphorous solubilization with a solubilization index ranging between 2.2 to 4.1 on Pikovskaya·s agar medium plates [9]. Similarly, Midekssa *et al*. (2016) characterized 52 bacterial isolates from chickpea rhizosphere for phosphate solubilization and observed that solubilization index (SI) of these isolates varied from 1.40 (PSBC126) to 3.06 (PSBC02) [10]. Zafar *et al*. (2017) characterized 17 chickpea rhizobial isolates and reported that all isolates except one were found to solubilize phosphate ranging between 4.53-12.33 µg/ml in broth [3]. Recently, Dhull *et al*. (2018) [6] observed that 50% of clusterbean rhizobial isolates solubilised phosphate on Pikovaskaya‟s agar medium plates [6].

Zinc (Zn) gets fixed in the soil matrix upon application and makes the soil deficient in zinc. Zinc deficiency is a serious constraint to crop production in many parts of the world and this could only be compensated by the application of costly chemical fertilizers, either as foliar or soil applications. Alternatively, numerous microorganisms, especially those associated with roots, have the ability to increase plant growth and productivity by increasing the supply of mineral nutrients of low mobility in the soil like Zn and P [11, 12]. Shahab *et al*. (2008) [13] studied the zinc phosphate solubilization efficiency of ten soil bacteria for various parameters like carbon sources, temperature, pH, variable concentrations of sodium chloride and glucose and found that pH 7 was the most favorable pH for solubilisation [13]. Isolates CMG851 (*Acinetobacter lwoffi*) and CMG852 (*Pseudomonas* sp.) showed enhanced solubilization in presence of 1% sodium chloride. Desai *et al*. (2012) [14] isolated *Azotobacter*, *Azospirillum*, *Bacillus* and *Pseudomonas* strains under *in vitro* conditions from diverse crop production systems and evaluated them for solubilization of 'Zn' and 'P' *in vitro* from insoluble zinc (ZnO, $ZnCO_3$) and phosphorus [tricalcium phosphate (TCP)], respectively [14]. After 15 days of incubation, 15 strains solubilized zinc and produced >50 cm^2 solubilization zone on solid media. Ramesh *et al*. (2014) [15] isolated *Bacillus aryabhattai*-related bacterial isolates with zinc-solubilizing abilities and assured that the strains MDSR7 and MDSR14 produced substantially higher soluble zinc content [15]. They concluded that these strains substantially influenced mobilization of zinc and its concentration in edible portion, yield of soybean, wheat and can be utilized as bio-inoculants for biofertilization and biofortification. Sharma *et al*. (2014) [16] isolated forty eight endophytic bacterial isolates from soybean (43) and summer mungbean (5) rhizosphere and screened them for zinc solubilizing ability. Endophytes 1J (*Klebsiella spp*.) and 19D (*Pseudomonas spp*.) were found to be promising bacterial isolates as they solubilised zinc with both inorganic sources of zinc [16].

Potassium (K) is an essential micronutrient which is also known to encourage plant photosynthesis. Potassium deficiency causes slow plant growth, incomplete root development and burned leaf appearance. Potassium solubilizing microorganisms acts as natural bio-agents which solubilize fixed and unavailable forms of potassium to plant available forms by different mechanisms including chelation and production of organic acids. Similarly, a total of 30 different isolates were isolated from rhizosphere of different crops from Indo Gangetic Plain (IGP) of India and characterized for potassium solubilization. Among these, only 12 were found to make a clearance zone indicating K-solubilization [17]. Fifty five isolates were characterized for their ability to solubilize P and K, based on the zone formation. The Solubilization Index (SI) showed that 55 bacterial isolates were capable to solubilize P and 12 capable to solubilize K [18]. In another report, 5 bacterial isolates were selected exhibiting highest potassium solubilisation. The highest solubilization (46.52 µg/ml) was observed in isolate KSB-1 and was identified as *Bacillus licheniformis* by Biolog system and was followed by KSB-3 (42.37 µg/ml) which was identified as *Bacillus subtilis* [19].

Conclusion

These multi-solubilizer rhizobial isolates are of potential value from the biofertilization point of view. Inoculation of legume and other crop plants with these rhizobial isolates is a better alternative to farmers who cannot afford to buy expensive inorganic fertilizers to overcome mineral deficiencies in crop plants. This process is environmental friendly and also provide renewable source of nitrogen, phosphorus, zinc and potassium which can replace the use of chemical fertilizers. So, isolation and characterization of rhizobial isolates for solubilization of phosphorus, zinc and potassium can proved be an important tool in sustainable agriculture for improvement of legume and cereal production.

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