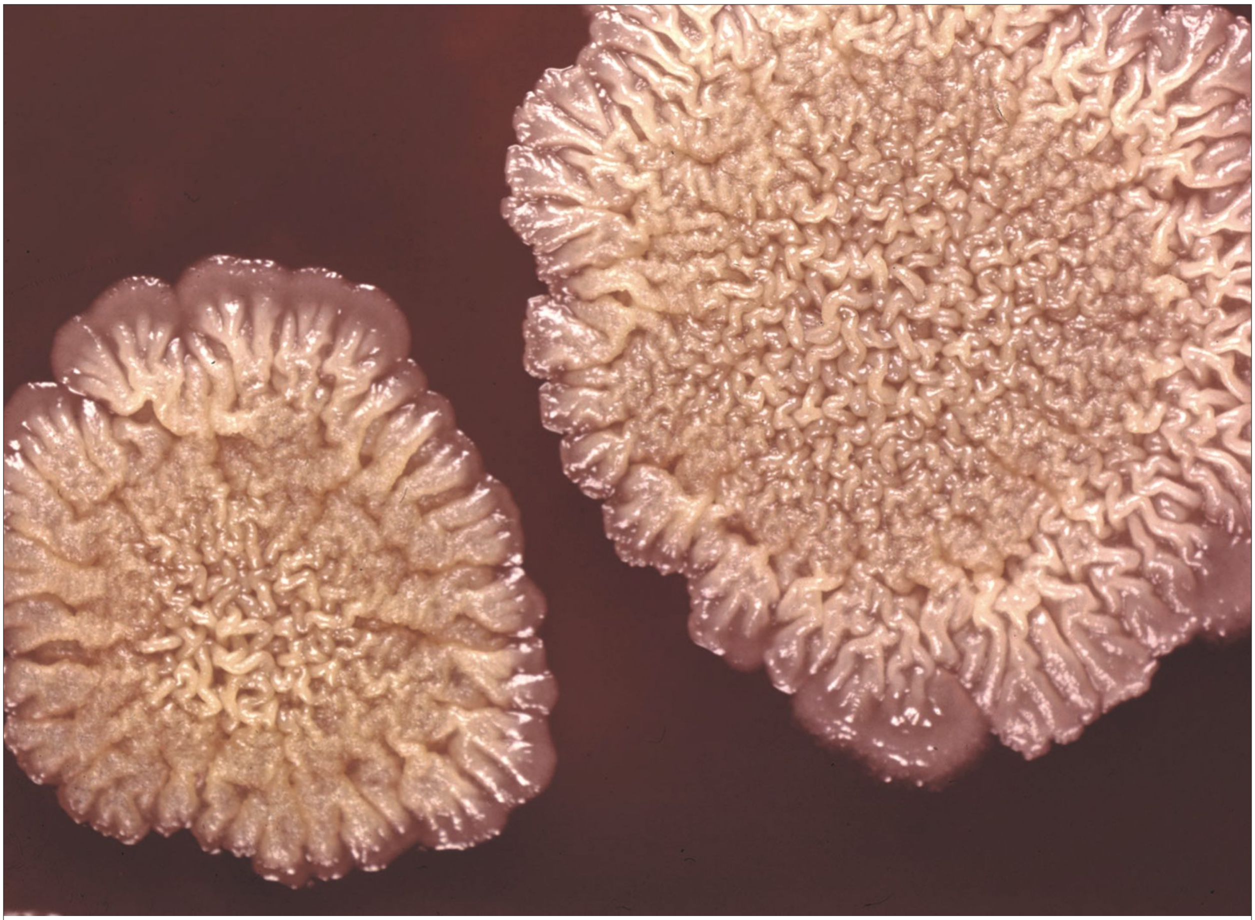

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Selection of Effective Indigenous *Rhizobium* Strain for Seed Inoculation of Chickpea (*Cicer aritenium* L.) Production

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ABSTRACT

Effective indigenous *Rhizobium* strains were selected and evaluated their ability for enhanced nodulation; grain yield and nutrient uptake of chickpea (*Cicer aritenium* L.) at eastern Uttar Pradesh region. Fifty *Rhizobium* strains were isolated from healthy chickpea root nodules. Eight *Rhizobium* strains were selected from out of fifty *Rhizobium* strains, on the basis of visual observations of effective nodules number and fast growth pattern on YEMA medium for field experiment. One reference strains USDA-3378 were selected for field experiment. In field experiment, the indigenous *Rhizobium* strain BHURC04 was found to be highly effective with significant increase in nodules number (73.53%) plant^{-1}, dry weight of nodules (78.07%) plant^{-1} and grain (31.76%) and straw (24.37%) yields followed by BHURC05, USDA-3378 and BHURC03 over uninoculated control. Similarly uptake of N and P by grain (46.93 and 93.26%) and straw (47.01 and 79.56%), respectively maximum in seed inoculation of *Rhizobium* strain BHURC04 over uninoculated control. Indigenous *Rhizobium* strain BHURC04 was found to be highly effective symbiotic nitrogen fixer for uptake of nutrient content and grain yield of chickpea (*Cicer arietinum* L.).

Key words: Root nodule, field experiment, nitrogen, phosphorus

INTRODUCTION

Chickpea (*Cicer arietinum* L.) is a leguminous crop. India is the largest chickpea producing country accounting for 64% of the global chickpea production. Pulses occupy a very important place in Indian diet because they constitute the major source of protein to the predominantly vegetarian population. Many legumes have the ability to form nitrogen (N_2) fixing root nodules with soil bacteria, collectively called rhizobia (Sprent, 2001) and thus contribute to the biological fixation of N_2. Legume and their rhizobia are often introduced to agricultural ecosystems to improve soil fertility and farming systems flexibility (Brockwell and Bottomley, 1995; Sessitsch *et al.*, 2002).

Nitrogen is known to be an essential nutrient for plant growth and development. Intensive farming practices that achieve high yield require chemical fertilizers, which are not costly but may also create environmental problems. The extensive use of chemical fertilizers in agriculture is currently under debate due to environmental concern and fear for consumer health. Consequently, there has recently been growing level of interest in environmentally friendly sustainable agricultural practices and organic farming system (Rigby and Caiceres, 2001; Lee and Song, 2007). Increasing and extending the role of biofertilizers such as *Rhizobium* would reduce the need for chemical fertilizers and decrease adverse environmental effects. Therefore, in the development and implementations of sustainable agriculture techniques, biofertilization is great importance in alleviating environmental pollution and the deterioration of nature (Elkoca *et al*., 2008).

Rhizobium symbiosis with legumes species is of special importance, producing 50% of 175 million tons of total biological nitrogen fixation annually worldwide (Sarioglu *et al*., 1993). Chickpea and *Rhizobium leguminosarum* sub sp. *ciceri* association annually produce up to 176 kg N ha^{-1} depending on cultivar, bacterial strain and environmental factors (Rupela and Saxena, 1987; Beck *et al*., 1991). However, *Rhizobium* species producing nodules in chickpea are specific only to this species and thus inoculation with effective strains is advised in soils with no or weak bacterial presence (Rupela and Saxena, 1987). The symbiosis between chickpea and its specific rhizoia has been recently studied in several countries-Tunisia (Aouani *et al*., 2001), Morocco (Maatallah *et al*., 2002), Canada (Kyei-Boahen *et al*., 2002), Turkey (Icgen *et al*., 2002) and Portugal (Laranjo *et al*., 2002)-because of the promising agricultural usefulness of this crop as a grain legume for human and animal nutrition and because of the interesting extreme host specificity of its rhizobia. The most studied and longest exploited PGPR are the rhizobia (including the *Allorhizobium*, *Azorhizobium*, *Bradyrhizobium*, *Mesorhizobium*, *Rhizobium* and *Sinorhizobium*) for their ability to fix N_2 in their legume hosts (Sawada *et al*., 2003). The symbiotic association is highly specific in chickpea, with a unique group of rhizobia necessary for formation of nodules and nitrogen fixation. Nodulation problem in chickpea attributed to the rhizobial symbiont may be due to absence of appropriate strains, low population, low ineffectiveness, poor survival in soil, or competition amongst strains of rhizobia. Inoculation trials in the eastern region of U.P. indicate yield response of cultivars to application of appropriate rhizobial strains, but the importance of adequate strain testing for their suitability and effectivity prior to an inoculation effort in a given aria is emphasized. The objective of the current work was selection of effective indigenous *Rhizobium* strain for seed inoculation of chickpea (*Cicer aritenium* L.) production.

MATERIALS AND METHODS

Isolation of indigenous *Rhizobium* strains: Effective root nodules were collected from chickpea rhizosphere soils of 50 villages from Varanasi, Mirzapur, Jaunpur and Azamgarh districts of Uttar Pradesh during December, 2006. The uprooted plants were washed in running water carefully and healthy nodules were separated from root with the help of blade and forceps. We sterilized nodule sample by soaking them in 0.1% $HgCl_2$ for 3 min and in 70% ethyl alcohol for 1 min, followed by rinsing in sterile distilled with sterilized water of each step. The well surface sterilized nodules were kept in to sterilized glass vial containing 90 µL sterile water and crushed with the help of sterilized glass rod and made serial dilution up to 10^{-7} was made (Schmidt and Coldwell, 1967) and spread on yeast extract mannitol agar (YEMA) medium (Vincent, 1970). After spreading, Petri dishes were kept in B.O.D. incubator for 2 to 3 days at 28±2°C. Further, sub-culturing of milky and sticky colonies on other new YEMA medium was done by the streaking method to find single and pure colonies of *Rhizobium* strain. The *Rhizobium* strains were isolated and transferred on YEMA containing test tube and stored at 4°C in the refrigerator. Eight *Rhizobium* strains were selected from out of 50 *Rhizobium* strains, on the basis of visual observations of effective nodules number and colour on the root and fast growth pattern on YEMA medium for further testing in field conditions.

Culture, media and growth condition: Effective nitrogen fixing *Rhizobium* strain BHURC01, BHURC02, BHURC03 BHURC04, BHURC05, BHURC06, BHURC07, BHURC08 one reference strains USDA-3378 were grown in yeast extract mannitol (YEM) broth medium. *Mesorhizobium ciceri* strain USDA 3278 was obtained from Agricultural research services patent culture collection, United States Department of Agriculture, America. The culture were maintained by periodic transfer and stored in the refrigerator for further studies. All media component was purchased from Himedia Pvt Ltd., Mumbai.

Host seeds: Seeds of chickpea (*Cicer arietinum* L.) cv. Radhey were obtained from Indian Institute of Pulse Research (IIPR), Kalyanpur, Kanpur, Uttar Pradesh, India.

Seed bacterization: The *Rhizobium* strains were grown in YEM broth by incubation for 120 rpm at 28±2°C for 48 h. Healthy seeds weighed for each plot of 5 m^2 (@ 100 kg ha^{-1}) were separately inoculated as per treatments in plastic bags with 5 mL of 7 days old broth cultures grown in specific media of respective I noculants (mixed in 1:1 ratio for combined treatments) along with 1 mL of 1% (w/v) sticker solution of gum acacia to ensure bacterial population in the range of 10^7 to 10^8 CFU $seed^{-1}$. After drying for one hour in shade, uninoculated seeds were sown first followed by inoculated seeds just to avoid contamination.

Field experiment for selection of indigenous *Rhizobium* strain: Field experiments, one for selection of most appropriate indigenous *Rhizobium* strain were conducted during winter season of October to March, 2006-2007 at Agricultural Research Farm, Banaras Hindu University, Varanasi. The first experiment was conducted with 10 treatments and 3 replication of 8 *Rhizobium* strain (BHURC01, BHURC02, BHURC03 BHURC04, BHURC05, BHURC06, BHURC07, BHURC08) and an exotic strain (USDA-3378) and one uninoculated control on chickpea (*Cicer arietinum* L.) cv. Radhey. The plot size was 12 m^2 and spacing 25 cm between row and 10 cm between plants. The physico-chemical properties of initial soil of filed experiment was sandy clay loam in texture with 40.83% water holding capacity, neutral in reaction (pH 7.25) and electronic conductivity 0.155 dS m^{-1}, organic carbon (0.778%) (Walkley and Black, 1934); available N (213.24 kg ha^{-1}) (Subbiah and Asija, 1956); P_2O_5 (27.22 kg ha^{-}) (Olsen, 1954) and K_2O (254.76 kg ha^{-1}) (Jackson, 1967); respectively in soil. The microbial population of total bacteria, fungi and actinomycetes (4.5×10^{-8}, 3.1×10^{-8} and 3.4×10^{-8} CFU g^{-1} soil) (Aneja, 2003), respectively.

Assessment of nodulation plant growth and yield: Study of nodulation and plant growth attributes was done 70 days after sowing (DAS). Five plants were randomly uprooted from each plot. Plant roots with the lump of the soil were washed in running water. Nodules were separated carefully. Different parameters like number of nodules and oven dried weight of nodules root and shoot $plant^{-1}$ were recorded. Physiologically matured crop of both experiments was harvested during March.

Chemical analysis plant sample: The nodules, plants and grains samples were digested in 4:1 of HNO_3: $HClO_4$ for total P (Vanadomolybdophosphoric acid yellow colour methods) (Jackson, 1967) in diacid mixture of 9:1 of H_2SO_4: $HClO_4$ for the analysis of total N (Nessler's reagent method) (Jackson, 1973) and Fe in nodules (Atomic Absorption Spectrophotometer method) (Jackson, 1967).

Experimental design: The experiment was arranged in a randomized block design and was replicated three times. Statistical analysis was conducted using one-way Analysis of Variance (ANOVA) using SPSS 12.0 software. Comparisons of means were performed by the Fisher's Protected LSD test at $p \leq 0.05$.

RESULTS

Effect of indigenous *Rhizobium* strain on nodulation, growth and yields: Results pertaining to performance of different indigenous rhizobial isolates used in field conditions had shown variability in nodulation and plant growth (70DAS) and yield of chickpea at harvesting stage (Table 1). The isolate BHURC04 was found to be most effective with maximum values of nodules number (73.53%) and dry weight (78.07%) of nodules dry weight of root (44.00%) and shoot (69.47%) per plant followed by reference strain USDA-3378 and BHURC05 significant increase over uninoculated control. The high nodulation and dry matter accumulation have shown competency of these three isolates in establishing symbiosis for nitrogen fixation which was very much conform from the iron content of 30.2, 26.95 and 24.69%, as the major constituent of nitrogenase enzyme in nodules caused by BHURC04, USDA-3378 and BHURC05, respectively. Therefore the stains BHURC04, USDA-3378 and BHURC05 showed significantly higher grain yield (31.76, 30.64 and 30.16%), respectively and straw yield BHURC04, USDA-3378 and BHURC05 (24.37, 26.02 and 19.17%), respectively as compare to control (Table 1). The other isolates which could not show better response in terms of nodulations growth and yield might be due to their competition for nutrition with native *Rhizobium* or their poor survivability and low infectivity in the soil.

Effect of indigenous *Rhizobium* strain on content and uptake of N and P: The effect of indigenous *Rhizobium* strain on the fractions of N and P (Table 2) was varying in the range of 3.50 to 4.06% and 0.31 to 0.52% in grains and 0.44 to 0.56% and 0.11 to 0.21% in straw, respectively. The maximum significant uptake of N and P in grain (46.93 and 93.26%) and straw (79.56 and 47.01%), respectively over uninoculated control at harvesting stage was recorded in *Rhizobium* strain BHURC04, followed by reference strain USDA-3378 and BHURC05. The

Table 1: Effect of indigenous *Rhizobium* strains on nodulation, growth and yield of chickpea

	Nodules plant^{-1}		Fe %	Dry weight plant^{-1} (g)		Yield (q ha^{-1})	
Treatments	Number	Dry wt. (g)	in nodule	Root	Shoot	Grain	Straw
Control	34 ± 2.49^{a}	0.107 ± 0.06^{a}	38.11 ± 2.03^{ad}	0.25 ± 0.08^{ab}	1.90 ± 0.42^{a}	21.25 ± 2.95^{a}	19.41 ± 0.05^{a}
BHURC06	52 ± 2.08^{b}	0.113 ± 0.04^{ad}	44.76 ± 3.73^{c}	0.28 ± 0.03^{ab}	2.59 ± 0.63^{ab}	21.76 ± 4.28^{a}	17.46 ± 0.09^{b}
BHURC01	32 ± 10.6^{a}	0.107 ± 0.06^{a}	32.45 ± 4.89^{a}	0.24 ± 0.07^{a}	1.87 ± 0.09^{a}	21.00 ± 2.65^{a}	18.92 ± 0.07^{c}
BHURC07	29 ± 1.51^{a}	0.097 ± 0.05^{a}	28.47 ± 2.96^{b}	0.23 ± 0.06^{a}	1.78 ± 0.49^{a}	20.65 ± 1.51^{a}	16.53 ± 0.27^{d}
BHURC08	26 ± 1.78^{a}	0.073 ± 0.04^{a}	23.10 ± 2.96^{b}	0.21 ± 0.07^{a}	1.75 ± 0.44^{a}	19.55 ± 3.10^{a}	18.06 ± 0.14^{e}
BHURC05	58 ± 2.64^{b}	0.173 ± 0.03^{bc}	47.52 ± 5.20^{bd}	0.36 ± 0.02^{b}	3.20 ± 0.79^{b}	27.66 ± 0.76^{c}	23.13 ± 0.36^{f}
BHURC04	59 ± 1.06^{b}	0.190 ± 0.03^{bc}	49.62 ± 10.26^{bd}	0.36 ± 0.06^{b}	3.22 ± 1.36^{b}	28.00 ± 1.80^{d}	24.14 ± 0.14^{g}
BHURC02	53 ± 1.79^{b}	0.130 ± 0.04^{ac}	46.59 ± 4.44^{ad}	0.30 ± 0.02^{a}	2.72 ± 0.54^{ab}	23.16 ± 2.57^{ac}	18.15 ± 0.05^{c}
BHURC03	56 ± 2.00^{b}	0.150 ± 0.01^{ac}	47.34 ± 3.51^{bd}	0.33 ± 0.06^{a}	3.16 ± 0.44^{b}	24.55 ± 3.59^{ab}	20.78 ± 0.05^{h}
USDA-3378	58 ± 3.93^{b}	1.830 ± 0.02^{bc}	48.38 ± 6.192^{bd}	0.35 ± 0.11^{b}	3.21 ± 0.63^{b}	27.76 ± 1.25^{b}	24.46 ± 0.14^{i}

HURC: Indigenous *Rhizobium* strain; USDA-3378, Exotic strain of *Rhizobium*; wt, dry weight; g, gram; Fe, iron; Values are the mean±SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD ($p \leq 0.05$)

Table 2: Effect of indigenous *Rhizobium* strains on content and uptake of N and P by chickpea

	Grain (%)		Straw (%)		Grain uptake (kg ha^{-1})		Straw uptake (kg ha^{-1})	
Treatments	N	P	N	P	N	P	N	P
Control	3.62 ± 0.33^{a}	0.36 ± 0.01^{ac}	0.40 ± 0.05^{ab}	0.13 ± 0.03^{a}	77.43 ± 15.78^{a}	7.57 ± 0.99^{a}	9.21±1.18a	2.25 ± 0.29^{ac}
BHURC06	3.67 ± 0.01^{ac}	0.40 ± 0.01^{b}	0.48 ± 0.08^{ab}	0.13 ± 0.04^{a}	79.75 ± 15.68^{a}	8.67 ± 1.73^{ab}	8.36 ± 1.43^{ac}	2.39 ± 0.74^{ac}
BHURC01	3.60 ± 0.23^{a}	0.34 ± 0.01^{c}	0.47 ± 0.04^{ab}	0.13 ± 0.03^{a}	5.68 ± 11.73^{a}	7.03 ± 0.87^{ab}	8.84 ± 0.79^{a}	2.35 ± 0.42^{ac}
BHURC07	3.56 ± 0.19^{a}	0.31 ± 0.05^{d}	0.45 ± 0.05^{a}	0.12 ± 0.04^{a}	73.30 ± 1.86^{a}	6.37 ± 0.26^{a}	7.44 ± 0.73^{a}	1.98 ± 0.69^{a}
BHURC08	3.50 ± 0.21^{a}	0.26 ± 0.01^{c}	0.44 ± 0.02^{a}	0.11 ± 0.05^{a}	67.96 ± 6.76^{a}	5.08 ± 0.76^{b}	7.91 ± 0.42^{a}	2.07 ± 0.81^{a}
BHURC05	3.96 ± 0.13^{bc}	0.45 ± 0.01^{bc}	0.53 ± 0.07^{ab}	0.18 ± 0.06^{ab}	109.38 ± 0.72^{b}	12.34 ± 0.45^{b}	12.18 ± 1.78^{b}	3.23 ± 0.56^{c}
BHURC04	4.06 ± 0.11^{b}	0.52 ± 0.01^{f}	0.56 ± 0.04^{b}	0.21 ± 0.03^{b}	113.77 ± 9.47^{b}	14.63 ± 0.80^{c}	13.54 ± 0.89^{b}	4.04 ± 0.06^{b}
BHURC02	3.92 ± 0.02^{ab}	0.41 ± 0.01^{e}	0.50 ± 0.05^{ab}	0.14 ± 0.04^{ab}	90.68 ± 10.06^{c}	9.55 ± 1.24^{b}	9.02 ± 0.89^{c}	2.45 ± 0.55^{ac}
BHURC03	3.97 ± 0.10^{bc}	0.43 ± 0.01^{e}	0.51 ± 0.08^{ab}	0.15 ± 0.04^{ab}	97.37 ± 13.37^{b}	10.55 ± 1.54^{b}	10.59 ± 1.69^{c}	3.25 ± 1.04^{c}
USDA-3378	3.97 ± 0.28^{bc}	0.49 ± 0.01^{g}	0.54 ± 0.03^{bc}	0.19 ± 0.07^{bc}	109.96 ± 2.82^{b}	13.49 ± 0.47^{c}	13.23 ± 1.14^{b}	3.64 ± 0.88^{b}

Values are the mean±SD, Mean values in each column with the same superscript (s) do not differ significantly by LSD ($p\leq0.05$)

significant accumulation of N-fraction and its uptake by grain and straw relative to uninoculated control and some of the other inoculated treatment were excellent indicator of symbiotic effectiveness with at par values of isolates in order to BHURC04>USDA-3378>BHURC05>BHURC03>BHURC02. The relative differences of N-accumulation in grains by these isolates were 46.69, 42.2, 41.26, 25.57 and 17.11% over uninoculated control. Considering all the above facts, the *Rhizobium* strain BHURC04, USDA-3378 and BHURC05 were considered to be effective and most synergistic seed inoculation of chickpea.

DISCUSSION

The plant rhizosphere is a major soil ecological environment for plant microbe interactions involving colonization of different microorganisms in and around the roots of the growing plant. This colonization may either result in associative, symbiotic, neutralist or parasitic interactions, depending upon the plant nutrient status in the soil environment.

Seed inoculation of chickpea with *Rhizobium* strains BHURC04 was found more significant nodulation, biomass production and grain yield over uninoculated control.

Rudresh *et al.* (2005) has been reported that inoculation of seed with *Rhizobium* has been enhanced nodulation, growth and yields response of legumes. The similar pattern of grain and straw yields of chickpea was also observed. Increase in gain and straw yield of chickpea due to effective *Rhizobium* inoculation has been also reported by Romdhane *et al.* (2007) and Wani *et al.* (2007). More significant nodule number and nodule dry weight were responsible for significant increased in yield of grain and straw due to high rate of atmospheric nitrogen fixation and its translocated in grain and straw. This finding agreed with the finding of Kyei-Boahen *et al.* (2002) who have found positive correlation between more number and weight of effective nodules and yield of chickpea. Thibodeau and Jaworski (1975) have reported that nitrate reductase activity peaks at early flowering and decreases very rapidly at pod filling stage, while nitrogenase peaks at early pod filling with a less rapid decline in activity. *Rhizobium* strains BHURC04 was found more significant content of nitrogen and phosphorus in grain and straw, respectively and also increase uptake of nitrogen and phosphorus in grain and straw, respectively followed by USDA-3378 and BHURC05 as compare to uninoculated control. Synergestic relationship was found between N and P uptake by grain and straw of chickpea as also reported by Andrew (1977), Arya *et al.* (2007) and Verma *et al.* (2010) in legumes.

CONCLUSION

In this study, isolation and selection of indigenous *Rhizobium* strain BHURC04 was found to be highly effective symbiotic nitrogen fixer followed by USDA-3378 and BHURC05 for uptake of nutrient content and grain yield of chickpea (*Cicer arietinum* L.). Overall study of field experiment showed that *Rhizobium* strain BHURC04 may be effective symbiotic nitrogen fixing bacteria for chickpea production.

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