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Assessment of benign bacterial isolates for plant growth promotion of coriander (*Coriandrum sativum*) crop

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Abstract

The purpose of this work was to isolate and identify native benign rhizobacteria with the ability to promote growth of Coriander (*Coriandrum sativum* L. cv Acr-1) from spice plants collected in the State of Rajasthan, India. Six bacterial isolates were identified that produced indole acetic acid (4.72–32.20 µg mL^{-1}) and shown tricalcium phosphate solubilization potential. The phosphate solubilization index of these benign rhizobacteria ranged from 1.18 to 2.45. All the rhizobacterial isolates were evaluated based on their ability to promote growth of coriander. Plants inoculated with *B. megaterium* ISB-28 showed an increase in plant height and no. secondary branches, whereas inoculation with *bacillus subtilis* NRCSS-II hastened germination of coriander seeds as well as increased the no. of primary branches. However, treatment of *Pseudomonas stutzeri* ISB-9 did not resulted into significant positive effect on growth and yield of coriander plants. Maximum seed yield (32.16 g $plant^{-1}$) was recorded with *B. megaterium* ISB-28 followed by *Azotobacter vinelandii* ISB-5 and minimum seed yield was observed with control which was at aper with *Pseudomonas stutzeri* ISB-9. The data showed that native *B. megaterium* ISB-28 and *B. subtilis* NRCSS-II have potential to be used as growth promoting microorganism for coriander, particularly in the state of Rajasthan, India.

Key words : *Bacillus* spp. growth-promoting rhizobacteria, indole acetic acid, phosphate solubilization, rhizobacteria.

Introduction

Coriander (*Coriandrum sativum* L. 2n = 22) is herb, generally grown as spice crop in India. It shows broad adaptation as crop, growing well under many different types of soil and weather conditions (Simon, 1990), even at extreme latitudes and elevations. All parts of the plant are edible, but the fresh leaves and dried seeds are most commonly used in cooking. The dried seeds are mainly used either whole or in ground form as spice for adding taste and flavour in different food stuffs whereas, green leaves are sprinkled to garnish a variety of dishes. This edible plant is non-toxic to humans, and the *C. sativum* essential oil is thus used in different ways, viz., in foods and in pharmaceutical products as well as in perfumes (Mandal and Mandal, 2015). Linalool is the main constituent of essential oil which constitutes more than 50% of the total essential oil. During 2015-16, coriander was cultivated on 6,20,000 ha with coriander seed production of 5,60,000 tonnes in India with average yield of 903 kg ha^{-1} (Annonymous, 2017). With in India, Rajasthan, Gujarat, Madhya Pradesh and Uttar Pradesh states are mainly producers of coriander crops as seed however cultivation of coriander for green leafy purpose is thoroughly distributed in entire India. About one-third of total production of coriander in Rajasthan comes from of south western Rajasthan. The variety Ajmer coriander-1(Acr-1) of coriander is cultivated as a most popular variety due to its resistance towards stem gall disease. Coriander is not only a spices crop but a main source of farm income in low-input agro-ecosystems in Rajasthan, India (Malhotra and Vashitha, 2007).

Plant-associated bacteria that are able to colonize roots are called rhizobacteria and can be classified into beneficial, deleterious, and neutral groups on the basis of their effects on plant growth. Rhizobacteria that stimulate plant growth are usually referred to as Plant- Growth-Promoting Rhizobacteria (PGPR), a group that includes different bacterial species and strains belonging to genera such as *Acetobacter*, *Azospirillum*, *Azotobacter*, *Bacillus, Burkholderia, Herbaspirillum,* and *Pseudomonas* (Glick, 1995). There benign bacterial isolates may promote growth directly, e.g. by fixation of atmospheric nitrogen, solubilization of minerals such as phosphorus, production of siderophores that solubilize and sequester iron, or production of plant growth regulators i.e. hormones (Grover *et al.,* 2009). Microbiological profile of coriander (*Coriandrum sativum* L.) crop rhizosphere in Rajasthan was studied with an aim to isolate native plant growth promoting rhizobacteria (Mishra *et al.,* 2013). Inoculation of crop plants with different strains of plant growth promoting rhizobacteria increased the yield and quality of

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several medicinal herbs including spice crops (Kumar *et al.*, 2013; Sahay and Patra, 2014, Mishra *et al.*, 2016.). Because of the importance of *Coriandrum sativum* in India, the purpose of this work was to isolate and identify native bacteria benign that have the ability to promote growth of coriander crop.

Material and methods

To carry out this work, rhizobacteria were isolated from roots of *Coriandrum sativum* L., *Foeniculum vulgare* Mill. and *Plantago ovata* Fossrk. collected from Rajasthan, India. To assay the Indole acetic acid (IAA) production, approximately 1×10^8 CFU mL^{-1} of each bacterium was inoculated in Luria–Bertani (LB) supplemented with 50 µg mL^{-1} tryptophan as precursor of IAA. Samples were incubated (30 °C, 180 rpm, 72 h), centrifuged (3000×*g*) for 15 min, and 1 mL of each supernatant was mixed with 1 mL of Salkowski reagent (1.5 mL of 0.5 M $FeCl_3 \cdot 6H_2O$ in 80 mL of 60 % H_2SO_4). Samples were incubated in the dark at room temperature for 30 min, a pink color indicated a positive test. IAA concentrations were determined at 535 nm using a standard curve of IAA (Himedia, India). To evaluate the phosphate solubilizing activity, 0.8 µL of bacterial culture at 1×10^{-8} CFU mL^{-1} was point inoculated onto NBRIP's medium and incubated at 30 °C for 7 days (Nautiyal, *et.al.*, 2000). Isolates that acidified the medium (change in color from blue to yellow) and produced clear zones around colonies were chosen. Phosphate solubilizing capacity of each isolated was calculated using the solubilization index (SI), SI = A/B, where A is the total diameter (colony diameter + halo diameter), and B is the colony diameter.

Micro plot trial was carried out in experimental farm of Indian council of agricultural Research (ICAR)-National Research Centre on seed Spices, Ajmer, Rajasthan, India, using a randomized block design with four replicates. The soil under study was sandy loam in texture classifed as Typic Ustorthents (USDA), EC 0.8 dSm-1, pH 8.1 and organic carbon 0.23%. The major available nutrients like N, P_2O_5 and K_2O were quantified as 118.0, 18.50 and 135 kg ha^{-1}, respectively. Unsterile field soil was used to evaluate the plant growth promotion potential of the selected six rhizobacteria using coriander (*C. sativum* cv. Acr-1) as a host plant (Malhotra and Vashitha, 2007) Each treatment was raised in 12 rows, each of 4 m length 3 m width, with an inter row spacing of 25 cm. Inoculums of rhizobacterial isolates were prepared by growing the culture up to 48 h at 30°C till 8 log CFU mL^{-1}. Surface-sterilized coriander seeds were then soaked with this bacterial suspension for 4 h at 30°C on a shaker at 100 rpm in a BOD incubator cum shaker. Control seeds were soaked in 0.85% saline. Harvesting was carried out after 115 days of sowing and data was recorded at the same time. Values for shoot lengths (cm), number of umbels, umbellets and number of seed umbellate^{-1} are the mean of 10 plants with four replicates. Values for the seed yield plant^{-1} (g) are the mean of 10 plants of each treatment with four replications. To identify bacterial isolates by 16S rDNA, bacterial DNA was amplified using the standard eubacteria primers and then nucleotide sequences were compared using GenBank data from NCBI (www.ncbi.nlm.nlh.gov) and submitted to GenBank for accession.

Results and discussion

In present investigation six promising benign rhizobacteria were selected from sub humid to semi-arid climate region of Rajasthan, India (Mishra *et al.*, 2013). In order to identify the rhizospheric bacterial strains, 16S rDNA was amplified obtaining amplicons of ~1.5 kbp, which were sequenced and compared with GenBank data in NCBI. As such, we identified Azotobator vinelandii (one isolate) *B. subtilis* (two isolate), *B. megaterium* (one isolates), and *Pseudomonas stutzeri* (one isolates) (Table 1).

One of the key aspects of plant growth promoting rhizobacteria is the production of plant hormones. In this regard, one of the most common and naturally-occurring plant hormone is the indole acetic acid (IAA). Recently, it has been demonstrated that *Bacillus* strains isolated from rice synthesized IAA in concentrations from 0.1 to 30 µg mL^{-1} (Beneduzi *et al.*, 2008). Bacterial strains isolated in this work produced IAA in a range from 4.72 to 32.2.3 µg mL^{-1}, which are within the range reported for rhizobacteria isolated from rice roots. *B. subtilis* NRCSS-II, *B. megaterium* ISB-28 and *B. subtilis* NRCSS-I showed higher IAA production, with values of 32.20, 26.85, and 18.23 µg mL^{-1}, respectively, which were significantly higher than those of the other isolates (Fig. 1**)**. It has been reported that IAA production by bacteria can vary among different species and strains, and it is also influenced by culture condition, growth stage and substrate availability (Mohite, 2013). Moreover, isolates from the rhizosphere are more efficient auxin producers than isolates from the bulk soil (Sarwar and Kremer, 1992).

The six rhizobacterial isolates described in this study did not show significant difference in the phosphate solubilization index, from 1.18 to 2.45 except *Azotobacter vinelandii* ISB-5 (Fig. 2). This index is higher compared with that reported in rhizobacterial isolates from rice (Badía *et al.*, 2011). Nevertheless, the solubilization index of *Bacillus* strains in this work were lower than those obtained

Table 1. Identification of rhizobacteria isolated from *Coriandrum sativum* seedling using16S rDNA sequence analysis.

Rhizobacterial isolates	Size sequence (bp3)	Identity	
		Similitude (GenBank number)	% sequence similarity
ISB-5	1433	*Azotobacter vinelandii* (gb| KF365886.1|)	99
Cor-15	1456	*Bacillus aerophilus* (gb|KU508625.1|)	99
NRCSS-I	1364	*B. subtilis* (gb|KU508624.1|)	99
NRCSS-II	1459	*B. subtilis* (gb|KU508626.1|)	99
ISB-28	1349	*B. megaterium*(gb| KF365888.1|)	99
ISB-9	1340	*Pseudomonas stutzeri* (gb| KF365887.1|)	99

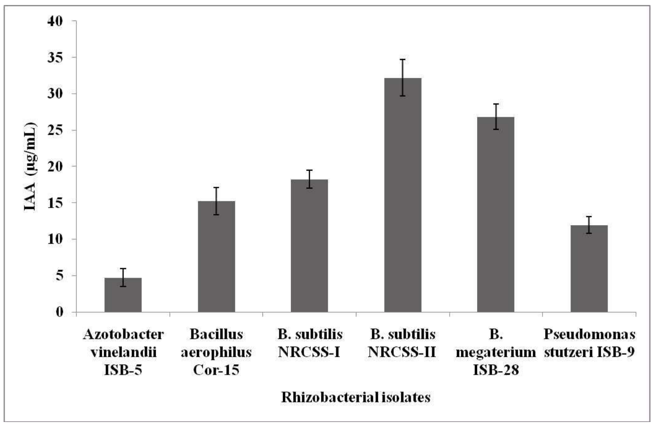

Fig. 1. Indole acetic acid (IAA) production by the rhizobacterial isolates. Vertical bars indicate the standard errors (n = 3).

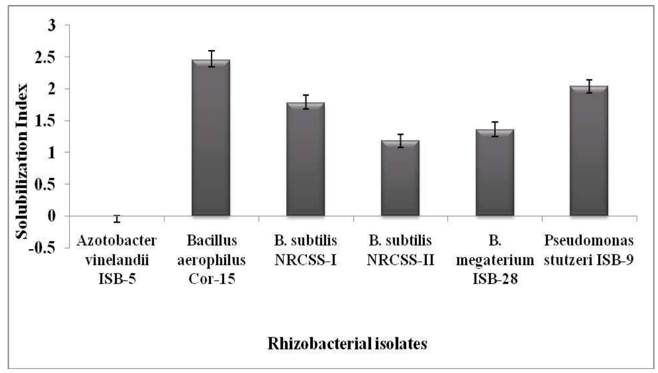

Fig. 2. Phosphate solubilization index of the rhizobacterial isolates. Vertical bars indicate the standard errors (n = 3).

with species of *Bacillus* that promote growth of cotton (Qureshi *et al.*, 2012)

The effects of the six benign rhizobacteria on the growth of *C. sativum* plants were evaluated with control. Significant differences in some of the variables were observed. Plants inoculated with isolate *B. megaterium* ISB-28 showed significantly higher ($P < 0.05$) plant height, whereas plants inoculated with *B. subtilis* NRCSS-II showed higher number primary branches. *B. megaterium* ISB-28 showed significantly more number of secondary branches along with umbels plant^{-1}, umbellets umbel^{-1} and no. of seed umbellets^{-1} compared with the control (Table 2). In contrast, a negative effect was observed on bacterial isolates *Pseudomonas stutzeri* ISB-9 on umbels per plant,

Table 2. Effect of Rhizobacterial isolates on the plant growth and yield of coriander plants

Rhizobacterial isolate	Plant height (cm)	No. Pri. branches plant^{-1}	No. Sec. branches plant^{-1}	Umbels plant^{-1}	Umbellates umbel^{-1}	Seeds umbellate^{-1}	Seed yield (g plant^{-1})
Azotobacter vinelandii ISB-5	76.74	7.16	59.24	77.00	5.05	7.95	22.99
Bacillus aerophilus Cor-15	79.82	8.08	63.16	82.30	5.20	8.30	11.89
B. subtilis NRCSS-I	97.24	7.99	67.91	86.20	5.30	7.95	15.37
B. subtilis NRCSS-II	97.24	19.08	68.07	90.45	8.50	8.20	18.56
B. megaterium ISB-28	110.33	14.20	70.41	106.05	8.55	12.45	20.91
Pseudomonas stutzeri ISB-9	80.65	7.33	62.80	64.20	3.72	5.50	10.91
Control	76.25	6.50	54.20	70.23	3.40	5.80	8.95
SEm ±	5.62	0.66	5.50	6.19	0.12	0.35	3.21
CD(0.05)	17.34	2.05	16.95	19.07	0.37	1.08	1.37

in comparison to control, which is one of the important attributes of seed yield in coriander (Table 2). Previous studies have shown the positive effect of PGPR to promote the germination and growth of *C. sativum* as well as other seed spices crops of India (Mishra *et al.*, 2016 and Mishra *et al.,* 2017). Similar results were found with the inoculation of *B. megaterium* ISB-28 and *B. subtilis* NRCSS-II, and the effect might be attributed to the synthesis of IAA as well as phosphate solubilization. Coriander yield attributes are in accordance with the Kauim *et al.,* (2015) but yield is higher in present investigation which may be due to varietal difference as well as agro-ecological variation. Interestingly, some strains (e.g. Cor-15, ISB-9 and ISB-5) that produce IAA and solubilize phosphate were not able to significantly promote growth of coriander plants for better seed yield. This may be due to some other factors also responsible for the growth-promoting effect benign rhizobacterial isolates. Kalidasu *et al.,* (2008) observed that that inoculation of coriander with microorganisms *Azospirillum,* Phosphobacteria and FYM @ 5 t ha^{-1} applied along with recommended dose of inorganic Nitrogen @ 100 % N had shown significant influence on growth parameters when compared with absolute control. However, the influence of microorganisms is significant only on number of secondary branches and number of umbels $plant^{-1}$ when compared to 100% N. Plant height, number of primary branches, number of umbels $plant^{-1}$ and number of umbellets $umbel^{-1}$ were significantly more in treatment with 100 % N in combination with *Azospirillum*, PSB and FYM @ 5 t ha^{-1} (66.4 cm, 6.2 and 15.4 respectively) than control (58.3 cm, 4.4 and 8.7 respectively). More number of secondary branches were recorded with *Azotobacter*, PSB and FYM @ 5 t ha^{-1} (15.4) which is significantly superior to control (8.7) and 100% N alone (10.2). Maximum number of umbels $plant^{-1}$ was recorded in 100 % N in combination with *Azospirillum*, PSB and FYM @ 5 t ha^{-1} (26.9) which is significantly superior to 100% N alone (19.8) and control (13.3).

Patidar *et al.*, (2016) reported the response of coriander (*Coriandrum sativum* L.) to the application of biofertilizers in combinations with different doses of chemical fertilizers. Plant height, number of primary and secondary branches, number of umbel $plant^{-1}$, number of umbellet $umbel^{-1}$, number of seeds $umbellet^{-1}$, total seed yield $plot^{-1}$, chlorophyll 'a', 'b' and total chlorophyll were found maximum in treatment Azotobacter + AM + PSB + 100% RDF which was at par with Azotobacter + AM + PSB + 80% RDF while it was found minimum in treatment having only biofertilizer. The treatment Azotobacter + AM + PSB + 80% RDF was found superior for better growth, yield, and quality of the plants while taking into consideration of long term beneficial effects of organic sources of plant nutrition.

Therefore, it may be concluded that the isolated and identified six native rhizobacterial species have capability to synthesize IAA and solubilize phosphate. These both parameters appear to be linked to the growth promoting effect on *C. sativum*. Our data shown that *Bacillus megaterium* ISB-28 and *B. subtilis* NRCSS-II have potential to be used as a beneficial rhizobacteria, particularly for organic cultivation of *C. sativum* L. cv Acr1 in the state of Rajasthan, India.

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