

## Isolation and evaluation of phosphate solubilizing microorganisms from fennel (*Foeniculum vulgare* Mill.) rhizospheric soils of Rajasthan

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### Abstract

Fennel plant and soil samples were collected from Ajmer, Pali, Jhalawar, and Kota Districts of Rajasthan for isolation of rhizospheric bacteria on selective nutrient agar medium. Total fourteen soil samples were analyzed for EC, pH and phosphate solubilizing bacteria count. In present investigation of fennel soil samples the EC ranged between 1.02 to 0.15 dS/m whereas pH of collected fennel field soil samples of different districts of Rajasthan state in India ranged from 8.8 to 7.6. Sixteen Phosphate solubilizing microorganism were isolated on Pikovskaya medium containing Tricalcium phosphate and these were further screened on National Botanical Research Institutes phosphate (NBRIP) broth with decolouring of bromophenol blue as indicator of acid production. Quantitative assay of Phosphate solubilization was done in NBRIP broth. The Maximum phosphate solubilization in broth assay was observed in bacterial isolates FEN-14 which was at par with FEN-1 and FEN-5.

**Key words:** Fennel, phosphate solubilizing bacteria, PSB, rhizosphere.

### Introduction

Fennel (*Foeniculum vulgare* Mill.) belongs to family Apiaceae with chromosome number  $2N=22$  (Masoud *et al.*, 12) and is cultivated as an annual crop in the winter months. Fennel is a native of Southern Europe and Mediterranean Region and one of popular major seed spice in India mainly grown in Rabi season. It is also known as *Saunf* in Hindi and there are many other popular regional names. It is widely cultivated throughout the temperate and subtropical regions of the world and major growing countries are Romania, Russia, Germany, France, Italy, India, Argentina and USA. Major fennel producing states in India are Gujarat Rajasthan, Karnataka, Maharashtra, U.P., Punjab and Bihar and among these, Gujarat and Rajasthan are major fennel producing states. The trend of area, production and productivity of fennel indicate that area and production of fennel is continuously increasing in India. The major importing countries of Indian fennel are USA, EU, Middle East, South East Asia, Japan, Malaysia and Indonesia. As a medicinal plant, fennel seed has been used as an antispasmodic, carminative, diuretic, expectorant, laxative, stimulant, and stomachic been used to stimulate lactation, as a remedy against colic, and to improve the taste of other medicines (Simon *et al.*, 16). The entire plant is a rich source of aromatic oils (10% fats), besides being a rich source of vitamins;

hence, used in food items and is an important ingredient in allopathic and ayurvedic medicine, especially against infections.

Soil microorganisms play important role in soil quality and plant productivity. The development of effective methods for studying the diversity, distribution, and behaviour of microorganisms in soil habitats is essential for a broader understanding of soil health. Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media designed to maximize the recovery of diverse microbial populations. However, only a small fraction (<0.1%) the soil microbial community has been accessible with this approach (Hill *et al.*, 8). Phosphorus (P) an essential element for plant nutrition can only be assimilated as soluble phosphate. It is found in soil as insoluble calcium, iron or aluminum phosphates and organic forms are derived from the decaying plants, animals and microorganisms. A number of soil microorganisms, which include bacteria, fungi and actinomycetes, are known to solubilize unavailable form of calcium bound P through metabolic activity by excreting organic acids, causing acidity in the medium. Phosphatases are known to play a major role in transforming organic forms of phosphorus into plant available inorganic forms. Soil application of such phosphate solubilizing biofertilizers (PSB) would result into the solubilization of naturally

abundant phosphatic compounds in a more environment friendly and sustainable manner. P solubilization by PSB is very common under *in-vitro* conditions but the field level performance of PSB has been sometimes contradictory. Numerous reasons have been suggested for this but none of them have been conclusively investigated. PSB are widely applied in agronomic practices in order to increase the productivity of crop while maintaining the health of soil, despite the variation in their performance (Gaur, 7). Plants suffer nutrient deficiency stress when the availability of soil nutrients (and/or the amount of nutrients taken up) is lower than required for sustaining metabolic processes in a particular growth stage. Factors vital for the differential capacities of plant genotypes to access soil nutrients includes plant genotypes, access to soil nutrients, differences in the surface area of contact between roots and soil and the composition and amount of root exudates and rhizosphere microflora. These various factors eventually lead in differences in the chemistry and biology of the rhizosphere. Several investigations have been made on agronomical aspects to increase the yield of fennel on normal and sodic soils (Avatar and Mahey, 2; Rai *et al.*, 15). The plant response to microbial inoculation, measured as vigour index showed highest values in fennel crop (1200–1300), with 40–80% increase over controls (no inoculation) was recorded (Kumar *et al.*, 10). Traditionally, the analysis of soil microbial communities has relied on culturing techniques using a variety of culture media designed to maximize the recovery of different microbial species. There are numerous examples where these techniques have revealed a diversity of microorganisms associated with various soil quality parameters such as disease suppression and organic matter decomposition (Maloney *et al.*, 11). Although there have been recent attempts to devise suites of culture media to maximize the recovery of diverse microbial groups from soils it has been estimated that less than 0.1% of the microorganisms found in typical agricultural soils are culturable using current culture media formulations (Atlas and Bartha, 1). This is based on comparisons between direct microscopic counts of microbes in soil samples and recoverable colony forming units. The ability of a few soil microorganisms to convert insoluble forms of phosphorus to an accessible form is an important trait in plant growth-promoting bacteria for increasing plant yields. The use of phosphate solubilizing bacteria as inoculants increases the P uptake by plants. Keeping these information's under consideration, the present investigation was conducted

for isolation and evaluation of phosphate solubilizing microorganisms from fennel (*Foeniculum vulgare* Mill.) rhizosphere soils.

## Materials and methods

Fennel plant and soil samples have been collected from Ajmer, Pali, Jhalawar, and Kota Districts of Rajasthan for isolation of rhizospheric bacteria. Soil samples were collected from various fennel cropped sites and about 50 g of soil sample was taken from the upper 30 cm of the soil profile. All the fennel field soil samples have been analyzed for electrical conductance (EC) and pH. The serially diluted soil samples were inoculated by pour plating technique on standard agar medium (pH 6.8–7.0) containing 5 g of tricalcium phosphate (TCP) as sole phosphorus source for selectively screening the bacteria which have the ability to release inorganic phosphate from tricalcium Phosphate (Nautiyal, 14). After 3 days of incubation at 30°C, bacterial colonies developed clear zones around colonies were selected. Colonies with clear zones were further purified by replating on agar medium supplemented with TCP. Sixteen phosphate solubilizing bacterial strains thus isolated were selected for further analysis. These fennel crop rhizospheric and soil isolates were designated as FEN-1 to FEN-16.

For the quantitative evaluation of P-solubilization potential these phosphate solubilizing bacteria were inoculated into 100 ml NBRIP broth containing tricalcium phosphate. After incubation for 3-days, pH of the medium was recorded with a pH meter equipped with glass electrode. Dissolved phosphate concentration in the culture filtrate was determined by vanado-molybdate method as described by Kaushik *et al.*, (9) and expressed in terms of µg /ml phosphorus released in culture broth. Based on diameter of radial growth of bacterial isolates and zone of clearance on Pikovskaya medium agar plates phosphate solubilization index [= the ratio of the total diameter (colony + halo zone) to the colony diameter] was calculated (Edi Premono *et al.*, 6). All the PSB isolates were further screened for their pH tolerance.

## Result and discussion

The standard soil chemical analyses used for determining the concentration and availability of nutrients in the bulk soil have at best only indirect relevance to nutrient availability at the root surface where uptake into the root cells takes place. In addition, the fraction of soil nutrients available to various plant species and genotypes differs widely suggesting a limited value of soil chemical analyses that attempt to determine plant available nutrients. Soil electrical

conductivity (EC) is a measurement that correlates with soil properties that affect crop productivity including soil texture, cation exchange capacity (CEC), drainage conditions, organic matter level, salinity, and subsoil characteristics. Soil pH measurement is useful because it is a predictor of various biochemical activities within the soil. In present investigation of fennel soil samples the EC ranged between 1.02 to 0.15 dS/m whereas pH of collected fennel field soil samples of Rajasthan ranged from 8.8 to 7.6. Maximum EC (10.2 dS/m) was recorded for fennel soil samples collected from Khanpura locality in District Jhalawar while minimum EC (0.15 dS/m) was observed with samples of KVK Pali-A. In case of soil pH, maximum pH was observed with fennel field soil samples from Sarwara in District Ajmer whereas minimum pH was recorded with sample of Sanchore in district Jalore of Rajasthan (Table 1). In present investigation, high pH was not always corresponded with high EC as shown in Table 1. Similarly, Mishra *et al.*, (13) have analyzed twenty five coriander plant soil samples collected from Ajmer, Baran, Jhalawar and Kota Districts of Rajasthan in India and reported that maximum EC 1.12 dS/m was observed in Siliya, Kota and minimum EC 0.21 dS/m was recorded in Ummedganj, Kota while maximum pH 8.6 was recorded in Kekari, Ajmer and minimum pH 7.88 was recorded in Bherupur, Jhalawar. Microbial characteristics of soils are being evaluated increasingly as sensitive indicators of soil health because of the clear relationships between microbial diversity, soil and plant quality, and ecosystem sustainability (Doran *et al.*, 5). The availability of nutrients in the rhizosphere is controlled by the combined effects of soil properties, plant characteristics and the interaction of roots with microorganisms.

Generally, phosphate solubilizing potential of microbes is directly related with production of organic acid and thereby making the insoluble phosphate to solubilize. For assessing the acid production capacity of PSB isolates, change in pH was recorded for all the strains in NBRIP broth after 3 and 7 days of incubation at 30 °C in BOD incubator. Irrespective of PSB strain, there was reduction in pH of liquid growth medium at 3 days as well as at 7 days. Highest pH reduction (pH 3.22) was recorded in PSB strain FEN-3 and lowest (pH 5.23) was observed with PSB strain FEN-1. Similar trend was recorded after 7 days of growth of PSB strain in liquid NBRIP broth and maximum pH reduction was observed for FEN-8 which was at par with FEN-2, FEN-3, FEN-4, FEN-10 and FEN-15 where as minimum pH reduction was recorded with FEN-16 (Table 2).

**Table 1.** List of Fennel plant Soil Samples from different districts of Rajasthan.

Village/ District	EC (dS/m)	pH
Kekari, Ajmer	0.34	8.6
Nayakedh, Ajmer	0.43	8.3
NRCSS,Tabiji -A, Ajmer	0.38	8.4
NRCSS,Tabiji -B, Ajmer	0.38	8.5
NRCSS,Tabiji -C, Ajmer	0.37	8.5
NRCSS,Tabiji -D, Ajmer	0.38	8.4
NRCSS,Tabiji -E, Ajmer	0.36	8.6
Sarwara, Ajmer	0.46	8.8
Khanpura, Jhalawar	1.02	8.3
Ummedganj farm, Kota	0.32	8.0
KVK, Pali -A, Pali	0.15	8.1
KVK, Pali -B, Pali	0.22	8.1
KVK, Pali -C, Pali	0.23	8.0
Sanchor, Jalore	0.69	7.6

**Table 2.** Change in pH during growth of PSB isolates in NBRIP broth (control pH- 7.0)

Culture No.	pH after 3 day's	pH after 7day's
FEN -1	5.23	3.21
FEN -2	4.57	3.06
FEN -3	3.22	3.18
FEN -4	4.20	3.08
FEN -5	3.25	4.86
FEN -6	3.38	5.54
FEN -7	4.53	3.82
FEN -8	5.35	3.05
FEN -9	4.75	4.82
FEN -10	4.15	3.08
FEN -11	3.32	3.52
FEN -12	3.78	4.67
FEN -13	4.12	3.89
FEN -14	3.70	4.02
FEN -15	4.17	3.08
FEN -16	5.12	5.01
SEm	0.08	0.08

Phosphate solubilization index as calculated on the basis of bacterial colony diameter and zone of clearance on Pikovskaya agar medium revealed the difference among various PSB strains isolated from fennel rhizosphere and soil samples. The maximum P-solubilization index (2.4) was recorded for FEN-6 which was at par with FEN-1 and FEN-2. The minimum P-

**Table 3.** Screening of selected bacterial isolates for Tricalcium Phosphate solubilization

Bacterial isolates	Phosphate solubilization Index	Tricalcium Phosphate solubilization ( $\mu\text{g/ml}$ )
FEN -1	2.2	51.30
FEN -2	2.2	34.82
FEN -3	2.1	18.86
FEN -4	1.6	23.92
FEN -5	1.9	50.68
FEN -6	2.4	22.77
FEN -7	1.6	38.04
FEN -8	2.0	30.62
FEN -9	1.9	31.67
FEN -10	1.4	27.92
FEN -11	1.3	47.26
FEN -12	1.8	25.94
FEN -13	1.4	38.21
FEN -14	1.7	51.66
FEN -15	1.2	41.13
FEN -16	1.3	15.83
SEm	0.08	1.07
CD 0.05	0.20	2.90

solubilization index was recorded for FEN-15 which was at par with FEN-16 (Table 3). Quantitative screening for phosphate solubilization was assayed with NBRIP broth containing tricalcium phosphate in liquid growth medium and released soluble phosphate was estimated. The

highest P-solubilization was recorded for PSB isolate FEN-14 which was at par with FEN-1 and FEN-5 although these isolates were not showing highest P-solubilization index. However, the minimum P-solubilization was recorded for PSB isolate FEN-16 which was showing least P-solubilization index (Table 3). These isolated PSB strains were tested for their ability to grow on different pH on nutrient agar medium. All the PSB isolates were able to grow in pH range of 6.0 to 8.0 and only few PSB isolates were able to grow on either pH 5.0 or pH 9.0 (Table 4).

Similarly, Chen *et al.*, (4) studied the isolation, screening and characterization of 36 strains of phosphate solubilizing bacteria (PSB) from Central Taiwan and mineral phosphate solubilizing (MPS) activities of all isolates were tested on tricalcium phosphate medium by analyzing the soluble-P content after 72 h of incubation at 30°C. P-solubilizing activity of these strains was associated with the release of organic acids and a drop in the pH of the medium. HPLC analysis detected eight different kinds of organic acids, namely: citric acid, gluconic acid, lactic acid, succinic acid, propionic acid and three unknown organic acids from the cultures of these isolates. An inverse relationship between pH and P solubilized was apparent from their study.

Phosphorous is the second most essential elements for plant growth after nitrogen and it plays a very major role in numerous physiological and biochemical plant actions like photosynthesis, transformation of sugar to starch,

**Table 4.** Screening of PSB isolates to grown at different pH of growth medium

PSB isolates	Genus	pH 5	pH 6	pH 7	pH 8	pH 9
FEN-1	<i>Bacillus</i> sp.	+(w)	+	+	+	+(w)
FEN -2	<i>Bacillus</i> sp.	-	+	+	+	-
FEN -3	<i>Bacillus</i> sp.	-	+	+	+	-
FEN -4	<i>Pseudomonas</i> sp.	+(w)	+	+	+	+(w)
FEN -5	<i>Pseudomonas</i> sp.	+(w)	+	+	+	+
FEN -6	<i>Pseudomonas</i> sp.	+	+	+	+	+(w)
FEN -7	<i>Pseudomonas</i> sp.	+	+	+	+	-
FEN -8	<i>Pseudomonas</i> sp.	+	+	+	+	+
FEN -9	<i>Bacillus</i> sp.	+	+	+	+	+(w)
FEN -10	<i>Bacillus</i> sp.	+	+	+	+	-
FEN -11	<i>Bacillus</i> sp.	+	+	+	+	-
FEN -12	<i>Pseudomonas</i> sp.	+(w)	+	+	+	+
FEN -13	<i>Bacillus</i> sp.	+	+	+	+	-
FEN -14	<i>Bacillus</i> sp.	+	+	+	+	+(w)
FEN -15	<i>Bacillus</i> sp.	+	+	+	+	+(w)
FEN -16	<i>Bacillus</i> sp.	+	+	+	+	+(w)

transporting of the genetic traits. Based on the phosphorous content, soils are classified as low P soils, medium P soils and high P soils (Bunemann *et al.*, 3). Majority of Indian soils are either low or medium P soils and the fennel soil samples collected from different parts of Rajasthan are no exception. Therefore, phosphate solubilizing bacteria as Biofertilizers may be considered among the most effectual plant assistants to supply phosphorous at a favorable level and which have the highest efficiency to enhance plant growth by giving those nutrients in a readily useable form. These PSB-biofertilizers should be produced on the basis of selection of native beneficial soil microorganisms and results obtained in present investigation may be valuable for formulation of native PSB biofertilizers for seed spices crops especially grown in semi-arid regions of India.

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### **References**

1. Atlas, R. M. and Bartha, R. 1998. *Microbial Ecology: Fundamentals and Applications*. Benjamin/Cummings, Redwood City, CA, 694p.
2. Avatar, S. and Mahey, R. K. 1994. A review on agrotechnology of fennel (*Foeniculum vulgare* Mill). *Madras Agric. J.* 81: 655–657.
3. Bunemann, E. K., Bossio, D. A., Smithson, P. C., Frossard, E. and Oberson, A. 2004. Microbial community composition and substrate use in a highly weathered soil as affected by crop rotation and P fertilization. *Soil Biol. Bioche.* 36:889-901.
4. Chen Y. P., Rekha P. D., Arun A. B., Shen F. T., Lai W. A. and Young C. C. 2006. Phosphate solubilizing bacteria from subtropical soil and their tricalcium phosphate solubilizing abilities. *Appl. Soil Ecol.* 34: 33–41.
5. Doran, J. W., Coleman, D. C., Bezdicek, D. F. and Stewart, B. A. 1994. *Defining Soil Quality for a Sustainable Environment*. American Society of Agronomy, SSSA Special Publication No. 35, Madison, WI, USA.
6. Edi Premono, M., Moawad, A. M. and Vlek, P. L. G. 1996. Effect of phosphate-solubilizing *Pseudomonas putida* on the growth of maize and its survival in the rhizosphere. *Indones. J. Crop Sci.* 11: 13-23.
7. Gaur, A. C. 1990. Phosphate solubilizing microorganisms as biofertilizer. *Omega Scientific Publishers*, New Delhi, 176p.
8. Hill, G. T., Mitkowski, N. A., Aldrich-Wolfe L., Emele L. R., Jurkonie D. D., Maldonado-Ramirez S., Lynch S. T., Nelson E. B. and Ficke A. 2000. Methods for assessing the composition and diversity of soil microbial communities. *Appl. Soil Ecol.* 15: 25-36.
9. Kaushik, B. D., Saxena, A. K. and Prasanna, R. 2004. *Techniques in Microbiology: A laboratory manual for post graduate students*, Indian Agricultural Research Institute, New Delhi, India, 60 p.
10. Kumar M., Prasanna R., Bidyarani N, Babu S, Mishra B. K., Kumar A., Adak A., Jauhari S., Yadav Kuldeep, Singh Rajendra and Saxena A. K. 2013. Evaluating the plant growth promoting ability of thermotolerant bacteria and cyanobacteria and their interactions with seed spice crops. *Scientia Horticulturae*, 164: 94–101.
11. Maloney, P. E., van Bruggen, A. H. C. and Hu, S. 1997. Bacterial community structure in relation to the carbon environments in lettuce and tomato rhizospheres and in bulk soil. *Microb. Ecol.* 34:109–117.
12. Masoud S., Kalhor-Home N. and Poorneydane A. 2007. Cytogenetic study of some populations of *Foeniculum vulgare* (Umbelliferae) in Iran. *Caryologia*, 60: 257-261.
13. Mishra, B. K., Sharma Ashutosh, Aishwath, O. P., Sharma, Y. K., Kant, K., Vishal, M. K., Saxena, S. N. and Ranjan, J. K. 2013. Microbiological profile of coriander (*Coriandrum sativum* L.) crop rhizosphere in Rajasthan and screening for auxin producing rhizobacteria. *International J. Seed Spices*, 3:59-64.
14. Nautiyal, C. S. 1999. An efficient microbiological growth medium for screening phosphate solubilizing microorganisms. *FEMS Microbiol. Lett.* 170: 265-270.
15. Rai, S. K., Katiyar, R. S., Singh, S. P., (2002). Effect of nitrogen and phosphorus on the growth and yield of *Foeniculum vulgre* on the sodic soil. *J. Med. Arom. Plant Sci.* 24: 65–67.
16. Simon, J. E., Chadwick, A. F. and Craker, L. E., 1984 —Herbs: An Indexed Bibliography. 1971-1980, the Scientific Literature on Selected Herbs, and Aromatic and Medicinal Plants of the Temperate Zone. Archon Books, Hamden, CT. 770 p.

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