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Isolation, characterization and identification of bacteria isolated from rhizosphere of *Vicia faba* L.

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Abstract

In the present study, seventeen isolates of bacteria were isolated from the rhizosphere of *Vicia faba* L. The isolates were identified on the basis of morphological, biochemical and molecular characterization. Only two isolates were selected for molecular characterization. Phylogenetic analysis was done on the basis of 16S rDNA sequence analysis. Almost all isolates promote plant growth.

Keywords: *Vicia faba* L., Rhizosphere, 16S rDNA gene sequence, plant growth promotion

1. Introduction

Plants are very important for our environment because they balance nature. It is impossible to imagine our environment without plants as they benefit society in various ways like food, feed, shelter, clothing, oxygen, medicine, agriculture etc. ^[1] Legumes are very important for human diet as they are rich in carbohydrates, proteins, vitamins and dietary fiber ^[2].

Faba bean is botanically known as *Vicia faba* L. which belongs to family Fabaceae. It is one of the protein-rich leguminous plants of the Fabaceae family and the third most common grain legume in the world. It is known by several names in India which include Bakala, Kala matar, Raj-rawan, Anhuri, Kadu huralikayee etc. It is an important protein source for both humans and animals ^[3].

The diversity of microorganisms plays a very important role in maintaining the functions of the ecosystem, which support life on earth. According to the World Data Centre for Microorganisms over 1.7 million strains are registered. The interactions between plants and microorganisms are helpful in the field of biofertilizers, bioinoculants, bioprocessing and biofilming. Both plant and types of soil play an important role in the diversity of microorganisms and structural community in the rhizosphere ^[4].

2. Materials and methods

2.1 Collection of seeds

Seeds of *Vicia faba* L. (variety HFB-1 and Vikrant) were taken from Birsa Agricultural University, Kanke, Ranchi, Jharkhand.

2.2 Isolation of bacterial isolates

Plants were grown in pots to collect rhizospheric soils. After flowering, the rhizospheric soils were collected. By serial dilution method the isolates of bacterial culture were obtained from rhizospheric soil. The diluted samples (10^{-1} - 10^{-9}) were then spread on the YEMA plates and were incubated at 28 ± 2 °C for 24 hours. After 24 h colonies were picked up and streaked again on the YEMA plates and were incubated again at 28 ± 2 °C for 24 hours to obtain the pure culture ^[5, 6].

2.3 Morphological characterization of isolates

For morphological characterization the size, form, elevation, margin and pigmentation of bacterial isolates were determined by observing the colonies on YEMA plates grown overnight at 28 ± 2 °C ^[7, 8]. Gram staining technique was used for microscopic observation of the isolates ^[9, 10].

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2.4 Biochemical characterization of isolates

The biochemical tests used for bacterial isolates were starch hydrolysis, casein hydrolysis, gelatin hydrolysis, catalase test, indole test, methyl red test, voges-proskauer test, citrate utilization test, triple sugar-iron agar test, H₂S production test and nitrate reduction test^[10].

2.5 Molecular identification

Total seventeen isolates of bacteria were isolated from the rhizosphere of *Vicia faba* L. Two isolates were selected for molecular characterization (HFB-1NS-VI and VKNS-VII). The quality of extracted DNA from the isolates was evaluated on 1.0% Agarose Gel. By using specific primers the fragment of rDNA gene was amplified by resolving on Agarose gel a single discrete PCR amplicon band of 1500 bp was observed. Forward and reverse DNA sequencing reaction of PCR amplicon was carried out using BDT v3.1 Cycle sequencing kit on ABI 3730xl Genetic analyzer with forward primer and reverse primer.

The rDNA gene sequence was used to carry out BLAST with the database of NCBI Genbank database for the identification of isolates of bacteria. On the basis of maximum identity score, the first ten sequences were selected and aligned using Clustal W. Software. The phylogenetic tree was constructed using MEGA XI.

The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2-

parameter model. On the basis of 1000 replicates the percentage of replicate trees was evaluated using the bootstrap resampling method^[11-13].

3. Results

3.1 Morphological characterization

A total of 17 isolates of bacteria were isolated from the rhizosphere of *Vicia faba* L. Gram staining result showed that 2 isolates were positive and 15 were negative. Colony morphology of the isolates was studied, which revealed that the size was pinpoint, small and moderate, the form was circular, the elevation was flat, raised and convex, the margin was entire and the pigmentation was off white and off yellow.

3.2 Biochemical characterization

Various biochemical tests were conducted, which revealed that only 1 isolate was positive for the starch hydrolysis test, 11 of the isolates were positive for casein hydrolysis test, 5 of the isolates were positive for gelatin hydrolysis test, 11 of the isolates were positive for indole test, 5 of the isolates were positive for methyl red test, 12 of the isolates were positive for citrate utilization test, 4 of the isolates were positive for TSI test, 7 of the isolates were positive for nitrate reduction test, all the isolates were positive for catalase test and all the isolates were negative for VP-test and H₂S production test.

Table 1: Morphological characterization of isolated bacteria from rhizosphere of *Vicia faba* L. (variety HFB-1)

Sl. No.	Isolates	Morphological characterization of isolated bacteria from rhizosphere of <i>Vicia faba</i> L. (variety HFB-1)				
		Size	Form	Elevation	Margin	Pigmentation
1.	HFB-1NS-I	Pinpoint, Small	Circular	Raised	Entire	Off White
2.	HFB-1NS-II	Pinpoint, Small	Circular	Raised	Entire	Off White
3.	HFB-1NS-III	Small, Moderate	Circular	Flat	Entire	Off White
4.	HFB-1NS-IV	Pinpoint, Small	Circular	Raised	Entire	Off White
5.	HFB-1NS-V	Pinpoint, Small	Circular	Raised	Entire	Off White
6.	HFB-1NS-VI	Pinpoint, Small, Moderate	Circular	Flat	Entire	Off Yellow
7.	HFB-1NS-VII	Pinpoint, Small, Moderate	Circular	Raised	Entire	Off White
8.	HFB-1NS-VIII	Pinpoint, Small	Circular	Flat	Entire	Off Yellow

* HFB-1 (Variety of *Vicia faba* L.); NS Rhizospheric soil

Table 2: Morphological characterization of isolated bacteria from rhizosphere of *Vicia faba* L. (variety Vikrant)

Sl. No.	Isolates	Morphological characterization of isolated bacteria from rhizosphere of <i>Vicia faba</i> L. (variety Vikrant)				
		Size	Form	Elevation	Margin	Pigmentation
1.	VKNS-I	Pinpoint, Small	Circular	Raised	Entire	Off White
2.	VKNS-II	Pinpoint, Small	Circular	Raised	Entire	Off White
3.	VKNS-III	Pinpoint, Small, Moderate	Circular	Raised	Entire	Off White
4.	VKNS-IV	Pinpoint, Small	Circular	Raised	Entire	Off White
5.	VKNS-V	Pinpoint, Small	Circular	Raised	Entire	Off White
6.	VKNS-VI	Pinpoint, Small, Moderate	Circular	Convex	Entire	Off White
7.	VKNS-VII	Moderate, Large	Circular	Flat	Entire	Off White
8.	VKNS-VIII	Pinpoint, Small, Large	Circular	Convex	Entire	Off White
9.	VKNS-IX	Pinpoint, Small	Circular	Convex	Entire	Off White

* VK (Variety of *Vicia faba* L.); NS Rhizospheric soil

Table 3: Biochemical characterization of isolated bacteria from rhizosphere of *Vicia faba* L. (variety HFB-1)

Sl. No.	Biochemical Tests	Isolates (Variety HFB-1)							
		HFB-1NS-I	HFB-1NS-II	HFB-1NS-III	HFB-1NS-IV	HFB-1NS-V	HFB-1NS-VI	HFB-1NS-VII	HFB-1NS-VIII
1.	Gram Staining	-ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
2.	Starch Hydrolysis	-ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve
3.	Casein Hydrolysis	+ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve
4.	Gelatin Hydrolysis	-ve	-ve	+ve	-ve	-ve	-ve	-ve	-ve
5.	Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	Indole test	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve

7.	Methyl Red test	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve
8.	Voges-Proskauer test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9.	Citrate Utilization test	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve
10.	TSI test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve
11.	H ₂ S Production test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12.	Nitrate Reduction test	+ve	+ve	-ve	+ve	+ve	-ve	+ve	+ve

* HFB-1 (Variety of *Vicia faba* L.); NS Rhizospheric soil; - = negative result, + = positive result

Table 4: Biochemical characterization of isolated bacteria from the rhizosphere of *Vicia faba* L. (variety Vikrant)

Sl. No.	Biochemical Tests	Isolates (Variety Vikrant)								
		VKNS-I	VKNS-II	VKNS-III	VKNS-IV	VKNS-V	VKNS-VI	VKNS-VII	VKNS-VIII	VKNS-IX
1.	Gram Staining	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
2.	Starch Hydrolysis	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
3.	Casein Hydrolysis	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve
4.	Gelatin Hydrolysis	-ve	-ve	+ve	-ve	-ve	-ve	+ve	+ve	+ve
5.	Catalase test	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve
6.	Indole test	-ve	+ve	-ve	+ve	+ve	+ve	-ve	+ve	+ve
7.	Methyl Red test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
8.	Voges-Proskauer test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
9.	Citrate Utilization test	+ve	-ve	+ve	-ve	+ve	-ve	-ve	+ve	+ve
10.	TSI test	-ve	-ve	-ve	-ve	+ve	-ve	-ve	+ve	+ve
11.	H ₂ S Production test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve	-ve
12.	Nitrate Reduction test	-ve	-ve	-ve	-ve	-ve	-ve	-ve	+ve	-ve

* VK (Variety of *Vicia faba* L.); NS Rhizospheric soil; - = negative result, + = positive result

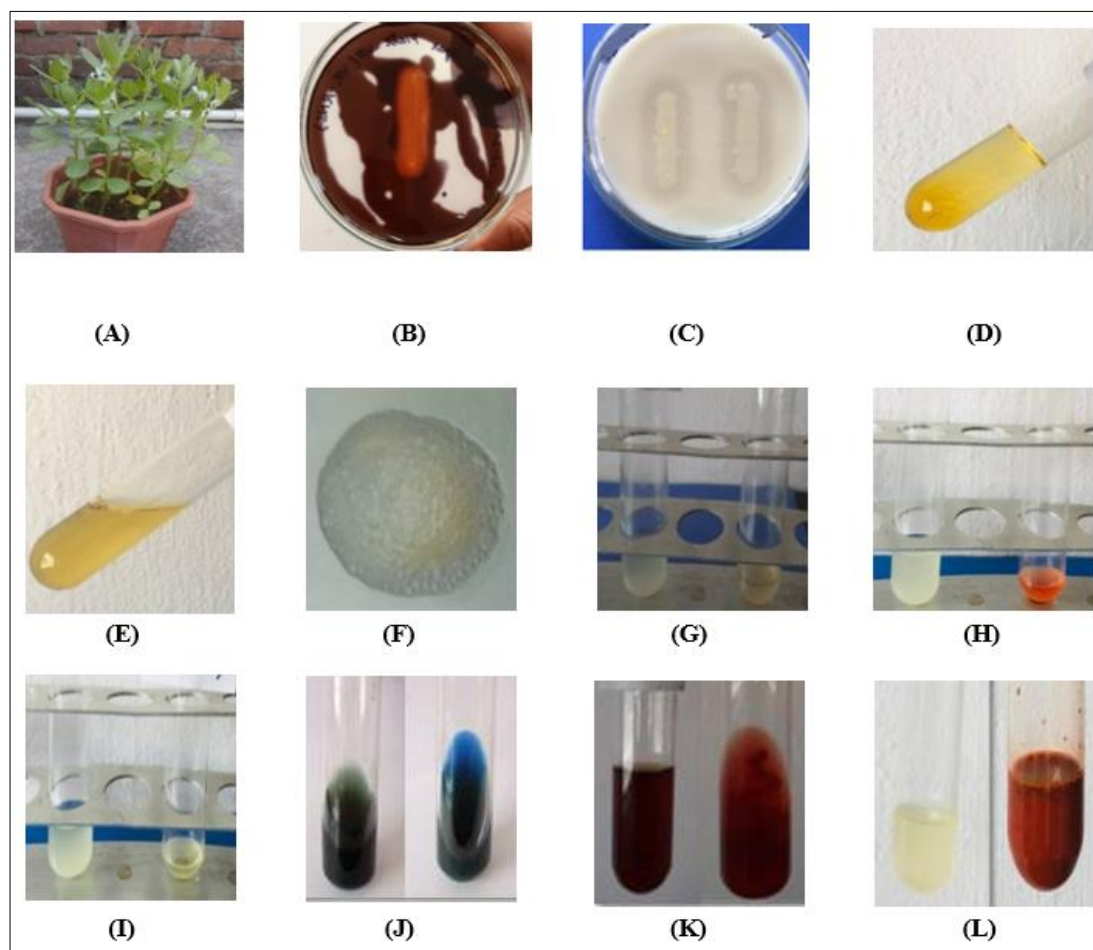


Fig 1: (A) A plant of *Vicia faba* L. (B) Starch hydrolysis test (C) Casein hydrolysis test (D) Gelatin hydrolysis test(negative) (E) Gelatin hydrolysis test(positive) (F) Catalase test (G) Indole test (H) Methyl red test (I) VP-test (J) Citrate utilization test (K) TSI test (L) Nitrate reduction test

3.3 Identification and molecular characterization

The identification of some isolates was made on the basis of biochemical tests performed and also according to Bergey's Manual and it was found that they were *Rhizobium* species. According to 16S rDNA sequencing, two isolates were

identified. BLAST and Phylogenetic tree analysis revealed that one isolate i.e. HFB-1NS-VI showed similarities of 96.77% with *Fictibacillus phosphorivorans* and another isolate i.e. VKNS-VII showed similarities of 90.27% with *Delftia rhizosphaerae*.

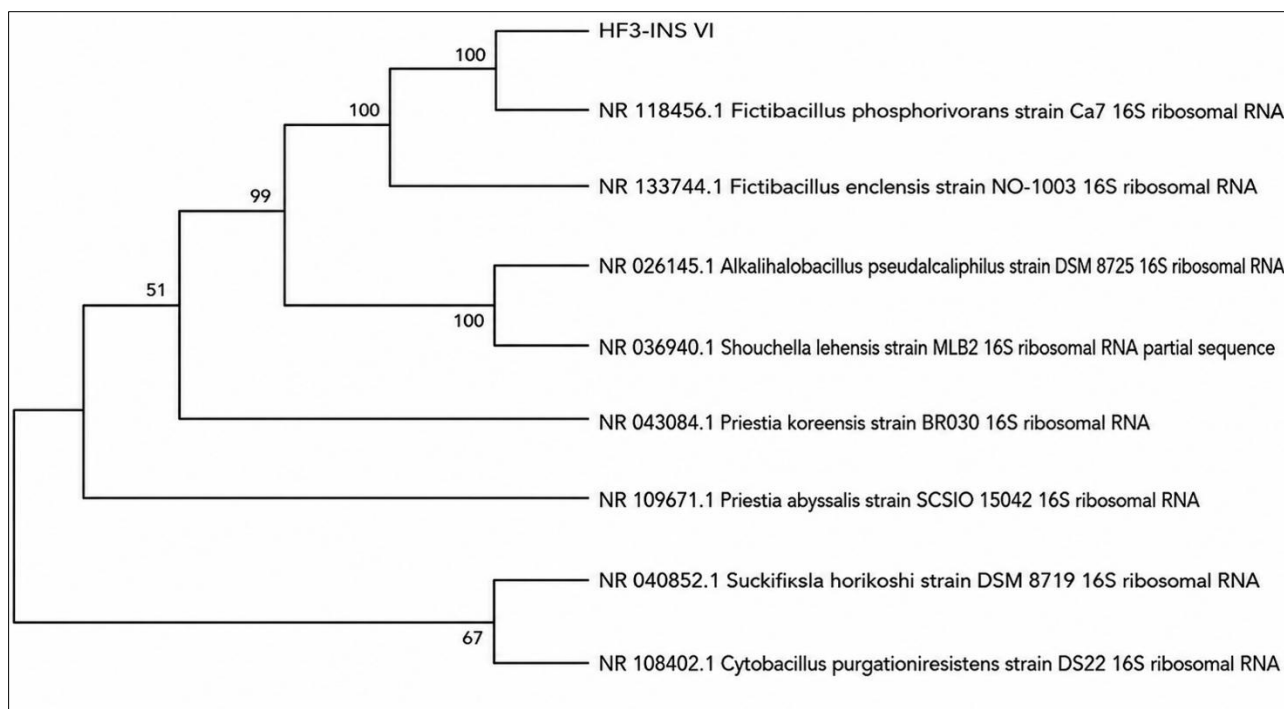


Fig 2: Phylogenetic tree based on 16S rRNA gene sequence of isolate of bacteria i.e. HFB-1NSVI from the rhizosphere of *Vicia faba* L. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2- parameter model. Evolutionary analyses were conducted in MEGA 11

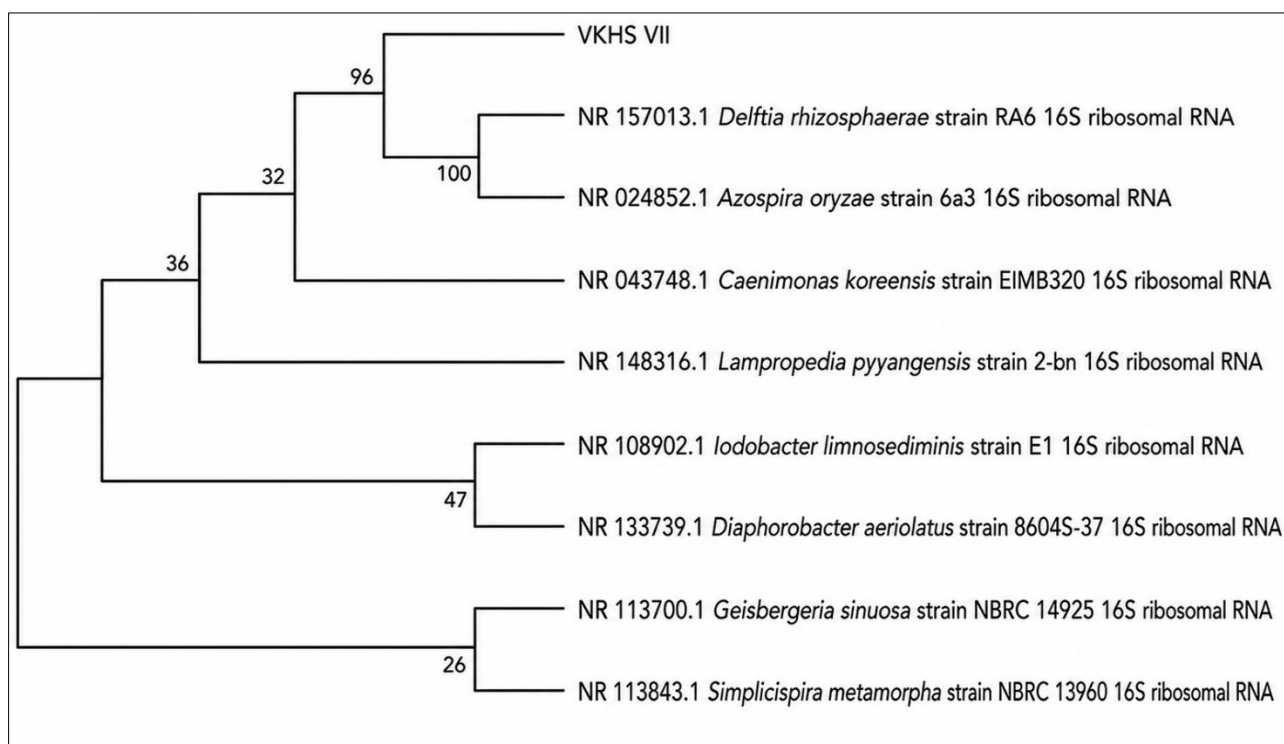


Fig 3: Phylogenetic tree based on 16S rRNA gene sequence of isolate of bacteria i.e. VKNS VII from the rhizosphere of *Vicia faba* L. The evolutionary history was inferred by using the Maximum Likelihood method based on the Kimura 2- parameter model. Evolutionary analyses were conducted in MEGA 11

4. Discussion

The present study deals with investigating the bacteria present in the rhizosphere of *Vicia faba* L. In the rhizosphere the interactions between plants and microbes occur due to rhizo-deposition, which contains several chemical compounds exuded from the roots of plants. (reference from paper 90 67) The isolation and purification of bacterial isolates collected from the rhizospheric soil of both varieties (HFB-1 and Vikrant) of *Vicia faba* L. The

isolates were identified and classified according to their cultural properties, biochemistry etc. A total of 17 isolates were isolated from the rhizospheric soil of both the variety (HFB-1 and Vikrant) of *Vicia faba* L. All the isolates were morphologically characterized on YEMA media plates. Colony morphology of the isolates was studied, which revealed the size, form, elevation, margin and pigmentation. The 2 isolates were found to be gram positive and 15 were gram negative.

The results of biochemical characterization are presented in Tables 3 and 4. Various biochemical tests were performed like starch hydrolysis test, casein hydrolysis test, gelatin hydrolysis test, catalase test, indole test, methyl red test, VP-test, citrate utilization test, TSI test, H₂S production test and nitrate reduction test.

On the basis of morphological and biochemical characterization, and 16S rRNA sequencing the isolates were identified as *Rhizobium* spp., *Fictibacillus phosphorivorans* and *Delftia rhizosphaerae*.

5. Conclusion

Through this research work many bacteria were identified which will be helpful in the field of agriculture. The bacteria found were nitrogen fixing and also help in plant growth promotion. The fertility of the soil can be maintained by effective nitrogen fixing bacteria. Further research may help to increase crop production with the help of beneficial bacteria.

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