International Journal of Science and Research Archive
eISSN: 2582-8185
Cross Ref DOI: 10.30574/ijsra
Journal homepage: https://ijsra.net/

(RESEARCH ARTICLE)

Check for updates

Assessment of bacteria isolated from root nodules and rhizosphere of *Vicia faba* L.

Ghazala Shaheen [1,*], Anita Tudu [1], Vinay Oraon [2] and Latika Sharan [1]

[1] *Department of Botany, Ranchi University, Ranchi, Jharkhand, India.*
[2] *Biotechnology, Department of Botany, Ranchi University, Ranchi Jharkhand, India.*

International Journal of Science and Research Archive, 2024, 12(01), 2185–2192

Publication history: Received on 11 April 2024; revised on 29 May 2024; accepted on 01 June 2024

Article DOI: https://doi.org/10.30574/ijsra.2024.12.1.0901

Abstract

In this present study bacteria were isolated from the root nodules and rhizosphere of *Vicia faba* L. A total of 23 isolates were obtained which were studied morphologically. All isolates were identified on the basis of biochemical tests performed. The isolates obtained in this study can be used to decrease the need of chemical fertilizers and helpful in the production of biofertilizers.

Keywords: *Vicia faba* L; Root nodules; Rhizosphere; *Rhizobium*

1. Introduction

Vicia faba L. (Faba bean) is one of the important leguminous food crops of family fabaceae. They are native to North Africa and Southwest Asia. Faba beans are known by various names like Fava bean, Broad bean, Horse bean, Windsor bean, Tick bean, Bakela, Kala matar, Bakala etc. At present faba beans are grown in 58 countries according to FAO[1]. About 130-160 kg N/ha is fixed by Faba beans[1]. It plays an important role in both crop rotation and in symbiosis[2]. As compared to soybean and maize *Vicia faba* L. contain more ability of rhizospheric acidification[3]. They are rich in protein, energy and fibre[4]. *Pseudomonas, Azospirillum, Azotobacter, Klebsiella, Enterobacter, Alcaligenes, Arthrobacter, Burkholderia, Bacillus and Serratia* are examples of some beneficial bacteria which can enhance the growth of plants[1].

Rhizobia are gram-negative bacteria which help in symbiotic nitrogen fixation in legumes. Legumes contain protein, fat, carbohydrates, bone building minerals and vitamins which are essential for good health. For sustainable long term soil fertility the contribution of fixed nitrogen play a major role in low input agricultural system[5]. The need of chemical fertilizers can be reduced by rhizobia and are good for environment[6]. The temperature of soil required by rhizobia are 25°C to 30°C[7]. The amount of root nodule bacteria is more in rhizospheric soil[8]. The roots of plant contain heterogenous group of microbes called Plant growth promoting rhizobacteria (PGPR)[9]. They play an important role in the stimulation of plant growth and are also used as biofertilizers[10].

2. Materials and methods

2.1. Collection of *Vicia faba* L. seeds (Variety-HFB-1 and Vikrant)

Seeds were collected from Birsa Agricultural University, Kanke, Ranchi, Jharkhand.

* Corresponding author: Ghazala Shaheen

2.2. Surface sterilization and isolation of bacterial strains from root nodules

Root nodules were taken from freshly uprooted plants. After removing all the impurities from the roots of plant, healthy root nodules were selected for the isolation of bacteria. For sterilization, root nodules were properly washed with water and then put in 70% ethanol for 30 seconds. Then they were treated with 3-5% H_2O_2 for 2 minutes and then they were successively washed 3-4 times with sterile distilled water[11-14].

Root nodules were crushed with the help of pestle and mortar and then the contents were spread on Yeast Extract Mannitol Agar plates. Then all the plates were incubated at 28± 2˚C for 24 hours. After 24 h colonies were picked for sub-culturing to obtain the pure culture.

2.3. Isolation of bacterial strains from rhizospheric soil

Isolation was done by the serial dilution method (Dilutions- 10^{-1}-10^{-9})[15]. The dilutions were inoculated on YEMA plates and incubated at 28± 2˚C for 24 hours. Then the diluted samples were spreaded for the separation of microbial colonies from each other in order to get single isolated colony in a mixed culture plate. Then streaking method is used to get the single type of culture on to the Yeast Extract Mannitol Agar plate.

2.4. Culture characterization

2.4.1. Morphological Characterization

For the morphological characterization size, form, elevation, margin, pigmentation and appearance in YEMA media were observed.

Gram Staining

The isolated strains of the bacteria were observed under the microscope using Gram Staining[16].

2.4.2. Biochemical Characterization

For biochemical characterization test like starch hydrolysis, casein hydrolysis, gelatin hydrolysis, IMViC(indole test, methyl red test, voges-proskauer test, citrate utilization test) test, triple sugar-iron agar test, H_2S production test, nitrate reduction test and catalase test were also done[17-21].

3. Results and Discussion

The results of the present study were shown in Tables and figures.

Table 1 Morphological Characterization of the bacteria isolated from *Vicia faba* L. (Variety-HFB -1)

S.No.	Strain	Morphological Characterization of the bacteria isolated from *Vicia faba* L. (Variety-HFB-1) in YEMA media				
		Size	**Form**	**Elevation**	**Margin**	**Pigmentation**
1	HFB-1RN	Large	Circular	Convex	Entire	White
2	HFB-1-(I)	Moderate, Large	Irregular	Convex	Entire	Off White
3	HFB-1-(II)	Small, Moderate, Large	Circular	Raised	Entire	Off Yellow
4	HFB-1-(III)	Small, Moderate,Large	Irregular	Flat	Entire	Off Yellow
5	HFB-1-(IV)	Pinpoint, Small	Circular	Raised	Entire	Off White
6	HFB-1-(V)	Small, Moderate, Large	Irregular	Raised	Entire	Off White
7	HFB-1-(VI)	Pinpoint, Small, Moderate	Circular	Raised	Entire	Off Yellow

8	HFB-1-(VII)	Pinpoint, Small, Moderate	Circular	Raised	Entire	Off Yellow
9	HFB-1-(VIII)	Pinpoint, Small, Moderate	Circular	Raised	Entire	Off Yellow
10	HFB-1-(IX)	Small, Moderate	Circular	Convex	Entire	Off Yellow
11	HFB-1-(X)	Pinpoint, Small	Circular	Raised	Entire	Off White

(HFB-1RN:- Bacteria isolated from root nodule of *Vicia faba* L. of variety HFB-1; HFB-1-(I-X):- Bacteria isolated from rhizosphere of *Vicia faba* L. of variety HFB-1)

Table 2 Morphological Characterization of the bacteria isolated from *Vicia faba* L. (Variety- Vikrant)

S.No.	**Strain**	**Morphological Characterization of the bacteria isolated from *Vicia faba* L.(Variety-Vikrant) in YEMA media**				
		Size	**Form**	**Elevation**	**Margin**	**Pigmentation**
1	VKRN	Small	Circular	Convex	Entire	White
2	VK-(I)	Pinpoint, Small	Circular	Flat	Entire	Off White
3	VK-(II)	Pinpoint, Small	Irregular	Flat	Undulate	Off White
4	VK-(III)	Pinpoint	Circular	Raised	Entire	White
5	VK-(IV)	Pinpoint, Small	Irregular	Flat	Entire	Off White
6	VK-(V)	Pinpoint, Small	Circular	Raised	Entire	Off White
7	VK-(VI)	Pinpoint, Small	Circular	Raised	Entire	Off White
8	VK-(VII)	Moderate, Large	Irregular	Flat	Entire	Yellow
9	VK-(VIII)	Pinpoint, Small	Circular	Convex	Entire	Off White
10	VK-(IX)	Pinpoint, Small, Large	Circular	Convex	Entire	Off White
11	VK-(X)	Pinpoint, Small	Circular	Raised	Entire	Off White
12	VK-(XI)	Pinpoint, Small	Circular	Raised	Entire	Off White

(VKRN: - Bacteria isolated from root nodule of *Vicia faba* L. of variety Vikrant; VK-(I-XI):- Bacteria isolated from rhizosphere of *Vicia faba* L. of variety Vikrant)

Table 3 Biochemical Characterization of the bacteria isolated from *Vicia faba* L. (Variety-HFB -1)

S.N	Strain	Biochemical Characterization of the bacteria isolated from *Vicia faba* L.(Variety-HFB-1)											
		Gram Staining	Starch Hydrolysis	Casein Hydrolysis	Gelatin Hydrolysis	Catalase test	Indole test	Methyl Red test	Voges-Proskauer test	Citrate Utilization test	TSI test	H_2S Production test	Nitrate Reduction test
1	HFB-1RN	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
2	HFB-1(I)	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
3	HFB-1(II)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
4	HFB-1(III)	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve
5	HFB-1(IV)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve
6	HFB-1(V)	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	-ve	+ve
7	HFB-1(VI)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
8	HFB-1(VII)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
9	HFB-1(VIII)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
10	HFB-1-(IX)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
11	HFB-1-(X)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve

(HFB-1RN:- Bacteria isolated from root nodule of *Vicia faba* L. of variety HFB-1; HFB-1-(I-X):- Bacteria isolated from rhizosphere of *Vicia faba* L. of variety HFB-1)

Table 4 Biochemical Characterization of the bacteria isolated from *Vicia faba* L. (Variety-Vikrant)

S.No.	Strain	Biochemical Characterization of the bacteria isolated from *Vicia faba* L.(Variety-Vikrant)											
		Gram Staining	Starch Hydrolysis	Casein Hydrolysis	Gelatin Hydrolysis	Catalase test	Indole test	Methyl Red test	Voges-Proskauer test	Citrate Utilization test	TSI test	H_2S Production test	Nitrate Reduction test
1	VKRN	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	+ve	+ve
2	VK-(I)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve
3	VK-(II)	-ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve
4	VK-(III)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
5	VK-(IV)	-ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve
6	VK-(V)	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve

7	VK-(VI)	-ve	+ve	+ve	+ve	+ve	-ve	-ve	-ve	+ve	+ve	-ve	+ve
8	VK-(VII)	-ve	-ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
9	VK-(VIII)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
10	VK-(IX)	-ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	+ve	-ve	+ve
11	VK-(X)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve	+ve	-ve	+ve
12	VK-(XI)	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	+ve	+ve	-ve	+ve

(VKRN: - Bacteria isolated from root nodule of *Vicia faba* L. of variety Vikrant; VK-(I-XI):- Bacteria isolated from rhizosphere of *Vicia faba* L. of variety Vikrant)

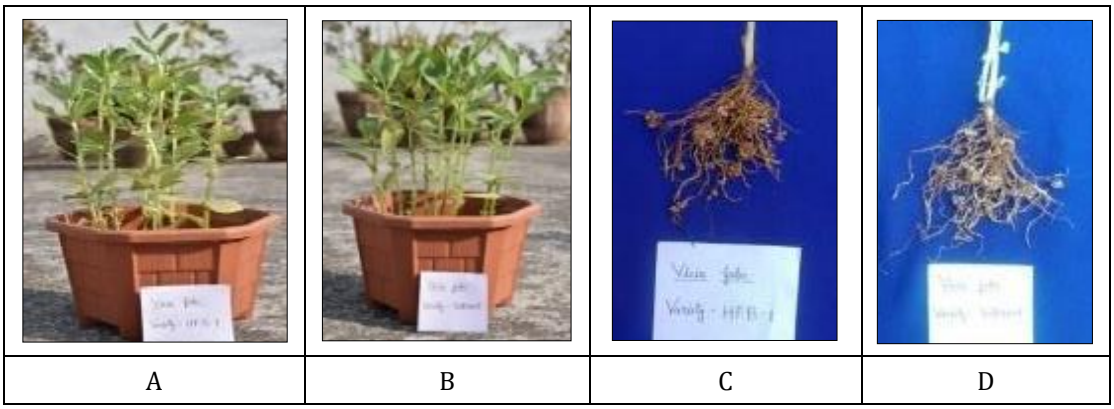


Figure 1 A. Plants of *Vicia faba* L. Variety (HFB-1); B. Plants of *Vicia faba* L. Variety (Vikrant); C. Root Nodules of *Vicia faba* L. Variety (HFB-1); D. Root Nodules of *Vicia faba* L. Variety (Vikrant)

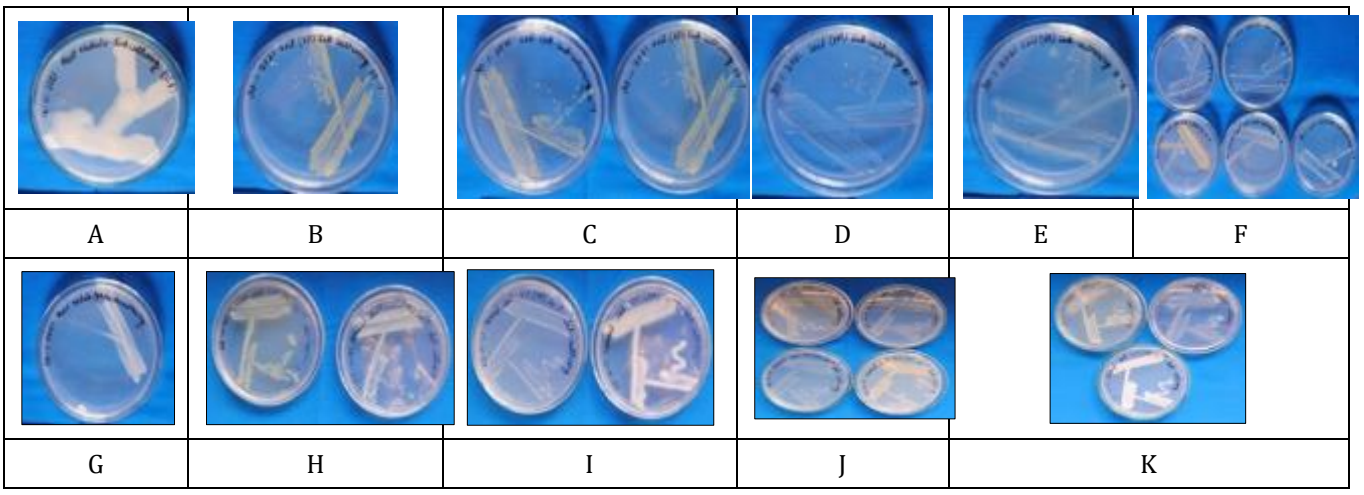
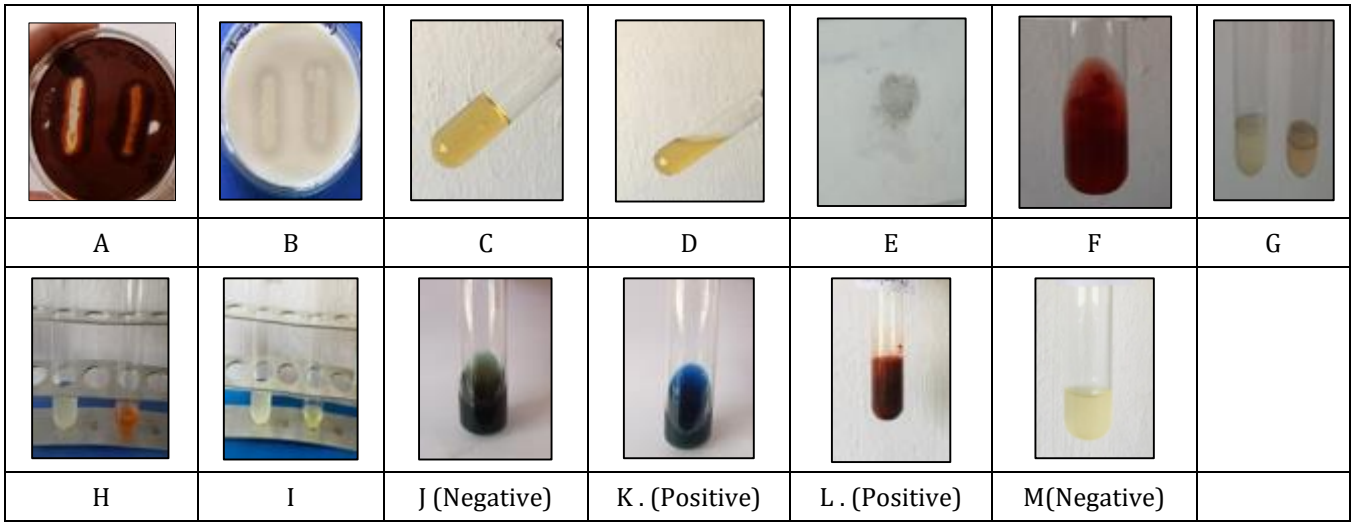


Figure 2 A: Pure culture of bacteria isolated from root nodules of *Vicia faba* L. (Var. HFB-1) B-F: Pure culture of bacteria isolated from rhizosphere of *Vicia faba* L. (Var.HFB-1) G: Pure culture of bacteria isolated from root nodules of *Vicia faba* L. (Var.Vikrant) H-K: Pure culture of bacteria isolated from rhizosphere of *Vicia faba* L. (Var. Vikrant)



A	B	C	D	E	F	G
H	I	J (Negative)	K . (Positive)	L . (Positive)	M(Negative)	

Figure 3 A- Starch Hydrolysis test ; B- Casein Hydrolysis test; C-D- Gelatin Hydrolysis test; E- Catalase test; F-TSI test; G- Indole test; H- Methyl Red test; I- VP test; J-K- Citrate Utilization test; L-M- Nitrate Reduction test

In the present investigation, 23 isolates of bacteria were isolated from root nodules and rhizosphere of *Vicia faba* L. All isolates were Gram negative, 18 isolates showed starch hydrolysis test positive, all isolates showed casein hydrolysis test positive, 22 isolates showed gelatin hydrolysis positive, 15 isolates showed indole test positive, 14 isolates showed methyl red test positive, no isolates showed positive for voges-proskauer,16 isolates showed citrate utilization test positive, 21 isolates showed triple sugar-iron agar test positive, 2 isolates showed H_2S production test positive, 19 isolates showed nitrate reduction test positive and all the isolates showed catalase test positive.

Identification was made on the basis of biochemical tests performed which showed that isolates HFB-1RN, HFB-1-IV, HFB-1-X, VKRN, VK-I, VK-V, VK-VIII, VK-IX, VK-X, VK-XI, are *Rhizobium* species[13,22,23], isolates VK-III, VK-VI are *Bradyrhizobium* species[19,24], isolates HFB-1-II, HFB-1-VI, HFB-1-VII, HFB-1-VIII, HFB-1-IX are *Azotobacter*[25] species, isolates HFB-1-I, HFB-1-V, VK-II, VK IV are *Azospirillum* species[26] and isolates HFB-1-III and VK-VII are *Pseudomonas* species[27,28] .

4. Conclusion

The present research showed the morphological and biochemical characterization of the isolated bacterial strains from the root nodules and rhizosphere of *Vicia faba* L. By the help of isolated nitrogen fixing bacteria the fertility of the soil can be improved which helps to reduced the use of chemical fertilizers and are better choice for the development of biofertilizer.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Singh, Anil Kumar; Bharati, R.C.; Manibhushan, Naresh Chandra and Pedpati, Anitha. (2013). An assessment of Faba bean (Vicia faba L.) current status and future prospects. African Journal of Agricultural Research 8(50):6634-6641.

[2] Zou, Lan; Chen, Yuan Xue; Penttinen, Petri; Lan, Qin; Wang, Ke; Liu, Ming; Peng, Dan; Zhang, Xiaoping; Chen, Qiang; Zhao, Ke; Zhong, Xiang and Xu, Kai Wei.(2016). Genetic diversity and symbiotic efficiency of nodulating rhizobia isolated from root nodules of faba bean in one field. PLoS One 11(12).

[3] Zhou, L.L.; Cao, J.; Zhang, F.S.; and Li, L. (2009). Rhizosphere acidification of faba bean, soybean and maize. Sci.Total Environ. 407(14):4356-62.

[4] Khazaei, Hamid and Vandenberg, Albert. (2020). Seed mineral composition and protein content of faba beans(Vicia faba L.) with contrasting tannin contents. Agronomy 10(511).

[5] A.M.E.,Rugheim and M.E.,Abdelgani.(2012). Effects of microbial and chemical fertilization on yield and seed quality of faba bean (Vicia faba). International Food Research Journal.19 (2):417-422.

[6] Shaheen, Ghazala; Tudu, Anita; Oraon, Vinay and Sharan, Latika. (2023). Morphological and Biochemical characterization of bacteria isolated from root nodules and rhizosphere of Glycine max L. IJIRT. 9(11):452-459.

[7] Shaheen, Ghazala; Oraon, Vinay and Sharan, Latika.(2022). Relationship between Legume - Rhizobia and Rhizosphere from Special Reference to Vicia faba and Glycine max – A Review. IJARESM.10(1):671-676.

[8] Tuzimura, Katura and Watanabe, Iwao.(1962). The effect of rhizosphere of various plants on the growth of Rhizobium. Soil Science and Plant Nutrition 8(4):13-17.

[9] Kamal, Brahma. (2020). Isolation and impacts of rhizobacteria from Saussurea obvallata (D.C.). Biologica Nyssana 11(1):35-44.

[10] Gomare, K.S.; Mese, M. and Shetkar, Y.(2013). Isolation of Rhizobium and cost effective production of biofertilizers. Indian J.L.Sci.2(2):49-53.

[11] BD.,Gachande and GS, Khansole. (2011). Morphological, cultural and biochemical characteristics of Rhizobium japonicum and Bradyrhizobium japonicum of soybean. Bioscience Discovery, 2(1).

[12] Poonia, Shefali. (2011). Rhizobium: A Natural Biofertilizer. International Journal of Engineering and Management Research, 1(1), 36-38.

[13] Panwar, Alka; Choudhary, Sharda; Sharma, Manoj; Sharma, Y.K.; Meena, R.S.; Malhotra, S.K.; Mehta, R.S. and Aishwath,O.P. (2012). Morphological and biochemical characterization of Rhizobium isolates obtained from fenugreek (Trigonella foenum). Seed Res.,40(2),196-200.

[14] B, Naga, Nirmala, Kumari; B, Nagaraju and K, Mallikarjuna. (2018). Biochemical characterization and protein profile by sodium dodecyl sulphate-polyacrylamide gel electrophoresis of French bean (Phaseolus vulgaris L.) associated Rhizobia. Innovare Journal of Agriculture Science,6(1).

[15] Hossain, Akbar; Gunri, Sunil, Kumar; Barman, Manashi; Sabagh, Ayman, El and Teixeira da Silva, Jaime, A. (2019). Isolation, characterization and purification of Rhizobium strain to enrich the productivity of groundnut (Arachis hypogaea L.). Open Agriculture, 4,400-409.

[16] Oraon, Vinay and Singh, Kiran.(2014). Genetic Biodiversity of Nitrogen-Fixing Bacteria from Agricultural Soil of Madhya Pradesh, India. Advances in Life Science and Technology, 24.

[17] Oraon, Vinay and Singh, Kiran. (2015). Assessment of phenotypic and genotypic diversity of Rhizobium bacteria in Madhya Pradesh. Journal of Biology, Agriculture and Healthcare, 5(7).

[18] Deshmukh, V.V.; Mane, S.S.; Gade, R.M.; Ingle,R.W. and Joshi, M.S. (2013). Biochemical studies of Bradyrhizobium japonicum isolates. American International Journal of Research in Formal, Applied & Natural Sciences, 4(1),53-57.

[19] Shoaib, Muhammad; Muzammil, Iqra; Hammad, Muhammad; Bhutta, Zeeshan, Ahmad and Yaseen, Ishrat. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. IJRP.ORG., 54(1).

[20] Singh, Baljinder; Kaur, Ravneet and Singh, Kashmir. (2008). Characterization of Rhizobium strain isolated from the roots of Trigonella foenumgraecum (fenugreek). African Journal of Biotechnology,7(20),3671-3676.

[21] Datta, Abhinav; Singh, Ravi, Kant and Tabassum, Shahina. (2015). Isolation, characterization and growth of Rhizobium strains under optimum conditions for effective biofertilizer production. Int.J.Pharm.Sci.Rev.Res. 32(1), 199-208.

[22] Nagananda, G.S.;Das, Arijit; Bhattacharya, Sourav and Kalpana, T. (2010). In vitro studies on the effects of biofertilizers (Azotobacter and Rhizobium) on seed germination and development of Trigonella foenum-graecum L. using a novel glass marble containing liquid medium. International Journal of Botany, 6(4),394-403.

[23] Anandrao, Pawade Vitthal and Arshiya, Minhaj. (2022). Impact of Rhizobium on seed germination of selected leguminous crop plants. Int.J.Adv.Res.Biol.Sci.,9 (4),58-66.

[24] Gachande, Bhagwat D. and Khansole, Gopinath S. (2011). Morphological, cultural and biochemical characterization of Rhizobium japonicum syn and Bradyrhizobium japonicum of soybean. Bioscience Discovery, 2(1).

[25] Painkara, Hemlata; Chowdhury, Tapas and Verma, Narayan Prasad. (2019). Characterization and screening of native isolates of PSB and Azotobacter under in vitro conditions.Int.J.Curr.Microbiol.App.Sci.,8 (5), 2058-2068.

[26] Rasool, L.; Asghar, M.; Jamil, A. and Rehman, U. (2015). Identification of Azospirillum species from wheat rhizosphere. The Journal of Animal & Plant Sciences. 25(4), 1081-1086.

[27] Zhao, H; Ma, Y; Wu, X and Zhang, L. (2020). Pseudomonas viciae sp. nov., isolated from rhizosphere of broad bean. Int. J. Syst.Evol.Microbiol.70 (9):5012-5018.

[28] Alemu, Fekadu. (2016). Isolation of Pseudomonas flurescens from rhizosphere of faba bean and screen their hydrogen cyanide production under in vitro study, Ethiopia. American Journal of life sciences. 4(2):13.