



**International Journal of Biology, Pharmacy  
and Allied Sciences (IJBPAS)**

*'A Bridge Between Laboratory and Reader'*

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**A REVIEW ON CHARACTERIZATION AND ANTIMICROBIAL  
ACTIVITY OF NITROGEN FIXING BACTERIA FROM LEGUMINOUS  
PLANT (FENUGREEK)**

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Received 22<sup>nd</sup> Jan. 2021; Revised 25<sup>th</sup> Feb. 2021; Accepted 26<sup>th</sup> March 2021; Available online 1<sup>st</sup> April 2021

<https://doi.org/10.31032/IJBPAS/2021/10.4.1049>

**ABSTRACT**

Nitrogen is normally considered one of the major limiting nutrients in plant growth. Symbiotic nitrogen fixation is a key to the nitrogen nutrition to the legumes. The biological mechanism of converting molecular nitrogen to ammonia is referred to as nitrogen fixation. The bacteria of the genus *Rhizobium* are the most effective agents for symbiotic nitrogen fixation. They invade the root hairs of leguminous plants and form nodules on the roots, where nitrogen fixation takes place. *Rhizobium* is a biocontrol agent that inhibits pathogen growth and promotes plant growth by fixing nitrogen from the atmosphere. The secretion of secondary metabolites is responsible for the biocontrol effect. The current review explores characterization and antimicrobial activity of nitrogen fixing bacteria from leguminous plant (fenugreek).

**Keywords:** Leguminosae, Fenugreek, Root nodules, nitrogen fixing, *Rhizobium*, Antimicrobial activity, Effecting factor

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## INTRODUCTION

### **Fabaceae or Leguminosae:**

The Fabaceae or Leguminosae commonly known as the legume or the fruit, seed of such a plant. India is largely a vegetarian nation adhering to a largely vegetarian diet, where in legumes in the form of vegetable beans or grains afford ample amount of fiber, fat, carbohydrates, proteins, vitamins, and minerals [1]. They are nutritionally rich, provide proteins (20– 45%) with essential amino acids, complex carbohydrates ( $\pm 60\%$ ) and nutritional fiber (5–37%) and are the second most important food source after cereals [2]. Additionally, being fixers of atmospheric nitrogen into the soil throughout symbiotic association with soil Rhizobium species, they play an important role in sustaining the soil fertility and therefore, grown in traditional crop rotation, mixed cropping and intercropping systems[3]. In the earth, among major families of flowering plant, Leguminosae is the third largest family of flowering plants on the world, with 19,400 species divided into 730 genera. *Astragalus* (over 2,400 species), *Acacia* (over 950 species), *Indigofera* (around 700 species), *Crotalaria* (around 700 species), and *Mimosa* (around 700 species) are the largest genera (around 500 species). Plants in this family can be found all over the world, in a variety

of habitats and climates. The Fabaceae is the most common family found in tropical rainforests and in dry forests [4].

### **Fenugreek plant:**

*Trigonella L.* that (Fenugreek), locally known as Methi [5]. *Trigonella L.* (Fabaceae) is a genus of annual and perennial herbs with pinnate leaves, trifoliate, fragrant flowers, and legumes. *Trigonella* species are widely distributed in the dry regions around Mediterranean, West of Asia, Europe, North and South Africa, North America, and South Australia [6]. Fenugreek, also known as fenugreek, is one of the oldest multipurpose medicinal herbs. An yearly plant, fenugreek grows to a normal height of two feet. The leaves and seeds, which grow into long pods, are used to produce medicinal extracts or powders [7]. its yellowish-white triangular flowers, it was given the name *Trigonella* from Latin, which means "little triangle." Like other legumes, fenugreek is an excellent source of dietary protein for both humans and livestock. It was and still is known as Greek hay because it was used as medicine, seasoning, and cattle fodder by the Greeks (and the Romans) since ancient times. Fenugreek seeds are used as a yellow dye, cosmetics, and for medicinal purposes. Fenugreek is an excellent soil renovator and

is often used as a green manure [8]. There has been much controversy regarding the number of species, which includes the genus, Fazli considered 97 species, Vasil'chenko recognized 128 species, Hector and Hutchinson recorded about 70 species. In recent studies, included approximately 100 species recognized about 93 species. However, the exact number depends on accepted synonymies [9].

#### **Root nodules:**

Nitrogen-fixing bacteria belonging to the genus *Rhizobium* cause root nodules in leguminous plants. The root is surrounded by nodules, which form a collar-like structure. The central infected tissue in these nodules and the peanut type is uniform, missing the uninfected cells seen in fenugreek nodules and many indeterminate including peas and clovers [10].

#### **Rhizobium:**

*Rhizobium* is a bacterium that lives in symbiotic courting with the basis nodules of leguminous plants. Nitrogen fixation is not feasible on its own. That is why *rhizobium* requires a plant host [11]. *Rhizobium* is an important source of nitrogen for agricultural soils, particularly in arid areas. Dinitrogen is converted to ammonia by them. Since ammonia is poisonous in nature, it is quickly absorbed by organic compounds [12].

#### **Rhizobium Bacteria Classification:**

*Rhizobium* can be categorised based on the plant types with which it is associated, as well as its rate of growth. Few species of *Rhizobium* bacteria include:

- *Rhizobium leguminosarum*
- *Rhizobium alamii*
- *Rhizobium lantis*
- *Rhizobium japonicum*
- *Rhizobium trifolii*
- *Rhizobium phaseolii*
- *Rhizobium smilacinae* [13]

#### **Nitrogen fixing biofertilizers:**

One method of transforming elemental nitrogen into a shape that plants can use is biological nitrogen fixation. Nitrogen-fixing bacteria (NFB) are bacteria that convert inert atmospheric nitrogen (N<sub>2</sub>) into organic compounds. A living fertiliser made up of microbial inoculants or groups of microorganisms that can fix atmospheric nitrogen is known as a nitrogen fixer or N fixer organism [14]. They are grouped into free living bacteria (*Azotobacter* and *Azospirillum*) and the blue green algae and symbionts such as *Rhizobium*, *Frankia* and *Azolla* [15]. Nonlegume-associated N<sub>2</sub>-fixing bacteria include *Achromobacter*, *Alcaligenes*, *Arthrobacter*, *Acetobacter*, *Azomonas*, *Beijerinckia*, *Bacillus*, *Clostridium*, *Enterobacter*, *Erwinia*, *Derxia*,

*Desulfovibrio*, *Corynebacterium*, *campylobacter*, *Herbaspirillum*, *Klebsiella*, *Lignobacter*, *Mycobacterium*, *Rhodospirill*. While several genera and species of N<sub>2</sub>-fixing bacteria have been isolated from the rhizosphere of various cereals, members of the *Azotobacter* and *Azospirillum* genera have been extensively tested in the field to increase cereal and legume yields. [16].

#### **Nitrogen fixing biofertilizers Rhizobium:**

Belongs to the Rhizobiaceae family, is symbiotic in nature, and only fixes nitrogen 50-100 kg/ha with legumes. It colonizes the roots of called root nodules, which acts as factories of ammonia production. *Rhizobium* has ability to fix atmospheric nitrogen in symbiotic association with legumes and certain non-legumes like *Parasponia* [17]. Six *Rhizobium* species have been described based on cross-inoculation studies and the legume host(s) with which they nodulate. A cross-inoculation community is a set of leguminous species that produce nodules on every other member of that plant group. Therefore, a single cross inoculation group ideally includes almost all host species which are infected by an individual bacterial strain [18].

#### **Antimicrobial activity of root nodules:**

The process of destroying or inhibiting disease-causing microbes is known as

antimicrobial activity. This is accomplished using a variety of antimicrobial agents. Antimicrobials are antibacterial, antifungal, and antiviral in nature [19]. They all work in various ways to keep the infection at bay. All root nodules isolates showed antibacterial activity against *psudomonas*. Antifungal activity against *Aspergillus niger* [20]. It is a pharmaceutical preparation used to treat or prevent fungal infections like Mycosis (fungal infection of animals including humans), These isolates had antibacterial activity and could be used in the development for pharmaceutical or agricultural purposes [21].

#### **The Factors Affecting Rhizobia, Legume and Nitrogen Fixation:**

Nitrogen fixing rhizobia cannot express their full potential in fixing nitrogen if the environment and the plant is in poor state. The process of nitrogen fixation depends much on the functional state of the legume plant and the optimum environmental conditions supporting the macro and microsymbionts [22]. The nitrogen fixing rhizobia vary in their tolerance to major environmental factors. Environmental stresses can affect the host plant and symbiotic rhizobia [23]. The maximum threatening environments for rhizobia features are marginal lands with low rainfall,

acidic soils with bad water keeping capacity, nutrient strain and temperature extremes. The proposed important ranges of mineral requirement for effect [24].

#### **Temperature:**

Temperature has a significant impact on rhizobial strain survival and durability in soil. For example, fenugreek rhizobia from the india grow at 25° to 30°C, and more than 90% of the strains isolated from this region grew well to 40°C. The influence of temperature on rhizobia appears to be both strain and soil dependent [25]. For example, *Bradyrhizobium* sp. was less susceptible than *R. leguminosarum* bv. trifolii to high soil temperatures, but in sandy soils, the addition of montmorillonite and illite solved the problem. Soil temperature also greatly influences competition for nodulation. This effect may, in part, be due to temperature-induced delay in nodulation or the restriction of nodules to the sub-surface region. The effect of higher temperatures and more water on CO<sub>2</sub> exchange and nitrogen fixation in nodulated alfalfa plants was studied [26]. They showed that plant and nodule dry weight affected by temperature. Relatively high-root temperature has also been shown to influence infection, N<sub>2</sub>- fixation ability, and legume growth and has a strong influence on specific strain and cultivar interactions every

legume and Rhizobium combination appears to have a preferred temperature range, which is around 30°C for clover and pea, 35–40°C for soybean, peanut, and cowpea, and 25–30°C for common bean legume development [27].

#### **Drought:**

Rhizobia and legumes can coexist in low-moisture soils, with the lowest population densities recorded in the most desiccated conditions. Drought decreases the number of rhizobia in soils, preventing nodulation and N<sub>2</sub> fixation [28]. The nitrogen fixation process is extremely vulnerable to soil moisture deficiency. In Tanzania and Africa as a whole, the population of Rhizobia in relation to drought has received little attention. The majority of Africa's dry lowlands have a low moisture content and a wide annual temperature range. As a result, obtaining good rhizobia candidates for creating successful symbioses in drought environments useful for the development of common bean and other legumes would almost certainly result from successful isolation of rhizobia from such environments [29].

#### **Soil Acidity and Related Stress:**

Soil acidity and related problems of manganese and aluminum toxicity as well as calcium deficiency seriously affect

nodulation, N<sub>2</sub> fixation and plant growth. In neutral or slightly acidic soils, rhizobia can fix nitrogen for most leguminous plants. Most legume species, according to researchers, are unable to nodulate at pH levels below 5.0 because they cannot tolerate the acidic environment. The pH range for fenugreek is 5.3-8.2. Common beans (*Phaseolus vulgaris*) can be nodulated by rhizobia from both low pH (acidic soils) and high pH soils, according to a study conducted in Kenya. Rhizobia isolated in Egypt developed at pH levels ranging from 6 to 8, with some strains tolerant of acidic pH of 3.5 to 4.0 and alkalinity at pH levels of 9 to 10. Strain 042B was stated to be adapted to alkaline soils with a pH of 10.7 and a wider temperature range of 10 to 42 degrees Celsius in China, and that it can grow faster in unfavourable environments and use a wider range of carbohydrates than other strains nodulating soybeans, providing an important commercial benefit for industrial inoculum development [30]. Since soil characteristics vary with altitude and rhizobia diversity, further research into the relationship between rhizobia population and soil pH in Tanzania and Africa as a whole is still required. As a result, obtaining successful native rhizobia resistant to low and high soil pH requires isolation of

rhizobia from a number of locations with a wide range of soil pH [31].

## CONCLUSION

This Study of Isolation Microorganism Characterization and Antimicrobial Activity of Nitrogen Fixing Bacteria from Leguminous Plant (Fenugreek) that helps to study the tolerance of nitrogen-fixing rhizobia to significant environmental factors varies. The host plant and symbiotic rhizobia may be affected by environmental stresses. Marginal lands with low rainfall, acidic soils with low water keeping ability, nutrient stress, and temperature extremes are the most hazardous conditions for rhizobia functions. The different types of *Rhizobium spp.* could be effectively used as a biocontrol agent in the form of bio-inoculant against bacterial pathogen but enhancement in its antibacterial properties would prove to be more efficacious. Therefore efforts are required to understand biocontrol mechanism of rhizobia against bacteria. Genetic engineering approach can also be used to introduce the genes coding for the synthesis of antimicrobial metabolites into rhizobial strains selected for use in biocontrol.

## Acknowledgement

First, I thank full of my guide for sharing her knowledge and encouragement during the study. I also want to thank to department of

microbiology of parul institute of applied science and my lab partners for each and everything helps.

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