Vol:2, Pg: 38-48, Yr: 2021-AJABTR

Microbes aiding plant growth: Probiotics in plants.

Pranab Roy* and Surupa Basu

Institute of Child Health

11, Dr Biresh Guha Street, Kolkata – 700017

*Corresponding: pranabroy@rediffmail.com

Abstract

Micro-organisms, including viruses, bacteria and fungi are generally perceived as pathogens, causing different diseases in plants and animals including human beings. However, there are many microbes known which not only help in the growth of their host organisms but are essential for their survival. Plants have symbiotic relationship with many microbes which are essential for the survival for both the host and micro-organisms. Nitrogen fixing bacteria which associate with the roots of leguminous plants can fix atmospheric Nitrogen gas into ammonia which is essential for plant growth. These bacteria, generally known as Plant Growth Promoting Rhizobcteria (PGPR) derive their food from the host leguminous plants, mutually benefitting each other. Some common examples of PGPR genera exhibiting plant growth promoting activity are: *Pseudomonas, Azospirillum, Azotobacter, Bacillus, Burkholdaria, Enterobacter, Rhizobium, Erwinia, Mycobacterium, Mesorhizobium, Flavobacterium,* etc. Viruses and fungi are also known to have such relationships with plants, eg. Vesicular Arbuscular Mycorrhiza (VAM).

Keywords: Plant Probiotics, Plant Bacterial Symbiosis

1. Introduction

Leguminous plants have root nodules which harbour various bacteria including *Rhizobium* species which can fix atmospheric Nitrogen into plant assimilable ammonia, thus reducing their dependence on externally applied fertilizers. Similarly, Inorganic phosphate is made available to plants by various *Pseudomonads* which solubilize the rock phosphate in soils to make it available to plants [1,2].

2. Materials & Methods

In our search for bacteria affecting the growth of a leguminous plant, Methi (Fenugreek), field grown plants with root nodules were taken at three months stage, the root nodules were

surface sterilized with Hypochlorite and homogenized with mortar and pestle. On plating in YEM (Yeast extract, Mannitol agar) medium and incubating at 30C for 48 hours, various colonies of bacteria were obtained. Three mucoid colonies were taken and plated on Nitrogen-free medium. Isolated colonies were studied for their biochemical characterization and molecular identification using 16S rRNA sequencing and comparing to the NCBI database.

3. Results & Discussion

A. The maximum sequence homology showed the species to be :

Table 1: Molecular identification and characterization.

Sample Code	Source	Accession Numbers	Identified Organisms
R1	Root nodules	KX687556	*Enterobacter cloacae*
R10	Root nodules	KX687557	*Pantoea dispersa*
R12	Root nodules	KX687554	*Enterobacter ludwigii*
A1	Rhizospheric soil	KX687553	*Pseudomonas sp*
P1	Rhizospheric soil	KX687555	*Pseudomonas sp*

B. The biochemical characterization of the isolated microbes were done using routine tests :

Test	R1	R10	R12
1.Catalase	-	-	-
2.Citrate	++	++	++
3.Oxidase	++	++	++
4.Indole	-	-	++
5.MR	-	-	-
6.VP	++	-	-
7.Gelatinase	-	-	-
8. Growth on Glucose peptone agar	++	++	++
9.Motility	+++	+++	+++
10.TSI Agar test	Acid formation with no gas formation and all the three sugars were fermented	Acid formation with gas formation and only glucose fermented	Acid formation with no gas formation and all the three sugars were fermented

Table 2:The routine biochemical tests for each of the three isolates were carried out.

C. The Gram staining of the microbes were done and indicated that all the three isolates were Gram negative short rods under 100X magnification.

To verify if the isolated microbes can initiate nodule formation by inoculating it in roots of Fenugreek seedling, we followed the process under microscope and observed as follows :

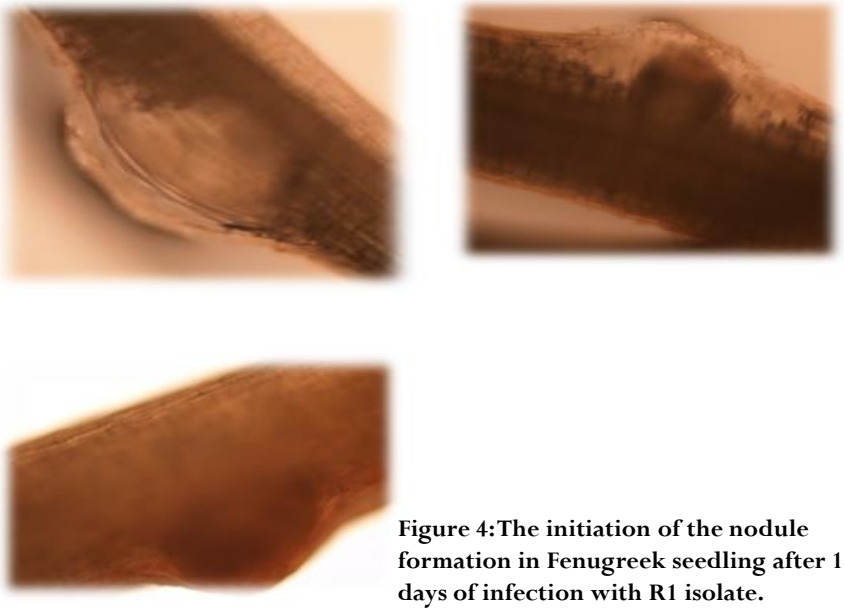
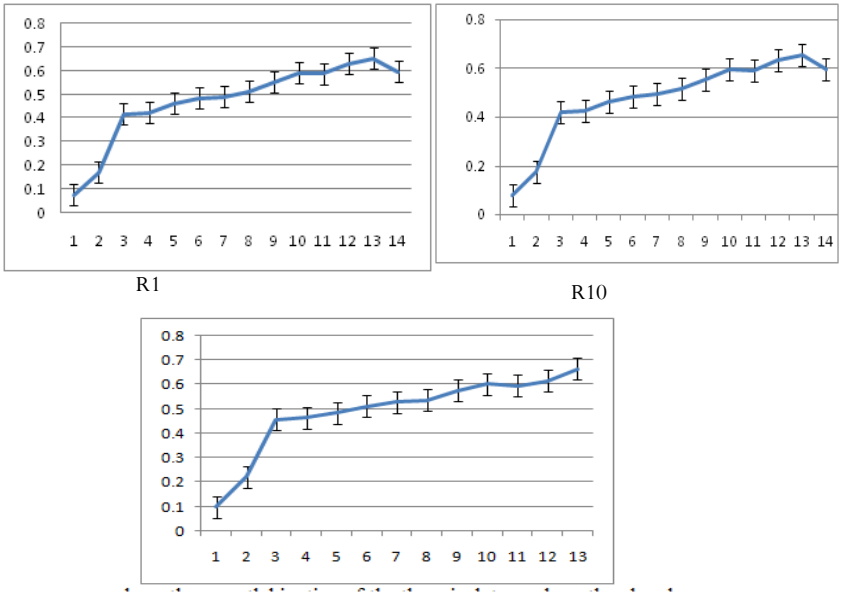
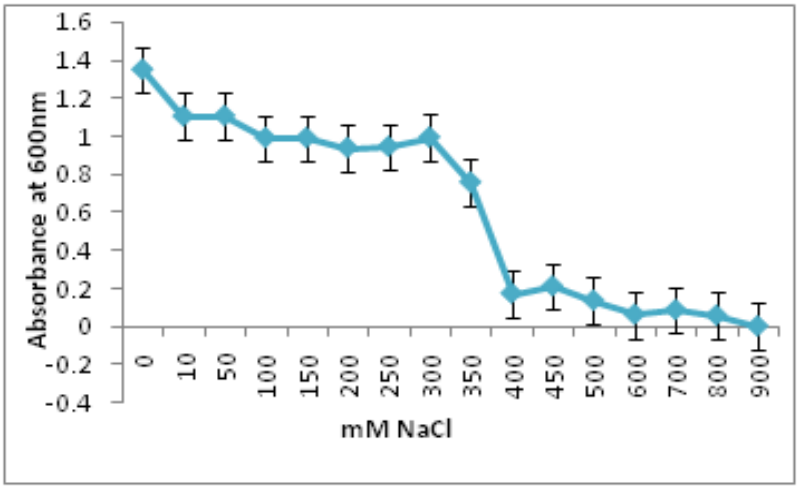


Figure 4:The initiation of the nodule formation in Fenugreek seedling after 15 days of infection with R1 isolate.

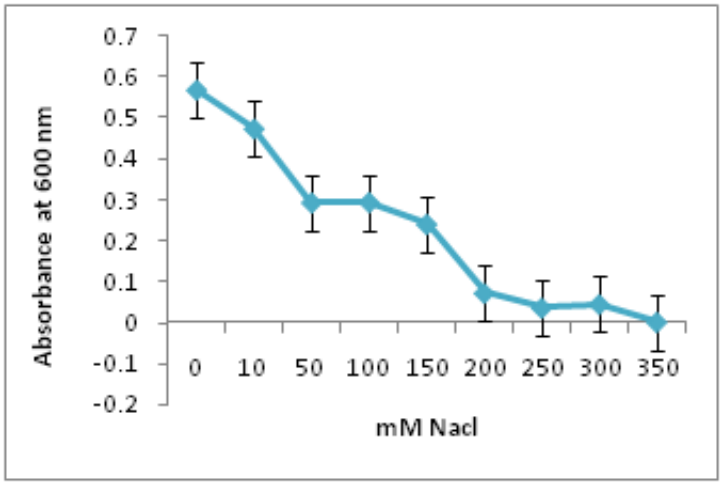
D. Looking at the growth curves of the three isolated bacteria at 30C with aereation, and plotting the absorbance at 590 nm vs. time of growth in hours, the following curves were observed :



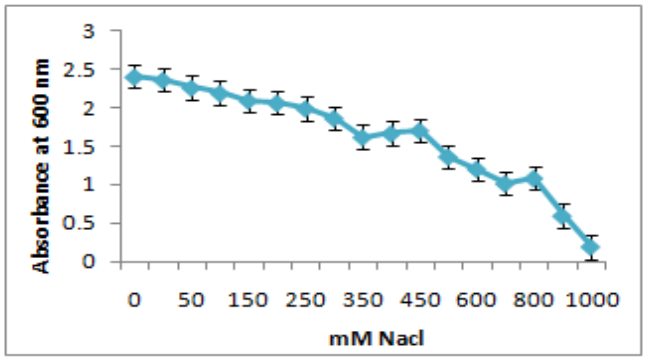
E. Next, we examined the tolerance of the microbes against salt stress by growing them in medium having varying salt (NaCl) concentrations:



7a. R1 Isolate: Isolate R1 shows 50% inhibition at 350 mM NaCl salt concentrations.



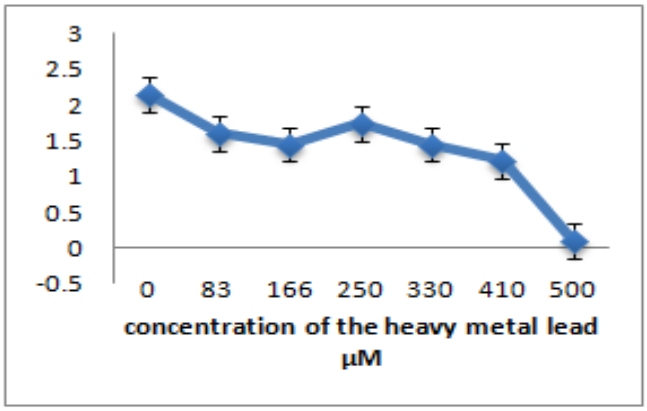
R10 isolate: Isolate R10 shows 50% inhibition at 50 mM NaCl salt concentrations.



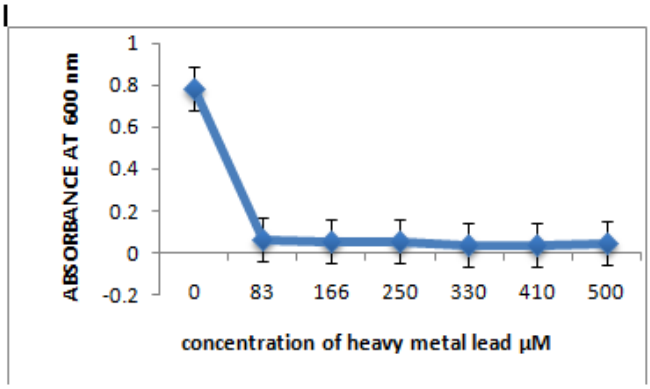
R12 isolate: Isolate R12 shows 50% inhibition at 500mM NaCl salt concentration and proves to be highly salt tolerant.

So, the three bacteria had wide ranging tolerance to salt, R12 being the most salt tolerant showing 50% inhibition of growth at 500mM NaCl, whereas R1 had 350mM and R10 had 50 mM respectively.

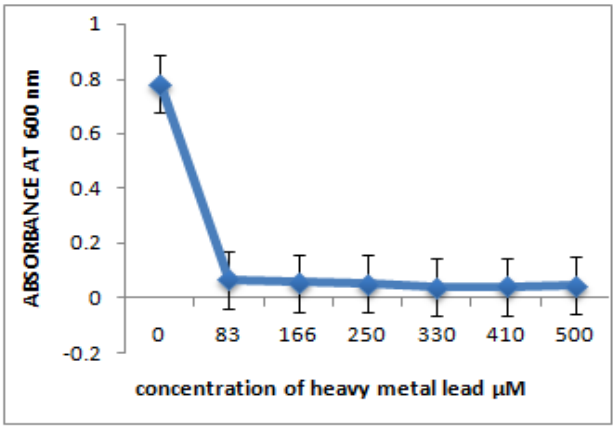
F. To examine the temperature tolerance of the three bacterial species, these were grown in liquid medium as well as agar plates at 30C, 37C and 42C temperatures. Growth diminished with higher temperature and 42C was inhibitory to R10 and R12.Next, the metal ion toxicity to the growth of the individual microbes were studied by varying the concentrations of metal ion, Pb(II) in liquid medium and shaken at 30C for 48 hours. Whereas R1 was tolerant to lead upto 410 uM concentration, R10 and R12 were sensitive and failed to grow even at 83 uM lead concentration.



A: R1 Isolate: (*Enterobacter cloacae*)



B: R10 Isolate: (Pantoea dispersa).



C: R12 Isolate: (Enterobacter ludwigii)

G. Looking at the antibiotic sensitivity of the three bacteria against the common antibiotics like Ampicllin, Gentamycin, Vancomycin etc, all of them showed identical sensitivity or resistance as shown in the Table

Table 4: Antibiotic sensitivity.

Antibiotics	R1	R10	R12
Ampicillin	Sensitive	Sensitive	Sensitive
Gentamycin	Resistant	Resistant	Resistant
Trimethoprim	Resistant	Resistant	Resistant
Tetracycline	Resistant	Resistant	Resistant
Streptomycin	Resistant	Resistant	Resistant
Vancomycin	Sensitive	Sensitive	Sensitive
Cloxacillin	Sensitive	Sensitive	Sensitive

H. The beneficial micro-organisms aid the growth of the host plant by producing one of the plant growth hormones, Indole acetic acid, essential for apical elongation of roots and shoots. The liquid medium supplemented with Tryptophan, the precursor of IAA were inoculated with the individual bacterial isolate and grown at 30C with shaking. The production of the hormone IAA was measured with the help of Seliwanoff reagent. It was found that R10 and R12 were very efficient in IAA production but a negative control, E.coli could not produce any.

Indole acetic acid production
The three isolated bacteria R1, R10 and R12 were able to produce indole acetic acid when recorded spectrophotometrically whereas a negative control *E. coli* did not produce IAA

R1	R10	R12
++	+++++++	++++

I. Ethylene, another plant hormone is inhibitory to growth and induces senescence in plants, is produced from the precursor, 1-amino, 1-carboxylic cyclopropane (ACC). So, to minimize the inhibitory effect of ethylene, the precursor is diverted to other product by an enzyme, ACC Deaminase which are produced by these beneficial microbes. This is illustrated in the following Table.

ACC Deaminase activity

The three isolates were found to have ACC Deaminase activity when plated on DF minimal media supplemented with ACC as the sole carbon and nitrogen source. Colonies appeared on the plates; also colorimetrically it had been shown to grow in DF liquid medium whereas a negative control showed no growth in this minimal medium. ACC Deaminase is the enzyme which reduces the biosynthesis of ethylene, a plant hormone inhibiting plant growth.Thus these bacteria act as plant growth promoters

R1	R10	R12
+++	+++++	++++++

4. Conclusion

Just like probiotics in animal feeds and human foods, plants have a cocktail of different microbes in the soil rhizosphere aiding the growth of the plant. There is specificity of interaction between the host and the microbe and are not general in their actions. Both symbiotic Nitrogen fixers and free living Nitrogen fixing micro-organisms are known. Phosphate solubilizing microbes help in accessing the insoluble rock phosphate in soil by the plants. Siderophores are chelating agents produced by some bacteria in the rhizosphere, eg. Pseudomonas sp. which chelate Iron ions to be assimilated by the plants.

In this paper, we demonstrated the plant root associated nodular microbes in Fenugreek, identified these by molecular technique and characterized them. The different abiotic stresses like salt, high temperature and toxic metal ion effects were studied with individual bacterial isolates. The production of the plant growth hormone, IAA and inhibition of the senescence hormone Ethylene by these microbes were studied and shown their probiotic effects on the host plant.

References:

[1] B R Glick, C L Patten, G Holguin and D M Penrose, Biochemical and genetic mechanisms used by plant growth promoting bacteria, Imperial College Press, London. 1999.

[2] A R Podile and K Kishore, Plant growth-promoting rhizobacteria. In: Plant associated

bacteria. Springer Netherlands, pp. 195–230, 2007.