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# Characterisation of *Rhizobia* on the Basis of Antibiotic Responses

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Abstract

*Rhizobium* spp. were isolated from root nodules of 10 cultivated & three wild legume hosts. All the isolated *Rhizobia* were individually tested for their antibiotic response. Biodiscs of five antibiotics having three different concentrations viz: 0.5%, 1% & 1.5% were tested against the isolated *Rhizobia* using plate culture method. The affectivity of the antibiotics was revealed in terms of zone formation & was measured in mm.

## Introduction

Nitrogen is the major limiting nutrient for most plant species (Green wood, 1982). Plants require nitrogen from soil or from atmosphere by symbiotic nitrogen fixation (Vance, 1990). Legume capable of fixing atmospheric nitrogen in symbiotic association with *Rhizobium* improves soil nitrogen content in uncultivated land (Sanginsg et. al. 1988). Inoculation of legume crop with *Rhizobium* strain having better symbiotic crop productivity (Singh & Singh 1983a, 1983b). Antibiotic resistance is found in antibiotic producing microorganisms which need the mechanism for self protection. (Gray & fitch 1983: Trieo-cuot et al 1987a). Cole & Elkan 1973,1979 repotted that resistances of antibiotics were plasmid born. The core replicons of self transmissible plasmid involved in antibiotics resistance gene transfer were devoid of resistance determinants in the bacteria (Hughes & Datta, 1983). The bacteria possess a differential response towards the antibiotics as some show resistance & some susceptibility for a particular antibiotic. Marker antibiotics are evaluated as confirmatory test of their identity as well as control. (Prasuna, 1987). The present investigation has been undertaken to study antibiotic resistance towards different *Rhizobium* spp.

## Material and Methods

*Rhizobia* were isolated from root nodules of 10 cultivated viz – *Phaseolus aureus (PA-$R_1$), Phaselus vulgaris (PV-$R_2$), Arachis hypogaea (AH-$R_3$). Dolichos lablab (DL-$R_4$) Glycine max (GM-$R_5$), Trigonella foenum graecum (TFg-$R_6$). Cicer arietinum (CA-$R_7$), Vigna ungiculeta (VU-$R_8$), Pisum sativum (PS-$R_9$). Lathyrus sativa (LS-$R_{10}$)* & 3 wild plants viz : - *Mimosa pudica (MP-$R_{11}$), Desmodium triflorum (DT-$R_{12}$), and Tephrosia purpurea (TP-$R_{13}$)*, Isolations were made from nodules by standard procedures taking only healthy nodule from root of each plant (Vincent 1970). Nodules were selected & surface sterilized with 0.1% (w/v) mercuric chloride ($Hgcl_2$) for one minute & washed thoroughly several time with glass distilled water (Sloger 1969). Pure *Rhizobia* isolates were transferred on yeast extract mannitol agar (YEMA) slant. Susceptibility test was performed by using the paper disc diffusion assay. Five antibiotics were used viz – streptomycin, Penicillin-G, Erythromycin, Teramycin & Norfloxacin. The biodiscs were prepared in three different concentration viz : 0.5%, 1.0% & 1.5%. Bacterial suspensions were prepared in nutrient broth medium. Each test tube containing 10 ml medium & one loop full *Rhizobia* were inoculated. One ml of bacterial suspension & 10 ml of nutrient agar medium were inoculated in petriplate. After plating & solidification of the medium biodiscs were placed upon the surface of solidified nutrient agar medium. The plates were incubated for 24 hrs. at 28±1˚C . The zone formation was measured in mm (Prasuna, 1987).

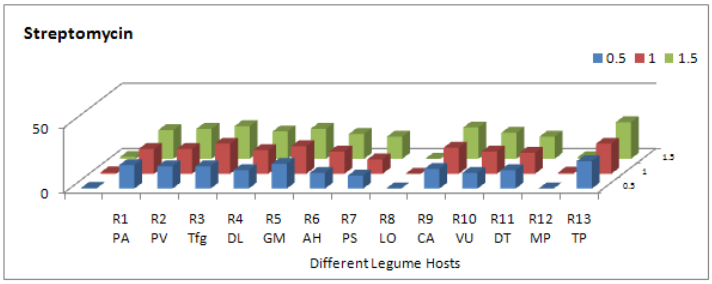
## Result and Discussion

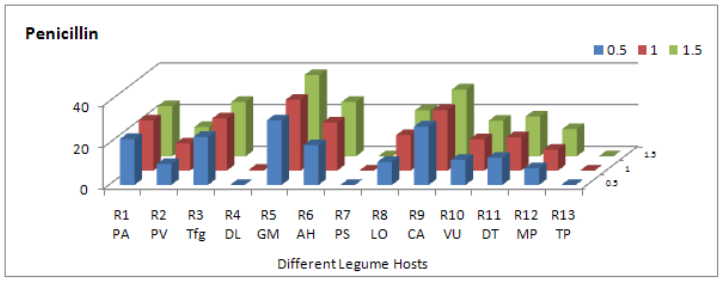
The study of antibiotics sensitivity tests showed that amongst cultivated hosts streptomycin was sensitive in *Rhizobium sp*. Isolated from $R_6$ (25 mm) followed by $R_7$, $R_2$ = $R_5$, $R_1$, $R_4$, $R_8$, $R_3$, $R_9$. Streptomycin could not inhibit the growth of *Rhizobium* spp. Isolated from $R_{10}$ (No zone formation). The inhibition zone indicate sensitivity of different isolates. $R_6$ was susceptible & $R_{10}$ showed resistance towards streptomycin. Sensitivity can be expressed as $R_6$> $R_7$> $R_2$= $R_5$> $R_1$> $_4$> $R_8$> $R_3$> $R_9$> $R_{10}$.

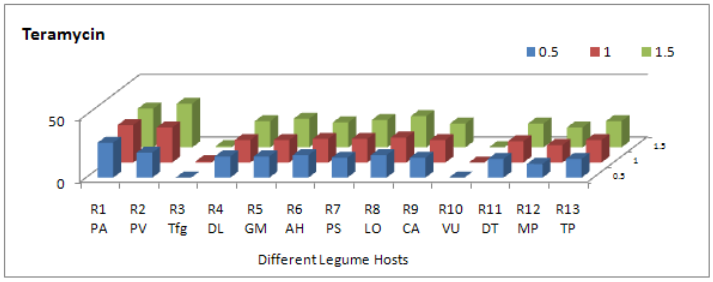
Penicillium was highly sensitive towards $R_5$ (39 mm) followed by $R_7$, $R_1$, $R_6$= $R_3$, $R_{10}$, $R_8$, $R_2$, was least sensitive towards $R_4$ (0.5 mm) & $R_9$ was resistance towards Penicillin (No zone Formation). The sequence of sensitivity can be expressed as $R_5$ > $R_7$> $R_1$> $R_6$ = $R_3$> $R_{10}$> $R_8$> $R_2$> $R_4$> $R_9$. Teramycin was highly sensitive towards $R_3$ (35mm) followed by $R_1$, $R_{10}$, $R_5$, $R_9$, $R_4$, $R_3$, $R_7$, $R_6$, Teramycin could not inhibit the growth of *Rhizobium* spp isolated from $R_8$. Response of Teramycin was in the order of $R_2$> $R_1$> $R_{10}$> $R_5$> $R_9$> $R_4$> $R_3$> $R_7$> $R_6$> $R_8$. Erythromycin was highly sensitive towards $R_3$ (26 mm) followed by $R_7$, $R_6$ = $R_2$, $R_9$, $R_{10}$= $R_5$, $R_1$, $R_8$ - Erythromycin could not inhibit the growth of $R_4$. Sensitivity can be expressed as $R_3$> $R_7$> $R_6$ = $R_2$> $R_9$> $R_{10}$= $R_5$> $R_1$> $R_8$> $R_4$. Norfloxacin was highly sensitive towards $R_7$ followed by $R_9$ = $R_1$, $R_6$, $R_3$, $R_4$, = $R_5$, $R_5$; $R_{10}$ & was least sensitive towards $R_8$ (22mm).

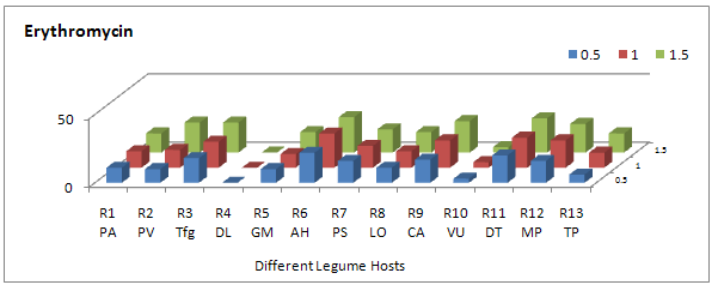
Table 1 : Effect of Antibiotics on the growth of *Rhizobium* Species

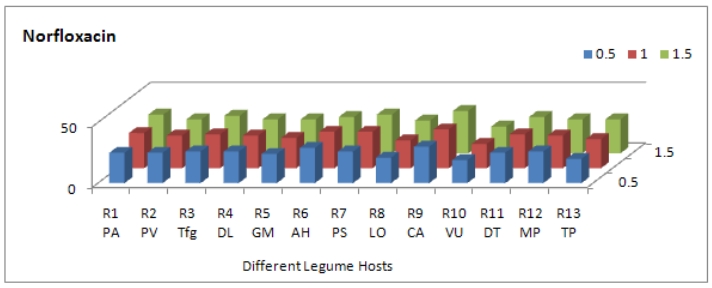
| Name of Antibiotics | Concentration | Name of legume hosts | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Cultivated | | | | | | | | | | Wild | | |
| | % | PA | PV | Tfg | DL | GM | AH | PS | LO | CA | VU | DT | MP | TP |
| (1)Streptomycin | 0.5 | 18 | 17 | 17 | 14 | 19 | 12 | 10 | - | 15 | 12 | 14 | - | 21 |
| | 1 | 19 | 19 | 23 | 18 | 21 | 17 | 11 | - | 20 | 17 | 16 | 0.8 | 23 |
| | 1.5 | 22 | 23 | 25 | 21 | 23 | 19 | 17 | - | 24 | 20 | 17 | 1.2 | 28 |
| (2)Penicillin | 0.5 | 22 | 10 | 23 | - | 31 | 19 | - | 11 | 28 | 12 | 13 | 8 | - |
| | 1 | 24 | 13 | 25 | 0.4 | 34 | 23 | - | 17 | 29 | 15 | 16 | 10 | - |
| | 1.5 | 24 | 14 | 26 | 0.5 | 39 | 26 | - | 22 | 32 | 17 | 19 | 13 | - |
| (3)Teramycin | 0.5 | 28 | 20 | - | 17 | 17 | 18 | 16 | 18 | 16 | - | 15 | 11 | 15 |
| | 1 | 30 | 28 | 1 | 18 | 18 | 19 | 19 | 20 | 18 | - | 17 | 14 | 18 |
| | 1.5 | 31 | 35 | 1 | 21 | 23 | 20 | 22 | 25 | 19 | - | 19 | 16 | 21 |
| (4)Erythromycin | 0.5 | 11 | 10 | 18 | - | 10 | 22 | 16 | 11 | 17 | 3 | 20 | 16 | 6 |
| | 1 | 12 | 13 | 19 | - | 10 | 25 | 16 | 12 | 20 | 4 | 22 | 20 | 11 |
| | 1.5 | 14 | 22 | 22 | - | 15 | 26 | 17 | 15 | 23 | 4 | 25 | 21 | 14 |
| (5)Norfloxacin | 0.5 | 25 | 25 | 26 | 26 | 24 | 29 | 26 | 21 | 30 | 19 | 25 | 26 | 20 |
| | 1 | 29 | 27 | 28 | 27 | 25 | 30 | 30 | 23 | 32 | 20 | 28 | 27 | 24 |
| | 1.5 | 32 | 28 | 31 | 28 | 28 | 30 | 32 | 27 | 35 | 22 | 30 | 28 | 28 |






1- Effect of antibiotics on the growth of different *Rhizobium* spp

However zone formation of $R_4$, $R_5$ & $R_2$ was equal. Respons of norfloxacin was in the order of $R_7$> $R_9$ = $R_1$> $R_6$> $R_3$> $R_4$= $R_5$= $R_2$> $R_{10}$> $R_8$.

In wild Legume hosts streptomycin & Teramycin weres highly sensitive in $R_{13}$ followed by $R_{12}$ & was least sensitive in $R_{11}$. In Penicillin, Erythromycin & norfloxcin was highly sensitive in $R_{12}$ followed by $R_{11}$ & was least sensitive in $R_{13}$. However penicillin could not inhibit the growth of *Rhizobium* spp. Isolated from $R_{13}$ (No zone formation) & zone formation of $R_{13}$ & $R_{11}$ was equally found in Norfloxacin. The finding of the present study revealed that norfloxacin is more effective followed by Penicillin, Teramycin, Erythromycin & Streptomycin. Norfloxacin is a flurequinolence antibacterial agent effective against several Gram negative bacteria (Kamath et al. 1992). The bacteria possess a differential response towards the antibiotics as some show resistance & some susceptibility for a particular antibiotic. Any sign of growth inhibilion was scored as sensitivity to that antibiotic. which means that “resistance” was very strictly defined so that no organism with any sign of sensitivity would be classified as resistant.

## References


Cole, M.A. & Elkan, G.H. (1973): Transmissible resistance to penicillin G, neomycin & chloramphenicol in *Rhizobium japonicum. Antimicrobial Agents & Chemofhempy.* 4, 248-253.

Cole, M.A. & Elkan, G.H. (1979) : Multiple antibiotic resistance in Rhizobium japonicum. Applied & Environmental microbiology, 37,867-870.

Gray, G.S. & Fitch, W.M. (1983) Evolution of antibiotic resistance genes : the DNA sequence of a kanamycin resistance gene from staphylococcus aureus. Molecular Biology & Evolution, 1, 57-66.

Green wood D.J. (1982) Nitrogen supply & Crop yield : The Global Scene : *Plant soil* : 67, 45-49.

Hughes, V. M. & Datta, N. (1983) : Conjugative plasmids in bacteria of the "pre antibiotic" era, Nature, 302, 725-726.

Kamath, R.U. Singh, U.V. & Udupa. N. (1992) : Indian *J Pharm Sci*., 4,148. J7'. Prasuna, P.L. (198?') : Legume Rhizobium nitrogen fixing Symbiosis. Ph.D. Thesis Ravishankar University, Raipur (C. G.).

Sanginga N. Mulongoy K & Ayanoba A (1988) Response of leucaena/Rhizobium symbiosis to mineral nutrients in south western Nigeria plant & boil 112 : 121-127.

Singh, B.P. & Singh, H.G. (1983a) comparative efficacy of S on production of green matter, mineral composition & uptake of mustard on vertisols. -. Forage Research. 9:37-41.

Singh, B.P. & Singh, H.G. (1983b) Inter relationship between soil & foliar applied sulphur on oil percent in seed & oil yield of mustard Crop Physiology. 1:63-76.

Sloger C, (1969) Plant Physical, 44 : 1966.

Subba Rao, N.S. (1988) : Biofertilizers in Agriculture. Oxford & IBH Pub.Co.Pvt.Ltd., New Delhi. PP. 248.

Trieu-Cuot, P. Arthur, M. & courvalin, P. (1987) Origin, evolution & dissemination of antibiotic resistance genes. Microbiological sciences, 4, 263-266.

Vance, C.P. (1990) Symbiotic nitrogen fixation : recent genetic advance. In *The Biochemistry of Plants : Intermediary Nitrogen Metabolism Mifin* B. J. Lea P.J. eds. vol. 16 pp. 48-88, Academic press, Sen Diego.

Vincent, J.M. (1970) : "A Manual for the Practical Study of Root Nodule Bacteria" Blackwell Scientific Publication, Oxford&Edinburg.