Research Article

Isolation and Biochemical Characterization of *Rhizobium* strains from Maize

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ABSTRACT
Rhizobium, a nitrogen fixing bacteria can live either in soil or within the root nodules of host legumes. A NF bacterium can exist freely or in symbiosis and in either case entraps atmospheric nitrogen and converts the unreactive N_2 molecule to NH_3, a form that is readily utilize by plants. *Rhizobium* was isolated from rhizosphere soil of maize in Lalgudi Taluk their morphological, biochemical characters and survival efficiency. This study confirms the presence of *Rhizobium* in the rhizosphere soil of non leguminous plants.

Keywords: Maize, *Rhizobium,*nitrogen fixation, non-legume.

INTRODUCTION

Soil contains many types of microorganisms such as bacteria, actinomycetes, fungi and algae, which are important because they affect the physical, chemical and biological properties of soil. Among the soil bacteria, unique group called Rhizobia has a beneficial effect on the growth of plants. Non leguminous plants like rice, maize and wheat belonging to the poaceae family form staple food for the approximately 6.5 billion people around the world. An exponential rise in world population indicates the need for increased crop production. Nitrogen fertilization of non-leguminous crops is one of the most expensive inputs in agriculture. However, approximately 65% of the applied mineral nitrogen is lost from the plant-soil system, through gaseous emissions, runoff, erosion and leaching. Environmental impact of this loss ranges from greenhouse effects, diminishing stratospheric ozone and acid rain to changes in the global nitrogen cycle and nitrate pollution of surface and ground water. With growing environment related concerns, various alternatives are being harnessed to reduce the dependence on N fertilizer for plant nutrients. It is in this context, that the use of the nitrogen fixing bacteria in agricultural practices is gaining importance.

Various researches demonstrated the ability of *Rhizobium* to colonize the roots of non legumes[1] and act as phytoharmone producers, phosphate solubilizer and to some extent as nitrogen fixers[2].

MATERIALS AND METHODS

Rhizobial strains were isolated from rhizosphere soil of maize in 20 different places in Lalgudi Taluk, Trichy District. The soil samples were serially diluted and inoculated on YEMA medium and incubated at 30˚c for 24-72 hours.Fifty three Rhizobial strains were isolated. and characterized based on morphological and biochemical (Table-3) characters.

Production of indole was noticed in inoculated tryptophan broth after 7 days of incubation by adding Kovac's reagent. The reduction of methyl red and voges- prouskauer reaction examined in glucose phosphate broth by adding methyl red and α- napthol solution with KOH respectively. citrate utilization was observed by using simmon's citrate medium with bromothymol blue in basal medium. Liquefication of gelatin was tested in 10% gelatin agar medium[3]. Hydrolysis of starch was examined on starch with nutrient agar& iodine solution. Production of hydrogen sulphide gas examined by method described[4] by Zobell and Feltham (1934). Production of ammonia from urea was examined by Christensen urea agar with phenol red as an indicator. Fermentation of carbohydrates was tested by adding 10% Andrade's indicator in the basal medium containing peptone water and 2% sugar. The Gram's staining technique was followed[5,6,7].

Catalase activity was observed by stirring the culture in a drop of hydrogen peroxide (10% by w/v), while oxidase activity was tested according to Kovac's(1956).The survival efficiency of viable rhizobium isolates were calculated by the following formula: cfu/ml=(no of colonies x dilution factor/volume inoculated). All the positive cultures were streaked on CRYEMA and GPA medium for further confirmation.

RESULTS AND DISCUSSION

In the present study, 53 strains were isolated from the rhizosphere soil of maize plants (Table-1).

Twenty strains among 54 were found to be having circular colonies with regular borders, convex, whitish pink in colour and glistering. Under light microscope, all the 20 isolates were non motile and were Gram negative. They were non sporing forming and aerobic (Table-2).

The bacterium showed positive for Voges-Proskauer, citrate utilization, catalase, oxidase, TSI, carbohydrate fermentation(maltose, galactose, arabinose) and negative for Indole production, Methyl red, Urease, gelatin hydrolysis and starch hydrolysis (Table-3).

Survival efficiency of viable *Rhizobium* isolates from low moistured soil showed lower value of cfu/g as compared to colony count from high moistured soil (Table-4).Red colour colonies on CRYEMA medium and white colour colonies on GPA medium were observed.

CONCLUSION

This study concluded the presence of *Rhizobium* in the rhizosphere soil of non leguminous plants. *Rhizobium* Sp. can be isolated from the rhizosphere soil of the target area and to create awareness among farmers to cultivate non leguminous plants for better agriculture growth.

Table 1: Isolate number and origin from where *Rhizobium species* were collected

S.No	Isolate number	Origin
1.	MR1,MR2,MR3	Vanthalai
2.	MR4,MR5,MR6,MR7	Nallur
3.	MR8,MR9	Thachankuruchi
4.	MR10,MR11,MR12	Pullampadi
5.	MR13,MR14	Kannakudi
6.	MR15,MR16	P.K.Aharam
7.	MR17,MR18,MR19	Koodalur
8.	MR20,MR21	Perukalapur
9.	MR22,MR23	Nambukuruchi
10.	MR24,MR25,MR26	Varakuppai
11.	M27,MR28	Sirukalapur
12.	MR29,MR30	Thappai
13.	MR31,MR32,MR33,MR34	K.K.Nallur
14.	MR35,MR36	Azhunthalaipur
15.	MR37,MR38,MR39	Ootathur
16.	MR40,MR41,MR42	Neikulam
17.	MR43,MR44,MR45	Viduthalaipuram
18.	MR46, MR47	Kuruchi
19.	MR48,MR49 MR50,MR51	Siruvayalur
20.	MR52,MR53	Kallakudi

M – Maize, R – *Rhizobium* sp.

Table 2: Cultural & morphological characters of *Rhizobium* isolates

S.No	Characters	Observation
1.	Shape	Circular
2.	Color	Whitishpink &Glistering
3.	Elevation	Convex
4.	Margin	Regular
5.	Opacity	Opaque
6.	Motility	Non-motile
7.	Bacterium shape	Rod
8.	Oxygen demand	Aerobic
9.	Spore forming	Non-spore forming
10.	Gram's nature	Gram negative

Table 3: Biochemical Characters of *Rhizobium* isolates

S.No	Test	Results
1.	Indole production	Negative
2.	Methyl Red	Negative
3.	Voges Proskauer	Positive
4.	Citrate utilization	Positive
5.	Catalase	Positive
6.	Oxidase	Positive
7.	TSI	Positive with H_2S Production
8.	Urease	Negative
9.	Carbohydrate fermentation(Maltose)	Positive
10.	Carbohydrate fermentation(Galactose)	Positive
11.	Carbohydrate fermentation(Arabinose)	Positive
12.	Gelatin Hydrolysis	Negative
13.	Starch Hydrolysis	Negative

Table 4: Survival efficiency of similar *Rhizobium* isolates from different places

S.No	*Rhizobium* Isolate	Cfu/g $\times 10^{-5}$ml
1.	MR2	25
2.	MR7	72
3.	MR8	30
4.	MR11	42
5.	MR13	25
6.	MR16	70
7.	MR19	27
8.	MR20	32
9.	MR22	65
10.	MR25	80
11.	MR27	75
12.	MR30	68
13.	MR33	42
14.	MR35	36
15.	MR38	24
16.	MR41	38
17.	MR45	36
18.	MR47	85
19.	MR49	45
20.	MR53	35

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