CULTURE COLLECTION OF *Rhizobium* STRAINS OF IMPORTANT PULSES OF ETHIOPIA[1]

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ABSTRACT

Different *Rhizobium* strains of economically important Pulses in Ethiopia were isolated from nodules of host legumes grown in the major growing areas. 110 strains of *Rhizobium leguminosarum* from *Pisum sativum, Vicea faba,* & *Lens esculenta;* 20 strains of *Rhizobium phaseoli* from *Phaseolus vulgaris;* 328 strains of *R. japonicum* /cowpea type *Rhizobium* from *Glycine max,* and *Cicer areietinum;* and 34 strains of *R. trifoli* from *Trifolium* sp, were isolated at Nazret Research Station microbiology laboratory. The main objective of the collection was to obtain available germplasm resource for selection of effective strains of *Rhizobium* which can be used for inoculant production for regions where the soil has not enough *Rhizobium* cells due to some reasons.

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INTRODUCTION

Rhizobium are soil bacteria, rod shape and gram negative. In symbiotic relationship with legumes, they play a crucial role in balancing the soil nitrogen by converting atmospheric nitrogen to NH_3. The biological fixation of atmospheric nitrogen by *Rhizobium* / legume association was estimated to be 50 % of all nitrogen fixed on the earth (FAO, 1983)

High prices of chemical synthesis fertilizes has seriously affected the production of food crops in developing countries. Many farmers are becoming reluctant to pay for the increased cost of fertilizers. Increased awareness of an alternative to the use of increasingly expensive nitrogen fertilizer, has promoted scientists from diverse disciplines to consider enhancing the efficiency of symbiotic N-fixation in legumes.

The first step in the multi-stage strategy to select effective strains of *Rhizobium* is to collect local strains and make available germplasm resources for selection. Traditionally there are two types of *Rhizobium* collections: ' Classical ' and ' Working ' (Halliday, 1979).

The ' Classical ' collection is one in which the curator conserves only fully authenticated strains that have proven effective nitrogen-fixers with specific legumes under growth conditions of the curator's choice. Such collections have to house relatively small numbers of strains due to the man-hours required for authentication of isolates testing their effectiveness with a range of legumes and maintaing a current catalog of their origins and characteristics.

The ' Working ' collection, is one in which all isolates conforming to expected visual characteristics on primary isolation plates are presumed to be *Rhizobium* species. Such collections tend to be assembled by mission-oriented researchers whose goal is to select the most effective strain of *Rhizobium* for a particulat legume host, or for a specific soil stress.

The Biological Nitrogen Fixation (BNF) Work at Nazret Research Station, initiated in 1984 with funding from International Fund for Science (IFS). The BNF research activity has started with the ' Work ' collection of local strains of *Rhizobium* of the important legumes of the country. The objective of local culture collection of *Rhizobium* strains was to obtain available germplasm resource for selection of effective strains of *Rhizobium* which can be used for inoculant production for regions where the soil has not enough *Rhizobium* cells due to some reasons.

MATERIALS AND METHODS

1. Nodules Collection & Preservation

The most effective way to build up a collection of *Rhizobium* strains is to isolate those strains from fresh nodules found on indigenous legume. However it was not possible to use isolation techniques immediately after collecting nodules, due to the distance of the laboratory from areas where collected. Thuse nodule preservation method was used to bring the collected nodules to the laboratory.

At the end of 1983, nodules of the important legumes were collected from the farmers field in the major pulse growing areas. Healthy plants with deep green leaves were selected in the field, and the roots were carefully excavated from the soil. Nodules were detached by cutting the root at about 0.5 cm on either sides of the site of the nodule attachment to get root tails for manipulating the nodules with forceps, there by reducing the risk of damage to the nodule itself. The detached nodules were placed in the nodules preservation vials at the top of the cotton wool layer.

The nodules preservation vials were prepered by filling the bottom half of vials with silica gel (desicant) and then covered with a 1 cm layer of cotton wool. The vials were kept closed until used.

2. Isolation of *Rhizobium* Strains from Nudules

Isolation procedures used by Halliday (1979) was employed for this work. Desicated nodules from vials were washed with water to eliminate most of the soil. Then the nodules were immersed (1-5 seconds) in 95% ethanol, transfered to 0.1% acidified mercuric chloride solution (H_gCl_2 1gm, HCl 5ml, water to 1 liter), and left for 4 minutes.

After that nodules were rinsed in five changes of sterile water, making transfers with alcohol flamed forceps. The sterilized desicated nodules were allowed to rehydrate in sterile water for 5 minutes after the final rinse.

The rehydrated nodules were crushed in a minimal volume of sterile water with fine-pointed forceps, and loop-ful suspension was taken from each crashed nodules and streaked on culture medium plates, containing Congo red (1 ml/h of a $1/_{400}$ aqueous solution). Then, the streaked plates were incubated, in an inverted posion, at 27°C. After about 5 days single colonies appeared along the streak lines. Isolated colonies from the plates were picked-off and transfered to slant screw-top vials to obtain pure cultures of *Rhizobium* strains.

The isolated colonies from the slant screw-top vials were examined for some *Rhizobium* characteristics. Examining them under the microscope, those which were gram negative and rod shape were taken as collection of *Rhizobium* strains.

3. Preparation of Culture Medium

The basic culture medium used for isolation and routine growth of *Rhizobium* was a modified yeast mannitol medium containing:

Mannitol	10gm
Agar	15gm
K_2HPO_4	0.5gm
$MgSO_4 . 7H_2O$	0.8gm
Na Cl	0.2gm
$FeCl_3 . 6H_2O$	0.1gm
Yeast	0.5gm

The ingredients were mixed in 1000 ml of distilled H_2O and boiled for 10 min, then dispensed into a litre narrow necked flasks plugged with cotton and autoclaved at 120°C for 20 minutes. Conogo red indicator was also autoclaved separatly and 2.5 ml (1% W/V) added to 1000 ml YMA just before pouring the plates. Congo red indicator is useful in isolation, since rhizobia take up the dye sparingly, if at all, while many other organisms are readily distinguished by their intense pink to red coloration. It also has an inhibitory effect on gram positive organisms.

RESULTS & DISCUSSION

Culture collection of *Rhizobium* isolated from economical important pulses' nodule of Ethiopia obtained from farmers' field of different ecological zones is shown in Table 1. The bacteria isolated from collected nodules were: *R. leguminosarum, R. japonicum, R. trifolii* and cowpea type *Rhizobium*.

1. *R. leguminosarum*

Not just any *Rhizobium* will form nodules on any legumes. Thus *Rhizobium* are classified into species or inoculation groups on the bases of the legumes that they infect the host legumes. Faba bean, *(Visia)* peas *(Pisum)*, and lentil *(Lens)* are in the same cross inoculation groups, pea group (Alexander, 1976).

Our collections of *R. leguminosarum* strains were isolated from nodules of peas, faba beans and lentils. 80 strains from faba bean, 20 from peas and 10 from lentil nodules were isolated and recorded as *R. leguminosarum* strains collection.

Neither the differences nor the similarities of the strains were not studied for the objective of this work collection was to have enough strains for selection of effective strains. Halliday (1979) has stated that working collection success is favored by high numbers of strains being available in germplasm resource for screening and selection.

2. *R. phaseoli*

Haricot bean is important pulse crop in the Reft Valley of Ethiopia. 20 strains of *R. phaseoli* were isolated from collected nodules of haricot bean grown in the Reft Valley.

3. *R. japonicum* & Cowpea type *Rhizobium*

200 strains of *Rhizobium* sp were isolated form soybean nodules collected from Nazret, Awassa, Jima, Metu & Assosa. 128 strains of *Rhizobium* sp (cowpea type) were isolated from *Cicer areietinum* nodules collected from the main growing area of the host plant.

Soybean is generally assumed to be nodulated only by specific rhizobia designated *R. joponicum* (Baldwin & Fred 1929). However, at Assosa and Metu, nodules of soybean were found in the areas new to the crop. From the history of those areas, (Metu and Assosa) it was noticed that soybeans have never grown before there. Thus those strains isolated from nodules of soybean collected from Metu and Assosa were expected to be ' Cowpea miscellany '. Alexander (1976) has reported that organisms isolated from soybean nodules frequently infect cowpeas and vice versa. This fact could suggest that at least some of the cowpea rhizobia may be varieties of *R. japonicum*. Leonard (1923), Wilson (1944) and Nanzju (1980) have also noticed that many strains classified as members of the ' cowpea miscellany ' are capable of nodulating soybean, and the boundries between the two groups of strains are far from clear.

The strains isolated from soybean nodules collected from the three Research Stations (Nazret, Awassa and Jima) could be *R. japonicum*, because different inoculation trials have been conducted in those stations.

4. *R. trifolii*

34 strains of *R. trifollii* were isolated from nodules of clovers collected from different locations (Table 1).

Table 1. Strains of *Rhizobium* Collected different agro-ecolgical zones of Ethiopia.

Cross-inoculation group	*Rhizobium* species	Host legume	Area of module collected	Number of strains
Pea group	*R. leguminosarum*	Faba bean	Debre Tabor	21
		(Vicia)	Adyt	7
			Mota	14
			Markos	10
			Bichena	2
			Debre Tsege	5
			Holeta	5
			Gebre Guracha	11
			Welka Georgis	5
		Field pea	Holeta	5
		(Pisum)	Degen	5
			Goha Tseon	10
		Lentil	Debre Zeit	3
		(Lens)	Debre Tsege	2
			Chacha	3
			Dima	2
Bean group	*R. phaseoli*	haricot	Nazret	6
		bean	Awassa	3
		(Phaseolus)	Zewai	8
				3
Cowpea		soybean	Awassa	29
group		*(Glycine*	Jima	41
		max)	Bako	61
			Assosa	11
			Metu	7
			Nazret	51
		Chick pea	Ambo	16
		(Cicer	Weliso	28
		arietinum)	Debre Birhan	3
			Welkite	11
			Maksegnet	18
			Abasamuel	17
			Akaki	6
			Arerte	26
			Debre Zeit	2
			Fiche	1
Clover	*R. trifolic*	clovers	Gebre Guracha	10
		(Tifolium)	Debre Tsege	10
			Chacha	4
			Werota	10

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