Trans. Nat. Acad. Sci. Technol. 14: 455-476 .1992

NATURE OF INDIGENOUS RHIZOBIA FROM *Arachis hypogaea* L. IN SOME BICOL SOILS[1]

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ABSTRACT

Indigenous peanut rhizobia from some Bicol soils with different cropping histories were enumerated, isolated, and characterized based on morphology, cultural characteristics, intrinsic antibiotic resistance and effectiveness. The serological properties of the isolates were compared with the reference strains RP182-13, CB756, 3G4B20, and P3. Tolerance to acidity and competitiveness of selected effective isolates were also studied.

The promising isolates RPG 24, RPG 149, RPG 103, RPG 112, and RPG 211 were as competitive as the reference strains. They showed essential and desirable qualities of a competitive inoculum for a successful inoculation program.

Introduction

Groundnut or peanut (*Arachis hypogaea* L.), a leguminous crop grown in the Bicol Region, offers many combined advantages as a food and food ingredients. It produces highly nutritious seeds containing 20-30% protein and 46-50% oil (Varnell and McCloud, 1975). Although the demand for it is high, there is a low production level due to the inadequate technology practiced by the local peanut farmers.

One aspect of peanut production that needs vital attention is legume inoculation. Successful inoculation of peanuts will depend on the survival of introduced rhizobia in the soil. Osa-Afiana and Alexander (1979) noted that benefits from legume inoculation with *Rhizobium* are sometimes not evident in the season after inoculation because the introduced rhizobia do not persist in the soil in sufficient numbers to nodulate subsequent crops. Thus, the plants are often nodulated by inferior nitrogen-fixing strains or even by ineffective rhizobia from the indigenous soil population (Dowling and Broughton, 1986). Paterno (1982), working on

[1]Excerpt from the Ph.D. dissertation ("Nature of Indigenous Rhizobia From *Arachis hypogaea* L. In Some Philippine Soils") of the author as a scholar of the Department of Agriculture at the University of the Philippines at Los Banos. The author acknowledges the support given by Dr. Erlinda S. Paterno, Dr. Santiago N. Tilo, Dr. Ruben B. Aspiras, Dr. Faustino T. Orillo, and Ms. Fe G. Torres of UPLB.

peanut in a number fo Philippine soils, found that inoculant strains could form 10 to 30% of the nodules, and sometimes up to 60%, but still have no significant effect on the yield. This variability in nodule occupancy by the inoculant strains might be indicative of the high competitiveness of the native rhizobia even among related soils.

Many nodules are produced by mixed infections (Broughton, 1978; May and Bohlool, 1983) and many inoculant strains now commercially available were once but members of some indigenous rhizobial populations of some soils. They have been selected for their ability to adapt to prevailing soil conditions and to persist into subsequent cropping seasons (Dowling and Broughton, 1986).

This study was conducted to: (a) enumerate and isolate indigenous peanut rhizobia from some Bicol soils of different cropping histories; (b) characterize native peanut rhizobia in terms of IAR patterns and serological properties; (c) determine tolerance of native peanut rhizobia to acid-Al stress; (d) and evaluate the competitive ability fo the native peanut rhizobia.

The study was performed at the Department of Soil Science, University of the Philippines at Los Banos, College, Laguna.

Review of Literature

Legume-Rhizobium Symbiosis

Agricultural crops usually have a fairly high nitrogen (N) requirement. Elemental N is abundant in the atmosphere. However, most plants and animals still suffer from N-deficiency at some stage of their lives because green plants can not use gaseous N. On the other hand, the root nodule bacteria or rhizobia have the ability to take atmospheric N and convert it into a form that is usable by their host legume.

Nitrogen is an expensive fertilizer – its commercial manufacture is costly, adding to the high farm production inputs. In contrast, taking advantage of the symbiotically-fixed atmospheric N would be economical.

Methods Used in Characterizing Rhizobial Population

Plant-Infection Technique

It is generally accepted that the only reliable form of identification of any particular species of *Rhizobium* is based on the ability of the organism to form nodules on the roots of a host legume (Fuzimura and Watanabe, 1961; Brockwell, 1963; Vincent, 1970; Weaver and Frederick, 1972). The method involved is called the "plant-infection" technique (Hely et al., 1957).

Test plants are grown aseptically in test tubes and are inoculated with a series of soil dilutions. *Rhizobium* numbers are calculated from the proportion of

test plants forming nodules at each dilution. The rhizobial population of the sample is determined by using the Most Probable Number (MPN) method (Brockwell, 1963).

Intrinsic Antibiotic Resistance

Antibiotic resistance is one of the most common methods used for the identification of inoculum strains from nodules (Amarger and Lobreau, 1982; Hagedorn, 1979; Kremer and Peterson, 1982; Labandera and Vincent, 1975). However, little attention has been given to the resistance patterns of indigenous rhizobia which have had less opportunity for exposure to various antibiotics other than through contact with antibiotic-producing microorganisms inhabiting the same ecological niche (Hagedorn, 1978). From the works of several researchers it appeared that many antibiotics could be used for the selection of resistant rhizobial strains; however, Hagedorn (1978) advocated that caution must be exercised so that essential characteristics are not impaired.

Gel Immunodiffusion

The Gel immunodiffusion (GID) technique involves soluble antigens from the bacterial suspension and antibodies from the antiserum which diffuse into the agar gel (Vincent, 1970). When the antigen and the antibody meet at optimal concentrations, precipitates are formed in the shape of lines or bands. The number of precipitin bands formed is equal to the number of detectable antigens in the bacteria (Vincent, 1970). Dudman (1971) showed that unknown strains present in soybean root nodules were readily identified by immunodiffusion.

Enzyme-linked Immunosorbent Assay

According to Ramanan (1983), enzyme-linked immunosorbent assay (ELISA) is based on the interaction between an antigen and an antibody. The antigen will only react with a particular antibody which is specific for it and this determines the specificity of the technique. The antigen is bound in between the antibody and an enzyme-conjugate. An enzyme-substrate is then added and the substrate which initially is colorless develops a yellow color when acted upon by the enzyme. The amount of color developed is directly proportional to the quantities of antigen present in the sample.

Factors Affecting Rhizobial Growth

Soil Acidity

Acidity is known to affect the activity of *Rhizobium* in soils (Keyser et al., 1979) and methods have been developed recently for the preselection of *Rhizobium* strains tolerant of the acidity factors (Date and Halliday, 1978; Keyser and Munns, 1979). Ayanaba et al. (1983) formulated an agar plate method for the rapid, preliminary screening of large numbers of rhizobia for tolerance of acidity and aluminum (acid-Al). Using this method, they showed that strains that formed dry, pinpoint colonies were more sensitive to acid-Al than those that formed large, gummy colonies.

Competition

One of the most important factors that influence soil population of indigenous rhizobia is its ability to compete for infection sites on the host root with previously inoculated and newly inoculated strains. Nodule induction at high frequency by introduced rhizobia has been readily seen in soils where indigenous rhizobia are deficient.

Effectiveness of rhizobial strains for N-fixation can easily be measured (Vincent, 1970). However, the ability of *Rhizobium* to nodulate competitively is much more difficult to assess (Hardarson et al., 1981). Bromfield and Jones (1979) suggested a competitiveness screening method to accelerate evaluation of rhizobial strains for competitive ability. The competitive ability test usually includes the determination of the percentage of nodules attributable to each of two or more strains of *Rhizobium* that are simultaneously inoculated on a host. Then strains forming nodules may be determined by using antibiotic resistant mutants (Hagedorn, 1979; Hardarson and Jones, 1979).

Materials and Methods

Isolation of Indigenous Rhizobial Strains

Field Site Description

The sampling areas consisted of different locations in the Bicol Region. They were divided to include three cropping histories, namely: (1) field not planted to peanut, (2) field previously planted to peanut without inoculation, and (3) field with standing peanut crop without inoculation.

Soil Sampling and Preparation

From each field measuring about one-half to one hectare, forty 200-g soils samples with a depth of 20 cm were taken at random using a shovel. Composite soil samples were taken for chemical analysis, for the estimation of the native rhizobial populations, and for the isolation of indigenous rhizobial strains.

Estimation of Indigenous Rhizobial Populations

The indigenous rhizobial populations for each field were determined using the plant-infection technique (Brockwell, 1982). A tenfold serial dilution of the soil sample was done. Aliquots were used to inoculate test plants in a plastic growth pouch. The most probable number of nodule bacteria in the original sample was calculated from the proportion of test plants forming nodules at each dilution level. The estimate of rhizobial population was done using the MPN Table (Brockwell, 1982).

Isolation of Rhizobia from Nodules

Surface-sterilized nodule was crushed aseptically and the milky fluid that came out was streaked over the surface of yeast extract mannitol (YEM) agar containing Congo red. The plates were incubated at 28°C for 7 to 10 days.

Then typical isolated colonies were picked up for further restreaking and Gram-staining (Vincent, 1970).

Authentication of Isolates

Authentication of the isolates was done using the Plant Infection Method (Vincent, 1970). Peanut c.v. BPI-P9 seedlings grown under bacteriologically controlled conditions in growth pouches (Fig. 1) were inoculated with the presumptive rhizobial isolates. Plants were observed for their response to inoculation on the basis of plant height, color, and nodules formed. They were compared for nodulation response with the nodulation reaction of the reference strains from the Nitrogen Fixation and Mycorrhizae Program Laboratory at BIOTECH, namely : RP 182-13, CB756, P3, and 3G4B20 (Table 1). Negative controls consisted of uninoculated plants and uninoculated plants with N added to the nutrient solution at the rate of 140 kg N/ha or 70 ppm in the form of KNO_3 (Somasegaran and Hoben, 1985).

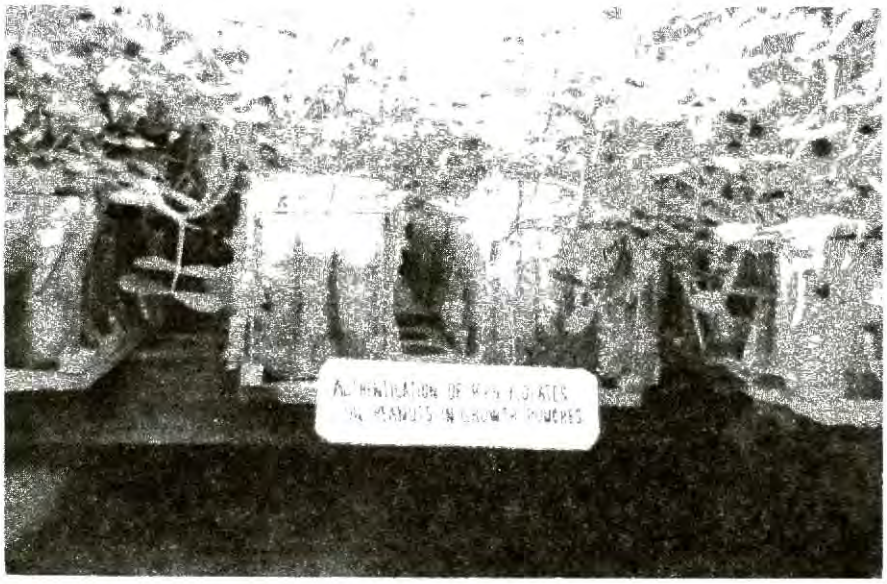

Figure 1. Authentication of indigenous peanut rhizobia in plastic growth pouches in the growth chamber.

Table 1. Reference strains used in the study.

Strain Designation	*Host of Derivation*	*Region of Origin*
RP 182-13	*Arachis hypogaea*	Mexico
CB 756	*Macrotyloma africanum*	Zimbabwe
P3	*Arachis hypogaea*	Philippines
3 G4B20	*Arachis hypogaea*	Zimbabwe

Determination of the Inoculum Level Using a Multiple Inoculator

A multiple inoculator was prepared and then was calibrated to determine the number of cells each prong would deliver (Fig. 2). There were 20 prongs.

Each tip of the prong was dipped into 1-ml yeast extract mannitol broth with rhizobial growth of the test strains. The dilution plating was done on plates of

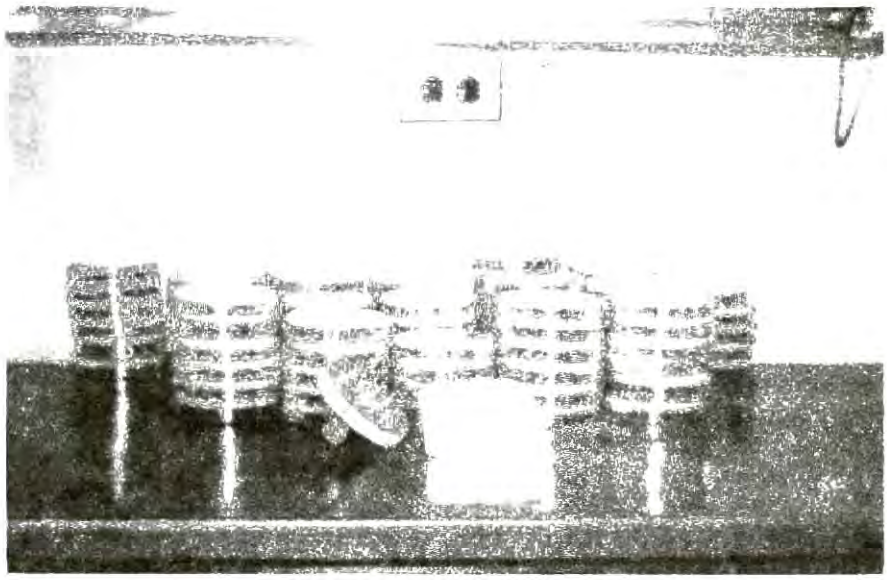

Figure 2. The multiple inoculator with 20 prongs (foreground).

YEM agar with Congo red. Colony growths were observed and number of colony-forming units per ml of stock culture delivered by each prong was computed.

Intrinsic Antibiotic Resistance

The Intrinsic Antibiotic Resistance (IAR) test was performed to determine the IAR patterns of the indigenous rhizobial isolates. The appropriate quantities of the lowest concentrations of the different antibiotics to which the reference strains were found to be resistant were added to the melted YEM agar. Then the plates were inoculated with cultures of the rhizobial isolates using the multiple inoculator. Plates were incubated at 28°C for 7 to 10 days, then scored accordingly.

Symbiotic Properties of the Promising Indigenous Rhizobial Strains

Test for Effectiveness

The test was a single factor in a Completely Randomized Design (CRD) with three replications. Each treatment was a plastic growth pouch with 3 peanut c.v. BPI-I89 plants.

The nodules and plant tops were harvested after six weeks of growth and were oven-dried at 70°C for 48 hours. Symbiotic effectiveness (SE) was calculated based on dry matter yield as:

$$\% \text{ SE} = \frac{\text{Dry weight of inoculated}}{\text{Dry weight of uninoculated + N}} \times 100$$

Data collected were analyzed statistically using the Pooled Analysis of Variance for CRD (Gomez and Gomez, 1984). The Duncan Multiple Range Test (DMRT) was used in the comparison of treatment means.

Serological Typing

Development of Antisera

The basic reagent for strain identification utilizing serological techniques is rabbit antiserum developed against *Rhizobium* (Somasegaran and Hoben, 1985). *Rhizobium* antigens are prepared and used for the development of antisera in rabbits.

Pure cultures of rhizobia were prepared and cell suspensions were centrifuged at 5000 rpm for 20 minutes at 15°C. The precipitate was washed with sterile saline solution for three consecutive times, then resuspended in 10 ml saline solution.

The prepared cell suspensions were injected on 4-month old rabbits at four different settings, namely: (1) day 1, (2) day 14, (3) day 28, and (4) day 35. On the 40th day, the rabbits were bled through cardiac puncture (Somasegaran and Hoben, 1985).

The blood was collected carefully, antiserum was purified, and its protein concentration was determined using an UV-visible spectrophotometer. Then the antiserum was standardized by having the appropriate enzyme conjugate combination.

Gel Immunodiffusion

Glass plates were prepared and hot melted Agarose was introduced on the surface of the plates and allowed to solidify. Using a stainless steel GID puncher, wells were formed on the agar (Fig. 3).

After dispensing the antisera and the antigens on the wells, the plates were incubated at room temperature in a water-saturated atmosphere to prevent moisture loss from the gel and its subsequent breaking up. Observation for the formation of precipitin bands was done after 48 hours (Vincent, 1970).

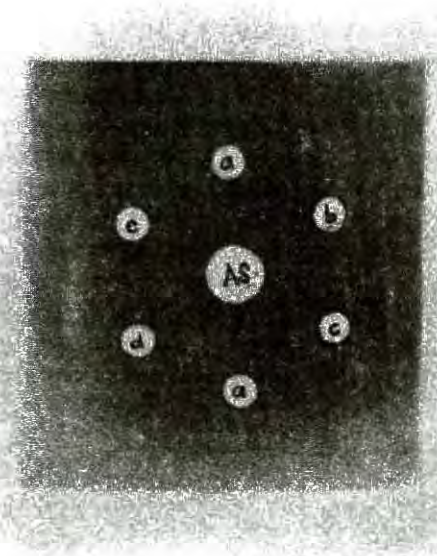

Figure 3. The hexagonal pattern of agar wells for GID test. Four unknown indigenous peanut rhizobia are compared with a standard strain. (AS) antiserum, (a) homologous antigen, (b) unknown 1, (c) unknown 2, (d) unknown 3, (e) unknown 4.

Enzyme-linked Immunosorbent Assay

Freshly prepared coating buffer solution was dispensed into the wells of ELIZA microtiter plates and nodules were crushed thoroughly in the respective wells. Plates were incubated at 4°C overnight. Then the crushed nodules were washed off from the plates with phosphate buffer solution, the antiserum was applied on the wells, and then incubated for 2 to 3 hours at room temperature. Washing with the buffer solution was again done for four more times. Then the enzyme-labelled antiglobulin was dispensed into the wells and the plates were incubated for another 2 to 3 hours at room temperature followed by four times washing with the buffer solution. This was followed by the application of freshly prepared substrate buffer solution into the wells, incubation for 30 minutes to one hour and the simultaneous observation for the positive yellow color development. Colorless or no yellow color development meant negative result or the antiserum was not related to the strain that nodulated the test plant.

Screening for Acid-tolerant Rhizobial Isolates

The indigenous rhizobial isolates were screened for acid tolerance following the method developed by Ayanaba et al. (1983). The dried agar plates were inoculated with the rhizobial isolates using the multiple inoculator. The plates were incubated at 28°C for 7 to 10 days and colony growth was observed. Likewise, change in color of the indicators on the agar media was noted.

Competition Among Rhizobial Strains

The rhizobial strains were inoculated in pairs into one-week-old peanut seedlings in growth pouches. After six weeks of growth, the nodules were tested for rhizobial invasiveness and nodule occupancy using ELISA (Ramanan, 1983) technique.

The parameter considered for evaluation was the number of nodules occupied by the isolates tested. Data collected were analyzed statistically using the Pooled Analysis of Variance for CRD (Gomez and Gomez, 1984). Statistical analysis was done on arc sine transformation of the data.

Results and Discussion

Soil Characteristics and Number of the Native Rhizobia

Field Not Planted to Peanut

There were three sampling sites for the field not planted to peanut. The soil samples were taken from the provinces of Camarines Sur, Masbate, and Sorsogon (Fig. 4). The soil sample from Camarines Sur falls on the order Vertisol and those from Masbate and Sorsogon are Ultisols (Mariano and Valmidiano, 1973). These soils have textural classes of clay and clay loam.

The pH of the soils ranged from 5.26 to 6.30, organic matter content was 1.07 to 3.92%, and total N was 0.08 to 0.29%. Native peanut rhizobia ranged from 0.40 x 10^2 to 4.20 x 10^2 cells/g soil.

Indigenous rhizobia were found in soils not planted to peanut, showing that they were adapted to their soil environment. However, the population was relatively low in an acid soil compared to a high population noted in a nearly neutral soil.

Presence of rhizobia in fields not planted to peanut is not entirely unusual since legume root nodule bacteria have the ability to live in soil in the absence of host plants (Hiltbold et al., 1985). They are facultative symbion s, and in the saprophytic state, are independent of their host legumes (Woomer et al., 1988).

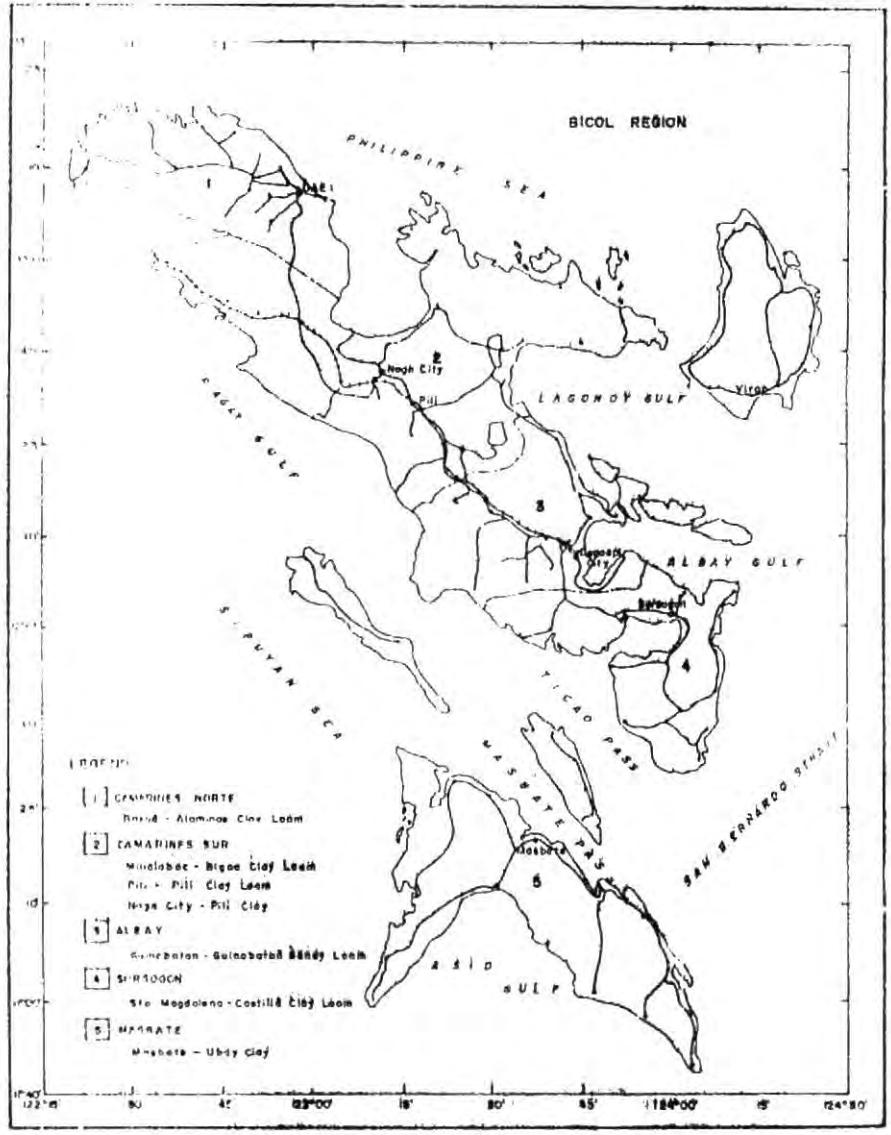

Figure 4. Soil Sampling Sites

Field Previously Planted to Peanut Without Inoculation

From three fields that were previously planted to peanut without seed inoculation, soil samples were collected. These samples were taken from selected sites in the provinces of Camarines Norte and Camarines Sur, including Naga City (Fig. 4). The soil from Camarines Norte is an Alfisol, that of Camarines Sur is a Vertisol, and that of Naga City is an Inceptisol (Mariano and Valmidiano, 1973). The soil textures were clay loam and clay.

Soil pH ranged from 4.80 to 6.20, organic matter content was 2.25 to 4.44%, and total N was 0.12 to 0.16%. Native rhizobia varied from 0.40×10^2 to 9.20×10^2 cells/g soil.

Indigenous rhizobia were likewise found in fields previously planted to peanut without inoculation, showing that they can persist in the soil even in the absence of a legume host.

Field with Standing Peanut Crop without Inoculation

One sandy loam soil sample was collected in Albay province. It is an Entisol (Mariano and Valmidiano, 1973) with a pH of 6.10, organic matter content of 1.27%, and total N of 0.07%. Native rhizobia was 14.70×10^2 cells/g soil.

Presumptive Test of Isolates on Different Media

Indigenous *Rhizobium* Strains

From the peanut nodules randomly selected, presumptive rhizobial isolates were collected. These cultures were designated RPG (Rhizobia Philippines Groundnut) isolates, which hereafter are used to refer to those indigenous rhizobia isolated from some selected Philippine soils.

Indigenous peanut rhizobial cells were seen to be Gram-negative, short-medium rods, and non-spore forming. They were observed to form colonies on YEM agar in 3 to 5 days for the fast growers and in 5 to 7 days for the slow growers.

There were two types of colony morphology, the dry and the wet (Fig. 5). In general, the fast growers formed semitranslucent colonies appearing smooth, watery, and wet. They were circular, raised, and with flat elevation. On the other hand, associated with the slow growers are colonies that were opaque, granular, paste-like, and dry. They were circular and convex with raised elevation.

Figure 5. Colony types of indigenous peanut rhizobia. dry = granular, opaque, wet = smooth, semi-transluscent, watery paste-like

Minimum Inhibitory Concentration of Antibiotics for Indigenous Peanut Rhizobia

Minimum inhibitory concentration (MIC) is the lowest concentration of antibiotics that would serve to distinguish among different strains or groups of rhizobia. The distinction is by differential response to antibiotics. One group of rhizobia may be inhibited by a given antibiotic while another group may not be affected by it. The levels of selected antibiotics that elicited the best differentiating response among groups of rhizobia are chosen and used to determine the intrinsic antibiotic resistance patterns of the isolates or strains under study.

From the response of the test isolates used, the selected minimum concentrations at which least resistance was observed (Figs. 6, 7, and 8) were 10 ug/ml for tetracycline (tet); 50 ug/ml for chloramphenicol (chl), kanamycin (kan), and streptomycin (str); and 300 ug/ml for penicillin (pen). These concentrations were used to develop the intrinsic antibiotic resistance patterns of the indigenous peanut rhizobia. They were chosen to elicit a range of responses from the three groups of rhizobial populations.

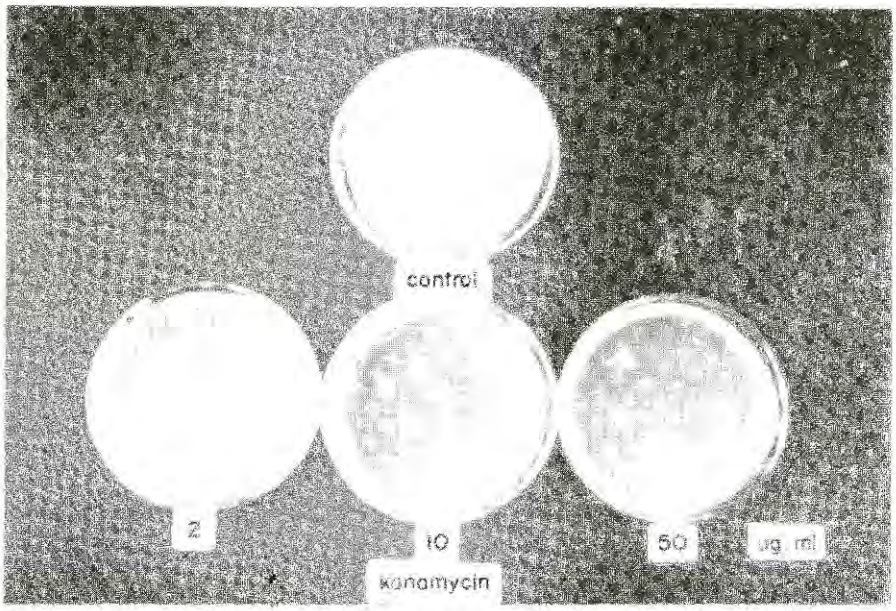

Figure 6. Determination of MIC level for kanamycin.

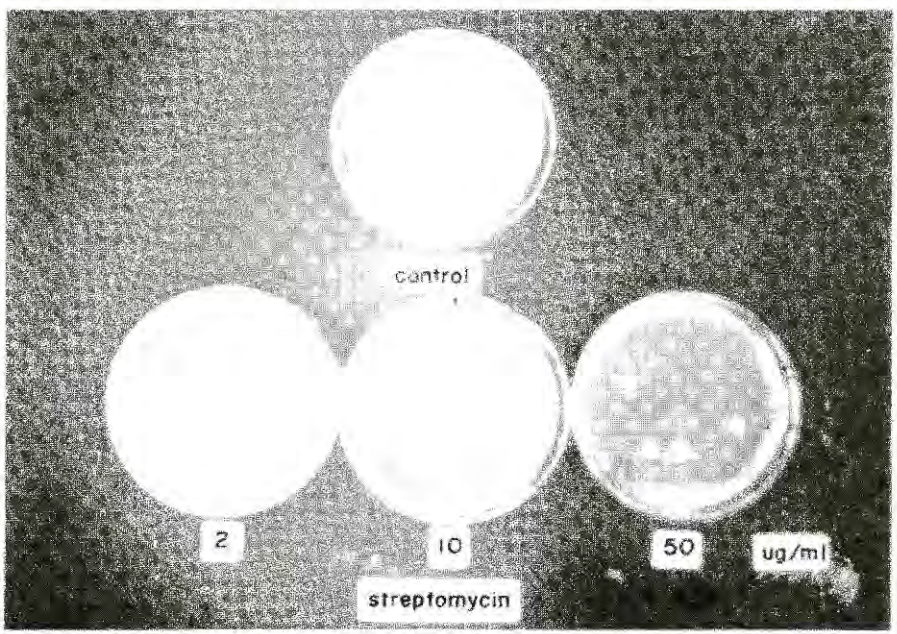

Figure 7. Determination of MIC level for streptomycin.

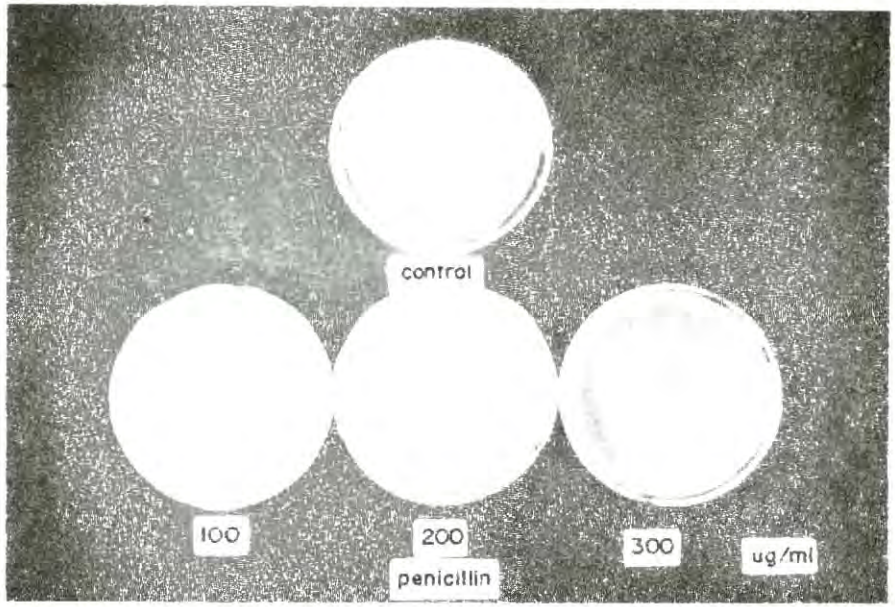

Figure 8. Determination of MIC level for penicillin.

Intrinsic Antibiotic Resistance (IAR) Patterns of Indigenous Peanut Rhizobia

The diversity of the isolates is evidenced by the number of IAR patterns exhibited by the group. Out of 31 possible IAR patterns, only 17 were obtained. There were 16 IAR patterns demonstrated by rhizobia from fields not planted to peanut, 7 for fields previously planted to peanut, and 8 for fields with standing peanut crop. The IAR patterns of rhizobia from fields not planted to peanut were distributed among the members with 15% showing resistance to chloramphenicol. On the other hand, the highest proportion (29%) of the isolates with the IAR pattern chl, kan, str, pen was observed in the fields previously planted to peanut.

From the fields with standing peanut crop the dominant IAR pattern noted was IAR-chl, str, pen.

Rhizobia from fields not planted to peanut were more diverse than those from fields previously planted to peanut and from fields with standing peanut crop. Likewise, rhizobia from fields with standing peanut crop were more diverse than those from fields previously planted to peanut. Their mean differences were statistically significant at 5% level ($x^2 = 16.17$).

Apparently then native rhizobia can persist in the soil regardless of the presence of a legume host. However, a more diverse group of rhizobia can survive better under legume than under previously cropped areas.

Symbiotic Effectiveness of the Indigenous Peanut Rhizobia

As a group, rhizobia from fields not planted to peanut had significantly lower nodules formed, dry matter yield, N content, N uptake, N fixed, and symbiotic effectiveness than rhizobia from fields with the other two cropping histories. In terms of dry matter yield, rhizobia from fields previously planted to peanut had significantly lower shoot weight than isolates from fields with standing peanut crop. But, these two groups of indigenous rhizobia did not differ signficantly in terms of symbiotic effectiveness. In general however, as groups, these indigenous rhizobia and significantly lower nodulation, N-fixation, and symbiotic effectiveness than the group of reference strains.

Selected promising isolates, evidently, were infective as reflected by their ability to nodulate the host plant. The highest dry matter yield was obtained with RPG 24 which was not significantly different from that of the uninoculated + N control but significantly higher than the reference strains. RPG 112, RPG 211, RPG 95, and RPG 149 produced dry matter yields similar to the reference strains.

RPG 24 had the highest mean N content at 2.28% which did not differ significantly from the uninoculated + N control. It was likewise observed that RPG 112, RPG 211, and RPG 95 produced high N content comparable to those produced by the reference strains.

The same trend of results was obtained with N uptake and N fixed. Isolates RPG 24, RPG 112, RPG 211, and RPG 95, showed good performance relative to the reference strains. The symbiotic effectiveness of the isolates ranged from 74.99 to 103.94% compared to 90.18 to 97.06% of the reference strains. RPG 24, RPG 112, RPG 211, and RPG 95 did not differ significantly from CB756, while RPG 149 did not significantly differ from RP182-13, CB756, P3, and 3G4B20.

Serological Properties as Determined by GID

Antigenic reactions showed that four indigenous rhizobia from field not planted to peanut reacted positively with antiserum RPG 24. Similarly, three native rhizobia from field not planted to peanut and two from field presently planted to peanut without inoculation exhibited relatedness with antiserum RPG 149 (Fig. 9).

In contrast, RPG 95 and RPG 103 antisera did not react positively with any of the isolates. Moreover they did not demonstrate relatedness with the reference strains like the rest of the native rhizobia from the three field categories.

These results indicated the diversity of the native rhizobial population as had been observed in their IAR patterns. Furthermore, it was noted that some isolates with the same IAR pattern did not consistently show the same antigenic properties exhibited by others.

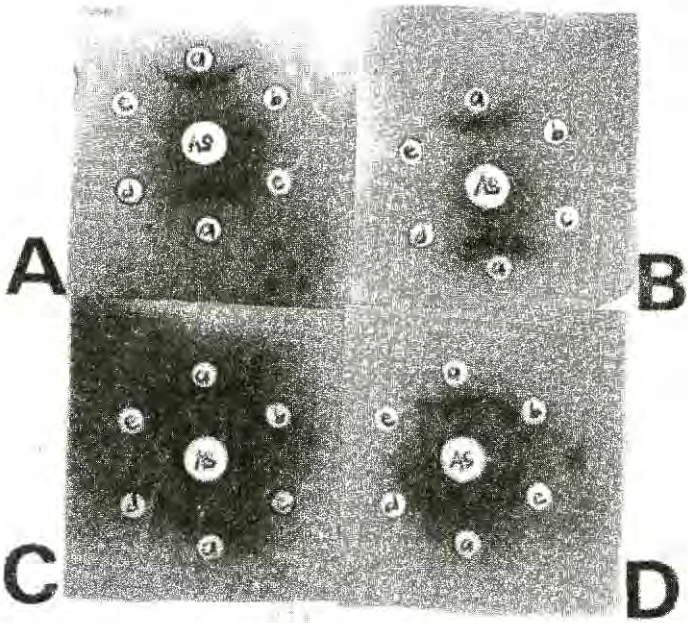

Figure 9. Gel immunodiffusion assay of indigenous peanut rhizobia.

- **A. Antiserum (AS) P3 showing antigenic unrelatedness to (b) RPG 24, (c) RPG 95, (d) RPG 103, and 9e) RPG 149.**
- **B. Antiserum (AS) CB756 showing antigenic unrelatedness to (c) RPG 24, (c) RPG 95, (d) RPG 103, and (e) RPG 149.**
- **C. Antiserum (AS) RPG 24 showing antigenic relatedness to (e) RPG 35, (d) RPG 37, (e) RPG 38, and unrelatedness to (c) RPG 103.**
- **D. Antiserum (AS) RPG 149 showing antigenic relatedness to (b) RPG 162, (c) RPG 15, (d) RPG 16, and unrelatedness to (e) RPG 24.**

Tolerance to Acid-Al Stress

The acid tolerant isolates were obtained from soils with pH ranging from 5.6 to 6.9. In general, the fast-growing rhizobia that formed large (1.0 to 1.5 mm dia), gummy, and wet colonies were more tolerant of acid-Al stress than the slow-growing rhizobia that formed small, pinpoint dry colonies (Fig. 10).

The four reference strains used were sensitive to acid-Al stress.

The proportion of native peanut rhizobia from the three cropping systems showed that more acid-Al tolerant isolates came from fields not planted to peanut and from fields previously planted to peanut without inoculation. However, there was no signficant difference in the proportion of acid-Al tolerant isolates between

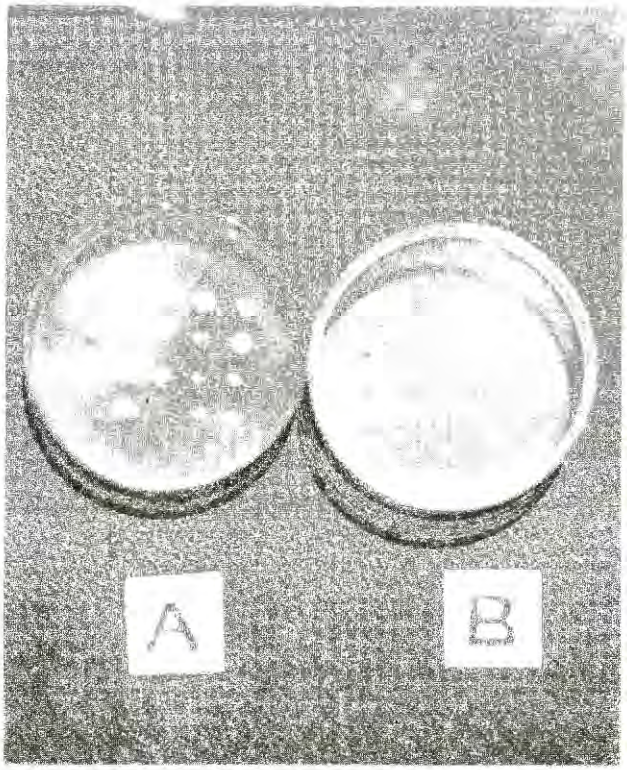

Figure 10. Agar plate screening of indigenous peanut rhizobia for acid-Al tolerance:

D. Growth in the control medium-change in pH indicated by color change of indicator in the agar
B. Growth in the acid-Al stress medium

the two cropping histories earlier mentioned. Results were statistically significant at 5% level ($x^2 = 12.272$).

It can be seen that occurrence of acid-Al tolerant rhizobia was not site specific. They can be found in soils with a wide pH range.

Effective acid-Al tolerant strains that can be used as seed inoculants would have an advantage over other acid, Al-sensitive strains in acid, Al-rich soils. Hence, the use of legumes and rhizobia adapted to acid-Al stress would reduce the need for expensive lime applications.

Competitiveness of Selected Indigenous Rhizobial Isolates

The ELISA test was used to assess competition of rhizobia in terms of nodule occupancy of the effective strain.

It was found that for all mixtures tested, one of the strains in each pair formed most of the nodules examined (Table 2). In all cases, however, the effective strain dominated the ineffective competitor.

Results indicated that RPG 24 and RPG 149 were as competitive as the four reference strains. On the other hand, RPG 103 and RPG 95 had similar competitive ability as P3 and CB756, respectively. RPG 95 was the least competitive among the test strains.

Table 2. Proportion of nodules formed by the indigenous rhizobia on peanut c.v. BPI-P9 grown in plastic growth pouches in the growth chamber.

Isolate Combination	*% Nodules Occupied*							
	Rhizobial Strain							
	RPG 24	*RPG 95*	*RPG 103*	*RPG 149*	*3G4B20*	*CB756*	*RP182-13*	*P3*
RPG 24 + 95	38.57	51.43ns						
24 + 103	40.13		49.87ns					
24 + 149	52.50*			37.50				
RPG 95 + 103		27.05	62.95**					
95 + 149		35.04		54.97**				
RPG 103 + 149			42.28	47.72ns				
RPG 24 + 3G4B20	51.60ns				38.41			
24 + CB756	52.27ns					37.73		
24 + RP182-13	41.68						48.32ns	
24 + P3	43.41							46.60ns
RPG 95 + 3G4B20		28.99			61.02**			
95 + CB756		38.57				51.43ns		
95 + RP182-13		24.02					65.98**	
95 + P3		34.41						55.59**
RPG 103 + 3G4B20			60.14**		29.86			
103 + CB756			37.76			52.24*		
103 + RP182-13			56.53**				33.47	
103 + P3			49.81ns					40.20
RPG 149 + 3G4B20				44.19	45.81ns			
149 + CB756				39.34		50.67ns		
149 + RP182-13				40.20			49.81ns	
149 + P3				39.20				50.80ns

** – Proportion is significantly different at 1% level
* – Proportion is significantly different at 5% level
ns – No difference in proportion at 5% level

Statistical analysis was done on arc sine transformation of the data.

It appears that under field conditions effective and ineffective indigenous rhizobia can be as competitive as the introduced inoculum strains. The ineffective rhizobia from the indigenous soil population could pose a problem in an inoculation system. They could hinder the establishment in the microcosm of the introduced effective soil inoculants. In such a case, it is important that the effective N-fixing strains be inoculated in a high density population to outcompete these more invasive members of the indigenous rhizobial microflora. They must be in great number in order to have a foothold on the nodulation niche exclusively. Weaver and Frederick (1974) suggested that in order for the inoculant strain to form 50% of the nodules, the inoculum should be supplied at 1000 times the level of the indigenous Rhizobium population.

Summary and Conclusion

From the results of this study, the following inferences are made:

1. There was lower indigenous peanut rhizobia in soils uncropped with peanut than in soils cropped with it. However, effective N-fixing rhizobia can persist in the soil, whether cropped or uncropped with legume.
2. There was low population density in soils with low pH value. Rhizobial number was positively correlated with soil pH ($r = 0.572$).
3. The MIC's for these indigenous rhizobia are 10 ug/ml for tetracycline, 50 ug/ml for chloramphenicol, kanamycin, and streptomycin; and 300 ug/ml for penicillin.
4. Some native peanut rhizobia showed tolerance of acid-Al stress. Moreover, they were not site specific.
5. The indigenous rhizobia can be as effective N-fixers as the reference strains. Moreover, symbiotic effectiveness was not influenced by cropping history.
6. Some native peanut rhizobia can effectively compete with the standard strains.

In the light of these findings, it is recommended that in the screening for inoculum strains, it is vital to select rhizobia that have the ability to compete effectively for nodulation sites. Moreover, they must have the ability to fix sufficient N to sustain a level of legume yield that is the same as, if not more than, its production when applied with nitrogenous fertilizer. Likewise, it is imperative that they survive and persist in the soil for a long period of time over different ecological stresses.

Furthermore, on any inoculation program, it is equally important that the initial prevailing cell density of the indigenous rhizobial population be deter-

mined and its effectiveness as N-fixer be assessed and evaluated to ensure a successful establishment of the introduced inoculum strain in the biological microcosm.

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