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NITROGEN FIXATION BY ASSOCIATIVE

GRASS-BACTERIA SYSTEMS

BY

THE UNIVERSITY OF FLORIDA

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INTRODUCTION

Highlights of our research on the nitrogen fixation potential of grass-bacteria systems are contained in this annual report. The main objectives of this contract with USAID are to investigate the nitrogen potential of nitrogen-fixing bacteria in association with grasses and cereal crop plants and to develop systems with a potential of reliably providing nitrogen to plants growing in nitrogen-deficient soils in developing countries. The principal organism used in these experiments was Azospirillum brasilense, although some other N_2-fixing organisms have also been investigated. The crop plants included pearl millet, sorghum and corn; the forage crops included sudangrass, bahiagrass, limpograss, and pearl millet x elephantgrass hybrids. The experiments ranged from axenic single bacterial strain and plant combinations to field experiments.

Several experiments were conducted to test the flow of N to the plant from the bacterium and/or soil, using ^{15}N as the tracer, and to evaluate N_2-fixation by ^{15}N-isotope dilution.

The major difficulty in conducting $^{15}N_2$ fixation experiments with grass-bacteria associations results from the relatively low level of nitrogenase activity. Long incubation periods in tightly sealed growth chambers are required. One of our major efforts during the year was continuing the design and construction of suitable growth chambers for long term $^{15}N_2$ fixation experiments. Several chambers described in last year's report were used to determine actual nitrogen fixation rates and nitrogen-transfer rates from the bacterium to the plant.

Several studies were completed in 1982-83 regarding ecological factors which affect the survival and activity of Azospirillum in the rhizosphere. In order to accomplish these experiments, a double marked antibiotic strain, CdSR, was engineered and dilution plate counts and most probable (MPN) techniques were used to determine bacterial numbers.

Field inoculation experiments continued in 1982-83 because of the need to verify Israeli reports of consistent yield increases when Azospirillum brasilense strain Cd was used. A summary of the three field experiments, conducted in Florida and in New Mexico in which strain Cd was used, is given.

Several experiments tested the effect of plant-derived and other carbon substrates on asymbiotic N_2-fixation. Acetylene reduction activities were 15 times greater when living grass roots were present compared to root-free cores. In addition, carbohydrate content of Hemarthria roots was studied under field conditions.

The group was extremely active in reporting research results and summaries of their research during the past year. Over twenty abstracts were prepared and either posters or presentations were made at the following meetings:

ASA (Anaheim) 2 presentations
N_2-Fixation (Banff) 2 presentations and 3 posters
Winter Symposium (Miami) 1 poster
ASM (New Orleans) 1 poster
SCSSF (Tallahassee) 2 presentations
SAAS (Atlanta) 2 presentations
Microbial Ecology (Lansing) 1 poster
ASA (Wash DC) 1 presentation and 2 posters
N_2-Fixation (Holland) 5 posters
Azospirillum Workshop (Beyreuth) 1 presentation

REPORTS

I. ESTABLISHMENT OF CONSISTENTLY REPRODUCIBLE, AXENIC PLANT-BACTERIA SYSTEMS.

a. Establishment of Associative N_2-Fixing Systems

Monoxenic root-bacteria associations were initiated in test tubes containing agar medium free of both nitrogen and carbohydrates. Seedlings of five sorghum genotypes were inoculated with four strains of bacteria. The degree of association was evaluated by visually rating the amount and location of bacterial growth and by assaying for acetylene reduction activity (ARA). Active ARA associations were established 83% of the time, in that the tubes with plants had higher ARA than the no-plant controls. Sterile controls remained sterile and had no ARA. The plants and intact agar-root systems were transferred aseptically to mason jar assemblies, containing autoclaved soil, to see whether the active, already established, associations would be maintained. Acetylene reduction assays were repeated three more times on the jar assemblies. The ARA rates in these pre-established systems were equivalent to two non-autoclaved, but highly active natural soils from Putnam and Madison Counties, which were included as standards because of their high ARA. Intact soil-plant cores from the mason jar assemblies were transplanted into a field with low ARA and were re-assayed for ARA two more times. Highest ARA was from the natural soil-plant transplants, with lower rates from all inoculated treatments. This suggests that the complex natural systems are better able to persist in competitive situations than the inoculated associations established from selected N_2-fixing strains.

b. Inoculation of Grasses With N_2-Fixing Bacteria in Autoclaved Versus Unautoclaved Soil.

Plant genotype and bacteria strain relationships were studied using axenic soil systems in greenhouse experiments. Both

I. (Continued)

glass and plastic jar assemblies were tested using sorghum plants, N_2-fixing bacterial inoculation, and appropriate controls. Based on acetylene reduction activities, bacteria were slow to become established in all autoclaved soil treatments. Among the autoclaved soil treatments, a significant yield increase was obtained from the treatment which used native soil-root microorganisms. Bacterial counts using the MPN technique in nitrogen free malate media showed significant differences among inocula. Highest count of N_2-fixing bacteria was 4.9×10^5 per gram of soil where Azospirillum brasilense strain Cd, was used as inoculum.

c. Factors Affecting Introduced Populations of N_2-Fixing Bacteria in the Rhizosphere.

Several factors affecting the introduction and maintenance of large numbers of N_2-fixing soil bacteria into the rhizosphere of selected crop plants were examined. Plants were grown in a greenhouse. Several methods were utilized to introduce N_2-fixing bacteria into the rhizosphere. Nitrogenase activity was estimated by C_2H_2 reduction. Organic material released by the roots was determined by ^{14}C labelling and bacterial populations were measured by dilution plate counts. Rates of nitrogenase activity were positively correlated to soil moisture. In monoxenic systems, the numbers of N_2-fixing bacteria were found to be positively correlated with soil organic matter content. Soil respiration and subsequent plant dry matter yields were higher with inoculation. Also, the amount of ^{14}C label in the rhizosphere was greater in the inoculated plants. General trends suggest that low soil moisture and organic matter will limit associative nitrogen fixation and growth of soil N_2-fixing bacteria.

d. Effect of Soil Conditions on Survival and Activity of Diazotrophic Bacteria.

Seedlings of Sorghum bicolor and Setaria italica were grown in nutrient solutions, sand, and sandy loam soil, and inoculated with strains of Azospirillum, Klebsiella and Azotobacter. Carbon substrates, pH, and oxygen levels were varied to determine their effects on survival and N_2 fixation activity of the bacteria. Some treatment combinations supported growth of the inoculation strains, but N_2 fixation activity was low in all cases. Further tests, comparing growth of the inoculant strains in cultivated and uncultivated soils, indicated that Azospirillum is more likely to become established in a rhizosphere which has not been disturbed by cultivation.

e. Evaluating Plant-Bacterial Interactions Which Affect Rhizosphere Nitrogen Fixation

Factors affecting the interactions of plants and N_2-fixing rhizosphere bacteria were studied. Different methods were used to inoculate bacteria into the rhizosphere. Nitrogenase activity was determined by C_2H_2 reduction, and bacterial populations were estimated by most probable number and dilution plate counts. The populations of inoculated N_2-fixing bacteria and nitrogenase activity were correlated positively with soil moisture and soil organic matter content. In axenic systems, growth of inoculated organisms was influenced by selected plant breeding lines. Introduced populations of N_2-fixing organisms increased in axenic systems, but their numbers were greatly reduced in non-sterile environments, suggesting that biotic factors are responsible for the
reduction in numbers of N_2-fixing bacteria. Nitrogen fertilizer did not alter N_2-fixing populations in axenic systems, although in sufficient quantity it did suppress nitrogenase activity. Low soil moisture or organic matter levels and microbial competition are much more significant than soil nitrogen levels in limiting populations of introduced N_2-fixing bacteria.

II. COLLECTION AND CHARACTERIZATION OF GRASS-RHIZOSPHERE INHABITING NITROGEN-FIXING BACTERIA

a. Nif Gene Location in the Megaplasmid Bacterium Azospirillum.

Each isolate of the two species of Azospirillum has previously been found to carry a different array of seven or eight DNA molecules separable by gel electrophoresis. The sizes in one strain range from 40 Mdal up to a minimum estimate of 1350 Mdal and add up to at least 4300 Mdal. A single prototrophic strain unable to reduce acetylene or grow in N-free medium had been isolated after serial curing treatments. Since the plasmids in this strain were indistinguishable, by gel electrophoresis, from the parental strain, no information was provided as to which of the molecules carried the nif genes. However, DNA homologous to the nif KDH DNA from Klebisella was seen on the same molecule in all strains examined when such a probe was applied to Southern blots of the separated molecules.

b. Genetic Studies with N_2-Fixing Enterobacter Strains F , the Rhizosphere of Grasses.

Enterobacteria form a substantial part of the microflora in the rhizosphere of grasses. Making them fix nitrogen seems a promising approach to save on nitrogen fertilizer. As a model system Enterobacter cloacae from the rhizosphere of Festuca heterophylla is being studied. Onto such bacteria, the gene group for N_2-fixation from Klebsiella was transferred, using

II. (Continued)

b. (continued):

the composite self-transmissible plasmid pRD1, and E. coli cells as donors. The plasmid with all genes could be stabilized in the recipient bacteria via integration into their chromosomes. Stable hybrids were nitrate reductase negative, hence integration was in a gene essential for the production or function of this enzyme. The hybrids fix more N_2 than the donor cells. In pot cultures, they compete successfully with wild type bacteria. However, they do not utilize nitrate, hence do not compete with the host plants for nitrate fertilizer added as a starter. A method for obtaining such hybrids from other Enterobacteriaceae is being developed which employs chlorate resistance as a means to select for nitrate reductase negative hybrid cells.

c. Partial Purification and Properties of an L-arabinose Dehydrogenase From Azospirillum brasilense

An L-arabino-aldose dehydrogenase responsible for the oxidation of L-arabinose to L-arabino-γ-lactone has been purified 59-fold from L-arabinose grown cells of Azospirillum brasilense. The dehydrogenase was found to be specific for substrates with the L-arabino-configuration at carbons 2, 3, and 4; K_m values for L-arabinose were 75 and 140 μM using NADP and NAD as co-enzymes, respectively. The enzyme had a pH optimum of 9.5 in glycine buffer and was stable when heated to 55°C for 5 min. No enhancement of activity was found in the presence of any divalent cation or reducing agent tested. L-Arabinose dehydrogenase had a molecular weight of 175,000 as measured by the gel filtration technique.

III. ECOLOGICAL FACTORS WHICH LIMIT OR ENHANCE PLANT-BACTERIAL RESPONSES.

a. Survival of Azospirillum brasilense Strain Cd After Field Inoculations in Florida and New Mexico

After inoculation with Azospirillum brasilense strain Cd in Florida and New Mexico, the bacterial population was monitored by MPN technique. The Florida location, a sandy soil, pH 6.0, with low organic matter content, showed a decline from 10^4 to 10^3 cells per gram of soil in three weeks, and to 10^2 cells by the fifth week. In New Mexico, on a sandy loam soil, pH 8.0, and medium organic matter content, bacterial populations were more erratic on samples taken on a tri-weekly basis.

III. (Continued)

b. Field Inoculation of Grasses with Azospirillum brasilense Strain Cd.

In 1981 inoculation experiments with Azospirillum brasilense strain Cd were conducted on Sorghum bicolor (L.) Moench (sorghum) and Pennisetum americanum (L.) K. Schum. (pearl millet) and in 1982 on pearl millet and its interspecific hybrid with P. purpurpeum Schumach. (napiergrass). In 1981, only the sorghum responded significantly, with a 11% dry matter yield increase due to inoculation. Acetylene reduction activity of inoculated plots was not increased over the natural uninoculated background rates and negatively correlated with yield. In 1982, neither species gave a statistically significant inoculation response in the first harvest, but in the second harvest the inoculated interspecific hybrid gave an 24% increase in dry matter yield and 39% increase in total nitrogen yield. Azospirillum populations were found to decline from 10^4 to 10^3 per gram of soil in about three weeks. Continued decline indicated that we did not establish the inoculated bacteria in the soil even when yield responses were observed.

c. Acetylene Reduction Activity and Non-Structural Carbohydrate Content of Hemarthria altissima cv. Bigalta, After Defoliation

Clipped vs. unclipped plants of Hemarthria altissima cv. Bigalta were studied to ascertain if the defoliated plants had significantly lower acetylene reduction activity (ARA) than unclipped plants. Non-structural carbohydrate determinations were made of dried root samples. Roots sectioned with a freezing microtome where observed to contain starch in the cortical cells in the center of the root. ARA per gram of root was significantly lower in the clipped plants ($p = .02$), and percent carbohydrate content was also significantly lower in the clipped plants 17 days after clipping ($p = .001$). Observations of root and shoot development indicated that clipped plants had increased shoot growth, but had initiated fewer new roots than the unclipped plants. Root carbohydrate content and ARA were not well correlated.

d. The Influence of Shading on Associative N_2-Fixation

The effect of reduced solar radiation on associative N_2-fixation and plant parameters was studied in three field experiments. 'Gahi-3' pearl millet (Pennisetum americanum (L.) K. Monch.) field plots were shaded with saran shade cloth that reduced solar radiation by 50% and 75%. Acetylene reduction activity (ARA) was reduced by shading in one of the three experiments. The two non-responding experiments were conducted on a well-drained, low-activity site (ARA means ranging 17-68 n moles ethylene core^{-1}h^{-1}), the responding experiment was conducted on a poorly drained, high-ARA site.

d. (Continued):

Shading affected the plants drastically, reducing fresh weight and dry matter yields up to 46% (50% shade) and 57% (75% shade). Shading also reduced dry matter percentage from 19.6 (no shade) to 15.3 (75% shade) and increased nitrogen content from 0.6% (no shade) to 1.53% (75% shading). However, shading did not affect protein yield. Inoculation with Azospirillum brasilense had no measurable effect on yield or acetylene reduction in the first two experiments.

In the third experiment, shading reduced mean ARA of inoculated plots over 100% but had no significant effect on control plots. Acetylene reduction activity was high, with means ranging between 208 and 465 n moles ethylene evolved $core^{-1}h^{-1}$. Soil moisture and millet growth stage also affected acetylene reduction activity.

e. Survival of Nitrogen Fixing Organisms in the Rhizosphere

Results of root and soil inoculation studies are frequently analyzed only in terms of favorable effects on the host plants. However, when these effects are not found it is impossible to identify reasons for failure of the experiments. It would be possible to do so at least in some instances, however, if data were collected to indicate survival and activity of the inoculant organism.

Our data from experiments with Azospirillum, Azotobacter, and Klebsiella suggest that failures in most cases indicate poor survival capability of the organism rather than its incapability to interact with the host plant genotype. Favorable effects on plant growth occur consistently when the inoculation strains proliferate in the rhizosphere. We continue to find, however, that these favorable effects occur even when N_2-fixation in the rhizosphere is not increased.

Further progress in developing useful bacterial strains to enhance yields of non-legumes may not be achieved until methods are identified to assure their growth in the rhizosphere. There is therefore an urgent need to identify environmental conditions and host plant characteristics which significantly enhance survival and activity of beneficial rhizosphere bacteria.

III. (Continued)

f. Effect of Plant-Derived and Other Carbon Substrates on Asymbiotic N_2-Fixation

Plant derived substrates released into the soil provide support for associative N_2-fixation. Acetylene reduction activities were 15 times greater when living grass roots were present compared to root-free cores. Certain soils from selected high ARA locations in Florida utilized a considerable portion of added substrate to fix N. The response to glucose and mannitol was greatest and the most active soil partitioned 52% of the added substrate to ARA. Maximum response to cellulose as a substrate was 21% partitioned to ARA (range 0.2-21%). Ground grass-root tissue and succinate had very low activities, about 1/10 that of cellulose. The initial bacterial population of the most active soil was about 7×10^7 (measured by MPN observing growth in 1/2 strength TSA). The addition of substrate did not increase this population and sometimes lowered it. Initial N_2-fixing bacterial populations measured by various substrates were: glucose and cellulose, 2×10^4; mannitol, 1×10^5; and succinate, 1×10^3 (measured by MPN observing ARA in N-free deeps containing specific substrate). These populations increased about 15 fold following the addition of substrate.

g. Effect of Tillage on N_2-Fixation in Soybeans

The effect of four tillage treatments on N_2-fixation of soybeans was studied. The treatments were 1) no tillage (NT), 2) subsoiling 35 cm deep in the plant row (SS), 3) conventional tillage (CT) and 4) conventional with subsoiling (CTSS). Neither tillage nor subsoiling affected ARA in the first two sampling dates but at the third date, after nodule senescence had begun, ARA was higher in NT. Root dry weights of NT plots were greater and the roots tended to be near the soil surface without good taproot penetration. Nodule weight correlated with ARA in the first two dates. Negative correlations noted between the third date ARA and the specific ARA of the other two dates indicated that plots with higher nodule ARA lost activity more rapidly, suggesting earlier nodule senescence. Plant N, top weight, pod weight and pod numbers were not changed, but seed yields were lowest in NT, and highest in CTSS.

h. Ecological Factors Affecting Survival and Activity of <u>Azospirillum</u> in the Rhizosphere

<u>Azospirillum</u> inoculants disappear rapidly when inoculated into the rhizosphere. We have attempted to identify conditions that would increase survival. After inoculating by various methods, we estimated N_2 fixation by C_2H_4 reduction, (organic material released by plant roots) by labelling with ^{14}C, and respiration by IR CO_2 analysis. Dilution plant counts and MPN

h. (Continued):

techniques were used to determine bacterial numbers. Nitrogenase activity and numbers of soil bacteria (including Azospirillum) were positively correlated with soil moisture. In monoxenic systems, Azospirillum populations were positively correlated with soil organic matter content. Soil respiration and subsequent plant dry matter yields were greater in some but not all cases after Azospirillum inoculation. Also, the amount of ^{14}C label in the soil solution was increased by inoculation.

We found that plant growth hormones produced by the inoculants often stimulated early growth of the host plants, but in both field and greenhouse investigations, population levels of the introduced bacteria were too low to fix amounts of nitrogen that would be significant in an intensively managed crop production system. Our data indicate that microbial competition limits Azospirillum growth and that suboptimal levels of moisture and carbon substrates in the soil limit nitrogenase activity. Further research on methods for increasing survival and growth of associative N_2-fixing bacteria in the soil is badly needed.

IV. CARBON DIOXIDE AND NITROGEN FIXATION BY PLANTS AND ASSOCIATED RHIZOSPHERE BACTERIA

a. Translocation of ^{15}N from Inoculated N_2-fixing Bacteria Into Zea Mays

The capacity of N_2-fixing, root-associated bacteria to provide reduced nitrogen to the plant is largely unknown. There is no direct mechanism for the transfer of the fixed nitrogen. We have started a long term study to examine the partitioning of nitrogen from N_2-fixing bacteria into the soil biomass and Zea mays.

Living cells of Azospirillum brasilense strain JM125A2 and Klebsiella pneumoniae were uniformly labelled with ^{15}N. The bacteria were inoculated into the rhizosphere of greenhouse-grown Zea mays (cv. Funks 509) at several levels of bacterial nitrogen. At maturity plants were harvested, the plant tissue separated into roots, shoots and grain. Plant dry weight, percent nitrogen, total nitrogen and ^{15}N: ^{14}N ratios were determined by Kjeldahl analysis and isotope ratio mass spectroscopy.

Soil microorganism populations were determined at plant maturity. Large populations of the inoculated bacteria were not maintained in the inoculated soils. The total population of N_2-fixing bacteria was equivalent to the non-inoculated controls by the end of the experiment. Plant dry weight yields were directly proportional to the rate of bacterial additions. Nitrogen content of the plant tissue increased

with increasing rates of bacterial addition, and ^{15}N was found in all of the plant tissue. That ^{15}N was incorporated into the plant tissue demonstrates that plants were able to take up nitrogen supplied by the root-associated microorganisms. The study indicates that 60-70 percent of the nitrogen incorporated into the bacterial biomass can be removed from the soil by plant growth. However, the reduction in N_2-fixing organisms indicates that insufficient N_2-fixing bacteria are present in the soil to provide reduced nitrogen in quantities required for agriculture.

b. Evaluation of N_2-Fixation in Bahiagrass by ^{15}N-Isotope Dilution and Other Techniques.

The potential for N_2-fixation in 21 genotypes of bahiagrass, Paspalum notatum Flügge, was investigated in a greenhouse study. Nitrogen fixation and the response due to inoculation with a mixture of seven diazotrophs was estimated by several techniques: yield, acetylene reduction (AR), nitrogen accumulation; and dilution of ^{15}N. An overall significant response ($P = 0.01$) to inoculation was observed for top dry weight (TDW), top N and plant N. AR was inconsistent with inoculation and ranged from 30 to 4420 nmol/(cylinder h). An overall response to inoculation (sign. only at $P = 0.09$) was observed for ^{15}N dilution. Inoculated and control plant-soil systems (PSS) accumulated 0.096 and 0.092 g N, respectively, of which 81% was in the soil and 76% of this portion was organic. Recovery of ^{15}N fertilizer for PSS and plant was 57% and 40% respectively.

c. Evaluation of Three Methods for Measuring the Potential of Associative N_2-Fixation of Corn.

A collection of 26 Zea mays L. genotypes were grown under field, greenhouse, and growth chamber conditions. Plants were grown in 2.2 L plastic jar assemblies in a greenhouse and axenically in test tubes in a growth chamber. Plants were inoculated with N_2-fixing bacteria in field and test tube experiments. The objectives of this study were to develop quick methods to screen corn genotypes for the ability to support associative N_2-fixation, compare these methods to core acetylene reduction assays of field-grown plants, and evaluate the potential of corn genotypes to support N_2-fixation. Quick screening procedures were difficult to evaluate because corn genotypes were not significantly different for ARA in field and greenhouse experiments. Significant differences among genotypes were observed within test-tube experiments but genotype ARA between experiments was generally inconsistent. In all studies ARA had large amounts of variation.

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36. Smith, Rex L., R. N. Gallaher, S. C. Schank, and M. E. Hassan. 1983. Effect of tillage on N_2-fixation in soybeans. Agron. Abstracts 75:114.

37. Smith, Rex L. and S. C. Schank. 1983. Associative grass-bacteria N_2-fixation systems. The Fifteenth Miami Winter Symposium, Department of Biochemistry, University of Miami. p. 76.

38. Smith, Rex L., J. R. Milam and S. C. Schank. 1983. Effect of plant derived and other carbon substrates on asymbiotic N_2-fixation. Abstract. Fifth International Symposium on N_2-Fixation, Noordwijkerhout, The Netherlands.

39. Wood, A. G. and D. E. Duggan. 1982. Nif gene location in the megaplasmid bacterium Azospirillum. 1982. Second International Symposium on N_2-Fixation with Non-Legumes, Banff, Canada. Program and abstracts. p. 17.

CROSS-LINKAGES

A. Domestic

In the U. S., important cross-linkages with other scientists are maintained by active participation in Regional Project S-130. This regional project concerns itself with plant-bacterial root association research, and includes scientists from USDA, Beltsville, Univ. of Georgia, Rutgers Univ., Louisiana State Univ., Texas A&M Univ., Univ. of Nebraska, Tuskegee Institute, as well as the Univ. of Florida. This project was extended to September 1983. A new regional project has been written and hopefully will be approved in the near future. Other important linkages with scientists are maintained at various national meetings, such as ASM (American Society of Microbiologists) and ASA (American Society of Agronomy), where members of our group regularly participate in symposia, or present papers.

Cooperative work with New Mexico State University (Dr. A. A. Baltensperger) was outlined and a full-scale inoculation experiment under four levels of N fertilization was planted at that location. Soil samples from the New Mexico experiment were sent to Florida, where bacterial counts of the CD strain of A. brasilense were accomplished. Details of this cross-linkage were reported in this annual report.

B. Overseas

(1) Australian scientists have continued to show interest and cooperation regarding grass-bacterial associations. Mr. Keith Weier of CSIRO Division of Tropical Plants and Pastures visited Florida for four days in 1982 and later he attended the Banff, Canada meeting.

(2) Cross-linkages with ICRISAT in India continued in the past year. Exchanges of preliminary research data, and other research findings is open with ICRISAT. Dr S. Wani visited our research group in Florida in August, 1982, prior to the Banff meeting. Dr. Purushothaman from Tamil Nadu University in India spent six months training with us in 1983.

(3) Dr. S. H. West has maintained cross-linkages with Egypt and C.I.D. (Consortium for International Development). Dr. West invited Dr. Victor S. Bedrous, from Cairo, Egypt to work with him in 1983. A visiting professor, Dr. Mohammed El-Helw concluded his year's sabbatical leave with Dr. Rex L. Smith in 1982. Dr. Mohamed Eid, from Cairo University, also visited our group for two weeks in July 1983.

(4) Dr. Manuel Quintero and Dr. Aquiles Solis, from Durango, Mexico, were provided a 4-week long training program in 1982.

(5) Dr. Hugh Popenoe, Director of University of Florida's International Programs has been invaluable in maintaining cross-linkages with BIFAD and other governmental agencies in Washington, D.C.

(6) Israeli contacts have primarily been through a commercial company known as BioTech General. This is the same group that Dr. Yaacov Okon has worked with in past years, and we welcome this added cross-linkage. A BARD proposal with Dr. Okon is still in the planning stages.

STUDENT, POST DOCTORAL, AND VISITING PROFESSOR
TRAINING PROGRAMS

AID/ta-C-1376
1977-1982

NAME	Country of Origin	Duration of Training	MS	PhD	Post Doc	Other
Mercedes Umali-Garci	Philippines	4 years		X		
Sharon Matthews	U. S.	3 years		X		
Alvin G. Wood	U. S.	5 years		X		
Gary Benzoin	U. S.	2 years	X			
Christine Carr Dykstra	U. S.	2 years	X			
Howard Berg	U. S.	2 years			X	
Robert Green	U. S.	3 years		X		
Wai-Jane Ho	Taiwan	2 years		X		
Elza Menezes	Brazil	4 years		X		
Norman Novick	U. S.	4 years		X		
Valerie Pence	U. S.	1 year			X	
Tran Minh Tien	Viet Nam	4 years		X	X	
Glen Weiser	U. S.	4 years	X	X		
Arden A. Baltensberger	U. S.	4 months				X
M. E. Mitchell	U. S.	1 year			X	
Mario Sotor	Brazil	1 year		X		
Chui-Chung Young	Taiwan	3 weeks				X
H. G. Diem	Senegal	3 months				X
J. H. Bouton	U. S.	4 years		X		
Joe Salvo	U. S.	2 years	X			
Prem Patel	India	Current		X		
Pushpam Shankar	India	Current		X		
Peggy Akins	U. S.	1 year			X	
Vilma Vasil	India	2 years			X	

(Continued)

NAME	Country of Origin	Duration of Training	MS	PhD	Post Doc	Other
Hart Spiller	Germany	1 year			X	X
Bruce Bleakley	U. S.	Current		X		
Cathy Meitin	U. S.	Current	X			
Brad Swedlund	U. S.	Current		X		
Mahammed El-Helw	Egypt	1 year				X
Duane Alphs	U. S.	Current	X			
Manuel Quintero	Mexico	4 weeks				X
Aquiles Solis	Mexico	4 weeks				X
D. Purushothaman	India	6 months			X	
Mohamed Eid	Egypt	2 weeks			X	
Jose diStefano	Costa Rica	Current		X		
Mohamed Hassan	Egypt	3 months			X	
Jiang You-Yi	China	1 year			X	
W. Klingmuller	Germany	3 months				X

AID/ta-C-1376-EQUIPMENT

Item	Date of Order	Date of Receipt	Price Paid	Location
Gas Chromatograph #1	03/25/77	05/09/77	$3,997.00	516 Bartram E.
Top Load Balance	09/08/77	10/15/77	$1,140.45	PSL
Transmitted Halogen Light Source for microscope	11/13/77	12/08/77	$2,269.00	McC 3197
Phase contrast and Dark Field Condensor	11/13/77	12/08/77	$1,530.00	McC 3197
Double Unit Microferm Fermentor	03/09/77	04/29/77	$8,688.00	516 Bartram E.
Gas Chromatograph #2	01/08/80	02/06/80	$6,960.00	PSL
Incubators (2)	01/07/80	02/08/80	$ 641.35	PSL
Water Purifier	01/07/80	02/05/80	$ 825.00	McC 3197
Water Bath Shaker	03/12/80	06/10/80	$1,221.50	PSL
Laminar Flow Hood	11/14/80	12/10/79	$2,154.00	PSL
Hygrometer	03/12/80	04/02/80	$ 550.40	PSL
Convection Oven	01/07/80	01/12/80	$ 769.00	McC 3197
Balance	03/12/80	04/01/80	$1,819.50	McC 2164
Dissolved oxygen controller & recorder	03/12/80	04/01/80	$ 824.65	516 Bartram E.
Shaking Water Bath	03/12/83	04/02/80	$1,221.50	120 MAE
Computer Terminal	12/03/81	03/16/80	$1,105.00	Bldg. 495
Commodore Floppy Disk Drive	12/03/81	01/26/82	$1,406.78	Bldg. 935
Smartface Interface			$1,054.00	Bldg. 495
Printer for CBM computer			$1,870.00	Bldg. 495
Rotohoe tiller & planter			$ 749.00	PSL
NEC Spinwriter (for apple)			$2,015.00	Bldg. 661
Tappan Microwave Oven			$ 300.00	Bldg. 495
Refrig/Freezer 21'			$ 700.00	Bldg. 495
GPI Growth Room			$4,850.00	Bldg.
Percival Biol. incubator			$2,675.00	113 MAE
Udy Grinder			$ 841.00	PSL
CBM Disk Drive			$1,560.00	Bldg. 495
Modem, Disk Drive & Printer			$1,597.00	Bldg. 495

Expenditures (by Outputs) 04/01/82 to 04/01/83	MICRO 89	AGRON 90	SOILS 91	TOTAL
A. Establishment of axenic plant-bacteria systems.				
B. Collection, characterization and modification of grass rhizosphere N_2-Fixing bacteria.				
C. Examination of bacterial-strain genotype specificity.				
D. Ecological factors that limit or enhance plant-bacteria interactions.				
E. Nitrogen and CO_2 fixation by plants and associated rhizosphere organisms.				
F. Association of N_2-fixing bacteria with plant tissue culture material.				
Actually Spent				
Overhead				
Total				

EXPENDITURES BY OUTPUT
(04/01/82 to 03/30/83)

	OUTPUT	Department			
		MICROBIOLOGY & CELL SCIENCE	AGRONOMY	SOILS	TOTAL
A.	Establishment of axenic plant-bacteria systems.	$ 6,170	$11,168	---	$ 17,338
B.	Collection, characterization and modification of grass rhizosphere N_2-Fixing bacteria.	$ 28,793	$ 3,722	---	$ 32,515
C.	Examination of bacterial-strain genotype specificity.	$ 2,057	$ 7,446	---	$ 9,503
D.	Ecological factors that limit or enhance plant-bacteria interactions	$ 2,057	$26,059	$ 7,619	$ 35,735
E.	Nitrogen and CO_2 fixation by plants and associated rhizosphere organisms.	---	$26,059	---	$ 26,059
F.	Association of N_2-fixing bacteria with plant tissue culture material.	$ 2,057	---	---	$ 2,057
	Actually Spent	$ 41,133	$74,455	$ 7,619	$123,207
	Overhead	$ 17,958	$21,956	$ 3,507	$ 43,421
	Total	$ 59,091	$96,411	$11,126	$166,628

WORK PLANS AND CURRENT RESEARCH NEEDS
AID/ta-C-1376 1983

The contract with USAID is scheduled to terminate on 30 November 1983, and therefore the work plans for the remaining few months will follow the previously outlined procedures. Emphasis will be given to finishing up the research objectives. Our multidisciplinary group will continue with the same major goal, overall purpose and the same six research objectives as were originally presented.

A. Establishment of axenic plant-bacteria systems.

1. Test a seedling assay for bacterial stimulation of grasses using inert plant support medium and nutrient solutions.

2. Search for nif$^-$, bacterial phytohormone (bph$^-$) mutants and compare them with non-mutated lines under axenic systems.

B. Collection, modification and characterization of grass rhizosphere N_2-fixing bacteria.

1. Evaluate a system based on photometric determination of N isotopes as a method for quantify low levels of N_2-fixation by root associated organisms.

2. Further test effects of bacterially produced phytohormones for long-term effects on plant growth and yield.

3. Identify field isolates from rhizospheres with high nitrogenase activities.

4. Study processes by which nitrogenase is required in Klebsiella pneumoniae and Azospirillum brasilense utilizing modern biochemical and genetic techniques.

(a) Klebsiella pneumoniae

(i) Isolate mutants that are blocked in the metabolism of NO_2^- to NO/N_2O; NO to N_2O and NO_2^- to NH_4^+.

(ii) Map the various genes involved and study the regulation of these genes.

(iii) Characterize these mutations biochemically.

(iv) Examine the effect of these mutations in the NO_2^- metabolic pathway on nitrogenase biosynthesis.

(b) A. brasilense

(i) Isolate and characterize nif$^-$ and hup$^-$ mutants (to determine the role of hup in N_2 fixation)

5. Separate plasmids of Azospirillum sp. by gel electrophoresis to characterize the bacterial strains.

6. Conduct microbiological analysis of plant experiments to determine total number of bacteria, including total numbers of N_2-fixing and denitrifying bacteria.

7. Act as curator for the collection, purification, maintenance (liquid nitrogen) and distribution of associative N_2-fixing bacteria in an effort to establish standardized strains for other investigators (national and foreign).

C. Examination of bacterial strain and plant genotype specificity.

1. Quantify N_2 fixation and N uptake efficiency of sorghum and/or wheat genotypes by using ^{15}N (on low N medium).

2. Mass produce inoculum strains for greenhouse and/or field experiments.

3. Analyze a number of pearl millet genotypes for carbohydrate loss, bacterial infection, and nitrogenase activity.

4. Continue development and evaluation of simple systems for large-scale testing of interactions between plant and root-associated bacterial genotypes.

5. Follow-up study of plant genotype-bacteria associations in most active soil cores collected from various counties in Florida. (moisture, OM, micronutrients, and isolations).

6. Study the role of non-soluble plant substrates in associative N_2-fixation, especially cellulose. Attempt to isolate and characterize cellulose using N_2-fixing systems.

7. Look for bacteria that will interact with roots to give special plant responses such as root hair curling, root lysis, root stimulation or modification.

D. Definition of ecological factors that limit or enhance plant-bacteria interactions.

1. Determine (to the extent possible) effects of the variables itemized below on population changes among nitrifyers, denitrifyers, and N_2-fixers in a partially defined model soil-root system. Variables: plant genotypes, levels of N in various forms, temperature, soil aeration, levels of various energy substrates.

2. Study microbial population dynamics in an Azospirillum-inoculated sorghum rhizosphere.

3. Establish inoculation procedures that may enhance grass-bacteria associations and monitor persistence of inoculated bacteria.

4. Study hydrolytic enzymes produced by bacteria.

E. Quantification of CO_2 and N_2 fixation.

1. Investigate root exudates in relation to associated, N_2-fixing soil microorganisms.

2. Study the relationships of nitrogen metabolism and possible cycling between crop plants and soil microorganisms.

3. Use $^{14}CO_2$ and $^{15}N_2$ combined labelling to determine, in a model associative system, their transfer between plants and bacteria.