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AGENCY FOR INTERNATIONAL DEVELOPMENT  
WASHINGTON, D.C. 20523

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MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18  
FROM: AID/SCI, Victoria Ose *VO*  
SUBJECT: Transmittal of 1 /SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 6. 233  
PK July 87 - Jan 88

Attachment

PW 1155 10  
6. 233

PROGRESS REPORT ON THE COLLABORATIVE RESEARCH PROJECT:  
"GENETIC ENGINEERING OF RHIZOBIA FOR NODULATION EFFICIENCY"

U. S. Agency for International Development Grant No.  
DPE-5542-G-ss-6039-00

Prepared by Dr. Nasir Malik, Battelle Memorial Institute  
Columbus Ohio, and Dr. M. H. Ahmad, Department of  
Biochemistry, University of West Indies, Kinsington,  
Jamaica, for the period between July 1987- January 1988

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Report for the U.S.A.I.D project No. DPE-5542-G-ss-6039-00

### Summary

This report covers the period between July 1987- January 1988 during which time Dr. Steve Vesper was in charge of this project. During this time, although the technical progress remained slow, various essential aspects of our settlement into Battelle Memorial Institute at Columbus were worked out. Experiments have now been started to accomplish various objectives of this project. Preliminary results of these experiments are encouraging, and we plan to increase our efforts on this project in the coming months.

The work in Jamaica has continued uninterrupted. Dr. Ahmad and his student continued with the screening and characterization of rhizobial isolates collected from Jamaican soils.

## Technical Progress:

Dr. Steve Vesper was in charge of this project during the period of this report. The transfer of equipment and supplies from Kettering was completed in August of 1987, but most of these materials, including the plant growth chamber, were placed in a storage room due to limited laboratory space. Dr. Vesper tried hard to grow soybean plants in a room with controlled temperature and light conditions, but was unsuccessful due to lack of humidity control and proper air circulation. Locating a space for the plant growth chamber took much longer than anticipated. In addition, the growth chamber was damaged during various shipments and could not be brought into proper operation before the end of the year. At that time, Dr. Vesper decided to leave Battelle.

During the course of transfer, bacterial culture also became contaminated. New cultures were purchased from Nitragin, and Dr. Vesper isolated the two colony types before his departure. He also made one attempt to conjugate inefficient strain with the available gene bank of 61A76. However, at the recommended kanamycin concentrations (20-30 ug/ml), the wild type rhizobia (61A76) were resistant to the antibiotic effect. Thus, a high background made the selection of transconjugants quite difficult.

So far, the only objective achieved in this project is the construction of a gene bank of 61A76 in the PLAFR1 cloning vector. The characterization of the gene bank, however, still remains to be done.

Since the beginning of this year, I have started to work on various aspects of this project. First of all, the growth chamber has been made operational and relocated at a convenient place closer to distilled water and sterile hood facilities. Thus, plant microbe interaction studies can be performed under optimum conditions with little difficulty. The small Conviron growth chambers usually require constant maintenance, so I have hired a technician who is also good in fixing minor mechanical problems.

I have also studied the resistance of wt 61A76 (both colony types) and the cloning vector (PLAFR1) to different concentrations of kanamycin. It appears that at 50-100ug/ml kanamycin in growth medium, the vector-containing bacteria grows normally while the growth of wt 61A76 is completely inhibited. Thus, in the future, selection of transformants will be performed at higher concentrations of the antibiotic, rather than the recommended concentration of 25-30 ug/ml of kanamycin. I have also discussed this project with a number of experts in the area of symbiotic nodulation, particularly in the genetic aspects of

symbiosis. It is generally believed that the cloning vector PLAFR3 is a superior vector than PLAFR1, because PLAFR1 tends to integrate with the chromosomal DNA making it harder to isolate it back from the nodule. I have already obtained PLAFR3 cosmid cloning vector from Dr. Verma. Since this cosmid has a tetracyclin resistance gene instead of kanamycin resistance gene, I have, therefore, tested the resistance of wt 61A76 rhizobia and the E.coli containing PLAFR3 to various concentrations of tetracyclin to see if this cosmid can be used as a cloning vector for 61A76 genome. Our results show that this vector can indeed be used to isolate efficiency genes from the 61A76 genome.

My strategy for the future work is as follows. First, I will characterize the existing gene bank to see if it has good size inserts, and then will use it for transformation and selection experiments as outlined in the original proposal. In the mean time, however, I will also start to make another genomic library in PLAFR3. The construction of another genomic library is a good idea because it will be in a better vector. In addition, it will provide a strong fallback position if the existing gene bank is found to be deficient in some respects.

Progress report from Dr. M. Ahmad for the research conducted at the University of West Indies:

Nearly seventy strains of indigenous cowpea rhizobia have been isolated from the nodules of local cultivars of cowpea (Vigna unguiculata c.v. Laura B) grown in different localities in Jamaica. Four of these strains have distinctly dry colony morphology, while the remaining isolates produce gummy colonies on agar plates containing yeast extract and mannitol. A majority of these isolates are resistant to high concentration of antibiotics, such as neomycin, gentamicin, nalidixic acid, and tetracyclin.

All of the rhizobial isolates were examined for nodulation ability using disposable plastic growth pouches. [I learned the technique of determining nodulations frequency on the roots of cowpea plants grown in plastic growth pouches during my visit to Battelle in August of 1987. This technique has now been routinely used in my laboratory.] Most of the isolates were found to be inefficient nodulators above the RT mark (position of the root tip at the time of inoculation), but two of the isolates were clearly efficient nodulators in those tests. Only one isolate showed high nodulation efficiency both above and below the RT mark.

Soybeans are not normally cultivated in Jamaica, therefore, isolation of native strains of Bradyrhizobium japonicum from Jamaican soils has been very difficult. So far only seven strains of native B. japonicum have been isolated. There is little difference in the colony morphology or in nodulation

efficiency of these strains. All of these isolates, are also resistant to high concentrations of a number of antibiotics, such as streptomycin, rifampicin, tetracyclin, and nalidixic acid.