PN-ABA-567

AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 4/8/88

MEMORANDUM

TO: AID/PPC/CDIE/DI, room 209 SA-18

FROM: AID/SCI, Victoria Ose

SUBJECT: Transmittal of AID/SCI Progress Report(s)

Attached for permanent retention/proper disposition is the following:

AID/SCI Progress Report No. 1. 20 2
last tech report

Attachment

1 cy

3 to P.O. Federal

7. 2022

First Technical Report

AID/SCI Specific Support Grant No. **DPE-5542-G-SS-7077-00**
Title: Molecular Identification of Frankiae Using Cross Inoculation Group-Specific DNA Sequences

Grantee: Yale University School of Forestry and Environmental Studies
Collaborating Institution: BNF Resource Center for South-East Asia
Co-principal investigators: Dwight Baker (Yale), Nantakorn Boonkerd (BNFRC)

This report covers the period: 1 October 1987 - 28 March 1988

During the first quarter of this project, the major tasks were to 1) establish the subcontract between Yale University and the BNF Resource Center, 2) to initiate the collaboration by the arrival of Miss Aphakorn Nittayajarn for her one year training at Yale University, and 3) undertake preliminary studies to identify regions of Frankia DNA that may be host specificity group related.

In regard to task 1, the subcontract between Yale University and the BNF Resource Center was signed on 2 March with minor modifications to the subcontract budget. The terms of the subcontract as well as the modified budgets were submitted to USAID for approval. Dr. Nantakorn Boonkerd visited Yale University on 1 and 2 March (enroute from an international meeting) to discuss strategies for the research objectives and to discuss implementation of the Thailand experiments.

In regard to task 2, Miss Aphakorn Nittayajarn arrived at Yale University in late February and is now actively working in the laboratory of Dwight Baker in the School of Forestry and Environmental Studies. She is undertaking experiments in Frankia isolation, culturing and strain characterization. These protocols will be valuable in her studies upon return to the BNFRC in Bangkok in 1989. Miss Nittayajarn brought with her 10 strains, tentatively identified as Frankia, which were isolated over the past year in Thailand. She is now conducting morphology, physiology and host infectivity studies to confirm that these strains are in fact Frankia. She is also initiating some ELISA studies of Frankia strains, using polyclonal antibodies prepared at the BNFRC. Some preliminary data from ELISA tests using these antibodies indicate that a serological approach may permit us to separate strains into major groups. If the major serological groups prove to be the same as host specificity groups, the simple ELISA technique may complement our studies of DNA probes. At present, a representative number of Frankia strains has not been tested, and thus further studies must be conducted before absolute conclusions can be made.

In regard to task 3, DNA was isolated from 19 strains of Frankia which represent the currently understood diversity of the Frankiaceae. Yields of DNA were relatively quite good (see attached table) and this DNA will serve as a stock for future studies. In addition, a clone library of Frankia DNA originally made by James Ligon, was obtained for initial studies of sym genes. Heterologous probing of this clone library was undertaken in collaboration with Dr. Beth Mullin, University of Tennessee, our consultant on this project. Frankia DNA sequences were identified as homologous to nif K gene probes from Klebsiella. We will now use the Frankia DNA to probe the DNA of other Frankia strains in an effort to see if genogroups can be identified. Other strategies for obtaining the host specificity probes were also discussed at length with Dr. Mullin after reviewing current studies being undertaken in other laboratories. Personal

communication from Dr. Ann Hirsch (Wellesley College) suggests that heterologous probing of Frankia DNA with nod sequences of Rhizobium may not be possible. This approach had been one of our objectives as originally proposed. We are now reviewing the more recent DNA studies of Frankia to establish the most appropriate strategy for DNA probe identification. Studies using our own Frankia-derived probes will proceed concurrently with studies of heterologous probes.

Within the second quarter of this project (1 April - 28 September 1988) the following tasks are identified:

1) continuation of Thai Frankia isolation and characterization,

2) experimentation with ELISA tests to validate their use in classifying Frankia strains into major groups,

3) subcloning of Frankia nif probes for homologous probing of Frankia strain DNA,

4) construction of additional Frankia clone libraries for development of probes.

First Financial Report

AID/SCI Specific Support Grant No. **DPE-5542-G-SS-7077-00**
Title: Molecular Identification of Frankiae Using Cross Inoculation Group-Specific DNA Sequences

Grantee: Yale University School of Forestry and Environmental Studies
Collaborating Institution: BNF Resource Center for South-East Asia
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This report covers the period: 1 October 1987 - 28 March 1988

Category	Budget	Expended to Date	Funds Remaining
Salaries	$ 25,267.00	$ 6,375.04	$ 18,891.96
Fringe Benefits	4,633.00	1,561.92	3,071.08
Tuition	24,100.00	4,560.00	19,540.00
Materials/Supplies	22,839.00	1,793.79	21,045.21
Subcontract	35,200.00	14,800.00	20,400.00
Indirect Costs	37,961.00	3,521.33	34,439.67
Totals	$ 150,000.00	$ 32,612.08	$ 117,387.92

Report Submitted by:

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Date: 3/25/88