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AGENCY FOR INTERNATIONAL DEVELOPMENT
WASHINGTON, D.C. 20523

DATE: 5/27/87

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. C5-002
2 copies
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C5-002

AID Scientific Report.
Report from September 1986 - January 1987.

Title: The Double-Stranded RNA Viruses of *Rhizoctonia solani* and the Regulation of Plant Pathogens.

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Cooperative Investigator: Dr. Albert Ofuso-Asiedu
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Rec'd in SCI JUN 24 1987

Introduction Virulent strains of *Rhizoctonia solani*, a soil borne plant pathogenic fungus, contain double-stranded RNA viruses. Two dsRNA segments with mol. wt. of 1.45 & 1.32 x 10 daltons have been extracted from the virus particles (Finkler *et al.*, 1985). Full-length viral transcripts have been synthesized *in vitro* using the viral dsRNA as template. These transcripts hybridized to the viral dsRNA segments. The transcripts also directed the synthesis *in vitro* of a major protein (= capsid protein) and perhaps a number of additional minor proteins. In addition to those segments found in the virus particles, the dsRNA segments extracted from the fungal mycelium occasionally include species with mol. wt. of 1.7, 1.6 and 1.25 x 10 . The relation of these species to the viruses was examined; also, the existence of small quantities of these segments in the viral genome was tested. Studies have also been directed during the last six months to test the relatedness between the different dsRNA segments of a specific virus and towards the assignment of proteins to specific segments of dsRNA. In addition, efforts are directed to characterize the mechanism of virus RNA transcription and replication.

End-labelling of dsRNA. The 3' ends of the double-stranded RNA, extracted from the fungal mycelia and virus particles, was labelled with P-pCp and T4-RNA ligase, as described by Bruenn & Brennan (1980). It appears that the viral genome contains only two dsRNA segments with mol. wt. of 1.45 & 1.32 x 10 . No other

bands could be detected by end-labelling of dsRNA in the virus particles. Thus, additional dsRNA segments detected in the mycelium must be unencapsidated species.

Purification of dsRNA segments. Individual segments of dsRNA of R. solani have been purified for the use in cross-hybridization and in vitro translation. Ds-RNA segments have been purified by an agarose gel. Elution of dsRNA from the agarose has been carried out by two basic techniques: (a) The binding of dsRNA segments to BND-cellulose followed by elution with 1M NaCl (Sedat et al., 1967). (b) By binding of dsRNA to DEAE-cellulose NA45 paper followed by an overnight elution at 37 C with 1M Nacl (Dretzen et al., 1981). Effieciency of recovery of purified dsRNA segments is greater with method (b).

Cross-hybridization of dsRNA segments. The homology between the different dsRNA segments of R. solani was tested by hybridizing P-labelled purified segments to a blot of total dsRNA, in stringent conditions (4xSSC at 65 C). It appears so far that no cross-hybridization occurs between the 1.45 & 1.25. The results related to the 1.6 segment are as yet unclear. Since purification of the segment is difficult due to their proximity, extra care is needed to prevent contamination with nearby bands that lead to false cross-hybridization.

In vitro translation. Following the completion of identification of viral encoded proteins from the viral transcripts the specific assignment of functions to specific segments of dsRNA is performed by in vitro translation of purified segments. Experiments are now in progress to complete this task. The main

problem encountered is the difficulty to denature the dsRNA prior to translation. The dsRNA molecules are extremely stable as double-stranded molecules and conditions used in other dsRNA viruses do not apply in the case of the *Rhizoctonia* viruses.

The mechanism of transcription and replication. Transcription and replication of the virus of *R. solani* may be conservative like that of reovirus (Silverstein *et al.*, 1976) or semiconservative like that of 06 (Van Etten *et al.*, 1980) and some fungal viruses (Buck, 1975; Ratti & Buck, 1978). Conservative transcription and replication in reovirus result in the uncoupled synthesis of plus and minus strands. Semi-conservative replication in 06 and *Aspergillus foetidus* virus S result from the synthesis of a new plus strand that displaces the parental plus strand in the template viral dsRNA. The mechanism of transcription and replication of *R. solani* virus will be resolved by (a) identification of the plus and minus strands (+ strand = mRNA) by hybridization of the viral transcripts with the separated strands of the viral dsRNA. (b) Non-radioactive virions incorporate low levels of P from [- P]UTP into dsRNA during transcription reactions. Based on strand separation electrophoresis, this incorporation may be detected in the minus strand of dsRNA in the case of conservative replication as in reovirus and *Saccharomyces cerevisiae* (Numeroff & Bruenn, 1986).

Strand separation electrophoresis of *R. solani* dsRNA, following basically the techniques described by Thiele & Leibowitz (1982) with some modifications, are currently used. The method includes

heating 3'-end-labelled dsRNA at 90 C for 2 min in the presence of 30% DMSO, followed immediately by cooling on ice for 2 min. Samples are loaded on 5% polyacrylamide gels and run for 18-24 hrs at 150-500 V (the voltage was varied in different experiments). Another method currently examined is electrophoresis through denaturing polyacrylamide gels containing 7M urea, as described by Maniatis *et al.* (1982). So far, strand separation of *R. solani* dsRNA was unsuccessful. It is suspected that the denaturation techniques used regularly for other dsRNA viruses may be ineffective and more potent chemicals should be used, such as methyl mercury hydroxide.

Description of cooperation. Dr. Ofuso-Asiedu visited once again our laboratory in October 1986 to further discuss and communicate our views regarding the work which has been initiated in Ghana. In the Forest Research Institute in Kumasi, Ghana, a system for distinguishing virulent and hypovirulent strains of the wood-rotting fungus, *Pseudophaeolus bandorii*, is being developed. The strains are being classified with respect to their level of virulence. These strains will be used to test for biological control of the disease caused by *Pseudophaeolus* affecting the forest trees in Ghana. At a later stage these isolates will be examined for the presence of dsRNA and virions in an effort to test the relations between virulence and these viruses.

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