Final Report

Project title:

Association of N_2-fixing Cyanobacteria and Plants: Towards Novel Symbioses of Agricultural Importance

Principal investigator

Miroslav Gantar, Florida International University. The original PI, Jeff Elhai, directed a subcontract of the project at University of Richmond. He has been PI in the second and third years.

Period covered by report

1 April 1996 to 31 May 1997

Reprint removed for separate processing

Recipient organization

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DOE award number

DE-FG02-96ER12188

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Unexpended funds at the end of the first year (Mar 31, 1997)

Approximately $36,000. This amount resulted from the fact that the research associate began work November 1, 1996 instead of April 1, 1996, as budgeted. It was impossible to gain his services in the spring with such short notice (we didn't know for certain that the grant would be approved until 1996). The unpaid salary and benefits ($18,667), along with $600 travel, an estimated $5,000 in other direct costs, and $12,133 in indirect costs, will be effectively carried through the second and third year to support seven months work after the third year. In addition there will be approximately $2,500 unspent from the FIU budget, owing to synergistic projects that could not take place between Gantar and the research associate during the first seven months of the project. This money will be carried over to support such interactions during the second year.

Progress during the first 12 months of the grant period

The goal of this project is to characterize an association that takes place between the roots of wheat and the nitrogen-fixing cyanobacterium *Nostoc* 2S9. By understanding how the association takes place and the extent to which it permits the growth of the plant without exogenous nitrogenous fertilizer, it may prove possible to increase the benefits of the association and to extend them to other plants of agrinomic importance.

A. DNA Transfer

One of the major goals of the first budget period was to find a means of transferring DNA into the nitrogen-fixing cyanobacterium *Nostoc* 2S9, a strain capable of infecting the roots of wheat. This ability would enable us to genetically mark the strain in such a way that we could readily follow the course of infection and the physiological state of the cyanobacterium. Although the member of the group primarily responsible for this part of the project began work only in the eighth month of the budget period, substantial progress has been made nonetheless.

Our initial attempts to transfer DNA into *Nostoc* 2S9 were not successful, but extensive characterization of the strain has suggested the reason for the failure. *Nostoc* 2S9 was found to possess at least four distinct restriction systems, each of which damage the DNA upon entry into cells. Data we obtained with DNA transfer efficiencies with other cyanobacteria permitted an estimate as to the efficiency of transfer into *Nostoc* 2S9 in the face of the four restriction systems: restriction was more than sufficient to explain the absence of observed DNA transfer.

With the knowledge that restriction must be overcome to achieve DNA transfer into *Nostoc* 2S9, we have focused our attention towards developing a means of protecting transferred DNA against destruction by the cyanobacterium. We have thus far designed and constructed a plasmid vector composed of modified DNA, immune to the effects of virtually all restriction activities. We've recently demonstrated that the vector is capable of withstanding restriction by the bacterium *Escherichi coli*, and we will soon test it on *Nostoc* 2S9 and other bacteria. If successful, we will be able to introduce foreign genes into *Nostoc* for purposes described in the research proposal. Moreover, the method should prove of general utility to geneticists who wish to introduce DNA into the large number of bacteria who also possess restriction activities.

B. Introduction of *Nostoc* 2S9 into plant tissue

We previously showed that the cyanobacterial strain *Nostoc* 2S9 in a mixture of bacteria is able to colonize the inner space of wheat roots. We have now purified the strain from the accompanying bacteria and found that it retained its colonization ability. The process of colonization is therefore a property of the cyanobacterial strain itself.

In order to assess the maximum extent to which internal *Nostoc* 2S9 can support the nitrogen requirements of plants, we have sought conditions that yield as high a number as possible of wheat-associated cyanobacteria. The plant hormone 2,4D is capable of inducing on roots structures called paranodules. Since others have reported that bacteria can infect paranodules, we investigated whether they may increase the association of *Nostoc* 2S9 with wheat. We found that hormone treatment led to heavy colonization by the cyanobacterium in the region where para-nodules formed on the roots. Quantitatively, the mass of cyanobacteria associated with wheat roots more than doubled. *Nostoc* was also observed in stem tissue, even though they were applied only to the soil, thus the cyanobacterium can enter through the roots and travel up the plant.

One might also obtain wheat with maximal association with *Nostoc* by regenerating the plant from cells cocultivated with the cyanobacterium. We tested this idea with tobacco cells, since tobacco plants are considerably easier to regenerate than wheat. Tobacco cells cultured in the presence of *Nostoc* 2S9 formed calli with visible growth of cyanobacteria on the surface. Electron microscopy revealed that cyanobacteria had penetrated the calli, thus the integration of cyanobacteria and tobacco cells was obtained at this stage. Cyanobacteria were no longer visible upon regeneration of a mature tobacco plant from calli. It may well be that they survive within the plant, but methods to answer that question await the molecular techniques whose development is described in Section III.A.

While the tobacco regeneration experiment was progressing, we also attempted to introduce *Nostoc* 2S9 into wheat by similar methods. As with tobacco, wheat calli are formed with cyanobacteria visible on their surfaces. We have not yet determined whether the cyanobacteria are able to penetrate the wheat calli. Plant regeneration has proven more difficult with wheat than with tobacco, as expected, and is still in progress.