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Interim Technical Report
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"Study of Nodulation of Aeschynomene by an Unusual Endophyte"
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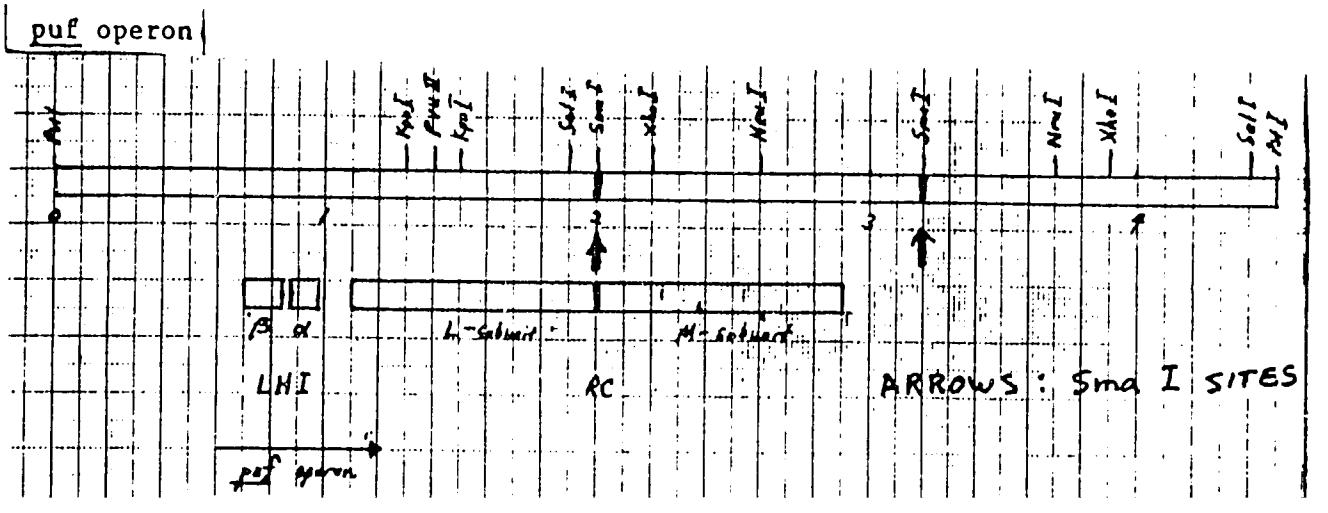
A. Molecular Biology of Aeschynomene Rhizobium BTAi 1. Dr. Shanmugasundaram and Dr. Suguna spent about three months at Wright State University and about a month in the laboratory of Dr. Paul Coussens at Michigan State University this summer. Their objective was to isolate and sequence the gene for the M subunit of the photosynthetic reaction center from BTAi 1. This gene is of particular interest for several reasons. BTAi 1 has many characteristics of the aerobic photosynthetic bacteria, which have been studied in greatest detail by Japanese workers. The aerobic photosynthetic bacteria which have been described are incapable of photosynthetic electron transport under anaerobic conditions. There is some evidence that this may be because the primary electron-accepting quinone has an unusually high midpoint redox potential, as a result of which it is reduced (and so unable to accept an electron from the photoexcited bacteriochlorophyll dimer) when oxygen is not present. The reason for this property is unknown. The primary quinone is bound largely to amino acids of the M subunits of the reaction centers whose crystal structures have been determined. These amino acids influence its redox behavior. Reaction center genes from aerobic photosynthetic bacteria (and from rhizobia) have not thus far been sequenced, so the information obtained could provide insight into why these bacteria are unable to grow phototrophically under anaerobic conditions. In addition, a comparison of the sequence of the BTAi 1 M subunit gene with the sequences of the genes from other photosynthetic bacteria could furnish hints about the phylogenetic relation between the photosynthetic rhizobia and other photosynthetic bacteria.

As described in our last progress report, Dr. Shanmugasundaram demonstrated that BTAi 1 contains DNA homologous to the M subunit gene from Rhodobacter sphaeroides. DNA from BTAi 1 was cut with the restriction enzymes Pst I and Sal I, electrophoresed on an agarose gel and blotted onto a Nytran membrane. DNA of the puf operon from Rb. sphaeroides was cut with the restriction endonuclease Sma I, which cuts the operon into three fragments, one of which consists almost entirely of the M subunit gene. The BTAi 1 blot was probed with radiolabelled M subunit DNA, and hybridization to several bands was observed (I have since probed the blot with the Sma I fragment which contains the L subunit gene and obtained a similar, but not completely identical, hybridization pattern).



BTAi 1 DNA was cut with Pst I and with Sal I. Two procedures were employed for preparing a gene library. In the first. total DNA was ligated into Bluescript KS (plus) and transformed into Epicurian coli. In the second, the cut BTAi 1 DNA was first hybridized with a Nytran blot of Sma I-cut puf operon DNA and DNA which hybridized to the M subunit band was collected and used for transforming E. coli. The cells were plated, transformed colonies containing the plasmid were transferred to a nitrocellulose membrane, lysed and probed with M subunit DNA. Duplicates of colonies giving positive hybridization were cultured, the plasmids isolated and the inserts excised and tested for hybridization to M subunit DNA. By the end of the September the isolation procedure had not yielded clones in which the inserts gave an unequivocally positive response to the M subunit probe. Dr. Shanmugasundaram is continuing the work in India.

B. Light Regulation of Formation of the Photosynthetic System in BTAi 1. In their original studies, W. R. Evans and P. Pyati found that BTAi 1 cultures form photosynthetic pigments when they are grown under an alternating light/dark regimen but not when they are grown in constant light or constant darkness. We learned last summer that cultures exposed to light for a single day can form as much bacteriochlorophyll as cultures grown under a 16 hr light/8 hr dark cycle for a week. A maximum amount of pigment is formed if the cultures are illuminated on the fourth day after inoculation with a 1% inoculum, as the cultures are entering stationary phase.

Cultures have now been illuminated for different lengths of time on the fourth day after inocuation, harvested on day 7 and their bacteriochlorophyll contents determined. Illumination for 8 hours resulted in maximal pigmentation.

Pigment formation was complete by the second day after the cultures had been returned to the dark.

In a first attempt to determine the action spectrum of pigment induction, cultures were exposed for 8 hours on day 4 after inoculation to white light (unfiltered tungsten light), blue light (through a Corning 4-96 filter, which transmits at wavelengths less than 640 nm), red light (through a Corning 2-64 filter, which transmits at wavelengths beyond 660 nm) and infrared light (through an RG 830 filter, which transmits at wavelengths beyond 830 nm). The cultures were then incubated in the dark for two days and examined by eye. Pigment had formed in the cultures which had been exposed to white or red light, but not in those exposed to

blue or infrared light. It appears that the receptor for light induction of pigmentation absorbs light at wavelengths between 640 and 830 nm.

Since cultures do not form pigment during illumination with white light, it appears that light can suppress pigment formation as well as induce it. Cultures were grown for 7 days under continuous white, blue, red or infrared light and their bacteriochlorophyll content determined on day 7. The culture grown under red light was highly pigmented and contained a significant amount of bacteriochlorophyll; the other cultures did not. It appears that the receptor which mediates light suppression of pigmentation absorbs light at wavelengths below 660 nm. In stem nodules, the endophytes are shaded by chloroplasts in the nodule cortical cells. These would be expected to absorb light in the blue wavelength range which suppresses pigment formation, but to transmit light in the red range which induces pigmentation. Thus formation of the photosynthetic system seems to be reciprocally regulated by two photoreceptors. My suspicion is that the purpose of this regulation is to prevent formation of the photosynthetic system when cells are outside the nodules where the cells might be damaged by light if oxygen availability is not appropriately regulated, for example by leghemoglobin, which has been shown to be present in the stem nodules.

C. Examination of leaf nodules. If a photosynthetic bacterium is going to form a symbiotic association with a plant, I would have expected it to form leaf rather than stem nodules, since leaves should be able to position themselves in such a way that the bacteria are exposed to maximal light intensity. There are in fact a number of bacteria which form leaf nodule symbioses. In most instances the roles of the symbionts are poorly understood. Some of these symbioses are being studied by Dr. Iain Miller, who, along with William Evans, discovered the photosynthetic properties of BTAi 1 while he was a visiting scientist at the Kettering Laboratory. Dr. Miller is now in the Biology Department at Wright State. Together with Dr. Gerald Alter of the Biochemistry Department, we examined the near-infrared spectrum of endophytes obtained from leaf nodules of Artis crispa (coralberry), a plant which possesses bacterium-containing nodules along the leaf margins. Photoacoustic spectroscopy is able to measure spectra of highly-scattering samples such as nodules, and has allowed us to detect bacteriochlorophyll in single Aeschynomene stem nodules. Unfortunately, we were unable to detect photosynthetic pigments in the coralberry endophytes. We plan to examine other leaf-nodulated plants.