RESEARCH PROPOSAL FOR 2001

Submitted to:
SLV Research Center Committee
and the
Colorado Potato Administrative Committee (AreaII)

TITLE: Genome Sequencing of the Bacterial Ring Rot Pathogen

PROJECT LEADER(S): Carol A. Ishimaru and Dennis L. Knudson
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PROJECT JUSTIFICATION:

Bacterial ring rot is one of the most feared diseases in the US potato industry because the disease spreads quickly and can easily destroy crops. Direct economic losses from bacterial ring rot occur in seed and table stock production. Since the disease is present in the US, exportation of US seed to countries free of bacterial ring rot is severely restricted.

Bacterial ring rot is caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *sepedonicus (Cms)*. Very little is known about the disease-causing genes in the pathogen. *Cms* grows slowly and can not be studied with the traditional genetic methods that work for other plant pathogens. Genome sequencing is the most straightforward means of identifying the disease-causing genes in the pathogen. The expected benefits to the potato industry are:

- Improved diagnosis of latent bacterial ring rot infections.
- Novel targets for agrochemical or cultural control.
- New approaches to the establishment of resistant potato genotypes, which would enhance the national (and potentially international) sustainability of this crop.

PROJECT STATUS: New

OBJECTIVES FOR 2001:

1. Submit proposals to federal granting agencies (USDA and Department of Energy) for funding of the complete genome sequence of *Cms*.
    - A proposal submitted last year to USDA/CSREES/IFAFS on genome sequencing of *Cms* and the related pathogen on tomato was given the rank of "outstanding". We were encouraged to resubmit a revised proposal in 2001.
    - A letter writing campaign initiated in January 2001 has obtained over 30 letters to date from growers and scientists supporting our efforts to obtain the complete genome sequencing of *Cms*.
    - The sequencing and annotation of *Cms* will be conducted in collaboration with a sequencing center capable of high throughput sequencing of microbial genomes. We are currently considering and in discussions with sequencing facilities for this project.
2. Sequence the ends of 1,000 cosmid clones from a genomic library of *Cms*.
    - A genomic library of *Cms* representing about a 13X coverage of the genome has been constructed. The ends of about 70 cosmids have already been sequence through funds supplied by a USDA Potato Special Research Grant.
    - The end-sequencing of 1000 cosmid clones will provide immediate sequence information and molecular markers that can become targets for further genome studies.
    - The sequence information from 1000 cosmid clones will be especially valuable as a tool in the final, gap filling stage of the complete *Cms* sequencing project.

FUNDING REQUEST:

2001 Request: $17,000

- Up to $5,000 will be used to support travel of C. Ishimaru and D. Knudson to sequencing centers in the US. Brazil, or Europe, for the purpose of negotiating contracts for the genome sequencing and annotation of *Cms*.
- $7,000 will cover the costs of reagents ($7/per sequencing reaction) for end-sequencing of the cosmid clones. Funding for staff **is not** requested.
- The remainder of the funds ($5,000 or more) will be used to finance the cost of the sequencing effort, which is estimated to be about $750,000. The financial contributions of the Area II CPAC will be noted in proposals submitted to USDA and DOE as an indicator of industry support for this work.