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Cms-DNA Chips: A Vision for CPAC's Economic Prosperity

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A. Nature of the Proposed Research

This project will provide cutting-edge resources for the Colorado seed potato industry. We will apply the latest technology in pathogen detection to identify strains of the ring rot pathogen, *Clavibacter michiganensis* subsp. *sepedonicus* (*Cms*). This chip technology will provide solutions for one of the most challenging disease problems facing the seed potato industry.

B. Objective

To produce a *Cms*-DNA chip representing all the genes in the pathogen's genome.

C. Scope

Bacterial ring rot is one of the most feared diseases in the seed potato industry. There is a zero tolerance for ring rot in all certified seed industries in the U.S., Canada, and the European Union. Early and accurate detection of *Cms* in certified seed production is critical to disease prevention and the goal of eradication. There are several methods currently available for diagnosing ring rot. These range all the way from visual field inspections to sensitive DNA tests that can detect as few as 100 cells of the pathogen. Some of the DNA-based tests have been especially difficult to integrate into seed certification programs in the US. There is clearly a need for better and more robust diagnostic techniques.

Several areas in the world including Colorado have attempted and failed to eradicate bacterial ring rot. The primary reasons for ring rot's reoccurrence are latent tuber infections and contaminated equipment and storage facilities, both of which are difficult to detect. The certification practices developed over the years have been largely successful in eliminating ring rot in the seed industry, especially in Colorado. Unfortunately, breakdowns can and do occur even with the best of practices. The consequences of even a single ring rot diagnosis are enormous. It can mean economic disaster for an individual grower, and can also damage the reputation of the regional seed potato industry.

Ring rot is a disease of global importance that significantly impacts the US seed potato export market. Countries place restrictions on seed imports from countries like the US where the disease is present. The burden of demonstrating seed is free of bacterial ring rot is costly and a missed diagnosis even more so. No one wants his or her seed farm identified as having a ring rot history. Likewise, regions and countries that do not have bacterial ring rot want to keep it that way. This year's identification of bacterial ring rot in a seed lot in Wales made international news with good reason; the disease had not been reported previously in the UK. This particular case also points out the economic consequences a positive ring rot diagnosis can have on a grower because there was no federal bailout for the grower in Wales. His only recourse is to file a lawsuit against the supplier of the seed.

We propose to apply the latest, cutting-edge technology to ring rot diagnosis with the additional goal of using this technology to identify strains of *Cms*. A *Cms*-DNA chip could be particularly valuable for tracing introductions of ring rot into the San Luis Valley and for monitoring the spread of strains within the valley.

Previous investments in ring rot research. Our long-term commitment to ring rot research at CSU has led to remarkable outcomes. Through our efforts, we have become a recognized world leader in ring rot research. In 2001, we were awarded $400,000 from the USDA Microbial Genome Sequencing Program to obtain the complete genome sequence of the type strain of *Cms*. This work is nearly completed and has supplied a tremendous resource to the national and international research communities.

Ishimaru is the Project Director on the grant and the sequence has been generated in collaboration with J. Parkhill, The Wellcome Trust Sanger Institute, U.K. The project website is www.sanger.ac.uk/projects/C_michiganensis. At present there are 55,801 DNA sequencing reads totaling 29.324 Mb and giving a theoretical coverage of 99.98% of the genome. A thorough analysis of the genome is planned for the Spring 2004. The project is scheduled for completion by September 2004.

In addition, we have developed an international collaboration with R. Eichenlaub and A. Puhler, University of Bielefeld, Germany to conduct genomic comparisons between *Cms* and the tomato pathogen *C. m.* subsp. *michiganensis*. We have obtained access to the unpublished genome sequence of *Cmm*. We have learned that while the sequence similarity between *Cmm* and *Cms* is extremely high, there are significant differences between the two genomes. These studies are allowing us to identify genes that are specific for *Cms* and for its ability to infect potato.

Investments in DNA technologies for ring rot research. Several pieces of state-of-the-art equipment are available for ring rot research in our laboratories at CSU. These include a DNA chip writer and reader, a DNA sequencer and a real-time PCR machine. Altogether this represents an investment of about $300,000. In addition, we are members of a Campus-wide Genomics Initiative Committee and this group is responsible for upgrading our equipment resources by $1.6 million. Last week, an RNA Laser Capture Microdissection unit arrived on campus. This $150,000 unit will allow us to examine localized gene expression of *Cms* infected materials by using a laser to dissect cells from tissue and to isolate the cellular RNA. This RNA will be used to probe the *Cms*-DNA chip so that we may produce a gene expression profile under a variety of experimental conditions.

D. APPROACH

This proposal focuses on the development of a *Cms*-DNA chip that can be used to detect genetic differences among *Cms* strains. We envision DNA chip technology will be as valuable in ring rot diagnosis as it has for other high profile cases, such as the anthrax letters. We see this as a tool for tracking the origin of strains. Its value in this regard will depend on the kinds and sizes of DNA differences occurring among *Cms* strains. In other work, we identified genetic differences between strains of *Cms* (Brown et al, 2002). The *Cms*-DNA chips will enable us to follow up on these previous findings and to expand current ring rot diagnosis to the level of strain. It is possible to make a *Cms*-DNA chip only because we have been successful in obtaining the *Cms* genome sequence.

The *Cms*-DNA chip set will be designed by us, and the 70-mer oligos will be synthesized by Operon-Qiagen. The oligonucleotide sets will be designed around 70mers (each probe on the chip will be 70 bases of DNA). We will be responsible for printing, quality control, use of the chips, and database management of gene expression and strain variation data. The complete *Cms* gene set will be comprised of all the genes in the sequenced strain. Currently this set is expected to encode for about 3,500 genes. Any unique genes identified in the genome of related phytopathogens currently being sequenced, such as *Leifsonia xyli* and *C. michiganensis* subsp. *michiganensis*, will also be incorporated into the arrays.

Each array set will include both negative and positive universal hybridization controls along with a set of probes that allow for dynamic range and dye effects to be normalized. These controls are used to normalize experiments so that experiments can be controlled in terms of inter-slide and intra-slide variation and does not impact statistical analysis of the overall experiment. Specific methods for microarray fabrication will essentially follow those reported elsewhere and used routinely at CSU. All microarray experiments will be documented in compliance with Minimum Information About Microarray Experiment (MIAME) recommendations.

E. RELATIONSHIP OF THE PROPOSED RESEARCH TO OVERALL PROBLEM

A vision for the future for ring rot research (or what is in this work for CPAC?). The proposed research will provide the means for evaluating the latest technology for tracking and identifying pathogens. The *Cms*-DNA chip can be used to address several questions affecting the seed industry. How

can we improve diagnosis of latent infections? What DNA differences can be used to confidently identify the sources of ring rot outbreaks? What is the genetic nature of latent infections? How does the ring rot pathogen regulate its virulence in plants? What genes are important in pathogenicity? Some outcomes that will have direct impacts on CPAC include:

- Identification of *Cms*-specific and strain-specific genes.
- Specialty DNA chips for pathogen detection or strain tracking.
- Identification of genes expressed during latent or active infections.
- New, more robust methods for ring rot diagnosis; for example litmus paper tests that can be performed with limited training and technical resources.
- Ways to confirm that equipment and storage facilities are properly disinfected.

F. POTENTIAL FOR LEVERAGING RESEARCH RESULTS TO OBTAIN OUTSIDE FUNDING

We are confident that the return on investment in chip technology will be large. The Area II Colorado Potato Administrative Committee has invested $71,000 in our bacterial ring rot research program since 1990. In that time period, we obtained $884,155 in contracts and grants for ring rot research, which does not include the contributions of the Colorado Agricultural Experiment Station or equipment grants. We know of several excellent opportunities for leveraging the *Cms*-DNA chip for funding at the national and international level. Some that we know of include:

- USDA-National Research Initiative in Functional Genomics June 2004
- Possibility of receiving matching funds from Canadian Food Inspection Agency (S. De Boer)
- NRI and NSF plant genome programs to study potato gene expression during ring rot infections

G. TIMELINE OF PROPOSED RESEARCH

Within one year, we will make the first *Cms*-DNA chips. The longer-term outcomes are listed above under section G.

- The DNA fragments (oligos) will be produced by Operon-Qiagen as soon as the genome project with the Sanger Institute is completed (expected completion early fall 2004).
- Once we receive the oligos from Operon-Qiagen, we will begin making the *Cms*-DNA chips.

H. DETAILED ANNUAL BUDGET

Personnel	0
Materials and supplies	44,800
Travel and equipment	0
TOTAL	**$44,800**

I. BUDGET JUSTIFICATION

Funds will be used to pay Qiagen to produce the oligo set of the *Cms* genes. We will design the oligos at no charge. The cost for the oligos is $0.16 per base or $11.20 per 70mer. We project there will be 3500 genes in *Cms* plus 500 genes of other phytopathogens.