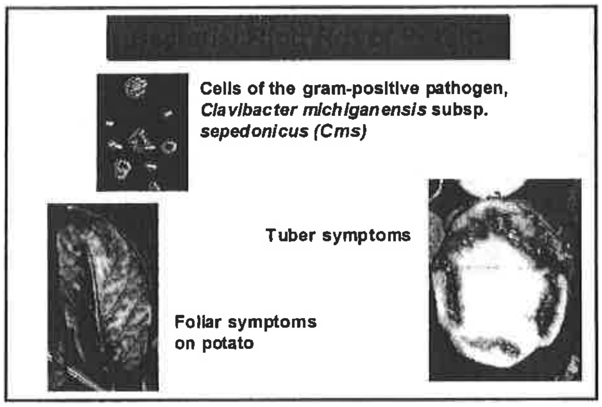
1

Genomics-
Revolutionizing the 21st Century

Structural	Functional	Uses
Cytogenetics	Bioinformatics	Targets of susceptibility
Genetic, physical maps	Gene expression analysis	Novel diagnostics
DNA sequences	Gene inactivation	Genome organization
		Gene Circuits
		Genetic diversity, polymorphisms
		New products

A. K. Vidaver, 2000



[illegible]

Cells of the gram-positive pathogen, *Clavibacter michiganensis* subsp. *sepedonicus (Cms)*

Tuber symptoms

Foliar symptoms on potato

Bacterial Ring Rot

- ***Clavibacter michiganensis*** **subsp. *sepedonicus***
- **vascular pathogen**
- **zero tolerance in seed industry**
- **certified seed, limited generation**

Genomic Analysis of the Plant Pathogenic Coryneform *Clavibacter michiganensis* subsp. *sepedonicus:*

“Genomics on a Shoestring”

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A Genomic Approach to *Cms* Molecular Biology

❶ DNA fingerprinting of genome

❷ Create a Sequence Tagged Connector (STC) Resource

- ➔Create recombinant libraries
- ➔DNA Fingerprint recombinant clones
- ➔Sequence ends of recombinant cosmid clones
- ➔Functional analysis through similarity searches against sequence databases

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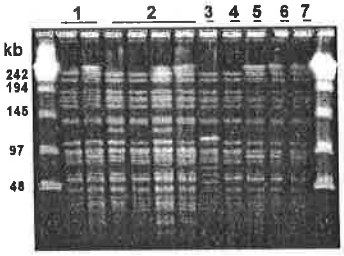
Genomic fingerprints of *Clavibacter michiganensis* subsp. *sepedonicus*



*Eco*R1

1=P45 2= Cs3 3= CIC31 4= CIC8
5= ATCC9750 6 = ATCC33111 7= ATCC33113

Genomic Fingerprint Summary

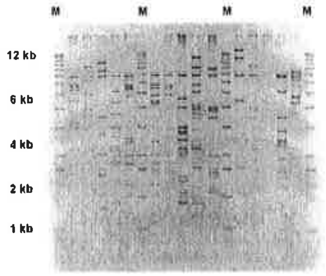
- Fingerprints vary among virulent and avirulent strains
- *NotI* cuts genome frequently
- Genome consists of a chromosome and a large cryptic plasmid (pCS1)
- Minimal genome size is 2.6 Mb

Genomic Approach cont.

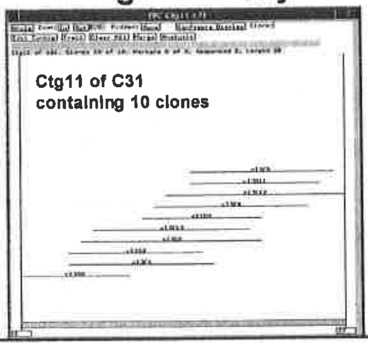
❶ DNA fingerprinting

❷ Create a Sequence Tagged Connector (STC) Resource
- Create recombinant libraries
- DNA fingerprint recombinant clones
- Sequence ends of recombinant cosmid clones
- Functional analysis through similarity searches against sequence databases

Cms Cosmid *NotI* DNA Fingerprints: Random Clones



FPC Contig Assembly C31

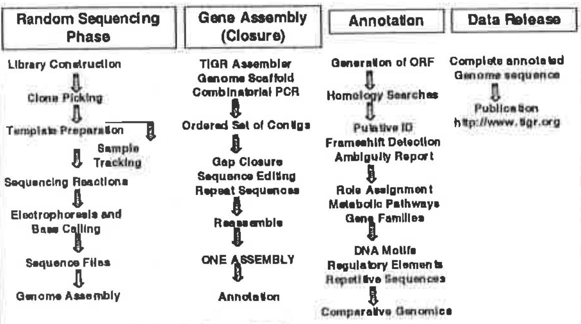


Ctg11 of C31
containing 10 clones

Summary of End-Sequencing

- Number of high quality end sequences obtained is 70
- Number of trimmed sequence is 30,311 b
- 74% of *Cms* sequence hits sequence in data banks

A Microbial Genome Sequencing Project



Random Sequencing Phase

Library Construction
⇩
Clone Picking
⇩
Template Preparation
⇩
Sequencing Reactions
⇩
Electrophoresis and Base Calling
⇩
Sequence Files
⇩
Genome Assembly

Sample Tracking

Gene Assembly (Closure)

TIGR Assembler
Genome Scaffold
Combinatorial PCR
⇩
Ordered Set of Contigs
⇩
Gap Closure
Sequence Editing
Repeat Sequences
⇩
Reassemble
⇩
ONE ASSEMBLY
⇩
Annotation

Annotation

Generation of ORF
⇩
Homology Searches
⇩
Putative ID
Frameshift Detection
Ambiguity Report
⇩
Role Assignment
Metabolic Pathways
Gene Families
⇩
DNA Motifs
Regulatory Elements
Repetitive Sequences
⇩
Comparative Genomics

Data Release

Complete annotated
Genome sequence
⇩
Publication
http://www.tigr.org

From: The Institute for Genomic Research (TIGR)

Clavibacter michiganensis

Subspecies	Hosts	Inoculated	Symptoms

adapted from: Metzler *et al.*, 1997

[redacted]

- **CLASS:** **Actinobacteria (high G+C bacteria)**
  Streptomyces, Mycobacterium
- **Order:** **Actinomycetales**
- **Suborder:** **Micrococcineae**
  Brevibacterium
- **Family:** **Microbacteriaceae**
  Curtobacterium, Rathayibacter, Microbacterium

2001 SLV Research Proposal

Carol A. Ishimaru
Dennis L. Knudson

Objectives

1. Submit proposals for complete genome sequencing of bacterial ring rot pathogen
2. Sequence ends of 1000 cosmid clones.

SLV Funding Request
$17,000 Total

- Up to $5,000 for travel to negotiate contracts for genome sequencing and annotation.
- $7,000 to cover reagent costs for end-sequencing 1000 clones.
- Remainder ($5,000 or more) to contribute to cost of complete sequencing effort.

Acknowledgments

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Colorado Agricultural Experiment Station
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