

**Microbial Genomic Sequencing
Perspectives of the
American Phytopathological Society
(Revised 2003)**



Healthy Plants • Healthy World

The American Phytopathological Society
3340 Pilot Knob Road
St. Paul, MN 55121-2097
Phone: +1.651.454.7250 Fax: +1.651.454.0766
Email: aps@scisoc.org *APSnet*: www.apsnet.org

Microbial Genomic Sequencing Perspectives of the American Phytopathological Society

Background

Microorganisms play a critical role in plant health. Depending on the organism, they can cause multiple diseases or prevent them. Yet, on a genomic level, we know little about them (*The Microbe Project*, 2001, National Science and Technology Council, Office of Science and Technology Policy, Washington, D.C., 29 pg.). Genomic analyses of plant associated microorganisms are as essential to understanding the development and suppression of plant diseases. Analyses of microbial genomes will complement those done on plant genomes (e.g. for Arabidopsis, rice, etc) by providing new insights into the nature of plant-microbe interactions. The APS has consulted its members and constituencies on priority setting of microorganisms that should be sequenced. A first list was compiled in 2000-2001 which had significant impact in stimulating efforts leading to the sequencing of a number of microbial genomes, particularly bacterial and fungal. Nevertheless, there remains a critical need for greater information in microbial genomics. For example, to date no plant associated nematode genome sequence has been determined nor is such a project underway. Thus it is with a sense of continued urgency that this revised list, created with extensive input from the membership of The American Phytopathological Society during 2002-2003 is presented. Plants for food, fiber, forestry and sport and amenity are subject to losses caused by a variety of physical and biological factors. Biological agents that cause losses estimated at 20-35%, over \$200 billion annually, are insects and microbes. Yet the biotic and abiotic variables that contribute to these losses are not fully understood. To date, much has been learned about the microbes that cause and suppress plant diseases, but genomic analyses will be needed to fully understand the nature of their interactions with plants. Due to multiple factors, such losses will continue to be a challenge. These factors include global climate change, plants grown in new ecosystems, mutation or gene transfer in pathogens, and spread of pathogens by nature or by commerce (shipping, transport, and storage). Such losses are considered unsustainable in view of projected needs of human and animal populations. Using both traditional and innovative genetic approaches, plant breeding programs will continue to make a significant impact on reducing crop losses. However, little consideration is currently given to the diverse microbes that colonize plant tissues under field conditions. Continued huge economic and nutritional losses incurred are considered unsustainable given current projections for the growth of the human population and their increasing demand for food.

A complete understanding of disease susceptibility and resistance will require understanding the interactions between plant hosts and pathogen, and beneficial microorganisms. Sequence data from the genes for such microorganisms is essential to advance our knowledge of infection and the interaction of pathogens and host gene products, and will provide knowledge on how these organisms reproduce and spread. Effective control will then be achieved through developing host resistance or through means that combat the pathogen directly or indirectly. Studies of microorganisms with biocontrol capacities may lead to further development of environmentally safe chemical controls for plant pathogens. The use of genomics has already been demonstrated through

enhancing disease resistance by using viral components that can be incorporated into a host with current technology. Identifying critical genes in other pathogens will permit similar strategies to be developed for disease control. Similar strategies are receiving significant support for microbes of medical importance, as such efforts are identifying targets for drugs, and suggesting new targets for drug development and diagnostics. The genetic potential and sensitivity of pathogens must be understood to effectively combat current and future disease threats.

It should be noted that while not included in these lists parasitic plants such as members of the genera *Striga*, *Orobanche*, *Cuscuta* and *Viscum* represent some of the most destructive pests in world agriculture. Their non-inclusion here is primarily to maintain a focus on microbes. However, the society supports the sequence analysis of these important organisms.

Priority Setting and Criteria

A variety of organisms cause and prevent plant diseases. Fungi and viruses are the most significant and widespread. It is estimated that there are more than 10,000 pathogenic species of fungi and viruses, many of which are economically important. It is clear that it is not possible to obtain genomic sequences of all of them, at least based on current and anticipated technology. Thus, we suggest focusing on a representative set of significant microorganisms. Because of the taxonomic diversity of plant associated microbes and because the economic and/or research importance of specific species, continued microbial genomic sequencing is essential. To assist with this endeavor the following lists are a current consensus of up to 10 species of “Immediate Priority” and up to an additional 25 species considered of “high priority” within each biological category.

There have been additional substantial changes to the lists from the first version. Due to their relatively small genome size it is not useful to list individual plant viruses for sequencing, rather it is within technical and fiscal reach to sequence all known plant viruses, which of course would provide an extraordinary research resource to the scientific community. Additionally, because some genomes on the earlier list have been completed, are in progress, or substantial funding is in place, a list of these organisms follows each of the pertinent sections.

The listing has been developed using these criteria, essentially as outlined by the USDA (RFP for IFAFS, 2000):

- Economic importance and relevance to U.S. (and world-wide) plant health.
- Unique biological or environmental features.
- Broad interest to a significantly sized community of scientists or agriculturists.
- Genetic tractability, i.e. the ease with which genetic studies, such as crosses, genome modifications, etc. can be performed.
- Availability of tools and other biological resources, such as gene libraries, genetic maps, genetic tractability of the host so that the system could be addressed from both sides of the host-pathogen interaction, etc.

It is recognized that some organisms may be of critical agricultural importance but not easily cultured or subjected to genetic analysis, and therefore may be candidates for sequencing.

The taxonomic position of species of interest is recognized as of considerable significance in the choice of organisms to sequence. A broad representation of genomes of diverse taxa within each group is thought by many to be of importance. Thus sequencing of multiple closely related species prior to collection of data from the diversity of the group should be considered with due caution. This topic is discussed with focus on fungi and oomycetes in an article under review for *Phytopathology*: Goodwin, S.B. "Minimum Phylogenetic Coverage: A Guide for the Selection of Microbial Pathogens for Initial Genomic Sequencing Efforts".

Needs

Additional and new funding is of critical need. A growing number of research programs are at a major impasse due to lack of information about pathogen and biocontrol genomes. Cloning and characterization of individual genes is no longer cost effective. Microorganisms in general have compact genomes, in most cases a fraction of the size of plants and animals. Costs of genomic sequencing are still high, but decreasing. Functional and structural genomics will be the next phase of analysis. Some investment has occurred in the private sector, but relatively little. Very little has been publicly available for plant pathogens. The lack of sequence information for agriculturally important microorganisms in public databases has other serious consequences, such as those related to human resources, trade (phytosanitary) issues, and agroterrorism. Finally, although this list is primarily focused on initial structural genomics for these organisms continued funding is universally recognized by the membership to be essential for taking full advantage of this information. A plant-associated microbe genome initiative has been published (Leach *et al. Phytopathology*, 93:524-527) as a result of a workshop held in 2002 that recommends additional new funding for needed further research and education to fully leverage the value of the structural data obtained as advocated in this document. Supplementary supporting documentation pertinent to species on the "Immediate Priority" list can be found at www.apsnet.org/media/ps/MicrobialGenomicsSequencingWhitePaperSupplement9_03.pdf

PLANT PATHOGENIC FUNGI AND OOMYCETES

**10 - IMMEDIATE PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

Species	Common Name	Genome Size	Rationale /Significance
<i>Alternaria brassicicola</i>	black spot disease of crucifers	28 Mb	a) Relative to a number of other important pathogens. b) Necrotrophic fungal pathogen model.
<i>Botrytis cinerea</i>	gray mold	ca. 22Mb	a) Economically very significant pathogen. b) taxonomically distinct discomycete. Botryotinia teleomorph
<i>Cochliobolus heterostrophus</i>	southern corn leaf blight	35 Mb	a) Necrotrophic pathogen model with classical and molecular genetic tractability and an historically significant disease. b) Representative of the <i>Cochliobolus/Alternaria</i> group of pathogens, notorious for production of host selective toxins c) Loculoascomycete representative
<i>Colletotrichum graminicola</i>	anthracnose stalk rot of corn	48 mb	a) Representative species of a genus affecting a broad range of important crops. b) A serious pathogen of cool season turfgrasses as well as maize. Glomerella teleomorph
<i>Fusarium verticillioides</i>	Fusarium stalk rot of corn	46 Mb	a) Major pathogen of maize worldwide, causes both kernel and stalk rot. b) Produces fumonisin mycotoxins. Gibberella teleomorph
<i>Mycosphaerella graminicola</i>	Septoria blotch	38-40 Mb	a) Largest genus of plant pathogenic fungi (including anamorphs).

			<ul style="list-style-type: none"> b) Economically significant disease of wheat worldwide. c) Model system for the order Dothideales. d) Large, international research community.
<i>Phytophthora infestans</i>	potato late blight	237 Mb	<ul style="list-style-type: none"> a) Most important pathogen of potato b) Model system within the oomycetes c) Advanced genetics and transformation system and libraries
<i>Puccinia graminis</i>	stem rust	67 Mb	<ul style="list-style-type: none"> a) Causes rust disease on several agriculturally important cereal crops (barley, oat, rye and wheat) and turfgrasses used for sport and amenity. b) Distributed worldwide.
<i>Pyrenophora tritici-repentis</i>	tan spot of wheat	27 Mb	<ul style="list-style-type: none"> a) Broad distribution of disease and researchers worldwide. b) Produces proteinaceous host-selective toxins.
<i>Sclerotinia sclerotiorum</i>	white mold	26-44 Mb	<ul style="list-style-type: none"> a) Number 2 cause of yield loss in soybean in US. b) Major pathogen of canola, sunflower, edible beans, vegetable crops and more than 400 plant species.

PLANT PATHOGENIC FUNGI AND OOMYCETES

**≤ 25 SPECIES - HIGH PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

Species	Common Name	Genome Size	Rationale /Significance
<i>Aphanomyces euteiches</i>	Aphanomyces root rot	50 Mb???	<p>a) One of the most economically important pathogens on a diverse group of organisms including: pea, lentils and sugarbeet as well as fish and crayfish.</p> <p>b) A taxonomically primitive oomycete so genomic work done on other oomycetes such as <i>Phytophthora</i> species is not directly comparable. <i>Aphanomyces</i> can thus serve as the model species for primitive oomycetes such as <i>Saprolegnia</i> and <i>Achlya</i>.</p>
<i>Armillaria mellea</i>	Armillaria root rot		
<i>Armillaria ostoyae</i>	Armillaria root rot	55 Mb	<p>a) In many forests, damage from pathogenic <i>Armillaria</i>, either directly or through predisposition to insect attack, accounts for a greater loss in forest productivity than that from any other forest pathogen.</p>
<i>Blumeria graminis</i>	powdery mildew	30-50 Mb	<p>a) Economically important pathogen of grain crops.</p> <p>b) Model for biotrophic fungal-plant interactions.</p>
<i>Bremia lactucae</i>	lettuce downy mildew	50 Mb	<p>a) Most important disease of lettuce. Lettuce is the eight most valuable crop in the US.</p> <p>b) Biotrophic member of the Oomycetes.</p> <p>c) Most experimentally tractable and studied of the downy mildews. Smaller genome size than some other oomycetes.</p>
<i>Cercospora kikuchii</i>	Purple seed stain	28.4 Mb	<p>a) Significant foliar and seed pathogen of soybean</p> <p>b) World-wide in occurrence.</p>
<i>Cronartium ribicola</i>	White pine blister rust		<p>a) Introduced biotroph; ecologically and economically the most significant pathogen of North American conifers.</p> <p>b) Gene-for-gene and quantitative relationships with two different hosts: 5-needle pines and <i>Ribes</i>.</p> <p>c) Environment triggers several transitions in life cycle,</p>

			allowing gene expression studies.
<i>Cryphonectria parasitica</i>	chestnut blight	Ca. 45 Mb	a) Causal agent of the economic extinction of the American chestnut. b) Model for fungal-mycovirus interactions, signal transduction and hypovirulence.
<i>Epichloe sp.</i>	grass endophyte	29-35 Mb	a) Endophytes provide benefits to forage grasses but often reduce livestock productivity. b) The model endophyte, <i>E. festucae</i> is the most genetically tractable, plant-mutualistic fungus and has numerous agronomically important genes such as for bioprotective alkaloids.
<i>Fusarium oxysporum</i>	Fusarium vascular wilt	43 MB	a) A model for soil-borne fungal diseases, a pathogen of <i>Arabidopsis thaliana</i> . b) Causes economically important diseases of a broad range of crops and ornamentals including tomato, banana, palm, cotton and many others. c) One of the dominant fungal species found in soil and endophytic on plant roots.
<i>Glomus sp.</i>	mycorrhizal fungus	100 Mb	a) Important symbiotic mycorrhizal fungus
<i>Heterobasidium annosum</i>	Annosum/ Annosus root rot		
<i>Ophiostoma novo-ulmi</i>	Dutch elm disease	30-40 Mb	a) Responsible for the pandemic which has been decimating elm populations in North America and Europe since the 1960's.
<i>Penicillium</i>			
<i>Phakopsora meibomia</i> *	soybean rust	70 Mb	a) A major soybean pathogen, the less virulent species of Phakopsora.
<i>Phakopsora pachyrhizi</i> *	soybean rust	70 Mb	a) A major soybean pathogen, listed as one of the "Agricultural Select Agents" by USDA and CDC.
<i>Rhizoctonia solani</i>	Rhizoctonia damping off and canker, etc.	37-46 Mb	a) Has a wide geographic distribution. b) Is an economically important pathogen of many plant species, including maize, potato, rice, soybean and wheat and

			turfgrasses used for sport and amenity.
<i>Tilletia caries</i>	Common bunt/ stinking smut		Causes Common bunt/stinking smut
<i>Venturia inaequalis</i>	apple scab		

PREVIOUSLY LISTED FUNGAL AND OOMYCETE PLANT PATHOGENS SEQUENCED/OR FUNDED (additional funding for completion may be needed):

<i>Aspergillus flavus</i>		~ 30 Mb	Important producer of aflatoxins, extremely potent carcinogenic mycotoxin.
<i>Fusarium graminearum</i>	wheat head scab fungus	40 Mb	a) Causes one of the most important plant diseases in US, wheat head scab. b) Produces trichothecene mycotoxins. c) Studied by large number of investigators worldwide.
<i>Magnaporthe grisea</i>	rice blast fungus	40 Mb	a) Most devastating fungal disease of rice and a serious pathogen of turfgrasses used for sport and amenity. b) Amenable to classical and molecular genetics
<i>Ustilago maydis</i>	corn smut fungus	20 Mb	Important basidiomycete model system for the smuts which are economically important disease agents.

PLANT PATHOGENIC BACTERIA AND MOLLICUTES

**<10 - IMMEDIATE PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

Organism	Strain	Genome Size	Rationale /Significance
<i>Burkholderia cepacia</i>	ATCC 25416	7.9 Mb incl 3.5, 3.1, 1.1, and 0.2 Mb replicons	Causes sour skin of onion, cavity disease of mushroom, brown spot of orchids, and rot of caladium bulbs. Strain ATCC 25416 is a plant-pathogen representative of genomovar I strains, which have rarely been isolated as human pathogens. Opportunity for direct genome comparison to genomovar III strains, which are more typically associated with cystic fibrosis patients exhibiting symptoms of Cepacia syndrome.
<i>Clavibacter michiganensis</i> subsp. <i>insidiosus</i>	NCPPB 1109	ca. 3.0	Causes bacterial wilt, an economically important disease of alfalfa. Very little known of its molecular biology due to the difficulty in genetic manipulation of the pathogen. Recent advances in the manipulation of related pathogens and the soon-to-be-available genome sequences of <i>Clavibacter michiganensis</i> subsp. <i>sepedonicus</i> , <i>C. michiganensis</i> subsp. <i>michiganensis</i> and <i>Leifsonia xyli</i> will enable genomic and functional comparisons across phylogenetically-related vascular pathogens.
Clover Phyllody Phytoplasma		ca. 0.8 Mb	Clover phyllody is widespread in North America and Europe where it is responsible for diseases in fruits and vegetables. Host plants damaged by this phytoplasma include <i>Trifolium</i> spp., <i>Fragaria</i> spp. (strawberry), <i>Olea</i> spp. (olive), <i>Poa pratensis</i> , <i>Anemone</i> spp., <i>Ranunculus</i> spp., and <i>Vitis</i> (grapevine). This phytoplasma is a member of subgroup C in group I (the aster yellows group) and is a distinct species from aster yellows phytoplasma.
<i>Pantoea citrea</i>	1056R	ca. 5 Mb	Causes pink disease of pineapple, resulting in \$130,000,000 loss per annum. Next to mangos (#1) and bananas (#2), pineapple is the third most consumed fruit in the world. Genomics would help us identify novel genes involved in invasion of the fruit, repression of the hypersensitive reaction in the host, induction of genes involved in glucose oxidation which leads to pink coloration, etc. Opportunity for genome-scale comparison with enterobacterial pathogens (virulence factors and evolution of pathogenesis).

<i>Pectobacterium carotovorum</i> subsp. <i>carotovorum</i>	Ecc71	ca. 5 Mb	Causes bacterial soft rot and related diseases of numerous crops worldwide, under temperate as well as tropical conditions and under field conditions, in storage and in transit. Opportunity for genome-scale comparison with enterobacterial pathogens (virulence factors and evolution of pathogenesis). Because this pathogen has world-wide economic importance and has been a principal model organism for molecular studies of plant-microbe interactions, the scientific community who would benefit from the genome sequence is particularly large.
<i>Ralstonia solanacearum</i> Race 3 Biovar 2	UW551	ca. 5.8 Mb	Causes potato brown rot, the third leading source of potato yield losses in the developing world. It is also a virulent pathogen of geraniums and tomatoes, causes latent infections of weeds and native plants, and is a quarantine pest and an agroterrorism Select Agent for the US. It is a model organism for understanding pathogenesis and systemic vascular colonization of host plants. Comparative genomics with <i>Burkholderia cepacia</i> and Race 1 of <i>R. solanacearum</i> , a model organism for molecular studies, will elucidate host specificity and microevolution. Large, international research community.
<i>Spiroplasma citri</i>	BR3-3X	1.6 Mb	Spiroplasmas are unique bacteria characterized by tiny cell and genome sizes, lack of a cell wall, helical morphology, and an unusual genetic code. <i>Spiroplasma citri</i> causes disease in a number of plant species including citrus, and also colonizes its insect vectors. <i>S. citri</i> strain BR3-3X, isolated from brittle-root diseased horseradish plants in Illinois, has been the model system for U.S. research on spiroplasma-vector interactions for over 20 years
<i>Xanthomonas axonopodis</i> pv. <i>malvacearum</i>		ca. 5.0-5.2 Mb	Causes bacterial blight, a worldwide problem on cotton resulting in yield losses to 50%, with 10-30% losses occurring commonly. The pathogen has served as a model bacterial gene-for-gene system with the highest level of race specificity of any xanthomonad (over 20 races described). The African strains are of particular interest because they are quarantined pathogens in the U.S., and they are capable of defeating most of the known bacterial blight resistance genes in cotton. The pathogen is amenable to standard microbial genetic tools, and the histology, biochemistry and physiology of the disease have been very well described, giving enormous leverage to the sequence information.

<i>Xanthomonas oryzae</i> pv. <i>oryzicola</i>	BLS256	ca. 5.0-5.2 Mb	Causes bacterial leaf streak of rice, emerging as serious disease in Asia. Model monocot-(parenchymal) bacterial pathosystem. By comparison with closely-related rice vascular pathogen <i>X. oryzae</i> pv. <i>oryzicola</i> , superb model to determine genetic requirements for mesophyll parenchyma colonization. Important genus for comparative genomics due to extensive host-pathovar specificity and sequenced <i>Xanthomonas</i> and <i>Xylella</i> genomes. Important for understanding xanthan gum and pigment biosynthesis and utilization of plant compounds. International research community. Tractable for molecular studies, many tools and resources available.
<i>Xanthomonas oryzae</i> pv. <i>oryzae</i>	PXO86	ca. 5.0-5.2 Mb	Causes bacterial blight of rice, a serious disease in Asia. Model monocot-(parenchymal) bacterial pathosystem. By comparison with closely-related rice vascular pathogen <i>X. oryzae</i> pv. <i>oryzae</i> , superb model to determine genetic requirements for mesophyll parenchyma colonization. Important genus for comparative genomics due to extensive host-pathovar specificity and sequenced <i>Xanthomonas</i> and <i>Xylella</i> genomes. Important for understanding xanthan gum and pigment biosynthesis and utilization of plant compounds. International research community. Tractable for molecular studies, many tools and resources available.
<i>Xanthomonas campestris</i> pv. <i>vesicatoria</i>	75-3	Ca. 5.0 Mb	Causes bacterial spot of tomato and pepper, a significant disease constraining tomato and pepper production worldwide, especially in tropical and subtropical regions. Model organism for molecular studies on plant-microbe interactions, especially host-pathogen specificity because the <i>hrp</i> system has been most extensively characterized in this species of <i>Xanthomonas</i> . Important genus for comparative genomics due to extensive host-pathovar specificity and sequenced <i>Xanthomonas</i> and <i>Xylella</i> genomes. Large international research community.

PLANT PATHOGENIC BACTERIA AND MOLLICUTES

**≤ 25 SPECIES - HIGH PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

<i>Acidovorax avenae</i> subsp. <i>avenae</i>	CAa4		Infects many important crops causing diseases such as bacterial stripe of rice, bacterial stalk rot and blight of corn, leaf blight of oats, and red stripe of millet and sugarcane. These diseases have become very important in Asia, especially in seedlings. The closely related <i>A. avenae</i> subsp. <i>citrulli</i> causes severe losses in watermelon and other cucurbits.
<i>Clavibacter michiganensis</i> subsp. <i>nebraskensis</i>	NCPPB 2581	ca. 3.0	Causes Goss's bacterial wilt and blight of corn. Very little is known of its molecular biology. Availability of the <i>C. michiganensis</i> subsp. <i>nebraskensis</i> genome sequence would enable genomic and functional comparisons across phylogenetically-related vascular pathogens, which specifically infect either monocots or dicots.
Elm Yellows Phytoplasma		ca. 0.8 Mb	Elm Yellows Phytoplasma is widespread in North America and Europe. This phytoplasma is lethal to American elm (<i>Ulmus americana</i>). A large percentage of American elm tree deaths that were originally attributed to Dutch elm disease are now believed to have been caused by the EY phytoplasma. This phytoplasma is also a major cause of the loss of our native American elm. EY phytoplasma is a member of group V subgroup A and is being named 'Candidatus Phytoplasma ulmi'.
<i>Lysobacter enzymogenes</i>	C3	6.5 Mb	Common soil and water inhabitant with antagonistic activity towards various microorganisms, and demonstrated biocontrol activity towards several plant diseases. Producers of multiple forms of chitinases and glucanases and other extracellular, depolymerizing enzymes. Also produces antibiotics and contains a type III secretion system. No <i>Lysobacter</i> spp. has been sequenced to date.
Potato Witches' Broom Phytoplasma		ca. 0.8 Mb	Potato witches' broom phytoplasma is responsible for serious disease in potato in North America. This phytoplasma is a member of group VI and represents a distinct species that is only distantly related phylogenetically to aster yellows and clover phyllody phytoplasmas.
<i>Ralstonia solanacearum</i> Race 2	Molk2	ca. 5.8 Mb	Causes Moko disease of banana & plantain, key cash & carbohydrate crops. A threat to US growers and a source of major economic and social impacts in the developing world. Insect-transmitted. Model organism for understanding

			pathogenesis and systemic vascular colonization of host plants. Comparative genomics with <i>Burkholderia cepacia</i> and Race 1 of <i>R. solanacearum</i> , a model organism for molecular studies, will elucidate host specificity & microevolution.
<i>Rathayibacter toxicus</i>	ATCC 49908		Infects annual Wimmera ryegrass in South Australia. It is carried by a nematode (Aguina species) into developing seed heads and produces corynetoxin, which is a relative of tunicamycin, an antibiotic that blocks glycosylation. The toxin kills livestock grazing on the infected wild ryegrass. Control is to use herbicides to kill the ryegrass. <i>R. toxicus</i> distorts the seed heads (swelling) and produces an orange exudate. It could therefore be considered a plant pathogen of a weed grass, but <i>R. toxicus</i> is more economically important to the cattle and sheep industry, which would also benefit from having <i>R. toxicus</i> ' genome sequenced.
<i>Rhodococcus fascians</i>	D188	5.8 Mb	Causes leafy gall formation on a broad array of monocot and dicot plants. It induces de novo cell division in cortical plant cells, leading to the formation of numerous shoot meristems. It is an adapted endophyte that possibly also enters the plant cells. Strain D188 has been the model strain in molecular studies. As a close relative to <i>Rhodococcus equi</i> (virulent on horse) and <i>Mycobacterium</i> species (known human and animal pathogens), it is a good model for understanding general and specific pathogenesis factors from Actinomycetes, from both plant and animal pathogens.
<i>Spiroplasma melliferum</i>		ca. 1.5 Mb	Although not a plant pathogen, <i>S. melliferum</i> is a pathogen of bees, and thus is similar to plant pathogenic spiroplasmas in replicating in insects. The genomic sequence will be useful for comparative genomics to identify genes that are potentially involved in plant pathogenesis.
<i>Xanthomonas phaseoli</i>			Causes common bacterial blight (CBB) on <i>Phaseolus vulgaris</i> (common dry and snap beans). CBB is one of the most economically important and widespread diseases of <i>P. vulgaris</i> , constraining both yield and seed quality. CBB has been reported to cause significant yield losses in Michigan (10%-20%), in Canada (38%) and in Colombia (45%). Management of CBB is very difficult due, in part, to the pathogen's persistence. Although CBB is seed transmitted, clean seed programs have been only partially effective in controlling it in the U.S. and Canada.

PROKARYOTIC PLANT PATHOGENS BEING SEQUENCED (additional funding for completion may be needed):

<i>Agrobacterium vitis</i>	S4	ca. 5.7 Mb	Causes crown gall of grapevine, a serious disease in vineyards and nurseries worldwide. It also causes a tissue-specific necrosis on grapevine, and a hypersensitive response on non-host plants. Certain non-tumorigenic strains can be used as biological control agents to prevent grape crown gall. Biochemical tests indicate a close relationship to certain <i>Rhizobium</i> spp. Sequencing would promote research on mechanisms of host-specificity, disease and HR and would provide insight into the evolution of Rhizobiaceae.
Aster Yellows phytoplasma	AY-WB	0.8 Mb	Aster yellows phytoplasmas are the most widespread of the phytoplasmas occurring worldwide, causing chronic, systemic diseases that affect over 300 species in 38 families of broad-leaf, herbaceous and woody fruit crops. There are more than 100 diverse isolates of aster yellows phytoplasmas. AY-WB was purified from field-collected lettuce plants (Celeryville, OH) and belongs to the aster yellows subgroup A cluster (16SrIA).
Aster Yellows phytoplasma	MBSP	ca. 0.8 Mb	Maize bushy stunt phytoplasma (MBSP) and <i>Spiroplasma kunkelii</i> often co-infect maize plants causing the corn stunting or "Achaparramiento" disease. <i>S. kunkelii</i> and MBSP infections are major constraints to maize production throughout Central and South America, and occasionally in the southern US. Of the two mollicutes, MBSP causes more severe stunting and proliferation of shoots. MBSP is classified in the aster yellows subgroup B.
<i>Erwinia amylovora</i>	Ea273	ca. 4.5 Mb	Causes fire blight, the most important bacterial disease of apples and pears worldwide. Serves as a model pathosystem for study of plant-microbe interactions. Opportunity for genome-scale comparison with enterobacterial pathogens (virulence factors and evolution of pathogenesis).
<i>Pantoea stewartii</i> subsp. <i>stewartii</i>		ca. 5 Mb	Causes Stewart's disease (also called bacterial wilt) of corn, which can cause yield losses ranging from 40-90 percent in susceptible varieties. Insect transmission, lack of an epiphytic phase, and the prominent role of a quorum-sensing controlled capsular polysaccharide (CPS) in virulence are distinctive characteristics of the pathogen. Opportunity for genome-scale comparison with enterobacterial pathogens (virulence factors and evolution of pathogenesis).

<i>Pseudomonas syringae</i> pv. <i>phaseolicola</i>	1448A	ca. 6.0 Mb	Causes halo blight of bean (<i>Phaseolus vulgaris</i>), which is a disease of world-wide importance and an established experimental model. The genetics of avirulence and pathogenicity have been extensively studied in this pathovar.
<i>Pseudomonas syringae</i> pv. <i>syringae</i>	B728a	6.08 Mb	Causes bacterial brown spot on bean and is pathogenic on Arabidopsis. The strain is a colonist of leaf surfaces as well as a virulent pathogen, is active in ice nucleation at warm subfreezing temperatures, and serves as an important model in molecular, ecological and epidemiological studies. Opportunity for comparative genomic studies with <i>P. syringae</i> pv. <i>tomato</i> DC3000, whose complete genomic sequence is known.
<i>Spiroplasma kunkelii</i>	CR2-3X	1.7 Mb	<i>Spiroplasma kunkelii</i> coinfects maize plants with Maize bushy stunt phytoplasma (MBSP) causing the corn stunting or "Achaparramiento" disease. <i>S. kunkelii</i> and MBSP infections are major constraints to maize production throughout Central and South America, and occasionally in the southern US.
<i>Streptomyces scabies</i>	87.22	8.0 Mb	Causes common scab of potato and other root crops; the disease results in significant losses to potato farmers in the US every year. <i>S. scabies</i> has a well-characterized pathogenicity island and is a model organism for molecular genetic analysis of pathogenicity and its evolution within the deeply rooted cluster of high G+C, gram positive prokaryotes. Scientists interested in the regulation of secondary metabolism and the evolution of pathogenity will utilize the <i>S. scabies</i> genome sequence, in combination with the existng <i>S. coelicolor</i> genome to unravel many important research questions. The total community is likely to be at least 800 scientists, worldwide.
<i>Xanthomonas axonopodis</i> pv. <i>aurantifolii</i>	B and C	ca. 5.0-5.2 Mb	Cause Cancrosis B and C of citrus, respectively. Cancrosis B is a disease of lemons in Argentina, Paraguay, and Uruguay; Mexican lime, sour orange, and pummelo are also susceptible to Cancrosis B. The only known hosts for Cancrosis C are Mexican lime and sour orange.
Western X phytoplasma		ca. 0.8 Mb	An insect-transmitted fastidious bacterial pathogen of stone fruits. It causes significant yield losses of stone fruits in the US.

PLANT ASSOCIATED BENEFICIAL PROKARYOTES AND EUKARYOTES

**≤10 - IMMEDIATE PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

Organism	Strain	Genome Size Mb	Rationale/Significance
<u>Prokaryotes:</u>			
<i>Bacillus subtilis</i>	GB03	ca. 4.3	One of the most widely distributed bacterial species in agricultural systems. This rhizosphere isolate used in biocontrol of soilborne root diseases. Well-established commercial applications. Excellent opportunity for genome-scale comparisons with non-functional saprophyte and human pathogens of same genera.
<i>Pantoea agglomerans</i>	C9-1	ca 4.0	Common plant epiphyte. Biological control strain for fire blight. Opportunity for genome-scale comparison with enterobacterial pathogens.
<u>Eukaryotes:</u>			
<i>Trichoderma harzianum</i>	T22	ca. 35	Active ingredient in commercial biocontrol products used on multiple crops. Subject of extensive genetic and ecological studies. Opportunity for genome-scale comparison with <i>T. reesei</i> strain which is currently being sequenced.

PLANT ASSOCIATED BENEFICIAL PROKARYOTES AND EUKARYOTES

**≤25 - HIGH PRIORITY SPECIES:
(Alphabetically, arranged - not ranked within the list)**

Prokaryotes:

<i>Bacillus cereus</i>	UW85	ca. 5.2	Common soil inhabitant involved in biological control of plant pathogens. Opportunity for genome comparisons with close relatives for which genome sequences have already been obtained, i.e. <i>B. anthracis</i> and a clinical isolate of <i>B. cereus</i> .
<i>Burkholderia ambifaria</i>	AMMDR1	ca. 7.2	Common rhizosphere organism. Biocontrol of soilborne oomycetes. Opportunity for genome-scale comparison with human pathogenic strains of the <i>Burkholderia cepacia</i> complex.
<i>Pseudomonas fluorescens</i>	A506	ca 5.5	Common plant epiphyte. Commercial biological control strain for fire blight. Opportunity for genome-scale comparison with other fluorescent pseudomonads.
<i>Pseudomonas fluorescens</i>	Q8r1	ca 5.5	Aggressive rhizosphere colonist and biological control agent of root diseases. Type strain for D genotype of DAPG producers. Biovar distinct from <i>P. fluorescens</i> Pf-5 which is currently being sequenced. Opportunity for comparisons with other fluorescent pseudomonads.
<i>Pseudomonas aureofaciens</i>	30-84	ca 5.5	Phenazine producer and model strain for genetic studies of biological control and quorum sensing. Opportunity for genome-scale comparison with other fluorescent pseudomonads.

PLANT PATHOGENIC NEMATODES

≤10 - IMMEDIATE PRIORITY SPECIES:

(Alphabetically, arranged - not ranked within the list)

Organism (by category)	Common Name	Genome Size	Rationale or Significance
<i>Aphelenchoides fragariae</i>	Foliar nematode	-	A leaf-inhabiting nematode that is a pathogen on ornamentals.
<i>Ditylenchus dipsaci</i>	Stem nematode	-	One of the most devastating nematodes on a wide range of crops worldwide. Feeds on the shoots of plants as a migratory endoparasite.
<i>Globodera pallida</i>	Potato cyst nematode	1×10^8	Most economically important nematode in Europe. Sedentary endoparasite of potato.
<i>Heterodera glycines</i>	Soybean cyst nematode	9.2×10^7	Most economically important pathogen on soybean. Chosen as a model nematode for plant parasites. Sedentary endoparasite.
<i>Meloidogyne hapla</i>	Northern root knot nematode	5.5×10^7	Problems on wide range of crops such as alfalfa, vegetable; cool weather nematode. Has BAC library from inbred diploid strain.
<i>Meloidogyne incognita</i>	Southern rootknot nematode	5.1×10^7	Most economically important nematode worldwide. A sedentary endoparasite that has an extremely wide host range.
<i>Nacobus abbesans</i>	False root knot nematode		Problems in Western US on potato, same family as <i>Pratylenchus</i> and <i>Radopholus</i> but sedentary.
<i>Pratylenchus penetrans</i>	Lesion nematode	-	An economically important migratory endoparasite. Wide host range with a different feeding habit.
<i>Radopholus similis</i>	Burrowing nematode	-	An economically important migratory endoparasite on citrus and ornamentals in the Americas.
<i>Rotylenchulus reniformis</i>	Reniform nematode	-	Widely distributed sedentary semiendoparasitic nematode in the tropical and subtropical countries that is an important parasite with a wide host range.

- Compiled by the nematology committee

PLANT VIRUSES

Because the genome of plant viruses is small compared to cellular microbes the aps membership believes that rather than listing individual viruses a great deal of progress can be made by accomplishing the goal of sequencing all known plant viruses. Below is a statement prepared to this effect by the aps virology committee.

The biodiversity among and between viruses is staggering. Viruses are microbes known to infect a majority of life forms. Among their genomes are single-stranded and double-stranded, linear and circular, DNA and RNA representatives. Viruses use a diversity of mechanisms to replicate in host cells, elucidation of such mechanisms has led to many major advances in biology. Yet, our understanding of viral genomes, driven by interests in human medicine and agriculture, has been limited mostly to economically important viruses. Still, the proportion of recognized viruses for which the genome has been completely sequenced is impressive. Completion of the genome sequencing of recognized viruses is within our grasp. An effort to complete the sequencing of the genomes of recognized plant viruses will have immediate significance for biotechnology, for molecular and cellular biology, and for virus diagnostics.

Given the importance of viral sequences to biotechnology, molecular biology, and virus detection and identification, it is surprising that virus sequencing projects have been under-funded by the Microbial Genome Sequencing Program. The poor representation of viruses is due in part to the ease with which single virus sequences can be obtained and in part to the lack of an organized effort of obtaining complete coverage of a virome, such as the plant virome. An organized effort to determine the genomic sequences of viruses most diverged from those whose genomes have been already completely sequenced is needed. The plant viral genomes are eukaryotic in nature because they function in eukaryotic cells. However, selection for small genome sizes resulted in a high gene density, more dense than found in other microbes, including eukaryotic microbes. With rare exceptions there are no introns, no highly repeated sequences and no large intergenic regions. Often one finds overlapping genes. Thus, the yield per million base pairs (Mbp) of useful molecular biological information from a virome project should be far greater than from other genome projects.

Therefore, the long range goal of the proposed work is to obtain complete genome sequences of all recognized viruses. Understanding that reaching this goal efficiently requires considerable organization and cooperation, we propose to demonstrate the feasibility of the larger goal by concentrating on viruses that infect plants. The defined number of recognized but unsequenced viruses, combined with the relatively small size of most of their genomes makes this an ideal pilot project. Sequencing the complete genomes of all known plant viruses would take minimal effort and resources in comparison to sequencing the complete genomes of two bacterial species, while providing enormous amounts of useful data.