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in the present work. The authors point out the possibility that another FtsZ protein, which does not cross-react with the antibody used, forms part of the outer ring, but their results suggest that the insoluble 56-kD protein is the principal ring component, and insoluble properties have not been reported previously for FtsZ proteins.

However, as in the case of *Fucus*, it cannot be assumed that green plants necessarily will share features of red algae, which belong to the protist phylum Rhodophyta. In transgenic *Arabidopsis* plants, expression of either *AtFtsZ1* or *AtFtsZ2*, both of which encode proteins related to cyanobacterial FtsZ sequences, reduces the number of chloroplasts in mature leaf cells from 100 to 1, indicating that both genes are required for chloroplast division in higher plant cells (Osteryoung et al., 1998). It is reasonable to hypothesize that FtsZ2 constitutes part of the outer PD ring, because, unlike FtsZ1, it lacks a chloroplast transit peptide and is not transported into isolated chloroplasts.

The cell cycle of higher plants contains unique features, aspects of which may be ripe for discovery in *Arabidopsis*. For example, molecular identification of the 56-kD protein component of the outer PD ring in *C. merolae* will lead to the immediate identification of homologs in the completely sequenced genome of *Arabidopsis*. It also should

be possible to identify all of the CDKs in *Arabidopsis* and begin a process of systematically investigating their possible functions in cell cycle control and other plant growth processes. The three articles highlighted here should stimulate renewed interest in comparative studies of cell cycle control among higher plants, algae, and other eukaryotes.

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## Agricultural Microbes Genome 2: First Glimpses into the Genomes of Plant-Associated Microbes

These are extraordinary times for the biological sciences. With the advent of genomics, the entire hereditary blueprint of an organism can be determined, and a deep transition into

genome-enabled biology is occurring. This new era of research allows a comprehensive study of gene function that has already delivered tremendous insights into the basic biology

of living organisms. Even though microbiologists were at the forefront of this paradigm shift, plant microbiologists had to be patient and for years watched with envy (and at times despair)

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the ever-growing list of genome sequences of model and animal-associated microbes. Due to, among other things, an unfavorable funding climate, the first completed genome of a plant-associated microbe, that of the citrus pathogen *Xylella fastidiosa*, was reported last year by a Brazilian team (Simpson et al., 2000) five years after the completion of the genome sequence of a human pathogenic bacteria (Fleischmann et al., 1995).

Fortunately, the tide has turned. Plant microbiologists attending the 2nd Agricultural Microbes Genome Conference, held from January 17 to 19, 2001, in San Diego, were greeted not only by the warm and sunny climate of southern California but also by the revitalizing news of the exponential accumulation of genome sequence data for plant-associated microbes. The gathering, which

followed the popular Plant and Animal Genome Conference, was put together by an organizing committee chaired by Peter Johnson (U.S. Department of Agriculture [USDA]; Washington, DC) and Stephen Heller (USDA; Beltsville, MD) and was attended by more than 120 participants. The program, which consisted of ~25 talks and 25 posters, was focused essentially on the genomics of microbes relevant to various aspects of agricultural sciences and also included a bioinformatics workshop.

In this report, we highlight selected talks and posters on plant-associated microbes presented at the 2nd Agricultural Microbes Genome Conference. Meeting abstracts are archived on the World Wide Web at <http://www.intl-pag.org/agm>. A list of resources on the genomics of plant-associated microbes is shown in Table 1.

## PLANT PATHOGENIC BACTERIA

Christian Boucher (Institut National de la Recherche Agronomique–Centre National de la Recherche Scientifique, Castanet Tolosan, France) discussed the virtually complete genome sequence of *Ralstonia solanacearum*, a wide host range bacterial pathogen that causes the bacterial wilt disease in more than 200 plant species from more than 40 botanical families, which was obtained in collaboration with the French genomics center Genoscope. The selected strain, GMI1000, infects several solanaceous crops, such as potato, tomato, and eggplant, as well as the model plant *Arabidopsis thaliana*, and induces a hypersensitive response on tobacco. The *R. solanacearum* GMI1000 genome consists of two replicons, a

**Table 1.** Internet Resources on Genomics of Plant-Associated Microbes

Taxonomic Group/Microbe Species <sup>a</sup>	URL <sup>b</sup>
Bacteria; Proteobacteria: Alpha subdivision; Rhizobiaceae group	
<i>Sinorhizobium meliloti</i>	<a href="http://sequence.toulouse.inra.fr/meliloti.html">http://sequence.toulouse.inra.fr/meliloti.html</a>
<i>Bradyrhizobium japonicum</i>	<a href="http://www.genome.clemson.edu/~twood/projects/brady.html">http://www.genome.clemson.edu/~twood/projects/brady.html</a>
<i>Mesorhizobium loti</i>	<a href="http://www.kazusa.or.jp/rhizobase">http://www.kazusa.or.jp/rhizobase</a>
<i>Rhizobium</i> sp strain NGR234	<a href="http://genome.imb-jena.de/other/cfreiber/pNGR234a2.html">http://genome.imb-jena.de/other/cfreiber/pNGR234a2.html</a>
Beta subdivision; Ralstonia group	
<i>Ralstonia solanacearum</i> strain GMI1000	<a href="http://sequence.toulouse.inra.fr/R.solanacearum">http://sequence.toulouse.inra.fr/R.solanacearum</a>
Gamma subdivision; Xanthomonas group	
<i>Xanthomonas axonopodis</i> pv <i>citri</i>	<a href="http://genoma4.iq.usp.br/xanthomonas">http://genoma4.iq.usp.br/xanthomonas</a>
<i>Xylella fastidiosa</i> Citrus Variegated Chlorosis strain	<a href="http://onsona.lbi.ic.unicamp.br/xf">http://onsona.lbi.ic.unicamp.br/xf</a>
<i>X. fastidiosa</i> Pierce's disease Temecula strain	<a href="http://onsona.lbi.ic.unicamp.br/xf-grape">http://onsona.lbi.ic.unicamp.br/xf-grape</a>
<i>X. fastidiosa</i> almond/oleander strains	<a href="http://spider.jgi-psf.org/JGI_microbial/html">http://spider.jgi-psf.org/JGI_microbial/html</a>
Gamma subdivision; Pseudomonadaceae	
<i>Pseudomonas syringae</i> pv <i>tomato</i> DC3000	<a href="http://www.tigr.org/tdb/mdb">http://www.tigr.org/tdb/mdb</a>
Bacteria; Firmicutes: Bacillus/Staphylococcus group	
<i>Bacillus subtilis</i> strain 168	<a href="http://genolist.pasteur.fr/SubtilList">http://genolist.pasteur.fr/SubtilList</a>
Mollicutes	
<i>Spiroplasma</i> spp	<a href="http://www.oardc.ohio-state.edu/spiroplasma">http://www.oardc.ohio-state.edu/spiroplasma</a>
Eukaryota: Fungi; Ascomycota	
<i>Magnaporthe grisea</i>	<a href="http://www.cals.ncsu.edu/fungal_genomics">http://www.cals.ncsu.edu/fungal_genomics</a>
Stramenopiles; Oomycetes	
<i>Phytophthora</i> spp	<a href="http://www.ncgr.org/research/pgi">http://www.ncgr.org/research/pgi</a>

<sup>a</sup> Phylogenetic subdivisions follow NCBI Taxonomy Database (<http://www.ncbi.nlm.nih.gov/Taxonomy>).

<sup>b</sup> One representative URL is provided for each organism based essentially on information obtained at the conference.

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chromosome of 3.6 Mb that bears most of the housekeeping genes and a megaplasmid of 2.1 Mb that essentially contains genes encoding adaptive functions. *R. solanacearum* is known to develop a natural competence state during infection (Bertolla et al., 1999), and the genome can be expected to contain numerous sequences acquired through lateral gene transfer. Indeed, several regions, ranging in size from 1 to 30 kb, exhibit aberrant G + C contents and are associated with sequences similar to those of genetically mobile elements.

Boucher and collaborators also used a series of criteria, such as similarity to known virulence factors, linkage to candidate virulence genes, promoter motifs, and sequence motifs characteristic of eukaryotic proteins, to identify ~150 novel candidate pathogenicity genes. Some of these candidates encode homologs of type IV pili proteins, nonfibrillar adhesins, and hemolysin that may suggest previously unknown mechanisms of interaction between *R. solanacearum* and its hosts. Remarkably, nine genes, related to the previously characterized *popC* (Gueneron et al., 2000), showed significant similarity to the eukaryotic family of leucine-rich repeat plant disease resistance genes. In addition, ~50 genes harbored promoters with PIP box motifs, which are thought to play a role in the transcriptional activation of virulence genes (Fenselau and Bonas, 1995; Zhu et al., 2000). Knockout mutants of the candidate genes are now being generated. Mutagenesis experiments with one of the candidate virulence genes, *PopB*, indicated that this gene mediates avirulence of *R. solanacearum* GMI1000 in petunia and virulence in *Arabidopsis*.

The Gram-negative *Xanthomonas* species infect a variety of crop plants, including rice, cotton, citrus, grape, and bean. In the wake of the success of the *Xylella* genome project, a network of laboratories from São Paulo State, Bra-

zil, embarked on the genomic analysis of xanthomonads. Fernando Reinach (Universidade de São Paulo, Brazil) discussed the complete genome sequence of *Xanthomonas axonopodis* pv *citri* (formerly known as *Xanthomonas citri* and the cause of the economically important citrus canker disease) and the 8X coverage genome sequence of *Xanthomonas campestris* pv *campestris*, a well-studied pathogen of crucifers. *X. a. citri* harbors a 5,175,422-bp chromosome with a large number of mobile elements and ~300 scattered regions containing putative pathogenicity genes. Analyses of the 4266 open reading frames (ORFs) revealed a large number of genes encoding degradative enzymes that may function in parasitism. For example, 70 genes encoding different classes of extracellular proteases were identified. In addition to the chromosome, two interdependent low copy number plasmids, pXa33 (33 kb) and pXa65 (65 kb), also occur in *X. a. citri*. Each of these plasmids contains two copies of *pthA*, a multifunctional gene characterized by 102-bp tandem repeats and previously demonstrated to mediate host specificity, pathogenicity, and avirulence (Swarup et al., 1992). pXa65 also was found to contain a homolog of the avirulence gene *AvrPphE* from the bean pathogen *Pseudomonas syringae* pv *phaseolicola*.

Preliminary comparative genomic analyses of the two *Xanthomonas* species revealed a number of insertions and/or deletions and noncolinear regions, suggesting significant genome fluidity in xanthomonads. Notable differences also were observed between the *X. a. citri* genome and that of the phylogenetically related *Xylella fastidiosa*. In particular, the *X. a. citri* genome bears a larger number of genes involved in macromolecule degradation and has fewer integrated phage sequences. Clearly, these new resources should prove useful in understanding the variety of *Xanthomonas*-host interactions of economic consequence.

The xylem-limited plant pathogen *Xy. fastidiosa* consists of a complex group of strains with different plant hosts that are vectored by homopteran insects, particularly xylem-feeding sharpshooter leafhoppers (Cicadellinae). The mechanism of plant infection by *Xylella* is poorly understood. It is unclear whether *Xylella* simply clogs up the xylem tubes, subsequently affecting water transport and causing starvation, or if disease is caused by other means. The genome sequence of an *Xy. fastidiosa* strain that causes citrus variegated chlorosis (CVC), a devastating disease that affects ~30% of all citrus trees in Brazil, was reported last year (Simpson et al., 2000). *Xy. fastidiosa* strain CVC harbors a 2,679,305-bp chromosome with 2782 ORFs. Analyses of the genome sequence revealed a number of candidate genes that may function in virulence. For example, several genes were identified that encode extracellular polysaccharide synthesis enzymes, such as fimbriae, afimbrial adhesins, and hemagglutinins, which may function in clumping of bacteria and adhesion to xylem tubes and sharpshooter leafhopper foreguts.

Another important disease caused by *Xy. fastidiosa* is Pierce's disease (PD) of grapevine, an emerging problem that is threatening the wine industry on the West Coast of the United States (Purcell and Saunders, 1999). Even though *Xy. fastidiosa* strains that cause PD have been known in California for many years, the disease has turned epidemic since the introduction of the glassy-winged sharpshooter (*Homalodisca coagulata*), which is capable of faster and longer distance movement into vineyards than other sharpshooters, reaches higher population numbers, and actively feeds on dormant grapevines in winter, resulting in chronic *Xylella* infections (see <http://www.cnr.berkeley.edu/xylella>). Marie-Anne van Sluys (Universidade de São Paulo, Brazil) presented an advanced sequence draft of the genome of an *Xy. fastidiosa* PD strain isolated

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recently from the Temecula wine-producing region of southern California. Similar to the *Xy. fastidiosa* CVC strain, isolate Temecula contains numerous phage-derived sequences and bears no avirulence-like genes or bacterial type III secretion system genes. However, one genomic region that contains putative virulence and hemagglutinin genes in the CVC strain appears deleted in the Temecula isolate.

Additional genomics resources for *Xylella* are available as well. Dan Drell (United States Department of Energy, Germantown, MD) discussed the first microbial sequencing month held in October 2000 by the Department of Energy's Joint Genome Institute (JGI). This initiative resulted in 8X drafts of the genome sequences of 15 microbes, including two *Xy. fastidiosa* strains isolated from almond and oleander ([http://www.jgi.doe.gov/tempweb/JGI\\_microbial/html](http://www.jgi.doe.gov/tempweb/JGI_microbial/html)). Future comparative genomic analyses of *Xylella* should reveal genes involved in host specificity and pathogenesis.

Understanding the molecular basis of *Xy. fastidiosa* pathogenesis and interaction with its insect vectors has been limited by the lack of genetic tools. In a poster presentation, Magalie Guilhabert (University of California, Davis) described progress in generating transposon insertions in two *Xy. fastidiosa* PD strains after electroporation of a hyperactive Tn5 transposase-transposon complex (transposome system; Epicenter Technologies, Madison, WI). Guilhabert detected *Xy. fastidiosa* transformants with single, independent, and stable insertion events and will use them in phenotypic analyses.

A poster presentation by Alan Collmer (Cornell University, Ithaca, NY) and collaborators introduced the objectives of a multi-institutional sequencing effort of the genome of the tomato pathogen *Pseudomonas syringae* pv *tomato* strain DC3000. This project, recently funded by the National Science Foundation Plant Genome Research Pro-

gram, will also include comparative genomic analyses of pseudomonads as well as functional analyses and transcriptional profiling of *P. syringae* interaction with tomato.

Kenneth Bell (Scottish Crop Research Institute, Dundee, Scotland) presented a poster on the sequence of two bacterial artificial chromosomes (BACs) of *Erwinia carotovora* subsp *atroseptica*, an important pathogen that causes soft rot on potato. The sequence revealed many potential pathogenicity genes, such as an entire *hrp* gene cluster, pectinolytic genes, homologs of the *Erwinia* virulence genes *dspE*, *hacAB*, and *pecSM*, homologs of *Xylella* hemolysin genes, and homologs of *Agrobacterium tumefaciens* pathogenicity genes. These data suggest that *E. c. atroseptica* pathogenesis may be more complex than previously thought.

Members of the genera *Spiroplasma* and *Phytoplasma* (class Mollicutes) have been associated with more than 200 plant diseases worldwide and are dispersed by homopteran insects. *Spiroplasmas* and *phytoplasmas* are restricted to the phloem tissue of plant hosts and, interestingly, replicate in their insect vectors as well. Members of the class Mollicutes arose from a Gram-positive Clostridium-like ancestor and are characterized by a lack of cell wall, small genome size (530 to 2200 kb), and low G + C content. The genome sequences of several human and animal pathogenic mollicutes have been determined, including that of *Mycoplasma genitalium*, which at 580,070 bp represents one of the smallest genomes of a cellular organism (Fraser et al., 1995). To date, the complete genome sequence of a plant pathogenic mollicute has not been determined. A poster presentation by Saskia Hogenhout (Ohio State University, Wooster) summarized the results of sample genome sequencing of one of the three plant pathogenic *spiroplasmas*, the corn stunt *spiroplasma* (CSS; *Spiroplasma kunkelii*). *Spiroplasmas* are primarily in-

sect pathogens, but they also include epiphytes such as *Spiroplasma floricola* and three plant pathogens vectored by phloem-feeding leafhoppers. Comparative genomic analyses of *spiroplasmas* and *mycoplasmas* revealed that the 1600-kb CSS genome harbors a surprisingly large number of virus-derived sequences. Moreover, the CSS genome clearly bears more genes commonly found in genomes of Gram-positive bacteria, confirming earlier findings that *spiroplasmas* did not experience extensive gene loss, as did most members of the mollicute class. Genes predicted to confer the spiral-shaped cell structure unique to *spiroplasmas* were identified as well.

Whereas *spiroplasmas* can be cultured, *phytoplasmas* cannot grow outside their insect or plant hosts. Despite the difficulty of obtaining sufficiently pure *phytoplasma* DNA, Lia Liefertink and Bruce Kirkpatrick (University of California, Davis) presented a poster on the successful cosmid cloning of a large proportion of the 670-kb genome of Western X disease *phytoplasma*, a significant pathogen of peach and cherry trees in California. This library will provide the basis for obtaining the first large sequence data set for a *phytoplasma* genome, which in turn will aid other *phytoplasma* genome sequencing initiatives. Genome comparisons among epiphytic, insect, and plant pathogenic *spiroplasma* species, as well as between *spiroplasmas* and *phytoplasmas*, will reveal genes involved in plant pathogenesis and insect transmission of bacteria from Gram-positive ancestry.

## BENEFICIAL BACTERIA

Plant-associated bacteria are not always detrimental; they also can have beneficial effects on plants, for example, by enhancing nutrient uptake and plant growth. The alpha bacterium *Si-*

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*norhizobium meliloti* (formerly known as *Rhizobium meliloti*) has taken such a role to a high level of sophistication. *S. meliloti* can establish intimate symbiotic interactions with legume plants, inducing the formation of root nodules and allowing nitrogen fixation and uptake by the plant (Viprey et al., 2000). Even though *S. meliloti* has been studied intensively at the genetic level, Sharon Long (Stanford University) illustrated how the complete genome sequence revealed unexpected aspects of the biology of this bacterium. *S. meliloti* strain 1021 carries a tripartite genome consisting of a 3.65-Mb chromosome and two plasmids, pSymA (1.35 Mb) and pSymB (1.68 Mb), known as the symbiotic plasmids because they carry genes required for symbiosis. A team of European and North American scientists completed the sequence of the three replicons, and an annotation jamboree was held in Toulouse, France, in September 2000. Analyses of the relation among the three replicons suggested that the pSym plasmids form an integral part of the *S. meliloti* genome. For example, pSymB, which is known to be required for growth, turned out to carry a *tRNA-Arg* gene that is missing in the chromosome. pSymA carries numerous genes with predicted functions as diverse as denitrification, nitrate respiration, and opine catabolism. The overall analyses suggest that the pSym plasmids encode multiple functions and contribute not only to the establishment of symbiosis but also to survival in the soil and other stages of the life cycle of *S. meliloti*.

Long also discussed how the complete genome sequence of *S. meliloti* was compared with sequences from other rhizobacteria, such as *Bradyrhizobium japonicum*, *Mesorhizobium loti*, and *Rhizobium* sp strain NGR234. Unlike *Bra. japonicum* and *Mes. loti*, symbiosis genes in *S. meliloti* are dispersed and not clustered in gene islands. Several symbiosis and nitrogen

fixation genes that have not been identified previously by classic genetic screens were revealed by similarity searches. For example, pSymA contains orthologs of the genes *nodV* and *nodW*, which encode a two-component regulatory system that positively regulates *nod* gene expression in *Bra. japonicum* in response to plant isoflavone signals (Loh et al., 1997).

In *Rhizobium* sp NGR234, a gene cluster encoding the components of a bacterial type III secretion system was identified recently, and mutant analyses of two of these genes showed that they are required for normal nodule induction on several legume plants (Viprey et al., 1998). Interestingly, genes for the type III secretion system were absent in *S. meliloti*, indicating that perhaps a different secretion mechanism is used in this species. Indeed, the overall message from the early comparative genomic analyses of rhizobacteria is that each species has developed unique solutions and adaptations to the symbiotic lifestyle.

The family of plant-beneficial bacteria includes an illustrious member, the spore-producing *Bacillus subtilis*, which is the best studied Gram-positive bacterium and is a classic model organism for the study of developmental processes in prokaryotes. A little known fact about *B. subtilis* is that it leads a normal life outside the laboratory in soil habitats, where it is known to attach to roots and enhance plant growth. Indeed, strains closely related to *B. subtilis* are established members of the so-called plant growth-promoting rhizobacteria (Andrews and Harris, 2000), and dozens of commercial formulations of these bacteria are available to farmers. Colin Harwood (University of Newcastle upon Tyne) discussed how his research team is taking *B. subtilis* back to its roots. It appears that *B. subtilis* strain 168, the classic laboratory strain that was used in the 1997 genome sequencing project (Kunst et al., 1997), exhibits biocontrol and plant growth enhancement in to-

mato, possibly through the production of fungicides and plant hormones and/or the relocation of metal ions and phosphates. Analyses of the more than 4000 ORFs of *B. subtilis* revealed a number of candidate genes that could modulate plant growth promotion. For example, a number of *B. subtilis* genes, such as *yqkF*, are related to auxin-regulated genes from tobacco and Arabidopsis and may manipulate hormonal processes in plants. Other genes show similarity to chitinases or are predicted to mineralize phosphates and may help *B. subtilis* compete with fungi and other microbes in the rhizosphere niche. Harwood and collaborators have initiated high throughput mutagenesis experiments with the objective of creating knockout mutants for all *B. subtilis* genes. Mutants in the identified candidate genes will then be tested for alteration of plant growth promotion and other phenotypes. In addition, Harwood's team has initiated signature-tagged mutagenesis experiments (Hensel et al., 1995) using a *B. subtilis* strain marked with the green fluorescent protein to identify mutants affected in various test environments, such as the rhizosphere. Recovery and identification of these mutants is expected to be accelerated significantly by the availability of the complete genome sequence.

## PLANT PATHOGENIC FUNGI

Diseases caused by fungi are well established as major constraints to food and crop production. Rice blast, caused by the ascomycete fungus *Magnaporthe grisea*, is one of the most devastating diseases of crop plants. In addition to its economic importance, *M. grisea*, a relative of the model fungus *Neurospora crassa*, has emerged as a model experimental system for the genetic analysis of plant pathogenic fungi. Driven by a dynamic community

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of researchers, a number of tools for classic and molecular genetic manipulation have been developed for *M. grisea*. Ralph Dean (North Carolina State University) discussed the goals of the recently funded USDA-Initiative for Future Agriculture and Food Systems (IFAFS) project that officially launches the rice blast genome project. *M. grisea* has a 40-Mb genome divided into seven chromosomes. Dean plans to generate a 5X draft sequence for chromosome 7, which at 4.2 Mb is the smallest chromosome of *M. grisea*. A BAC-by-BAC approach will be taken, based on the availability of a complete physical map of chromosome 7 (Zhu et al., 1999). In addition, Daniel Ebbole (Texas A&M University), a co-principal investigator in the IFAFS project, will generate 35,000 expressed sequence tags (ESTs) from several in vitro stages to develop a unigene set for the rice blast pathogen and help design gene prediction algorithms for analysis of the genomic sequence. This project should prove an important stepping-stone for the completion of the entire genome sequence of *M. grisea*.

### PLANT PATHOGENIC OOMYCETES

Oomycetes, such as Phytophthora, downy-mildews, and Pythium, form a unique branch of eukaryotic plant pathogens with an independent evolutionary history (Kamoun et al., 1999b). Among the oomycetes, Phytophthora species cause some of the most destructive plant diseases in the world, and are arguably the most devastating pathogens of dicot plants (Erwin and Ribeiro, 1996). For example, *Phytophthora infestans*, the Irish potato famine fungus, causes late blight, a disease that results in multibillion dollar losses in potato and tomato production, and *Phytophthora sojae* causes root and stem rot of soybean, a disease that can result in up to 12% losses in crop

yields. Brett Tyler (University of California, Davis) introduced another recently funded USDA-IFAFS project that significantly extends the efforts of a community-based initiative known as the Phytophthora Genome Initiative ([www.ncgr.org/research/pgi/whitepaper.html](http://www.ncgr.org/research/pgi/whitepaper.html)). Building on the successful completion of pilot cDNA sequencing projects (Kamoun et al., 1999a; Qutob et al., 2000), Tyler and collaborators plan to generate an additional 50,000 ESTs from a series of in vitro and infection stages of *P. infestans* and *P. sojae*. These sequences will be compiled in an expanded and improved version of the Phytophthora Genome Initiative (PGI) database (named the Phytophthora Genome Consortium or PGC), which is expected to be released during 2001.

In addition to cDNA sequencing, coordinated efforts in the genomic sequencing of Phytophthora are emerging. Poster presentations by Stephen Whisson (Scottish Crop Research Institute) and collaborators and by members of the Tyler laboratory described progress in the physical mapping of both the *P. infestans* and *P. sojae* genomes. BAC contigs spanning avirulence gene clusters were described as prime candidates for targeted genomic sequencing.

With the accumulation of sequence data for Phytophthora, the challenge is shifting to functional analyses. Sophien Kamoun (Ohio State University, Wooster) described the use of potato virus X (PVX)-mediated gene expression to carry out functional screens of *P. infestans* genes in plants. The transient nature of the PVX expression system and the use of a recently developed Agrobacterium binary PVX vector (Baulcombe, 1999; Takken et al., 2000) allowed functional analyses of pathogen genes at an unprecedented high throughput rate. By combining EST data mining with PVX-based functional screens, Kamoun and collaborators identified a battery of novel Phytophthora genes that trigger hypersensitive-like necrosis in Nicotiana and tomato.

### LESSONS FROM OTHER GENOME PROJECTS

Several talks illustrated the impact of genome-enabled research on the understanding of microbial physiology and evolution. Claire Fraser (The Institute for Genomic Research, Rockville, MD) provided an overview and update of more than 30 microbial genome sequences. In prokaryotes, genomic analyses revealed the tremendous impact of lateral gene transfer on genome evolution. For example, there is evidence for massive gene exchange between bacterial and archaeal thermophiles (Aravind et al., 1998; Nelson et al., 1999). One can expect that with the accumulation of sequence data, the theme of lateral gene transfer among plant-associated microbes and their hosts will become prevalent in the coming years.

Fraser also discussed how the genome sequence of the bacterial pathogen *Neisseria meningitidis*, the cause of human meningitis, was used to identify promising vaccine candidates. This variable pathogen occurs as five pathogenic serogroups, and vaccines are not available against some of the serogroups, such as serogroup B. In a major study published last year, Pizza and co-workers (2000) systematically screened the genome sequence of a virulent strain of *N. meningitidis* serogroup B for surface-associated proteins that are conserved in other *N. meningitidis* strains and in the closely related pathogen *Neisseria gonorrhoeae*. They successfully expressed 350 of the identified ORFs in *Escherichia coli* and used the recombinant proteins to immunize mice. Of these, seven proteins evoked effective antibacterial antibody responses and emerged as prime candidate vaccine antigens. This study clearly illustrates how combining sequence mining with high throughput functional assays can accelerate the identification of genes with desirable characteristics.

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Lively discussions centered on the value of partially versus fully sequenced genomes. Fraser and others warned that even with multiple genome coverage, incomplete genome sequences might lack genes that are meaningful to the full understanding of the biology of the examined organism. Nonetheless, incomplete genomes can be useful. For example, mining for surface proteins of *N. meningitidis* was conducted while the sequencing project was still in progress (Pizza et al., 2000). Stanley Malow (University of Illinois, Urbana-Champaign) gave a spirited illustration of how he is using sample sequencing, at 3X to 4X genome coverage, of *Salmonella* serovars adapted to different animal hosts to gain insight into the genetic basis of host specificity. Differences in host specificity between these serovars are expected to involve multiple loci and do not include characterized pathogenicity gene islands or other known virulence genes. Preliminary analyses of two *Salmonella* serovars identified ~60 polymorphic insertions and/or deletions that are candidates for functional assays. It will be interesting to see how sample sequencing will help unravel this complex problem and to determine whether similar approaches can be applied to plant-associated microbes that also show a great level of diversity in host specificity.

## FUTURE DIRECTIONS

The exponential accumulation of sequence data creates a formidable challenge for the community of plant microbiologists to acquire new skills and expertise in handling and mining large data sets. For example, excellent opportunities exist in mining the sequence data generated through the JGI Microbe Month program for candidate genes relevant to fundamental questions on the association of microbes with plants. It is hoped that several plant microbe research groups will collabo-

rate with bioinformaticists or computer science students to take advantage of the available sequence information.

Some of the sequenced plant microbes interact with model plant species with completed or ongoing genome projects. For example, both *X. c. campestris* and *R. solanacearum* infect the model dicot *Arabidopsis*, and *S. meliloti* can form symbiotic nodules on the model legume plant *Medicago truncatula*. In the coming years, the integration of microbe and plant genomic data should revolutionize the study of plant-microbe interactions. Of particular interest is the determination of interaction transcriptomes, the sum of transcripts that are produced by the host and the microbe during their association (Birch and Kamoun, 2000). High throughput methods for profiling interaction transcriptomes will allow a comprehensive and coordinated study of gene expression during plant-microbe interactions.

As stated in closing comments by the co-chair of the organizing committee, Peter Johnson, it seems unlikely that the Agricultural Microbes Genome Conference will continue in its current format. Future challenges will be to maintain an identity for the agricultural microbes genome community while coordinating with other microbial genomics gatherings, such as The American Society for Microbiology and The Institute for Genomic Research Conference on Microbial Genomes that was held this year in Monterey, California, from January 29 to 31, 2001. Whatever the future of the Agricultural Microbes Genome Conference will be, the plant microbe community has now fully embraced the era of genomics.

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## Medicago truncatula on the Move!

Plant biology is moving rapidly. With the recent completion of the sequence of the Arabidopsis genome (The Arabidopsis Genome Initiative, 2000), researchers have turned their attention to the genomes of other crop plants (Adam, 2000). Many agronomically important crop plants are legumes, such as soybean, pea, and alfalfa. However, the size and complexity of these genomes makes them unwieldy and has slowed progress on the genetic characterization of these crops. *Medicago truncatula* recently emerged as a model plant for legume genetics and genomics (Cook, 1999).

The small diploid genome, autogamous genetics, and ease of transformation make this close relative of alfalfa (*Medicago sativa*) a good model system. The recent development of genetic and genomic tools for *M. truncatula* research has propelled *M. truncatula* into the forefront of legume research as an ideal legume model. Advances in *M. truncatula* genomics were the subject of a recent workshop preceding the 9th Annual Plant and Animal Genome Meeting in San Diego, California, on January 11 and 12.

The international scope of this workshop underscored the interest in *M. trun-*

*catula* as a model system for studying plant–microbe interactions, in particular rhizobial and mycorrhizal symbioses. More recently, interest has turned to *M. truncatula* as a system for examining the very rich production of secondary metabolites by legumes and legume-specific disease resistance (Cook et al., 2000; Harrison, 2000). This workshop, which started out three years ago as a small band of National Science Foundation (NSF) genome grant collaborators sponsored by the NSF Plant Genome Project (award number 9,872,664), has grown into an international effort spanning national boundaries and legume species.