

Meeting report

Post-genomic *Pseudomonas*

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A report on the *Pseudomonas* 2001 Meeting, Brussels, Belgium, 17-21 September 2001.

The genus *Pseudomonas* is renowned for its ecological diversity, its pathogenic potential in a range of hosts (insects, nematodes, plants and humans) and its potential for use in degrading environmental contaminants (bioremediation) and preventing infections of important food crops (biocontrol). With the release of the *Pseudomonas aeruginosa* genome one year ago and the near-completion of the genome sequences of *Pseudomonas putida* (reported on at this meeting by Burkhard Tümmler, Medizinische Hochschule Hannover, Germany), *Pseudomonas fluorescens* and *Pseudomonas syringae*, the challenge to the community of *Pseudomonas* researchers is to determine the function of as-yet-uncharacterized open reading frames (ORFs; these make up 46% of *P. aeruginosa* ORFs). It was evident from the meeting that the tools and techniques required for post-genomic studies have been developed and are now being implemented. There were excellent presentations on a wide range of areas including genomics, post-genomics, quorum sensing (the mechanism by which bacteria detect population density), bacteria-host interactions, protein secretion - all of which we discuss in more detail below - as well as biotechnological applications and biodiversity. (Unfortunately, because of the events of September 11th many of the US speakers and registered participants did not attend.) In this report, we focus on reports of post-genomic studies as well as the highlights of research that arose from the availability of the *P. aeruginosa* genome sequence.

Transposon mutagenesis

Transposon-insertion libraries are an essential post-genomic tool for determining the function of unknown genes as they allow rapid mutagenesis and gene identification. A poster

from the lab of Frederick Ausubel (Harvard Medical School, Boston, USA) and a talk from Laurence Rahme (at the same school) described approaches to identifying *P. aeruginosa* genes that are necessary for pathogenesis in a number of hosts. Transposon mutants were constructed and tested for virulence in high-throughput insect, plant and nematode models of infection. The Ausubel lab intends to identify each transposon insertion site and ultimately produce a non-redundant library of approximately 3,000-5,000 *P. aeruginosa* mutants that will be made publicly available.

Signature-tagged mutagenesis (STM) is an alternative mutagenesis method that is based on the negative selection of unique oligonucleotide-tagged transposon mutants so as to identify genes that are essential during infection *in vivo*. STM was adapted for use in *P. aeruginosa* and described in posters presented by Roger Levesque (Université Laval, Sainte-Foy, Canada) and by Tümmler and colleagues. The main modifications of STM for use in *P. aeruginosa* include the number of unique tags used; the choice of antibiotic resistance cassette and promoterless gene reporter present within the transposon; hybridization or PCR-based methods for the detection of tags present in pools of genomic DNA *in vitro* and *in vivo*; and the model of infection used *in vivo*. Levesque's group used STM technology to identify genes essential for survival in a rat chronic lung infection model, whereas Tümmler's group was interested in identifying genes essential for survival of a particularly virulent *P. aeruginosa* strain that invades polymorphonuclear granulocytes. These studies represented technical advances for characterizing novel *P. aeruginosa* virulence genes and are en route to providing important insights.

Microarrays and proteomic approaches

DNA microarrays provide the opportunity to study the global expression and regulation of the whole genome in a single experiment. The Affymetrix GeneChip® *P. aeruginosa* Genome Arrays have recently been made available through

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the US Cystic Fibrosis Foundation Therapeutics, Inc. (CFFTI, University of North Carolina, Chapel Hill, USA). One of us (R. H.) reported on the results of microarray experiments using the Affymetrix GeneChip® technology. Nearly 500 *P. aeruginosa* genes were found to be either up- or down-regulated by at least fourfold when the bacterium was starved of Mg²⁺. This Mg²⁺-dependent regulation acts in part through the two-component regulatory system PhoPQ to influence virulence and survival of *P. aeruginosa* in the presence of polycations, including polymyxin B, aminoglycosides and cationic antimicrobial peptides.

A number of presentations reported on proteomic approaches to characterizing expressed proteins under specific conditions or within a specific subcellular localization. The proteomic method used involved the separation of proteins using two-dimensional gel electrophoresis followed by trypsin digestion of isolated proteins and peptide-mass characterization via matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy. Genome sequence data are a requirement for proteomic studies because the identification of proteins on the basis of peptide mass is dependent on the construction of theoretical two-dimensional mass maps. Stuart Cordwell (Macquarie University, Sydney, Australia) described studies in which his laboratory successfully used such an approach to characterize the *P. aeruginosa* membrane subproteome, showing that it consists of approximately 200 protein spots and includes porins and other outer membrane proteins. He has compared the extracellular subproteomes of invasive and cytotoxic strains, revealing dramatic differences in the type and number of virulence proteins secreted, and has also determined that mutations in the LasIR and RhlIR quorum-sensing systems result in a tenfold reduction in total secreted proteins. The LasIR system was reported to control the production of a novel putative virulence factor, an aminopeptidase. This technique allowed reproducible comparisons of two-dimensional gel patterns and revealed a significant degree of post-translational modification among secreted proteins, but it is limited by its inability to identify hydrophobic proteins with many transmembrane domains.

Biofilm formation and quorum sensing

The ability to grow in a biofilm adhering to a surface is now recognized as an important property of *P. aeruginosa* in chronic lung infections in people with cystic fibrosis; the biofilm lifestyle is also adopted by several pseudomonads in other environments. Several talks and posters addressed the physiology and other levels of regulation involved in biofilm development. For example, a poster from the lab of Volker Brozel (University of Pretoria, South Africa) reported the use of two-dimensional gel electrophoresis to compare the proteomes of *P. aeruginosa* cells growing as biofilms while adherent to glass wool to those of non-adherent planktonic cells. Dramatic differences in the protein patterns of planktonic and

biofilm cells were observed, and studies to identify and determine the role of proteins induced or repressed during biofilm formation are ongoing.

A common theme in the meeting was the characterization of novel genes, which can be identified through bioinformatic analyses or large-scale screens of transposon insertion libraries. For example, Alain Filloux (Université de Rouen, France) identified a novel fimbrial adhesin gene cluster, independent of type IV pili, that is involved in bacterial cell-surface interactions that take place in the early stages of biofilm formation. A novel type II secretion pathway, which is mediated by homologs of the general secretory pathway genes *xcp*, was also identified and shown to play a role in the secretion of proteins under phosphate starvation conditions.

In the area of gene regulation, Dieter Haas (Université de Lausanne, Switzerland) described the importance of a unique post-transcriptional regulatory system based on a regulatory RNA, termed RsmZ, that sequesters the RNA decay factor RsmA away from target mRNA. In the absence of RsmA binding, the target mRNA has an increased stability. The global two-component signaling system GacAS is involved in regulating this post-transcriptional regulatory system. Although this work studied the regulation of the cyanide biosynthetic operon in *P. fluorescens*, there was evidence that a similar system operates in *P. aeruginosa*. Morten Hentzer (Technical University of Denmark, Lyngby, Denmark) demonstrated that halogenated furanone compounds derived from the marine macroalgae *Delisea pulchra* were capable of inhibiting quorum-sensing biosensors and thereby attenuating *P. aeruginosa* virulence in a chronic lung infection model. Paul Williams (University of Nottingham, UK) reported on the pathogenic role of the *P. aeruginosa* quorum-sensing signals *N*-(3-oxododecanoyl)-L-homoserine lactone (C₁₂-oxo-HSL) and *N*-butyryl-L-homoserine lactone (C₄-HSL). According to their model, the two signals are produced at different times throughout growth, and each signal has a unique role in establishing an infection. C₁₂-HSL is produced early in stationary phase and is thought to play a role in establishing a host niche by eliminating competitor bacteria such as *Staphylococcus aureus*; C₄-HSL is produced later in stationary phase, concomitantly with several known *P. aeruginosa* virulence factors.

The *P. aeruginosa* genome, at 6.3 Mb, is one of the largest sequenced bacterial genomes. The availability of sequence data is proving to be a tremendous benefit to the community of *Pseudomonas* researchers by providing insights into the pathogenic and environmental diversity of this organism. The post-genomic era is upon us, and as other *Pseudomonas* genome projects come to completion, these types of studies can be extended to the whole *Pseudomonas* genus. We can expect to see the benefits for human health and for basic science in the coming decade.