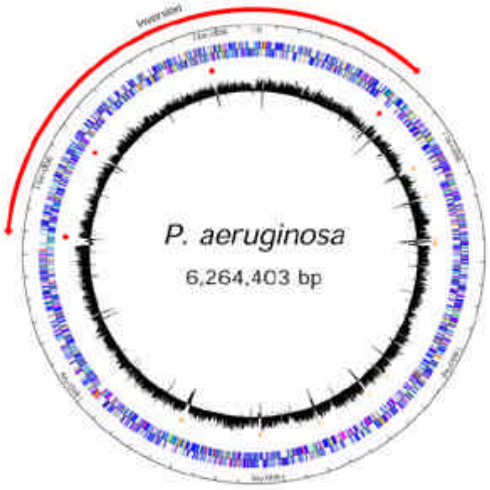
Functional Genomics of *Pseudomonas aeruginosa*

Pseudomonas aeruginosa is an important bacterial pathogen in Western society; it is the third leading cause of hospital-associated (nosocomial) infections, causing many deaths in hospitalized patients, and is the leading cause of eventually fatal lung infections in patients with the genetic disease, cystic fibrosis. It is difficult to treat infections caused by this pathogen largely because antibiotics can be ineffective. For more than two decades we have been involved in a broad range of projects on this organism, in particular with respect to its outer membrane permeability, intrinsic resistance to antibiotics and outer membrane proteins. However since the 2001 the sequencing of the *Pseudomonas* genome we have dramatically changed our approach to studying this organism. Thus we now utilize a series of functional genomic approaches.

Genomics

We were associated with the original genome sequencing effort, in particular the annotation of the *Pseudomonas aeruginosa* PAO1 reference genome. In particular we were involved in coordinating a *Pseudomonas aeruginosa* research community annotation project called PseudoCAP. This is described elsewhere at http://cmdr.ubc.ca/bobh/PAAP.htm. The lab is also, with the assistance of Fiona Brinkman at SFU, hosting the *Pseudomonas aeruginosa* web page. Since the genomics publication we were involved in establishing a series of tools to permit post genomic studies. These included the establishment of microarrays to probe transcription of all genes in P. aeruginosa, the development of a compendium of the secreted proteins of this organism through 'PhoA fusion technology, and development of a mutant library using lux-fusion technology.



Outer membrane proteins

We originally demonstrated that low outer membrane permeability due to the poor functioning as a pore of porin OprF, accounted substantially for generalized antibiotic resistance in *P. aeruginosa*. A combination of biophysical-biochemical, molecular genetic, structural, bioinformatic and genomic approaches have been utilized to study the structure:function relationships of major porin and adhesin OprF, the phosphate-selective porin OprP, the PhoPQ-regulated protein OprH, the 18-member OprD family of substrate-selective porins, and the 19-member family of homologs of the efflux porin OprM. Certain of these porins also have a role in antibiotic uptake/efflux, e.g. OprD in specific imipenem uptake/resistance, OprF in general passage of antibiotics, and OprM together with its cognate linker & pump components MexAB in intrinsic resistance to antibiotics. As part of our contribution to the Pseudomonas Genome Project, we have set up a reference web site listing known and putative *P. aeruginosa* outer membrane proteins.

Analysis of the recently-published *P. aeruginosa* genome sequence indicated several large families of proteins most of which are paralogs of genes which we have studied intensively, e.g. the beta-lactam imipenem-selective porin OprD [19 proteins], the multidrug-efflux outer membrane protein OprM [18 proteins], the iron-regulated outer membrane proteins [32 proteins], and the ECF sigma factor family [21 proteins] homologous to SigX which regulates expression of the major outer membrane porin OprF.

More recently we defined the functions of many members of the family of porins related to OprD. The substrate specificities of 7 OprD homologues were inferred using studies of the growth specificities of mutants, the regulation of encoding genes and the genomic context. The specificities determined were as follows: OpdK – vanillate, OpdP - glycine-glutamate, OpdC - histidine, OpdB - proline, OpdT - tyrosine, OpdH - *cis*-aconitate, and OpdO - pyroglutamate.

Virulence

Access to a broad series of functional genomic tools has permitted us to perform detailed studies on certain aspects of virulence. In addition to our studies of two component regulators that are described in more detail under “Two Component Regulators of Resistance”, we have been particularly interested in motility (especially swarming motility) and adherence.