American Society for Microbiology: Pseudomonas 2007

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The Pseudomonas 2007 Conference, organised by the American Society for Microbiology, C.S. Harwood, R. Parales, J. Loper and S. Lory, took place in Seattle (Washington, USA) from 26–30 August 2007. This conference is held every 2 years and focuses on the Pseudomonads. It was attended by some 300 participants from all regions of the world. The proceedings were divided into 7 presentation sessions concerning the following topics: signal transduction and gene regulation, cell to cell communication, secondary metabolism, cell surfaces, pseudomonas physiological and metabolic versatility, type II and type III secretion and transport; and 2 poster sessions grouping the previous topic areas and including two extra topics, plant associated pseudomonads and *Pseudomonas aeruginosa* infections and treatment options.

The meeting started on the Sunday evening with an inaugural lecture on "*Pseudomonas aeruginosa* in the real world" by M. Olson (USA), a presentation providing an overview of *Pseudomonas aeruginosa* and its biodiversity. This gave rise to the following question: are we really seeing what is going on in the real world in using our laboratory tools? It was emphasised that *Pseudomonas* research should join forces with cancer research, since these two fields share a close conceptual relationship. At the end of his presentation, M. Olson encouraged young researchers to pursue challenging applied research in academia.

On the Monday, during the signal transduction and gene regulation session, we heard a number of outstanding presentations. One was by U. Blaesi (Austria) on the use of RNomics to find new regulatory non-coding small RNAs in *Pseudomonas*, and how to find their targets by the use of bioinformatics, transcriptomics, proteomics and microarray analysis. S. Dove (USA) presented a study on *cupA* fibrial gene expression regulators and the role of anaerobiosis on *cupA* activation. D. Haas (Switzerland) presented his group's advances in the study of post-transcriptional regulation in *Pseudomonas* mediated via the Hfq pathway and the Rsm/Csr pathway. Speaking on regulation of the expression of virulence genes, S. Lory (USA) showed that the *retS* gene, a pleiotropic regulator of gene expression, is required for acute and chronic infection. He demonstrated that the second messenger cyclic-di-GMP is involved in biofilm formation. By the use of high-throughput pyrosequencing technology, M. J. Filiatrault (USA) analysed the transcriptome of *Pseudomonas syringae* pv *tomato* strain DC3000 and showed that this technique represents an efficient approach to transcript mapping in bacteria. G. Rampioni (Italy) reported his discovery that the RsaL protein is a global regulator of *P. aeruginosa* physiology and virulence. N. Boes (Germany) identified and characterised 5 universal stress proteins in *P. aeruginosa* produced in response to oxygen limitation. In the afternoon followed talks on *Pseudomonas* and its hosts. L. Rahme (USA) presented his studies on *Drosophila melanogaster* infection by *P. aeruginosa* and the role of skeletal muscle in this infection. D. Hogan (USA) described the novel regulator GbdR, which controls *P. aeruginosa* biofilm formation and subsequently the interaction of the bacterium with the fungus *Candida albicans*. B. Tummler (Germany) presented his work on the genotyping-phenotyping of multiple *P. aeruginosa* strains, showing that *P. aeroginosa* is a ubiquitous organism, that CF (cystic fibrosis) strains adapt in CF lungs and that the course of chronic infection is dependent on the host genetic background. B. N. Kunkel (USA) studied the role of coronatine, a phytotoxin, in *P. syringae* pathogenesis and its functional analogy with jasmonic acid (a plant hormone). I. Valley-Gely (France) reported his discovery of a new entomopathogen *P. entomophila*, and studies on its multifactorial virulence using Drosophila as a model. A. E. Clatworthy (USA) demonstrated the use of zebrafish embryos as a new model for study of *P. aeruginosa* pathogenicity and identification of potential therapeutics. R. C. Levesque (Canada) compared 4 *P. aeruginosa* strains: PA14, PAO1, PAK and LESB58 and showed that the hypervirulent CF strain LESB58 produces higher amounts of biofilm compared with other strains and presents mobility defects.

The rest of the afternoon was devoted to the poster session, where I presented a poster entitled "Post-transcriptional regulation of the *hcnABC* genes by the Gac/Rsm system in *Pseudomonas fluorescens* CHA0". *Pseudomonas fluorescens* CHA0 is a rhizosphere bacterium that protects plant roots against the action of pathogenic fungi by synthesising diverse extracellular products such as antibiotics, exoproteases and hydrogen cyanide (HCN) at the end of exponential growth. The biosynthesis of these compounds is strictly regulated by the two-component signal transduction system GacS/GacA. The hydrogen cyanide biosynthetic genes *hcnABC* are indirectly regulated via GacA. At low cell density, expression of the *hcnABC* genes is repressed at a post-transcriptional level by two RNA binding proteins, RsmA and RsmE. When the cellular density increases, the GacS/GacA system is activated and induces the expression of small non-coding regulatory RNAs (RsmX, RsmY, RsmZ) which sequester RsmA and RsmE, thereby allowing translation of HCN synthase, thus resulting in HCN production. Previous studies have shown that point mutations at or near the ribosome binding site (RBS) of *hcnA* mRNA could diminish or abolish GacA and RsmA control, suggesting that RsmA as well as RsmE may bind to the *hcnABC* RBS region (Blumer C., Heeb S., Pessi G. and Haas D. 1999, Proc. Natl. Acad. Sci. USA 96: 14073-14078). We have conducted a systematic analysis of the untranslated *hcnA* leader to identify sequence elements and secondary structures which participate in RsmA and/or RsmE interaction. Lead(II) cleavage analysis allowed us to identify two stem-loop structures in the *hcnA* leader mRNA which contained potential RsmA/

RsmE binding sites. Moreover, footprinting studies identified two RsmE binding sites, one of which overlaps with the *hcnA* RBS. Mutations were introduced into the *hcnA* leader sequence which altered five potential RsmA/RsmE binding sites. β-galactosidase assays of *hcnA'-'lacZ* translational fusions and mobility shift assays demonstrated that the RsmE binding site overlapping with the *hcnA* RBS is necessary, but not sufficient, for correct Gac/Rsm regulation, and that additional upstream motifs are involved. Unfortunately, time was lacking to study this session's other posters since they had to be removed at the end of the afternoon.

On Tuesday, in the cell-to-cell communication session, R. Hancock (USA) investigated genes involved in the swarming motility of *P. aeruginosa*. P. Williams (England) unravelled the interaction of the RsmA protein in homoserine lactone, quinolone and c-di-GMP signalling pathways. S. E. Lindow (USA) demonstrated that quorum sensing in *P. syringae* is related to iron availability and that bacterial crosstalk plays a role in plant disease control. M. Schuster (USA) presented the social cheating behaviour of a *P. aeruginosa lasR* mutant in not producing quorum-controlled factors and taking advantage of their production by the group. V. S. R. K. Maddula (USA) demonstrated the complexity of the phenazine regulatory network in *P. chlororaphis*. A. Doetsch (Germany), using mathematical modelling, showed that microcolonies promote intraspecific cooperative behaviour and maintain inter-colony communication. A. M. Misic (USA) presented the crystal structure of *P. aeruginosa* PilT (type IV pilus retraction motor) and its utilisation of an asynchronous mechanism for pilus retraction.

In the afternoon session on the topic of secondary metabolism, P. Cornelis (Belgium) explained that fluorescent pseudomonads produce siderophores such as pyoverdine (high iron affinity), and secondary siderophores with low iron affinity which they are currently investigating. E. Pesci (USA) demonstrated the importance of 4-quinolones and quinolone signalling to *P. aeruginosa*, and that quinolone signalling could be a good target for the development of novel therapeutics designed to decrease *P. aeruginosa* virulence. J. Raajmakers (Netherlands) presented the genes and regulatory mechanisms in cyclic lipopeptide surfactant biosynthesis. L. Thomashow (USA) reported studies of phenazine and 2,4-diacetylphloroglucinol (DAPG) biosynthesis, diversity and environmental implications. J. O. Morrissey (Ireland) presented his work on DAPG evolution and mode of action. J. E. Loper (USA) presented the results of his genomic study on *P. fluorescens* PF-5 secondary metabolite production. C. Chen (USA) characterised osmoregulatory transporters in *P. syringae* and *P. aeruginosa*. The second poster session presented material on plant-associated pseudomonads, plasmid transcriptomes in different host bacteria, the discovery of the histidine-phosphotransfer protein HptB, of FleQ, a transcriptional regulator coordinating the switch between motility and biofilm formation of *P. aeruginosa,* of *AlgJ*, a periplasmic protein required for alginate O-acetylation, and of the cytoplasmic heme binding protein (Phus). Posters on transcriptome and genomics studies of *P. aeruginosa,* on the Gac system and quorum sensing control, were also present.

On Wednesday's cell surfaces session, J. S. Lam (Canada) gave a presentation on membrane proteins involved in B-band polysaccharide assembly and surface diversity. M. Whiteley (USA) presented data on the formation of extracellular membrane vesicules derived from the cell outer membrane, and demonstrated the role of quinolone signal (PQS) in the formation of these vesicules. D. J. Wozniak (USA) demonstrated the reciprocal control of exopolysaccharide and alginate, and showed that the exopolysaccharide gene *psl* is a structural component of the biofilm matrix. B. I. Kazmierczak (USA) described the use of a yeast two-hybrid screen and the interaction of FimX (a regulator of pilus assembly) with other proteins localising to the bacterial pole. J. Nogales (Spain) reported his discovery of the GalT/GalP two-component transport system of gallic acid in *P. putida*. L. F. Wood (USA) presented studies on the roles of intracellular proteases in the cell wall stress stimulon in PAO1, which includes genes for alginate biosynthesis. C. L. Giltner (Canada) presented data on the role of minor pilins of the type IV pilus system on twitching motility and type II secretion of *P. aeruginosa*. The afternoon session was devoted to the physiological and metabolic versatility of *Pseudomonas*. S. Fetzner (Germany) spoke on the biodegradation of quinolines and quinolones. H. J. Heipieper (Germany) presented evidence that the conversion of *cis* to *trans* unsaturated fatty acids is an adaptative mechanism of *Pseudomonas* to environmental stress. K.-E. Jaeger (Germany) presented his work on lipase regulation. L. Johansson (Sweden) presented the 3,4-dimethylphenol system of *P. putida* demonstrating that bacteria can adjust their transcriptional capacity in order to adapt to their current environment. H. Nojiri (Japan) presented the structural basis of the substrate specificity of rieske non-heme iron oxygenase, carbazole 1,9 α-dioxygenase. D. Jendrossek (Germany) identified and characterised proteins involved in acyclic terpene utilisation and the leucine/isovalerate utilisation pathway. The day was rounded off with a cruise and dinner to the Kiana Lodge on Seattle's waterfront.

On Thursday, the last day of the congress, type II and type III secretion and transport were discussed with A. Filloux (France) presenting the molecular mechanisms of the type II system and discussing the mechanistic relationship between the type II secretion system and type IV pili assembly. J. Engel (USA) presented a role for ExoT, Crk and paxillin in cytokinesis. T. Yahr (USA) investigated the mechanism of transcriptional activation by ExsA, the master regulator of type III secretion system. J. Chang (USA) screened and identified type III effectors from different *P. syringae* species. A. Prince (USA) presented his work on the type III secretion system and showed that ExoS in particular facilitates invasion and permeability of airway epithelial cells. M. L. Hayes (USA) presented data concerning the role of cell contact and host signals in the type III secretion system in *P. syringae*. Finally M. L. Vasil (USA) reported on his discovery of PlcH, a phospholipase which inhibits wound healing during sepsis and is a promising angiogenesis inhibitor.

At the end of the proceedings it was announced that the next conference, Pseudomonas 2009, will be organised by Prof. B. Tümmler in Hannover, Germany.

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