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General

Bacterial biofilms of importance to medicine and bioterrorism: proteomic techniques to identify novel vaccine components and drug targets

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Biofilms are highly ordered microbial communities enmeshed in a carefully sculpted matrix designed for survival of organisms either in multi- or mono-genus/species in a specific microniche. In human disease, biofilm infections are some of the most recalcitrant to treat. Even with rigorous antibiotic regimens, some biofilms, such as those within the thick airway mucus of cystic fibrosis (CF) patients, persist throughout the course of the disease process. In this editorial, discussion will cover the utility of using advanced proteomic techniques to help identify potential weaknesses in the already impressive defensive armamentarium of biofilm bacteria. Two biofilm systems will be discussed herein, one of which is that of *Pseudomonas aeruginosa* biofilms within CF airway biofilms. The other is referred to as persistent 'bioterrorist agent biofilms' in which *Francisella tularensis* can grow on surfaces where environmental amoeba can phagocytose them, allowing for growth of *F. tularensis* within the amoebae.

Keywords: biofilms, *Francisella tularensis*, mass spectrometry, proteomics, *Pseudomonas aeruginosa*

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1. Introduction

A myriad of both prokaryotic and eukaryotic organisms are capable of forming well-structured communities on various substrata or cells, which are termed biofilms (for review, see [1]). In the Gram-negative rod *Pseudomonas aeruginosa*, two types of biofilm can be formed on inanimate surfaces or cells. One type involves direct adherence through one of the many structures of *P. aeruginosa* that allows it to adhere directly to surfaces. These are discussed below. Another biofilm form is when bacteria are encased in a thick matrix where direct attachment to surfaces or cells does not occur. These two forms and the mode by which the bacteria survive in them are the subject matter of section 3 below.

Another version of biofilm that will be discussed is directly related to increased concerns about bioterrorism in the US and abroad. As a result of the events of 11 September 2001, the world has become increasingly aware of bioterrorism and the agents that could potentially be disseminated during such an attack. The most recent attacks were highly covert in the sense that finely milled spores of *Bacillus anthracis*, the causative agent of anthrax, were delivered in sealed envelopes to government and post offices in several highly populated areas around the US. Five people died and another eleven developed inhalational anthrax. An estimated

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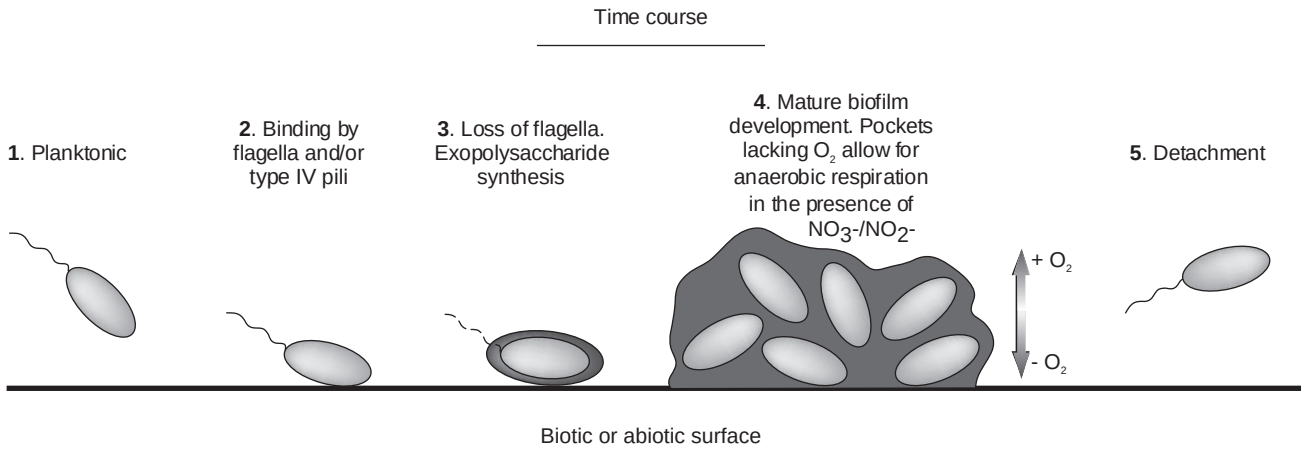

Figure 1. Model of the steps in the formation of a *P. aeruginosa* biofilm. On biotic or abiotic surfaces **1**. Free-swimming (planktonic) bacteria. **2**. Attachment of bacteria by flagella/type IV pili. **3**. Loss of flagellum and initiation of EPS production. **4**. Cell division and accumulation of EPS, leading to the development of an oxygen gradient. **5**. Detached cell synthesises a new flagellum and migrates to new site for colonisation.
EPS: Exopolysaccharide.

32,000 potentially exposed individuals were placed on antibiotics. Typically, 8000 – 10,000 anthrax spores are required for 1_{LD50} equivalent (a dose causing death in 50% of cases). In contrast, the Gram-negative coccobacillus *Francisella tularensis* (Ft), which causes highly lethal tularaemia, requires only 10 organisms for infection [2]. Ft is capable of rapidly replicating in human alveolar macrophages, ultimately leading to dissemination through the bloodstream and eventual death from liver and/or pancreatic failure. The source of such infections is controversial, and previous studies were only able to locate Ft in animal, tick or mosquito hosts. However, when these animal reservoirs of infection die, the organisms are thought to survive for extended periods of time in streams, soils and lakes. More recently, Ft has been found to survive and replicate in the environmental amoeba *Acanthamoeba castellanii* [3], and the authors' own research, discussed below, has indicated that Ft in biofilms are preferentially taken up by amoebae. Amoebae use very similar machinery to kill invading bacteria as do human macrophages. Thus, Ft in environmental biofilms could serve as a 'lure' for voracious amoebae, with the phagocytic event leading to propagation of hundreds of Ft per amoeba. Ultimately, growth within amoebae is likely to 'prime' the virulence properties of Ft within human alveolar macrophages [3,4].

2. *Pseudomonas aeruginosa* biofilms: two very distinct forms

P. aeruginosa can form biofilms on multiple substrata that include environmental surfaces as well as teeth, bones, organs, arteries/veins, skin, catheters and prostheses. Many laboratories worldwide have examined biofilms of strain PAO1 on multiple animate and inanimate substrata. Gene products that are required for biofilm formation and/or development include:

- polyphosphate kinase [5]
- the *las* quorum sensing circuit [6]
- the catabolite repressor control protein [7]
- flagella and type IV pili [8]
- the global regulator GacA [9]

Other unknown factors that do not appear to be related to the production of surface appendages also appear to be important for *in vitro* biofilm formation [10]. Recently, Finelli *et al.* [11] used in-biofilm expression technology to examine genes involved in overall biofilm development. They found that inactivation of a porin homologue, a putative alcohol dehydrogenase and a homologue of the *Streptomyces griseus* developmental regulator *adpA* were essential for optimal biofilm development. A model of biofilm formation on biotic or abiotic surfaces using either static or flow-through systems is depicted in Figure 1 (from Hassett *et al.*, [12,13]). However, this model is restricted to biofilms formed on surfaces.

P. aeruginosa biofilms formed in the thick cystic fibrosis (CF) mucus are actually enmeshed in spherical macro-colonies (for model see Figure 2, Hassett *et al.*, [12]). In fact, steep hypoxic gradients in the mucus [14] prompt *P. aeruginosa* to undergo anaerobic metabolism [12]. Strict anaerobic conditions could occur in CF mucus by the following mechanisms. First, unlike normal airway epithelia that have a thin, hydrated and highly aerated mucus layer on top of a periciliary liquid layer, CF airway epithelia have a thick, inspissated mucus layer (Figure 2C). The raised O_2 consumption by CF epithelia [15] that is associated with accelerated CF ion transport and increased Na^+/K^+-ATPase activity does not generate gradients in thin airway surface liquid films. In Figure 2D, *P. aeruginosa* are deposited onto the mucus layer and can penetrate the mucus via flagella or type IV pili (twitching motility) and/or passively (due to mucus turbulence) into hypoxic zones within

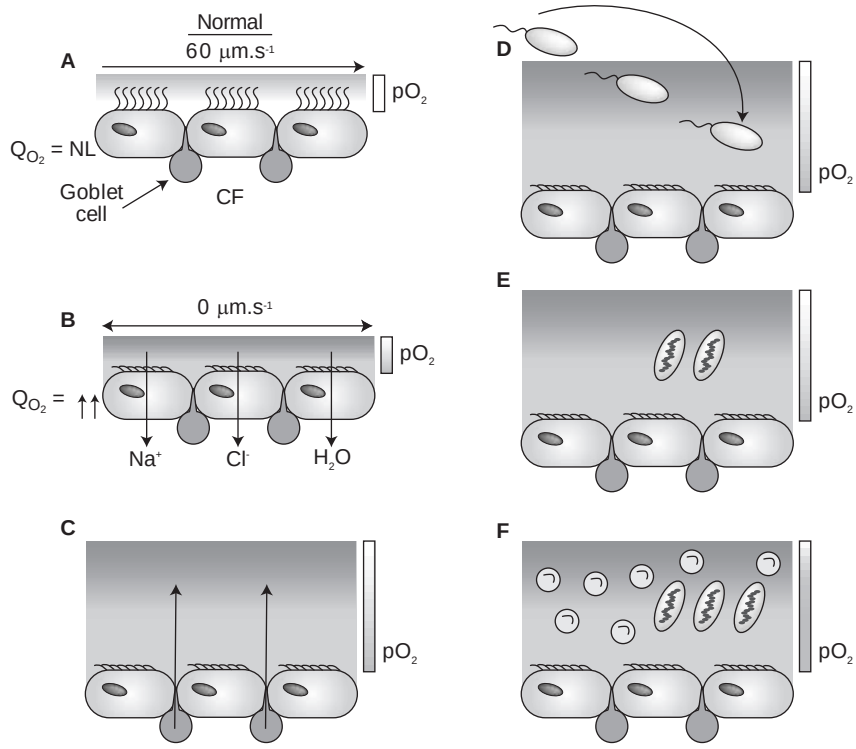

Figure 2. Model of *P. aeruginosa* "biofilms" in hypoxic/anaerobic CF mucus relative to normal mucus. Redrawn from Worlitsch *et al*. [14] and from Hassett *et al*. [12]. For a description of each caption, see section 2.
CF: Cystic fibrosis; NL: Neutral loss.

the mucus masses. *P. aeruginosa* easily adapts to such a niche by undergoing a rapid transition from aerobic to anaerobic respiration, the latter process involving utilisation of the alternative electron acceptors, nitrate or nitrite, that are present in CF airway mucus. In Figure 2F, macrocolonies resist secondary defences, including neutrophils and many first tier antibiotics (ceftazidime, ticarcillin, tobramycin, ciprofloxacin), setting the stage for chronic infection.

3. *Francisella tularensis* biofilms: 'lures' for environmental amoeba and other protists?

Unlike *P. aeruginosa*, the potential weapon of bioterrorism, Ft, has no flagella. Pilin homologues have been found within the genome sequence, but there is currently no evidence that Ft expresses functional pili. Thus, in theory, one might predict that Ft would form little or no biofilm on various environmental substrata. The authors have performed preliminary biofilm studies using an avirulent version of Ft, designated *F. tularensis* subsp. *novicida*. Unlike *P. aeruginosa* biofilms, which are highly organised and very thick, Ft forms a thin but well-structured mosaic-like biofilm (Figure 3; Hassett, Klaus & Nan, unpublished data). It was then postulated that such biofilms are actually 'lures' for environmental amoebae and other protists. Note in Figure 3 how the environmental amoeba *A. castellanii* creates a swath within the Ft biofilm, essentially engulfing not only the bacteria, but also the biofilm matrix. Given the importance of both *P. aeruginosa* and Ft biofilms in the context of human disease, it is important now to embark on studies designed to better understand the genes and proteins required for biofilm formation and survival. In the case of Ft, it is necessary to begin to determine the genes/proteins involved in intra-amoebal survival and how these relate to those required for intra-macrophage survival. As RNA expression using microarray technology does not always correlate with protein expression, described below are some recent advances in proteomic research that may help us unravel such mysteries.

4. Proteomics

Modern proteomics now offers researchers a multitude of approaches that can be used in basic research associated with vaccines and drug targets. The more traditional approach of two-dimensional gel electrophoresis separation of proteins followed by their identification with mass spectrometry is still used for the characterisation of differentially expressed proteins. However, one drawback of this approach is the time and large sample size required for a complete proteomic characterisation of a defined system. More recently, chromatographic approaches have replaced gel electrophoresis as the preferred separation method, as such approaches can be directly coupled with electrospray ionisation mass spectrometry. The newest developments in quantitative proteomics

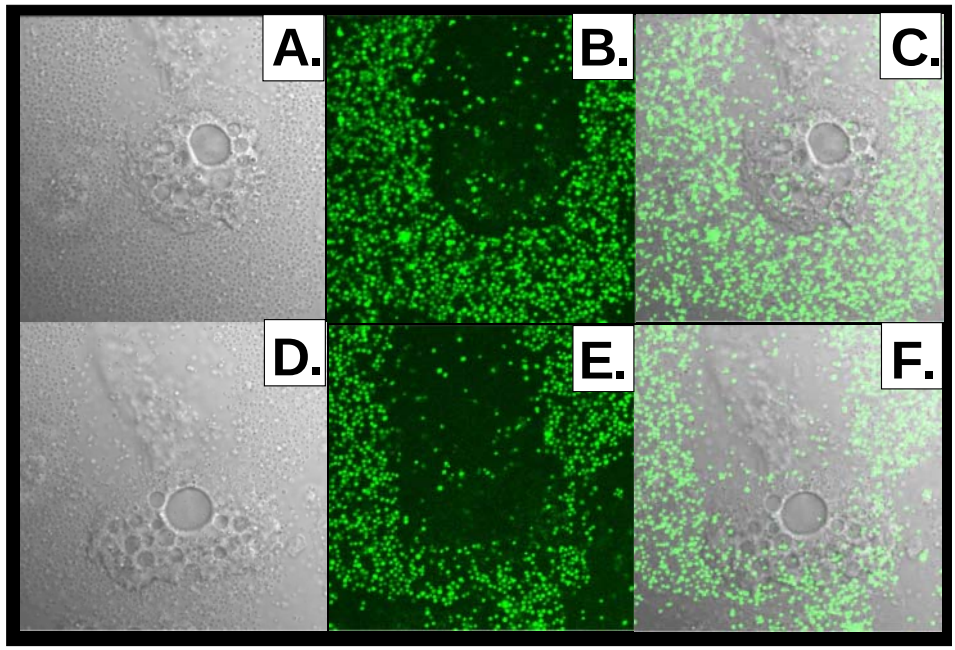

Figure 3. *A. castellanii* devouring *F. tularensis* subsp. novicida biofilms. Ft expressing GFP were grown as biofilms for 7 days at 37°C in a cover-slip bottomed chamber and then washed three times with 0.9% saline. Dual GFP-DIC time lapse images were acquired on a Zeiss LSM510 confocal mated to a Zeiss Axiovert microscope with a 63x 1.2NA water immersion objective at 2-second intervals for a total of 4 minutes.

DIC: Differential interference contrast; Ft: *Francisella tularensis*; GFP: Green fluoresence protein.

involve the use of high-end mass spectrometry instrumentation, which permits the relative quantitation of protein amounts in a single analysis without the use of labels or internal standards.

4.1 Processing of bacteria from representative biofilms or from lysed amoebae

For advanced proteomic analyses, the quality of protein preparation is critical. The authors routinely take organisms grown under a specific condition and pour bacterial suspensions over crushed ice to halt protein synthesis, finally diluting them in ice-cold 0.1 M NH_4HCO_3-1 mM DTT-0.05% CHAPS (buffer A). The bacteria are harvested by centrifugation at 13,000 × *g* for 10 min at 4°C, quick-frozen in dry-ice-ethanol, thawed on ice and again resuspended in buffer A. The bacteria are typically lysed twice by extrusion through a small aperture at 12,000 psi, 4°C using a French press. The samples are then treated with 20 U/ml of both DNase and RNase containing 10 mM $MgCl_2$ for 15 min on ice. At this point, 1 mM EDTA is then added. Debris is removed by centrifugation at 4°C for 15 min at 13,000 × *g*, the supernatant is saved and an aliquot removed to check protein concentration. Finally, the protein can be stored at -80°C. To enhance recovery of membrane proteins, 0.3 M NaCl can be added to the 0.1 M NH_4HCO_3-1 mM DTT-0.05% CHAPS. Samples are then subjected to ultracentrifugation at 150,000 × *g* for 60 min at 4°C. The supernatant is the soluble fraction and the pellet is the membrane fraction. After dialysis of all samples against 100 mM ammonium acetate (pH 8.9), bacterial protein, 500 µg (10 nmol), in 500 µl of 100 mM ammonium acetate (pH 8.9) is digested with trypsin (20 µg) overnight at 37°C.

4.2 Protein digestion and mass spectroscopy

Once the protein extract is obtained, it is then digested with a protease, typically trypsin. The resulting mixture of peptides is then analysed by liquid chromatography-mass spectrometry (LC-MS). For bacterial proteomes, either one-dimensional reverse-phase high performance liquid chromatography (RP-HPLC) or two-dimensional anion-exchange/RP-HPLC is used to separate the peptides before their introduction into the mass spectrometer for analysis. As the chromatographic system is coupled directly to the mass spectrometer, the entire characterisation can be carried out online without subsequent operator intervention.

Although a number of mass spectrometric approaches can be used, the authors believe that the use of a Fourier transform ion cyclotron resonance (FTICR) mass spectrometer provides state-of-the-art capabilities for whole-bacterium proteomic analysis. For example, Figure 4 (top) are representative proteomic analyses performed on samples from *P. aeruginosa* grown anaerobically and aerobically. Comparative proteomics is done by merely subtracting the LC-MS runs between experiments. As seen in this example, a number of peptides eluting around 8 min in the aerobic sample were over-represented as compared with those from the anaerobic sample. By keeping instrument conditions identical among LC-MS experiments,

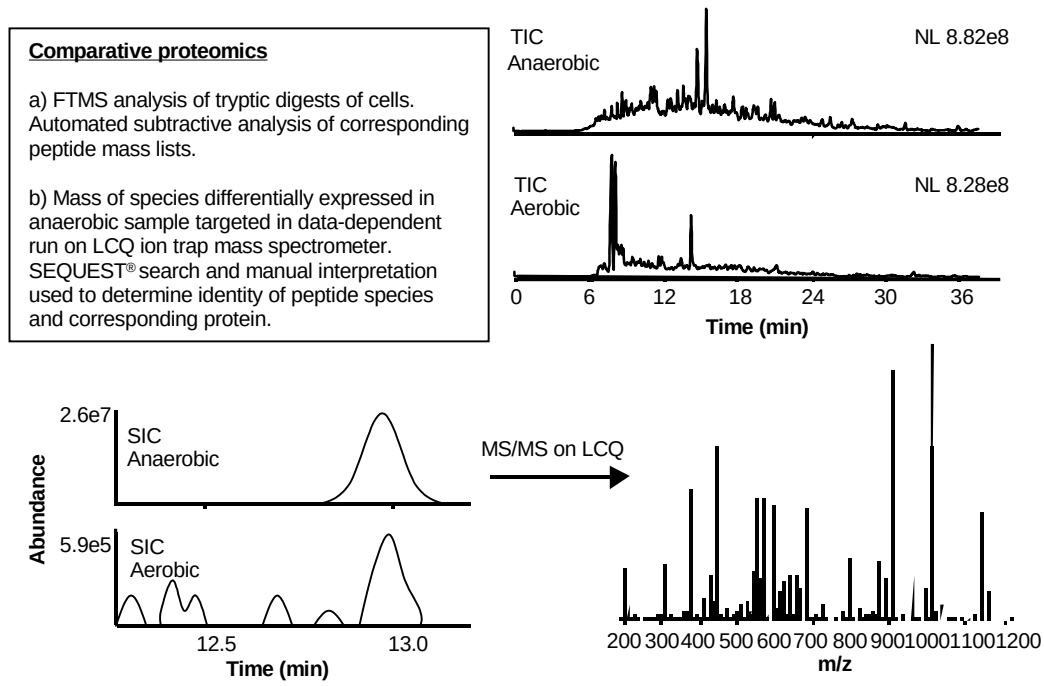

Figure 4. Protocol with representative data used for the identification of proteins differentially expressed by *P. aeruginosa* under aerobic and anaerobic conditions.

FTMS: Fourier transform mass spectrometry; MS: Mass spectrometry; m/z: Mass to charge ratio; NL: Neutral loss; SIC: Selected ion chromatogram; TIC: Total ion chromatogram.

the only differences detected will be due to differences in protein abundance. The significant advantage of this approach is that no additional labelling or internal standards are required to obtain relative differences in protein expression.

Initial FTICR-MS (e.g., Figure 4) was done using a lower-field (7 T) superconducting magnet. Under these conditions, a second set of LC-MS experiments is required to identify the various peptides through generation of partial sequence tags that can be identified by searching protein or genomic databases. However, as with nuclear magnetic resonance, the use of higher-field magnets improves performance in FTICR-MS. As demonstrated by R Smith and colleagues at Pacific Northwest Laboratories [16,17], when one has the technology to measure peptide masses extremely accurately, a second set of LC-MS experiments is no longer necessary.

5. Expert opinion

How then can proteomic studies of the two disease scenarios described above, namely *P. aeruginosa* development and growth in anaerobic biofilms in the thick CF mucus and Ft biofilm growth and survival within environmental amoebae, lead to the identification of novel vaccine components and drug targets. In *P. aeruginosa*, most candidate vaccines comprise antigenically accessible surface components, including outer membrane proteins (OprF, OprI-OprF fusion proteins, etc.), lipopolysaccharide O-antigen, flagella, pili and the exopolysaccharide alginate [18-20], although live attenuated cell vaccines have been considered [21]. Many animal models systems, as well as human challenges, have been used to test the efficacy of these potential vaccine candidates. For example, the alginate vaccine in mice and rabbits generated a good opsonic response, but failed to help humans [18]. One way in which proteomics can assist in vaccine discovery is to identify new surface antigens for use as vaccines. Thus, for example, the proteome of the outer membrane of *Pseudomonas* (particularly in bacteria growing in biofilm mode) would be anticipated to include novel protein vaccine candidates. A second area that proteomic approaches would contribute to is drug discovery. The ideal drug target would be:

- relatively accessible (to subvert the issues of transport into and efflux out of cells that have plagued many antibiotic candidates)
- essential (such that its inhibition would cause cell death)
- relatively well conserved (such that inhibitors would have broad spectrum activity)

The latter two issues for *P. aeruginosa* can be determined by reference to knockout libraries that will become available from the University of Washington and Harvard (whereby essential genes are those that cannot be knocked out) and BLAST comparisons contained on the *Pseudomonas* genome web page [101]. However, the accurate determination of accessible targets requires experimental studies of the cell envelope proteome.

Regarding Ft, a live attenuated Ft vaccine strain ('Strain 15') was developed in the Soviet Union from a virulent Ft subsp. *holarctica* (type B) strain and was shown to be harmless and to induce protective immunity [22,23]. This strain was among a mixture of potential live vaccine strains (LVSs) transferred to the US in 1956. From this mixture, an isolate was derived and passed through mice, then tested for protective immunity against Ft (type A) challenge. This is the LVS that was subsequently shown to protect against aerosolised type A (Schu4 strain) challenge in human studies [24-27]. In these limited studies, the Ft subsp. *holarctica* (type B) LVS strain appeared to be an effective vaccine against aerosolised Ft (type A) challenge. However, this vaccine has never received FDA approval for human use, in part due to problems with standardisation. The greatest problem with using a LVS as a vaccine is that the nature of the attenuating mutation(s) in this strain is completely unknown and, thus, there is a significant danger of reversion. Since Ft has no flagella or pili, these obvious vaccine candidates are not available for this organism. The lipopolysaccharide of Ft LVS has been shown to protect against infection with the LVS strain [28]. Even so, as is the case for *P. aeruginosa* biofilms, a knowledge of the surface proteins expressed during infection of these hosts and the proteins required for survival in such settings will help us make significant strides towards identifying drugs targets and new vaccine candidates.

6. Conclusion

Given the potential inherent in new mass spectrometry technologies, it would seem important to perform comparative proteomics on biofilm *P. aeruginosa* and on Ft grown inside amoebae using LC-FTICR-MS. Subtractive analysis will provide quantitative information and the knowledge of peptide masses and HPLC retention times will permit the unambiguous identification of the parent proteins. Providing that the scientist using such technology is very hard working, thorough and dedicated, it is estimated that ~ 500 proteins/month can be identified and quantified using this approach.

Bibliography

1. O'TOOLE GA: To build a biofilm. *J. Bacteriol.* (2003) 185:2687-2689.
2. EIGELSBACH HT, DOWNS CM: Prophylactic effectiveness of live and killed tularemia vaccines I: production of vaccine and evaluation in the white mouse and guinea pig. *J. Immunol.* (1961) 87:415-425.
3. ABD H, JOHANSSON T, GOLOVLIOV I, SANDSTROM G, FORSMAN M: Survival and growth of *Francisella tularensis* in *Acanthamoeba castellanii*. *Appl. Env. Microbiol.* (2003) 69:600-606.
4. TITBALL RW, JOHANSSON A, FORSMAN M: Will the enigma of *Francisella tularensis* virulence soon be solved? *Trends Microbiol.* (2003) 11:118-123.
5. RASHID MH, KORNBERG A: Inorganic polyphosphate is needed for swimming, swarming, and twitching motilities of *Pseudomonas aeruginosa*. *Proc. Natl. Acad. Sci. USA* (2000) 97:4885-4890.
6. DAVIES DG, PARSEK MR, PEARSON JP, IGLEWSKI BH, COSTERTON JW, GREENBERG EP: The involvement of cell-to-cell signals in the development of a bacterial biofilm. *Science* (1998) 280:295-298.
7. O'TOOLE GA, GIBBS KA, HAGER PW, PHIBBS PV JR, KOLTER R: The global carbon metabolism regulator Crc is a component of a signal transduction pathway required for biofilm development by *Pseudomonas aeruginosa*. *J. Bacteriol.* (2000) 182:425-431.
8. O'TOOLE GA, KOLTER R: Flagellar and twitching motility are necessary for *Pseudomonas aeruginosa* biofilm development. *Mol. Microbiol.* (1998) 30:295-304.
9. PARKINS MD, CERI H, STOREY DG: *Pseudomonas aeruginosa* GacA, a factor in multihost virulence, is also essential for biofilm formation. *Mol. Microbiol.* (2001) 40:1215-1226.
10. DEZIEL E, COMEAU Y, VILLEMUR R: Initiation of biofilm formation by *Pseudomonas aeruginosa* 57RP correlates with emergence of hyperpiliated and highly adherent phenotypic variants deficient in swimming, swarming, and twitching motilities. *J. Bacteriol.* (2001) 183:1195-1204.
11. FINELLI A, GALLANT CV, JARVI K, BURROWS LL: Use of in-biofilm expression technology to identify genes involved in *Pseudomonas aeruginosa* biofilm development. *J. Bacteriol.* (2003) 185:2700-2710.
12. HASSETT DJ, CUPPOLETTI J, TRAPNELL B *et al.*: Anaerobic metabolism and quorum sensing by *Pseudomonas aeruginosa* biofilms in chronically infected cystic fibrosis airways: rethinking antibiotic treatment strategies and drug targets. *Adv. Drug Deliv. Rev.* (2002) 54:1425-1443.

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13. HASSETT DJ, OCHSNER UA, DE KIEVIT TR *et al.*: Genetics of quorum sensing circuitry in *Pseudomonas aeruginosa*: implications for control of pathogenesis, biofilm formation and antibiotic/biocide resistance. In: *Modern Microbial Genetics, 2nd Edition*, Streips U & Yasbin R (Eds), Wiley-Liss Publications (2002):261-272.
14. WORLITZSCH D, TARRAN R, ULRICH M *et al.*: Reduced oxygen concentrations in airway mucus contribute to the early and late pathogenesis of *Pseudomonas aeruginosa* cystic fibrosis airway infection. *J. Clin. Invest.* (2002) 109:317-325.
15. STUTTS MJ, KNOWLES MR, GATZY JT, BOUCHER RC: Oxygen consumption and oubain binding sites in cystic fibrosis nasal epithelium. *Pediatr. Rev.* (1986) 20:1316-1320.
16. SMITH RD, ANDERSON GA, LIPTON MS *et al.*: An accurate mass tag strategy for quantitative and high-throughput proteome measurements. *Proteomics* (2002) 2:513-523.
17. SMITH RD, PASA-TOLIC L, LIPTON MS *et al.*: Rapid quantitative measurements of proteomes by Fourier transform ion cyclotron resonance mass spectrometry. *Electrophoresis* (2001) 22:1652-1668.
18. THEILACKER C, COLEMAN FT, MUESCHENBORN S, LLOSA N, GROUT M, PIER GB: Construction and characterization of a *Pseudomonas aeruginosa*

mucoid exopolysaccharide-alginate conjugate vaccine. *Infect. Immun.* (2003) 71:3875-3884.

19. HANCOCK REW, LAM JS: *Pseudomonas aeruginosa*: infection and immunity. In: *Encyclopaedia of Immunology, 2nd Edition*, Delves PJ (Ed.), Academic Press, London (1998) 4:2042-2045.
20. HOLDER IA: *Pseudomonas* vaccination and immunotherapy: an overview. *J. Burn Care Rehabil.* (2001) 22:311-320.
21. PRIEBE GP, MELULENI GJ, COLEMAN FT, GOLDBERG JB, PIER GB: Protection against fatal *Pseudomonas aeruginosa* pneumonia in mice after nasal immunization with a live, attenuated aroA deletion mutant. *Infect. Immun*. (2003) 71:1453-1461.
22. SJOSTEDT A, NORTH RJ, CONLAN JW: The requirement of TNFα and IFNγ for the expression of protective immunity to secondary murine tularemia depends on the size of the challenge inoculum. *Microbiol.* (1996) 142:1369-1374.
23. TIGERTT WD: Soviet viable *Pasteurella tularensis* vaccines: a review of selected articles. *Bacteriol. Rev.* (1962) 26:354-373.
24. ERICSSON M, SANDSTROM G, SJOSTEDT A, TARNVIK A: Persistence of cell-mediated immunity and decline of humoral immunity to the intracellular bacterium *Francisella tularensis* 25 years after natural infection. *J. Infect. Dis.* (1994) 170:110-114.
25. SANDSTROM G: The tularemia vaccine. *J. Chem. Technol. Biotechnol*. (1994) 59:315-320.
26. SASLAW S, EIGELSBACH HT, PRIOR JA, WILSON HE, CARHART S: Tularemia vaccine study II: respiratory challenge. *Arch. Intern. Med*. (1961) 107:702-714.
27. SASLAW S, EIGELSBACH HT, WILSON HE, PRIOR JA, CARHART S: Tularemia vaccine study I: intracutaneous challenge. (1961) *Arch. Intern. Med.* 107:689-701.
28. DREISBACH VC, COWLEY SC, ELKINS KL: Purified lipopolysaccharide from Francisella tularensis live vaccine strain (LVS) induces protective immunity against LVS infection that requires B cells and gamma interferon. *Infect. Immun*. (2000) 68:1988-1996.

Website

101. http://www.pseudomonas.com *Pseudomonas* genome web page.

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