Proteome analysis for antimicrobial drug development and target validation

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Introduction

Earlier this year, I provided a review of the technologies involved in studying microbial physiology using proteomics [1]. This global analysis of protein expression can be utilised to discover novel targets for vaccines, antimicrobial therapeutics and diagnostics, as well as to determine the cellular effects and interaction partners of newly synthesized compounds. Furthermore, the technology has also been applied to comparisons of bacterial strains that differ in their resistance to commonly used antibiotics [2].

Proteomics can also be used to monitor the cellular response of bacteria to antimicrobial compounds, firstly to provide a database of responses to known compounds, and then to begin screening the functions of new compounds in an attempt to characterise their mode of action.

Vaccine discovery

Proteomics is particularly amenable to approaches aimed at elucidating immunogenic proteins from amongst complex mixtures. Two-dimensional gel electrophoresis (2-DE) combined with mass spectrometry (MS) and Western blotting using patient sera is a highly effective method of predicting potential cell surface or secreted vaccine targets (Figure 1) [3, 4].

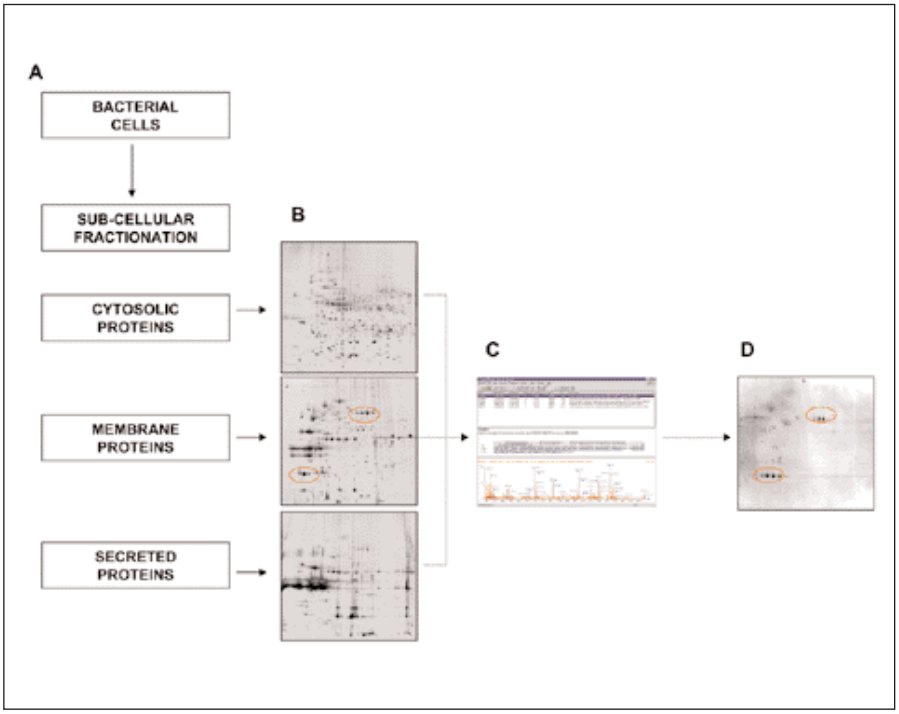
However, this approach often discovers highly abundant cytosolic proteins as immunogenic constituents, yet their role in pathogenicity remains controversial. It seems likely that proteins such as the chaperone GroEL and enzymes involved in glycolysis become immunogenic in the host perhaps only following cell autolysis

A much better approach is to attempt to enrich for surface exposed and secreted proteins [5, 6]. This pre-fractionation approach, combined with 2-DE/MS and Western blotting, may provide much more focused information. Furthermore, new methodology is becoming available that utilises the surface display of pathogen genomic peptide libraries fused to *Escherichia coli* outer membrane proteins that are screened against patient sera with high antibody titre [7]. This method also allows the identification of specific epitopes that interact with antibody. Interestingly, in *Staphylococcus aureus* 60 antigenic proteins were characterised and all were predicted surface or secreted proteins [7].

Proteomic signatures

A proteomic signature is "the subset of proteins whose alteration in expression is characteristic of a response to a defined condition or genetic change" [8], and therefore may contain members of a single pathway, proteins with similar functions and/or those with shared regulation (Figure 2). The response of bacteria to several antimicrobial compounds of both known and unknown mode-of-action can be monitored via

Figure 1. **Immunogenic proteins detected by Western blotting.**
A: Bacterial cells are fractionated via sub-cellular location
B: Cytosolic, membrane and secreted protein enriched fractions are separated by 2-DE
C: Proteins of interest are analysed by MS
D: Replicate 2-D gels of each fraction are screened by Western blotting and the proteins mapped back to those identified in C.



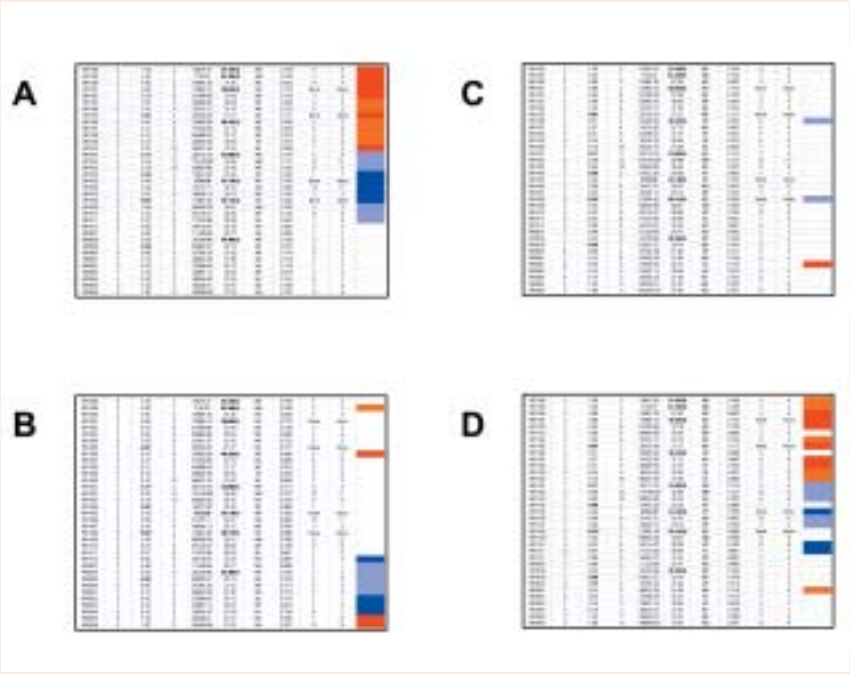
protein expression using 2-DE and MS. The patterns of the expressed proteins under these conditions can then be used to create a database of responses.

Each proteomic signature is then compared and similar 'signatures' used to predict the targets and mode-of-action of a given compound, both alone and in comparison to those with known targets. For example, proteomic signatures have been published for the *S. aureus* response to cell wall-active antibiotics, including oxacillin [9]. This study showed that cell-wall-active antibiotics induced or repressed similar proteins (including peptide methioinine sulfoxide reductase [MsrA] indicative of oxidative stress and GroES indicative of protein synthesis stress) to each other, yet antibiotics with different mechanisms caused wholly different alterations to the protein expression profile. For screening many compounds, this allows recognition of expression patterns across many hundreds or thousands of synthetic and natural compounds, and therefore the grouping of compounds into particular modes-of-action, based upon their proteomic signatures.

Drug interactions and validation

It is particularly difficult to use standard proteomics tools, such as 2-DE and MS, to determine the interactions of drugs with their specific microbial targets. Some predictions, as described above, can be made depending on the physiological response of the organism, as viewed via expression changes in metabolic and regulatory pathway proteins.

Figure 2. **Database of proteomic 'signatures' for characterizing the mode-of-action of compounds with both known and unknown targets. Each row represents statistical data on a given Open Reading Frame (ORF), colour indicates response of ORF to an antimicrobial compound; red/orange, up-regulation; blue/violet, down-regulation.**
A: known compound
B-C: unknown compounds
Characterisation of proteomic signatures for each compound indicates D is likely to have same target as A, while B and C appear to have no functional relationship to A.



However, another approach may be to use the drug itself as an affinity ligand for molecular 'fishing' of interacting proteins prior to MS. For example, one group has used vancomycin derivatives bound to sepharose as the basis of an affinity experiment to select for vancomycin-binding proteins in an *E. coli* model [10]. These researchers determined that at least six enzymes involved in peptidoglycan assembly were the targets of this compound. This model remains an effective approach for studying interacting partners of other antimicrobial compounds.

Conclusions

Proteomics using both 2-DE/Western blotting and affinity approaches prior to MS is an excellent method for determining potential new vaccines and drug targets, as well as for the micro-characterisation of drug-protein interactions. As such, it will be a critical technique in the coming years to aid both drug design and to validate new compounds in conjunction with biological models.

References

1. Cordwell SJ (2002). *Microbiology Australia* 23:10-11.
2. Cordwell SJ, Larsen MR, Cole RT & Walsh BJ (2002). *Microbiology* 148:2765-2781.
3. Grandi G (2001). *Trends Biotechnol.* 19:181-188.
4. Vytvytska O, Nagy E, Bluggel M, Meyer HE, Kurzbauer R, Huber LA & Klade CS (2002). *Proteomics* 2:580-590.
5. Cullen PA, Cordwell SJ, Bulach DM, Haake DA & Adler B (2002). *Infect. Immun* 70:2311-2318.
6. Sabarth N, Lamer S, Zimny-Arndt U, Jungblut PR, Meyer TF & Bumann D (2002). *J. Biol. Chem.* 277:27896-27902.
7. Etz H, Minh DB, Henics T *et al.* (2002). *Proc. Natl. Acad. Sci* USA 99:6573-6578.
8. VanBogelen RA, Schiller EE, Thomas JD & Neidhardt FC (1999). *Electrophoresis* 20:2149-2159.
9. Singh VK, Jayaswal RK & Wilkinson BJ (2001). FEMS *Microbiol. Lett.* 199:79-84.
10. Sinha Roy R, Yang P, Kodali S *et al.* (2001). *Chem. Biol.* 8:1095-1106.