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Mass spectrometry as a tool for bacterial identification

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The identification of microorganisms is of considerable importance in clinical medicine, food production, biotechnology, biological warfare and numerous other areas. The identification of bacteria by current techniques is often time consuming with positive identification typically taking hours or even days. This has led to the development of a variety of mass spectrometric techniques for the identification of microorganisms.

In this context phospholipids were often utilized in chemotaxonomic studies, due to the fact that they are present at relatively high concentrations in all living cells. The development of matrix-assisted laser desorption mass spectrometry (MALDI-MS) and electrospray mass spectrometry has made possible high molecular weight analysis of proteins, glycoproteins, oligosaccharides and oligonucleotides and therefore makes possible direct identification of microorganisms.

We have used matrix-assisted laser desorption mass spectrometry for the analysis of whole bacterial cells; this methodology has the potential for high sensitivity detection, due to the abundance of proteins, up to 60-70% dry weight in E.Coli and Bacilli. Present methodology requires the bacterial cells to be removed from the growth medium and to be washed[1,2].

A variety of gram-positive and gram-negative intact bacterial cells have been analysed by MALDI-MS and shown to provide fingerprint mass spectra with discrete peaks being observed over the mass range from 3 to 40kDa. The spectra show both more peaks and peaks at a higher mass/charge ratio than have hitherto been reported for these microorganisms and would appear to provide a profile of cellular proteinaceous material. The spectra are shown to be reproducible over variable time periods of up to three months. The procedure, which requires minimal sample preparation, yields results in 10-20 minutes and allows visual identification of species- and strain-specific biomarkers for the characterization of the organisms[3].

Washing has been shown to selectively release components from the bacteria. However it still has to be established whether those components are exclusively from the cell surface and are not a consequence of cell lysis. For the solvents we have used to date, no cell lysis has been shown to occur.

We have found differences to occur from choice of matrix. To optimise an analytical protocol will involve the examination of combinations of solvent, matrix, pH and salt concentrations. Such parameters must be evaluated before a choice of biomarkers and library criteria are established. The importance of accurately defining sample preparation methodologies is central to the ability of the technique to generate reliable and reproducible data. The strategy outlined is currently being pursued and the results obtained to date will be reported together with consequent recommendations for the choice of biomarkers to be employed in library search techniques.

References

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