

LC/MS/MS as a Potential Method for Characterizing Bacterial Contamination During Spacecraft Assembly

Introduction

Exploration of outer bodies of interest to life's origins requires stringent measures to prevent contamination of these bodies with Earth life forms. Procedures are taken to vigorously clean spacecraft before launch. We are exploring MS to measure contamination during spacecraft assembly. MALDI and ES-LC/MS create unique protein profiles from bacteria whole cells and cell lysates. Under ideal circumstances, these methods work well, but fail to yield consistent results on samples from different sources and handling techniques. Our more robust approach uses LC/MS/MS of the mixture of peptides obtained from the trypsin digestion of whole cell bacterial lysates.

Methods and Instrumentation

Bacteria cells were processed by a lysis procedure followed by removal of cellular debris and digestion of the cytoplasmic components. LC/MS/MS analyses were performed using a custom built capillary HPLC system coupled to an ion trap mass spectrometer. MS/MS spectra were collected in a data dependant fashion and then matched to known protein sequences using the SEQUEST database search program.

Preliminary Data

The described procedure depends on obtained fragment ion spectra of sufficient number of the most abundant and readily ionizable peptides present in the cytoplasm for that particular organism. The technique was found to work well for organisms whose genome has been sequenced. For *Bacillus subtilis*, whose genome is in the database, we obtained specific peptide matches to expected proteins such as chaperonins, metabolic enzymes and ribosomal proteins. While the complex bacterial digestion mixture did not yield the same set of results each time different aliquots or different concentrations were analyzed, the majority of high scoring matches were assigned to *Bacillus subtilis* proteins. Analysis of samples from organisms not in the database failed to correlate well to any organism.

Novel Aspect

allows identification of bacteria regardless of growth conditions and history of the sample