An Examination of the Issues Associated with Analyzing Complex Microbial Communities

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Global proteomics offers a unique opportunity to study the relationships between microorganisms by examining changes in the protein expression on a community level. This examination process has, however, proven to be difficult due to many levels of complexity.

A high throughput proteomic method for peptide identification detecting species-specific protein expression (using MS/MS) was explored in two ways. Initially through an in-silico study, and second through experimental measurement.

The method application was applied to a current bioremediation site. Samples were taken from a contaminated Colorado River sediment community on a former uranium ore-processing site, before and after bio-stimulation.

While results from our in-silico study illustrated the feasibility of detecting an organism against a background of other organisms in a microbial community, the applied approach demonstrated many complexities which hampered the ability to discern individual peptide identities.

• Introduction

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Methods

Peptide identification by MS/MS

• In theory, species-specific peptides can be detected within a community and differentiation of proteins is possible on a community level, as demonstrated with an artificial 23-gene organism study.

• An enzymatic bacterial extraction technique by Bockelmann, et al. (2005) was deployed for this study. An aliquot of each sample was first collected into a lysis buffer containing protease inhibitors. Samples were then transferred to a high-speed centrifuge and spun at 14,000 RPM for 10 minutes to pellet the bacteria. The supernatant was then collected and stored at -70°C until analysis.

• The actual application of this approach, however, demonstrated some significant problems. It was necessary to introduce the extracted protein into a mass spectrometer for analysis, followed by database searching, before the peptide sequences can be identified in the presence of other peptide identities.

Soil Bacteria Extraction Procedure (3)

• Certain diatomaceous earth microorganisms are capable of producing low molecular weight compounds that can be used to contaminate surrounding environments. The precipitation of heavy metals decreases due to the high pH, which is caused by lowering its ability to leach into waterways. (1)

• In order to study the microbial processes that make bioremediation possible, the bacteria must be removed from the sediment, the proteins extracted and digested into peptides and then analyzed on a mass spectrometer.

• The purpose of the in-silico study was to assess the utility of peptide identification methods for detecting a single species within a community of species.

• For the in-silico study, proteins predicted from annotated genomes that corresponded to 20 microorganisms were compiled, virtually digested, and analyzed to determine whether a particular peptide was detectable in the presence of the other peptide identities.

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Informatics Challenges:

• Complexity - Multiple copies of the same protein introduce redundancy of peptide to protein information, and multiple organism protein files are generally appended together in database searching, in effect blurring the scores associated with the peptides identified and reducing the number of possible matches. In terms of peptide counts, a multi-organism community can easily achieve a presence of over 40,000 proteins – the number currently detected in many eukaryotic systems.

• Lack of database - As protein databases only which are present in the sample, therefore, without an annotated eukaryotic database, it is hard to make a good identification. A match is only as good as the database.

• In-silico
Peptide identification by MS/MS  • In theory, species-specific peptides can be detected within a community and differentiation of proteins is possible on a community level, as demonstrated with an artificial 23-gene organism study. (2)

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Conclusions

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