Proposal to present #154i – Meta-proteomics as a tool to study microbial communities: Effect of cadmium exposure

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Introduction

Microbial communities are the basis for engineered environmental bioprocesses. Current characterization methods are incapable of providing information on the functions of the members of the community, which thus remains a black box. To date, microbial community genomics, based on DNA extraction from a particular environment, has been the preferential method used to study communities; however, knowledge of community structure does not necessarily lead to useful information on functions such as metabolic capacity, control of population dynamics, and sensitivity to variable environmental conditions. On the other hand, microbial community proteomics has the potential to detect proteins expressed in an environment under different conditions, revealing the current functional state of the community being studied.

Despite its possible advantages, the use of meta-proteomics has been almost completely unexplored. Here a mixed culture can be viewed as a meta-organism, in which population shifts are a form of functional response. In this project, proteomics is used as a tool to obtain functional information about the response of a microbial community to cadmium stress. Specifically, we demonstrate the ability to obtain two-dimensional electrophoresis gels from a microbial community sample, to identify protein markers of significant environmental change, and to use mass spectrometry to obtain putative protein identifications. Cadmium stress was chosen as a model perturbation to which a significant physiological response was expected.

Materials and methods

The mixed culture inoculum was taken from a continuous-flow wastewater treatment bioreactor that was fed a mixture of organic chemicals and added to 10 mg/L tryptic soy broth. The culture flasks were shaken at room temperature overnight, and then 10 mg/L cadmium was added to half of the flasks. Treated and control cultures were harvested after 0.25, 1, 2 and 3 hours of cadmium presence, and the protein content was extracted. Overall protein profiles were studied using 18-cm narrow-range (pH 4 to 7) immobilized pH gradients strips for isoelectric focusing, and 16 x 18 cm SDS-PAGE gels. Mass spectrometric analysis was performed to identify proteins that play central roles during the cadmium shock. After analyzing the gel patterns, differentially expressed proteins were chosen for identification and digested with trypsin. Digested peptides were analyzed by MALDI-TOF/TOF and the results were searched for peptide mass fingerprinting and ion search.

Results and discussion

Comparison of the two-dimensional gels from Cd-exposed and control cultures revealed that the community “reacts” by changing its protein profile. At each time point,

cadmium exposure resulted in both up- and down-regulation of proteins relative to the controls showing that cadmium can have both stimulatory and inhibitory effects. An important observation is that the protein levels of at least 100 proteins had changed (by at least three fold) within 15 min of exposure to cadmium. A similar number of proteins with altered expression levels relative to the control were also noted after longer exposure, but comparison of the proteomes after different time points revealed major differences, indicating that the cadmium shock led to rapid physiological responses as well as longer term changes. When the temporal expression patterns were tracked for each of the differentially expressed proteins, several different trends were evident. Gel spots chosen for MS analysis generated good quality mass spectra (peptide mass fingerprints) but some yielded no statistically significant matches in the database searches. Since it is likely that none of the species in this consortium have been sequenced, this lack of matching is not surprising and is consistent with the experiences of others. More successful results for unsequenced species were obtained using MS/MS data.

In conclusion, proteomic analysis revealed significant shifts in the microbial community physiology after short cadmium exposure, a rapid change not detectable using the phylogenetic profiling tools common to molecular microbial ecology. Furthermore, the proteome of the cells exposed to cadmium for a longer time was significantly different from that of the cells exposed for 15 min, suggesting that the community's short- and medium-term responses to this stress were different. These results support the promise of proteomics to reveal insights into the functional responses of a microbial community, in spite of current limitations for protein identification.