Poster G-26

Bioinformatic analysis of Gluconacetobacter diazotrophicus proteome

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Short Abstract: A molecular analysis of the G. diazotrophicus metabolism was performed and we have mass-spectrometry identified more than 600 housekeeping and 50 regulated proteins, which could help validation of genome predicted ORFs and hypothetical proteins, assignment of non-predicted ORFs, start and stop codons definition, information about post-translational modifications and protein processing.

Long Abstract:

Gluconacetobacter diazotrophicus is a nitrogen-fixing bacteria found as an endophyte in roots, stems and leaves. It was first isolated from sugar cane, but has been found in association with coffee plant, sweet-potato and pineapple. G. diazotrophicus is of great interest not only due its capacity to assimilate N2, but because it is a good model to study symbiosis of the bacterium with plant. Moreover, besides its economical importance to increase crop productivity, a better understanding of its physiology will allow the development of more efficient methods for its inoculation into vegetal species and others biotechnological approaches.

In order to generate an extensive proteomic portraiture of G. diazotrophicus PAL5 a 2D-GE and Mass Spectrometry were used to analyze whole cell lysate proteins. The almost 600 proteins identified make up a reference map, which could be used in a comparative proteomic study to identify members of the nitrogen fixation process in G. diazotrophicus, at logarithmic and stationary phases of liquid cultures, both, in under nitrogen fixing and non-fixing conditions.

Computer-aided analysis of 2D gels, revealed complex proteomes, with a differential expression of about 40 proteins between the logarithmic and stationary phases of growth, both in fixing and non-fixing conditions. Moreover, at least 15 proteins were specifically induced in nitrogen fixing condition and 20 proteins in the non-fixing condition, in logarithmic phase of growth. Membrane associated processes appears to be of major importance for the bacterial metabolism, because, in all samples tested, many outer membrane proteins were detected by SDS-PAGE.

Mass spectrometry has allowed us to identify about 80 proteins from 2D gels, including a gluthatione synthetase, an important enzyme of aminoacids metabolism, and ModC, an ATPase of the molybdate transport system, which may helps couple of ATP hydrolysis to active molybdate transport. Most of identified proteins were categorized as members of energy and aminoacid metabolism and translation. Nitrogen fixation regulatory proteins, a transketolase and a bacteriocin were also found.

Proteomic analysis has permitted the validation of genome predicted ORFs and hypothetical proteins. Moreover, tryptic peptide sequences could be used not only to safely establish open reading frames but, also, translational start and stop codons, what greatly helps

genome annotation. Additionally, proteomic analysis by two dimensional gel electrophoresis (2D-GE) followed by mass spectrometry (MS) provides information on post-translational modification of proteins, such as phosphorylation, acetylation and glycosylation.
Finally, a complementary structural investigation was performed by homology modeling of identified sequences, for instance, we propose structural models of almost all proteins involved in the glutamate biosynthesis pathway. It is important to note that the determination of Gluconacetobacter diazotrophicus complete genome sequence (project in progress) will contribute to a better proteome annotation and study of proteins from the point of view of their structure and expression.