Volume 54(1):59-73, 2010
Acta Biologica Szegediensis
http://www.sci.u-szeged.hu/ABS

DISSERTATION SUMMARIES

Monitoring the biogas producing microbes

Norbert Ács

Department of Biotechnology, University of Szeged, Szeged, Hungary, Institute of Biophysics, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary

Nowadays, biogas is one of the most important renewable energy carrier. It is produced in many countries and many facilities to treat biological wastes and to produce heat, biofuel and electricity from it. There is significant potential in replacing fossil fuels with biogas in various areas of our energy consumption, particularly as it combines the benefits of organic waste treatment with renewable energy production.

Biogas is produced by a special microbial population, which can be classified into three groups. The first one is the hydrolyzing bacteria; they cut the long biopolymers into smaller pieces. The acetogenic bacteria comprise the second group. They use mono- or oligosaccharides, lipids and amino acids to produce volatile fatty acids and hydrogen. Finally, the methanogenic archeabacteria utilize the volatile fatty acids and the product is biogas, *i.e.*, a mixture of CH_4 and CO_2. In order to increase biogas production and to improve the economical viability of this technology, it is very important to understand the relationship between these microbial populations and the rate limiting molecular events. This information can be collected via molecular biological techniques. Several approaches are employed. First, we developed a method for quantitative identification of a single bacterium in the biogas generating microbial population that invokes Real-Time PCR. The target microbe was the thermophilic bacterium, *Caldicellulosiruptor saccharolyticus*. Two unique genes, which code for proteins characteristic of this organism were selected. These were Ech (similar to *Escherichia coli* hydrogenase-3), and the Cel (cellulase). Successful experiments were carried out with both targeted genes from samples, originated from biogas fermentors. The other bacterium for our studies was the mesophilic eubacterium, *Enterobacter cloacae*. In this case the target gene was coding for one of the large subunit hydrogenase of this microbe, HycE. The detection of this bacterium was also possible, using whole extracted DNA from the liquid samples.

We have also shown that T-RFLP in capillary gel electrophoresis, combined with the conventional cloning-sequencing is a promising way for quantitative and qualitative monitoring of the biogas producing consortia.

Metagenomic methods are used for the identification of novel genes and pathways implicated in biomass degradation and biogas formation. In order to achieve a high yield of prokaryotic DNA, bacteria are extracted from the anaerobic fermentation using methods already available. The DNA samples are independently pooled and used for DNA sequencing and for the construction of metagenomic libraries. DNA sequences are used to identify the biodiversity of genes involved in organic substrate degradation. Metagenomic mass sequencing also lowers the amount of sequencing of clones isolated from metagenomic libraries. For sequencing we use a strategy based on pyrosequencing in order to obtain long (average 400-500) nucleotides, combined with sequencing using SOLiD and Solexa platforms that yield a huge number of short high-quality sequences. Beside the sequence based searches, we will also perform functional screening. Metagenomic sequencing will result in a large database that will include genes and pathways interesting for other biotechnological application. These databases will be screened to search for genes encoding esterases, lipases, proteases, phytases, cellulases, lignolytic enzymes involved in the decomposition of organic waste streams.

Bagi Z, Ács N, Bálint B, Horváth L, Dobó K, Perei K, Rákhely G, Kovács KL (2007) Biotechnological intensification of biogas production. Appl Microbiol Biotechnol 76:473-482.

Bagi Z, Perei K, Rákhely G, Kovács KL (2004) Biotechnological procedure for the intensification of biogas production in a thermophilic system. Hungary. Patent number: P0402444/2004.

Kovács KL, Bagi Z, Ács N, Perei RK, Imre E, Telekes G, Bartha I (2009) Biotechnological methods to increase landfill gas production and degradation of organic waste. Proc. 3rd Int. Workshop "Hydro-Physico-Mechanics of Landfills". Braunschweig, Germany, March 10-13 (2009)

Supervisor: Kornél L. Kovács
E-mail: acsn@brc.hu

Expression analyis of the *hup* genes encoded a NiFe hydrogenase in *Thiocapsa roseopersicina*

Tímea Balogh

Department of Biotechnology, University of Szeged, Szeged, Hungary

Thiocapsa roseopersicina is Gram-negative, phototrophic purple sulphur bacterium, which belongs to the family of Chromatiaceae. There are four active [NiFe] hydrogenases in the cells, which differ in their function, localization and stability. Two of them are membrane-associated [NiFe] hydrogenases (Hyn and Hup), while the other two are soluble hydrogenases (Hox1, Hox2). HynSL shows extraordinary stability and it catalyzes either H_2 uptake or H_2 evolution. The other membrane-associated hydrogenase (Hup) plays a role in hydrogen uptake (hup=hydrogen uptake) exclusively. The soluble hydrogenases of *Thiocapsa roseopersicina*, Hox1 and Hox2 are bidirectional NAD^+