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DISSERTATION SUMMARIES

Monitoring the biogas producing microbes

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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.

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

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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^+

reducing hydrogenases (Kovács et al. 2005; Maróti et al. – in press). Furthermore the bacterium possesses nitrogenase activity, and the atmospheric N_2 fixing is accompanied by H_2 evolution.

The *hup* locus consists of seven genes (*hupSLCDHIR*). Some of these genes are well characterized, *hupC* encodes a cytochrome b-type protein involved in electron transfer (Palágyi-Mészáros et al. 2009), while *hupD* codes for an endopeptidase which plays a role in the maturation of the large subunit (HupL). HupR is a regulatory protein, a part of a transcriptional regulatory system. However little is known about the transcriptional organization and regulation of *hup* gene, function of *hupH* and *hupI* gene products.

According to the literature, HupH protein is required for the translocation of the H_2ase stuctural protein to the membrane by bonding to the small subunit, while HupI is a rubredoxin-type protein plays a role in the electron transfer (Manyani et al. 2005).

In order to investigate the role of *hupH* and *hupI* genes in frame deletion mutants were created and the phenotypical effects of the mutations were analyzed by measuring *in vivo* and *in vitro* hydrogenase enzyme activity. Results showed that the absence neither of HupI nor HupH cause a significant decrease in Hup uptake activity.

The transcription of *hup* genes was investigated by reverse transcription coupled PCR, the results showed that *hupSLCDHIR* genes transcribe as a whole transcript.

Expression level of the *hup* genes was measured by quantitative real-time PCR in cells grown on various medium. Under nitrogen fixing conditions an enhanced *hup* mRNA level was observed, which indicates that the physiological function of Hup is somehow linked to the activity of nitrogenase enzyme complex. In standard non-nitrogen fixing growth conditions the *hupSL* transcription downregulated by both thiosulfate and succinate and upregulated by the inactivation of HupC. Therefore it was hypothesized that the redox status of the membrane/quinone pool controls the expression level of Hup hydrogenase.

To identify regulatory proteins which control the *hup* expression, mini Tn5 transposon based mutagenesis was carried out. A screening procedure was developed for identification of strains having Hup hydrogenase activity when the quinone pool is overreduced. *In vitro* hydrogenase uptake activity measurments were showed an appreciably increased Hup activity in the mutant and this points to the fact that the insertion of the transposon inactivated a gene which encodes a protein likely involved in the redox control of the expression of Hup hydrogenase.

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Clarifying the mechanism of T-cell apoptosis induced by cell-derived or low and high concentration of soluble recombinant galectin-1

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Galectin-1 (Gal-1) is a mammalian lectin with β-galactoside binding activity. It is expressed by numerous cell types and binds to cells and extracellular matrix components presenting glycoconjugates of N-acetyl-lactosamine. The most prominent biological function of Gal-1 is its anti-inflammatory effect which is predominantly exerted by induction of apoptosis of Th1 cells (1). Many studies have emerged analyzing Gal-1 signal transduction mechanism during T-cell apoptosis. However these data have resulted confusing knowledge due to using soluble recombinant protein although Gal-1 exerts its physiological function bound to the producing or neighboring cells or extracellular matrix components.

We have aimed to resolve this controversy by comparing cell death induced by low (1.8 μM, lowGal-1) and high (18 μM, highGal-1) concentration of soluble Gal-1. We show that lowGal-1 and highGal-1 trigger phosphatidylserine exposure, generation of rafts and mitochondrial membrane depolarization. In contrast, lowGal-1 but not highGal-1 are dependent on the presence of p56lck and ZAP70 and activates caspase cascade. The results allow the conclusion that the cell-death mechanism strictly depends on the concentration of Gal-1 (2).

Recombinant Gal-1 is always manipulated during purification and in apoptosis assays since it has to be in reduced form for functional conformation. To avoid this process we analyzed the role and mechanism of cell-derived Gal-1 in the apoptotic process. In co-culture system Gal-1 remains as a native, functional protein without any chemical modification and the apoptosis assay also avoids addition of reducing agent. We applied co-cultures of various cell lines producing Gal-1 as effectors and T-cells (activated peripheral blood cells or