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Biohydrogen production using the cellulose containing plant biomass

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There are many serious gobal problems caused by mankind in the last dacades. Among these, two have oustanding importance: a) the enviromentally friendly, biological degradation of the large amounts of organic waste poduced by the industrial sector; b) as well as the reinforcement of the energy supply by renewable energy source and the substitution of the current energy carriers by environmentally sound fuels. Hydrogen is considered as the best candidate for the future energy carriers, since just pure water is formed during its oxidation. There are biological tools for producing hydrogen and hydrogen evolving photosynthetic or fermentative microbes are primarily involved in these processes. In the dark fermantative processes, usually biopolymers of agricultural origin used to be the substrate, which have to be first converted to simpler monomers being fed to the hydrogen producing bacteria. Strains capable to convert and utilize complex biopolymers are of extrme importance. One of the best candidates is the Gram positive, hyperthermophilic, anaerobic *Caldicellulosiruptor saccharolyticus.* (Bagi et al. 2007) Its biotechnological importance is that it is capable to degrade cellulose-based biomasses, such as paper, or energy plants (which are found in the large quantities) and has hydrogenases for removal of excess electrons formed during the fermentative metabolism. The genome of this bacterium is available and the strain was shown to possess not only numerous glycosidases required for the hydrolysis of different kinds of polysaccharides, but also hydrogenase enzymes responsible for hydrogen production.

In our group an enviromentally friendly biological method have alredy been developed by wich we waste of animal origin could be transformed to hydrogen. (Bálint et al. 2005.)

In my work I aim at adapting this process to cellulose-based waste of plant origin. Morover,on the basis of the known genome of this organism, I intend to create a genetically modified strain which is capable of degrading the avaible biomass (currently filter paper) more efficiently and thereby producing significantly higher amounts of hydrogen.

According to our aims, I studied the hydrolysis of untreated filter paper in batch fermentation conditions in the presence of six various kinds of sugar using minimal media containing no other carbon sources. In paralel, I monitored the hydrogen evolution from sugars and filter paper alone and various combinations.

My results clearly showed, that *C. saccharolitycus* was able to degrade the untreated filter paper and to produce abundance hydrogen from this material in the presence of minimal amount of sugar. From the sugar specificity, it is likely that these sugars are basically necessary for the induction of cellulase enzymes., thus paper decomposition can be promoted by sugars.

For better understanding the molecular background of the events and for further improvement of the process genetically modified strains should be constructed. However, no protocol is available for gene transfer into *C. saccharolyticus*. In order to introduce foreign DNS into the cells, a functional genetic system has to be developed. As a first step, the efficient plating technique was ameliorated and numerous suitable vectors (replicative and non replicative) were collected and constructed to achieve sustainable vector replication or to modify the given part of the genome. Furthermore the development of an efficient method for introduction of foreign DNA is also requisite.

My future plans are the optimization of the paper decomposition conditions, getting deeper insight into the the molecular mechanism of the paper/sugar metabolism → hydrogen conversion.

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

Bálint B, Bagi Z, Tóth A, Rákhely G, Perei K, Kovács KL (2005) Utilization of keratin-containing biowaste to produce biohydrogen. Appl Microbiol Biotecnol 69:404-410.

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Construction and characterization of synthetic genetic oscillator in yeast

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The design of gene expression systems with both spatial and temporal regulation has been an area of intense scientific interest during the last ten years. Our aim is to create a synthetic genetic circuit in yeast based on transcriptional – translational feedback loops mimicking the structure (input, oscillator, output) and function (self-sustaining oscillation and resetting) of eukaryotic circadian clocks. The circuit will serve as an ideal test system for mathematical modeling describing oscillatory mechanisms (eg. circadian clocks), since all components are known, well characterized and can be easily modified/adjusted in order to test predictions from the model. The main requirements for such a system are: a genetic network with well defined components, which do not interfere with the physiology of yeast; option to set/modify the expression level/turn-over rates of components, proper input/resetting mechanism and easily measurable output (in vivo, real-time).

First we created and tested components for the input pathway of the oscillator. In the circadian systems of eukaryotes, external signals (e.g. light and/or temperature) reach the oscillating genetic network through the input pathway and cause an acute change in the expression

or stability of one or more oscillator components, which consequently resets the phase of the oscillation. In our system, light act as a resetting signal via an artificial light switch. The switch is based on the light-dependent interaction between the plant photoreceptor phytochrome A (PHYA) and its specific interacting protein FAR-RED ELONGATED HYPOCOTYL 1 (FHY1) [Hiltbrunner, 2005]. The activator and the DNA-binding domain of the GAL4 transcription factor are fused to FHY1 and PHYA, respectively, so transcription from GAL4-dependent promoters is activated only by the physical interaction between PHYA and FHY1 in mutant yeast cells lacking the endogenous GAL4 protein. Red light converts PHYA to its active form, which interacts with FHY1; however, far-red light diminishes the interaction, because it converts PHYA to its inactive conformer. To test the function of the light switch we measured expression of the GAL1 promoter driven luciferase reporter gene (GAL1:LUC+). We showed that luciferase activity is tightly controlled by red or far-red light pulses indicating the proper function of the light switch.

The core components of the oscillator (termed "Yeascillator") are represented by two artificial genes whose gene products can mutually regulate transcription of each other. Expression of the positive protein [Gari, 1997] is driven by a modular promoter, which contains cis-elements for copper induction and for binding of the negative component protein. The basal activity of this promoter is controlled by copper in the media. The positive protein is a fusion between the tetracycline-responsive transactivator (tTA) and the YFP proteins. tTA-YFP is able to bind to a specific cis-element (*tetO*) built in the promoter of the second gene encoding the negative protein. Binding of tTA-YFP can be controlled by doxycycline and results in the activation transcription. The negative protein consists of the DNA-binding domain of the bacterial LexA protein, the yeast transcriptional repressor SSN6 and the CFP proteins. LexA-CFP-SSN6 binds to the modular promoter of the first gene via specific LexA binding sites and represses transcription. The different fluorescent protein tags (YFP and CFP) allow simultaneous detection and quantification of the positive and negative protein components. The output of the oscillator is represented LUC+ reporter gene controlled by a promoter, which responds to the positive component only. Our preliminary results indicate that the two genes can regulate each other as expected, but detection of oscillation will require more optimizations.

Garí E, Piedrafita L, Aldea M, Herrero E (1997) A set of vectors with a tetracycline-regulatable promoter system for modulated gene expression in Saccharomyces cerevisiae. Yeast 13:837-848.

Hiltbrunner A, Viczian A, Bury E, Tscheuschler A, Kircher S, Toth R, Honsberger A, Nagy F, Fankhauser C, Schafer E (2005) Nuclear accumulation of the phytochrome A photoreceptor requires FHY1. Curr Biol 15:2125-2130.

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Regulation of single spike initiated feed-forward networks through 5-HT-2 receptors in the human and rat cerebral cortex

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The performance of the human cerebral cortex is unparalleled by the nervous system of other species and this is presumably supported by refined, but largely unknown features of the human microcircuit. We have shown that single action potentials in pyramidal cells can trigger reliable and stereotyped series of multiple postsynaptic potentials in simultaneously recorded pyramidal cells and interneurons in the human cerebral cortex. These polysynaptic event series are composed of alternating excitatory and inhibitory postsynaptic potentials lasting up to tens of milliseconds (Molnar, Olah et al. 2008).

We tested how these complex network events could be affected by the endogenous neurotransmitter serotonin known to be involved in several physiological processes, and implicated in many psychiatric disorders (Jones and Blackburn 2002). We recorded from pairs, triplets and quadruplets of neurons in slices of human association cortices looking for mono- and polysynaptic connections. Nanomolar concentrations of serotonin reversibly supressed single pyramidal spike activated di- and polysynaptic events, and this effect could be mimicked by the 5-HT-2 receptor agonist alpha-methylserotonin. Similarly, alpha-methylserotonin was effective in eliminating axo-axonic cell triggered polysynaptic but not disynaptic events.

We then investigated the effect of serotonin on monosynaptic unitary connections between various types of layer 2/3 neurons. We found that serotonin and alpha-methylserotonin decreased the amplitude of EPSPs between pyramidal cells and from pyramidal to various types of interneurons including fast-spiking basket and axo-axonic cells but little or did not change the amplitude of IPSPs from fast-spiking to pyramidal neurons.

To examine the mechanism by which serotonin might modulate excitatory transmission, we analysed the percentage of failures to evoke an EPSP and the coefficient of variation of unitary EPSP amplitudes with and without serotonin and alpha-methylserotonin. Both serotonin and alpha-methylserotonin increased the failure rate and the coefficient of variation suggesting a presynaptic site of modulation of serotonin in the glutamatergic synaptic transmission.

Finally, we found that therapeutic concentrations of the serotonin reuptake inhibitor fluoxetine, a widely prescribed medication for treatment of depression, could enhance the effect of serotonin on excitatory synaptic transmission.