Screening for Bio-H_2 production by cyanobacteria isolated from the Baltic Sea and Finnish lakes

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Cyanobacteria are capable of harnessing solar energy and converting it into chemical energy of carbohydrates in oxygenic photosynthesis. Under specific conditions, they can use solar energy to produce also molecular hydrogen. Cyanobacteria have three different enzymes that are directly involved in H_2 production.

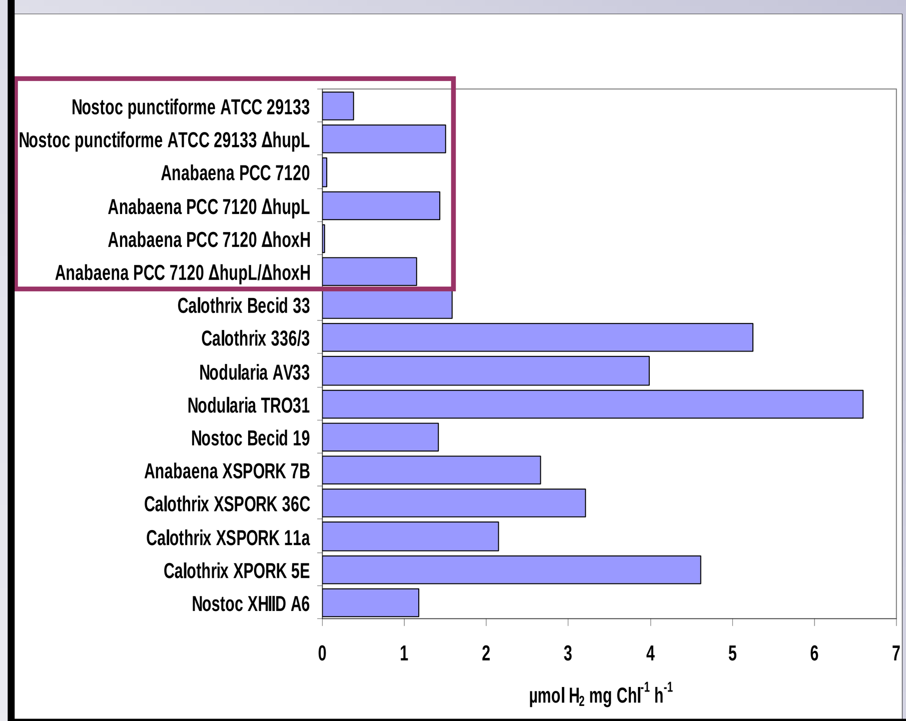
Bidirectional hydrogenase (*hox*) has the ability to function both in uptake and in production of H_2. Bidirectional hydrogenases are pentameric, made of the hydrogenase moiety (HoxYH) and the diaphorase moiety (HoxEFU). The HoxEFU subunits are homologous to subunits of complex I of mitochondrial and bacterial respiratory chains. Uptake hydrogenases consisting of HupS and HupL subunits are present in all nitrogen fixing cyanobacteria strains. The uptake hydrogenase recycles the molecular hydrogen produced by nitrogenase in nitrogen fixation.

Nitrogenase produces hydrogen as a byproduct in nitrogen fixation. Mo-nitrogenase is made up of two parts, dinitrogenase consisting of two subunits each of NifD and NifK, and dinitrogenase reductase, consisting of two subunits of NifH. *Anabaena variabilis* possesses two Mo-containing nitrogenases and also alternative vanadium(V)-containing nitrogenases (*vnf*).

Biodiversity among cyanobacteria for natural H2 production has not been efficiently screened despite extensive research on H2 production by microorganisms in general. Here we report the screening for H2 production of 400 cyanobacterial strains isolated from the Baltic Sea and Finnish lakes. Approximately 50% of these strains produced detectable amounts of H2. Each of the ten best H2 producers belongs to N2-fixing filamentous strains, eight of them are benthic and only two are planktonic strains.

The effect of different environmental factors, such as light intensity, cell density, pH and temperature on H2 production rate was studied in the two good H2 producers, *Calothrix* strains 336/3 and XPORK 5E. Notably, changing of environmental factors had differential effects on H2 production depending on the strain.

The rate of H_2 production of the ten best H_2 producers discovered during the screening process



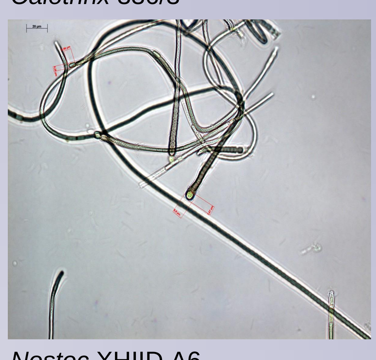
Most of the H_2 producers were N_2-fixing filamentous strains, of 100 best producing strains 51 were planktonic, 46 benthic, and 3 soil/lichen associated. Only 1 unicellular and 1 non-N_2 fixing filamentous strain produced H_2. The highest H_2 production rates generally occurred under microaerobic/light conditions.

Figure demonstrates the rate of H_2 production of the 10 best H_2 producers discovered during the screening process. Uptake hydrogenase mutants of Nostoc punctiforme ATCC 29133[(1)] and Anabaena PCC 7120[(2)] were used as positive controls in experiments.

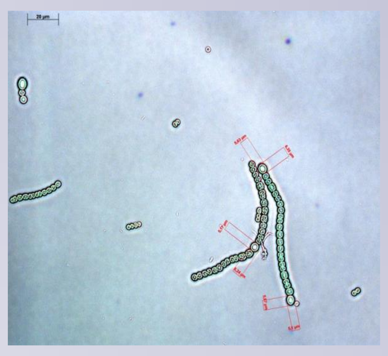
The origin of top 10 'superior' H_2 producers

Strain	Type	Origin	Habitat	Medium
Calothrix 336/3	filamentous	L. Enäjärvi, Laukilanlahti,	benthos	Z8X
Calothrix BECID 33	filamentous	Gulf of Finland, the Baltic Sea	benthos	Z8X
Nodularia AV33	filamentous	Brackish water, the Baltic Sea	plankton	Z8XS
Nodularia TRO31	filamentous	Brackish water, the Baltic Sea	plankton	Z8XS
Nostoc Becid 19	filamentous	Vuosaari, the Baltic Sea coast	benthos	Z8X
Nostoc XHIID A6	filamentous	L. Hiidenvesi, Kirkkojärvi	benthos	Z8X
Calothrix XPORK 5E	filamentous	Porkkala cape, the Baltic Sea coast	benthos	Z8X
Calothrix XSPORK 11A	filamentous	Porkkala cape, the Baltic Sea coast	benthos	Z8XS
Anabaena XSPORK 36C	filamentous	Porkkala cape, the Baltic Sea coast	benthos	Z8XS
Anabaena XSPORK 7B	filamentous	Porkkala cape, the Baltic Sea coast	benthos	Z8XS

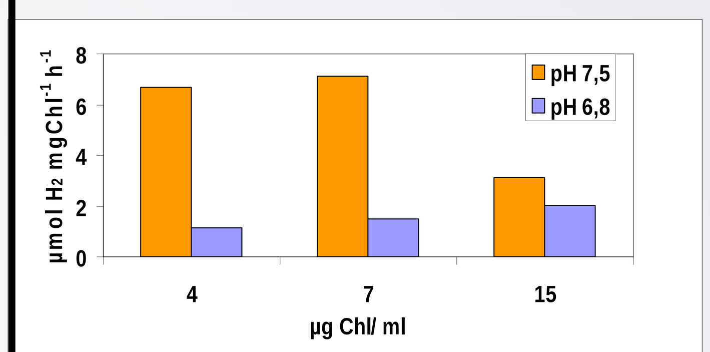
Calothrix 336/3



Nostoc XHIID A6

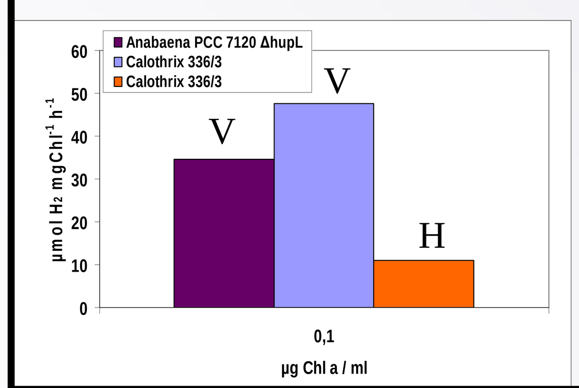


The effect of pH on the H_2 production rate of *Calothrix* XPORK 5E



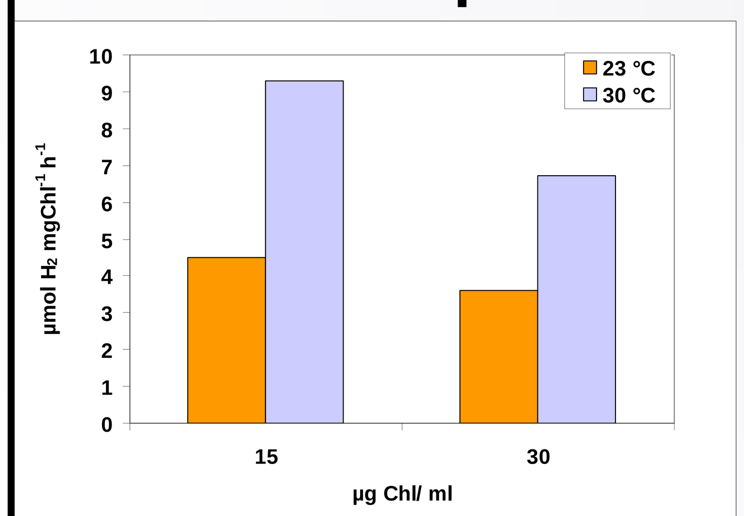
Calothrix XPORK 5E strain did not grow at pH 8.2 and grew relatively slowly at pH 6.8. At pH 6.8 Calothrix XPORK 5E produced much less H_2 compared to that at optimal pH 7.5.

The H_2 production rate of *Calothrix* 336/3 after optimizing the conditions



Calothrix 336/3 produced H_2 in much higher rate in vials incubated in horizontal position with larger surface area for light perception, more equal light distribution and more intensive mixing of the gas phase than in vials in vertical position.

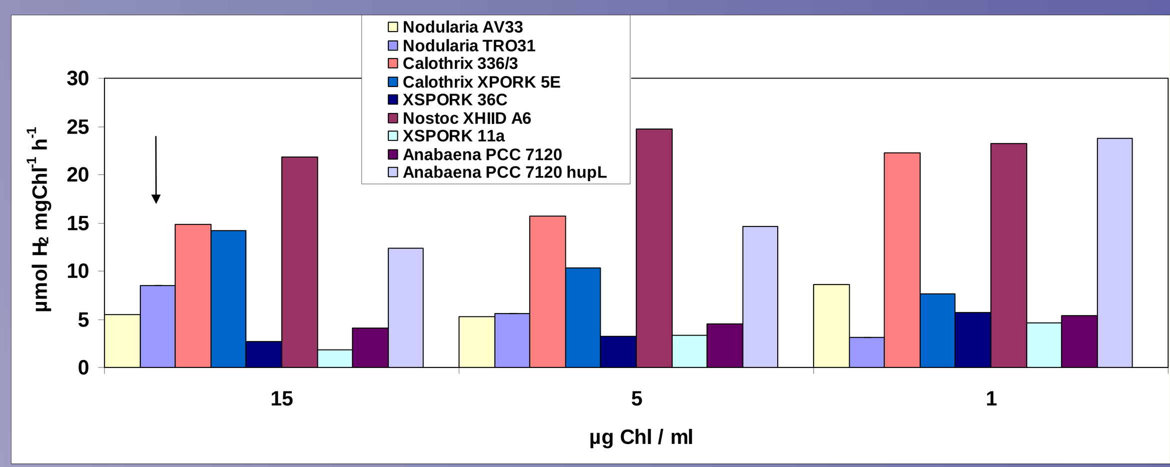
The temperature effect on the H_2 production rate in *Calothrix* 336/3



By increasing the temperature of the H_2 determination assay from 23°C to 30°C the H_2 production rate of this strain was stimulated almost two fold.

Ref: 1) Lindberg P., Schutz K., Happe T. & Lindblad P. Int. J. Hydrogen Energy 2002: 27, 1291-1296
2) Masukawa H., Mochimaru M. & Sakurai H. App. Microbiol. Biotech. 2002: 58, 618-624

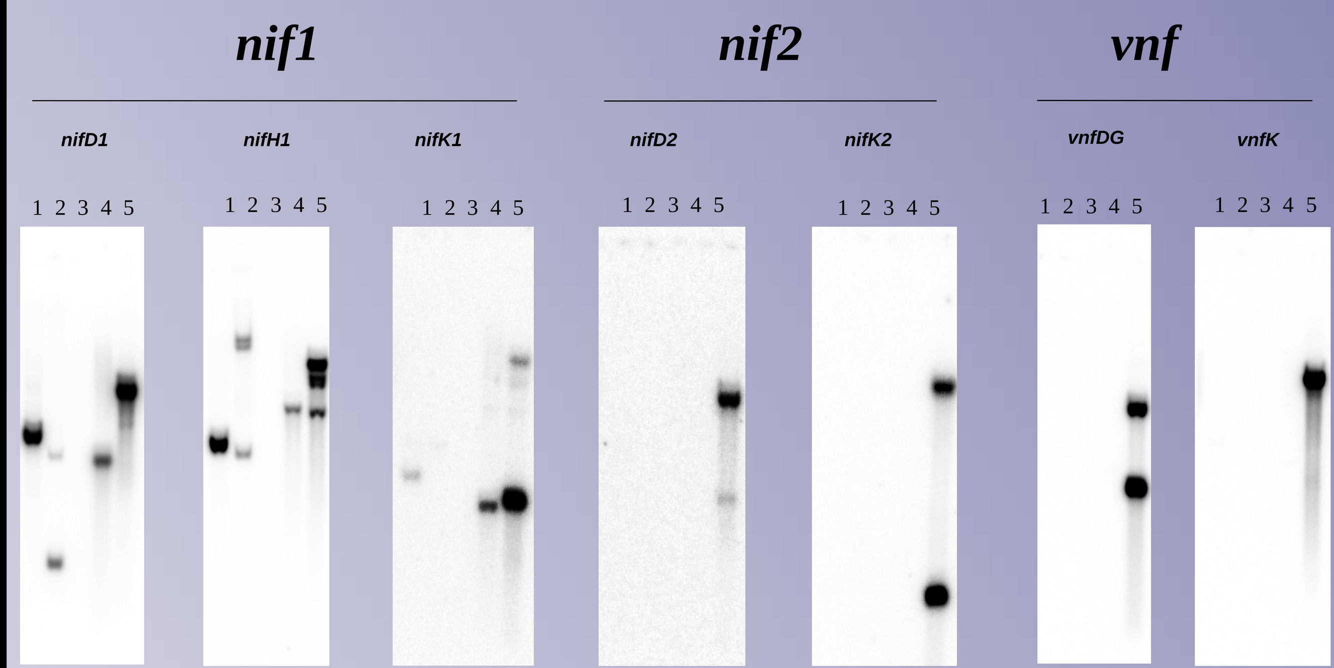
The effect of decreasing cell density on the H_2 production rate in more optimized conditions



The H_2 production rate of most of the strains so far examined increases with lowering the cell density of the cultures. Nevertheless, some planktonic strains, like Nodularia TRO31 demonstrated different behavior and lowering of the cell density of the cultures did not change or even lowered the H_2 production rate due to sensitivity to increased light intensity.

Detection of *nif1, nif2* and *vnf* genes by Southern hybridization

Southern hybridization of genomic DNA with nif1, nif2 and vnf probes showed the presence of nif1 Mo-nitrogenase in both Calothrix XPORK 5E and Calothrix 336/3, and absence of nif2 and Vanadium nitrogenase in both strains.



1- *Calothrix* 336/3

2- *Calothrix* XPORK 5E

3- *Synechocystis* 6803, negative control

4- *Anabaena* 7120

5- *Anabaena varialibilis*, positive control

Detection of *hup* and *hox* genes by Southern hybridization

M – 1 Kb ladder
1- *A.variabilis* intact DNA
2- *A.variabilis* digested
3- *Calothrix* 336/3 intact DNA
4- *Calothrix* 336/3 digested
5- *Calothrix* XPORK 5E intact DNA
6- *Calothrix* XPORK 5E digested

1- *A.variabilis* ATCC 29413
2- *Calothrix* XPORK 5E

Southern hybridization of genomic DNA with hup and hox probes showed the presence of uptake hydrogenase in both Calothrix 336/3 and Calothrix XPORK 5E, and the presence of bidirectional hydrogenase only in Calothrix XPORK 5E.

Future perspectives

Most of the superior strains with high H_2 production capacity, discovered by screening of the University of Helsinki Cyanobacteria Culture Collection (UHCC), are benthic microorganisms with high capacity to adhere to the solid substrates. In collaboration with Dr. Tsygankov's group (Institute of Basic Biological Problems, Pushchino, Russia) we will apply immobilization and continues culture techniques to these strains in order to further improve their H_2 production activity.

We are taking a systems biology approach to characterise these strains in detail for maximising the H_2 producing capacity. The sequencing of the genomes of the two novel 'superior' strains, will be performed by submission them to the next-generation sequencing system SOLiD™ in collaboration with researchers in the Center of Biotechnology (BTK), Biocity, Turku. In long run, this will give us an opportunity to further improve the H_2 production capacity of these strains by genetic engineering techniques.