Isolation of strains from a bio-hydrogen plant: Novel [FeFe]-hydrogenases for Exploitation in Biotechnology

Isolation of strains from a bio-hydrogen plant: Novel [FeFe]-hydrogenases for Exploitation in Biotechnology

Francesca Valetti[1]*, Simone Morra[1], Mariaconcetta Arizzi[1], Veronica Sarasso[1], Sheila J. Sadeghi[1], Barbara La Licata[2], Fabio Sagnelli[2], Paola Zitella[2] and Gianfranco Gilardi[1]

[1]Department of Life Sciences and Systems Biology, University of Torino, Torino, Italy. [2]Bioenergy Lab, Enviroment Park, Torino, Italy

*francesca.valetti@unito.it

INTRODUCTION

Hydrogen is considered a promising fuel that can replace fossil fuels in the future. The ability of various microorganisms to produce hydrogen from low cost waste materials is of high interest (Kapdan 2006) and several bioreactors and pilot plants are being tested (La Licata 2011). Nevertheless, very often the microbiological consortium remains uncharacterized. Moreover, there is much interest in the identification of new [FeFe]-hydrogenases, enzymes responsible for H_2 evolution. [FeFe]-hydrogenases can then be used as very efficient catalysts for the development of bio-hybrid devices that produce H_2 (Cracknell 2008).

The main aim of this study is to characterize the culturable bacteria in the sludge from a pilot plant fed with waste materials that produces efficiently high amount of hydrogen. The best hydrogen producers are then selected for gene expression studies. Five new previously uncharacterized [FeFe]-hydrogenases are cloned, recombinantly purified and exploited in the construction of a biotechnological device.

PILOT PLANT

The pilot plant was designed and built to produce biohydrogen from waste materials taking into consideration the environmental and economical feasibility.

It was fed with a mixture of fruit and vegetables scraps from unsold stocks. The optimised process granted high percentage of hydrogen with good yield.

Figure 1. Pilot plant.

Average Productivity	0.72 L/L day
Productivity	0.9 L/h
Temperature	Room temp.
HRT	30 h
H_2 max	54 %
Yield	45 mL H_2/g VS
pH	5-6

Table 1. Parameters and yield for optimized dark fermentation.

BACTERIAL CONSORTIUM CHARACTERIZATION

The culturable bacteria were isolated and identified by means of 16S rDNA gene analysis. Each species was tested for its ability to produce hydrogen.

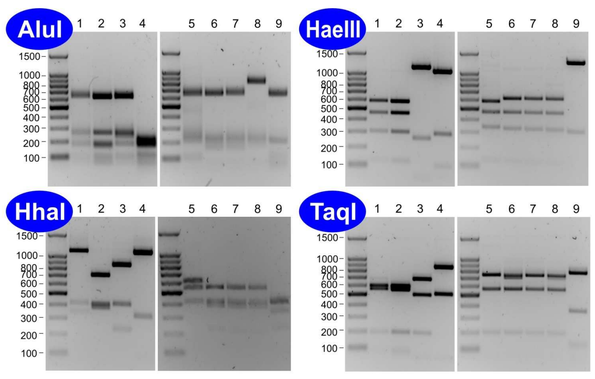


Figure 2. 16S rDNA gene RFLP of the 9 isolated bacteria.

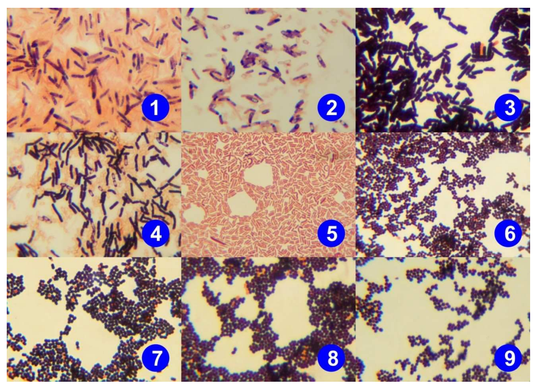
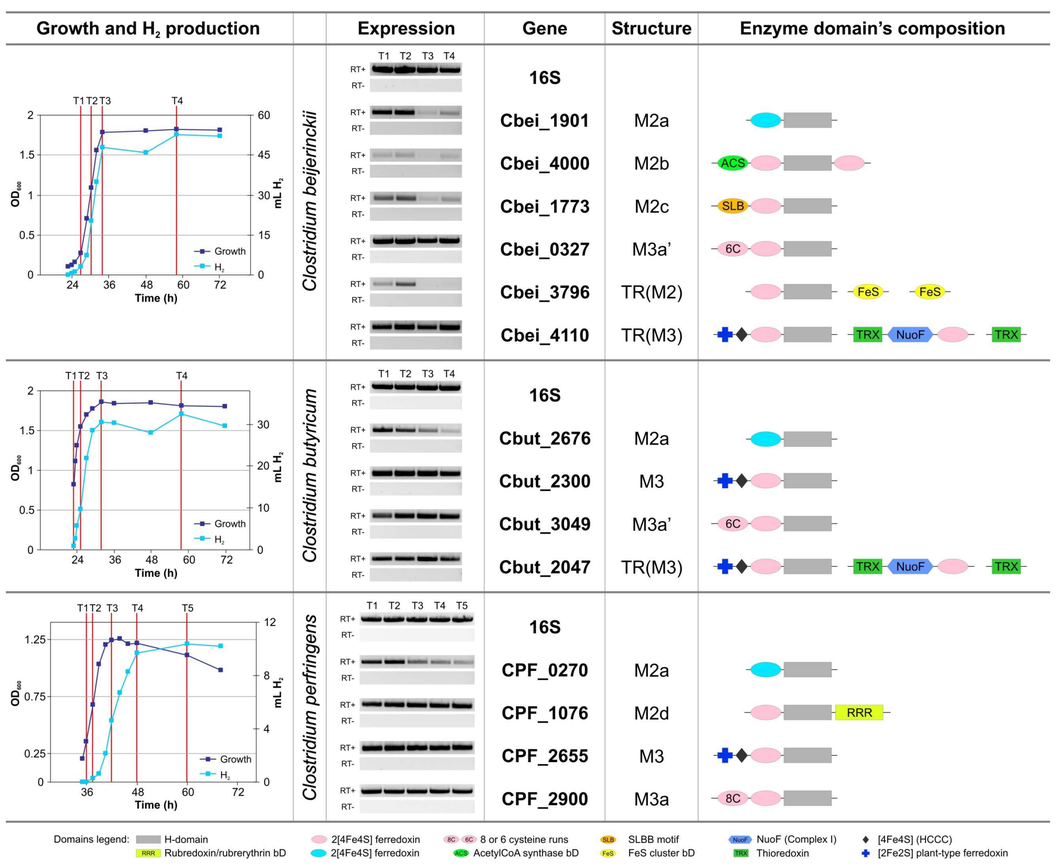


Figure 3. Gram staining of the 9 isolated bacteria.

ID	Species	Morphology	Gram	O_2	Kan	mL H_2/g glucose
1	*Clostridium beijerinckii*	bacilli	+	-	+	247.2 ± 9.6
2	*Clostridium butyricum*	bacilli	+	-	+	136.3 ± 4.6
3	*Clostridium perfringens*	bacilli	+	-	+	130.6 ± 3.0
4	*Clostridium bifermentas*	bacilli	+	-	-	97.1 ± 9.2
5	*Lactobacillus plantarum*	bacilli	+	+	+	n.d.
6	*Enterococcus sp.*	cocci	+	-	-	n.d.
7	*Enterococcus devriesei*	cocci	+	-	-	n.d.
8	*Enterococcus sp.*	cocci	+	+	+	n.d.
9	*Staphylococcus hominis*	cocci	+	+	-	n.d.

Table 2. Summary of the 9 bacteria identified in the H_2 producing sludge. n.d. = not detectable.

[FeFe]-HYDROGENASE GENES EXPRESSION STUDY



[FeFe]-HYDROGENASE CLONING AND RECOMBINANT EXPRESSION

Five genes encoding for previously uncharacterized [FeFe]-hydrogenases were cloned into a specific vector suitable for the recombinant expression in *E. coli* (King 2006): Cbei_1773, Cbei_0327, Cbei_1901, Cbei_4110 (from *C. beijerinckii*) and CPF_2655 (from and *C. perfringens*).

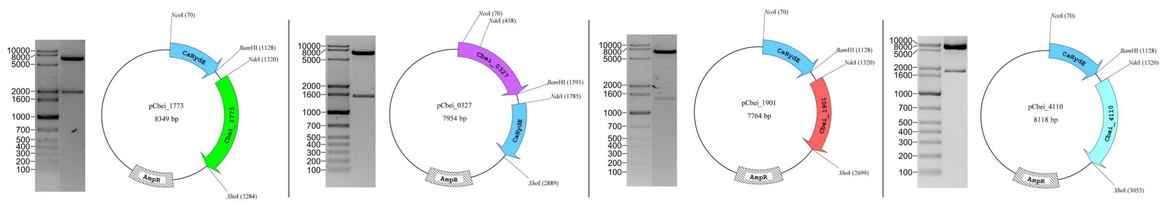


Figure 4. Expression vectors with the five cloned genes.

Three of these genes could be readily expressed in the active form and two of them have been purified by affinity chromatography under strict anaerobic conditions. The two purified proteins catalyzed H_2 evolution at a rate of: CPF_2655 and Cbei_1773.

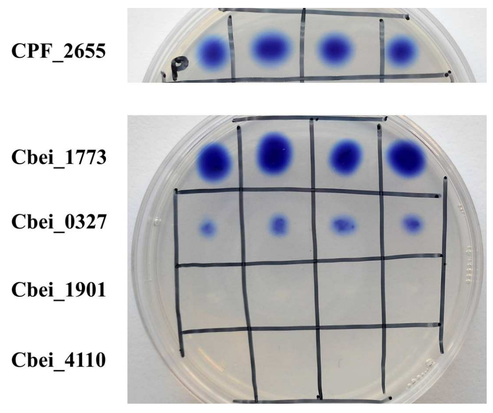


Figure 5. Activity test of the five cloned genes in the *E. coli* recombinant expression system.

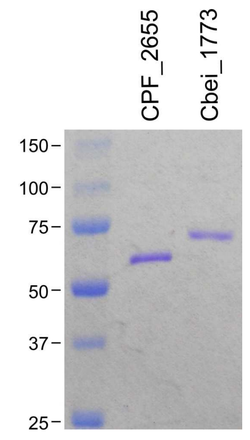


Figure 6. Coomassie stained SDS-PAGE of the two purified enzymes: CPF_2655 (64.7 kDa) and Cbei_1773 (73.1 kDa).

EXPLOITATION: ENZYME IMMOBILIZATION ON TiO_2 ELECTRODES

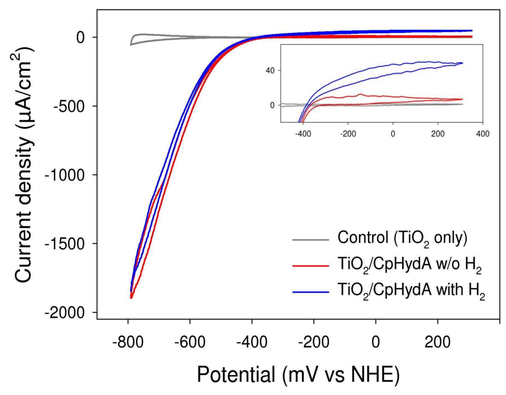


Figure 7. Cyclic voltammetry of CpHydA on TiO_2 electrodes. Voltammograms were acquired under nitrogen atmosphere in the presence or in the absence of H_2 to observe both reductive and oxidative catalytic currents (inserts).

CpHydA was immobilized by adsorption on TiO_2 electrodes. Cyclic voltammetry was used to demonstrate CpHydA immobilization, with specific electron transfer; in particular, high reductive catalytic currents due to H^+ reduction into H_2 were observed.

Hydrogen evolution was demonstrated applying a fixed potential (-741 mV vs NHE) to the electrode for 2 hours. Analysis of the gas phase by gas chromatography showed H_2 production with very high efficiency: 98±1 %.

CONCLUSIONS AND FUTURE PERSPECTIVES

The sludge from a pilot plant producing hydrogen in high percentage (up to 54%) from waste materials was found to contain a consortium of 9 different bacterial strains, 4 of which were hydrogen producing *Clostridia*. The expression of all the [FeFe]-hydrogenases of *C. beijerinckii, C. butyricum* and *C. perfringens* was studied by RT-PCR during the growth. Five previously uncharacterized [FeFe]-hydrogenases were cloned in a vector for the recombinant expression in *E. coli*. CPF_2655 and Cbei_1773 were affinity purified and CPF_2655 was exploited to develop a bio-inspired biotechnological cathode able to produce H_2 with extremely high efficiency.

In the future all the five enzymes will be purified and characterized to test their potential as *in vitro* H_2 producing catalysts.

REFERENCES

Broda DM *et al.,* **2000**, *J. Appl. Microbiol.*, 88, 107-116.
Cracknell JA *et al*., **2008**, *Chem. Rev.*, 108, 2439-2461.
King PW *et al*., **2006**, *J. Bacteriol.*, 188, 2163-2172.
Vignais PM *et al.*, **2007**, *Chem. Rev.*, 107, 4206-4272.
Calusinska M *et al*., **2011**, *Lett. Appl. Microbiol.*, 53, 473-480.
Kapdan IK *et al*., **2006**, *Enzyme Microbial Technol.*, 38, 569-582.
La Licata B *et al*., **2011**, *Int. J. Hydrogen Energy*, 36, 7861-7865.
Wang X *et al.,* **2007**, *J. Appl. Microbiol.*, 103, 1415-1423.

ACKNOWLEDGEMENTS

The Authors are grateful to Dr. P.W. King for the generous gift of the plasmids for expression systems in *E.coli.* A. Cordara and A. Belluati are acknowledged for partecipation in the Cbei_1773 purification.
The study was funded by projects BIOH2 and HyStrEM (EU structural funds N.1083/2006 P.O.R. "Competitività regionale e occupazione" F.E.S.R. 2007/2013 Asse 1 «Innovazione e transizione produttiva » Misura I.1.3 Innovazione e PMI)