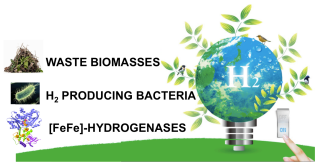
Characterisation of novel hydrogenases from waste biomasses for applications as H_2 producing catalysts

Mariaconcetta Arizzi
Università degli Studi di Torino, Dipartimento di Scienze della Vita e Biologia dei Sistemi
mariaconcetta.arizzi@unito.it; arizzi.mc@gmail.com
Tutor: Dr.ssa Francesca Valetti



Bio-hydrogen (bioH_2) is a promising alternative energy to the existing fossil fuels [1]. Today, several bioreactors are based on the ability of various microorganisms to produce bioH_2 during dark fermentation from low cost waste materials [2]. One of the most fascinating challenges for future applications is the identification, characterisation and engineering of microorganism and of their enzymes involved in this process. The [FeFe]-hydrogenases (H_2ase) are key enzymes in the H_2 metabolism [4] and represent a promising source of inspiration for artificial catalysts for high efficiency H_2 production and in fuel cells [3, 4]. Oxygen (O_2) sensitivity is the main factor hindering the applicative exploitation of H_2ases as, generally, exposure to O_2 leads to irreversible inactivation in few seconds. Several hundreds of sequenced genes can potentially express enzymes of this class, but so far, only a few of them have been characterised. In this perspective the aims of this project are: *i*) Production of bioH_2 from low cost materials or waste biomasses by dark fermentation; *ii*) Isolation of H_2-producingbacteria; *iii*) Identification and characterisation of novel H_2ase.

i) Compost samples and the raw material of composting process (green agricultural wastes), were tested as feedstock for H_2 and CH_4 productions. The materials were supplied by AgriNewTech srl (co-funding the study with CRT/Lagrange Scholarships 2014-15). The compost samples or green wastes could produce H_2 and/or CH_4, without any pre-treatment or inoculum. The water amount played a key role in the process, as it can push towards the alternative production of H_2 or CH_4 [5].

ii) The culturable microflora from autumnal green wastes able to produce H_2 was isolated and characterised. Two highly efficient H_2 producers *Clostridium beijerinckii* AM2 and *Clostridium tyrobutyricum* AM6 were identified. *C. beijerinckii* has 6 H_2ase genes annotated, *C. tyrobutyricum* as only one reported. These H_2ase have not been studied yet. The spring green waste was enriched with *C. beijerinckii* AM2 and *C. tyrobutyricum* AM6. The correlation between bioH_2 production and H_2ase genes expression was studied by RT-qPCR. The results showed that *C. beijerinckii* AM2 prevailed over *C. tyrobutyricum* AM6 and a high expression modulation of the 6 different *C. beijerinckii* H_2ase genes occurred in the first 23 hours, suggesting a complex interplay of alternative pathways involving H_2ase with different time, and mode of expression.

iii) Five previously uncharacterised H_2ases from *C. beijerinckii* SM10 [6] (CbA5H, CbB3H, CbB2H, CbA8H) and *C. tyrobutyricum* AM6 (CtA2H) were cloned, expressed in *E.coli* [7] and tested by *in vivo* activity assays. CbA5H, CbB3H and CtA2H were readily expressed in active form. The most interesting was CbA5H, where we found that O_2 present in air does not cause any irreversible damage to the enzyme. The catalytic active state H_{ox} can switch to the O_2-insensitive inactive state H_{inact}. The transition is reversible and can be performed several times without any activity loss or spectral influence. The use of CbA5H (or engineered enzymes with similar properties) would make the exploitation in real applications much simpler and pave the way for highly efficient biocatalysts-based H_2-producing devices [8].

Reference:

[1] Armaroli Balzani, 2011, Chem. Sus. Chem., 4, 21-36.
[2] Kapdan *et al.*, 2006, Enz. Microb. Technol., 38, 569-582.
[3] Esselborn *et al.*, 2013, Nat. Chem. Biol. 9, 607.
[4] Lubitz *et al.*, 2014, Chem. Rev., 114, 4081–4148.
[5] Arizzi *et al.*, 2016, Waste Manag., 56, 151-7.
[6] Morra *et al.*, 2014, Int. J. Hydr. Enerrgy, 39, 9018-9027.
[7] King PW et al., 2006, J. Bacteriol., 188, 2163-2172
[8] Morra*, Arizzi* *et al.*, 2016, Biochemistry 55, 5897–5900 53.