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Bacillus sp. strains to produce bio-hydrogen from the organic fraction of municipal solid waste

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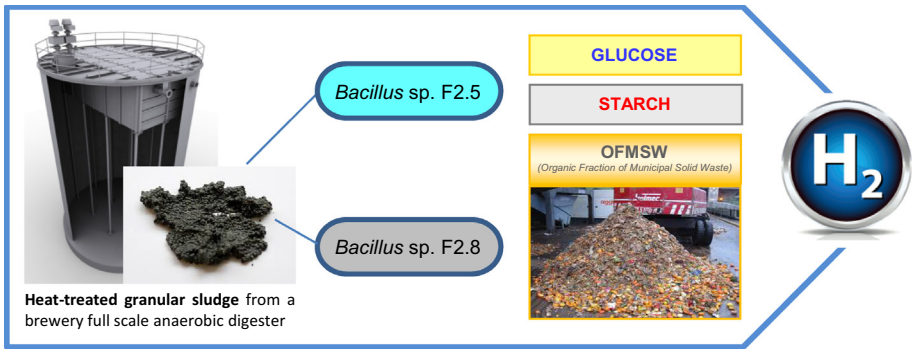
HIGHLIGHTS

- For the first time pure microbial cultures produced bio-hydrogen from organic waste.
- Two *Bacillus* sp. strains were selected for high amylolytic activities.
- The strains produced high H_2-yields from glucose and soluble starch.
- Promising H_2 production was confirmed also from organic waste.

GRAPHICAL ABSTRACT



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ABSTRACT

Bio-hydrogen, obtained by fermentation of organic residues, is considered a promising source of renewable energy. However, the industrial scale H_2 production from organic waste is far to be realized as technical and economical limitations have still to be solved. Low H_2 yields and lack of industrially robust microbes are the major limiting factors.

To look for bacteria with both interesting hydrogen fermentative traits and proper robustness, granular sludge from a brewery full scale Upflow Anaerobic Sludge Blanket (UASB) digester was selected as trove of microbes processing complex substrates. One hundred and twenty bacterial strains, previously isolated from heat-treated granular sludge and genetically identified by 16S rDNA sequencing, were screened for extracellular hydrolytic enzymes on cellulose, hemicellulose, starch, pectin, lipids, protein. The most interesting hydrolytic strains were assessed for their H_2 production from glucose and soluble starch. Two *Bacillus* sp. strains, namely F2.5 and F2.8, exhibited high H_2 yields and were used as pure culture to convert Organic Fraction of Municipal Solid Waste (OFMSW) into hydrogen. The strains produced up to 61 mL of H_2 per grams of volatile solids and could be considered as good candidates towards the development of industrially relevant H_2-producing inoculants. This is the first successful application of pure microbial cultures in bio-hydrogen production from OFMSW.

1. Introduction

Biological hydrogen production from organic waste represents both an energy production process and a first stage of stabilization for organic biomass since it degrades complex substrates to readily

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biodegradable compounds or to metabolites of commercial interest (i.e. organics acids and solvents) [1–3].

Organic waste and low-cost organic by-products of food-processing industry have been already investigated as promising renewable materials to be converted into hydrogen and other fuels, polymers, enzymes and bulk chemicals [4–13]. However, to guarantee the economical sustainability of the organic waste-to-hydrogen route, one of the main requirements is linked to the availability of efficient H_2 producing microbes with proper robustness to be used at industrial scale [1]. In order to obtain suitable inoculants, methanogens and hydrogen-consuming bacteria should be inhibited. To this purpose, several methods for pre-treatment of inocula have been proposed, including heat-treatment, aeration, irradiation, freezing, addition of chemical inhibitors such as acid, alkali, chloroform, etc., as extensively reviewed in [14–18].

The Organic Fraction of Municipal Solid Waste (OFMSW), characterized by high moisture and high biodegradability due to a large content of food waste, kitchen waste and leftovers from residences, cafeterias and markets, has been previously evaluated for H_2 production through the addition of heat-treated inocula [5,19–21]. Although heat shock pre-treatment contributed to good H_2 performances in short lab scale operations, increasing evidences show that a stable H_2 production and methanogens repression is not possible for long-term continuous mode [1,14,22]. Further research is also needed to establish whether the additional technical complexity of heat-treating the inoculum at industrial scale is cost-effective. Pragmatically thinking, heat shock of inocula is technologically more difficult during scale-up as compared to other pre-treatments [16]. Moreover, the use of exogenous inocula does not allow to guide properly the fermentation process [5,14]. To address this issue, recent research advances have been reported indicating that OFMSW itself could produce high H_2 yields, without any external inoculum supplementation [5]. Natural decomposition occurs to food waste when left for few days at room temperature due to the presence of indigenous microorganisms. In case of no or very low oxygen concentration, fermentation of organic matter takes place and methane production may also occur with time. Therefore, some species of indigenous microbial population of organic waste may have good characteristics for the hydrolysis of complex substrates and for an efficient conversion into H_2. As a result, food waste could serve both as substrate and source for H_2 production and H_2-producing bacteria, respectively [5,23]. This novel approach paves the way for the development of inoculants to produce H_2 from OFMSW relying on the indigenous microbes.

Another recent research strategy is the use of selected microbe (s) for the conversion of organic waste into H_2 [21,24]. The main advantages of using pure cultures over mixed microflora are that metabolic changes are easier to detect/tune and more information on the conditions that promote H_2 production can be disclosed [17,18,25]. Furthermore, even in non-sterile environments, pure cultures may be useful in bioaugmentation to achieve higher gas outputs [16,18,23,26]. The possibility to select strain(s) for their hydrolytic and fermenting abilities according to the main complex substrates available in the food waste makes this avenue very effective. However, it remains still unexplored as pure cultures have been so far mostly applied for H_2 production from simple sugars (i.e., glucose, sucrose and xylose) or laboratory-grade soluble starch [14,17,27]. Thus, more researches using pure cultures for H_2 production from organic waste are recommended [17,18,25].

In this paper, to look for microbes with both high hydrogen production potential and proper robustness, granular sludge from a brewery full scale Upflow Anaerobic Sludge Blanket (UASB) digester was selected as promising source because of processing complex substrates at industrial scale. One hundred and twenty bacterial strains, previously isolated from heat-treated granular sludge and selected for their high H_2 production [28], were screened for extracellular hydrolytic profile on cellulose, hemicellulose, starch, pectin, lipids and protein. The isolates exhibited a broad range of hydrolytic activities and the most interesting strains were assessed for their H_2 production from glucose. The top H_2-performing microbes were evaluated using starch as main carbon source. Two *Bacillus* sp. strains showed high H_2 levels and were evaluated also on OFMSW, mainly composed by starch, lipids and protein. The microbes gave promising H_2 yields and could be considered as good candidates towards the future development of industrially relevant microbes for the processing of organic waste into H_2. This is the first successful application of pure microbial cultures in bio-hydrogen production from OFMSW.

2. Materials and methods

2.1. Microbial strains

One hundred and twenty microbial strains were previously isolated from granular sludge samples heat-treated (100 °C) with increasing residence times in order to inhibit indigenous methanogenic bacteria. All the strains were identified by 16S rDNA sequencing [28].

2.2. Screening for the production of extracellular hydrolytic enzymes

Calibrated suspensions (A_{600} = 0.9, corresponding to an average concentration of 10^6 cells per mL) of bacterial cells, grown for 24 h at 37 °C in NB (Nutrient Broth) broth at 100 rpm, were used to inoculate plates containing the appropriate media described below and purified agar (Sigma, Italy). Petri dishes were checked for the presence of enzymatic activity described below, after aerobic incubation at 37 °C for 3 days. No discrepant results were recorded in repeated experiments.

2.2.1. Cellulase activity (CelA)

Cellulase production was detected on Hankin and Anagnostakis Medium containing 5 g/L carboxymethyl-cellulose (CMC). After cell growth, the presence of cellulolytic activity (CelA) was detected by Congo red method [29].

2.2.2. Lipolytic activity (LipA)

Strains were tested on tributyrin agar medium containing (g/L): peptone, 5; yeast extract, 3; tributyrin, 10; agar, 15; pH 6.0. Lipase activity (LipA) of the strains were indicated by a clear halo around the colony in an otherwise opaque medium as previously described [30].

2.2.3. Pectinolytic activity (PecA)

The secretion of extracellular pectic enzymes was tested on polygalacturonic acid medium (g/L): yeast nitrogen base, 6.7; glucose, 5; polygalacturonic acid (Fluka, Italy), 7.5; pH 7.0 [31]. The screening was performed using polygalacturonic acid medium with or without glucose (10 g/L). After cell growth, plates were flooded with a solution of 6 N HCl. The appearance of a degradation halo around bacterial colony was considered an indication of the polygalacturonic acid hydrolysis [32].

2.2.4. Proteolytic activity (PrA)

Extracellular protease production was determined on protein medium with skim milk (Difco, Italy), pH 6.5. A clear zone around the colony indicated protease activity (PrA) as described in literature [31,33].