Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/he

Biohydrogen production by the psychrophilic G088 strain using single carbohydrates as substrate

Cecilia Lizeth Alvarez-Guzmán [a], Edén Oceguera-Contreras [a], José Tomás Ornelas-Salas [b], Victor E. Balderas-Hernández [a], Antonio De León-Rodríguez [a,*]

[a] División de Biología Molecular, Instituto Potosino de Investigación Científica y Tecnológica, A.C. Camino a la Presa San José 2055, Col. Lomas 4a Sección, San Luis Potosí, SLP, C.P. 78216, Mexico

[b] Universidad Autónoma de Guadalajara, Facultad de Ciencias, Av. Patria 1201, Lomas del Valle 3a Sección, Zapopan, Jalisco, C.P. 45129, Mexico

ARTICLE INFO

Article history:
Received 24 July 2015
Received in revised form
22 October 2015
Accepted 24 November 2015
Available online 31 March 2016

Keywords:
Biohydrogen
Psychrophilic bacteria
Potential hydrogen producers
Carbohydrate metabolism

ABSTRACT

The production of biohydrogen by psychrophilic G088 strain ([EU636029]), closely related to *Polaromonas rhizosphaerae* ([EF127651]) was evaluated using xylose, glucose, fructose, galactose, lactose or sucrose as a carbon source. Biohydrogen production was performed in 120 ml serological bottles with a production medium containing 2.75 g/l tryptone, 0.25 g/l yeast extract, and 20 g/l of each carbohydrate. Results showed that the G088 strain produced biohydrogen using all the evaluated substrates, ranging from 91.7 to 439.8 ml for lactose and glucose, respectively. However, glucose was the substrate with the highest consumption rate, accompanied by the maximum values of biohydrogen production rate and a biohydrogen yield of 50.1 ml/l/h and 1.7 mol H_2/mol glucose, respectively. Analysis of the secreted metabolites showed that ethanol and organic acids were the main by-products. Our results demonstrate that the G088 strain has potential to be used for developing new biotechnological processes for biohydrogen production.

Introduction

Environmentally friendly energy carriers and sources are the most outstanding topics in the energy and environmental sector. The current global energy demand is mostly dependent on reserves of fossil fuel uses [1]. In recent years, various studies have been conducted to obtain a sustainable source of energy that can replace fossil fuels and its negative impact on the environment. In this regard, hydrogen was found as a promising clean and environmental friendly energy carrier [2], furthermore its energy value is 122 kJ/g, which is 2.75-times higher than hydrocarbon fuels [3] and upon oxidation hydrogen produces water [4]. In addition, handling of hydrogen gas is safer in comparison to other known natural gases. These features make hydrogen an ideal candidate to replace fossil fuels [5].

Hydrogen is a valuable energy carrier, an important feedstock to the chemical industry, and useful in detoxifying a wide range of water pollutants. As an energy carrier, it is especially attractive due to its potential to be used to power chemical fuels. In industry, hydrogen is used for the

* *Corresponding author.*
E-mail addresses: aleonr@me.com, aleonr@ipicyt.edu.mx (A. De León-Rodríguez).
http://dx.doi.org/10.1016/j.ijhydene.2015.11.189

hydrogenation of many products, including heavy oils in gasoline production, foods, and ammonia for fertilizer. Nowadays, hydrogen is mainly produced by reforming fossil fuels, 40% hydrogen is produced from natural gases, 30% from heavy oil and naphtha, 18% from coal, 4% from electrolysis and about 1% from biomass. Therefore, hydrogen is currently neither renewable nor carbon-neutral. Instead, hydrogen manufacturing has a large greenhouse-gas footprint.

Among various hydrogen production processes, the biological method is known to be less energy intensive; compared with the chemical processes for hydrogen production, biological hydrogen production by fermentative process can be operated at ambient temperatures and normal pressures [6,7]. There are different biological methods of hydrogen production, such as photosynthetic and fermentative processes. Dark fermentation is a process in which microorganisms utilize carbohydrates to produce biohydrogen in anaerobic fermentation conditions. However, low yields and production rates have been the main barriers for practical applications [8]. Most of the studies addressing fermentative hydrogen production operate on anaerobic digesters at mesophilic (24–40 °C), thermophilic (40–65 °C) or hyperthermophilic (>80 °C) [5] temperatures. Whereas, to our knowledge, only two studies have been reported on biohydrogen production using psychrophilic bacteria [9,10].

Most microorganisms isolated from cold environments are either psychrotolerant or psychrophile strict. Psychrotolerant organisms grow well at temperatures close to the freezing point of water, but have the fastest growth rates above 20 °C, whereas psychrophile strict organisms grow faster at temperatures of 15 °C or lower, but are unable to grow above 20 °C. Irrespective of how they may be defined, 'psychro' microorganisms are cold-adapted and exhibit properties which are distinctly different from other thermal classes [11]. These microorganisms have slower metabolism rates and higher catalytic efficiencies than mesophiles [12], the high activity of psychrophilic enzymes at low and moderate temperatures offers potential economic benefits due to the substantial energy savings in large-scale processes that would not require the expensive heating of reactors [13]. In addition, the temperature range prevents the risk of microbial contamination [12]. These advantages make the psychrophilic bacteria a good candidate for biohydrogen production. Currently, these microorganisms are being exploited as cell factories for the production of unstable compounds as well as for bioremediation of polluted cold soils and wastewaters. Furthermore, their enzymes have already found useful applications in various domains such as molecular biology, medical research, industrial food or feed technologies, detergents or cosmetics [14]. However, in the biofuels field their application has not been widely explored due to only a few studies addressing hydrogen, methane and biodiesel production having been reported [9,10,15–18].

In this study, the effectiveness of biohydrogen production from single carbohydrates using a psychrophilic G088 strain closely related to *Polaromonas rhizosphaerae* was assessed. This microorganism was isolated from samples of glacier sediment from Antarctica [19]. The carbohydrates evaluated were glucose, xylose, fructose, galactose, lactose and sucrose. Currently there is only one study reporting biohydrogen production from psychrophilic bacteria isolated from Antarctica, which was reported by our research group [9].

Material and methods

Strain and culture media

In this study, the G088 strain obtained from samples of glacier sediment from Antarctica was used. The accession number EU636050 and closest relativity of this strain according to NCBI is *P. rhizosphaerae* [EF127651] [16]. The strain was grown routinely in YPG agar plates [0.25 g/l Bacto-tryptone (Difco), 0.25 g/l yeast extract (Difco), 0.25 g/l glucose (Sigma) and 15 g/l Bacto-agar (Sigma)] and maintained at 4 °C. Six carbohydrates were used as substrates (xylose, glucose, fructose, galactose, sucrose or lactose). Biohydrogen production experiments were done in a rich production medium containing 2.75 g/l Bacto-tryptone (Difco), 0.25 g/l yeast extract (Difco) and 20 g/l of the corresponding carbohydrate mentioned above (Sigma) [20].

Biohydrogen production experiments

To evaluate hydrogen production by the G088 strain, preinocula were grown in a rich production medium under anaerobic conditions at 20 °C. Cells were harvested, centrifuged, washed and inoculated into 120 ml anaerobic serological bottles (Prisma, DF, Mex) containing 110 ml of production medium with 20 g/l of the respective carbohydrate supplemented with 1 ml/l of trace elements solution (0.015 g/l $FeCl_3 \cdot 4H_2O$, 0.00036 g/l $Na_2MoO_4 \cdot 2H_2O$, 0.00024 g/l $NiCl_2 \cdot 6H_2O$, 0.0007 g/l $CoCl_2 \cdot 6H_2O$, 0.0002 g/l $CuCl_2 \cdot 2H_2O$, 0.0002 g/l Na_2SeO_3, 0.01 g/l $MgSO_4$). The cultures were started at an optical density at 600 nm (OD_{600nm}) of 1, pH was adjusted at 6.8 and were incubated at 20 °C and 150 rpm [21]. All experiments were carried out in triplicate.

Analytical methods

Hydrogen produced was measured by water displacement with NaOH 1 N in an inverted burette connected to serological bottles with rubber tubing and a needle and validated by Gas chromatography using a thermal conductivity detector as described elsewhere [21]. All the experiments were carried out in triplicate. Samples of 1 ml were taken at different times during fermentation, they were then diluted and filtered through a 0.22 mm membrane (Millipore, Bedford, Massachusetts, USA) [9]. Concentrations of xylose, glucose, fructose and galactose and several metabolites such as succinic acid, lactic acid, acetic acid and butanol were analyzed by High Performance Liquid Chromatography (HPLC, Infinity LC 1220, Agilent Technologies, Santa Clara CA, USA) using a Refraction Index Detector, a column Phenomenex Rezex ROA (Phenomenex, Torrance, CA, USA) at 60 °C, and 0.0025 M H_2SO_4 as mobile phase at 0.41 ml/min. Sucrose was analyzed by the colorimetric method for determination of sugars and related substances [22] and lactose was analyzed by the 3, 5-dinitrosalicylic acid (DNS) method [23]. Ethanol, butyric acid, propionic acid, and acetone were analyzed in a Gas