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Biohydrogen production using psychrophilic bacteria isolated from Antarctica

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ABSTRACT

The growing interest in hydrogen as a fuel has intensified the search of novel approaches for new production processes, among which biohydrogen stands out. Currently mesophilic and thermophilic microorganisms have been used as inocula, and practically no information on the use of psychrophilic microorganisms is available. In this study, the capability of producing biohydrogen by 14 cultivable psychrophilic bacteria, which were isolated from samples of Antarctica, was evaluated. Microorganisms were typified using molecular techniques, and biohydrogen production screening 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 glucose as carbon source. Results showed that all psychrophilic strains produced biohydrogen from 34.8 to 253.3 ml. Maximum production and production rate of 253.3 ml and 16.64 ml/l/h, respectively were attained by GA051, which is closely related to *Janthinobacterium agaricidamnosum* (Y08845) with an identity of 98%, whereas the maximum biohydrogen yield of 1.57 mol H_2/mol glucose was for the GA024 strain, closely related to *Polaromonas jejuensis* strain JS12-13 (NR044379) with an identity of 99%. Metabolites were also tested and the main byproducts were organic acids and ethanol. Our results demonstrate that psychrophilic bacteria isolated from Antarctica have a high potential to be used for developing new biotechnological processes for biohydrogen production.

Introduction

The climate-driven changes have intensified the search for alternative energy sources, among which hydrogen stands out. It is an attractive option because is a renewable energy source, its combustion generates only water and heat, and has a high-energy yield of 122 kJ/g [1]. One of the important options to produce hydrogen is through microbial fermentation

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(biohydrogen), which can be classified as biophotolysis, dark fermentation, photofermentation, and microbial electrolysis cell [2]. Among these, dark fermentation and photofermentation technologies are processes that are being studied widely. Compared with photofermentation, anaerobic dark fermentation has the advantages of not requiring solar input and accepting a variety of substrates, such as organic waste, agricultural crops, or their byproducts, and using a very simple reactor technology [2].

Although the current biohydrogen yields are low, it is expected that with improvements in technology and genetic engineering, the amount of generated biohydrogen could be enhanced tremendously [3]. Highly efficient biohydrogen-producing strains still need to be screened. Anaerobic fermentative microbial species may be obtained from the natural environment and screening in the laboratory [4]. Most full-scale anaerobic digesters operate under mesophilic or thermophilic conditions and most rarely in the psychrophilic temperature ranges. However, it is desirable to find psycrophilic microorganisms that improve biofuel production and reduce the energy consumption for heating of the digesters [5–8]. On this particular issue, some authors have also noticed that biohydrogen could be produced at low temperatures [9,10].

The harsh environmental conditions of continental Antarctica have done that organisms face severe conditions such as low water and nutrient availability, extremely cold temperatures, oxidative stress, frequent freeze–thaw cycles, periods of prolonged darkness in winter, and exposure to high levels of ultraviolet radiation in summer. Therefore, Polar Regions such as the Antarctica represent a vast resource of novel psychrophilic microorganisms [11,12].

Psychrophilic bacteria and their enzymes are of commercial interest because their possibility of use at low temperatures and to scientific interest due to their relationship between protein structure and thermal stability [13,14]. The main areas of potential applications are food technologies, medical uses, bioremediation and environmental sciences [14]. However, biotechnological applications of psychrophile for biofuel production, such as biohydrogen, have not been assessed. Thus, the capability of cultivable psychrophilic microorganisms for biohydrogen production was studied. To our knowledge this is the first work of Antarctic bacteria in the production of biohydrogen, with the exception of a previous report by our own group [15].

Material and methods

Sample collection

Samples from glacier sediment (GS), seaside mud (SM), glacier melted ice (GI) and *Deschampsia antarctica* rhizosphere (DAR) were collected in the Collins glacier at Fildes Peninsula, King George Island, Antarctica (62°10′S, 58°55′W).

Strains

Psychrophilic microorganisms were isolated in a solid YPG culture medium and single colonies were used for amplification of 16S rDNA gene, which was digested and sequenced. Classification of microorganisms was done according to the identity percentage of 16S rRNA gene sequence using the criteria of Drancourt as described elsewhere [16,17]. From our collection of 54 psychrophilic strains [16], only those that were cultivable at 25 °C were selected for biohydrogen production screening (Table 1).

Table 1 – Taxonomic classification and biohydrogen production parameters for the psychrophilic bacteria cultured with 20 g/l of glucose, pH 6.5 and 25 °C.

Strain	Accession number	Closest relative according to the NCBI	Identity (%)	Sample origin[a]	Biohydrogen production (ml)	Production rate (ml/l/h)	Yield ($molH_2/mol_{glucose}$)
M02	EU636032	*Sejongia marina* (EF554366)	97.9	GI	34.8	3.99	0.32
L2	HQ226068	*Bacillus simplex* (EU236732)	99.9	SM	48.5	3.87	1.06
G088	EU636029	*Polaromonas rhizosphaerae* (EF127651)	98.7	GS	141.5	11.38	0.62
GA0G	EU636051	*Pseudomonas antarctica* (AJ537601)	99.4	GS	47.83	4.35	0.82
G024	EU636026	*Polaromonas jejuensis* strain JS12-13 (NR044379)	99.0	GS	70.17	5.61	1.57
G057	EU636044	*Janthinobacterium agaricidamnosum* (Y08845)	98.6	GS	42.33	3.57	1.07
N92	EU636058	*Rhodobacter ovatus* (AM690348)	96.1	GS	228.83	14.29	0.88
N25	EU636053	*Pseudomonas frederiksbergensis* (AJ249382)	98.6	GS	195.5	11.11	0.89
R19	EU636063	*Pedobacter aurantiacus* (DQ235228)	98.4	DAR	178	12.30	1.19
A02	EU636035	*Devosia limi* strain R-21940 (NR042324)	98.0	DAR	236.3	15.42	0.80
N04	EU636031	*Flavobacterium limicola* (AB075230)	95.8	GS	219.67	14.96	0.74
GA0L	EU636046	*Actimicrobium antarcticum* (HQ699437)	97.7	GS	43.67	5.08	0.52
GA051	EU636048	*Janthinobacterium agaricidamnosum* (Y08845)	98.0	GS	253.33	16.64	0.86
GA0F	EU636050	*Pseudomonas meridiana* (AJ537602)	99.7	GS	151.5	9.05	0.51

[a] Origin of samples: glacier sediment (GS), seaside mud (SM), glacier melted ice (GI), *D. antarctica* rhizosphere (DAR).