Bioresource Technology 157 (2014) 98–106

Contents lists available at ScienceDirect

Bioresource Technology

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

Predominance of cluster I *Clostridium* in hydrogen fermentation of galactose seeded with various heat-treated anaerobic sludges

Jeong-Hoon Park [a,b], Sang-Hoon Lee [a], Jeong-Jun Yoon [b], Sang-Hyoun Kim [c], Hee-Deung Park [a,*]

[a] School of Civil, Environmental and Architectural Engineering, Korea University, Seoul 136-714, South Korea
[b] IT Convergence Materials R&D Group, Korea Institute of Industrial Technology, Chungnam 330-825, South Korea
[c] Department of Environmental Engineering, Daegu University, Gyeongbuk 712-714, South Korea

HIGHLIGHTS

- Bacterial community compositions were explored in hydrogen fermentation of galactose.
- Cluster I *Clostridium* was ubiquitous and predominant in dark fermentation.
- The abundance of cluster I *Clostridium* was proportional to hydrogen production.
- Cluster I *Clostridium* was the key in hydrogen fermentation of galactose.

ARTICLE INFO

Article history:
Received 14 November 2013
Received in revised form 17 January 2014
Accepted 20 January 2014
Available online 28 January 2014

Keywords:
Biohydrogen
Clostridium
Galactose
Marine red algae
Pyrosequencing

ABSTRACT

To identify the key bacterial populations in hydrogen fermentation of galactose, a fermentor seeded with a heat-treated sludge was operated. After 27 h of fermentation, the proportion of butyric acid increased to 69.4 wt.% and the gas production yield reached 1.0 mol H_2/mol galactose. In the pyrosequencing of 16S rDNA, an increase of the proportion of the phylum *Firmicutes* from 4.2% to 92% (mostly cluster I *Clostridium*) was observed. To verify the predominance and the ubiquity of the cluster, five fermentors seeded with different heat-treated anaerobic sludges having different feedstock compositions and digestion temperatures were investigated using qPCR analyses. The abundance of the cluster increased >100-fold during the fermentation, regardless of the inocula. Moreover, the abundance was negatively correlated with the lag time of hydrogen production and positively correlated with the hydrogen production rate, demonstrating the relevance of the cluster to hydrogen production. Taken together, the results clearly revealed the importance of cluster I *Clostridium* in the hydrogen fermentation of galactose.

1. Introduction

Hydrogen gas is a promising future energy carrier mostly due to its attractive features including its high conversion rate to usable power, generation of low amounts of pollutants, and high energy efficiency (Hallenbeck and Ghosh, 2009). Currently, approximately 98% of hydrogen is produced from fossil fuels such as coal and methane. To overcome these drawbacks, researchers have proposed diverse biohydrogen production methods, including direct bio-photolysis of water (Gaffron and Rubin, 1942), indirect bio-photolysis photosynthetic bacteria, and dark fermentation (Lo et al., 2008). Even in biohydrogen production, several issues are associated with the feedstock including a low amount of available fermentable substrates, the food versus fuel problem, and destruction of forests (Panagiotou and Olsson, 2007). Particularly, if the cost related to the processes for the mechanical pre-treatment of biomass, fermentable sugar production by hydrolysis, and post-treatment of fermentation residue in the life cycle assessment, biohydrogen production would be more expensive than hydrogen production from fossil fuels (Singh and Olsen, 2011).

During the last decade, marine red algae have been proposed as sustainable and environmentally friendly feedstock for biohydrogen production that uses dark fermentation (John et al., 2011), although the algae could be used for the production of high value products (e.g., pharmaceuticals, pigments, animal feed, fertilizers, and polyunsaturated fatty acids, etc.) and fuels (e.g., biodiesel, ethanol, and methane, etc.) (Šoštarič et al., 2012). They can be easily cultivated in the sea and have high capacity of carbon dioxide capture. In addition, most red algae do not include lignin, which reduces the cost associated with their pretreatment for the production of easily metabolizable substrates (Park et al., 2012). Despite the myriad advantages of marine red algae, a problem involved in their use remains unresolved. Marine red algae mainly consist

* Corresponding author. Tel.: +82 2 3290 4861; fax: +82 2 928 7656.
E-mail address: heedeung@korea.ac.kr (H.-D. Park).

http://dx.doi.org/10.1016/j.biortech.2014.01.081
0960-8524/

of galactan, a polymer of galactose (Jol et al., 1999; Park et al., 2011). To metabolize galactose for hydrogen fermentation, galactose should initially be converted into glucose-1-phosphate through the Leloir pathway, which entails the expense of chemical energy (Servinsky et al., 2010). Glucose-1-phosphate is then metabolized to generate various organic acids and hydrogen similarly to glucose-based substrates (Hallenbeck and Ghosh, 2009). The conversion of galactose is therefore regarded as a reason for slow hydrogen production compared with hydrogen production based on glucose.

Applications using molecular biological techniques have been attempted to identify the key bacterial populations producing hydrogen. The studies revealed that the bacterial populations belonging to *Clostridiales* and *Enterobacteriacae* predominated in dark fermentation (Hung et al., 2007; Im et al., 2012; Kim et al., 2006b; Wang et al., 2007a). Most of the studies used relatively simple molecular techniques such as fingerprinting or clone library analysis. These methods were useful for identifying major bacterial species and the changes in bacterial community compositions in response to the operational conditions in biohydrogen production, although the methods provided limited and non-quantitative information. More detailed and quantitative approaches (e.g., high-throughput sequencing and quantitative PCR (qPCR)) are required for a comprehensive analysis of biohydrogen productions. On the other hand, most of the previous studies on biohydrogen production from galactose focused on hydrogen production pathways and performances using pure-culture strains or unknown mixed cultures (Pan et al., 2008; Park et al., 2011; Rosales-Colunga et al., 2012). To the best of the author's knowledge, bacterial populations responsible for hydrogen fermentation of galactose have not been reported. It is therefore necessary to investigate bacterial community compositions in detail to better understand and improve the hydrogen fermentation of galactose.

The objective of this study was to identify and quantify the key hydrogen producing bacteria during hydrogen fermentation of galactose as the primary substrate, and compare these key bacterial groups to those found in the hydrogen fermentation of other substrates. To achieve this objective, a fermentor seeded with a heat-treated anaerobic sludge was operated and bacterial community composition was investigated using 454 pyrosequencing of the 16S rRNA gene (16S rDNA). Phylogenetic analysis was conducted to identify the populations producing biohydrogen and to design qPCR primers quantifying these populations. Using the qPCR with the newly designed primers, the predominance and ubiquity of the hydrogen producing populations were evaluated for the other five fermentors seeded with different anaerobic sludges.

2. Methods

2.1. Inocula

The inoculum of the fermentor identifying the bacterial populations for hydrogen production from galactose was obtained from an anaerobic digester in the Kyungsan (K1) wastewater treatment plant (WWTP) located in Kyungsan, Korea. The inocula of the other fermentors testing the ubiquity of the hydrogen producing populations identified from the fermentor seeded with K1 sludge were obtained from five different anaerobic digesters in Seonam 1 (S1), Jungnang 2 (J2), Jungnang 3 (J3), Nanji 2 (N2), and Tancheon 1 (T1) WWTPs. All of the WWTPs are located in Seoul, Korea. The operational conditions of each anaerobic digester were described in a previous study (Lee et al., 2012). The solids content of these sludges is summarized in Table 1. Before inoculation, the anaerobic sludges were heated at 90 °C for 10 min to inactivate all microorganisms, except for heat-resistant spore-forming microorganisms (Kim et al., 2006a).

2.2. Batch test for hydrogen production using various anaerobic sludge inocula

Hydrogen fermentation was conducted in a 7 L of fermentor (Fermentec Co., Cheongju, South Korea) with a 4 L working volume. Galactose (Acros, Morris Plains, NJ, USA) was used as the substrate because galactose is the main monomeric sugar of marine red algae (Park et al., 2011). Nutrients were supplied based on a previous studies (Park et al., 2011) as follows: 0.96 g/L NH_4Cl, 0.22 g/L KH_2PO_4, 0.12 g/L $MgSO_4{\cdot}7H_2O$, 0.01 g/L $CaCl_2$, 0.01 g/L $MnSO_4{\cdot}H_2O$, and 0.043 g/L $FeCl_2$. The predetermined volume of the heat-treated sludge was added to the fermentors (all fermentors were adjusted to 10 g VSS/L, and the fermentor was filled with distilled water to 4 L). Subsequently, the fermentors were purged with N_2 gas for 5 vvm (volume gas (L) per volume fermentor (L) per time (min)), and then agitated at 150 rpm and incubated at 35 ± 0.1 °C. The initial pH of all fermentors was around 7.5. During the fermentation, pH was maintained at over 5.5 ± 0.1 using 3 M NaOH automatically.

2.3. Analyses of biogas, organic acids, and solids

The hydrogen content was measured using gas chromatography (series 580, Gow-Mac Instrument Co., Bound Brook, NJ, USA), with a previously described protocol (Park et al., 2011). Organic acids (C2–C6) and sugars were analyzed using high performance liquid chromatography (YL9100 series, Yong Lim Co., Inchon, South Korea) using a refractive index detector, an ultraviolet detector (210 nm), and a 300 × 7.8 mm of Aminex HPX-87H ion exclusion column with 5 mM H_2SO_4 as the mobile phase. The liquid samples were filtered through a 0.45 μm membrane filter before injection into the HPLC. The chemical oxygen demand (COD) and volatile suspended solid (VSS) were measured according to the Standard Methods (AHPA, 1997).

2.4. Kinetic analysis of hydrogen production

The hydrogen production with time was fitted to the values estimated by a modified Gompertz equation (Eq. (1), (Zwietering et al., 1990)), to estimate maximum H_2 volume, maximum H_2 production rate, lag time of H_2 production, and H_2 production yield:

Table 1
Characteristics of seed sludges from various anaerobic digesters.

Digesters	TSS[a] (g/L)	VSS[b] (g/L)	VSS/TSS (%)	Feedstock composition	Digestion temperature (°C)
K1	35.2 ± 0.9	25.6 ± 0.8	73.1 ± 0.4	Waste sludge + food waste	37.0 ± 1.2
T1	26.5 ± 0.1	15.0 ± 0.2	56.5 ± 0.5	Waste sludge	40.2 ± 1.7
S1	27.8 ± 0.7	18.1 ± 0.8	65.1 ± 1.0	Waste sludge	51.2 ± 1.6
J2	21.4 ± 0.2	16.2 ± 0.4	76.1 ± 1.2	Night soil	48.0 ± 2.4
J3	23.6 ± 0.9	15.9 ± 0.1	67.4 ± 3.0	Waste sludge	38.4 ± 0.7
N2	18.4 ± 0.9	13.2 ± 0.1	72.0 ± 3.7	Waste sludge	37.0 ± 1.1

[a] Total suspended solid (TSS).
[b] Volatile suspended solid (VSS).