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Short Communication

Hydrogen production with *R. faecalis* RLD-53 isolated from freshwater pond sludge

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ABSTRACT

The *Rhodopseudomonas faecalis* strain RLD-53 was isolated from freshwater pond sludge and was demonstrated it could produce hydrogen. This study to investigate their ability of hydrogen production under some conditions in batch culture experiments. At pH 7.0, temperature 35 °C and light intensity of 4000 lux, the H_2 yield was 2.64 mol-H_2/mol-acetate, 2.34 mol-H_2/mol-propionate, 1.75 mol-H_2/mol-lactate and 3.55 mol-H_2/mol-malate, respectively. The maximal H_2 production rate of 32.62 ml-H_2/l/h and H_2 yield of 2.84 mol-H_2/mol-acetate were achieved when beef extract was used as nitrogen source. Light intensity is the most important factor for H_2 production, H_2 production yield and rate decreased with increasing light intensity and reached highest under light intensity of 3000–5000 lux. Result indicated the strain RLD-53 was a high efficient bacteria for hydrogen production.

1. Introduction

Hydrogen is considered to be an ideal, renewable and clean energy resource. Until now, photo-hydrogen production have achieved more advancing for high theoretical conversion yield of substrate, ability to use a wide wavelength of light, and various organic substrates for hydrogen generation, and lack oxygen evolving activity (Akkerman et al., 2002). Therefore, photo-hydrogen production is attracting extensive attention.

Among purple non-sulfur phototrophic bacteria (PNSPB), some strains of *Rhodobacter sphaeroides* (Sasikala et al., 1991), *Rhodobacter capsulatus* (Hillmer and Gest, 1977), *Rhodopseudomonas palustris* (Barbosa et al., 2001) and *Rhodospirillum rubrum* (Miyake et al., 1982) have been studied widely for photo-hydrogen production, and can produce hydrogen from the organic substrate or wastewater as carbon source. However, up to now, hydrogen production by the strain of *Rhodopseudomonas faecalis* has not been reported. In this work, a new strain of photo-hydrogen production was isolated and designated as *R. faecalis* strain RLD-53, and capability of hydrogen production was investigated.

Many PNSPB can utilize the short chain organic acid as electron donors to produce hydrogen. In former study, the malic acid and lactic acid are considered as the best substrate for hydrogen production of photosynthetic bacteria (Eroglu et al., 1999; Koku et al., 2002). The recent studies have shown that some photosynthetic bacteria can utilize the acetate for hydrogen production in a suitable light intensity and produce higher hydrogen (Chen and Chang, 2006; Fang et al., 2005; Oh et al., 2004).

To improve hydrogen production by PNSPB, researchers adopt different ways, such as the selection of strain (Mao et al., 1986) and mutant (Willison et al., 1984; Colbeau et al., 1990; Zorin et al., 1996) with higher yield hydrogen, the optimization of the many parameters of inoculant age, carbon to nitrogen ratio (C/N), light intensity, and so on. Nitrogen source may lead to high yield of hydrogen generation using PNSPB. Sasikala et al. (1995) found that the early stationary phase of microorganism growth for providing nitrogen sources is suitable for H_2 production. The effect of various nitrogen sources on the hydrogen production was obvious. Among yeast extract, glutamate and NH_4Cl, yeast extract was the best nitrogen source for higher H_2 production, NH_4Cl was greatly inhibited H_2 production (Oh et al., 2004). The C/N was found to be an important factor for bio-hydrogen production. The optimum C/N was 70 mM/2 mM (Eroglu et al., 1999). Photo-hydrogen production under a low light intensity results in an increase of light efficiency and a decrease in H_2 yield and H_2 production rate (Barbosa et al., 2001; Otsuki et al., 1998). Initial cell concentrations of 0.6–1.2 g/l and light intensities of 3000 lux or higher were suitable for H_2 yield and light efficiency (Shi and Yu, 2005).

This study isolated *R. faecalis* strain RLD-53 with a high capability of hydrogen production from a freshwater pound sludge. The effects of some factors on hydrogen yields were investigated in order to determine the optimal conditions for the higher hydrogen yields.

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2. Methods

2.1. Isolation and culture medium for photo-hydrogen producing bacteria

The photosynthetic bacteria are isolated from freshwater pond sludge in the north of Songhua River in Harbin, China. Bacteria were isolated and purified as described by Kantachote et al. (2005). ATYP medium (Zhang et al., 2002) was used as the enrichment culture medium of photosynthetic bacteria. Two percentage agar is added in the enrichment culture medium as isolation medium. To examine the photo-assimilation of organic substrates, various organic substrates of 20 mM/l replaced acetate in ATYP medium as sole carbon source.

2.2. 16S rRNA sequential and hylogenetic analysis of isolated bacteria

DNA was extracted using the Bacterial DNA Mini Kit (Watson Biotechnologies, Inc). The 16S rRNA gene complete sequence was amplified by previously described methods (Xing et al., 2006). The PCR products were purified using Gel Extraction Mini Kit (Waston Biotechnologies, Inc), and cloned with pMD19-T plasmid vector system (Promega). Sequence alignment and analysis of the similarity of the 16S rRNA gene were performed with the clustal w program in GenBank.

2.3. Batch culture reactors and medium

All batch experiments were performed at 35 °C in 100 ml blood serum bottles sealed rubber plug. The bottles contained 80 ml liquid medium for hydrogen production. The top area of the bottles was filled with argon to maintain anaerobic conditions. The bottles with liquid medium were sterilized at 121 °C and steam pressure 1.05 kg/cm^2 for 15 min. The gas was collected by draining method. The bottles were shaken on the constant temperature incubation oscillator at 120 rpm. The light intensity of outside surface of the bottles was maintained at 4000 lux by incandescent lamps (60 W). The medium for hydrogen production consists of CH_3COONa 50 mM/l, KH_2PO_4 0.5 g/l, K_2HPO_4 0.5 g/l, $MgSO_4$ $4H_2O$ 0.2 g/l, $CaCl_2$ 0.08 g/l, NaCl 0.1 g/l, glutamate 10 mM/l, EDTA-Na 0.1 g/l, $FeSO_4$ $7H_2O$ 0.012 g/l, L-cysteine HCl H_2O 0.5 g/l, pH 7.0, the trace element and vitamin solution, which is the same as medium for enrichment culture.

2.4. Factors on photo-hydrogen production

The initial pH of the medium in the range 4–9 was adjusted using 1 mol/l HCl or 1 mol/l NaOH solution. The other operation conditions were as follows: inoculant volume 10%, inoculant age 24 h, temperature 35 °C; concentration of acetate 50 mM/l, concentration of glutamate 10 mM/l.

The inoculant volume was varied in the range of 1%–20% (v/v). The initial pH of the medium was set to 7.0 while the rest of the parameters were as above.

Different carbon sources of 50 mM/l were used keeping all the parameters constant (viz. temperature 35 °C, pH 7.0, glutamate concentration 10 mM/l, inoculant volume 10% and inoculant age 24 h).

Different nitrogen sources of 10 mM/l were used keeping all the parameters constant (viz. temperature 35 °C, pH 7.0, acetate concentration 50 mM/l, inoculant volume 10% and inoculant age 24 h).

Different light intensity (1000, 3000, 5000, 7000 and 9000 lux) was used as illumination. The other operation conditions were same as above.

2.5. Analytical methods

The glucose in supernatant of the culture broth was determined by oxidase method. The volatile fatty acids in supernatant of the culture broth, and H_2 analysis in evolved gas were determined according to the method of Xing et al. (2008). The light intensity was measured by using a digital luxmeter (TES1330A, Junkai Co.). Cell concentration and the absorption spectrum (300–900 nm) of intact cells were determined by an Amersham pharmacia biotech ultrospec 34300 UV/Vis spectrophotometer.

3. Results and discussion

3.1. Isolation and identification of the photosynthetic bacteria

The strain RLD-53 was selected as a high-yield hydrogen producer. The microscopic studies revealed that strain RLD-53 was a Gram-negative, vibrioid or peanut shaped cells, 0.6–1 μm wide and 1.3–2 μm long. After growth under anaerobic-light conditions, cell suspensions were red. The strain grew well in medium with acetate, malate, lactate, succinate, pyruvate, mannose and maltose, and grew only slightly with fructose and propionate. Benzoate, sorbitol, citric acid, tartaric acid, xylose, aspartate, glucose, cellulose, butyrate and ethanol were not utilized as electron donors for growth. About 1.5 kb 16S rRNA gene complete sequence was PCR amplified and submitted to GenBank database (Accession no.EU410078). A BLAST search of GenBank database showed RLD-53 most resembled *R. faecalis* strain gc^T (99.5% similarity). The physiological and morphological characteristic shows that the strain RLD-53 was most closely allied to *R. faecalis* although strain RLD-53 has not been shown to utilize glucose and butyrate under anaerobic light conditions. The characteristic absorption peaks of strain RLD-53 are 388, 465, 498, 528, 592, 806 and 864 nm. Results indicated the presence of carotenoids of normal spirilloxanthin series and bacteriochlorophyll *a*. The peaks in the strain RLD-53 were similar to that of *R. faecalis* reported in literature (Zhang et al., 2002). So we characterized it as a new strain within the species *R. faecalis* and designated as *R. faecalis* strain RLD-53.

Capability of H_2 production of *R. faecalis* strain RLD-53 was affected by initial pH, inoculant volume, and light intensity, carbon and nitrogen source.

3.2. Effect of initial pH and inoculant volume

At initial pH 4.0, 5.0 and 6.0, the bacteria can not grow and produce hydrogen. Lower pH result in lower level of ATP in the cell and inhibit the growth of bacteria (Bowles and Ellefson, 1985). Maximum H_2 production yield and hydrogen content (%) were 2.71 mol-H_2/mol-acetate and about 80% at pH 7.0, respectively (Table 1). H_2 production was greatly inhibited at pH 8.0–9.0, and no hydrogen was produced at pH 9.0.

The inoculant volume changed from 1% to 20% (Table 1). The highest H_2 production yield at inoculant volume of 10% occurred after a lag phase of about 24 h. At inoculant volume between 15% and 20%, H_2 production yield and the growth of cell were little, it was probably because high inoculant volume leads to lower light penetrability and result in lower light intensity for the growth of bacteria.

3.3. Effect of carbon and nitrogen source

Different carbon sources have effects on H_2 production of the RLD-53 obviously (Table 1). Acetate, propionate, malate, and lactate are suitable electron donors. The hydrogen yield obtained in acetate, propionate, malate and lactate were 2.64 mol-H_2/mol-acetate, 2.336 mol-H_2/mol-propionate, 3.55 mol-H_2/mol-malate and 1.75 mol-H_2/mol-lactate, respectively. Highest H_2 production rate and substrate conversion efficiency was 26.56 ml/l/h and 66%,