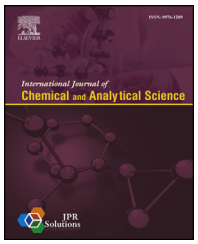
Available online at www.sciencedirect.com

SciVerse ScienceDirect

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



Original Article

Gas chromatography analysis for hydrogen production from *Bacillus subtilis* MKMP 2013

*Chinnasamy Mohanapriya, Marimuthu Krishnaveni**

Department of Biochemistry, Periyar University, Salem 636 011, Tamil Nadu, India

ARTICLE INFO

Article history:
Received 23 July 2013
Accepted 14 August 2013
Available online 21 September 2013

Keywords:
Bacteria
Fuel
Gas chromatography
Hydrogen
Lignocelluloses

ABSTRACT

Aim: Hydrogen is an uncontaminated energy carrier as it releases only water and no green house gas is released. Hence, bio-hydrogen production was aimed from *Bacillus subtilis*, a gram positive bacteria isolated near solid waste dumped place, Namakkal.

Methods: The isolated strain was confirmed by 16srRNA sequencing. Phylogenetic tree analysis was performed to know the nearest sequences. The isolated strain was allowed to grow in production medium containing beef, yeast extract, peptone, sodium chloride and sugarcane baggase for 16 days. Since, most of the hydrogen producing bacteria grow at broader pH, no pH control was opted during the study. After incubation period, the gas collected was analysed for hydrogen production by gas chromatography.

Results: The obtained sequence was submitted to genbank under the accession number KF484756. Phylogenetic analysis revealed the strain as *B. subtilis*. The hydrogen gas produced by *B. subtilis* using sugarcane bagasse was in the range of 1.3–3.3 mol H_2/mol substrate.

Conclusion: From this result, we can conclude that the isolated strain is able to produce hydrogen to certain extent and can be improved by gene modification.

1. Introduction

Hydrogen is the first element in the periodic table, making it the lightest element on earth. Since, hydrogen gas is so light, it rises in the atmosphere and therefore rarely found in its pure form H_2.[1] Generation of hydrogen is achieved through several ways but production by biological way is a cheap, eco-friendly way. Waste materials can be used as a substrate for the production of hydrogen which is economical, at the same time it will be very difficult to meet the demand, as it is a time consuming process and producing hydrogen in mass is very difficult. Organic waste materials when subjected to dark fermentation is a potential route of bio-hydrogen production requiring no light, no oxygen, on the other hand giving high rate of cell growth. Lignocellulose wastes such as sugarcane bagasse are mount up every year in large quantities causing ecological, environmental problems. The principal polysaccharide present in plant cell wall is cellulose 35–50%, 20–35% hemicellulose and 10–25% lignin.[2] Hence, *Bacillus subtilis*, a gram positive bacterium was isolated from waste dumped soil at Namakkal. This isolated bacterium was inoculated in to the production medium containing sugarcane baggase for the observation of hydrogen production.

* *Corresponding author*. Tel.: +91 9894829823.
E-mail address: logasarvesh@gmail.com (M. Krishnaveni).

0976-1209/$ – see front matter
http://dx.doi.org/10.1016/j.ijcas.2013.08.002

Fig. 1 – Phylogenetic analysis showing nearest sequences.

2. Material and methods

2.1. *Isolation of bacterial culture*

Isolation of soil bacteria was done by serially diluting 1 g soil sample collected from the experimental site. 0.1 ml of sample at 10^{-6} was transferred aseptically to nutrient agar plates and incubated at 37 °C for 24 h. Appearance of cream colour colonies confirms the presence of *Bacillus* sp. The bacterial isolates were further subcultured to obtain pure culture. The obtained pure culture was analysed for biochemical test,[3] amplification of 16srRNA sequencing. The gene was amplified using universal primer. The constituted reaction was denatured at 95 °C for 5 min. Cycling began with denaturing at 95 °C for 30 s, annealing at 52 °C for 30 s and extension for 4 min at 60 °C and the cycle repeated for a total 30 cycles in an MWG thermocycler. The reaction was then purified on sephadex plate (Edge Biosystems) by centrifugation to remove unbound labelled and unlabelled nucleotides and salts. The purified reaction was loaded on to 96 capillary ABI 3700 DNA analyzer. The cycle sequencing reaction was performed using Big Dye terminator V3.1 cycle sequencing Kit containing Ampli Tac DNA polymerase (from Applied Biosystems, P/N: 4337457). The sequencing reaction mix was prepared by adding 1 μl of Big Dye v 3.1, 2 μl of 5× sequencing buffer and 1 μl of 50% DMSO. To 4 μl of sequencing reaction mix was added 4 pmol of primer (2 μl). Sequences were compared to those in GenBank by using the BLAST function of National Center for Biotechnology Information.

Server, National Institutes of Health, USA. The sequence obtained was submitted to gen bank for accession number.

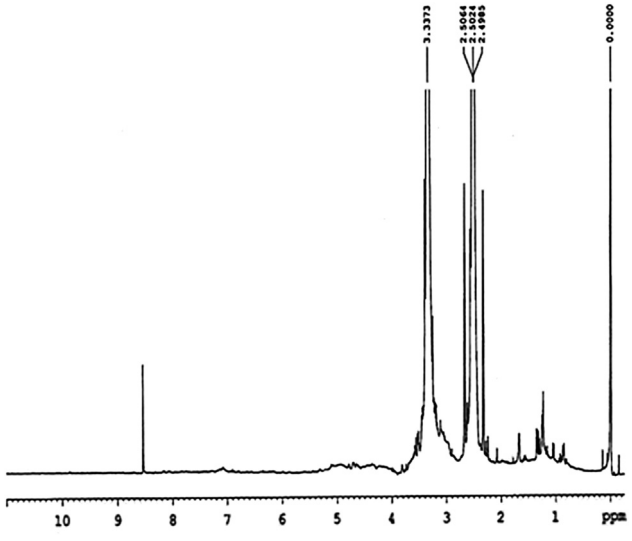


Fig. 2 – Gas chromatography analysis showing hydrogen production from *B. subtilis*

2.2. *Screening for hydrogen production by* B. subtilis *KF484756*

The isolated strain *B. subtilis* was inoculated in 100 ml peptone broth containing peptone 0.5 g and yeast extract 0.75 g and incubated for 24 h. After incubation period, 100 μl of peptone broth culture was inoculated in to 100 ml MYG medium containing malt extract 0.5 g, yeast extract 0.5 g, glucose 1.0 g. 500 μl of MYG culture was used to inoculate 250 ml production media containing beef extract 0.5 g, yeast extract 1.25 g, peptone 1.25 g, sodium chloride 5.12 g, sugar cane bagasse 1.5 g. Heat treatment for 1 h at 80 °C was done to remove the lignin present in bagasse. The inoculated culture was allowed to grow for a period of 16 days in a setup which can collect gas in to the empty space, thereby pushing the water up. After 16 days, the produced gas that has been collected through water displacement method was sent for gas chromatography analysis.

3. Results and discussion

3.1. *Identification of hydrogen producing isolates by 16srRNA gene sequencing*

The isolated soil bacteria were identified as *B. subtilis* with accession number **KF484756**. The strain was named MKMP 2013. Phylogenetic analysis of nucleotide sequences revealed that it is very close to *B. subtilis* (Fig. 1) and showed 99% similarity to *B. subtilis* subsp. *Subtilis* (NR102783.1).

3.2. *Screening for hydrogen production*

The isolated strain *B. subtilis* was observed for hydrogen production by allowing it to grow in production medium for a period of 16 days. After 16 days, the gas collected was sent for gas chromatography analysis at Royal Bio Research Centre, Chennai. From the obtained results, the hydrogen recovery was found to be between 1.5 mol H_2 and 3.3 mol H_2/mol substrate (Fig. 2). According to Vander Voort et al, *Bacillus* spp. are able to adjust their metabolism, respiratory activities according to their existing stressful environment, likewise in the presence of limited oxygen, genes of hydrogen generation from pyruvate get induced.[4]

4. Conclusion

The isolated strain *B. subtilis* was able to produce hydrogen within 1.5–3.3 mol H_2/mol substrate. From this results, we can conclude, that sugarcane bagasse acted as a better source of substrate in producing hydrogen by *B. subtilis*.

Conflicts of interest

All authors have none to declare.

Acknowledgement

The author thank Managing Director, Mr. D. Jagadeesh Kumar, Chrompark Research Centre, Namakkal for providing lab facilities to carry out the research and Managing Director, Dr. Sankarapandian Selvaraj, Helini Biomolecules, Chennai for helping us in doing bioinformatics work.

REFERENCES

1. Altork LN, Busby JR. Hydrogen fuel cells: part of the solution. *Technol Eng Teach*. 2010;70:22–27.
2. Saha BC. Hemicellulose bioconversion. *J Ind Microbiol Biotechnol*. 2003;30:279–291.
3. Cappuccino James G, Sherman Natalie. *Microbiology a Laboratory Manual*. 7th ed. Dorling Kindersley (India) Pvt. Ltd; 2009.
4. Van der Voort M, Abee T. Transcriptional regulation of metabolic pathways, alternative respiration and enterotoxin genes in anaerobic growth of *Bacillus cereus* ATCC 14579. *J Appl Microbiol*. 2009;107:795–804.