Available online at www.sciencedirect.com

SciVerse ScienceDirect

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

Original Article

Hydrogen production by *Pseudomonas stutzeri* JX442762 isolated from thermal soil at Mettur power station, Salem district, Tamil Nadu, India

S. Penislus Shiyan [a], *M. Krishnaveni* [b,*]

[a] *Department of Biochemistry, Periyar University, Salem 636011, Tamil Nadu, India*
[b] *Assistant Professor, Department of Biochemistry, Periyar University, Salem 636011, Tamil Nadu, India*

ARTICLE INFO

Article history:
Received 21 August 2012
Accepted 6 November 2012

Keywords:
Biological hydrogen production
Mango juice effluent
SSKVM 2012
Starch
Sucrose

ABSTRACT

Background: Carbohydrate based substrates presents a promising route of biological hydrogen production compared with chemical routes. Pure substrates including starch, sucrose as well as different organic waste materials can be used for hydrogen production. Among a large number of microbial species, strict anaerobes and facultative anaerobic chemoheterotrophs, thermoanaerobacter species are efficient producers of hydrogen. Since hydrogen is a high-energy fuel than hydrocarbon fuel, it is essential to find an alternative source.

Objectives: To isolate hydrogen producing organism from thermal soil sample, to identify the organism as it is a cost effective way for biological hydrogen production. The hydrogen production was assessed in pure starch, sucrose and also in mango juice effluent obtained from Krishnagiri dist., to find a better substrate.

Methodology: Morphological, biochemical characterization of the isolate was evaluated and finally confirmed by 16S rRNA gene sequencing. Hydrogen production was measured by simple water displacement method for the selected substrates at 70 °C, initial pH 4.0 as well as for the effluent.

Results: 16S rRNA gene sequencing confirms the organism as *Pseudomonas stutzeri* which was deposited in gene bank under the accession number JX442762 and the identified strain was named as SSKVM 2012. The G + C content of the strain *P. stutzeri* was found to be 53 mol%. The obtained results showed that the maximum hydrogen production was observed with pure starch (255.98 ± 0.76 ml) but in sucrose it was found to be 212.82 ± 0.57 ml. The mango juice effluent showed 190.03 ± 0.81 ml hydrogen. Even though it was less when compared to hydrogen production by starch and sucrose, mango juice effluent could be a better substrate for the identified organism.

Conclusion: The identified strain confirms that it can use effluent as a good source for the hydrogen production as its initial pH is 6.0 and also freely available in the environment.

* *Corresponding author*. Tel.: +91 9894829823 (mobile).
E-mail addresses: krishnavenim2011@gmail.com, logasarvesh@gmail.com (M. Krishnaveni).

0974-6943/$ – see front matter
http://dx.doi.org/10.1016/j.jopr.2012.11.024

1. Introduction

The current global demand for H_2 was estimated to be approximately 45 million tons/annum.[1–3] Current estimates indicate that the global energy demand would continue to increase from 536 EJ in 2008, to 653, in 2020, and 812 EJ by 2035.[4] On the other hand, the United Nations Statistics show that the global CO_2 emissions increased 44% between 1990 (20.69 billion metric tons) and 2008 (29.86 billion MT).[5] Progressive depletion of non-renewable energy sources worldwide, together with the fact that their use has resulted in environmental deterioration and public health problems, has led to development of new renewable energy harvesting technologies.[6,7] Hydrogen is considered an ideal alternative fuel to the current energy scenario due to its high-energy content and non-polluting nature.[8–11] It is a clean and environment friendly fuel that produces only water when combusted with oxygen. It is a high-energy fuel (122 kJ/g) than hydrocarbon fuel.[12] Approximately 95% of commercially produced hydrogen comes from carbon containing raw materials, primarily fossil in origin.[13] Moreover, the petroleum reserves of the world are depleting at an alarming rate.[14] Due to the depletion of fossil fuel and emission of greenhouse gas (CO_2) during conventional hydrogen production process, biological hydrogen production from biomass has been recognized as an eco-friendly and less energy intensive process to produce hydrogen compared to photosynthetic/chemical processes.[15] Thermophiles are organisms capable of living at high temperature. These organisms do not only survive but might even thrive in boiling water.[16] The ability of thermophilic bacteria to grow at high temperature and to produce stable extracellular enzymes was attributed to the probability of increasing their enzyme excoriation and activity by means of genetic manipulation. Therefore, these microorganisms were the first candidates for massive enzyme production for industrial applications.[17] Thermophilic anaerobic fermentation processes hold tremendous potential for the forthcoming generation as well as commercial production of hydrogen fuel.[18] Hence, in view of the above, we have isolated a *Pseudomonas stutzeri* from soil near thermal wells at Mettur power station, Salem, Tamil Nadu, India. The identified strain was studied for its ability to produce hydrogen using mango juice effluent as a preliminary study, in order to reduce the cost of hydrogen production by using synthetic source starch as well as sucrose.

2. Materials and methods

2.1. Sample collection

Thermal soil samples were collected from soil near thermal wells at Mettur power station, Tamil Nadu, India. One gram of thermal soil was dissolved in 100 ml distilled water. Serial dilution was carried out as per the standard procedure.[19] Serial dilution technique was used to obtain pure cultures. In order to be sure to obtain pure isolates, serial dilution steps were repeated several times.

2.2. Cultivation of isolate

The isolate was cultivated in the solid nutrient agar medium containing Peptone −1 g, Beef extract − 3.0 g, Sodium chloride − 5 g, Yeast extracts − 2.0 g, Distilled water − 1000 ml, pH 7.4 ± 0.2. Sterile nutrient agar was taken in petri dishes. A sterilized loop was dipped into the suspension of desired organism and was streaked on the surface of solidified agar plate. The plates were then incubated for 24–48 h to get the individual colonies. Bacteria grows on the surface nutrient agar, and is clearly visible as small colonies.

2.3. Isolation of strain

Thermal soil samples were inoculated in anaerobic liquid basal medium consisting of (g/l): NH_4Cl 0.5, Yeast extract 5, K_2HPO_4 0.25, KCl 0.002, $MgCl_26H_2O$ 0.125, NH_4CO_3 0.4, Peptone 1, $NH_4H_2PO_4$ 0.4, NaH_2PO_4 0.5. Trace element 1 ml, vitamin solution 1 ml.[20] Sucrose (10 g/l) was used as a carbon and energy source. All the culture bottles were incubated at 70 °C for 3 days and sub cultured after 3 days of incubation. All the sub cultures and diluted cultures were incubated at 70 °C under atmospheric pressure. Cells were observed under a light microscope and pure isolate was routinely cultivated in anaerobic liquid basal medium.

2.4. Morphological studies

Morphological characteristics were investigated. Gram staining was performed to confirm the gram reaction and spore position. Motility was determined by hanging drop method.[19]

2.5. Conventional identification tests

All isolates were evaluated by conventional tests for catalase, oxidase, indole, urease, methyl red, voges-proskauer, citrate utilization, triple sugar iron, starch hydrolysis, hydrogen sulphide and oxidative fermentative carbohydrate utilization.[19]

2.6. Identification of strain by using 16s rRNA gene sequence

Genomic DNA was extracted from the isolate using Pure Fast® Bacterial Genomic DNA isolation kit. 1 μL of genomic DNA was used as template and amplified by PCR using Master Mix Gene kit (HELINI biomolecules Chennai, India) with the aid of 16S rDNA primers (16S Forward Primer: 5-AGAGTRTGATCMTY GCTWAC-3 16S Reverse Primer: 5-CGYTAMCTTWTTACGR CT-3) with the programme consisted of denaturation at 94 °C for 1 min and subsequent 35 cycles of denaturation at 94 °C for 30 sec, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min followed by final extension at 72 °C for 5 min. Amplified product was sequenced using the Dye Deoxy Terminator Cycle sequencing kit (HELINI biomolecules Chennai, India) as directed in the manufacturer's protocol. The nucleotide sequencing of 16S rRNA gene of the isolate was compared with other related sequences using FASTA programme. Further, the nucleotide sequences of the isolate was aligned with closely related sequence using CLUSTAL W mega version-5.