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Photoproduction of Hydrogen by Anoxygenic Phototrophic Consortium Isolated from Pharmaceutical Industrial Effluents

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Abstract : Hydrogen can be used as a clean fuel in future. Photoproduction of hydrogen by anoxygenic photosynthetic bacteria is efficient and ecofriendly. A study of hydrogen production (ml/ 10 ml vessel) was carried out using anoxygenic phototrophic consortium isolated from pharmaceutical industrial effluents with different carbon and nitrogen sources and various growth factors. The carbon sourcemannitol could induce a maximum of 2.5± 0.2 ml hydrogen followed by 2.0 ± 0.2 ml in benzoate,2.0 ± 0.3 ml in acetate and 1.5 ± 0.3 incellobiose containing medium. Ammonium chloride as nitrogen source could induced maximum of 3.0 ± 0.2 ml hydrogenand alanine induced lowest amount of hydrogen (I.0 ± 0.4 ml)by the photosynthetic consortium. Other nitrogen sources thiourea induced 2.5 ± 0.1 ml,valine2.5 ± 0.2 ml and sodium nitrate 1.5 ± 0.2 ml of hydrogen. Of the growth factors tested, cyanocobalamine could induce highest amount of hydrogen (3.5 ± 0.1 ml) and niacin induced lowest amount (2.5 ± 0.4 ml) of hydrogen production. The growth factor riboflavin induced 3.0 ± 0.2 ml of hydrogen. Addition of growth factors showed an increase in hydrogen production by the phototrophic consortium.
Key words : hydrogen production, PNSB, carbon, nitrogen

Introduction

Anoxygenic phototrophicpurple non sulphur bacteria carry out photosynthesis that involves oxidation of molecules other than water. But, the general principles of energy transfer are the same in anoxygenic and oxygenic photosynthesis [1]. Purple phototrophic bacteria have the ability to trap and use sunlight efficiently as an energy source under anoxic conditions. By introducing oxygen into a bacterial culture growing phototrophically results in slower synthesis of the photosynthetic apparatus components and a change to another form of energy, generally derived from the degradation of organic compounds under aerobic conditions (chemoheterotrophy). Change from anaerobic (photosynthetic) to aerobic growth requires tight regulation of photosynthetic gene expression at the molecular level. Cohen-Bazire*et al*. [2] showed quite clearly that the regulation of photosynthetic gene expression was in response to two environmental stimuli. The most potent stimulus was oxygen; its presence shuts down production of photosynthetic pigments very rapidly. To a lesser extent, photosynthetic gene expression was sensitive to intensity of light. Less light intensity produced high levels of photosynthetic pigments; high light intensities caused a decrease, but the effect was less than that observed for oxygen.

The purple non-sulfur bacteria (PNS) are the most versatile of the phototrophic purple bacteria. Many PNSB phylogenitically branch within the α-proteobacteria, some are also found within the β- and γ-

Proteobacteria[3]. PNS bacteria can grow as photoautotrophs or chemoheterotrophs depending on the environmentalconditions available [4]. PNS bacteria have been isolated from freshwater, marine systems, plants, soil and activated sludge [5]. Oda*et al*. examined purple non sulphur bacterial genotypic and phenotypic diversity in two different aquatic sediments [6]. Purple non sulphur bacteria are able to produce hydrogen under various environmental conditions like anaerobic light and dark and aerobic dark [7,8].Swetha*et.al* [9] has reported the production of hydrogen with various carbon and nitrogen sources and growth factors by a photosynthetic consortium isolated from river water. Similarly, a marine photosynthetic bacterial consortium was found to produce cumulative hydrogen of 200 ± 67 mL using treated soy sauce wastewater and bagasse as a sole carbon source [10]. In the present work, a photosynthetic consortium isolated from pharmaceutical industrial effluents was studied for the production of hydrogen utilizing various substrates.

Materials and Methods

The phototrophic bacteria were isolated by inoculating into Beibl and Pfennig's medium and incubated anaerobically in the light (2000 lux). Identification keys provided in Bergey's Manual of Systematic bacteriology, 1989 [11] were adopted. The carbon sources used in this study are benzoate, acetate, mannitol and cellobiose. The nitrogen sources tested include alanine, sodium nitrate, valine, thiourea and ammonium chloride. The growth factors used are riboflavin, niacin and cyanocobalamine. The technique used in the hydrogen production wasof Vincenzini*et.al*. [12]. Statistical analysis was performed by Standard mean deviation and t-test.

Results and Discussion

Rhodopseudomonas palustris and *Rhodobacter sphaeroides* were the main bacteria found in the consortium along with other photosynthetic bacteria. With carbon sources as substrates ,mannitol could induce a maximum of 2.5 ± 0.2 ml hydrogen/10 ml vessel, followed by 2.0 ± 0.2 ml in benzoate and 2.0 ± 0.3 ml in acetate as carbon sources (Table 1). Lowest amount of hydrogen (1.5 ± 0.3) was produced in cellobiose containing medium. Benzoate followed by acetate induced 2.0 ± 0.2 ml and 2.0 ± 0.3 ml respectively. Similarly, the cultures of *R. rubrum* with whey as a substrate containing lactate as carbon sourceproduced hydrogen at an average rate of 6 ml/h per g (dry weight) of cells [13]. The highest hydrogen productivity of 0.8 mmol H2/lc.h and yield of 88% was obtained in the 40 mM/ 4 mM acetate/glutamate continuously fed photobioreactor for a period of 21 days with *Rhodobacter capsulatus* Hup- (uptake hydrogenase deleted strain) [14].

Nitrogen sources also play an important role in hydrogen production. A photosynthetic consortium isolated from sewage water showed 7.1ml/30ml and 6.9 ml/30 ml hydrogen production with histidine and ammonium nitrate respectively in Biebl and Pfennigs Medium [15]. In the present work, ammonium chloride induced highest amount of hydrogen/ 10 ml vessel (3.0 ± 0.2 ml) whereasalanine induced lowest amount (I.0 ± 0.4 ml) of hydrogen by the photosynthetic consortium (Table 2). Other nitrogen sources induced hydrogen per 10 ml vessel in the descending order as thiourea (2.5 ± 0.1 ml),valine (2.5 ± 0.2 ml) and sodium nitrate (1.5 ± 0.2 ml).

Table 3 shows the effect of growth factors on hydrogen production by the photosynthetic consortium. In the presence of cyanocobalamine highest amount (3.5 ± 0.1 ml) of hydrogen per 10 ml vessel was produced whereas niacin induced lowest amount (2.5 ± 0.4 ml) of hydrogen per 10 ml vessel. Riboflavin could induce 3.0 ± 0.2 ml of hydrogen per 10 ml vessel. Enhancement of hydrogen production by addition of growth factors like vitamin B12, para amino benzoic acid, biotin, nicotinic acid and riboflavin by a phototrophic bacterial consortium isolated from marine sediments was also reported [16]. There is no correlation between the biomass of the consortium (in terms of optical density) and the amount of hydrogen produced.

Table1: Effect of carbon sources on hydrogen production by phototrophic bacteria

Carbon Source(1.0%)	OD (at 660 nm)	Hydrogen Produced(ml/10 ml vessel)
Benzoate	0.59	2.0 ± 0.2
Acetate	0.80	2.0 ± 0.3
Mannitol	0.40	2.5 ± 0.2
Cellobiose	0.99	1.5 ± 0.3

Hypothesis: The Hydrogen Production from Carbon sources complies with the standard of 33%.

Level of Significance: 5%

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2	3.3
Variance	0.166667	0
Observations	4	4
Hypothesized Mean Difference	0	
Df	3	
t Stat	-6.36867	
P(T<=t) one-tail	0.003918	
t Critical one-tail	2.353363	
P(T<=t) two-tail	0.007835	
t Critical two-tail	3.182446	

Conclusion: The Hydrogen Production from Carbon sources does not comply with the standard of 33%.

Table 2: Effect of nitrogen sources on hydrogen production by phototrophic bacteria

Nitrogen Source(0.5%)	OD (at 660 nm)	Hydrogen Produced(ml/10ml vessel)
Alanine	0.88	I.0 ± 0.4
Sodium Nitrate	0.96	1.5 ± 0.2
Valine	0.82	2.5 ± 0.2
Thiourea	0.60	2.5 ± 0.1
Ammonium Chloride	0.90	3.0 ± 0.2

Hypothesis: The Hydrogen Production from Nitrogen sources complies with the standard of 33%.

Level of Significance: 5%

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2.1	3.3
Variance	0.675	0
Observations	5	5
Hypothesized Mean Difference	0	
Df	4	
t Stat	-3.26599	

P(T<=t) one-tail	0.015453
t Critical one-tail	2.131847
P(T<=t) two-tail	0.030906
t Critical two-tail	2.776445

Conclusion: The Hydrogen Production from Nitrogen Sources does not comply with the standard of 33%.

Table 3: Effect of growth factors on hydrogen production by phototrophic bacteria

Growth Factor(0.1µg/ml)	OD (at 660 nm)	Hydrogen Produced (ml/10 ml vessel)
Riboflavin	0.75	3.0 ± 0.2
Niacin	0.80	2.5 ± 0.4
Cyanocobalamine	0.85	3.5 ± 0.1

Hypothesis: The Hydrogen Production from Growth Factors complies with the standard of 33%.

Level of Significance: 5%

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	3	3.3
Variance	0.25	2.96E-31
Observations	3	3
Hypothesized Mean Difference	0	
Df	2	
t Stat	-1.03923	
P(T<=t) one-tail	0.203922	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.407843	
t Critical two-tail	4.302653	

Conclusion: The Hydrogen Production from Growth Factors does not comply with the standard of 33%.

Conclusion

The phototrophic consortium isolated from pharmaceutical industrial effluents could be used for hydrogen production as good amounts of hydrogen production was recorded using various carbon and nitrogen sources and growth factors. Out of all, a combination of mannitol as carbon source and ammonium chloride as nitrogen source could yield maximum amount of hydrogen. The utilization of growth factors further enhanced hydrogen production by the consortium.

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