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Research Article

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Photoproduction of hydrogen by anoxygenic phototrophic consortium isolated from Bhima Amarja River, Karnataka

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ABSTRACT

A major route for biological hydrogen production is purple non sulphur photosynthetic bacteria. In this study, we have analyzed the purple photosynthetic bacterial consortium isolated from Bhima amarja river of Karnataka. PNSB (Purple non sulphur photosynthetic bacteria) metabolize many different organic compounds as carbon sources and produce hydrogen. In presence of acetate, 2.0 ml of hydrogen was produced followed by cellobiose and lactate as carbon sources. Lowest amount of hydrogen was produced in benzoate and mannitol containing medium. In the presence of sodium nitrate as nitrogen source, more amounts of hydrogen was seen compared to other nitrogen sources. Ammonium chloride induced lowest amounts of hydrogen production by the photosynthetic consortium. In presence of cyanacobalamine as growth factor higher amounts of hydrogen was produced. Niacin induced lowest amounts of hydrogen. T-Test was done on two samples assuming unequal variances and the results are presented.

Keywords: Purple non sulphur bacteria, hydrogen production, carbon, nitrogen sources.

INTRODUCTION

Photosynthetic bacteria are known to have various biotechnological applications including hydrogen production and bioremediation [1]. Purple non-sulfur bacteria (PNSB) are distributed widely in natural habitats, particularly in those with large amounts of soluble organic matter, such as mangrove swamps, wastewater ponds, coastal lagoons and waste lagoons [2]. Purple photosynthetic bacteria are the most widely studied bacteria for their metabolic flexibility, biological hydrogen production and nature of their photosynthetic apparatus. Purple Non-Sulfur bacterial production of hydrogen and optimizing different cultural conditions for enhancing the production of hydrogen form this group of bacteria [3-17]. Environmental factors affect the production of hydrogen by these bacteria. Light, temperature, pH, carbon and nitrogen sources are the major factors affecting hydrogen production [18-20]. These organisms are wide spread and metabolically diverse and are in the forefront of laboratory research. In this study, consortium isolated from Bhima amarja river water sample from Karnataka state was studied for their hydrogen producing potential. The results obtained were statistically analyzed using t-test with the standard potential of production of hydrogen predicted to be at 33%. The significance of the above are discussed in this communication.

EXPERIMENTAL SECTION

Purple non sulphur anoxygenic phototrophic bacteria were isolated from Bhima amarja river of Karnataka. Samples were isolated by inoculating into BP medium and incubated under anaerobic (2000lux) light conditions. Bergey's Manual of Systematic Bacteriology (1994) was used for the identification of these bacteria. Based on different concentrations of electron donors, nitrogen sources and growth factors ten days old cultures of phototrophic bacteria of 1% (v/v) concentration were inoculated. The technique used for hydrogen measurement was water displacement method where as Gas Chromatography was used for gas analysis. Statistical analysis was done keeping the predicted amount of hydrogen at 33%.

RESULTS AND DISCUSION

Ten day active cultures were used to assess their probability of producing hydrogen. Photosynthetic bacterial consortium produced different amounts of hydrogen with various carbon, nitrogen and growth factors. The pH was maintained at 7.2 under anaerobic light. In presence of acetate, 2.0 ml of hydrogen was produced followed by cellobiose and lactate as carbon sources (Table 1). Lowest amount of hydrogen was produced in benzoate and mannitol containing medium. In the presence of sodium nitrate as nitrogen source more amounts of hydrogen was seen compared to other nitrogen sources (Table 2). Ammonium chloride induced lowest amounts of hydrogen production by the photosynthetic consortium. In presence of cyanacobalamine as growth factor more amounts of hydrogen was produced. Niacin induced lowest amounts of hydrogen (Table 3). T-Test was done on two samples assuming unequal variances and the results are presented in Tables 1a, 2a and 3a. Statistical analysis for the effect of various carbon, nitrogen and growth factors based on the hypothesis of standard at 33%, the results show that there was a deviation between the predicted and observed amounts of hydrogen production. Level of significance was 5%. The present study reveals that impact of various carbon, nitrogen and growth factors on the ability of phototrophic bacteria to produce hydrogen in anaerobic conditions. It is more economically attractive and suitable cheaper carbon and nitrogen sources should be searched for hydrogen production.

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

Carbon source (1%)	Growth (Optical density at 660nm)	Hydrogen produced (ml/10ml vessel)
Benzoate	1.59	1.0±0.2
Acetate	1.10	2.0±0.4
Mannitol	1.40	1.0±0.3
Cellobiose	0.99	1.5±0.3
Lactate	1.22	1.5±0.2

Table 1a: Statistical analysis of the effect of carbon sources on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	1.4	3.3
Variance	0.175	0
Observations	5	5
Hypothesized Mean Difference	0	
Df	4	
t Stat	-10.1559	
(T<=t) one-tail	0.000265	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.000529	
t Critical two-tail	2.776445	

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

Nitrogen source (1%)	Growth (Optical density at 660nm)	Hydrogen produced (ml/10ml vessel)
Alanine	0.88	2.5± 0.3
Sodium nitrate	0.96	3.0±0.3
Valine	0.82	1.5±0.2
Glutamic acid	0.60	2.0±0.4
Ammonium chloride	0.90	1.0±0.2

Table 2a: Statistical analysis of the effect of nitrogen sources on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2	3.3
Variance	0.625	0
Observations	5	5
Hypothesized Mean Difference	0	
Df	4	
t Stat	-3.67696	
P(T<=t) one-tail	0.01063	
t Critical one-tail	2.131847	
P(T<=t) two-tail	0.021261	
t Critical two-tail	2.776445	

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

Growth factors (100µl)	Growth (Optical density at 660nm)	Hydrogen produced (ml/10ml vessel)
Riboflavin	0.75	2.5±0.4
Niacin	0.80	2.0±0.4
Cyanacobalamine	0.80	3.0±0.2

Table 3a: Statistical analysis of the effect of growth factors on hydrogen production

t-Test: Two-Sample Assuming Unequal Variances		
	Variable 1	*Variable 2*
Mean	2.5	3.3
Variance	0.25	0
Observations	3	3
Hypothesized Mean Difference	0	
Df	2	
t Stat	-2.77128	
P(T<=t) one-tail	0.054638	
t Critical one-tail	2.919986	
P(T<=t) two-tail	0.109276	
t Critical two-tail	4.302653	

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