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Hydrogen uptake hydrogenase activities of two anoxygenic phototrophic bacteria isolated from Leather industry effluents

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Abstract: Two anoxygenic phototrophic bacteria *Rb.capsulatus* KU002 and *Rps.acidophila* KU001 was isolated from leather industry effluents and their hydrogenase activities were studied in presence of argon and nitrogen. Hydrogenase activity was more when argon was used as inert gas phase compared to nitrogen as gas phase. *Rps.acidophila* exhibited more amount of hydrogenase than *Rb.capsulatus*. Hydrogenase was found to be bound to the membrane bound. Significance of the above in the light of existing literature is discussed in this communication.
Key words: Purple non sulphur bacteria, hydrogenase, argon, nitrogen.

Introduction:

Purple non sulphur phototrophic bacteria are very well known for their hydrogen producing capabilities. Hydrogen production from this group of organisms depends on two enzymes namely nitrogenase and hydrogenase. Hydrogenase not only produces hydrogen but also has hydrogen reuptake activity. Isolation and characterisation of hydrogenase gene from hydrogen-producing bacterial strain *Enterobacter cloacae* IIT-BT 08 was investigated by Mishra *et al*[6]. Genes for uptake hydrogenase were knocked out to study their effect on hydrogen production[5]. Asada *et al.*[2] attempted to overexpress hydrogenase from *Clostridium pasteurianum* in a cyanobacterium, *Synechococcus* PCC7942. They also demonstrated that clostridial hydrogenase protein, when electro-induced into cyanobacterial cells was active in producing hydrogen by receiving electrons produced by photosystems[7]. Therefore, hydrogenase activity of two anoxygenic phototrophic bacteria was assayed and results are discussed.

Material and Methods:

The phototrophic bacteria were isolated from the effluent samples by enrichment techniques by inoculating into the medium and incubated anaerobically in the light (2000 lux). Bacteria thus isolated were identified with the help of cultural characteristics (colour, size and shape), carbon and nitrogen requirement, vitamin requirements, absorption spectra analysis, bacteriochlorophylls and carotenoids. Identification keys provided in Bergey's manual of systematic bacteriology (1994)[3] was adopted.

Hydrogenase activity and localisation:

Cell pellets were sonicated after centrifugation for 10,000 X g for 10 min. The soluble fraction and the particulate fraction were assayed for the presence of hydrogenase. Hydrogenase activity was assayed spectrophotometrically by the method described by Drutschamann and Klemme[4] with a modification. Stationary phase cultures of the photosynthetic bacteria were harvested by centrifugation at 10,000 X g for 10 min washed in 0.3% saline thrice. Thick bacterial suspension was made by resuspending the cells in the basal medium. Three ml of the basal medium containing carbon source (10 mM) and 0.2 M of methylene blue was taken in a rimless test tube and sealed with subaseal. The gas phase was replaced by oxygen free N_2 by flushing the tube with ultra pure nitrogen for 10 min with help of hypodermic syringe. To the gas phase 10% (v/v) oxygen free hydrogen was injected and the reaction was started by injecting 250 µl of thick bacterial suspension into the test tubes and this was followed by colorimetric analysis on a spectrophotometer at 578 nm using reagent blank. Hydrogenase activity was expressed in units (1 unit = 0.01 O.D).

Results and Discussion:

Hydrogenase activity was measured in succinate and glutamate instead of ammonium chloride as ammonium ions were known to repress the activity of hydrogenase[1]. Hydrogenase was found in the particulate fraction rather than the soluble fraction as soluble fraction could not reduce methylene blue. The presence of membrane bound hydrogenase was also reported by Colbeau *et al*.[1] in *Rhodobacter capsulatus* B10. Both the bacteria under investigation could produce hydrogenase but they differed significantly in the degree of activity which varied with the incubation period and gas phase (table 1). Since the activity of hydrogenase was found to be more at stationary phase (Tsyganakov *et al*.,1982) cells at stationary phase were selected. Hydrogenase activity increased with progress of incubation period tried. Hydrogenase activity was more when argon was used as inert gas phase compared to nitrogen as gas phase. *Rps.acidophila* exhibited more amount of hydrogenase than *Rb.capsulatus*.

Highest hydrogenase activity was observed after 40 minutes of incubation in both the bacteria. Since hydrogenase also has hydrogen reuptake activity more amounts of hydrogenase indirectly correlates with less amounts of hydrogen produced by these group of organisms. Inhibition of hydrogen reuptake activity of the enzyme could result in more amounts of hydrogen production.

Table 1 : Hydrogenase activity of two anoxygenic phototrophic purple non sulphur bacteria

Organism	Incubation (in min)	Nitrogen+10% hydrogen	Argon + 10% hydrogen
Rb.capsulatus	5	1.0	1.2
	10	1.4	3.8
	15	2.8	6.4
	20	4.6	14.6
	25	8.2	20.2
	30	12.4	26.4
	35	14.8	32.8
	40	18.6	40.2
Rps.acidophila	5	2.2	3.6
	10	4.8	6.8
	15	6.2	12.4
	20	8.6	18.4
	25	10.4	26.8
	30	16.2	34.5
	35	26.4	42.8
	40	29.6	52.4

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