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Extracellular enzymes 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 were isolated from leather industry effluents and extra cellular enzyme production by them was assessed and the results are discussed in this communication. Both the bacteria could produce cellulases, hemicellulases, amylases, proteases and lipases. *Rb.capsulatus* KU002 was comparatively more superior to *Rps.acidophila* KU001 in the production of these enzymes. Enzyme production started from the 4th day of incubation and prolonged for duration of 12 days. This study was done to assess phototrophic bacterial enzyme production which could be of possible use in industry and bioremediation.

KEYWORDS

Phototrophic bacteria;
Protease;
Amylase;
Lipase;
Cellulases.

INTRODUCTION

Interest in the production of industrially important enzymes using anoxygenic phototrophic bacteria has increased with the report of *Chloroflexus aurantiacus*[6] producing amylase on medium containing starch as carbon source. Amylases produced by *Chloroflexus auranticus* are reported to be more stable than other amylases. *Rhodocyclus gelatinosus* is reported to produce protease[8]. Srinivas *et al.*[9] has studied the production of proteases by four anoxygenic phototrophic bacteria. The study of protease production by anoxygenic phototrophic bacteria may provide some idea about the role of these bacteria in remediation of water pollution. Extensive research has been carried out to assess lipase producing capacity of heterotrophic bacteria, not much information is available on anoxygenic phototrophic bacteria. The above facts tempted the investigator to assess the extracellular enzyme production by two anoxygenic phototrophic bacteria.

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, bacteriochlo-

rophylls and carotenoids. Identification keys provided in Bergey's manual of systematic bacteriology[1] was adopted.

Cellobiohydrolase (C_1) activity was assayed by DNS method suggested by Miller[3]. Endoglucanase (Cx) activity was assayed viscometrically as suggested by Reese *et al.*[7]. Hemicellulases activity was assayed by the method described by Sreenath *et al.*[10]. Protease activity was assayed by the method of Keay and Wrildi[2]. α-Amylase activity was assayed by the method adopted by Mukherjee and Majumdar[4]. β-amylase activity was assayed by DNS method as suggested by Plummer[5]. Lipase activity was assayed by the method of Urs *et al.*[11] with some modifications.

RESULTS AND DISCUSSION

Rb.capsulatus secreted good amount of cellobiohydrolase which increased with maximum production on the 8th day of incubation. Endoglucanases production was maximum on the 12th day of incubation (TABLE 1). Similar trend was observed by *Rps. acidophila* in the production of cellobiohydrolase and endoglucanase. *Rb.capsulatus* was comparatively superior to *Rps.acidophila* in the production of cellulases. Both the organisms under investigation produced hemicellulases. Eight days incubation was optimum for the production of hemicellulases. *Rb.capsulatus* was comparatively superior in production of more protease than *Rps.acidophila* .When *Rb.capsulatus* opted for 12 days incubation for maximum production of the protease, *Rps.acidophila* opted 8 days. Such variation in protease production was also observed by Srinivas *et al.*[9] among the bacteria studied by them.

Bacteria under investigation could secrete dextrinising amylase by the end of eight days which decreased significantly during subsequent incubation period. Saccharifying amylase production was comparatively more by *Rb.capsulatus* than *Rps.acidophila*. The same trend was observed by both the bacteria in the production of Saccharifying amylases. *Rb.capsulatus* was more efficient in secretion of both the amylases than *Rps.acidophila*.

Lipase production by the two anoxygenic phototrophic bacteria reveals that both the bacteria could secrete lipases. Maximum lipase was produced by 8 days of incubation period. *Rb.capsulatus* produced

TABLE 1 : Extracellular enzyme production by two purple non sulphur phototrophic bacteria

Enzymes	Incubation Period (days)	*Rb.capsulatus*	*Rps.acidophila*
Cellobiohydrolase#	4	20	26
	8	48	34
	12	22	18
	16	14	8
Endoglucanase##	4	15.87	27.04
	8	42	36.18
	12	63.12	46.12
	16	58.06	50.54
Hemicellulase*	4	22	14
	8	25	22
	12	13	8
	16	3	2
Protease **	4	12.5	25
	8	22	30
	12	25	20
	16	14	12
Amylases			
Dextrinizing	4	0.128	0.132
amylase$	8	0.286	0.18
	12	0.162	0.085
	16	0.064	0.054
Saccharifying	4	0.612	0.308
amylase$$	8	0.929	0.418
	12	0.724	0.388
	16	0.464	0.118
Lipase^	4	8.2	6.4
	8	15.6	13.5
	12	9.8	7.2
	16	4.4	2.8

#Cellobiohydrolase activity expressed in µg/ml of glucose liberated in 6 hrs of incubation

##Endoglucanase activity expressed in relative viscometric units (RVU)

***Hemicellulase activity expressed in µg/ml of glucose liberated in 30 minutes of incubation**

****Protease activity expressed in units.One unit is that quantity of enzyme which produced TCA soluble fragments giving blue colour equivalent 0.5 µg/ml of tyrosine liberated under conditions of assay**

$Dextrinising amylase activity expressed in mg starch hydrolysed

$$Saccharifying amylase activity expressed in mg starch hydrolysed

^Activity expressed in units (A37,0.05 N NaOH required quantity was taken as 1 unit of enzyme activity)

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comparatively more amounts of lipase than *Rps. acidophila*. There was a decrease in enzyme production with an increase in incubation period.

The ability of *Rb.capsulatus* to produce more amounts of extracellular enzymes than *Rps.acidophila* could to be due to its more versatile nature. Not only remediation applications but phototrophic bacterial enzymes are also known to resemble eukaryotic enzymes in some aspects. Further studies for purification and characterisation of these enzymes are required for their potential applications in bioremediation.

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