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EXTRACELLULAR L-GLUTAMINASE PRODUCTION BY MARINE BACTERIA

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SUMMARY

Four species of bacteria which included Pseudomonas fluorescens, Vibrio cholerae and Vibrio costicola were observed to produce glutaminase both as extracellular and intracellular fractions. Comparatively both the fractions were higher in mineral media supplemented with 1% glutamine than in nutrient broth added with or without glutamine. Extracellular glutaminase production was about 2.6-6.8 times greater than the intracellular production by all the tested strains.

INTRODUCTION

Microbial L-glutaminases (L-glutamine amidohydrolase, EC.3.5.1.2) have received greater attention and significance since they were recognised as having antitumor activity along with L-asparaginase (El-Asmar and Greenberg, 1966; Roberts et al., 1970) and salt tolerant and heat stable glutaminases of fungi improved the taste of soysauce due to the increase in glutamate content as a result of their action (Yokotsuka, 1987). Except for the reports on the extracellular secretion of L-glutaminase by Aspergillus oryzae (Tomita et al., 1988; Yano et al., 1988), no information is available on bacteria.

Extracellular glutaminases are more advantageous than intracellular since they could be produced abundantly

in the culture broth under normal conditions and could be purified economically. Since glutaminase has dual importance both in cancer therapy and in food industry, a search for high enzyme yielding strains was made with marine samples which provide a source of potential micro-organisms from different environmental conditions. The present communication deals with a comparative study of the intracellular and extracellular secretion of L-glutaminase by marine Pseudomonas and Vibrio species which offer good potential for their application in industry.

MATERIALS AND METHODS

Four strains isolated from sediments and water of marine environments of Cochin (West Coast of India) and identified as Pseudomonas fluorescens (ACMR 43), Pseudomonas fluorescens (ACMR 171), Vibrio costicola (ACMR 267) and Vibrio cholerae (ACMR 347) were used in the present study. The cultures were isolated, maintained and grown in a mineral salts medium that contained 1 g of KH_2PO_4, 500 mg of $MgSO_4 7H_2O$, 100 mg of $NaNO_3$, 100 mg of $CaCl_2$, 100 mg of $FeCl_3$, 10 g of L-glutamine and 10 g of NaCl in one litre adjusted to a pH 7 ± 0.2.

The cells were grown for 24 hrs and harvested by centrifugation at 5000 rpm for 20 min. The supernatant obtained was partially purified by $(NH_4)_2SO_4$ fractionation (50-80% saturation) followed by extensive dialysis against phosphate buffer (0.2 M) at 4°C for 24 hrs. The purified fractions were analysed for extracellular glutaminase activity. Intracellular glutaminase production was tested according to Cedar and Schwartz (1967).

Enzyme assay: Glutaminase activity was assayed following the procedure of Imada et al., (1973). The reaction mixture containing 0.5 ml of enzyme preparation plus 0.5 ml of 0.2 M phosphate buffer of pH 6 or 8 (pH of the buffer varied with respect to the optimum pH of enzyme; pH 8 for the enzyme of P.fluorescens ACMR 171 and pH 6 for all other strains tested), 0.5 ml of 0.04 M L-glutamine and distilled water to a total volume of 2.0 ml was incubated for 15 min. at 40°C and the reaction was arrested by the addition of 0.5 ml of 1.5 M trichloroacetic

acid. The precipitated protein was removed by centrifugation at 10,000 rpm for 20 min. To 0.1 ml of this supernatant, 3.7 ml of distilled water and 0.2 ml of Nessler's reagent were added and the colour developed after 10 min. was measured at 450 nm in a UV-visible spectrophotometer. Enzyme and substrate blanks were separately included for each assay. One International Unit of glutaminase was defined as the amount of enzyme that liberates 1 μ mol of ammonia under optimal assay conditions.

RESULTS AND DISCUSSION

In the present investigation, species of P.fluorescens, V.cholerae and V.costicola were observed to produce glutaminase, both as extracellular and intracellular fractions, in ample amounts (Table 1). The extracellular glutaminases were in higher titres in all the 3 types of media tested. Comparatively both extracellular and intracellular fractions were higher in mineral media supplemented with 1% glutamine than in nutrient broth with or without glutamine. Extracellular glutaminase production was about 2.6-6.8 times greater than intracellular production by all the four strains. Mineral media added with glutamine yielded 2.5 fold higher yields of glutaminase than in nutrient broth added with glutamine. Nutrient broth substituted with 1% glutamine gave only meagre levels of intracellular fractions from all the strains.

When the organisms were grown in a mineral medium with glutamine as the only carbon and nitrogen source, it appears that the organism has secreted out the glutaminase which are otherwise intracellular. There was

Table 1: Production of extracellular and intracellular glutaminase by Pseudomonas sp. and Vibrio sp. (Enzyme production expressed as u/ml)

Media	P.fluorescens ACMR 43		P.fluorescens ACMR 171		V.costicola ACMR 267		V.cholerae ACMR 347	
	E.F.	I.F.	E.R.	I.F.	E.F.	I.F.	E.F.	I.F.
MMG	2.348	0.469	1.878	0.704	2.114	0.469	2.818	0.413
NBG	1.057	0.234	1.408	0.393	1.174	0.117	0.587	N.D.
NB	N.D.	N.D.	0.234	N.D.	0.469	N.D.	0.359	N.D.

MMG - Mineral media + 1% glutamine; NBG - Nutrient broth + 1% glutamine, NB - Nutrient broth; E.F. - Extracellular fraction; I.F. - Intracellular fraction; N.D. - Not detectable.

was minimal release of glutaminase in nutrient broth with glutamine.

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