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Synthesis of L-asparaginase by *Serratia marcescens* (Nima)

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Abstract. The production of L-asparaginase by two mutants of *Serratia marcescens* grown on 14 different media was studied. The enzyme content increased from trace levels to 2·4 international units per ml when the organisms were grown in glycerol-peptone yeast extract medium. Glucose was the best carbon source under aerobic conditions. The enzyme content increased when L-asparagine was present in the growth medium.

Keywords. L-asparaginase; *Serratia marcescens*; growth media.

Introduction

Several micro-organisms including *Serratia marcescens* produce L-asparaginases (EC. 3.5.1.1) with antitumor activity (Wriston and Yellin, 1973). Although extensive studies have been carried out on the isolation and on the antileukemia properties of this enzyme (Heinemann and Howard, 1969; Heinemann *et al*., 1970; Khan *et al*., 1970), very little information is available on the production of this enzyme by *S. marcescens* (Khan *et al*., 1970; Netrval, 1977; Singh *et al*., 1977). This paper describes the effect of changing the composition of the medium used for growing two mutants WF and 933 of *S. marcescens* on the levels of the enzyme in the medium.

Materials and methods

Growth of organisms

Two colourless mutants of *S. marcescens* WF, and 933, were used in these studies. Stock cultures were maintained at 27° C on agar slants containing 5 g beef extract, 5 g peptone, 5 g glucose and 20 g agar per litre medium, pH 6·8–7·0.

Organisms were grown aerobically on a rotary shaker at 37° C for 24 h in 500 ml Ehrlenmeyer flasks containing 100 ml of a 1% glycerol and 0·5% peptone medium.

Corn-steep-liquor (CSL) mediumwas prepared according to Roberts *et al.* (1968) To 50 ml CSL, 100 ml water was added and the pH was adjusted to 7·5. The precipitated material was removed by filtration, boiled and refiltered. The clear medium was diluted to 1·0 litre with water and sterilised.

Cells were harvested by centrifugation at 5,000 g for 20 min, washed with saline and suspended in distilled water. They were disrupted ultrasonically at 20 kHz for 3 min in a Branson sonifier. Cell-free extracts were obtained by centrifugation.

Enzyme assay

Asparaginase activity was assayed according to the method of Meister (1955) and ammonia liberated was estimated by direct Nesslerisation (Peter *et al*., 1970).

Units

One International Unit (I.U.) of L-asparaginase liberates 1 μmol ammonia from L-asparagine per min at 37° C. Enzyme content is expressed per 10 ml broth rather than on a dry weight basis.

Results

L-asparaginase production in various media

The effects of changing the media on L-asparaginase production are summarised in table 1. It can be seen that the enzyme production by the mutant WF was higher than by the mutant 933 in all the media.

The enzyme activity could not be detected when the mutants were grown in the synthetic medium. Nutrient broth and CSL medium were equally efficient. Maximum enzyme production occurred in both the mutants in glycerol peptone medium.

L-asparaginase activity was increased by the addition of L-asparagine to synthetic medium. Yeast extract further activated L-asparaginase production. In CSL medium, there occurred only marginal increase in enzyme production by the addition of L-asparagine, lactic acid or both in WF and 933. Maximal stimulation was observed when L-asparagine or yeast extract were added to glycerol-peptone medium. Semi-aerobic conditions created by the addition of agar and sodium thioglycollate had no effect on enzyme production. In view of the maximal activity observed, glycerol-peptone medium was chosen for further studies on enzyme production.

Effect of various carbon sources

It was observed that the enzyme synthesis was stimulated by the addition of glucose in glycerol-peptone medium while soluble starch and dextrin had no effect (table 2). In addition to glycerol (table 1), glucose and sucrose were the best carbon sources for enzyme production by the two mutants. Maltose and galactose were marginally favourable while fructose, mannitol and sorbitol were inhibitory for enzyme production by the two mutants. While lactose inhibited in WF, it enhanced the enzyme production in mutant 933.

Table 1. L-asparaginase activity in two mutants of *Serratia marcescens* in the various media.

Medium	L-asparaginase activity I.U./10 ml medium	
	WF	933
Synthetic medium (SM)[a]	Nil	Nil
Nutrient broth[b]	8·5	4·2
Glycerol-peptone (GP)[c]	12·0	6·8
Corn-steep-liquor (CSL)[d]	8·0	3·8
SM + L-asparagine[e]	2·9	1·3
SM + L-asparagine + yeast extract	5·0	2·4
GP + L-asparagine	21·0	15·8
GP + yeast extract	24·0	20·0
GP + L-asparagine + yeast extract	22·0	16·8
CSL + L-asparagine	9·0	3·8
CSL + lactic acid	9·0	4·6
CSL + lactic acid + L-asparagine	9·2	5·0
GP + agar + sodium thioglycollate	13·0	7·0
CSL + agar + sodium thioglycollate	9·0	3·9

[a] Synthetic medium consisted of $(NH_4)_2$ SO_4 5 g, KH_2PO_4 3 g, Na_2HPO_4 7 g, $MgSO_4$-$7H_2O$, 1·2g, NaCl 1 g, $FeCl_3$–$6H_2O$ 1 mg, $CaCl_2$–$7H_2O$ 0·05 g and glucose 10g in 1 litre, pH was adjusted to 6·8–7·2.

[b] Nutrient broth at pH 6·8–7·2 contained 1% beef extract, 0·5% yeast extract and 0·5% sodium chloride.

[c] Glycerol-peptone medium at pH 6·8–7·2 contained 1% glycerol and 0·5% peptone.

[d] Corn-steep-liquor medium was prepared according to Robert *et al*. (1968).

[e] Concentrations of compounds added to the various media were 0·3% L-asparagine, 0·6% lactic acid, 0·4% yeast extract, 0·2% agar and 0·5% sodium thioglycollate.

Effect of amino acids and amides

Supplementing the medium with amino acids like methionine, aspartic acid, glutamic acid and glutamine resulted in increased synthesis of L-asparaginase. The effect on 933 was more pronounced than on WF (table 3).

Effect of metabolic intermediates

Lactic, fumaric, oxalic, pyruvic and citric acids increased enzyme production in both the mutants, while succinic and malic acids had a marginal effect. α-Ketoglutaric acid slightly inhibited the enzyme production in WF but was marginally stimulatory in the mutant 933 (table 4).

Table 2. Effect of various carbon sources on L-asparaginase synthesis by two mutants of *Serratia marcescens*.

Additional carbon source	L-asparaginase I.U./10 ml medium	
	WF	933
None	10·6	6·6
Glucose	15·1	18·9
Fructose	6·7	5·2
Sucrose	15·6	16·5
Maltose	12·6	12·6
Lactose	8·8	17·6
Galactose	11·6	8·3
Mannose	12·1	8·5
D-Mannitol	6·1	5·1
D-Sorbitol	6·4	4·8
Soluble starch	9·8	6·8
Dextrin	10·2	6·3

The optimal concentrations for carbohydrates were predetermined in each case. The concentration of glucose was 0·2% in WF and 0·3% in the mutant 933. Concentration of sucrose, maltose, lactose and galactose were 0·3% in WF and 0·4% in the mutant 933. Other compounds were used at 0·3 % level for the growth of both the mutants.

Table 3. Effect of amino acids and amides on L-asparaginase synthesis by two mutants of *Serratia marcescens.*

Compounds added	L-asparaginase (I.U./10 ml medium)	
	WF	933
L-Methionine	13·6	13·4
L-Aspartic acid	20·0	22·2
L-Glutamic acid	19·8	15·1
L-Asparagine	25·0	18·1
L-Glutamine	20·7	16·8
None	11·0	6·7

The optimum concentrations of L-methionine, L-aspartic acid and L-glutamic acid were 0·1 %, 0·2% and 0·1% for WF and 0·1%, 0·1% and 0·3% for the mutant 933, respectively. LAsparagine and L-Asparagine and L-glutamine were used at 0·3 % and 0·2 % for the production of L-asparaginase by both the mutants.

Table 4. Effect of various metabolic intermediates on L-asparaginase synthesis by two mutants of *Serratia marcescens*.

Metabolite added	L-asparaginase (I.U./10 ml medium)	
	WF	933
Lactic acid	24·5	18·1
Fumaric acid	21·8	16·5
Succinic acid	12·0	8·3
Oxalic acid	18·0	9·1
Citric acid	16·6	10·4
Pyruvic acid	22·2	11·8
α-Ketoglutaric acid	9·3	7·8
Malic acid	14·1	7·1
Nil	11·8	6·6

The optimum concentrations of various metabolites were determined in each case initially. The concentrations used were 0·2%, 0·2%, 0·3%, 0·2%, 0·1%, 0·2%and 0·6% respectively for α-ketoglutaric acid, pyruvic acid, fumaric acid, succinic acid, oxalic acid, citric acid, malic acid and lactic acid for the mutant WF. In the case of the mutant 933, optimal concentrations were, α-ketoglutaric acid and citric acid 0·3%; pyruvic acid, fumaric acid and oxalic acid 0·2%; succinic acid and malic acid 0·1 % and lactic acid 0·6%.

Discussion

In these studies, the enzyme content is expressed per 10 ml of the medium rather than on a dry weight basis for the following reasons: (1) The aim of the study was to determine the effect of changes in the media on the total enzyme production and (2) the production of the enzyme was not always directly proportional to the biomass.

Aerobically grown *Escherichia coli* cultures yielded larger quantities of cells containing minimal amount of L-asparaginase, whereas the anaerobic culture produced less amount of cells that contained abundant enzyme (Boeck *et al*., 1970). Some others (Roberts *et al*., 1968; Netrval and Smekal, 1970) isolated L-asparaginase from cells grown in highly aerated cultures of *E. coli.* Cedar and Schwartz (1968) observed that synthesis of L-asparaginase in *E. coli* is inversely dependent on aeration. However, Bilimoria (1969) recommended growing the culture under mild aeration, while Boeck and Ho (1973) suggested a bipartite process that used both aerobic and anaerobic conditions.

Heinemann and Howard (1969) observed that mild agitation of culture was essential for optimal growth of *Serratia* and that L-asparaginase was produced only at very low levels of dissolved oxygen. On the contrary, Khan *et al.* (1970) have reported L-asparaginase production in *Serratia* under aerobic conditions. Our observations show that semiaerobic fermentation was not satisfactory.

The role of glucose in the synthesis of L-asparaginase is controversial. It is generally accepted as a catabolite repressor in the case of *E. coli* and *Erwinia aeroideae* at higher concentrations (Jeffries, 1976; Liu and Zajic, 1972). The increased production of L-asparaginase in the presence of glucose observed in the present study, although contrary to the above mentioned studies, is in agreement with the observation in other organisms like *Bacillus mesentericus* (Tyulpanova *et al*., 1972) and *Mycobacterium* (Pastuszak and Szymona, 1976).

In our studies, L-asparaginase synthesis was increased by the addition of L-aspartic acid, L-glutamic acid, L-asparagine and L-glutamine similar to the results obtained with *Pseudomonas* (Nikolayev *et al*., 1969, 1971) and *Erwinia corotovora* (Ozolins *et al*., 1973).

The beneficial effects of methionine and yeast extract are in agreement with the observations in *Serratia* (Khan *et al*., 1970), *E. coli* (Netrval, 1977; Jeffries, 1976), *Rhodopseudomonas capsulatus* (Tohan *et al*., 1971) and *Erwinia aeroideae* (Liu and Zajic, 1972).

Appreciable stimulatory effects of lactate, fumarate, oxalate, citrate, pyruvate and malate have been found in WF and 933. Succinate and α-ketoglutarate either have no effect or caused only marginal changes in enzyme production. In *E. coli*, (Netrval, 1973, 1977) stimulatory effects of lactate, pyruvate, malate and oxaloacetate are known. Fumarate, succinate and acetate have no appreciable effect. In *Bacillus* (Smirrous *et al*., 1976), α-ketoglutarate and oxalate exert stimulatory effects while citrate, pyruvate, succinate and fumarate were inhibitory. In Erwinia, L-asparaginase production is stimulated by citrate, fumarate, malate and succinate (Ozolins *et al*., 1973).

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