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L-Asparaginase Synthesis by *Erwinia aroideae*

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Maximum L-asparaginase activity was obtained when 1.0% lactose and 1.5% yeast extract were supplied as carbon and nitrogen sources, respectively. Glucose inhibited the enzyme formation. The diauxie phenomenon was observed with *Erwinia aroideae* NRRL B-138 grown in a medium containing glucose and lactose.

Erwinia aroideae (3) and *Erwinia carotovora* (5) have been reported to produce larger quantities of L-asparaginase than *Escherichia coli*. Thus, *Erwinia* species have potential for large-scale production of L-asparaginase, especially if the enzyme preparation from *Erwinia* species can be used to treat some of the obstinate tumors that cannot be cured with the enzyme from *E. coli*.

L-Asparaginase from *E. aroideae* has been produced under semi-pilot-plant conditions and has been partially purified (3). Published data concerning the factors controlling enzyme formation are limited. The purpose of the present study was to determine some of the factors controlling enzyme formation by *E. aroideae* NRRL B-138, which produces high yields of L-asparaginase.

E. aroideae NRRL B-138 used was obtained from Northern Regional Research Laboratory, U.S. Department of Agriculture. This culture was maintained on agar slants containing 0.1% K_2HPO_4, 0.5% tryptone, 0.5% yeast extract, and 2% agar. The same medium (without agar) was used to prepare inocula. Fermentations were conducted in 500-ml cotton-plugged Erlenmeyer flasks, each containing 100 ml of medium. The basal medium contained 0.1% K_2HPO_4 to which various nitrogen and carbon sources were added. Each flask was inoculated with 1 ml of a 12-hr-old vegetative inoculum and was incubated at 24 C (±2 C) for 17 hr unless otherwise indicated. Agitation and aeration were done by shaking the flasks on a rotary shaker (New Brunswick Scientific Co., model G53) at 200 rev/min. Cells were harvested by centrifugation (Sorvall, model RC2-B) at 10,000 rev/min for 15 min and were washed with 1/30 M phosphate buffer. Samples of washed cells were resuspended to their original volume in 0.1 M sodium borate buffer (*p*H 8.5).

L-Asparaginase activity was determined by the method of Meister (2). One international unit (IU) of L-asparaginase is that amount of enzyme which liberates 1 μmole of ammonia in 1 min at 37 C. Glucose content of the medium was determined spectrophotometrically with Somogyi (4) reagent. Determination of lactose was carried out by the spectrophotometric method of Malpress and Morrison (1).

When tryptone and yeast extract were used as nitrogen sources, lactose gave the highest yield (2.47 IU of L-asparaginase per ml of culture) of all C sources tested (Table 1).

The effect of this sugar on the yield of L-asparaginase was further studied. Addition of 0.1% lactose increased the enzyme synthesis

TABLE 1. *Effect of carbon compounds on formation of L-asparaginase*[a]

Carbon compound	L-Asparaginase (IU/ml)	Dry wt of cells (mg/ml)
Lactose	2.47	2.67
Mannose	1.56	3.49
Galactose	1.41	3.30
Fructose	0.47	3.76
Sucrose	0.41	2.57
Glucose	0.35	2.22
WSL[b]	0.89	1.46
Starches[c]	0.84-0.88	1.45-1.50
Control (no C added)	0.88	1.48

[a] Fermentation medium contained 0.5% carbon source, 0.1% K_2HPO_4, 0.5% yeast extract, and 0.5% tryptone. The initial *p*H was adjusted to 7.0. The cultures were incubated for 17 hr at 24 C.

[b] Two per cent (v/v) of waste sulfite liquor was used as carbon source.

[c] Soluble starch or wheat flour.

TABLE 2. *Effect of nitrogen sources on formation of L-asparaginase*[a]

Nitrogen source	L-Asparaginase (IU/ml)	Dry wt of cells (mg/ml)
Yeast extract	2.76	3.03
Tryptone-yeast extract[b]	2.33	2.15
Tryptone	1.19	1.68
Corn steep liquor[c]	0.53	1.58
Casein hydrolysate	0.47	0.44
Peptone	0.45	1.55
Promine-D	0	0.25

[a] The medium contained 0.5% lactose, 0.1% K_2HPO_4, and 1% nitrogen sources. The initial *p*H was adjusted to 7.0. The cultures were incubated for 17 hr at 24 C.

[b] Tryptone (0.5%) and 0.5% yeast extract were used as nitrogen sources.

[c] Two per cent (v/v) of corn steep liquor filtrate was used as nitrogen source.

TABLE 3. *Effect of glucose concentration on formation of L-asparaginase*[a]

Initial glucose concentration (%)	L-Asparaginase (IU/ml)		Glucose utilized (g/liter)		Final *p*H	
	11 hr	17 hr	11 hr	17 hr	11 hr	17 hr
0	1.94	2.29	0	0	7.5	8.1
0.1	0.88	1.28	1.0	1.0	7.2	8.1
0.2	0.59	1.12	1.6	2.0	7.0	7.9
0.4	0.53	1.09	2.7	4.0	6.3	7.7
0.8	0.50	0.67	2.8	7.5	6.2	6.6

[a] The medium contained 0 to 0.8% glucose, 0.1% K_2HPO_4, and 1.5% yeast extract, adjusted to *p*H 7.5 before sterilization. The cultures were incubated at 24 C on a rotary shaker at 200 rev/min.

TABLE 4. *L-Asparaginase formation and rate of lactose and glucose utilization by Erwinia aroideae*

Time of incubation (hr)	Medium 1[a]		Medium 2		
	Residual lactose (%)	L-Asparaginase (IU/ml)	Residual sugar (%)		L-Asparaginase (IU/ml)
			Glucose	Lactose	
0	1.00	0	0.50	1.00	0
12	0.76	2.32	0.22	1.00	0.47
17	0.35	3.66	0	1.00	0.86
24	0	3.54	0	0	1.05

[a] Medium 1 contained 1% lactose, 0.1% K_2HPO_4, and 1.5% yeast extract, initial *p*H 7.5. Medium 2 was medium 1 plus 0.5% glucose.

from 1.55 to 2.42 IU/ml. The optimum concentration of lactose was 1.0%, which gave 2.84 IU/ml enzyme activity.

Various sources of nitrogen were also investigated. With 0.5% lactose as carbon source, the L-asparaginase activities varied widely depending on the nitrogen source present (Table 2). No growth was observed in the flasks containing inorganic nitrogen sources, e.g., KNO_3, $(NH_4)_2HPO_4$, $(NH_4)_2SO_4$, $NaNO_3$, NH_4Cl, and NH_4NO_3, within 3 days of incubation. The optimum concentration of yeast extract was found to be 1.5%, which gave 3.41 IU/ml enzyme activity when 1.0% lactose was used as carbon source.

Further studies on the inhibition of L-asparaginase formation by glucose (as noted in Table 1) showed that the extent of inhibition increased with increasing concentration of glucose (Table 3). *E. aroideae* metabolized 0.28 and 0.75% of glucose in 11 hr and 17 hr, respectively, in the medium containing 1.5% yeast extract. During incubation with glucose, *p*H usually decreased to a minimum value in 11 hr (if lactose was used as carbon source, the minimum value was obtained in 16 to 18 hr) and increased thereafter. At 0.1% level of glucose, the final *p*H at 11 hr was 7.2 (Table 3), whereas the control (1.0% lactose, no glucose) gave a final *p*H of 7.1 and enzyme yield of 2.18 IU/ml. This suggests that the enzyme formation is not repressed at this *p*H.

The diauxie phenomenon was observed with *E. aroideae* NRRL B-138 grown in the medium containing glucose and lactose (Table 4). Without glucose (medium 1), 0.65% lactose was utilized in 17 hr and maximum enzyme yield of 3.66 IU/ml was synthesized. Addition of 0.5% glucose (medium 2) completely inhibited the utilization of lactose which did not occur until the glucose had been consumed. Glucose inhibited the synthesis of both L-asparaginase and the inducible lactose-hydrolyzing enzyme (β-galactosidase). Lactose utilization at this latter stage (17 to 24 hr) did not stimulate synthesis of L-asparaginase. Most of the enzyme was synthesized during the logarithmic and early stationary phases.

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