Factors Influencing L-Asparaginase Production by *Erwinia aroideae*¹

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Increased yields of L-asparaginase from *Erwinia aroideae* NRRL B-138 were obtained by medium enrichment techniques coupled with aeration optimization.

L-Asparaginase from *Escherichia coli* inhibits the growth of some forms of neoplastic cell disease in man (4, 5); however, some toxicity has been observed during clinical studies (2, 3). Although improved purification techniques helped lower the incidence of antigenic reactions exhibited by some patients, other sources of this enzyme are desired to provide an alternate immunologically distinct L-asparaginase which may allow continued therapy of an *E. coli* enzyme-sensitized patient (9).

*Erwinia aroideae* (Townsend) Holland NRRL B-138 was found to produce substantial quantities of L-asparaginase (6). Later studies indicated that enzyme yields up to 2 international units (IU)/ml could be readily obtained in 8 hr with this organism (7).

Because of the demand for quantities of pure L-asparaginase and a 60 to 95% activity loss associated with purification techniques (1, 8), an investigation was undertaken to increase yields of enzyme through optimization of substrates and aeration. The present communication describes the effects of various substrates and increased aeration on L-asparaginase production by *E. aroideae*.

*E. aroideae* NRRL B-138 was used throughout this investigation. The culture was maintained on TGY slants (glucose, 1.0 g; K₂HPO₄, 1.0 g; Difco yeast extract, 5.0 g; tryptone, 5.0 g; agar, 20 g; tap water to 1.0 liter; pH adjusted to 7.0); the same medium (without agar) was used in growth factor experiments. Unless noted, 50 ml of TGY was placed in a 300-ml Erlenmeyer flask, sterilized by autoclaving at 121 C for 10 min, and inoculated from a slant with *E. aroideae* NRRL B-138. The flasks were incubated at 28 C on a rotary shaker (250 rev/min) for 16 hr. Asparaginase activity was determined by nesslerization, as previously described (6).

A 2⁴ factorial experiment with two concentrations of each of the four components of TGY was used to determine the interactions or effects on L-asparaginase production. Enzyme production at these substrate concentrations was directly proportional to cell growth as determined by optical density (OD) at 540 nm. The flasks with higher level yeast extract (YE) produced significantly more L-asparaginase. A calculation of mean response to the four factors (Table 1) indicated a direct relationship between enzyme production and YE concentration. Changes in concentration of tryptone and K₂HPO₄ had little effect. A small difference associated with glucose concentrations would be statistically significant only at about a 1 in 7 probability level. Glucose inhibition is not evident at these levels.

Fermentation nutrients from several manufacturers were substituted for YE in the TGY medium, and L-asparaginase production was determined (Table 2). Cell growth was obtained with most substrates tested; however, except for Difco technical yeast extract (TYE) and Amber BYF100, enzyme levels were sub-

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### Table 1. Mean response to four factors affecting L-asparaginase production by *Erwinia aroideae*

| Factor      | Level (%) | IU/ml |
|-------------|-----------|-------|
| Tryptone    | 0.25      | 0.93  |
|             | 1.00      | 0.80  |
| YE          | 0.25      | 0.43  |
|             | 1.00      | 1.76  |
| Glucose     | 0.05      | 0.95  |
|             | 0.20      | 0.78  |
| K₂HPO₄      | 0.05      | 0.93  |
|             | 0.20      | 0.80  |
TABLE 2. Influence of various substrates substituted for YE in TGY medium on L-asparaginase production by Erwinia arorideae

| Substrate* | Per cent | IU/ml | Substrate* | Per cent | IU/ml |
|------------|----------|-------|------------|----------|-------|
| Difco YE   | 0.5      | 1.5   | NIBIN      | 0.5      | 0     |
|            | 2.0      | 3.1   |            | 5.0      | 0     |
|            | 5.0      | 3.4   | Pharmamedia* | 0.5    | 0.5  |
| Difco TYE  | 0.5      | 1.7   |            | 2.0      | 0.7   |
|            | 2.0      | 3.4   | Colab Y autolysate* | 0.5 | 0.25 |
|            | 5.0      | 0.25  |            | 2.0      | 1.25  |
| Amber BYF50c | 0.5 0.25 |       | Sheffield NZ Amine AT' | 0.5 | 0     |
|            | 2.0 1.9  |       |            | 2.0      | 1.25  |
|            | 5.0 0.37 |       | Sheffield NZ Amine BT | 0.5 | 0.5   |
| Amber BYF100 | 0.5 1.5  |       | Soy peptone T | 0.5 | 0.65 |
|            | 2.0 3.5  |       |            | 2.0      | 1.8   |
| Amber BYF300 | 0.5 0.19 |       | Fermm Amin | 0.5 | 1.1   |
|            | 2.0 1.9  |       | Type I     | 2.0      | 1.2   |
|            | 5.0 2.0  |       | Type II    | 2.0      | 2.5   |
| Amberex 1003 | 0.5 0.6  |       | Type III   | 0.5      | 0.5   |
|            | 2.0 2.2  |       |            | 2.0      | 1.25  |
|            | 5.0 0    |       | Type IV    | 0.5      | 0.9   |
| Amber WWBY | 0.5 0    |       | Edamin T   | 0.5      | 0.5   |
|            | 2.0 0.9  |       |            | 2.0      | 1.6   |
|            | 5.0 1.7  |       | Soytone    | 0.5      | 0.75  |
| OMHAP      | 0.5 0    |       | Cottonseed hydrolysate* | 0.5 | 1.4  |
|            | 2.0 0.65 |       |            | 2.0      | 1.25  |
| OM peptone | 0.5 0    |       | Staley’s amino A* | 0.5 | 0     |
|            | 5.0 0    |       |            | 2.0      | 0     |
| OMBHY      | 0.5 0    |       | Corn steep liquor* | 2.0 | 1.5  |
|            | 5.0 0    |       |            |          |       |

* Substituted for Difco yeast extract (YE) in TGY medium.
* Difco Laboratories, Detroit, Mich.; TYE, technical yeast extract.
* Amber Laboratories, Juneau, Wis.
* Trader’s Protein Division, Ft. Worth, Tex.
* Colab Inc., Chicago Heights, Ill.
* Sheffield Chemical, Norwich, N.Y.
* A. E. Staley Mfg. Co., Decatur, Ill.
* Grain Processing Corp., Muscatine, Iowa.

![Graph](image-url)

**FIG. 1.** Effect of increased aeration and agitation on L-asparaginase production by Erwinia arorideae NRRL B-138 in 300-ml shake flasks at 28 C.
substantially lower than those achieved with YE. Substrates containing TYE or BYF100 produced slightly more L-asparaginase than TGY at levels of 0.5 and 2.0% substrate concentrations; but at the 5.0% level, enzyme production was essentially absent because of diminished cell growth. A mixture of YE and TYE (1:1) at concentrations of 0.25 to 2.0% gave enzyme levels slightly higher than YE alone, and at 5.0% the l-asparaginase production was 3.8 IU/ml.

Since higher concentrations of YE appear to increase the viscosity of the medium and thereby may reduce the oxygen uptake, aeration rates were raised by using indented flasks and faster agitation. A comparison between L-asparaginase production under standard and increased aeration conditions is shown in Fig. 1. At 5.0% YE concentration, the higher aeration provided yields of 5.2 IU/ml in this experiment. L-Asparaginase levels up to 6.6 IU/ml were observed during these studies. With greater concentrations of YE, viscosity increases could not be compensated for, and subsequent enzyme levels were greatly reduced.

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