

# L-Asparaginase Production by *Streptomyces griseus*

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*Streptomyces griseus* ATCC 10137 synthesizes about 1 IU of L-asparaginase/100 ml of a 4% peptone medium. The enzyme has a pH optimum of 8.5 which is comparable to that of the L-asparaginase derived from *Escherichia coli* which has antitumor properties.

Since the demonstration by Broome (1) that L-asparaginase (L-asparagine aminohydrolase, EC 3.5.1.1) is capable of inhibiting the growth of various tumors, sources of this enzyme for medical use have been sought. Two reports indicate that some organisms in the *Actinomycetales* are incapable of producing L-asparaginase or produce it in extremely small quantities (6, 8), but it has been demonstrated that *Mycobacterium tuberculosis* produces an L-asparaginase capable of inhibiting tumors (5). During this investigation, Becker and Forster (Bacteriol. Proc., p. 9, 1971) reported that other organisms in the *Actinomycetales* are capable of producing L-asparaginase.

The media examined for ability to support L-asparaginase production by *Streptomyces griseus* ATCC 10137 included the yeast malt glucose (YMG) medium of Pridham et al. (4), the same medium without glucose (YM), the synthetic glucose-asparagine (GA) medium described by Waksman (7), and 3% solutions of Difco Casamino Acids, tryptone, peptone, and proteose peptone. Still another medium used was Casamino Acids at a concentration of 3% with 1.5% yeast extract.

L-Asparaginase activity of each sample was determined by placing 1-ml fractions of washed cells into the outer well of each of four microdiffusion dishes (Arthur Thomas Catalog no. 4472-F). To the inner well of each was added 2 ml of 0.1 M H<sub>2</sub>SO<sub>4</sub>. To the outer well of each of two dishes was added 0.05 M tris(hydroxymethyl)aminomethane (Tris)-hydrochloride buffer at pH 8.6, and to the outer well of each of the other two dishes was added 0.01 M L-asparagine (Matheson, Coleman and Bell) in 0.05 M Tris-hydrochloride buffer at pH 8.6. The plates were covered and incubated at 37 C for 60 min, after which 1 ml of saturated K<sub>2</sub>

CO<sub>3</sub> was added to the outer well of each dish. Incubation was continued at 37 C for 120 min, after which the ammonia in the sulfuric acid was determined by the Nessler test. The rate of reaction was determined to be linear with respect to enzyme concentration using known quantities of L-asparaginase (Worthington Biochemical Corp.).

Unless otherwise indicated, cultures were grown in 100 ml of broth in 500-ml Erlenmeyer flasks incubated at 30 C on a rotary shaker which described a circle of 2.5 cm and was set at a speed of 300 rev/min.

Results in Fig. 1 indicate that L-asparaginase production approximately parallels growth of the cells and is accompanied by an increase in the pH of the YMG medium. The quantity of L-asparaginase measured in the cells falls off rather rapidly after the maximal quantity has been reached, but no L-asparaginase could be detected in the medium to account for this loss.

The quantity of L-asparaginase produced by *S. griseus* ATCC 10137 in most media did not differ significantly; yields ranged from 0.6 to 1.2 IU of L-asparaginase in cells from 100 ml of broth. The GA medium supported only poor growth and poor L-asparaginase production. Heinemann and Howard (3) reported that addition of glucose to the medium of *Serratia marcescens* resulted in substantial reduction in the quantity of L-asparaginase produced. In our comparison of YMG and YM media, we found 1.0 to 1.2 IU of L-asparaginase, respectively, in cells from 100 ml of the media, with cell yields of 136 and 276 mg (dry weight), respectively, from 100 ml of the media. The 3% peptone supported cell growth with the highest specific L-asparaginase content.

When the concentration of peptone was

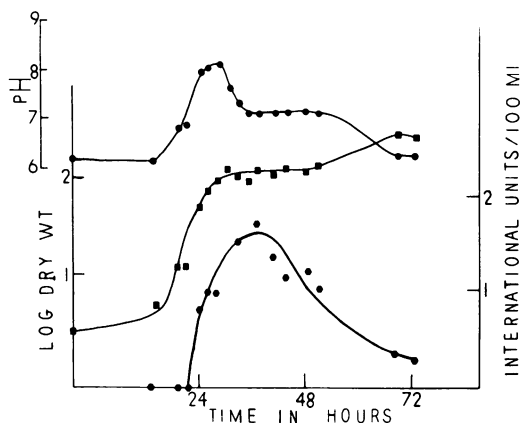


FIG. 1. Growth and L-asparaginase production of *Streptomyces griseus* ATCC 10137. (●) pH, (■) log dry weight, and (●) international units/100 ml.

varied from 1 to 5%, it was found that 1 and 2% concentrations supported poorest L-asparaginase production and that 3, 4, and 5% concentrations all resulted in about the same yield.

The 4% peptone medium, to which  $\text{KH}_2\text{PO}_4$  and Tris buffer were added to concentrations of 0.05 M, was adjusted to a variety of pH values ranging from 6.5 to 8.5. Little difference was noted in the production of L-asparaginase under these different conditions of pH.

To determine the effect of aeration on L-asparaginase production, ratios of broth (4% peptone) volume to flask volume of 1:1.25 to 1:10 were set up, and the flasks were shaken as before. Little difference was observed in the quantity of L-asparaginase synthesized under these conditions of aeration. Cells grown under conditions of greatest aeration had the highest specific L-asparaginase content.

According to Campbell et al. (2), the L-asparaginase from *Escherichia coli* which possesses anti-tumor activity has a pH optimum of 8.4 to 8.5. To assess the affect of pH on the enzyme in the cells of *S. griseus* ATCC 10137, the buffer used in the assay mixture was modified to include  $\text{K}_2\text{HPO}_4$  at a concentration of 0.05 M, and the pH of the mixture was adjusted to values from 5.5 to 9. The results of assaying the enzyme at the various pH values are shown in Table 1 and indicate that the

TABLE 1. Effect of pH of the assay mixture on the L-asparaginase activity in the cells of *Streptomyces griseus* ATCC 10137

pH	IU of L-asparaginase in cells from 100 ml of broth
5.5	0.11
6.0	0.11
6.5	0.05
7.0	0.22
7.5	0.24
8.0	0.89
8.5	1.17
9.0	1.08

enzyme is most active at pH 8.5.

It was found that the L-asparaginase activity could be quantitatively released from cells of *S. griseus* ATCC 10137 by treatment with lysozyme or a sonic oscillator.

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#### LITERATURE CITED

- Broome, J. D. 1961. Evidence that the L-asparaginase activity of guinea pig serum is responsible for its antilymphoma effects. *Nature (London)* **191**:1114-1115.
- Campbell, H. A., L. T. Mashburn, E. A. Boyse, and L. J. Old. 1967. Two L-asparaginases from *Escherichia coli* B. Their separation, purification, and antitumor activity. *Biochemistry* **6**:721-730.
- Heinemann, B., and A. J. Howard. 1969. Production of tumor-inhibitory L-asparaginase by submerged growth of *Serratia marcescens*. *Appl. Microbiol.* **18**:550-554.
- Pridham, T. G., P. Anderson, C. Foley, L. A. Lindenfelser, C. W. Hesseltine, and R. G. Benedict. 1957. A selection of media for maintenance and taxonomic study of *Streptomyces*. *Antibiot. Annu.* 1956-1957, p. 947-953.
- Reddy, V., V. Subba, H. N. Jayaram, M. Sirsi, and T. Ramakrishnan. 1969. Inhibitory activity of L-asparaginase from *Mycobacterium tuberculosis* on Yoshida ascites sarcoma in rats. *Arch. Biochem. Biophys.* **132**:262-267.
- Roberts, J., G. Burson, and J. M. Hill. 1968. New procedures for purification of L-asparaginase with high yield from *Escherichia coli*. *J. Bacteriol.* **95**:2117-2123.
- Waksman, S. A. 1967. *The Actinomycetes: a summary of current knowledge*, p. 245. The Ronald Press Co., New York.
- Zanin, V. V., T. T. Berezov, A. Y. Nikolaev, and A. K. Abdumalikov. 1969. Formation of L-asparaginase in some microorganisms. *Mikrobiologiya* **38**:432-436.