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# L-Asparaginase Production by Various Bacteria

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Of 123 species of bacteria surveyed for L-asparaginase synthesis, *Erwinia aroideae* NRRL B-138 provided the highest yields.

Although L-asparaginase inhibits tumor cell growth (1), studies of this phenomenon have been limited, usually because sufficient quantities of the enzyme were not available. For clinical trials, this enzyme has been obtained from microorganisms, *Escherichia coli* (4), *Serratia marcescens* (6), bakers' yeast (2), guinea pig serum (3), and chicken livers (5). Reported yields of this enzyme from bacterial sources have been 80 to 950 international units per g (dry weight) of cells.

Screening procedures were initiated at this laboratory in an effort to find organisms that would produce higher yields of L-asparaginase. In this screening, 123 bacterial species were examined for enzyme production. A 50-ml amount of TGY broth (glucose, 1.0 g; $K_2HPO_4$, 1.0 g; yeast extract, 5.0 g; tryptone, 5.0 g; tap water to 1.0 liter; *p*H adjusted to 7.0) in 300-ml Erlenmeyer flasks was inoculated with the organism to be tested. The organism was incubated on a Gump rotary shaker (200 rev/min) at 28 C for 24 hr. Cells were harvested by centrifugation at 10,000 $\times$ *g* for 15 min and were washed twice with 20-ml portions of phosphate buffer ($Na_2HPO_4$, 4.757 g; $KH_2PO_4$, 4.539 g; Triton X-100, 0.125 ml; water to 1.0 liter). The washed cells were suspended in 3 ml of 0.1 M sodium borate buffer (*p*H 8.5). In test tubes (10 by 75 mm), 0.1 ml of the suspension was placed with 0.9 ml of 0.1 M sodium borate buffer and 1 ml of 0.04 M L-asparagine. The mixtures were incubated at 37 C for 15 min, and the reaction was stopped by the addition of 0.5 ml of 15% (w/v) trichloroacetic acid. Precipitated proteins were removed by centrifugation, and the liberated ammonia was determined by nesslerization. One international unit equals that amount of enzyme which releases 1 $\mu$mole of ammonia in 1 min at 37 C.

Almost all of the bacterial species screened contained measurable quantities of L-asparaginase (Table 1). However, four strains of *Erwinia aroideae* and one of *Hydrogenomonas eutropha* produced substantially larger amounts under the test system described.

*E. aroideae* NRRL B-138 was selected for further production experiments. Inoculum was grown for 8 hr in 300-ml Erlenmeyer flasks containing 50 ml of TGY broth and then was trans-

TABLE 1. *L-Asparaginase activity of various species of bacteria*

| Microorganism | No. of strains examined | Range of activity[a] |
|---|---|---|
| *Aerobacter aerogenes* | 10 | 0-65 |
| *Aeromonas hydrophila* | 4 | 40-150 |
| *A. liquefaciens* | 1 | 130 |
| *A. salmonicida* | 1 | 35 |
| *A. sinuosa* | 1 | 100 |
| *Bacillus megaterium* | 3 | 0-35 |
| *B. subtilis* | 3 | 0-55 |
| *Erwinia amylovora* | 6 | 0-85 |
| *E. aroideae* | 5 | 55-770 |
| *E. atroseptica* | 1 | 150 |
| *E. carotovora* | 4 | 40-450 |
| *E. dissolvens* | 2 | 85-90 |
| *Escherichia coli* | 16 | 0-225 |
| *E. freundii* | 1 | 200 |
| *Hydrogenomonas eutropha* | 1 | 620 |
| *H. pantotropha* | 1 | 40 |
| *Photobacterium fischeri* | 1 | 0 |
| *Proteus americanus* | 1 | 160 |
| *P. mirabilis* | 4 | 120-170 |
| *P. morganii* | 1 | 110 |
| *P. paramericanus* | 1 | 115 |
| *P. pseudovaleriei* | 1 | 120 |
| *P. sphingides* | 1 | 110 |
| *P. vulgaris* | 9 | 65-370 |
| *Pseudomonas acidovorans* | 2 | 175-210 |
| *P. alliicola* | 1 | 85 |
| *P. ammoniagenes* | 1 | 5 |
| *P. asplenii* | 2 | 65-80 |
| *P. aureofaciens* | 6 | 130-300 |
| *P. caviae* | 3 | 50-100 |
| *P. convexa* | 1 | 125 |
| *P. dacunhae* | 1 | 30 |
| *P. fluorescens* | 2 | 140-240 |
| *P. geniculata* | 1 | 290 |
| *P. lemonnieri* | 1 | 330 |
| *P. pavonacea* | 1 | 300 |
| *P. putida* | 1 | 100 |
| *P. reptilivora* | 1 | 70 |
| *Pseudomonas* species | 1 | 150 |
| *P. stutzeri* | 2 | 0-35 |
| *P. synxantha* | 1 | 130 |
| *P. taetrolens* | 1 | 270 |
| *Serratia marcescens* | 14 | 100-335 |
| *Xanthomonas campestris* | 1 | 50 |

[a] Measured as international units per gram (dry weight) of cells.

ferred aseptically to 2.8-liter Fernbach flasks containing 500 ml of the same medium. The production flasks were incubated for 8 hr at 37 C on a Gump rotary shaker (200 rev/min). Yields of L-asparaginase were as high as 1,250 international units per g (dry weight) of cells. These results make *E. aroideae* an attractive source of L-asparaginase.

Serological tests with antibodies sensitive to the L-asparaginase from *E. coli* and *S. marcescens* proved the L-asparaginase from *E. aroideae* NRRL B-138 to be immunologically distinct. The availability of two or more different L-asparaginases would be advantageous in clinical trials. Further studies of the factors controlling L-asparaginase synthesis by *E. aroideae* should improve the yields still further. In this manner, the shortage of this enzyme, needed for clinical trials in antitumor therapy, may be alleviated.

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