

## IMPROVEMENT OF *BACILLUS SUBTILIS* FOR LYSINE PRODUCTION

BY

A. GUHA, A. K. MISHRA AND G. NANDA

Department of Microbiology, Bose Institute,  
93/1, A. P. C. Road, Calcutta-700 009

Induced mutation and strain selection techniques have been undertaken to increase lysine production by the S-(2-aminoethyl) L-cysteine resistant strain of *Bacillus subtilis*. Using physical and chemical mutagen in a stepwise fashion, the strain BUE129 has been selected which is 50% more productive than the parent strain.

### INTRODUCTION

Lysine, an essential amino acid, is required for balanced nutrition and development of human beings. Due to lack of adequate lysine in cereal (Bhattacharjee, 1970), attention has been paid to the microbial production of lysine, so that it can be used as a food supplement.

Lysine production has been investigated in S (2-aminoethyl) L-cysteine (AEC) resistant strain of *Corynebacterium glutamicum* (Nakayama, 1972), *Brevibacterium flavum* (Sano and Shio, 1970), *Bravibacterium lactofermentum* (Tosaka *et. al.* 1978) and *Bacillus subtilis* (Chaudhuri *et. al.* in press)

Here we describe isolation of hyper lysine producing strain of *B. subtilis* from AEC resistant strain by resort to induced mutation and strain selection technique.

### MATERIALS AND METHODS

**Bacterial strain:** The AEC resistant strain BAEC 29, used as the parent strain was isolated from a wild *B. subtilis* strain in presence of 20mM AEC after mutagenic treatment with N-methyl-N-nitrosoguanidine.

**Media:** The following media was used.

**Medium 1:** peptone 1.0%; beef extract 1.0%; NaCl 0.5%; adjusted to pH 7.2.

Medium 2 : glucose 1.0% ;  $K_2HPO_4$  0.05% ;  $KH_2PO_4$  0.05% ;  $MgSO_4$  0.025% ;  $NH_4Cl$  0.2% ;  $CaCl_2$  0.004% ; d-biotin 20  $\mu g/lit.$  pH adjusted to 7.2.

Medium 3 : Seed medium as used by Plachy' (1970).

Medium 4 : glucose 10% ;  $K_2HPO_4$  0.05% ;  $KH_2PO_4$  0.05% ;  $MgSO_4$  0.025% ;  $(NH_4)_2SO_4$  2.0% ;  $CaCO_3$  5.0% ; d-biotin 25  $\mu g/lit.$  ; thiamine.HCl 50  $\mu g/lit.$  pH was 7.5 after sterilization.

The concentration of agar, when employed, was 2.0%.

UV treatment : Cells grown in medium 1 was irradiated with a 4 Watt General Electric Germicidal (U.S.A.) UV lamp at 253.7 nm wavelength. The dose was 100 ergs/mm<sup>2</sup>/sec. After irradiation the cell suspension was diluted and plated on medium 2 plates. Colonies resulting from the surviving cells were replicated on AEC ( 20 mM ) containing medium 2 plates. AEC resistant colonies were randomly picked up for testing L-lysine production.

Ethyl methan sulfonate ( EMS ) treatment : 1.0% EMS was directly added to the washed cell suspension. After incubation at 30°C, cell suspension was diluted and plated on medium 1. Colonies appearing were replicated and tested as before.

Production of L-lysine : 5.0% (v/v) seed inoculum prepared in medium 3 was added to 20 ml medium 4 distributed in 250 ml Erlenmeyer flask, After 96 hours of incubation on a shaker at 30°C, L-lysine produced was estimated quantitatively following (a) the acidic ninhydrin method ( Chinard, 1952 ) and (b) the rapid microbioassay method ( Itoh *et al* 1974 ) using lysine auxotroph of *Escherichia coli* K<sub>12</sub> ( obtained from Dr. D. L. Deway, Dept. of Chemical Pathology, University College Hospital, London ) as the test organism.

## RESULTS AND DISCUSSION

Cells of strain BAEC29 was irradiated by UV rays for 30, 60, 90 and 120 seconds. Table 1 shows that the survival percent of the cells decreased with the increase in doses of UV irradiation. The plus variants ( those colonies producing more lysine than BAEC29 ) increased with the increase in doses of UV applied, reached maximum at a certain dose of the mutagen and then declined. Plus variants were maximum among those colonies which survived UV treatment for 60 seconds and the best strain BU109 producing 21 g/l of L-lysine was isolated from them.

Treatment of cells of strain BU109 with EMS for 15, 30, 45 and 60 minutes shows that survival percent of the cells decreased linearly ( Table 2 ) with the increase in time of treatment with 1.0% EMS. Here also plus variants ( those colonies producing more lysine than BU109 ) increased, reached, maximum then declined. Maximum plus variants existed among the colonies which survived EMS treatment for 30 minutes and the best strain BUE129 producing 24g/l of lysine was screened from them.

Similar increase and decrease of the percentage of plus variants with the dosage of the mutagen applied have been reported ( Alikhanian, 1962 ) after treatment of *Actinomyces subtropicus* with X-ray for increasing albomycin production and *Actinomyces olivaceus* with ethylimine for increasing B<sub>12</sub> production.

Table 1. Lethal and mutagenic effects of ultraviolet rays on the strain BAEC29

| Strain | Exposure time (sec) | Survival (%) | Plus variant (%) |
|--------|---------------------|--------------|------------------|
| BAEC29 | 0                   | 100.0        | —                |
|        | 30                  | 55.0         | 6.0              |
|        | 60                  | 2.2          | 18.0             |
|        | 90                  | 0.28         | 15.0             |
|        | 120                 | 0.11         | 11.0             |

Table 2. Lethal and mutagenic effects of 1.0% EMS on the strain BU109

| Strain | Time of treatment (min) | Survival (%) | Plus variant (%) |
|--------|-------------------------|--------------|------------------|
| BU109  | 0                       | 100.0        | —                |
|        | 15                      | 16.0         | 3.0              |
|        | 30                      | 2.9          | 10.9             |
|        | 45                      | 0.71         | 8.8              |
|        | 60                      | 0.23         | 5.7              |

Table 3. Integration of small increase in productivity than the previous strain by stepwise mutation

| Best strain of intermediate step | Mutagen including the strain | Lysine production g/l | Excess lysine production over previous strain (%) | Excess lysine production over parent strain (%) |
|----------------------------------|------------------------------|-----------------------|---------------------------------------------------|-------------------------------------------------|
| BAEC29 (parent)                  | —                            | 16.0                  | —                                                 | —                                               |
| BU109                            | UV                           | 21.0                  | 31                                                | 31                                              |
| BUE129                           | EMS                          | 24.0                  | 14                                                | 50                                              |

Table 3 shows that the integration of small increase in productivity from that of every previous strain by stepwise mutation have resulted the strain BUE129 which is 50% more productive than the parent strain BAEC29. Thus the strain BUE129 producing 24 g/l lysine have been developed by stepwise mutation.

#### ACKNOWLEDGEMENT

The authors wish to thank Prof. S. C. Bhattacharyya, Director of this Institute, for his interest in the work.

#### REFERENCES

- Alikhanian, S. I. (1962). Induced mutagenesis in the selection of microorganisms. *Adv. Appl. Microbiol.* 4, 1-50.
- Bhattacharjee, J. K. (1970). Microorganisms as potential sources of food, *Adv. Appl. Microbiol.* 13, 139-161.
- Chaudhuri, A., Mishra, A. K. and Nanda, G. Lysine excretion by S-(2-aminoethyl) L-cysteine resistant mutants of *Bacillus subtilis*. *Acta Microbiologica Polonica* ( in press )
- Chinard, F. P. (1952), Photometric estimation of proline and ornithine. *J. Biol. Chem.* 199, 91-95.
- Itoh, H., Morimoto, T., Kawashima, K. and Chibata, I. (1974). Improved method for rapid microbioassay of amino acids. *Agric. Biol. Chem.* 38, 869-870.
- Nakayama, K. (1972). Lysine and diaminopimelic acid. *In the microbial production of amino acids.* Yamada, K., Kinoshita, S., Tsunoda, T. and Aida, K. Eds. John Wiley & Sons. New York p, 369-397.
- Plachy, J. (1970), Effect of medium composition on the production of lysine by *Corynebacterium* sp. 9366-H-4<sup>c</sup>4. *Fol. Microbiol.* 15, 347-349.
- Sano, K. and Shio, I. (1970). Microbial production of L-lysine III. Production by mutants resistant to S-(2-aminoethyl) L-cysteine. *J. Gen. Appl. Microbiol.* 16, 373-391.
- Tosaka, O., Takinami, K. and Hirose, Y. (1978). L-lysine production by S-(2-aminoethyl) L-cysteine and  $\alpha$ -amino- $\beta$ -hydroxyvaleric acid resistant mutants of *Brevibacterium lactofermentum*. *Agric. Biol. Chem.* 42, 745-752.