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Short communication

Screening of UV-irradiated and S-2-aminoethyl-L-cysteine resistant mutants of *Bacillus megaterium* for improved lysine accumulation

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The screening of UV irradiated and aminoethyl-l-cysteine resistant (AEC^R) mutants of *Bacillus megaterium* strains for improved lysine accumulation was conducted. The mutants, MR-10 and MR-25 derived form irradiated cultures of *B. megaterium* SP 76, and MS-3 and MS-5 derived form *B. megaterium* SP 86 produced higher lysine levels than the parent strains. In contrast, however, lysine accumulation by MV-2 and MV-18 mutants of *B. megaterium SP* 14, was of low yield. In aminoethyl-l-cysteine resistant mutants, R-14 and R-76, derived from *B. megaterium* SP 14 and *B. megaterium* SP 76 respectively, lysine accumulation was enhanced.

Key words: L-Lysine, fermentation, screening, mutants, accumulation.

INTRODUCTION

Lysine is an essential, economically important amino acid used as food and feed supplement. It is not usually synthesized biologically in the body, and children and growing animals have a high requirement for lysine since it is needed for bone formation (Shah et al., 2002). Research on the possible utilization of wild strains revealed that many microorganisms, such as bacteria, yeast, filamentous fungi and actinomycetes accumulated amino acids in culture containing a supplementary source of nitrogen. Many efforts have been devoted to elucidating the mechanism of microbial production of amino acids, and the biosynthetic pathway of most amino acids are now well documented (Aida, 1972).

Most natural strains cannot produce industrially significant amounts of L-lysine in the culture broth due to various metabolic regulation mechanisms. However, alteration of these mechanisms can lead to L-lysine accumulation (Nakayama, 1972). This study aims at screening UV-irradiated and S-2-aminoethyl-L-cysteine resistant mutants of *Bacillus megaterium* for improved lysine accumulation.

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MATERIALS AND METHODS

Cultivation of *Bacillus megaterium* strains

The three *Bacillus* stains, SP 86, SP 76 and SP 14, were cultured for UV irradiation following the method described by Calton and Brown (1981). An Erlenmeyer flask (250 ml) containing 50 ml of Nutrient Broth (Oxoid) inoculated with two loopful of a 24 h growth culture of the *Bacillus* strain on solid agar was incubated at 30°C on a rotary shaker (120 rpm). After a 24 h period, broth culture was chilled on ice for 30 min to prevent further growth, and a 5 ml portion of it in a test tube centrifuged for 5 min at 5,000 x g. The pelleted cells were resuspended in 1 ml of sterile distilled water containing O. 1 ml $MgSO_4$, and then transferred to sterile glass Petri-plate (100 mm diameter). Duplicate plates of each *Bacillus* strain were prepared.

UV irradiation and mutant selection

Uncovered Petri-plate containing the cultivated bacterial suspendsion was placed at a distance of 50 cm from a UV light source (253.7 nm). After 8 min exposure, 0.1 ml of the irradiated cells was spread inoculated on Nutrient Agar (Oxoid) plate, and colonies which appeared after 24 h incubation at 30°C were subsequently plated out on minimal agar medium. Mutant strains selected from bacterial colonies on Nutrient agar, plate, which showed no growth on minimal medium, were stored on agar slants at 4°C.

Screening of UV-irradiated mutants for lysine production

The UV-derived mutants were screened for lysine accumulation foll-

Table 1. Lysine accumulation by UV irradiated and AEC^R mutants of *Bacillus megaterium.*

Organism	UV Irradiated Mutant	AEC^R Mutant	Lysine(mg/ml)
Bacillus megaterium SP 86	MS-3		3.84
	MS- 5		3.88
			3.22[a]
Bacillus megaterium SP 76	MR-10		2.05
	MR-23		4.80
	MR-27		2.76
		R-76	2.36
			1.93[b]
Bacillus megaterium SP 14	MV-18		2.04
	MV-2		2.07
		R-14	3.00
			2.16[c]

a, b and c: Controls for the parent strains.

owing the methods described by Ekwealor and Obeta (2005). 3 ml of a 24 h seed culture of the mutant was inoculated into a 250 ml flask containing 25 ml of a basal fermentation medium. The broth culture was assayed for lysine accumulation after 48 - 144 h incubation on a rotary shaker (160 rpm) at 30°C.

Selection of S-aminoethyl-L-cysteine resistant (AEC^R) mutants of *Bacillus* strains

Following a modified method of Tosaka et al. (1978), 20 ml of a seed medium (peptone, 1%; yeast extract, 1%; NaCl, 0.5%) in a 100 ml flask, inoculated with a loopful of a 24 h growth culture of the *Bacillus* strain on agar slant, was incubated at 30°C for 24 h on a rotary shaker (120 rpm). The broth culture (5 ml) was centrifuged and the cells washed with 0.5 ml of 0.1 M sodium phosphate buffer (pH 7.0) before resuspending in an equal volume of the buffer. The cell suspension (0.1 ml) was inoculated on minimal agar plates with varied concentrations (0.02, 1.0, 2.0 mg/ml) of S-aminoethyl-L-cysteine. Colonies of AEC^R mutants, after 3-4 days incubation of the plates at 30°C, were stored on agar slants at 4°C.

Screening AEC^R mutants for lysine production

AEC^R mutants were screened for lysine accumulation following the method described above. The basal fermentation medium inoculated with a seed culture of the mutant was incubated on a rotary shaker (160 rpm) for 96-144 h at 30°C. Lysine level in the broth culture was determined.

RESULTS AND DISCUSSION

The results (Table 1) of UV irradiated cultures of *Bacillus* strains show that two mutants each were derived from *B. megaterium* SP 86 and *B. megaterium* SP 14, while three mutants were produced from *B. megaterium* SP 76. As presented on Table 1, MS-3 and MS-5 of *B. megaterium* SP 86 and MR-10, MR-23 and MR-27 of *B. megaterium* SP 76 accumulated higher lysine yields than the parent strains. In contrast, MV-18 and MV-2 of *B. megaterium* SP 14 had poor lysine yields.

Two AEC^R mutants, designated R-76 and R-14, derived from *B. megaterium* SP 76 and *B. megaterium* SP 14, respectively, were isolated from the growth of the *Bacillus* strain on minimal agar plates containing 0.02 mg/ml of s-aminoethyl-L-cysteine (Table 1). Both AEC^R mutants had improved lysine accumulation.

In the course of screening UV irradiated cells of *Bacillus* strains for improved lysine yields, the derived mutants were observed to accumulate different quantities of lysine. Although Kinoshita et al. (1957, 1958) and Nakayama et al. (1961) reported a similar induction of nutriational mutants from various amino acid-accumulating strains for the production of ornithine, lysine and valine, it is, however, difficult to explain the reason for the high amino acid accumulation in some mutants or the low yield in others. This variation in amino acid accumulation may probably be as a result of the non-directional effect of the mutagen used.

The derivation of AEC^R mutants from *Bacillus* strains (Table 1) with increased lysine accumulation is similar with the reports of Adelberg (1958) and Karlstrom (1965). They noted that some mutants resistant to amino acid analogues are suitable as amino acid producers. The enhanced lysine production by AEC^R mutants is likely due to the mechanism of control operative in the biosynthesis of lysine in the parent stains being interfered with in the mutants, thus making them over-producers. This view is supported by the findings of Samanta and Bhattacharyya (1991), Crociani et al. (1991) and Bhatta-charyya and Samanta (1992). These researchers working with *Arthrobacter globiformis* and *Bacillus stearothermophilus,* noted the improved lysine yields of AEC^R mutants of these organisms. This study has shown the possibility of using UV irradiated and S-2-aminoethyl-L-cysteine resistant mutants of *B. megaterium* for improved lysine yields.

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