SHORT COMMUNICATION

Characterization of Mutants of *Bacillus subtilis* Resistant to *S*-(2-Aminoethyl)cysteine

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S-(2-Aminoethyl)-L-cysteine, an analogue of L-lysine, inhibits growth of *Bacillus subtilis*. Two different mutants resistant to this analogue were isolated. One overproduced lysine and the mutation mapped near *ilvC*. The other did not overproduce lysine and was unable to express resistance to the analogue in a medium containing threonine; it was linked to the *thr-5* marker.

INTRODUCTION

Mutants resistant to amino acid analogues are useful tools in the study of the regulation of amino acid biosynthesis in micro-organisms (Umbarger, 1971). Among lysine analogues, *S*-(2-aminoethyl)cysteine (AEC), in which the γ -methylene group of lysine is substituted by a sulphur atom, is one of the better investigated (Shiota *et al.*, 1961; Rabinovitz & Fisher, 1961; Work, 1962; Soda *et al.*, 1969; De Marco *et al.*, 1976; Jegede & Brenchley, 1976; Friedrich & Demain, 1977).

In this paper, we describe two mutants of *Bacillus subtilis* resistant to AEC. One of the mutants overproduces lysine and maps near *ilvC* while the other one does not overproduce lysine and maps in the region of the *thr-5* marker.

METHODS

Growth. Bacteria were grown at 37 °C in minimal medium (MM; Davis & Mingioli, 1950) supplied with 1 g asparagine l⁻¹; other growth factors, when required, were added at a final concentration of 25 µg ml⁻¹.

Isolation of resistant mutants. The parental prototrophic strain (SB19) was grown overnight in MM. Bacteria were then washed and spread on MM plates containing *S*-(2-aminoethyl)cysteine (Sigma; 100 µg ml⁻¹). Spontaneous resistant colonies were recovered after 24 h at 37 °C. Presumptive mutants were cloned on MM plates and tested for their resistance to the analogue.

Bioassay of lysine excretion. The excretion of lysine was tested by estimating the growth of the lysine auxotrophic strain PB1652 (*trpC lys-3 metB*) in the supernatant of strains under assay (Kelln & O'Donovan, 1976).

Genetic procedures. Transduction was carried out as described by Yamagishi & Takahashi (1968) and transformation as described by Young & Spizizen (1961).

Table 1. Mapping of *aec-56* and *aec-59* markers by transduction and transformation

Donor*	Recipient†	Selected phenotype	Recombinant class	No. of colonies scored‡	Implied order
FB56 (<i>aec-56</i>)	FB74 (<i>thr-5 ala-1</i>)	Thr ⁺	Thr ⁺ Ala ⁺ Aec ^r	169	<i>ala-1-aec-56-thr-5</i>
			Thr ⁺ Ala ⁺ Aec ^s	8	
			Thr ⁺ Ala ⁻ Aec ^r	171	
			Thr ⁺ Ala ⁻ Aec ^s	25	
FB59 (<i>aec-59</i>)	FB65 (<i>leu-8 ilvC</i>)	Leu ⁺	Leu ⁺ Ilv ⁺ Aec ^r	14	<i>aec-59-ilvC-leu-8</i>
			Leu ⁺ Ilv ⁺ Aec ^s	140	
			Leu ⁺ Ilv ⁻ Aec ^r	3	
			Leu ⁺ Ilv ⁻ Aec ^s	41	
		Ilv ⁺	Ilv ⁺ Leu ⁺ Aec ^r	15	
			Ilv ⁺ Leu ⁺ Aec ^s	94	
			Ilv ⁺ Leu ⁻ Aec ^r	15	
			Ilv ⁺ Leu ⁻ Aec ^s	67	

* Crosses involving FB56 were done by transduction and those with FB59 by transformation (see Methods).

† In the two recipient strains only the markers linked to the *aec* mutation are indicated: FB74 was *trpC metB leu-8 thr-5 purB ala-1*; FB65 was *trpC metB leu-8 thr-5 purB hisA ilvC*.

‡ Data from two experiments for the cross FB56 × FB74.

RESULTS

Mutants resistant to AEC. Two spontaneous mutants, FB56 (*aec-56*) and FB59 (*aec-59*), resistant to 700 µg AEC ml⁻¹ were isolated. These mutants were also resistant to another analogue of lysine in which selenium substitutes for the γ-methylene group of lysine (De Marco *et al.*, 1976) (data not shown).

In the wild-type strain (SB19), 5 µg lysine ml⁻¹ relieved the inhibition by 50 µg AEC ml⁻¹, while *meso*-2,6-diaminopimelic acid (100 µg ml⁻¹) did not.

Excretion of lysine by the mutants. The amount of lysine present in the supernatant of cultures of parental and AEC-resistant strains was determined. Mutant FB59 excreted about 8 µg (ml supernatant)⁻¹, while the parental strain SB19 and mutant FB56 excreted between 2 and 3 µg (ml supernatant)⁻¹.

Mapping of AEC mutations. Preliminary transduction experiments indicated that the *aec-56* mutation was cotransferred with the *thr-5* marker, and mutation *aec-59* was cotransferred with *leu-8*. Three-point transduction crosses (Table 1) indicated that *aec-56* was located between *ala-1* and *thr-5*. The map distance from the *thr-5* marker, computed from the transduction data of Table 1, was 16 units (the *ala-1* marker had previously been located between *argA* and *thr-5*, unpublished results).

The other marker, *aec-59*, was located by transformation (Table 1) to the left of *ilvC*, at 91 units from *ilvC* and 96 units from *leu-8*. During the scoring of transformants involving *aec-59*, we observed that recombinants of the class Leu⁺Ilv⁻Aec^r were resistant only to 100 µg AEC ml⁻¹, while all other resistant recombinants were able to grow in the presence of 700 µg AEC ml⁻¹.

Effect of threonine on AEC resistance. During the mapping experiments involving FB56 we did not find any recombinants of the class Thr⁻Aec^r when threonine was the unselected marker. This observation suggested a possible involvement of threonine in the AEC resistance mechanism. Subsequent experiments showed that the resistance of strain FB56 to AEC was completely lost when threonine (25 µg ml⁻¹) was added to the medium. In contrast, threonine had no effect on the resistance of mutant FB59. Other amino acids did not affect the resistance of the two mutants.

DISCUSSION

Two mutations causing resistance to AEC mapped in different regions indicating that they belong to two different genes. Mutant FB59 may be affected in a gene involved in the regulation of lysine biosynthesis as it overproduces lysine. Mutants resistant to AEC which overproduce lysine have been described previously by Masurekar & Demain (1974) in *Penicillium chrysogenum*. Furthermore, mutation *aec-59* is located near to *ilvC* and recombinants which carry both the *aec-59* and *ilvC* mutations showed a reduced level of resistance to AEC. These observations suggest a connection between lysine and branched-chain amino acid biosynthesis, as found in *Escherichia coli* by De Felice *et al.* (1977).

Mutant FB56 is unable to express resistance to AEC in a medium containing threonine. One possible explanation of the threonine effect could be that one (or more) of the threonine biosynthetic enzymes is involved in the resistance to AEC. Moreover, mutation *aec-56* maps in the same region as the *thr*, *tdm*, *hom* and *sprB* markers which carry mutations involved in the synthesis and utilization of threonine (Vapnek & Greer, 1971*a, b*).

The mutants described here could be useful in the study of the regulatory relationships involved in the biosynthesis of amino acids derived from aspartate.

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