RESISTANCE TO ACTINOMYCIN D AND TRANSFORMABILITY IN B.SUBTILIS

Erela Ephrati-Elizur

Department of Bacteriology
The Hebrew University-Hadassah Medical School
Jerusalem, Israel

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Studies on the nature of competency, the ability of bacteria to absorb DNA molecules and undergo genetic transformation, in the transformation systems of Streptococcus, Pneumococcus and Bacillus cereus, were reported recently. It was shown that a factor, probably a protein, isolated from competent cells, was capable of inducing competency in incompetent cells (Pakula & Walczak, 1963; Tomasz & Hotchkiss, 1964; Felkner & Wyss, 1964). Tomasz & Hotchkiss (1964) further showed that the transient nature of competency is due to the appearance of an inhibitor, which interferes with the propagation of competence. The nature of the mechanism determining competency in Bacillus subtilis is not yet understood. Some correlation between transformation and spore formation was indicated by Young & Spizizen (1961) and Nester (1964). Since mutants resistant to actinomycin D were reported to have lost sporulating ability (Zlotnik, 1959), a correlation between actinomycin resistance and transformability was looked for. It was of interest to search for mutants that have lost transformability and could serve as test organisms in the study of the factors participating in the induction of competency, or determining a potential stage of competency. The experiments presented here describe the isolation and some of the characteristics of such mutants.

EXPERIMENTAL

The strain used in these experiments was SB25, an indole and histidine

requiring B.subtilis 168 (try_2^-, his_2^-). Transformation experiments were carried out according to Anagnostopoulos & Spizizen (1961), with the modification reported by Mahler et al (1963). Mutants resistant to actinomycin were isolated by the gradient plate technique according to Szybalski & Bryson (1952). Spore formation was determined according to Schaeffer (1964).

Cultures developing stepwise increase in resistance to actinomycin were examined for transformability at each level of resistance. It can be seen in Table I that with rising resistance to actinomycin transformability was gradually lost. Cells resistant to as little as 0.5 μg/ml showed a 90-99% loss of transformability, with the exception of strain A9 which retains a high degree of transformability.

Table I

The correlation between developing resistance to actinomycin D and transformation in B.subtilis

	Strain	Actinomycin resistance level μg/ml	No. transformants per ml	Per cent of transformation*	Transformation per cent of control**
	A1	0.2	7.0×10^3	0.017	17
EXP.1	A3	0.5	5.0×10^2	0.001	1
	A5	1.0	1.0×10^2	0.0001	0.1
	A2	0.1	8.7×10^3	0.024	24
EXP.2	A4	0.5	4.0×10^3	0.01	10
	A6	1.0	1.3×10^2	0.0005	0.5
	A7	0.5	6.0×10^1	0.0001	0.1
EXP.3	A9	0.5	3.0×10^4	0.02	20
	SB25	0.02	6.5×10^4	0.1	

Resistant mutants to actinomycin were isolated from actinomycin plates and streaked on Tryptose Blood Agar Base (BAB). 18 hrs. cultures served as inoculum for the transformation experiments.

*Percent of transformation was calculated from the total viable count.

**Transformation of the parent strain SB25 served as control.

Repeated subcultures of the mutant strains on culture media devoid of actinomycin, resulted in partial loss of resistance accompanied in most cases by a considerable rise in transformability. Examination of colony morphology of the original mutants and the subcultured strains revealed that colonies isolated from actinomycin were large, smooth with an uneven margin (S), whereas the majority of the colonies found in the subcultured strains were rough (R). Both colony types differed from the actinomycin sensitive parent strain. Since actinomycin resistant strains (S) mutated with high frequency towards low resistance it was necessary to keep the strains on media containing actinomycin for further experiments.

Actinomycin resistant mutants were next tested for sporulating ability. It can be seen in Table II that the efficiency of sporulation of the mutant strains was much lower than that of the parent strain as manifested by the 100 fold decrease in sporulation in strain A7. Strain A9 is again an exception. This strain sporulated well even on BAB, a medium which usually supports sporulation poorly.

Table II

The correlation between resistance to actinomycin, spore formation and transformation in B.subtilis

Strain No.	Actinomycin resistance level µg/ml	No. heat-resistant spores/ml	Per cent spores*	Per cent transformation
A3	0.5	1.3×10^7	2.3	0.0006
A4	0.5	6.2×10^6	1.0	0.003
A5	1.0	7.3×10^6	1.2	0.00015
A6	1.0	1.0×10^7	1.7	0.0006
A7	0.5	1.2×10^6	0.25	0.00001
A9	0.5	8.0×10^7	18	0.03
SB25	0.02	8.8×10^7	23	0.05

Bacteria were grown in the transformation medium for 4 hrs. Samples were taken for transformation experiment. The rest of culture was centrifuged and resuspended in nitrogen free mineral medium supplemented with glucose and incubated with aeration for 20 hrs.(sporulation medium).The number of heat resistant spores was determined by heating the culture for 10' at 85°C and plating the appropriate dilutions on BAB.
*The per cent of spores was calculated out of the total viable count.

In order to find out whether the spores found in the mutant cultures were produced by the S-type mutant or by the small number of R-type revertants found in each culture, which might explain the low number of spores, examination of single colonies derived from heated spore suspensions was carried out. Inspection of several hundred colonies derived from germinating spores demonstrated the prevalence of S-type with very few R-type colonies present. This indicates that actinomycin resistant mutants S-type are capable of sporulation although with poor efficiency which did not increase upon repeated sporulation cycles. When R-type mutants were allowed to sporulate, the percentage of spores rose to 20-100% of control. The efficiency of transformation in these mutants reached in different strains 10-50% of that observed in the parent strain.

DISCUSSION

Mutants developing resistance to actinomycin, also show poor transformability and reduced ability to form heat resistant spores. The correlation between these three phenomena is not yet understood. Ceferri *et al* (1964), studying the mechanism of resistance to actinomycin in *B.subtilis*, suggested that resistance to actinomycin is due to impermeability of the bacterial cell wall to the drug. It is conceivable that changes in cell surface resulting in the appearance of resistance to actinomycin may also affect DNA absorption, thus causing a loss or reduction in transformability. Experiments are underway to examine this assumption. The correlation between spore formation and transformability is still ambiguous. Young & Spizizen (1961) reported on the poor transformability of a sporeless mutant. On the other hand, Schaeffer & Ionesco (1959) described sporeless mutants that are fully transformable. Actinomycin resistant mutants isolated in this study are not sporeless but sporulated poorly. Such mutants have been termed oligosporogenous by Schaeffer & Ionesco (1960). If a mutation of

this kind results in some changes in surface antigens, a correlation between spore formation, actinomycin resistance and transformation can be visualized. However, actinomycin resistance is not necessarily correlated with reduced efficiency of transformation and sporulation, as can be seen from the behaviour of strain A9.

In the light of the above observation, it is tempting to speculate on the possible connection between the resistance to actinomycin D in *E.coli* and its refractivity to transformation by DNA.

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