REGULATION OF THE SYNTHESIS OF PENICILLINASE IN DIPLOIDS OF *STAPHYLOCOCCUS AUREUS*

by

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Received December 21, 1967

Study of merodiploids of *Staphylococcus aureus*, involving the genetic region concerned in penicillinase synthesis, indicates that there is a positional effect in that a constitutive mutation present on a plasmid is not completely repressed by the presence in the same cell of a chromosomal gene for inducibility.

Pardee, Jacob & Monod (1959) demonstrated that the inducibility (i^+) gene of the *Escherichia coli* β-galactosidase system is dominant to the constitutive (i^-) gene. A similar result was obtained by Richmond (1965) with the i gene of the staphylococcal penicillinase system, when both i^+ and i^- were present on extrachromosomal particles (plasmids).

S. aureus PS80 (NCTC 9789) is believed to carry the genes responsible for the synthesis of penicillinase on its chromosome (Asheshov, 1966). This strain carries genes conferring resistance to arsenate ions (Asa^R), cadmium salts (Cd^R) and mercury

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salts (Hg^R) on a plasmid but, despite this, certain preparations of phage 80 propagated on PS 80 co-transduce penicillin resistance and the three metal-ion resistances with a high frequency (Asheshov, to be published). In the transductants the genes for penicillin resistance and metal-ion resistance are extrachromosomal.

PS 80 is inducible for penicillinase production (i^+p^+)(†). A semi-constitutive mutant (i^-p^+) was isolated from this strain after treatment with ethyl methane sulphonate. The inducible strain and the semi-constitutive mutant were grown at 43.5° and mutants sensitive to the three metal-ions were isolated from them. These mutants, PS 80 $i^+p^+Asa^{(del)}Cd^{(del)}Hg^{(del)}$(*) and PS 80 $i^-p^+Asa^{(del)}Cd^{(del)}Hg^{(del)}$ were used as recipients in transduction experiments where the donor strains were PS 80 $i^-p^+Asa^RCd^RHg^R$ and PS 80 $i^+p^+Asa^RCd^RHg^R$ respectively and the selected marker was cadmium resistance. Selection for cadmium resistance was carried out on agar plates containing 40 μM cadmium sulphate and 0.2% (w/v) soluble starch, and cadmium-resistant transductants were screened for penicillinase production by developing the plates with a benzylpenicillin-iodine mixture (Dyke, Jevons & Parker, 1966).

In the first experiments the donor was PS 80 $i^-p^+Asa^RCd^RHg^R$ and the recipient PS 80 $i^+p^+Asa^{(del)}Cd^{(del)}Hg^{(del)}$. A total of 409 cadmium-resistant transductants was obtained, of which more than 90% appeared to produce large amounts of penicillinase in the absence of inducer. Several of these were isolated, and

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(†) p = gene determining amino acid sequence of penicillinase

(*) (del) = deletion mutant

quantitative estimations of the average amount of penicillinase produced by each of them was measured under standard conditions (Richmond, Parker, Jevons & John, 1964). The results are shown in Table 1.

Table 1. Quantity of penicillinase produced by S. aureus PS 80, a semi-constitutive mutant and cadmium resistant transductants.

Culture	Genotype	Phenotype	Units penicillinase / mg. dry wt. bacteria	
			No inducer	Induced with methicillin
1. Donor	$i^-p^+Cd^R$	Constit.	90	185
2. Recipient	$i^+p^+Cd^{(del)}$	Inducible	6	140
3. Cd-resistant transductants	$\frac{i^-p^+Cd^R}{i^+p^+Cd^{(del)}}$	Constit.	63	625
4. $Cd^{(del)}$ mutants derived from Cd-resistant transductants	$i^+p^+Cd^{(del)}$	Inducible	8	136

One unit of penicillinase hydrolyses 1 μmole benzylpenicillin in one hour at 35° and pH 5.9.

Cadmium-sensitive mutants, isolated from three transductants after growth at 43.5°, reverted to an inducible phenotype and their penicillinase-producing ability was the same as the recipient (Table 1, lines 2 and 4). The transductants were, therefore, diploid and continued to carry the i^+ gene of the recipient. Nevertheless, a considerable amount of penicillinase was produced in the absence of inducer, indicating that the product of the chromosomal i^+ gene was only slightly repressing synthesis of penicillinase in this diploid. The reduction in the quantity of penicillinase produced by the diploid in the absence of inducer suggests that the i^- mutant is not of the O^c

type since there is some interaction between the chromosomal i^+ gene product and the plasmid penicillinase region.

In the reciprocal experiment the donor was PS 80 i^+p^+ $Asa^RCd^RHg^R$ and the recipient PS 80 $i^-p^+Asa^{(del)}Cd^{(del)}Hg^{(del)}$. Of the total of 1940 cadmium-resistant transductants, 92% were phenotypically inducible. The results of quantitative measurements of enzyme production on several of these transductants are presented in Table 2. Cadmium-sensitive mutants were selected from three of these transductants after growth at 43.5° and these were found to have reverted to a constitutive phenotype (Table 2). The transductants were therefore diploid for the genes controlling penicillinase production and continued to carry the i^- gene on the chromosome, where its presence was masked by the plasmid i^+ gene.

Table 2. Quantity of penicillinase produced by S. aureus PS 80, and cadmium-resistant transductants

Culture	Genotype	Phenotype	Units penicillinase / mg. dry wt. bacteria	
			No inducer	Induced with methicillin
1. Donor	$i^+p^+Cd^R$	Inducible	4	138
2. Recipient	$i^-p^+Cd^{(del)}$	Constit.	92	156
3. Cd-resistant transductants	$\frac{i^+p^+Cd^R}{i^-p^+Cd^{(del)}}$	Inducible	10	126
4. Cd-sensitive mutants derived from Cd-resistant transductants	$i^-p^+Cd^{(del)}$	Constit.	96	124

One unit of penicillinase hydrolyses 1 μmole benzylpenicillin in one hour at 35° and pH 5.9.

Revel & Luria (1963) and Markovitz & Rosenbaum (1965) have reported results where, despite the presence of an i^+ gene in the same cell as an i^- gene, greater than the basal amount of enzyme is synthesized. They propose explanations based either on the assumption that there are more copies of the plasmid genes than the chromosomal genes or that plasmid gene products are synthesized in greater amount than chromosomal gene products.

From the observations of Jacob, Brenner & Cuzin (1963), Cuzin & Jacob (1967 and Novick & Richmond (1965) we conclude that there is probably only one plasmid per chromosome. However, if replication of the chromosome and plasmid is initiated simultaneously and proceeds at the same rate, and if the chromosomal penicillinase i gene is far from the site of initiation then many cells can have two copies of the plasmid gene but only one copy of the chromosomal gene. Although such an explanation may account for the failure of the chromosomal i^+ gene to exert more than partial control over the level of enzyme synthesized in the absence of inducer, it does not account for the very high induced amount of enzyme obtained with the diploid in which the i^- mutation is on the plasmid.

Explanations of the observed positional effect based on the presence of more than one regulatory region for penicillinase synthesis are not, however, ruled out and are under investigation.

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