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Penicillinase synthesis in *Staphylococcus aureus*: the effect of inducer and of the cellular location of the genetic determinants on the amount of enzyme formed

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SUMMARY

A comparison was made of the amount of penicillinase produced by strains of *Staphylococcus aureus* carrying chromosomal penicillinase genes, plasmid penicillinase genes and strains carrying both chromosomal and plasmid genes. Plasmid genes were found to produce at least twice as much enzyme as homologous chromosomal genes. The results suggest that there are, on average, two plasmids per chromosome in exponentially growing cells.

Two inducers were used in these experiments—methicillin and 2-(2′-carboxyphenyl)-benzoyl-6-aminopenicillanic acid (CBAP). The latter proved a more efficient inducer than methicillin and this difference was most apparent when more than one i^+ gene was present, which suggests that the efficiency of induction reflects the ability of the inducer to react with repressor.

1. INTRODUCTION

Penicillinase produced by *Staphylococcus aureus* is an inducible enzyme whose synthesis is controlled by two closely linked genes—*pen*, the structural gene for the enzyme and *i*, an inducibility locus. Complementation studies have shown that the *i* gene contains at least two regulatory regions (Richmond, 1967). In most strains the *pen* and *i* genes are on plasmids, but in a few they are chromosomal (Asheshov, 1966, 1969; Poston, 1966; Sweeney & Cohen, 1968). Richmond (1965) studied the synthesis of penicillinase in strains carrying two penicillinase plasmids and showed that in such diploids the i^+ gene was dominant to its i^- allele, since strains carrying both an i^+pen^+ and an i^-pen^+ linkage group were phenotypically inducible. The i^+ gene thus appears to exert control on enzyme synthesis through a diffusible repressor. On the other hand Asheshov & Dyke (1968) measured penicillinase production in diploids in which one copy of the genes was on the plasmid but the other on the chromosome. When one copy was wild-type and the other constitutive, an i^+ gene on the plasmid, acting *trans*, reduced the uninduced enzyme level to that found in wild-type inducible strains. However, a chromosomal i^+ gene exerted only partial control on the uninduced level of enzyme synthesized.

This was explained by a gene dose hypothesis which suggested that there were more copies of plasmid than chromosomal genes.

The induced level produced by a diploid carrying i^- on the chromosome and i^+ on the plasmid was much the same as that produced by either haploid parent, but the diploids with i^+ on the chromosome and i^- on the plasmid synthesized almost twice as much penicillinase as the haploid parents. Both diploids carried the same number of *pen* genes and differed only in the number of copies of the i^+ gene and, presumably, in the amount of repressor present in the cell. This difference would not have been expected to affect the induced enzyme levels, if inducer had been able to react with all repressor molecules. If, however, inducer can react only with a limited amount of repressor, enough might remain in the diploids with i^+ on the plasmid to maintain the strain in a partially repressed state.

The inducer used in these experiments was methicillin, an almost completely gratuitous inducer that is hydrolysed only to a small extent by staphylococcal penicillinase (Novick, 1962). It has the disadvantage that it is antibacterial for staphylococci. Recently Leggate & Holms (1968) described a derivative of penicillanic acid—2-(2′-carboxyphenyl)-benzoyl-6-aminopenicillanic acid or CBAP—which is an efficient inducer of staphylococcal penicillinase. This compound is also a gratuitous inducer, but shows little antibacterial activity. Enzyme levels produced by various haploid and diploid strains have now been re-examined with CBAP as inducer.

2. METHODS

(*a*) *Strains of* Staphylococcus aureus

The strains used in these experiments are listed in Table 1. Most of them were derivatives of strain PS 80 (NCTC 9789), which carries the penicillinase genes on the chromosome and the genetic determinants for resistance to various metal ions on the π plasmid (Asheshov, 1966). When PS 80 is stored on nutrient agar, a duplication of the penicillinase genes occurs in some cells, one copy being retained on the chromosome and the other incorporated into the π plasmid (Asheshov, 1969). The strain thus changes from a penicillinase haploid to a penicillinase diploid strain and presumably the two copies of the penicillinase genes in the diploid are homologous. The diploids of PS 80 used in these experiments either occurred spontaneously as a result of this process of duplication or were constructed by transduction of the appropriate plasmid into a recipient that had lost the π plasmid after growth at 43·5 °C.

The chromosomal penicillinase genes in PS 80 are stable and it is therefore not possible to measure the level of penicillinase produced by plasmid genes in the absence of chromosomal genes in this strain. The appropriate strains were therefore constructed by transduction out of PS 80 to strain 258/N, a penicillinase-negative recipient.

The location of the penicillinase genes in each of the strains in Table 1 was confirmed by studying the transduction kinetics in experiments in which each strain was used as the donor of these genes to penicillinase-negative recipients.

Table 1. *Genotype and source of strains of* Staphylococcus aureus

Strain no.	Designation	Penicillinase genotype*	Source
1	PS 80 haploid	i^+pen^+	Wild type
2	PS 80 diploid	i^+pen^+ (i^+pen^+)	Spontaneous duplication in strain 1
3	PS 80 haploid constitutive	$i_1^-pen^+$	Mutant of strain 1 isolated after treatment with ethyl methane sulphonate
4	PS 80 diploid constitutive	$i_1^-pen^+$ ($i_1^-pen^+$)	Spontaneous duplication in strain 3
5	PS 80 heterozygous diploid	i^+pen^+ ($i_1^-pen^+$)	Transduction of plasmid from strain 4 to strain 1
6	PS 80 heterozygous diploid	$i_1^-pen^+$ (i^+pen^+)	Transduction of plasmid from strain 2 to strain 3
7	258/N	—	Penicillinase-negative recipient
8	258/A	i^+pen^+	Transduction of chromosomal genes from strain 1 to strain 7
9	258/B	(i^+pen^+)	Transduction of plasmid genes from strain 2 to strain 7
10	258/C	$i_1^-pen^+$	Transduction of chromosomal genes from strain 3 to strain 7
11	258/D	($i_1^-pen^+$)	Transduction of plasmid genes from strain 4 to strain 7

* Plasmid-borne genes are in parentheses.

(*b*) *Quantitative estimations of penicillinase*

Penicillinase was measured by the iodometric method of Perret (1954) as modified by Novick (1962). One unit of penicillinase = 1·0 μmol penicillin G inactivated/h at 30 °C at pH 5·9. Cultures were grown in CY medium (Novick, 1962) with shaking and were induced for 3 h using either methicillin (0·5 μg/ml) or CBAP (3·4 μg/ml). This concentration of methicillin was the highest that could be used without interfering with growth of the strain. CBAP showed little or no inhibition of growth when used at concentrations as high as 17·0 μg/ml. However, there was no increase in the induced enzyme level when concentrations greater than 3·4 μg/ml were used while lower concentrations were less effective.

3. RESULTS AND DISCUSSION

Table 2 shows the amount of penicillinase produced by the various strains listed in Table 1. The amount of enzyme formed by the four variants of strain 258/N indicates that plasmid genes produce at least twice as much enzyme as the homologous chromosomal genes. This is seen most clearly in the two constitutive strains —258/C and 258/D—in which enzyme estimations were made in the absence of inducer. The difference in enzyme levels in the inducible pair–258/A and 258/B—is seen only when CBAP is used to induce, which agrees with the observation of Leggate & Holms that CBAP is a more efficient inducer of staphylococcal penicillinase than methicillin. Although it might be argued that the difference in enzyme

levels produced by plasmid genes compared with chromosomal genes reflects differences in the rate of transcription or translation of these genes, a simpler explanation is that there are two copies of plasmid genes for each copy of chromosomal genes. Differences in the level of β-galactosidase produced by the *F'lac* episome compared to chromosomal *lac* genes in *Escherichia coli* were interpreted similarly by Jacob & Monod (1961).

Table 2. *Amount of penicillinase produced by derivatives of strain 258/N and PS 80 in the absence of inducer, and after induction by methicillin and CBAP*

Strain	Penicillinase genotype: Chromosomal	Penicillinase genotype: Plasmid	No. of genes: i^+	No. of genes: i^-	No. of genes: pen^+	Units of penicillinase mg. dry weight* with: No inducer	Methicillin	CBAP
258/A	i^+pen^+	—	1	0	1	2·8	50	91
258/B	—	(i^+pen^+)	2	0	2	3·2	67	213
258/C	$i_1^-\ pen^+$	—	0	1	1	127	140	137
258/D	—	$(i_1^-\ pen^+)$	0	2	2	235	315	276
PS 80 inducible haploid	i^+pen^+	—	1	0	1	2·8	60	90
PS 80 constitituve haploid	$i_1^-\ pen^+$	—	0	1	1	92	120	100
PS 80 inducible diploid	i^+pen^+	(i^+pen^+)	3	0	3	3	61	237
PS 80 heterozygous diploid	$i_1^-\ pen^+$	(i^+pen^+)	2	1	3	4·2	86	267
PS 80 heterozygous diploid	i^+pen^+	$(i_1^-\ pen^+)$	1	2	3	16	232	312
PS 80 constitutive diploid	$i_1^-\ pen^+$	$(i_1^-\ pen^+)$	0	3	3	315	400	346

* Figures given are the average of at least three determinations on separately induced cultures.

Assuming that the gene-dose hypothesis is correct and that plasmid genes are present in duplicate, one can calculate the number of i^+, i^- and pen^+ genes carried by each of the strains in Table 2 (see columns 4–6). The four penicillinase diploid strains are assumed to carry three copies of the genes, one on the chromosome and two on plasmids. They should, in theory, be able to produce three times as much enzyme as a strain carrying a single copy on the chromosome provided that (1) the presence of the plasmid does not interfere with expression of chromosomal genes and vice versa and (2) the cells are not restricted in the amount of penicillinase produced by factors other than the amount of messenger-RNA present.

The enzyme levels produced by these diploids showed considerable differences depending on the inducer. Methicillin again proved a less efficient inducer than CBAP except in the case of the constitutive diploid. Methicillin consistently induced higher enzyme levels in constitutive strains than CBAP. We do not know

the reason for this but it is possible that methicillin caused lysis of the cells which would tend to increase the enzyme level. Enzyme levels for the diploids induced by CBAP ranged from 237 units for the inducible diploid to 346 for the constitutive diploid. These strains presumably carry the same number of structural genes, so that the differences in the amount of enzyme produced can be attributed to the ratio of i^+ and i^- genes. Judging by the amount of penicillinase produced in the absence of inducer one may conclude that the product of two or three i^+ genes represses three *pen* genes better than the product of one i^+ gene, which suggests that there is not a large excess of repressor produced under the normal haploid conditions. A fixed amount of CBAP induced the diploid with one i^+ gene better than that with two i^+ genes, which suggests that this concentration of inducer is not saturating the binding sites for inducer on the repressor, but is much nearer to saturating them than is methicillin at the concentration used.

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