Naturally Occurring Constitutive β -Lactamase of Novel Serotype in *Staphylococcus aureus*

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Fusidic acid resistance was associated with a penicillinase plasmid in naturally occurring strains of *Staphylococcus aureus* (Lacey & Grinsted, 1972). The β -lactamase produced by two of these strains and by two others has been studied. It was produced constitutively in three of the strains and in all four the enzyme was of a serotype different from any described hitherto.

METHODS

Staphylococci. Strains FAR 4 and FAR 5 have been described previously (Lacey & Grinsted, 1972); strains FAR 13 and FAR 14 are also fusidic acid-resistant strains in which genes specifying fusidic acid resistance are linked to the penicillinase plasmid (R. W. Lacey and V. T. Rosdahl, in preparation). All four cultures were recently obtained as clinical isolates. Strain 524SC (Rogers, 1953), was used as the source of standard type A enzyme, and strain 22260/72 as that of type B. The latter was kindly supplied by Dr Asheshov, Cross Infection Reference Laboratory, Colindale, London.

Penicillinase assay. Penicillinase activity was measured quantitatively by the iodometric method of Perret (1954). One penicillinase unit is defined as the activity that destroys 1.0 μ mole benzyl penicillin/h at 30 °C and pH 5.9. Penicillinase concentrations are expressed as penicillinase units/mg dry wt bacteria. The bacteria to be tested were inoculated into 10 ml 1% CY medium (Richmond, 1963) containing 0.4% glucose (Richmond, Parker, Jevons & John, 1964) and shaken overnight at 35 °C. The next day 100 ml of prewarmed CY broth with glucose was inoculated with some of the 10 ml culture to give a density of 0.1 mg dry wt bacteria/ml. This culture was shaken for 1 h at 35 °C and then induced with methicillin 0.5 μ g/ml culture. Shaking was continued for a further 3.5 h. The basal level of enzyme activity was measured under similar conditions without methicillin.

Immunological definition of the enzyme. A portion of the induced culture in late exponential phase was centrifuged at 2500 g for 10 min, and the supernatant retained. Specific antiserum to type A exo-penicillinase was kindly supplied by Professor M. H. Richmond. The enzyme in the supernatant was titrated with the antiserum as described by Richmond (1963, 1965a) in order to measure the effect of the antiserum on enzyme activity.

RESULTS AND DISCUSSION

The basal levels of enzyme produced by the various strains were as follows (units/mg dry wt): FAR 4, 66; FAR 5, 0.9; FAR 13, 0.7; FAR 14, 43. After induction the levels were: FAR 4, 72; FAR 5, 81; FAR 13, 1.5; FAR 14, 85. Sixty to seventy per cent of the enzyme was extracellular in all cases, and duplicate experiments performed on different occasions showed good agree-

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Fig. 1. Stimulation of β -lactamase by anti-(exo-penicillinase type A) serum. (○), Type A enzyme from strain 524SC; (×), type B enzyme from strain 22260/72; and (△), enzyme from strain FAR 4. Antiserum was diluted 1:10 with saline before use.

ment. The induction ratios (Induced level/Basal level, see Richmond *et al.* 1964) were as follows: FAR 4, 1.1; FAR 5, 93; FAR 13, 2.3; FAR 14, 2.0. The enzyme produced by FAR 5 is inducible, but those produced by strains FAR 4, 13 and 14 appear to be constitutive.

Although constitutive mutants have been produced *in vitro* (Novick, 1963; Richmond, 1965*b*) naturally occurring constitutive strains of *Staphylococcus aureus* have not been described previously. FAR 4 and FAR 14 seem macroconstitutive, since each produced large amounts of enzyme whether induced or not. Strain FAR 13, which produced small amounts of enzyme under all conditions tested, is probably micro-constitutive.

The supernatant enzyme from strain FAR 4 (Fig. 1) was titrated with antitype A penicillinase serum. The titration curves of standard type A and type B enzyme are also shown. Type C enzyme activity is not stimulated by type A antiserum (Richmond, 1965*a*). Enzymes from strain FAR 5, 13 and 14 gave titration curves indistinguishable from FAR 4, but different from type A and B enzyme. The new naturally occurring type of exo-penicillinase (which is to be called D) is therefore easily distinguishable from the three previously described types. The maximum stimulation of type D enzyme with antiserum is 220 to 250% of the initial enzyme activity, while a similar value for type A is about 400%. Type B enzyme is stimulated about 150%.

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REFERENCES

- LACEY, R. W. & GRINSTED, J. (1972). Linkage of fusidic acid resistance to the penicillinase plasmid in *Staphylococcus aureus*. *Journal of General Microbiology* **73**, 501-508.
- NOVICK, R. P. (1963). Analysis by transduction of mutations affecting penicillinase formation in *Staphylococcus aureus*. *Journal of General Microbiology* **33**, 121-136.
- PERRET, C. J. (1954). Iodometric assay of penicillinase. *Nature, London*, **174**, 1012-1013.
- RICHMOND, M. H. (1963). Purification and properties of the exo-penicillinase from *Staphylococcus aureus*. *Biochemical Journal* **88**, 452-459.
- RICHMOND, M. H. (1965*a*). Wild-type variants of exo-penicillinase from *Staphylococcus aureus*. *Biochemical Journal* **94**, 584-583.
- RICHMOND, M. H. (1965*b*). Penicillinase plasmids in *Staphylococcus aureus*. *British Medical Bulletin* **260**-263.
- RICHMOND, M. H., PARKER, M. T., JEVONS, M. P. & JOHN, M. (1964). High penicillinase production correlated with multiple antibiotic resistance in *Staphylococcus aureus*. *Lancet* **i**, 293-296.
- ROGERS, H. J. (1953). Variant population within a hyaluronidase-producing culture of *Staphylococcus aureus*. *Journal of Pathology and Bacteriology* **66**, 545-551.