Temperature-dependent expression of the chromosomal \( \beta \)-lactamase gene in a strain of \textit{Pseudomonas aeruginosa} \\

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Summary. A strain of \textit{Pseudomonas aeruginosa} (3-Post) was resistant to cefsulodin and ceftazidime at 37°C but sensitive at 20°C. Resistance was mediated by chromosomally-encoded \( \beta \)-lactamase which was synthesised at a high level during growth above 30°C but at a low, inducible level during growth below 27°C. \\

Introduction \\
The regulation of chromosomally-encoded \( \beta \)-lactamase synthesis in \textit{Pseudomonas aeruginosa} is an important area of study because resistance to “anti-pseudomonal” \( \beta \)-lactam antibiotics (Slack, 1981) can arise by mutation to constitutive synthesis (King et al., 1983; Williams et al., 1984). This class C \( \beta \)-lactamase (Ambler, 1980; Jaurin and Grundstrom, 1981; Knott-Hunziker et al., 1982) is normally inducible by \( \beta \)-lactam compounds (Nordstrom and Sykes, 1974). Induction of \( \beta \)-lactamase synthesis in \textit{Citrobacter freundii}, apparently involves a positive control mechanism requiring two protein-encoding regulatory genes, termed \textit{ampR} and \textit{ampD} (Lindberg et al., 1985; Lindberg and Normark, 1986). Here we describe a mutant of \textit{P. aeruginosa} that displays properties consistent with the operation of a similar control system. \\

Materials and methods \\

Bacteria and sensitivity testing \\
Organisms used were \textit{P. aeruginosa} strain 3-Pre, and a cefsulodin-resistant mutant derived from it in vitro, strain 3-Post (Slack and Pitt, 1982; Slack et al., 1983). MIC values were determined on IsoSensitest Agar (Oxoid) by the plate-incorporation method, with an inoculum of c. 10⁶ cfu/spot. Disk tests were performed by Stokes’s method on IsoSensitest Agar. \\

Growth and harvesting of bacteria \\
Cells were grown in 80-ml volumes of the chemically-defined medium (pH 7-8) of Anwar et al. (1983) in 250-ml conical flasks with baffles, orbitally shaken at 170 rpm (32-mm diameter of orbit). Cells were harvested at \( E_{650} = 0.2 \) (0.098 mg dry wt cells/ml) which represented the early exponential phase of growth. Induction of \( \beta \)-lactamase was achieved by adding benzyl penicillin (200 mg/L final concentration) to the culture 2 h before harvesting the cells by centrifugation at 9400 g (rav 7.0 cm) for 10 min at 4°C. The supernate was discarded. For suspensions to which penicillin had not been added, the pellet was used as the source of \( \beta \)-lactamase. For induced cells, the first pellet was washed by resuspension in 35 ml of ice-cold 5 mM MgCl₂-25 mM 3-(N-morpholino)-propanesulphonic acid (MOPS) (pH 7-4) and re-centrifugation as above. The pellet of washed bacteria was used as the source of \( \beta \)-lactamase from induced cells. \\

Liberation of \( \beta \)-lactamase \\
Pellets were subjected to three cycles of freezing and thawing, resuspended in ice-cold 5 mM MgCl₂-25 mM MOPS (pH 7-4) and subjected to a further freeze-thaw cycle. This treatment should release more than 90% of the \( \beta \)-lactamase of \textit{P. aeruginosa} (Berks, 1977). A sample of the final freeze-thawed preparation of cells was centrifuged at 11000 g (rav. 9 cm) for 34 min at 4°C. \( \beta \)-Lactamase activity was assayed in the supernate. \\

Assays of \( \beta \)-lactamase activity \\
The hydrolysis of cephalosporin C was monitored spectrophotometrically at 260 nm at 37°C in 5 mM MgCl₂-25 mM MOPS (pH 7-4). The kinetic parameters, \( K_m \) and specific \( V_{\text{max}} \), were determined by automated (Nichols and Hewinson, 1987) half-time analysis of reaction progress curves (Wharton and Szawelski, 1982). \\

Results \\
As defined by disk-diffusion tests, \textit{P. aeruginosa} 3-Post was sensitive to cefsulodin and ceftazidime
at 20°C but was resistant at 37°C. *P. aeruginosa* 3-Pre, from which strain 3-Post was derived (Slack and Pitt, 1982), was sensitive to both antibiotics at both temperatures. The MIC of cefsulodin at 37°C for strain 3-Pre was 1.70-1.75 mg/L, and for strain 3-Post was 17-18 mg/L. However, at 17°C the MIC of cefsulodin for both strains was 0.25-0.5 mg/L.

The table shows the effects of growth temperature on β-lactamase synthesis in the two strains. The β-lactamase of strain 3-Post was synthesised at an elevated level at 37°C but at a 14-fold lower level at 21°C. The lower basal level (480 nmol/min/mg dry wt) was higher than that of the parent strain, 3-Pre, at either temperature (24 and 72 nmol/min/mg dry wt at 22°C and 37°C respectively). When strain 3-Post was grown at the lower temperature, β-lactamase synthesis was inducible.

The change from low-level to high-level β-lactamase synthesis in strain 3-Post occurred between the growth temperatures 27°C and 29.5°C (figure). The amount of β-lactamase synthesised in strain 3-Post decreased with rising temperature above 30°C.

**Discussion**

The different cefsulodin and ceftazidime sensitivities of *P. aeruginosa* 3-Post at room temperature and at 37°C were, at least partly, determined by the levels of β-lactamase synthesised at these two temperatures. We suggest that a protein involved in regulating β-lactamase synthesis is thermolabile in strain 3-Post, and that this explains the sharpness of the temperature dependence of the cell complement of β-lactamase (figure).

**Figure.** β-Lactamase activities of extracts of *P. aeruginosa* 3-Post grown at different temperatures. Assays of cephalosporin C hydrolysis were all made at 37°C. The enzyme kinetic parameter, specific $V_{\text{max}}$, was determined for each point by half-time analysis of reaction progress curves. Error bars show ±2 SEM determined from four to six replicate measurements. At 27°C and below, error bars were too small to draw.

In *C. freundii* there is a regulatory protein (the *ampR* gene product) that represses synthesis of the β-lactamase about 2.5-fold but that apparently acts as an activator of transcription of the β-lactamase

**Table.** β-Lactamase activities (substrate cephalosporin C) assayed at 37°C

<table>
<thead>
<tr>
<th>Strain</th>
<th>Mean growth temperature (°C)</th>
<th>Induced*/uninduced</th>
<th>K_m (μM) mean/SEM</th>
<th>Specific $V_{\text{max}}$ (nmol/min/mg dry wt) mean/SEM/n</th>
</tr>
</thead>
<tbody>
<tr>
<td>3-Pre</td>
<td>21.9 uninduced</td>
<td>18.2 1.8</td>
<td>24 2 3</td>
<td></td>
</tr>
<tr>
<td>3-Pre</td>
<td>37.0 induced</td>
<td>25.5 2.6</td>
<td>1260 200 6</td>
<td></td>
</tr>
<tr>
<td>3-Post</td>
<td>21.3 uninduced†</td>
<td>20.0 1.1</td>
<td>72 3 4</td>
<td></td>
</tr>
<tr>
<td>3-Post</td>
<td>37.0 induced</td>
<td>18.9 1.3</td>
<td>1240 40 4</td>
<td></td>
</tr>
</tbody>
</table>

* β Lactamase was induced by the addition of benzyl penicillin (200 mg/L) to the culture 2 h before harvesting the cells.
† The data for uninduced cells of 3-Post are included in the figure.
(ampC) gene on induction (Lindberg et al., 1985; Lindberg and Normark, 1986). Mutations to constitutive synthesis map outside the ampR–ampC region but the constitutive phenotype is only observed in the presence of a functional ampR gene product (Lindberg et al., 1985). The results of the experiments reported in the present communication are consistent with this system also operating in P. aeruginosa because a mutant of C. freundii has been isolated which demonstrates temperature-sensitive expression of β-lactamase with a phenotype that is very similar to that of strain 3-Post (Sawai et al., 1977).

Constitutive synthesis of the class C β-lactamase in P. aeruginosa confers resistance to cefsulodin and ceftazidime whereas basal, but inducible, synthesis does not (Livermore, 1986). The mechanism of this type of resistance has been the subject of debate as to whether hydrolysis (Livermore, 1985) or non-hydrolytic “trapping” (Sanders, 1984) by plasmid β-lactamase is responsible. The mechanism of resistance of strain 3-Post to cefsulodin at 37°C could be best explained by β-lactamase-catalysed hydrolysis of the antibiotic within the periplasmic space (Hewinson et al., 1985).

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REFERENCES


