JOURNAL OF BACTERIOLOGY, Dec. 1979, p. 1126-1128
0021-9193/79/12-1126/03$02.00/0

Vol. 140, No. 3

Response of *Pseudomonas cepacia* to β-Lactam Antibiotics: Utilization of Penicillin G as the Carbon Source

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Received for publication 31 August 1979

Pseudomonas cepacia utilized penicillin G as the sole source of carbon and energy. We report here an unexplained correlation between lysine auxotrophy and β-lactamase deficiency, resulting in loss of capacity to utilize penicillin.

Pseudomonas cepacia (*P. multivorans*) is recognized as the most nutritionally versatile of the pseudomonads (1, 11). As we report here it includes penicillin G (Pen) in its repertoire of utilizable substrates. All wild-type strains of *P. cepacia* we examined and certain strains of *P. marginata* and *P. caryophylli* utilized penicillin G (benzylpenicillin) as the sole source of carbon and energy (see Table 1 for a list of representative strains). The same strains failed to exhibit significant growth with the benzylpenicillin derivatives ampicillin or carbenicillin, but were resistant to these antibiotics. *P. cepacia* 249, the strain we studied in greatest detail, grew with a mean generation time of 6.5 h in inorganic salts medium containing 2% (wt/vol) penicillin G as the sole carbon source.

Neither the closely related *P. pickettii* (9, 10) strains listed in Table 1 nor the less closely related pseudomonads *P. aeruginosa* PU21-(RP1) Ilv$^-$ Pen^r (obtained from G. A. Jacoby, Massachusetts General Hospital, Boston, Mass.), *P. aeruginosa* PAO25(R68.45) Leu$^-$ Arg$^-$ Pen^r (obtained from W. R. Sistrom, University of Oregon, Eugene), *P. putida* AC811(FP2::Tn1) Met$^-$ Pen^r (obtained from A. M. Chakrabarty, University of Illinois Medical Center, Chicago), or *P. fluorescens* 13525 (obtained from D. H. Pope, Rensselaer Polytechnic Institute, Troy, N.Y.) were able to utilize penicillin G despite their resistance to the antibiotic action of this drug (tested as in Table 1). The *P. fluorescens* 13525 strain clearly differs in its behavior from the soil isolate identified as *P. fluorescens* by Johnsen (3), which has been reported to utilize benzylpenicillin as a carbon, nitrogen, and energy source.

When *P. cepacia* 249 was grown in the presence of penicillin G, extracts of the bacteria contained high levels of β-lactamase activity (see Table 2). The highest activity was noted in extracts of bacteria grown in inorganic salts medium containing 2% (wt/vol) penicillin G as the sole carbon source. The lowest level was with bacteria grown in inorganic salts medium containing 1% (wt/vol) Casamino Acids as the carbon source. Intermediate levels of enzyme were detected in extracts of bacteria grown in Casamino Acids medium supplemented with 350 μg of penicillin G per ml. Similar results were obtained with *P. marginata* (see Table 2) and with *P. cepacia* strains 104, 382, and 383 (results not shown).

High levels of β-lactamase activity were not in themselves sufficient for growth on penicillin. Extracts of *P. aeruginosa* PU21(RP1) and PAO25(R68.45), two strains which failed to utilize penicillin G as a carbon source, contained constitutive levels of β-lactamase (ca. 2,500 specific activity) that were almost as great as that of *P. cepacia* 249.

All four lysine-requiring strains in our collection of auxotrophic derivatives of *P. cepacia* 249 were deficient in β-lactamase (Table 3) and sensitive to penicillin G, ampicillin, carbenicillin, and cephalosporin C (tested as in Table 1). Other amino acid auxotrophs (including 249-13, a threonine-deaminase-deficient strain from which the lysine auxotrophs 13-4 and 13-5 were derived) had normal levels of β-lactamase, utilized penicillin G as carbon source, and exhibited the same pattern of resistance to penicillin G, ampicillin, carbenicillin, and cephalosporin C as did the wild type. Diaminopimelic acid (100 μg of a mixture of LL-, DD-, and *meso*- isomers per ml, obtained from Sigma Chemical Co., St. Louis, Mo.) failed to spare the lysine rquirement of the aforementioned mutants, suggesting that they are blocked late in lysine biosynthesis. We have been unable to obtain Lys$^+$ revertants of these strains, raising the possibility that they may be deletion mutants or perhaps that they have suffered loss of a plasmid present in the wild type. Penicillin G-resistant derivatives were obtained. None of these, however, had regained normal levels of β-lactamase nor the capacity to utilize penicillin G (or to synthesize lysine), suggesting a different mechanism of resistance from that of

TABLE 1. *Response of P. cepacia and related pseudomonads to penicillin*

Strains	Source	Penicillin G utilization[a]	Resistance to growth inhibition[b]			
			Penicillin G	Ampicillin	Carbenicillin	Cephalosporin C
P. cepacia						
249, 104, 382, 383	University of California, Berkeley; N. J. Palleroni	+	+	+	+	+
533, 546, 3487, N28-504C	University of Utah Medical Center; J. M. Matsen	+	+	+	+	+
PC22, PC23, PC24, PC25	University of California, Davis; M. P. Starr	+	+	+	+	+
K998, K1021, K1425, K1555	University of California, Los Angeles; M. J. Pickett	+	+	+	+	+
P. marginata						
PM106, PM107	Davis collection	+	+	+	+	+
PM2, PM11	Davis collection	−	−	−	−	+
P. caryophylli						
PC113	Davis collection	+	+	+	+	+
PC115	Davis collection	−	+	+	+	−
P. pickettii						
K232, K279, K286, K288	UCLA collection	−	+	+	+	+

[a] The bacteria were tested for growth on solid medium containing inorganic salts (4) supplemented with 2% (wt/vol) penicillin G as the sole carbon source. Incubation was for 60 h at 37°C.

[b] The bacteria were grown on inorganic salts medium containing 1% (wt/vol) Casamino Acids and 350 μg of antibiotic per ml. Incubation was for 48 h at 37°C.

TABLE 2. *β-Lactamase induction in P. cepacia and P. marginata cultures*

Growth medium[a]	β-Lactamase sp act[b]	
	P. cepacia 249	*P. marginata* 106
Casamino Acids (1% wt/vol)		
No other additions	10	11
Penicillin G (350 μg/ml)	250	143
Ampicillin (350 μg/ml)	197	945
Carbenicillin (350 μg/ml)	172	402
Cephalosporin C (350 μg/ml)	182	148
Penicillin G (2%, wt/vol) (sole carbon source)	6,300	6,930

[a] The bacteria were grown in inorganic salts medium (4) containing the indicated supplements. Cells from 50 ml of culture were collected by centrifugation, suspended in buffer, and disrupted by sonic treatment as reported earlier (5). The disrupted cell suspensions were centrifuged to remove unbroken cells and cell debris, and the supernatants were assayed for β-lactamase activity by using the procedure of O'Callaghan et al. (8). Protein was determined by the procedure of Lowry et al. (6).

[b] Expressed as nanomoles of chromogenic cephalosporin 87/312 cleaved per minute per milligram of protein at 24°C.

TABLE 3. *β-Lactamase deficiency in lysine auxotrophs of P. cepacia 249*[a]

Strain[b]	Phenotype	β-Lactamase sp act[c]
249	Wild type Pen^r Pdm^{+d}	10.0
249-1	Lys^- Pen^s	0.7
249-2	Lys^- Pen^s	1.0
249-13	Ile^- Pen^r Pdm^+	8.0
13-4	Ile^- Lys^- Pen^s	1.1
13-5	Ile^- Lys^- Pen^s	1.2

[a] The bacteria were grown in inorganic salts medium supplemented with 1% Casamino Acids as the carbon source.

[b] The lysine auxotrophs were all obtained from strain 249 after mutagenesis with UV light.

[c] Nanomoles of chromogenic cephalosporin 87/312 cleaved per minute per milligram of protein at 24°C.

[d] Ability to dissimilate and utilize penicillin as a carbon source is designated as Pdm^+.

the wild type.

We have explored the possibility that the joint loss of ability to synthesize lysine and utilize penicillin might be a consequence of loss of a plasmid. The procedure of Currier and Nester (2) was used to screen *P. cepacia* wild-type and auxotrophic strains for plasmid DNA. Both *P. cepacia* 249 and the four lysine auxotrophs derived from it contained 95-megadalton plasmid DNA (molecular mass estimated by agarose gel electrophoresis [7]). No other size classes of plasmid DNA were detected. No plasmids were de-

tected in *P. cepacia* strains 104 and 383, both of which utilized penicillin as the sole carbon source. Our current working hypothesis is that the joint loss of capacity to synthesize lysine and utilize penicillin is due to some event other than loss of a plasmid. However, it remains possible that *P. cepacia* contains a large plasmid, difficult to isolate by conventional procedures, that carries genes related to β-lactamase and lysine formation, or that there is more than one 95-megadalton plasmid in strain 249, one of which is missing in the lysine auxotrophs. We plan to test the latter possibility by determining whether restriction enzyme digests of plasmid DNA from the wild-type and mutant strains differ in their complement of DNA fragments.

We have examined whether transfer of plasmid RP1 from *P. aeruginosa* PU21(RP1) to *P. cepacia* 13-5 would restore the ability to utilize penicillin G. *P. cepacia* transconjugants were obtained by selecting for resistance to 300 μg of tetracycline per ml conferred by plasmid RP1. The donor strain was counterselected by omitting branched-chain amino acids from the medium and utilizing 0.5% (wt/vol) phthalate as carbon source. The transconjugant strains were screened for plasmid DNA and found to contain 36- and 95-megadalton DNA, corresponding to plasmid RP1 and the cryptic plasmid DNA of strain 13-5. Acquisition of the RP1 plasmid conferred on strain 13-5(RP1) the ability to form high levels of β-lactamase (ca. 2,600 specific activity) and resist the antibiotic action of penicillin, but failed to restore the capacity to utilize penicillin as carbon source. The results suggest either that *P. cepacia* β-lactamase possesses special properties, different from the RP1-encoded β-lactamase, which permit it to serve in penicillin dissimilation, or, more likely, that *P. cepacia* lysine auxotrophs are deficient in other enzymes, in addition to β-lactamase, required for penicillin utilization.

This work was supported by Public Health Service grant GM-21198 from the National Institute of General Medical Sciences.

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