Microbiology (1995), 141, 1081–1083 Printed in Great Britain

Division-inhibition capacity of penicillin in *Escherichia coli* is growth-rate dependent

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Growing bacteria are sensitive to various β-lactam derivatives due to their interference with peptidoglycan biosynthesis. At low concentrations, penicillin G (benzylpenicillin) blocks cell division without affecting mass growth rate. The MIC for division of *Escherichia coli* B/r (H266) was found to depend on the growth rate, which was modified by the nutritional conditions. Our hypothesis, that division sensitivity is proportional to the rate of peptidoglycan synthesis for septum formation, as well as to cell circumference, was thus confirmed.

Keywords: *Escherichia coli*, β-lactam, penicillin G, FtsZ ring, penicillin-binding proteins

INTRODUCTION

Various β-lactam derivatives have high specific affinities to their target molecules, penicillin-binding proteins (PBPs), which are involved in bacterial peptidoglycan biosynthesis (Spratt, 1983; Waxman & Strominger, 1983). Growing cells are therefore sensitive to these drugs. At high concentrations, most of them cause cell lysis in *Escherichia coli* by inactivating all the PBPs (Spratt, 1975, 1983; Waxman & Strominger, 1983). The time and rate of lysis are proportional, respectively, to the generation time (τ) and its inverse, growth rate (μ), determined by the medium composition (Boman & Eriksson, 1963; Tuomanen *et al.*, 1986).

At low concentrations, different β-lactams exert various physiological effects according to their relative affinities for the distinct PBPs (Botta & Park, 1981; Spratt, 1975; Wientjes & Nanninga, 1991). Blocking PBP-1, which is responsible for cell elongation (probably by priming peptidoglycan chains) results in cell lysis; blocking PBP-2, which governs cell shape, results in ovoid cells; blocking PBP-3, which is solely required for septal murein synthesis, results in filamentation. Furazlocillin, ampicillin, cephalexin and penicillin G (benzylpenicillin) at low concentrations bind preferentially to PBP-3, and they inhibit cell division without affecting net peptidoglycan synthesis, mass growth and DNA replication (Donachie, 1993; Spratt, 1975). The question thus arises whether the MIC for division depends on the nutritional conditions as does the MIC for lysis.

Abbreviation: PBP, penicillin-binding protein.

METHODS

E. coli B/r (H266) was cultivated in Luria–Bertani broth supplemented with glucose (0·4%; LBG), and in M9 minimal medium (Miller, 1972) supplemented with 1% (w/v) casein hydrolysate and tryptophan (20 μg ml^{-1}; Casa) or supplemented with 0·4% of either glucose, glycerol or acetate as the sole carbon source. Doubling times of 22–25, 29–31, 45–50, 68–70 and 120–140 min, respectively, were observed, as previously documented (Rosenberger *et al.*, 1978; Zaritsky *et al.*, 1979). Each experiment was performed with a steady-state growing culture (Fishov *et al.*, 1995), after diluting (10^{-3}) an overnight culture into the same medium (pre-warmed), vigorously aerated at 37 °C in a gyratory water-bath shaker (250 r.p.m.). Growth was monitored by cell number using Coulter Counter model ZM and by measuring OD_{450} using an LKB Ultrospec II spectrophotometer. Steady-state growth was maintained by appropriate dilutions in fresh, pre-warmed medium (below OD_{450} ~0·4) and demonstrated by the Kolmogorov-Smirnov test for size distributions in successive generations (Woldringh *et al.*, 1977). Treatment of each steady-state culture was initiated by dilution (to OD_{450} ~0·06) into medium containing penicillin G (Sigma) at the indicated concentrations. The rate of mass growth was not affected by these penicillin concentrations.

RESULTS AND DISCUSSION

The division-inhibition capacity of penicillin clearly depends on growth rate (Fig. 1). At 20 units ml^{-1}, penicillin blocks division in acetate culture completely, and blocks approximately 90, 80, 35 and 20% of division in glycerol-, glucose-, Casa- and LBG-grown cells, respectively. The corresponding penicillin concentrations that halve the rate of division (Pn_{50}) are 5·2, 10·0, 13·2, 25·4 and 47·5 units ml^{-1} (Fig. 2).

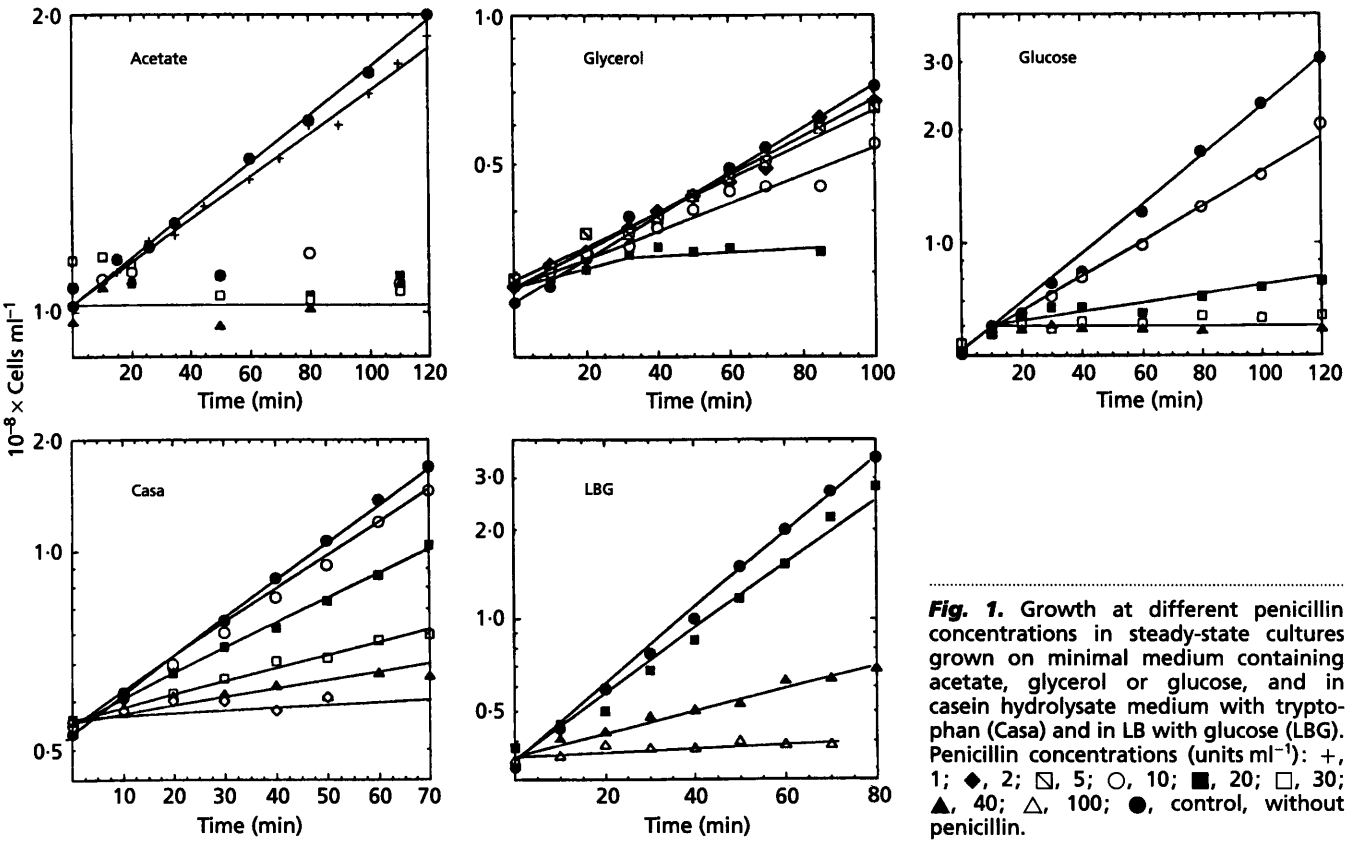

Fig. 1. Growth at different penicillin concentrations in steady-state cultures grown on minimal medium containing acetate, glycerol or glucose, and in casein hydrolysate medium with tryptophan (Casa) and in LB with glucose (LBG). Penicillin concentrations (units ml^{-1}): +, 1; ◆, 2; ⧅, 5; ○, 10; ■, 20; □, 30; ▲, 40; △, 100; ●, control, without penicillin.

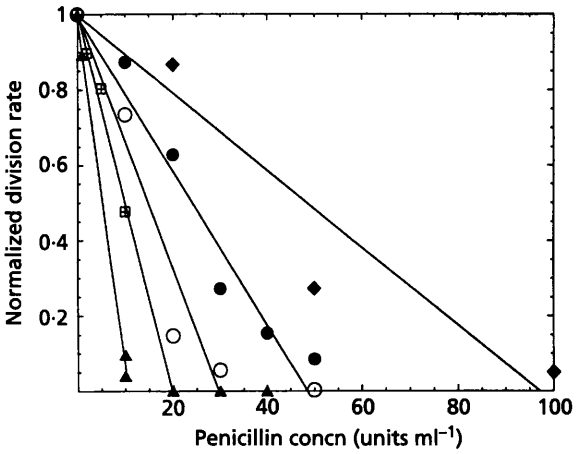

Fig. 2. Inhibition of cell division by penicillin in different growth media. Division rates (slopes from Fig. 1), normalized to 1·0 in each control experiment, were plotted against penicillin concentration. ◆, LBG ($y = 0{\cdot}975 - 0{\cdot}010x$, correlation coefficient $R = -0{\cdot}96$); ●, Casa ($y = 1{\cdot}009 - 0{\cdot}020x$, $R = -0{\cdot}98$); ○, glucose ($y = 0{\cdot}998 - 0{\cdot}034x$, $R = -0{\cdot}97$); ⊞, glycerol ($y = 1{\cdot}013 - 0{\cdot}051x$, $R = -0{\cdot}99$); △, acetate ($y = 1{\cdot}080 - 0{\cdot}096x$, $R = -0{\cdot}97$).

Bacterial cell division occurs a constant time after termination of DNA replication over a wide range of growth rates (Helmstetter *et al.*, 1968). Thereafter, cell division is no longer dependent on synthesis of macromolecules other then peptidoglycan for septum formation (Donachie, 1993). Septation is apparently initiated by self-assembly of the FtsZ ring in the membrane (Bi & Lutkenhaus, 1991) surrounding the DNA-free gap formed upon disassociation between the two sister nucleoids (Mulder & Woldringh, 1989; Woldringh *et al.*, 1990). The activity of PBP-3 as an enzyme involved in septation is expressed after FtsZ ring formation (Donachie, 1993), and would be inhibited by penicillin. The sensitivity of division at low penicillin concentrations will thus depend upon the length of that ring (cell circumference), as well as on the rate of peptidoglycan synthesis for septum formation, which is presumed to be proportional to μ. Cell circumference ($2\pi r$) is determined by cell radius (r), which is known to change regularly with growth rate (Rosenberger *et al.*, 1978; Woldringh *et al.*, 1977). At a faster μ (shorter τ), the cell must therefore produce a bigger cross-wall during a shorter time. If the density of PBP-3 involved in septation does not vary with growth rate, the penicillin concentration needed to reduce the rate of cell division to a certain extent will therefore be proportional to $r \times \mu$ (i.e. $k \times$ penicillin concn $= r/\tau$). This prediction is confirmed by the data (Table 1), as shown by the relatively constant proportionality coefficient ($k = 0{\cdot}00274 \pm 0{\cdot}00048$), and is consistent with the invariant duration (T period) of the process of cell constriction at different growth rates (Woldringh *et al.*, 1977).

Our data support the conclusion that PBP-3 plays a major role in the regulation of cell division, as indicated by the small number of molecules a cell contains (Spratt, 1977).

Table 1. Effects of growth medium on penicillin division-inhibition parameters

Growth medium	Generation time τ (min)	Pn_{50}* (units ml^{-1})	Cell circumference† (μm)	k‡ (μm min^{-1})/ (units ml^{-1})
LBG	23	47·5	2·90	0·00265
Casa	30	25·4	2·51	0·00319
Glucose	48	13·2	1·95	0·00308
Glycerol	70	10·0	1·79	0·00256
Acetate	130	5·2	1·51	0·00223

* Penicillin concentration that inhibits the rate of cell division by 50%; calculated from Fig. 2.

† Calculated from cell radius (r); taken from Woldringh *et al.* (1990).

‡ Penicillin activity coefficient (k); calculated by dividing the cell circumference by the doubling time τ and then dividing by Pn_{50} ($2\pi r/\tau/Pn_{50}$).

ACKNOWLEDGEMENTS

This study was supported in part by grant no. 91-00190 from the US–Israel Binational Science Foundation (BSF), Jerusalem (to A. Z.) and the Ben-Gurion Fellowship administered by the Ministry of Sciences and the Arts (to H. H.).

REFERENCES

Bi, E. & Lutkenhaus, J. (1991). FtsZ ring structure associated with division in *Escherichia coli*. *Nature* **354**, 161–164.

Boman, H. G. & Eriksson, K. G. (1963). Penicillin induced lysis in *Escherichia coli*. *J Gen Microbiol* **31**, 339–352.

Botta, G. A. & Park, J. T. (1981). Evidence for involvement of penicillin-binding protein 3 in murein synthesis during septation but not during cell elongation. *J Bacteriol* **145**, 333–340.

Donachie, W. D. (1993). The cell cycle of *Escherichia coli*. *Annu Rev Microbiol* **47**, 199–230.

Fishov, I., Zaritsky, A. & Grover, N. B. (1995). On microbial states of growth. *Mol Microbiol* **15**, (in press).

Helmstetter, C. E., Cooper, S., Pierucci, O. & Revelas, L. (1968). The bacterial life sequence. *Cold Spring Harbor Symp Quant Biol* **33**, 809–822.

Miller, J. H. (1972). *Experiments in Molecular Genetics*. Cold Spring Harbor, NY: Cold Spring Harbor Laboratory.

Mulder, E. & Woldringh, C. L. (1989). Actively replicating nucleoids influence positioning of division sites in *Escherichia coli* filaments forming cells lacking DNA. *J Bacteriol* **171**, 4303–4314.

Rosenberger, R. F., Grover, N. B., Zaritsky, A. & Woldringh, C. L. (1978). Surface growth in rod-shaped bacteria. *J Theor Biol* **73**, 711–721.

Spratt, B. G. (1975). Distinct penicillin binding proteins involved in the division, elongation, and shape of *Escherichia coli* K-12. *Proc Natl Acad Sci USA* **72**, 2999–3003.

Spratt, B. G. (1977). Properties of the penicillin-binding proteins of *Escherichia coli* K-12. *Eur J Biochem* **72**, 341–352.

Spratt, B. G. (1983). Penicillin-binding proteins and the future of β-lactam antibiotics. *J Gen Microbiol* **129**, 1247–1260.

Tuomanen, E., Cozens, R., Tosch, W., Zak, O. & Tomasz, A. (1986). The rate of killing of *Escherichia coli* by β-lactam antibiotics is strictly proportional to the rate of bacterial growth. *J Gen Microbiol* **132**, 1297–1304.

Waxman, D. J. & Strominger, J. L. (1983). Penicillin-binding proteins and the mechanism of action of β-lactam antibiotics. *Annu Rev Biochem* **52**, 825–869.

Wientjes, F. B. & Nanninga, N. (1991). On the role of the high molecular weight penicillin-binding proteins in the cell cycle of *Escherichia coli*. *Res Microbiol* **142**, 333–344.

Woldringh, C. L., de Jong, M. A., van den Berg, W. & Koppes, L. (1977). Morphological analysis of the division cycle of two substrains during slow growth. *J Bacteriol* **131**, 270–279.

Woldringh, C. L., Mulder, E., Valkenburg, J. A. C., Wientjes, F. B., Zaritsky, A. & Nanninga, N. (1990). Role of the nucleoid in the toporegulation of division. *Res Microbiol* **141**, 39–49.

Woldringh, C. L., Huls, P. G. & Vischer, N. O. (1993). Volume growth of daughter and parent cells during the cell cycle of *Saccharomyces cerevisiae* a/α as determined by image cytometry. *J Bacteriol* **175**, 3174–3181.

Zaritsky, A., Woldringh, C. L. & Mirelman, D. (1979). Constant peptidoglycan density in the sacculus of *Escherichia coli* B/r growing at different rates. *FEBS Lett* **98**, 29–32.

Received 14 October 1994; revised 28 December 1994; accepted 3 January 1995.