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NOTES

Temperature Effects on Minimum Inhibitory and Bactericidal Concentrations of Cell Wall Antibiotics in *Streptococcus faecalis*

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The minimal inhibitory and bactericidal concentrations were determined at 30 and 37°C for several antibiotics affecting cell wall biosynthesis. The test organism, *Streptococcus faecalis* ATCC 9790, possesses a single autolytic enzyme and does not produce penicillinase. Penicillin, methicillin, and, possibly, bacitracin appeared more effective at 37 than at 30°C. Vancomycin, ristocetin, and phosphonomycin appeared equally effective at both temperatures, and cycloserine appeared consistently more active at 30 than at 37°C.

An effect of temperature of incubation on the minimal inhibitory concentrations (MICs) or minimal bactericidal concentrations (MBCs) of several antibiotics has been reported for penicillin-resistant bacteria. For penicillin or methicillin-resistant staphylococci, greater killing is observed at 37 than at 30°C, apparently due to temperature sensitivity of a penicillinase-producing plasmid (2, 8). At the higher temperature the plasmid is inactivated, and the bacteria become increasingly susceptible to the antibiotic. In contrast, the MIC of methicillin-susceptible *Staphylococcus aureus* is unaffected by incubation temperature (1). Thermosensitive drug-inactivating plasmids have been reported also in *Proteus vulgaris* and *Escherichia coli* (4, 14).

We have determined MICs and MBCs for several antibiotics inhibiting cell wall biosynthesis in *Streptococcus faecalis* ATCC 9790. Although this strain is killed by penicillin (see below), it is moderately resistant when compared with other streptococci. In common with other enterococci, *S. faecalis* is not known to produce penicillinases (3). We have observed a difference between antibiotic concentrations required to inhibit or kill *S. faecalis* at 37 and 30°C. These differences do not appear to be related to either a plasmid-mediated drug inactivation or a loss of antibiotic activity after prolonged incubation.

S. faecalis was grown in a chemically defined medium (16) at 37°C for 7.5 generations to an adjusted optical density of 1,000 at 675 nm. The optical density was adjusted to agree with Beer's law (15). The cells were in mid-exponential phase at this point. A 1:1,000 dilution of cells into fresh chemically defined medium resulted in 10^6 cells (adjusted optical density) per ml. The cells were distributed into a series of tubes containing twofold dilutions of antibiotic. Tubes were incubated at 37 or 30°C for 18, 24, and 48 h, and the MIC was determined from the antibiotic concentration in the last clear tube in the series of twofold dilutions. The medium in the clear tubes of the MIC assay at 18, 24, and 48 h was streaked onto Trypticase-soy agar (Baltimore Biological Laboratory, Cockeysville, Md.) with an inoculating loop and incubated for 18 to 24 h at the temperature of the MIC assay. The MBC is the lowest concentration of antibiotic that kills the organism, as determined by absence of visible colonies on agar. Since dilutions of antibiotics were in geometric progression (twofold dilutions), results of at least three observations were recorded as the geometric mean; that is, the antilog of the arithmetic mean of the logarithms of the MIC or MBC. The geometric mean, $\bar{x}$, equals antilogarithm $(\Sigma \ln x)/n$, where x is the value of an item and n is the number of items (10).

Solutions of antibiotics were prepared by dissolving a weighed amount in double-distilled water. Stocks were stored in the frozen state and were not kept for more than 1 week. Ristocetin was obtained from Abbott Laboratories (North Chicago, Ill.), and vancomycin was ob-

tained from Eli Lilly & Co. (Indianapolis, Ind.). Potassium penicillin G was obtained from Wyeth Laboratories (Philadelphia, Pa.), bacitracin was from Pfizer, Inc. (New York, N.Y.), D-cycloserine was from Eli Lilly & Co., phosphonomycin (fosfomycin) was from Merck Sharp & Dohme (Rahway, N.J.). and sodium methicillin was from Bristol Laboratories (Syracuse, N.Y.). The antibiotics were tested immediately after preparation, and the MIC and MBC were determined at 18, 24, and 48 h (Table 1). In addition, in a separate series of experiments, MICs were determined at 24 h, using antibiotics that had been preincubated in the chemically defined medium at the specific temperature (37 or 30°C) for 48 h. These MICs were compared to the MICs obtained using freshly prepared antibiotics.

For penicillin G and methicillin, the MIC or MBC at 30°C was approximately two times higher than that at 37°C (Table 1). In other experiments, similar results were obtained after preincubation of the medium with the antibiotic (data not shown). At both temperatures the MICs determined by using preincubated media were approximately twice that obtained by using freshly prepared drugs, indicating that the results are not due to alterations in the relative stability of the antibiotics. *S. faecalis* was tested for penicillinase production by the method of Gots (5) and by a rapid slide test (J. E. Rosenblatt and A. M. Neumann, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother., 15th, Washington, D.C., Abstr. no. 388, 1975). Results were negative. Therefore, the possibility that the greater killing observed at high temperatures for the penicillins could be due to the presence of a thermosensitive plasmid coding for penicillinase can be discarded.

Bacitracin results yielded a complex time course, where both the MIC and the MBC increased with time of incubation (Table 1). However, in all instances the antibiotic appeared more effective at 37 than at 30°C. A twofold decrease in the MIC was obtained after preincubation of the antibiotic, but this effect was seen at both temperatures (data not shown).

An opposite effect was obtained with cycloserine, which appeared consistently more ac-

TABLE 1. *MICs and MBCs at 37 and 30°C*[a]

Antibiotic	H	MIC at:			MBC at:		
		37°C	30°C	37°C / 30°C	37°C	30°C	37°C / 30°C
Penicillin G	18	13 × ÷ 1.0	27 × ÷ 1.0	0.48	27 × ÷ 1.0	54 × ÷ 1.0	0.50
	24	27 × ÷ 1.0	54 × ÷ 1.0	0.50	27 × ÷ 1.0	54 × ÷ 1.0	0.50
	48	27 × ÷ 1.6	48 × ÷ 1.3	0.56	27 × ÷ 1.6	48 × ÷ 1.3	0.56
Methicillin	18	865 × ÷ 1.8	1,988 × ÷ 1.0	0.44	1,988 × ÷ 1.0	3,976 × ÷ 1.0	0.50
	24	1,672 × ÷ 1.4	3,976 × ÷ 1.0	0.42	1,988 × ÷ 1.0	3,976 × ÷ 1.0	0.50
	48	1,988 × ÷ 1.0	3,976 × ÷ 1.0	0.50	1,988 × ÷ 1.0	4,463 × ÷ 1.3	0.45
Bacitracin	18	2.9 × ÷ 1.5	2.3 × ÷ 1.3	1.26	3.9 × ÷ 1.6	4.7 × ÷ 1.3	0.83
	24	3.5 × ÷ 1.4	4.3 × ÷ 1.0	0.81	3.9 × ÷ 1.6	4.7 × ÷ 1.3	0.83
	48	4.6 × ÷ 1.5	5.0 × ÷ 1.4	0.92	5.4 × ÷ 1.9	5.9 × ÷ 1.5	0.92
Cycloserine	18	980 × ÷ 1.0	490 × ÷ 1.0	2.00	980 × ÷ 1.0	852 × ÷ 1.4	1.15
	24	980 × ÷ 1.0	696 × ÷ 1.5	1.41	980 × ÷ 1.0	823 × ÷ 1.4	1.19
	48	980 × ÷ 1.0	774 × ÷ 1.4	1.27	980 × ÷ 1.0	774 × ÷ 1.4	1.27
Phosphonomycin	18	2,621 × ÷ 1.3	1,876 × ÷ 1.4	1.40	3,201 × ÷ 1.6	2,230 × ÷ 1.4	1.44
	24	2,578 × ÷ 1.3	2,151 × ÷ 1.5	1.20	3,251 × ÷ 1.7	2,897 × ÷ 1.0	1.12
	48	2,897 × ÷ 1.6	3,128 × ÷ 1.3	0.93	4,099 × ÷ 1.5	3,382 × ÷ 1.4	1.21
Ristocetin	18	0.25 × ÷ 1.0	0.25 × ÷ 1.0	1.00	0.29 × ÷ 1.4	0.42 × ÷ 1.4	0.69
	24	0.25 × ÷ 1.0	0.25 × ÷ 1.0	1.00	0.35 × ÷ 1.5	0.42 × ÷ 1.4	0.83
	48	0.39 × ÷ 1.4	0.39 × ÷ 1.4	1.00	0.44 × ÷ 1.3	0.39 × ÷ 1.4	1.13
Vancomycin	18	0.22 × ÷ 1.0	0.19 × ÷ 1.4	1.16	0.22 × ÷ 1.0	0.19 × ÷ 1.4	1.16
	24	0.22 × ÷ 1.0	0.19 × ÷ 1.4	1.16	0.22 × ÷ 1.0	0.19 × ÷ 1.4	1.16
	48	0.25 × ÷ 1.3	0.22 × ÷ 1.0	1.14	0.25 × ÷ 1.3	0.22 × ÷ 1.0	1.14

[a] Values are geometric means and standard deviations in micromolar concentrations calculated from at least three observations.

tive at 30 than at 37°C (Table 1). For vancomycin, ristocetin, and phosphonomycin, the ratios of the MICs at 37 versus 30°C were near or slightly above 1 after the 48 h of incubation. In one of two series of experiments, this was the case for phosphonomycin as well (Table 1). There were small fluctuations between the MIC and MBC ratios in the cases of phosphonomycin and ristocetin.

It is hard to discern a unifying trend in these observations since the antibiotics tested possess widely different modes of action (6, 7, 9, 11–13, 17). However, the present observations indicate that a decrease in penicillin, methicillin, or possibly, bacitracin susceptibility can be found when *S. faecalis* is tested at 30°C. This decrease is not due to an alteration in penicillinase production, since the organism does not produce penicillinase. On the other hand, sensitivity to cycloserine is greater at 30 than at 37°C. For the remaining antibiotics, ratios of MICs or MBCs at 30 and 37°C approach unity after 48 h of incubation.

An interesting finding in this study is the similarity between the MICs and the MBCs (Table 1) for all antibiotics. This similarity suggests that, in *S. faecalis*, inhibition of wall growth and killing are closely related, possibly via a common mechanism.

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