ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1981, p. 487-489
0066-4804/81/030487-03$02.00/0

Vol. 19, No. 3

NOTES

Filament Formation of *Fusobacterium nucleatum* Cells Induced by Mecillinam

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Subinhibitory concentrations of mecillinam transformed *Fusobacterium nucleatum* cells into a marked filament form, quite different from a spherical form demonstrated in *Escherichia coli*.

It is generally accepted that the morphological responses of gram-negative rods to β-lactam antibiotics are filamentation at low concentrations and spheroplast formation at high concentrations (4, 9, 18). Piperacillin and cephalexin are known to induce only filamentation (7, 12). In contrast, mecillinam is reported to induce osmotically stable round cells (5, 10).

Fusobacteria are anaerobic, gram-negative rods found in natural cavities of humans and other animals (11). Morphologically these organisms are spindle-shaped rods, and *Fusobacterium nucleatum* is a typical species of this genus (11).

In the present paper, the morphological effects of mecillinam on *F. nucleatum* ATCC 10953 and ATCC 25586 were investigated and compared with those of piperacillin. *Escherichia coli* K-12 was used as a reference. Each organism was cultivated at 37°C for 18 h in brain heart infusion broth (Difco Laboratories, Detroit, Mich.) in an anaerobic chamber (Anaerobox, Type TE-HER; Hirasawa Works, Tokyo, Japan) filled with 80% N_2, 10% H_2, and 10% CO_2. Mecillinam and piperacillin were obtained from Takeda Chemical Industries Ltd. (Osaka, Japan) and Toyama Chemical Co. (Tokyo, Japan), respectively.

The minimum inhibitory concentrations (MICs) of mecillinam for *F. nucleatum* ATCC 10953 and ATCC 22586 in brain heart infusion broth with an inoculum of 10^6 cells per ml were 0.79 and 1.56 μg/ml, respectively, slightly higher than for *E. coli* K-12 (MIC, 0.2 μg/ml). On the other hand, the MICs of piperacillin for *F. nucleatum* ATCC 10953 and ATCC 25586 were 0.006 and 0.0125 μg/ml, respectively, indicating that these strains were quite susceptible to this antibiotic. The MIC of piperacillin for *E. coli* was 1.56 μg/ml. The MICs of mecillinam (12.5 and 25 μg/ml, respectively, for strains ATCC 10953 and ATCC 25586) for both strains tested with an inoculum of 10^8 cells per ml were markedly higher than those with an inoculum of 10^6 cells per ml. Thus, the changes in inoculum size had effects on MICs for *F. nucleatum* greater than those reported for *E. coli* (15).

The morphological changes of *F. nucleatum* ATCC 10953 cells grown in the presence of the subinhibitory concentration (1/4 MIC) of mecillinam and piperacillin were compared under a phase-contrast microscope (Fig. 1, panels 1, 2, and 3). Mecillinam-treated cells induced, surprisingly, long filamentation (Fig. 1, panel 2) as compared with nontreated cells (Fig. 1, panel 1), whereas mecillinam treatment transformed *E. coli* cells, used as a control, into a spherical form (not shown) as reported previously (5, 10). On the other hand, piperacillin treatment of *F. nucleatum* induced filament formation as observed with mecillinam treatment (Fig. 1, panel 3). Piperacillin caused *E. coli* to grow filaments (not shown) as reported previously (7).

Figure 1, panels 4 and 5 shows scanning electron micrographs of *F. nucleatum* ATCC 10953 in the presence of 1/4 MIC of mecillinam. Untreated control cells of the strain were longer than those of *E. coli*, and both ends were tapered (Fig. 1, panel 4). The subinhibitory concentration of mecillinam transformed cells of strain ATCC 10953 into a marked filament form at length, and the cell diameters slightly increased (Fig. 1, panel 5).

Table 1 summarizes the morphological changes of *F. nucleatum* ATCC 10953 and ATCC 25586 and *E. coli* K-12 cells by the addition of various concentrations of mecillinam. Higher concentrations (above MIC) caused collapsed cells and lysis in all strains tested. The concentrations of 1/2 to 1/4 MIC of mecillinam induced filament formation in both strains of *F.*

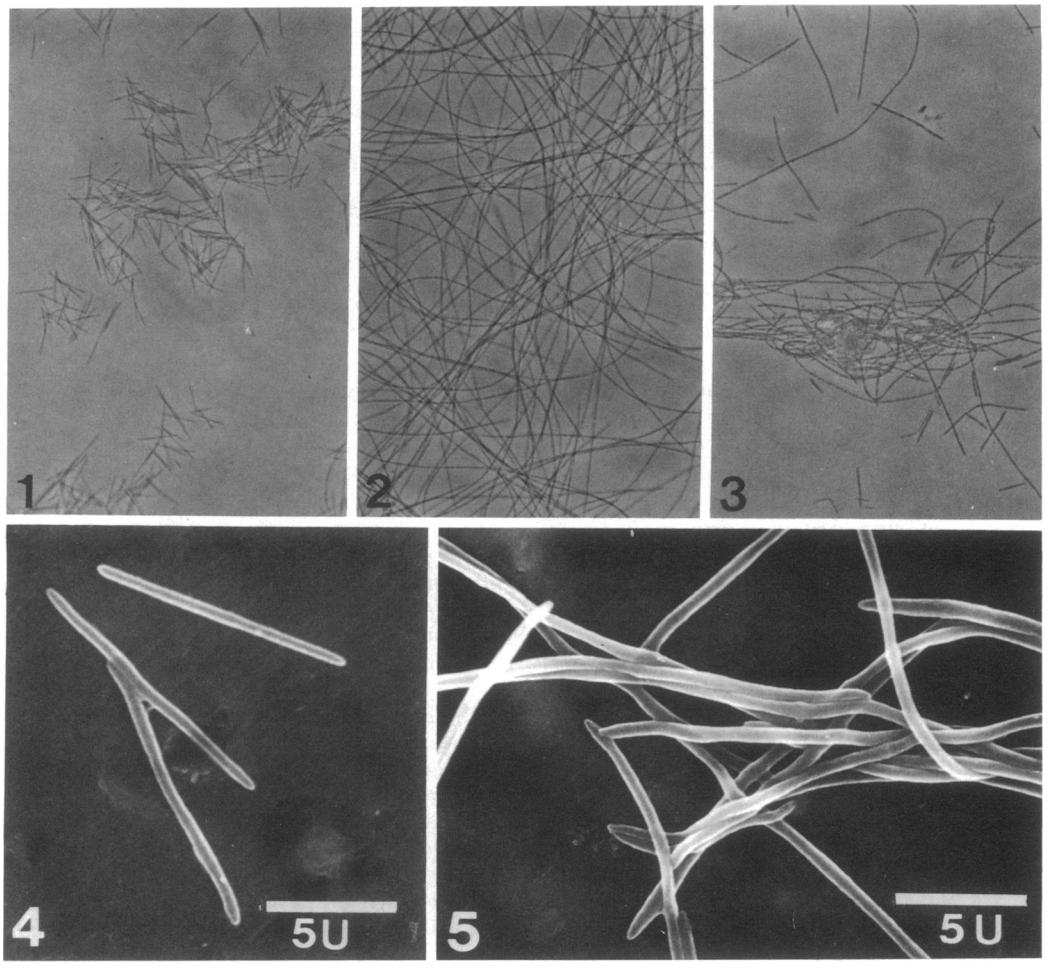

FIG. 1. *Morphological responses of F. nucleatum ATCC 10953 to mecillinam and piperacillin. The organism was grown in BHI broth in the absence of antibiotics (1 and 4) or in the presence of a subinhibitory concentration (1/4 MIC) of mecillinam (2 and 5) or piperacillin (3) with an inoculum size of 10^6 cells per ml for 18 h, and then the morphology of cells was examined with a phase-contrast microscope (1, 2, and 3) and a scanning electron microscope (4 and 5).*

TABLE 1. *Morphological changes of F. nucleatum ATCC 10953 and ATCC 25586 and E. coli K-12 cells by addition of mecillinam*

MIC	Morphological changes in cells of:		
	F. nucleatum		*E. coli* K-12
	ATCC 10953	ATCC 25586	
2	Collapsed	Collapsed	Collapsed
1	Collapsed	Collapsed	Round, collapsed
1/2	Long filament[a]	Filament	Round
1/4	Long filament[a]	Long filament[a]	Round
1/8	Filament	Long filament[a]	Round
1/16	Filament, normal	Long filament,[a] normal	Normal
1/32	Normal, filament	Normal, long filament[a]	Normal
1/64	Normal, filament	Normal, filament	Normal
1/128	Normal	Normal	Normal

[a] Indicates that cells were approximately 10-fold longer than normal cells.

nucleatum, and the length of each cell at 1/4 MIC was approximately 10-fold that of a normal cell. At concentrations of 1/16 to 1/64 MIC, filament cells were mixed with normal cells. In contrast, 1/2 to 1/8 MIC of mecillinam induced round cells in *E. coli*. The filament formation by mecillinam was also observed in laboratory strains of other *Fusobacterium* species: eight strains of *Fusobacterium nucleatum*, two strains of *F. varium*, one strain of *F. glutinosum*, one strain of *F. mortiferum*, one strain of *F. freundii*, and one strain of *F. necrophorum*. Moreover, cells of 24 clinical strains identified as *F. nucleatum* and 19 clinical isolates of other *Fusobacterium* spp. from human oral cavities were transformed into filaments (unpublished data). The filamentation effect by mecillinam, however, was completely absent in four strains of the genus *Bacteroides*, classified in the same family (*Bacteroidaceae*) as the genus *Fusobacterium* (6), and the response was round cell formation as seen in *E. coli*. The response of two strains of *Leptotrichia* sp., also classified in the same family (6), was filamentation (unpublished data). Details will be reported in a separate paper.

Recently, penicillin-binding proteins (PBPs) located on the cytoplasmic membrane of several bacteria have been detected, and functions of each PBP are being determined (1–3, 8, 13, 14, 16, 17–20). It was reported that at least seven PBPs were detected in *E. coli* (18), and that mecillinam bound exclusively to PBP 2 (18), whereas piperacillin bound to PBP 3 preferentially (7). PBPs of *F. nucleatum* were quite different from those of *E. coli* (unpublished data). Presumably, mecillinam and piperacillin would bind to the same PBP(s) in *F. nucleatum* with high affinity. Experiments on the detection and identification of PBPs of *F. nucleatum* by using various β-lactam antibiotics are now under way in our laboratory.

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