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Salt-Induced Filamentous Growth of a *Salmonella* Strain Isolated from Blood

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A strain of *Salmonella choleraesuis* subsp. *choleraesuis* serovar paratyphi-A isolated from the blood of a febrile patient grew into filaments on a nutrient agar containing various salts, such as NaCl, KCl, $MgCl_2$, NH_4Cl, $(NH_4)_2SO_4$, or $(NH_4)_2HPO_4$, at concentrations of 50 to 400 mM. The filamentous cells were nonseptate and multinucleate, and they had colony-forming ability. This mutant strain, however, did not show filamentous growth in liquid media which contained the same salts. On nutrient agar containing 20% sucrose but no salts, some of the cells formed large spheroplasts. Both ampicillin treatment and in vivo environment may in part be responsible for the induction of the mutant strain.

Filamentous forms of bacteria are often observed microscopically in precipitates of the urine or Gram-stained sputa (2) from patients who have received antibiotic therapy. Sub-MICs of β-lactam antibiotics are considered to produce filaments of rods in vivo as well as in vitro. In such cases, filamentous rods are formed only in the presence of antibiotics. Interestingly, however, the clinical isolate of *Salmonella choleraesuis* subsp. *choleraesuis* serovar paratyphi-A, which we present here, grew into long filaments on an agar plate that contained no β-lactam antibiotics but that did contain various salts.

Characterization of the organism. Bacterial strain SPA602 was isolated in Columbia broth with SPS (BBL Microbiology Systems) from the blood of a 13-year-old febrile boy. The patient was receiving aminobenzyl penicillin (200 mg/kg of body weight per day) intravenously at the time of blood collection. The organism was gram negative and motile. Biochemical characteristics by API 20E (Analytab Products) and Micro-ID (General Diagnostics, Div. Warner-Lambert Co.) systems and conventional manual media showed that the isolate corresponded to *S. choleraesuis* subsp. *choleraesuis* serovar paratyphi-A. Somatic and flagellar antigens of SPA602 were determined by slide agglutination tests with anti-O or anti-H immune sera (Denka Seiken, Tokyo, Japan), respectively. The antigenic formula of strain SPA602 was 1,2,12:a:-. Anomalous filamentous growth of the strain was noticed during subculture on a nutrient agar slant (Nissui Seiyaku Co., Ltd., Tokyo; containing 0.5% NaCl).

Nutrient broth (Difco Laboratories) was used as a standard medium; for the preparation of a solid medium, Bact agar (Eiken Chemical Co., Ltd., Tokyo) was added to a final concentration of 1.5% (wt/vol), and this will be referred to as nutrient agar hereafter.

The organism grew in filaments on nutrient agar supplemented with 100 mM NaCl but not on the same medium without NaCl. To examine the effect of various salts on filament formation, nutrient agar was supplemented with NaCl, KCl, $MgCl_2$, NH_4Cl, $(NH_4)_2SO_4$, or $(NH_4)_2HPO_4$ at a concentration of 50, 100, 200, or 400 mM. All salts used were effective for filament formation at some concentrations (Table 1). In particular, when cells were cultivated on the nutrient agar containing 200 or 400 mM NH_4Cl, more than 50% of the cells were elongated rods and developed into long filaments which were 10 to 50 times as long as the normal rod-shaped cells (Fig. 1). This filament formation was observed at 30 or 42°C and also under anaerobic conditions. No filaments developed under such conditions when nutrient agar without salts was used. Three other clinical isolates of *S. choleraesuis* subsp. *choleraesuis* serovar paratyphi-A were used simultaneously as controls, and it was confirmed that they did not show filamentous growth on the salt-containing nutrient agar under these conditions.

Localization of nucleoids in such filamentous cells was examined by Robinow's HCl-Giemsa staining technique (5). Most of the filaments were multinucleate (Fig. 2). Flagellar staining was performed by the method of Toda (8), and flagella were shown to be arranged indiscriminately over the whole bacterial cells. Thin sections of filamentous cells were examined by transmission electron microscopy. Plasmolysis was occasionally observed at the tip of the filamentous cells, but neither septumlike structures nor cytoplasmic constriction was observed in the cells.

Filamentous strain SPA602 cells were collected from the surface of the nutrient agar containing 100 mM NaCl and washed with distilled water. The suspension was inoculated onto nutrient agar containing no added salts and incubated at 37°C. Single filamentous cells were observed at intervals under a phase-contrast microscope. Cell division was first observed after incubation for about 100 min (Fig. 3). The divided cells gradually multiplied and then exhibited colonization during subsequent incubation.

Interestingly, filament formation was not observed in nutrient broth or other liquid media even if the media were supplemented with various salts at 50 to 400 mM.

To increase osmotic pressure, sucrose was added to nutrient agar to a final concentration of 20% (wt/vol). Fila-

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TABLE 1. Effect of salts on the filament formation of strain SPA602 on nutrient agar

Salt	Concn (mM)	Filament formation[a]
NaCl	50	+ +
	100	+ +
	200	+ +
	400	+ +
KCl	50	+
	100	+
	200	+
	400	+
$MgCl_2$	50	+ +
	100	+ +
	200	+
	400	NG
NH_4Cl	50	+
	100	+ + +
	200	+ + +
	400	+ + +
$(NH_4)_2SO_4$	50	+ +
	100	+ +
	200	+ +
	400	+ +
$(NH_4)_2HPO_4$	50	+
	100	+
	200	NG
	400	NG

[a] Colonies were suspended in saline after 24 h of culture on salt-containing nutrient agar, and the suspensions were photographed under a phase-contrast microscope. The cell lengths of 1,000 organisms were measured, and the ratio of cells more than 30 μm long was calculated. Symbols: + + +, >10%; + +, 1 to 10%; +, <1%; NG, no growth.

ment formation was not observed on such an agar plate if the medium contained no salts, but many spheroplasts were observed instead of filaments. By transmission electron microscopy, we found that the spheroplasts had defective cell walls.

The MIC of ampicillin for this strain was 2 μg/ml by the agar diffusion method. The value was identical to that of the three other clinical isolates of *S. choleraesuis* subsp. *choleraesuis* serovar paratyphi-A used as controls.

Discussion. As far as we know, there have been no reports showing the influence of salts on filament induction (6). Apparently, strain SPA602 has some defects in cell wall synthesis, and inhibition of septation by the salts is expressed on agar media but not in liquid media. The subcellular mechanism of the inhibition is not known. However, it seems likely that not only ionic strength but also some other factor(s) affects the process of septation. The fact that spheroplasts are produced from SPA602 on agar plates containing sucrose but no salt also suggests that cell wall synthesis is impaired in this strain.

Because the filamentous cells retain the ability to divide into normal rod forms after transfer to agar medium containing no salt, filamentous growth may not be a lethal event for the organism.

β-lactam antibiotics are known to induce abnormal or cell wall-deficient forms from bacteria both in vitro and in vivo (2). It is not known, however, whether ampicillin treatment is responsible for the induction of mutant strain SPA602, because abnormal forms (1, 3) or cell wall-deficient forms of bacteria (4) have also been isolated from patients or animals that did not receive β-lactam antibiotics. In a strain of *Salmonella typhi*, filamentous forms appear as a part of the L-cycle (7). It is possible that mutant strain SPA602 is a derivative of an L-form which was induced in vivo.

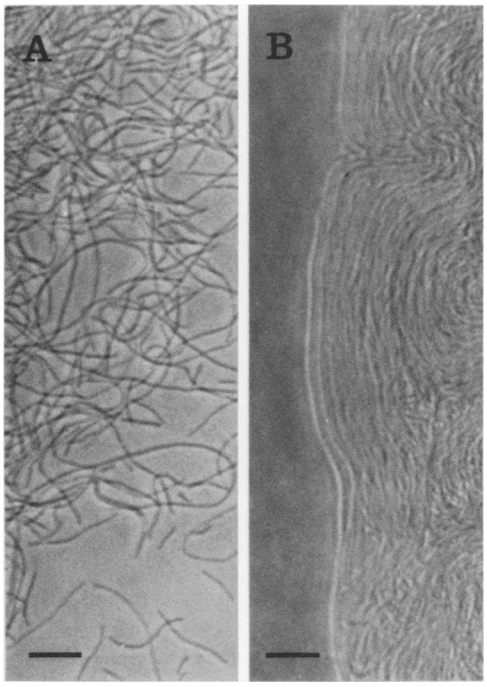

FIG. 1. Formation of filamentous cells 24 h after growth on nutrient agar containing 400 mM NH_4Cl. Phase-contrast microscopy of cell suspension in saline (A) and edge of the colony (B). Bars represent 10 μm.

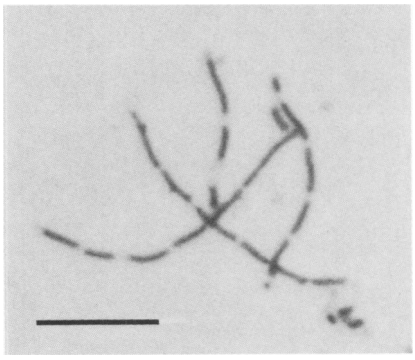

FIG. 2. Arrangement of nucleoids in filamentous cells of strain SPA602 revealed by HCl-Giemsa staining. Cells were grown for 24 h on nutrient agar containing 200 mM NH_4Cl. They were fixed for 45 min with OsO_4 vapor, treated with 1 N HCl at 60°C for 10 min, and stained with Giemsa. Bar represents 10 μm.

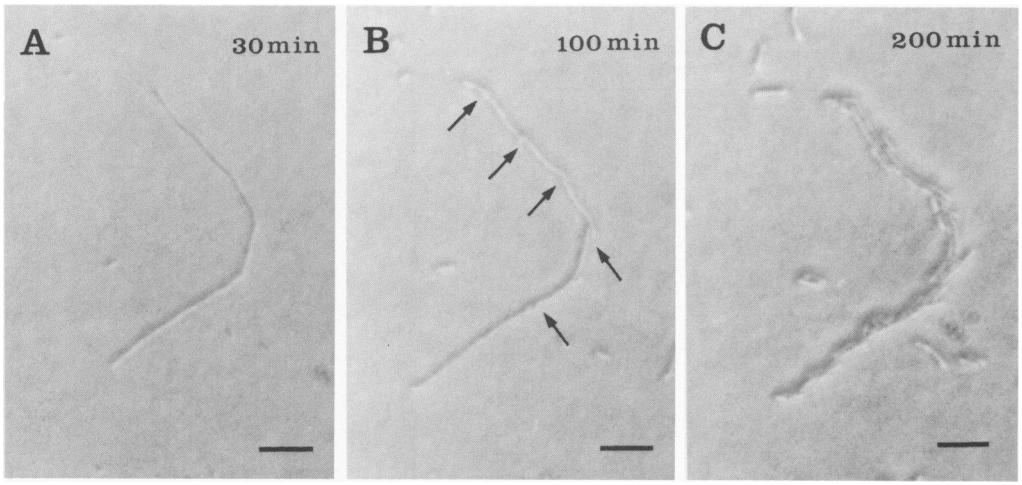

FIG. 3. Colony-forming ability of a filamentous cell. A suspension of filamentous cells was inoculated onto a nutrient agar plate containing no salt at 37°C, and one filamentous cell was observed at intervals under a phase-contrast microscope. (A) No septa were observed 30 min after inoculation. (B) Septation occurred at several points (arrows) 100 min after inoculation. (C) A microcolony was formed along the shape of the filamentous cell at 200 min. Bars represent 10 μm.

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LITERATURE CITED

1. **Domingue, G. J., and J. U. Schlegel.** 1977. Novel bacterial structures in human blood: cultural isolation. Infect. Immun. **15**:621–627.
2. **Lorian, V., A. Waluschka, and Y. Kim.** 1982. Abnormal morphology of bacteria in the sputa of patients treated with antibiotics. J. Clin. Microbiol. **16**:382–386.
3. **Louria, D. B., T. Kaminski, M. Grieco, and J. Singer.** 1969. Aberrant forms of bacteria and fungi found in blood or cerebrospinal fluid. Arch. Intern. Med. **124**:39–48.
4. **Mattman, L. H.** 1974. Cell wall deficient forms, p. 259–260. CRC Press, Inc., Cleveland.
5. **Robinow, C. F.** 1960. Morphology of bacterial spores, their development and germination, p. 207–248. *In* I. C. Gunsalus and R. Y. Stanier (ed.), The bacteria, vol. 1. Academic Press, Inc., New York.
6. **Slater, M., and M. Schaechter.** 1974. Control of cell division in bacteria. Bacteriol. Rev. **38**:199–221.
7. **Timakov, V. D., and G. Y. Kagan.** 1957. The production and cytological features of L-forms of typhoid bacilli. Zh. Mikrobiol. Epidemiol. Immunobiol. **5**:38–43.
8. **Toda, T., and K. Takeya.** 1979. Toda's new bacteriology, 27th ed., p. 714. Nanzando Co. Ltd., Tokyo.