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Author(s)	Kotani, Shozo; Kato, Keijiro; Matsubara, Toshiro et al.
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Letter to the Editor

Staphylolytic activity of a Culture Filtrate of a Flavobacterium species, isolated from Soil

A previous paper (Kotani *et al.*, 1959) has described results on the isolation from soil of bacteria which lyzed the cell walls of BCG, *Corynebacterium diphtheriae*, *Staphylococcus aureus* and *Streptococcus pyogenes* (Lancefield group A).

One of the three cell wall lytic bacteria isolated, a *Flavobacterium* sp. (L_{11} bacterium), was grown in 100 ml of fluid medium (0.1 per cent Bacto-Casamino Acids, Technical, 0.025 per cent K_2HPO_4 and 0.025 per cent $MgSO_4 \cdot 7H_2O$, pH 7.2) in shallow layers in 500 ml Roux's bottles. The lytic activity of the culture filtrates towards the cell wall and living cell suspensions of *Staph. aureus* (strains 209 P and Newman 1) was tested after various periods of incubation at 30°C. The cell wall preparations used were made as described in the previous paper and shown to be

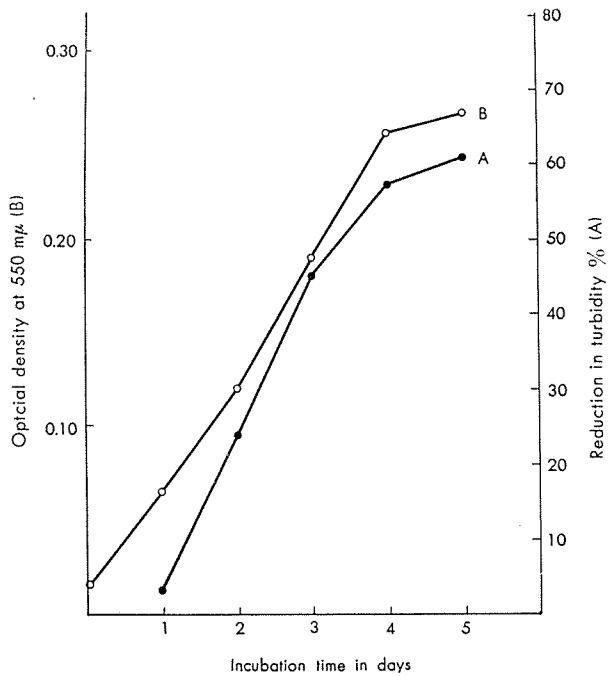

Fig. 1 Appearance in culture filtrates of the lytic activity towards *Staph. aureus* cell walls during exponential growth of a *Flavobacterium sp.*

Curve A. Lytic activity of two-fold dilution of culture filtrates as determined by per cent reduction in turbidity after 30 minute incubation at 37°C.

$$\% \text{ reduction in turbidity} = \left(1 - \frac{\text{Initial turbidity} - \text{Final turbidity}}{\text{Initial turbidity}}\right) \times 100$$

Curve B. Growth of L_{11} bacterium (optical density at 550 mμ).

completely resistant to egg white lysozyme at a final concentration of 1.0 mg/ml. Lytic activity was assayed at 37°C in Tris buffer (1/50 M, final concentration) by following the reduction in turbidity. Turbidity was determined in a Hitachi photoelectric colorimeter, Type EPO-B, using a No. 55 filter and glass-capped test tubes of 18 ± 0.5 mm external diameter. The initial turbidity of the reaction mixture was adjusted to about 0.3.

Fig. 1 shows that the lytic activity of culture filtrates increases during the exponential growth of L_{11} bacterium and that the filtrates of four to five day old cultures have strong lytic activity towards both cell walls and living cells of *Staph. aureus*.

The lytic principle in the culture filtrate was precipitated by $ZnCl_2$ at a final concentration of 0.25 per cent. The precipitate was dissolved in a small amount of 20 per cent $Na_2HPO_4 \cdot 12H_2O$ and dialyzed against a large volume of distilled water. Fig. 2 shows the lytic activity of the culture filtrate thus obtained, concentrated 8 times, towards both cell walls and living cells of *Staph. aureus*. Electron microscopy showed that the reduction in turbidity observed was due to the dissolution of the cell walls.

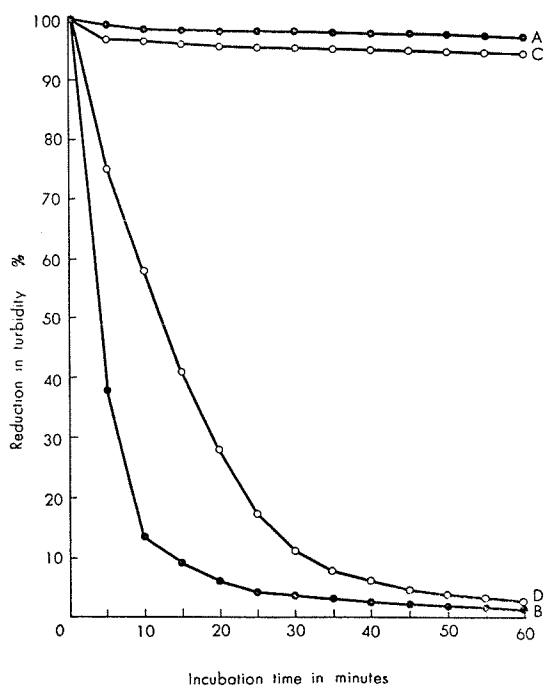

Fig. 2. Lysis of cell walls and living cells of *Staph. aureus* by concentrates of an L_{11} bacterium culture filtrate.

Curve A. Cell walls suspended in buffer.
Curve B. Cell walls incubated with the concentrated culture filtrate (1 : 4).
Curve C. Living cells suspended in buffer.
Curve D. Living cells incubated with the concentrated culture filtrate (1 : 4).

The lytic principle produced by L_{11} bacterium is non-dialysable. It is inactivated completely by heating at 60°C for 60 minutes at a neutral pH but is resistant to tryptic digestion. The optimum pH for the lytic activity is between 6.5 and 7.0 in Tris-maleate buffer, pH 5.0-9.0.

The lysis of both cell walls and living cells of *Staph. aureus* was almost completely inhibited by 1/3000 M diisopropyl fluorophosphate and 1 M NaCl. The lysis of living cells, but not of cell walls, was markedly inhibited by 0.025 per cent polyvinyl sulfate. On the other hand, the lysis of the living cells is increased by pretreatment with EDTA. No stimulation of the lytic activity of the concentrated filtrate was seen with divalent metal ions (Zn^{++}, Cu^{++} and Cd^{++} seemed to be inhibitory).

There is no evidence for significant lysis of cell walls of lysozyme-resistant bacteria other than *Staph. aureus*, such as BCG, *C. diphtheriae* and *Strep. pyogenes*, by the culture filtrate of L_{11} bacterium.

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Shozo Kotani
Keijiro Kato
Toshiro Matsubara
Teiji Hirano
Mikio Higashigawa

Department of Bacteriology,
Nara Medical College,
Kashiwara, Nara
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