"Protoplast" Formation in *Staphylococcus aureus* Using the Lytic Enzyme Produced by a Flavobacterium

In a letter to the Editors of this Journal (Kotani *et al.*, 1959), the authors reported on the production of a lytic principle from a *Flavobacterium sp.* (named L_{11} bacterium) active against the cell walls of *Staphylococcus aureus*. "Protoplasts" of *Staph. aureus* have been prepared using this lytic enzyme.

As test organism a young culture of strain Newman 1 was used, growing it at 37°C for 4 hours with shaking culture in a medium of a following composition (in per cent): Polypeptone 1, lactose 0.2, K_2HPO_4 0.5, $NaH_2PO_4 \cdot 2H_2O$ 0.2, NaCl 0.5 and dehydrated yeast extract 0.25, pH 7.2. The staphylolytic enzyme employed in this study was prepared from the culture filtrate of L_{11} bacterium grown at 30°C for about 5 days in a 0.1 per cent casamino acid dialysate medium. The lytic culture filtrate was concentrated 1,000-fold in volume and 100-fold in lytic activity by precipitation with $ZnCl_2$ and by salting out with $(NH_4)_2SO_4$ (to 0.5 saturation). Details of the methods employed will be reported elsewhere.

As shown in Fig. 1, washed suspensions of strain Newman 1 were incubated with the concentrated lytic enzyme solution in the presence of 0.0125 M Tris-HCl buffer (pH 7.2) with or without 15 per cent sucrose and/or 0.0025 M $MgSO_4$. The change in optical density of the reaction mixture at 37°C was followed for 120 minutes in a Hitachi photoelectric colorimeter (Type EPO-B) using a No. 55 filter. Whereas there was about 90 per cent reduction in the optical density of the cell suspension after 120 minutes incubation with the lytic enzyme in the absence of sucrose (curves C and E), only 20 per cent reduction was observed for the same period in a reaction mixture containing both sucrose and $MgSO_4$ (curve F). Although $MgSO_4$ at a final concentration of 0.0025 M slightly inhibited lysis of cells by the lytic enzyme (as indicated by comparison of curve E with curve C), the presence of this salt seems to be essential for "protoplast" formation by this enzyme, for there was much lysis of cells in a reaction mixture containing sucrose, but no $MgSO_4$ (curve D).

After 120 minutes incubation, each reaction mixture was centrifuged at 14,000 *g* for 20 minutes. The supernatant fluids were separated and examined by the ring test for their serological activity with sera of rabbits immunized with heat-killed *Staph. aureus* (strain Newman 1) or with *Staph. epidermidis* (strain Akamatsu) by the method of Julianelle and Wieghard (1935). All the supernatants of the reaction mixtures containing the lytic enzyme, regardless of the presence or absence of lysis, gave a very similar high precipitation titer against anti-*Staph. aureus* antiserum, but practically no reaction with anti-*Staph. epidermidis* antiserum. The antiserum specimens employed in this study specifically reacted with the cell wall lysates of homologous cocci. Therefore even in reaction mixtures, in which there was no reduction in optical density due to the presence of sucrose and $MgSO_4$, the walls of Staphylococcal cells were considerably affected and the serologically active cell wall materials were released into the medium under the action of the

lytic enzyme. On the other hand, when the cells sedimented from the reaction mixture containing both sucrose and $MgSO_4$ were resuspended in the original volume of distilled water, they lysed instantaneously giving a very viscous solution. The viscosity of the lysate completely disappeared on the addition of one drop of a crystalline DNase solution (100 γ/ml).

The observations described above clearly indicate that "protoplasts" of lysozyme-resistant *Staph. aureus* (strain Newman 1) may be prepared using the cell wall lytic enzyme produced by *Flavobacterium sp.* Experiments are in progress to see if the resulting "protoplasts" are completely devoid of cell wall materials and to prepare a biologically active cytoplasmic membranes from burst "protoplast" suspensions.

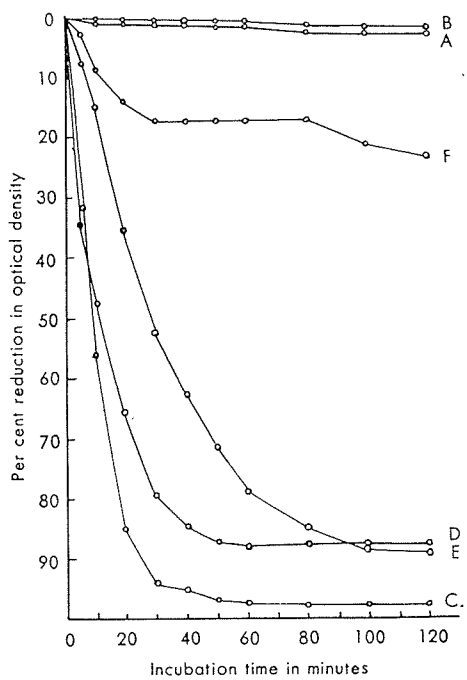

Fig. 1. The lysis of intact cells of *Staph. aureus* by the lytic enzyme of L_{11} bacterium in the presence and absence of sucrose and $MgSO_4$.

Reaction mixtures consisted of (1) 0.4 ml of the washed intact cell suspension of Newman 1, (2) 0.4 ml of the concentrated lytic enzyme solution or distilled water, (3) 1.0 ml of 0.05 M Tris-HCl buffer (pH 7.2), (4) 1.0 ml of 60 per cent sucrose solution or distilled water and (5) 0.2 ml of 0.05 M $MgSO_4$ solution or distilled water.

Curve A:	sucrose	(−),	$MgSO_4$	(−)	lytic enzyme (−)
Curve B:	〃	(+),	〃	(−)	
Curve C:	〃	(−),	〃	(−)	
Curve D:	〃	(+),	〃	(−)	lytic enzyme (+)
Curve E:	〃	(−),	〃	(+)	
Curve F:	〃	(+),	〃	(+)	

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KEIJIRO KATO
TOSHIRO MATSUBARA
YUJI MORI
SHOZO KOTANI

Department of Bacteriology
Nara Medical College
Kashiwara, Nara
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