Lysis of Radio-resistant Bacteria by Enzyme of *Achromobacter lunatus*

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Pseudomonas radiora) of radio-resistant bacterium is not lysed by egg white lysozyme (EC 3.2.1.17), several detergents, or the action of both lysozyme and detergent. Radio-resistant bacteria such as Micrococcus radiodurans, Micrococcus radioproteolyticus, Micrococcus sp. H55, and Arthrobacter radiotolerans) are hardly lysed by lysozyme either. Therefore, it has been difficult to extract gently intracellular components from cells of these microorganisms and to elucidate the structure of cell wall and the role of cell envelope to radio-resistance. The susceptibilities of these radio-resistant bacteria and some other strains of bacteria to the lytic enzyme of *Achromobacter lunatus* isolated by N. Tsumura and M. Ishikawa were examined.

Bacteria cells were prepared as follows: cells were inoculated into the medium containing 1.0% meat extract, 1.0% peptone, 0.5% yeast extract, 0.5% glucose and 0.2% sodium chloride at pH 7.0, and cultivated with shaking at 30°C, except Ar. radiotolerans was cultivated at 40°C. Then the cells of stationary-phase were harvested by centrifugation at 6000 rpm for 15min, and washed twice with 0.01 M phosphate buffer (pH 7.0) and once with deionized water. The washed cells were suspended in deionized water. The precipitate from the culture broth of Ac. lunatus was used as a crude enzyme, of which protein contents were determined by biuret method. The reaction mixture, in a total volume of 6 ml, was composed of 0.02 protein-mg/ml of enzyme, 0.01 M phosphate buffer (pH 7.0), 1.6 x 10^-4 M Triton X-100 (ε at 280 nm = 1670), and cell suspension. The optical density at 600 nm of the mixture was adjusted to about 0.7. The susceptibility of each bacterium to the lytic enzyme is expressed as the percentage decrease in optical density of the reaction mixture.

Most gram-negative bacteria are normally resistant to the lytic enzymes. As shown in Table I, gram-negative bacteria on incubation at 30°C were hardly lysed by enzyme alone, but gram-positive bacteria except for *Bacillus sphaericus* were easily lysed under the same conditions. Gram-negative bacteria incubated at 50°C were lysed by enzyme alone more than that at 30°C. Ps. radiora, even if incubated at 50°C, was difficult to be lysed by enzyme alone or detergent alone, but was found to be lysed by the cooperative action of the lytic enzyme and Triton X-100 of nonionic detergent. Gram-positive radio-resistant bacteria were lysed effectively as well as other gram-positive bacteria. Triton X-100 was found not to increase significantly a rate of lysis of these gram-positive bacteria.

Recently, it has been reported by K. Nakamura and one of authors (Y.O.) et al. that the cells of *M. radiodurans* were lysed by the action of both enzyme produced by *Ac. lyticus* and detergent, but were hardly lysed without detergent. The “R1 fraction” in enzymes produced by *Streptomyces albus G* has been also found to be lytic towards *M. radiodurans* in the presence of sodium dodecyl sulphate. However, the lytic enzyme of *Ac. lunatus* was able to lyse the cells of *M. radiodurans* without detergent (Fig. 1). The optimum temperature was above 50°C, but even if the cells were incubated at 20°C, the optical density of cell suspension became below five percent of the initial one after 60 min.

From the results of electrophoresis, at least four kinds of enzymes have been detected in the culture broth of *Achromobacter lunatus*. The presence of protease has been revealed in acetone precipitate from the broth, and the optimum pH of the protease is 8 to 9.5. Optimum
Table I. Susceptibilities of Various Bacteria to Lytic Enzyme of Actinobacter lunatus

The reaction mixture was composed of 1 ml of enzyme solution (0.12 protein-mg/ml), 1 ml of Triton X-100 (9.3 x 10^{-4} M), 1 ml of cell suspension and 3 ml of 0.02 M phosphate buffer (pH 7.0), and incubated at 30°C or 50°C for 60 min.

<table>
<thead>
<tr>
<th>Bacteria</th>
<th>Degree of lysis (% decrease in turbidity)</th>
<th>30°C</th>
<th>50°C</th>
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<tr>
<td></td>
<td>E (a)</td>
<td>T (b)</td>
<td>E+T (c)</td>
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<td>93</td>
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<td>11</td>
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<tr>
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</table>

(a) Percentage decrease in turbidity=(O.D. of reference - O.D. of reaction mixture) x 100/O.D. of reference. The reference which removed both enzyme and detergent was incubated at the same conditions as reaction mixture.

(b) Instead of Triton X-100, the same volume of water was added to the mixture.

(c) Instead of enzyme, the same volume of water was added to the mixture.

(d) Complete reaction mixture containing both enzyme and Triton X-100.

(e) Ps. radiotolerans and M. sp. H55 isolated by H. Ito et al. were kindly supplied from the isolator. M. sp. H55 was isolated from sawdust medium for mushroom cultivation, and its radio-resistance was almost the same as that of M. radiodurans.4)

(f) M. radiodurans isolated by A. W. Anderson et al. and Ar. radiotolerans isolated by T. Yoshinaka et al. were obtained from Institute of Applied Microbiology, Culture Collection Center.

(pH to living cells of M. radiodurans was 8.8. Therefore, protease will act predominantly to the walls of this organism. The outer layer of the walls of M. radiodurans contains significant amounts of lipoprotein and polysaccharide as is typical for gram-negative bacteria, and is little affected by lysozyme. The inner layer is composed of a mucoprotein which is solubilized by lysozyme.5) Therefore, it seems that the protease in lytic enzyme of Ac. lunatus may be able to decompose both layers of the walls.

It is concluded that the lytic enzyme of Ac. lunatus is available for lysis of radio-resistant bacteria. This observation will lead to the development of a suitable procedure for isolation of the intracellular components from these bacteria.

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