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Osmotic Fragility of Lysozyme- and Thiol-Treated *Lactobacillus plantarum*

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Reduced but not oxidized thiols increased the sensitivity of lysozyme-treated cells of *Lactobacillus plantarum* to lysis by osmotic shock.

Metabolic studies on mevalonate-derived lipids have been hindered by the lack of gentle methods for the lysis of *Lactobacillus plantarum*. This report describes the lysis of lysozyme-treated cells by reducing agents such as thiols, and ascorbate.

Growth of *L. plantarum* (ATCC 8014) and reagents were as previously described (1). Growth was determined turbidimetrically by measuring absorbancies at 660 nm. Cells grown in 1 liter of liquid culture for 12 hr (absorbancy 1.5 at 660 nm) were harvested by centrifugation at 4,500 × *g* for 15 min, and washed once with 120 ml of cold 0.03 M tris(hydroxymethyl)aminomethane (Tris)-hydrochoride buffer (*p*H 8.0). The cells (3 gm) were suspended in 120 ml of 1 M sucrose containing 0.03 M Tris-hydrochloride buffer (*p*H 8.0), lysozyme (0.25 mg/ml), and magnesium sulfate and reduced glutathione each to a final concentration of 10^{-3} M. A sample was diluted 10-fold in the above sucrose-Tris solution for reading the absorbancy. The suspension was incubated at 37 C with gentle shaking for 30 min, and the absorbancy was again measured. The suspension was then centrifuged at 33,000 × *g* for 15 min. The supernatant fluid was decanted, and the pellet was shocked with 5 ml of ice cold 0.01 M Tris-maleate buffer (*p*H 5.0). The extract was drawn through a pipette several times; subsequently, the extract was incubated with deoxyribonuclease (25 μg/ml) for 5 min at 30 C to decrease its viscosity. The extract was centrifuged at 33,000 × *g* for 15 min; the yield of soluble protein (2) was 50% of the total cell protein.

The per cent decrease in absorbancy of the bacterial suspension before and after treatment with lysozyme was calculated as follows: (original absorbancy − absorbancy after 30 min)/original absorbancy × 100. To determine total cell protein, 1 g of the wet pellet was dissolved in 50 ml of 1 N KOH and boiled for 1 min. A portion was then used for protein measurement (2). It was found that 1 g of wet cells contained 60 mg of protein.

When cells of *L. plantarum* were incubated with various concentrations of lysozyme, a decrease in absorbancy was observed. However, no lysis occurred as indicated by the low viscosity and the lack of soluble protein in the supernatant fluid. The additon of glutathione increased the osmotic fragility of lysozyme-treated cells, since subsequent shocking yielded very viscous suspensions. This viscosity could be reduced only by deoxyribonuclease. The soluble protein of such extracts was almost doubled by glutathione (Table 1). Although the protein yield varied by a factor of 2, the decrease in absorbancy did not vary significantly. Ethylenediaminetetraacetate slightly inhibited the action of lysozyme, but reduced the action of glutathione (Table 1). Glutathione alone did not lyse the cells. Furthermore, glutathione was ineffective as a pretreatment before lysozyme treatment. On the other hand, glutathione was equally effective whether added with lysozyme or added after treatment of the cells with lysozyme, thereby suggesting that glutathione was effective

TABLE 1. *Effect of glutathione and EDTA on lysis of Lactobacillus plantarum*

Additions	Per cent decrease in absorbancy	Soluble protein (mg/ml)
Lysozyme	30	9.6
Lysozyme + Mg^{2+}	30	9.6
GSH[a] + Mg^{2+}	0	0.0
Lysozyme + GSH	30	14.4
Lysozyme + GSH + Mg^{2+}	30	17.0
Lysozyme + EDTA[b]	36	8.1
Lysozyme + GSH + EDTA	25	6.4

[a] Glutathione.
[b] Ethylenediaminetetraacetate.

[1] This report is from a thesis by Z. Habbal in partial fulfillment of the requirements for the M.S. degree.

TABLE 2. *Effect of reductants and oxidants on lysis of Lactobacillus plantarum*

Additions	Per cent decrease in absorbancy	Soluble protein (mg/ml)
None	20	6.8
Ascorbate	65	14.4
BAL[a]	60	15.6
Cysteine	59	16.8
GSH[b]	43	16.0
GSSG[c]	43	5.8

[a] 2,3-Dimercaptopropane.
[b] Glutathione.
[c] Oxidized glutathione.

only after lysozyme had partly or completely digested the cell wall. Varying the concentration of glutathione between 0.001 M and 0.01 M did not improve the protein yield. Sucrose was still needed for the plasmolysis of the cells as no lysis occurred in its absence. Thioglycollic acid, L-cysteine, dithiothreitol, 2,3-dimercaptopropane, or ascorbate, but not oxidized glutathione, were as efficient as glutathione (Table 2).

Cells from cultures grown to an absorbancy of 1 to 1.5 were most susceptible to lysis. Cells grown on casein hydrolysate or yeast extract were equally sensitive. Since culture media contained Tween 80, which adversely affects the sensitivity of microorganisms to lysozyme, cells were grown in its absence. Such cells were still resistant to lysozyme alone but could be lysed when glutathione was included. Addition of albumin or casein (1 mg/ml) to lysozyme and glutathione completely blocked lysis.

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