Cell-Bound Lipase and Esterase of Brevibacterium linens

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The activities of glycerol ester hydrolase, lipase (EC 3.1.1.3) and carboxylesterase, and esterase (EC 3.1.1.1) were determined for whole cell preparations of Brevibacterium linens by using the pH-stat assay. The culture growth lipids were inactive against the three substrates, tributyrin emulsion, triacetin, and methyl butyrate. Cells washed in water had less activity than cells washed in 5% NaCl; the ratio of activities was close to 1:2 for all strains using tributyrin emulsion as the substrate. For the esterase substrates, this relationship varied widely and was strain dependent. The ability to hydrolyze the two esterase substrates varied independently of the level of lipase activity.

Three earlier investigations on the lipase activity of Brevibacterium linens have led to contradictory results. Albert et al. (1), using an agar plate technique with buffer fat emulsion, concluded that under such conditions the organism lacked lipolytic activity. However, butterfat is considered by Lawrence (4, 5) to be a poor substrate for bacterial lipase. San Clemente and Vadehra (7) demonstrated the presence of lipase in the culture growth liquor of their test strain. They used a pH-stat procedure with emulsified olive oil as the substrate. Singh (S. Singh, Ph.D. thesis, Graduate College of the University of Illinois, Urbana, 1968), using the modified agar well technique of Oterholm and Ordal (6) with a tributyrin substrate, obtained evidence of lipolytic activity with cell suspensions of B. linens ATCC 9174.

B. linens occurs commonly in the salt-containing slime of surface-ripened cheeses. In this environment of high fat content, it would seem natural for this organism to possess lipolytic activity. Singh (S. Singh, Ph.D. thesis) has demonstrated that fat hydrolysis is an important aspect of Limburger cheese aging. However, the surface slime also contains other bacteria and yeasts (3). This investigation was initiated to evaluate more thoroughly the lipase and esterase activities of B. linens.

Four strains from the American Type Culture Collection (ATCC; Rockville, Md.), B. linens ATCC 8377, 9172, 9174, and 9175, and B. linens I, an isolate from a Limburger cheese, were tested. The latter organism, whose parent strain is ATCC 9174, was characterized in this labora-

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there was a linear relationship between cell dry matter (DM) and optical density (650 nm) and the amount of DM and enzyme activity, specific activity was defined as nanoequivalents of fatty acid released per minute per milligram of DM. Microscopic examination demonstrated that the triglyceride emulsion globules were larger than the size of the individual bacterial cells. The emulsified substrate could, therefore, only be attacked by an enzyme on the outside of the cell wall. The recorded titrations never showed a lag and the reaction rate remained constant for long periods of time. This suggested that a transport mechanism was not involved. These results and other evidence (T. Sørhaug, Ph.D. thesis) indicate that the active principles (sites) are located in or on the bacterial cell wall.

The specific lipase and esterase activities using whole-cell preparations and the percentage values for these activities, related to the rate of lipolysis in 5% NaCl for each bacterial strain, are presented in Table 1. For each bacterial strain, tributyrin in emulsion was the substrate hydrolyzed at the most rapid rate. The activities of cells washed in 5% NaCl were higher than for those washed in water with a single exception, the hydrolysis of triacetin by cells of the ATCC 8377 strain. The occurrence of cell-bound lipase and esterase activities which are activated by NaCl is, therefore, common to the five strains of *B. linens*.

Although activities were always detected, the levels varied among strains, and the relationship between the hydrolytic rates when using the three substrates for one strain was different from that of another strain (Table 1). The ratio between lipase activity of cells in water to that of cells in 5% NaCl varied from 0.43 to 0.63, with 0.55 as the mean value (Table 1). The corresponding relationships using the esterase substrates varied widely.

The assay used by San Clemente and Vadehra (7) was reported with culture growth liquors of *B. linens* I. Olive oil emulsion served as substrate in the presence of sodium chloride, calcium chloride, and sodium taurocholate in the pH-stat titration. Extracellular activity was not detected. It is possible that the strain used by San Clemente and Vadehra (7) differs genetically from those tested in the present investigation. Troller (J. Troller, personal communication) has found both cell-bound and extracellular lipase activity for *Corynebacterium acnes*, another coryneform bacterium.

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