Wall Autolysin of *Lactobacillus acidophilus* Strain 63 AM Gasser*

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ABSTRACT: The autolysin of *Lactobacillus acidophilus* strain 63 AM Gasser has the specificity of an endo-*N*-acetylmuramidase. It hydrolyzes both *N*-acetylmuramic acid and *N*, *O*-diacetylmuramic acid linkages. It does not exhibit any amidase or endopeptidase action. It is present in both log-phase and stationary-phase cells.

In stationary-phase cells its action upon the wall peptidoglycan is inhibited.

Experiments carried out with several Gram-positive bacteria (Cole, 1965) and with *Escherichia coli* (Schwarz *et al.*, 1969) strongly suggest a zonal growth of the wall peptidoglycan at least during cell division. Biochemical study of the biosynthesis of the peptidoglycan also strongly suggests that the insertion of newly synthesized β-1,4-*N*-acetylglucosaminyl-*N*-acetylmuramyl peptide fragments requires the creation of nonreducing *N*-acetylglucosamine receptor sites in the glycan strands (for a review, see Ghuysen, 1968). Wall lytic endo-*N*-acetylmuramidases could thus play the role of providing those receptor sites a nd it may be that the safe enlargement of the peptidoglycan sacculus is due, in fact, to a strict coordination between the biosynthetic and the hydrolytic processes. So far is known, *Streptococcus faecalis* appears to be one of the simplest model with regard to the study of wall growth at the cellular level. Indeed, the active wall-bound autolytic system consists of a single enzyme that has the specificity of an endo-*N*-acetylmuramidase (Shockman *et al.*, 1967b). Moreover, it has been shown that the cell equator is the site where the active autolysin is located and the region where new wall material is inserted (Shockman *et al.*, 1967a; Shockman and Martin, 1968; Shockman and Cheney, 1969; Pooley and Shockman, 1969). In contrast to *S. faecalis* which exhibits a single plane of division, other spherical microorganisms that are characteristically arranged in clusters such as *Staphylococcus aureus* (Tipper, 1969), and rod-shaped bacteria such as *E. coli* (Weidel and Pelzer, 1964) and *Bacillus subtilis* (Young, 1966a,b) have complex autolytic systems. These systems contain enzymes such as amidases, endopeptidases, and endo-*N*-acetylglucosaminidases that do not appear to be consistent with a role in wall biosynthesis but that might be involved in other phenomena such as competence, excretion, and permeation of large molecules.

The experiments hereby presented show that *L. acidophilus* 63 AM Gasser is another simple model that may be useful for the study of wall expansion and cell division in a rod-shaped microorganism.

Materials and Methods

Growth conditions, analytic techniques (measurement of reducing groups, acetamido sugars, and N-terminal groups), and *walls preparation and structure* have been described (Coyette and Ghuysen, 1970).

Enzymes. Streptomyces F_1 endo-*N*-acetylmuramidase was used (Ghuysen, 1968).

Experimental Section

Autolysis and Bacterial Growth. Strains of *Lactobacilli* are known to autolyze (Knox and Brandsen, 1962). Preliminary experiments carried out with *L. acidophilus* 63 AM Gasser showed that the rate of autolysis of log-phase cells suspension was maximal in a 0.05 M citrate buffer, pH 5. The specific autolytic activity during growth of *L. acidophilus* was determined as follows: cells were harvested at various times and washed by centrifugations with cold distilled water. The pellets were resuspended in 0.05 M citrate buffer, pH 5, and the turbidity of each cell suspension was adjusted to an optical

* From the Service de Bactériologie, Liège, Belgium. *Received April 2, 1970.* This research has been supported in part by the Fonds de la Recherche Fondamentale Collective, Brussels, Belgium (Contracts 515 and 1000). This paper is from a dissertation submitted by J. C. in partial fulfilment of the requirements for a Ph.D. degree, University of Liège, Belgium (1969).

anisms may not be identical in these two microorganisms.

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