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Detection and Localization of a Muramidase type-2 Autolysin in Cell Walls of *Lactobacillus delbrueckii ssp. bulgaricus.*

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ABSTRACT

The presence of cross-reacting muramidase in *Lactobacillus delbrueckii* ssp. *bulgaricus* UL12 was shown by using monoclonal antibodies raised against an muramidase-2 of *Enterococcus hirae* ATCC 9790. The separation of protein by sodium dodecyl sulfate-polyacrylamide gel electrophoresis followed by Western immunoblot confirmed the presence of one cross-reacting band in *Enterococcus hirae* with an estimated molecular mass of 80 kDa. *L. bulgaricus* cultured cells harvested after 4 and 12 h were submitted to different autolysin releasing procedures and the liberated products were allowed to cross-react with muramidase-2 antibodies in order to estimate the efficiency of each treatment. Although the cultured cells harvested after 4 h yielded only a slight immuno-reaction in Western immunoblots against these enzyme monoclonal antibodies, a strong signal was observed for the cell walls obtained from the same experimental conditions and treated with Triton X-100 surfactant. The same phenomenon was also observed by light fluorescence microscopy. Immuno-labelling followed by optical and electron microscopy have shown that the muramidase-2 of *L. bulgaricus* UL12 was essentially localized in the innermost part of the cell wall.

INTRODUCTION

The bacterial autolysis ia a phenomenum by which the bacterial cell wall losses its integrity under the influences of different natural or artificial factors. The enzymes responsible of the autolysis are grouped under the general appellation of autolysins which comprised several classes of enzyme having different hydrolytic functions and acting on the peptidoglycan which is the main constituent of the Gram positive bacterial cell wall (1). Several studies have been carried to allow the characterization of either autolysis issued from different bacterial strains or the different factors inducing the autolysis of the cell (2, 3, 4). The leaking of the intracellular content of the lactic

bacteria resulting from the hydrolytic action of the autolysins is of major importance in the cheese aging process. A better knowledge of the autolysis biochemistry of the implicated lactic bacteria must lead to a better control of this industrial process.

Reference

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