Effect of medium composition on the ultrastructure of Lactobacillus strains

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Abstract. The growth of some locally isolated Lactobacillus strains forming D(--) or L(+) lactic acid, Lactobacillus helveticus ATCC 15009 and Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842 was examined in different media. L. helveticus and Lactobacillus LBL strains formed atypical protoplast-like cells in LAPT medium, sensitive to SDS and proteinase. Specific morphological changes in the cell wall structure of these variants were revealed by transmission and scanning electron microscopy. The effect of glucose and various salts on their appearance was investigated. The prevalent role of metal cations, especially of Mg²⁺, was established.

Key words: Lactobacillus — Medium composition — Metal cations — Electron microscopy — Protoplast-like forms

The resistance of the Lactobacillus cell wall to the usual lytic agents renders significant problems in the isolation of DNA by the conventional methods. The optimization of the procedures is usually aimed at improvement of the growth conditions and achievement of better lysis of the cells (Klaenhammer 1984; Bernard et al. 1991).

With the same aim, in the course of the genetic investigations of Lactobacillus strains, used in the production of Bulgarian yoghurt (Miteva et al. 1991, 1992), we compared different cultivation media. Here, the ultrastructural morphological changes mediated by variability of nutrients, revealed during these studies are presented.

Materials and methods

Strains, media and growth conditions

The Lactobacillus strains studied are: Lactobacillus delbrueckii subsp. bulgaricus ATCC 11842, LBD 5, 37, 144; Lactobacillus helveticus ATCC 15009; locally isolated Lactobacillus strains LBL.

4, 5, 9, 13, 25, 45, 49, 70, 149. LBD and LBL are designations of Lactobacillus strains from the Collection of LB Engineering, forming D(--) or L(+) lactic acid respectively. Two media were used: MRS (De Man et al. 1960) (g/l): peptone - 10, “Lab Lemco” powder - 8, yeast extract - 4, glucose 20, Tween-80 - 1, K₂HPO₄ - 2, CH₃COONa·3H₂O - 5, triammonium citrate - 2, MgSO₄·7H₂O - 0.2, K₂MnO₄ - 4H₂O - 0.05, final pH 6.2; or LAPT (Raibaud et al. 1961) (g/l): peptone - 15, tryptone - 10, yeast extract - 10, glucose - 10, Tween-80 - 1, final pH 6.2. The cells were cultivated anaerobically, using 0.5% milk inoculum, at 37 °C or 45 °C in two steps — dilution (1:2) in fresh broth medium after overnight growth.

Scanning electron microscopy

The cells were harvested, washed in 0.1 M cacodylate buffer, pH 7.2 supplemented with 0.1% MgSO₄·7H₂O and 7.5% sucrose and fixed in 4% glutaraldehyde for 2 h at 4 °C. After washing in the same buffer and dehydration in ethanol series the samples were dried, coated with 30 Å gold in an Edwards S 150 A vacuum apparatus and observed with a scanning device at 20 kV.

Transmission electron microscopy

The cells were fixed in 4% glutaraldehyde as described above. A second fixation was done in 1% OsO₄ for 2 h at room temperature, followed by dehydration in ethanol series and embedding in durecopan. Staining was done according to Reynolds (1963). Thin sections were examined on Opton 10 CM electron microscope.

Results

Growth in LAPT and MRS

The Lactobacillus strains studied were cultivated in LAPT and the growth and cell morphology were compared with that observed in the conventionally used MRS medium. Recently Amoroso and Manca de Nadra (1992) reported that L. bulgaricus grew well on LAPT supplemented with lactose or glucose. In our investigations the two carbohydrates were also equally suitable and glucose was used further.

The microscopic observations of the cells grown on LAPT showed that some of the strains (L. helveticus and...
the group of LBL strains) formed atypical cells with round form, while \( L. \) \textit{delbrueckii} subsp. \textit{bulgaricus} ATCC 11842 and the LBD group did not form such cells. Their amount in \( L. \) \textit{helveticus} was usually about 20\% and reached 30\%–50\% in LBL strains. \( L. \) \textit{bulgaricus} ATCC 11842 differed from both groups and formed very long thin cells less intensively Gram-stained.

Trying to clear up the nature of these morphological changes we undertook electron microscopy studies.

\textit{Scanning electron microscopy}

Samples from exponentially growing \( L. \) \textit{helveticus} and LBL strains, cultivated in LAPT were examined. Both types of cells – normal long rods and spherical ones usually attached to the rods were visualized (Fig. 1). The surface of the atypical cells was rough with invaginations, contrasting with the smooth wall of the normal cells. The picture was very similar to the one observed in protoplast formation (Cocconcelli et al. 1986). The round forms appeared at the distal edge of the cell, between two dividing cells or in the middle of the rod. In some cases they were in the form of buds, in others – as larger round cells attached to the mother cell and rarely as separate spherical particles. Possibly these are the stages of the formation of the abnormal cells. In addition, small buds were observed on the surface of the round cells (Fig. 1, B').

\textit{Transmission electron microscopy of thin sections}

The atypical cells were further examined in thin sections. Normal cells, spherical cells, devoid of cell walls and many intermediate forms were observed (Fig. 2).

The wall of the normal cells consisted of the usual three layers: an outside layer of low density known as S-layer, a more dense and thick intermediate layer and a most dense and thin inner layer (Fig. 2, A'). This typical structure has been found in \( L. \) \textit{helveticus} (Lortal et al. 1992), \( L. \) \textit{casei} (Watanabe et al. 1990) and other Gram-positives (Beveridge and Graham 1991). The presence of ribosomes and mesosomes in the cytoplasm was clearly revealed. The nuclear region was visualized as a more dispersed and less electron dense area.

At the same time bacillus shaped forms surrounded by a wall layer loosely associated with the cell could be seen both by scanning and transmission electron microscopy. Significant disruptions of the cell wall and partial detachment from the membrane had taken place. As a rule the ends of the disrupted walls were curved out (Fig. 2B, D). Some “budding” cells were revealed as well (Fig. 2E, F). These observations supposed the protoplast-like nature of the atypical forms. They could be classified as spheroplasts (osmotically fragile cells, which have partially lost their cell wall but retained the rod shape) and protoplasts (spherical forms without cell walls) as defined by Connell et al. (1988).

Fig. 1A–C. Scanning electron micrographs of \textit{Lactobacillus} LBL strains, cultivated in LAPT medium. A Bud on the cell surface. B–C Protoplast-like forms. B' Bud on a round form cell. Bar 0.5 \( \mu \text{m} \).