SHORT COMMUNICATION

Lactococcus lactis STRAINS ECTOPICALLY EXPRESS THE INTERNALIN-A PROTEIN FROM *Listeria monocytogenes* UNDER THE BACTERIAL SEPTUM REGION

*Cepas de **Lactococcus lactis** expressam ectopicamente a proteína Internalina-A de **Listeria monocytogenes** na região do septo bacteriano*

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Running title: Internalin-A Gene Expression in Lactococcus lactis

Abstract

In the present report, we describe how *Lactococcus lactis* strains ectopically express the internalin-A protein from *Listeria monocytogenes* at the cell surface, situated specifically under the bacterial septum region. Such findings provide relevant insights into the spatial expression pattern of exogenous genes in lactic bacteria.
Key-words: Internalin-A gene; *Lactococcus lactis*; Spatial expression.

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Resumo

No presente relato, nós descrevemos como cepas de *Lactococcus lactis* expressam ectopicamente a proteína internalina-A de *Listeria monocytogenes* na superfície celular, localizada especificamente na região do septo bacteriano. Tais descobertas proporcionam relevantes informações sobre o padrão de expressão espacial de genes exógenos em bactérias lácticas.

Palavras-chave: Gene internalina-A; *Lactococcus lactis*; Expressão espacial.

Several protein expression and targeting systems have also been designed to engineer the food-grade bacterium *Lactococcus lactis* for the intra- or extra- cellular production of numerous proteins of viral bacterial or eukaryotic origins (1, 2). All of these studies provide evidence of the powerful application of lactic bacteria for the synthesis and production of heterologous proteins. Within this context, the aim of this study was to investigate the spatial expression pattern of the internalin-A gene from *Listeria monocytogenes* in transforming *L. lactis* cells.

During the past decade, several questions have been raised concerning the molecular basis involved in mechanisms of cellular invasion unleashed by *L. monocytogenes* and it is already well established that two surface bound proteins, called internalin-A (*inlA*) and internalin-B (*inlB*), promote invasion into host cells (3). This process depends on the interaction between the bacterial surface protein internalin-A and the host protein E-cadherin, located below the epithelial tight junctions at the lateral cell-to-cell contacts (4).

To verify the ectopical expression of the *inlA* gene from *L. monocytogenes* in transforming *L. lactis* cells, we performed immunofluorescence assays using specific antibodies. Under our experimental conditions, plasmids pGM10 constructed originally from pUC19/pAT19 derivatives carrying the *inlA* promoter and gene from *L. monocytogenes* (5) were transferred by electroporation in *L. lactis* cells (MG1363, wild type strain). Transforming cells were plated on BHI agar plates containing erythromycin 5 μg/ml and were counted after a two-day incubation period at 30ºC (86ºF). The immunofluorescence assays of InlA surface protein expressed in *L. lactis* strains were performed according to the Lebrun et al. (6) method. Saturated cultures of *L. lactis* expressing inlA gene were grown anaerobically in Brain Heart Infusion broth (BHI, Difco Laboratories) at 30ºC (86ºF). Bacteria were fixed on glass slides with 3% paraformaldehyde. Slides were incubated in a 1/500 dilution of specific InlA antibody (mAlL7.7) (7). The slides were then incubated for 30 minutes at room temperature in fluorescence-labeled goat anti-mouse antibodies (1/300) (Biosystems). Cells were examined with an epifluorescent microscope (Nikon Eclipse TE200 equipped with a Nikon DXM1200 digital still camera).

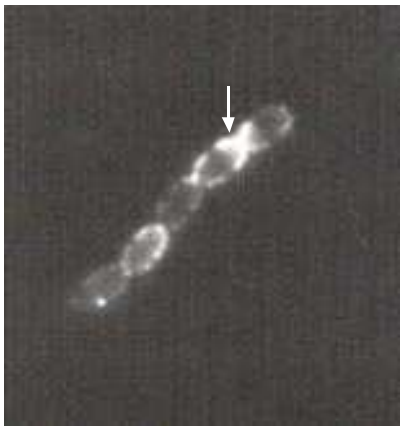

Figure 1. Immunodetection of the InlA protein from L. monocytogenes at the cell surface of L. lactis strains. Arrows indicate the marked expression of this gene under the bacterial septum region.

As observed in Figure 1, the InlA protein was properly and efficiently displayed at the cell surface of the *L. lactis* strains. Accentuated expression of the *inlA* gene was situated specifically under bacterial septum region (Figure 1). Such findings reinforce studies conducted by Lecuit et al. (5), which observed expression of internalin at a similar level on the surface of noninvasive bacterial strains of *Listeria innocua* and *Staphylococcus epidermidis* transformed with plasmid harboring the *inlA* gene under the control of the *spa* gene promoter.

Interestingly, our findings suggest that transforming *L. lactis* cells carrying the *inlA* promoter and gene from *L. monocytogenes* appear to be driving normal cell divisions (Figure 1).

Bacterial growth is an orderly increase in the quantity of cellular constituents and in most bacteria, it involves an increase in cell mass and the number of ribosomes, duplication of the bacterial chromosome, synthesis of the new cell wall and plasma membrane, partitioning of the two chromosomes, septum formation and cell division. As reported by Harry (8), septum formation is driven by a complex of several proteins, which localize to the division site prior to septal in-growth. The spatial and temporal regulation of cell division must be maintained and coordinated with chromosome replication to ensure equal partitioning of chromosomes into daughter cells (8). Therefore, further studies must be conducted in order to clarify the influence of exogenous gene expression on the process of cellular division in transforming bacterial strains.

In the present report, we describe relevant insights into the expression of the internalin-A protein in lactic bacteria (Figure 1). It is already well-established that InlA is necessary for the invasion of epithelial cells and that it is sufficient for reconstituting invasion when expressed in the non-pathogenic and noninvasive species *Listeria innocua* (5). Recently, Guimaraes et al. (9) showed that internalin-expressing *L. lactis* strains are able to invade the small intestine of guinea pigs and deliver DNA into mammalian epithelial cells. In this respect, we can also speculate that the findings presented in this report provide new evidence of powerful approaches using *L. lactis* cells for biotechnological purposes.

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