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Adaptive response of *Lactobacillus sakei* 23K during growth in the presence of meat extracts: A proteomic approach

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ABSTRACT

Lactobacillus sakei is a lactic acid bacterium mainly found in meat and meat products. In order to understand the factors favoring its adaptation to meat matrix, growth parameters and survival of the strain *L. sakei* 23K in the presence of sarcoplasmic or myofibrillar extracts were assessed. Cytosolic proteins putatively involved in the response of this strain to meat proteins were determined using 2D electrophoresis and the significantly regulated proteins were identified by Maldi Tof-MS analyses. From the 31 differentially expressed spots, 16 occurred in the presence of myofibrillar extract while 6 proteins were modulated by the sarcoplasmic extract. Two dipeptidases were overexpressed in the presence of sarcoplasmic proteins, in correlation to the protein degradation patterns obtained by SDS-PAGE. In the presence of the myofibrillar extract, *L. sakei* 23K overexpressed proteins related to energy and pyrimidine metabolism as well as ala- and tyr-tRNA synthetases, involved in translation, while others corresponding to general stress response, pyrimidine, vitamin and cofactor biosynthesis were down-regulated. The supplementary nutrients furnished by meat extracts modulated the overexpression of proteins related to translation, peptide/amino acid metabolism and energy production while the stress proteins were under regulated. The results obtained here suggest that meat proteins would not represent a stress environment *per se* for *L. sakei* 23K in contrast to the harsh conditions during meat processing. This study has extended the understanding of the molecular responses and growth mechanisms of *L. sakei* 23K in the presence of meat proteins. The transference of genomic information into useful biological insight is an important step for the selection of well-adapted strains for the achievement of high-quality fermented products.

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1. Introduction

Lactobacillus sakei is a ubiquitous lactic acid bacterium commonly associated with the food environment. Although this organism was isolated from various fermented vegetables such as sauerkraut and kimchi (Choi et al., 2003; Vogel et al., 1993) and fish products (Najjari et al., 2008), it was mostly isolated from the meat environment (Chenoll et al., 2007; Fontana et al., 2005, 2006). *L. sakei* represents the major population of many fermented meat products and vacuum-packaged raw meats and is recognized as an important component of the starter cultures used for fermented sausage production in Western Europe (Vignolo et al., 2010). In addition, *L. sakei* has been found to produce bacteriocins and consequently it has been used as bioprotective culture to preserve fresh and processed meat and fish (Castellano et al., 2008; Katla et al., 2001). More recently, it was shown that this species is also a transient member of the human gastrointestinal microbiota (Chiaramonte et al., 2009; Dal Bello et al., 2003).

Due to the ubiquitous occurrence, *L. sakei* displays notable differences in physiological and biochemical properties compared to other lactobacilli (Chaillou et al., 2005). The prevalence of *L. sakei* in a variety of habitats indicates its potential to adapt and/or to compete in such different ecosystems. Ecologically, meat represents a diverse and changing environment that influences the growth potential of different bacterial species during storage (Labadie, 1999). As bacterial substrate, meat represents a relatively poor source of carbohydrates but an important source of proteins. During ageing, endogenous proteolytic enzymes still act releasing amino acids from meat proteins. Consistently, *L. sakei* adaptation to meat has led to a lack of bacterial biosynthetic pathways for amino acid synthesis; this species being auxotrophic for all amino acids except for aspartic and glutamic acids (Champomier-Vergès et al., 2002a,b). Moreover, this species has psychrotrophic and osmotolerant properties and is able to grow at low temperatures as well as in 10% NaCl concentrations; these

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features being associated with the presence of genes coding for stress response proteins in *L. sakei* genome (Marceau et al., 2004). The analysis of *L. sakei* 23K genome revealed potential survival strategies as well as metabolic properties enabling it to effectively compete in the raw-meat environment (Chaillou et al., 2005). It has been argued that niche-specific genes showing greater expression in a particular ecosystem are more likely to contribute to better ecological performance than those equally expressed in a range of environments. A transcriptomic approach dealing with the detection of *L. sakei* 23K genes specifically induced during meat fermentation has been reported by Hüfner et al. (2007). As consequence, intraspecies genomic diversity may be required for successful adaptation in this complex habitat; the extent of *L. sakei* intraspecies variation has been recently reported (Chaillou et al., 2009).

Despite of the huge amount of DNA information provided by the completed genome sequencing projects, the biological function of the proteins encoded by the detected genes still remain to be revealed. Indeed as the next step in the post genomic era, proteomics focuses on the functionality of these genes contributing to establish the connection between genome sequences and their biological role. The introduction of bioinformatics to extract information from these genetic data constitutes a key tool of the post genomic era. In combination with transcriptional profiling expression, proteomics provides access to interesting candidate genes and proteins that can be further characterized. For lactic acid bacteria (LAB), cold adaptation and salt stress challenges have been illuminated by this approach (Cotter and Hill, 2003; van de Guchte et al., 2002). Moreover, a proteomic approach has been undertaken to elucidate adaptation mechanisms of *L. sakei* 23K to the fermented sausage environment by studying various stress factors such as high NaCl concentrations or cold temperatures (Champomier-Vergès et al., 2002a).

The knowledge of bacterial response to a specific environment is of paramount importance in the selection of the most appropriate functional starter cultures. The aim of this work was to evaluate the response of *L. sakei* 23K during growth on meat sarcoplasmic and myofibrillar proteins to better understand their impact on bacterial adaptation to the meat environment by using a proteomic approach.

2. Materials and methods

2.1. Bacterial strain and culture conditions

Lactobacillus sakei 23K, originally isolated from a French sausage, and plasmid cured is used as model strain (Berthier et al., 1996). A single colony was grown in MRS broth at 30 °C for 24 h, transferred to a chemical defined medium (CDM) (Lauret et al., 1996) and then grown at 30 °C for 16 h. This subculture was used to inoculate 250 mL of CDM containing sarcoplasmic (S-CDM) or myofibrillar (M-CDM) extracts at an initial $OD_{600} = 0.1$. A culture grown in CDM without meat extracts was used as control (C).

2.2. Meat protein preparation

Ten grams of meat (*Longissimus dorsi*) was homogenized with sterile phosphate buffer 20 mM (pH 6.0), 1:10 (w/vol) in a laboratory blender (Stomacher 400 London, UK) for 2 cycles of 4 min each. The supernatant obtained after centrifugation (10,000 g, for 20 min at 4 °C) containing the soluble fraction was filter sterilized (Steritop GP, BIOPORE, Buenos Aires, Argentina) and constituted the sarcoplasmic extract. Myofibrillar proteins were extracted from the pellet using a high-ion strength phosphate buffer (0.1 N Na_2HPO_4–NaH_2PO_4; 0.7 M KI; pH 6.0 containing 0.2 g/L sodium azyde) as reported previously (Sanz et al., 1999). Separately, both meat extracts were incubated at 30 °C during 5 days to allow endogen proteolysis to occur and then lyophilized and stored at 4 °C until used. The mixture of peptides and proteins obtained was independently used for CDM supplementation. Sarcoplasmic and myofibrillar lyophilized extracts were added to CDM medium at 0.19 g/L and 0.075 g/L of protein final concentration, respectively.

2.3. Bacterial growth and survival

Growth of *L. sakei* 23K cultured microaerobically in CDM at 30 °C during 72 h was followed by measuring OD_{600} and cell viability. Growth rates (μ) were estimated from the growth curve by fitting the data to the Eq. (1):

$$\mu = \text{Ln}N_t – \text{Ln}\ N_0 / \Delta t \tag{1}$$

μ: growth rate.

N_t and N_0 are the cell densities at time t_n and time zero on exponential phase, respectively.

For bacterial enumeration, decimal dilutions were prepared, plated on MRS agar (Merck, Darmstadt, Germany) and incubated at 30 °C for 48 h. Sample pH was determined by using a Metrohn 692 pH/Ion Meter (Metrohn Ltd, Herisau, Switzerland). Three independent cultures were used for each condition.

2.4. Sample preparation and SDS-PAGE

Cultures of 0 and 72 h were centrifuged (10,000 *g* for 15 min at 4 °C) and supernatants were subjected to protein precipitation with cold acetone (MERCK, Darmstadt, Germany) (1:3 v/v) and stored overnight at −20 °C. Protein pellet was separated by centrifugation (10,000 *g*, for 10 min at 4 °C), air-dried and solubilized with sample buffer containing glycerol, SDS, β-mercaptoethanol (SIGMA Chemical CO, St. Louis, MO, USA), 0.5 M Tris–HCl pH 6.8 and brome phenol blue (BIORAD, Richmond, UK). These protein samples were subjected to SDS-PAGE analysis using a Mini Protean 3 gel Unit (BIORAD; Richmond, CA) on 12% (w/v) polyacrylamide gels (Laemmli, 1970). Wide range protein markers (from 212 to 6.5 kDa) were used as molecular weight standards (BioLabs Inc, Hitchin Herts, UK). Proteins were visualized by Biosafe colloidal Coomassie blue (BIORAD; Richmond, CA) according to the manufacturer's instructions.

2.5. Cell-free proteins extraction

Two hundred and fifty milliliter-cultures grown to mid-exponential phase ($OD_{600} \cong 0.8$, 4.5 h for C and S-CDM, and 7 h for M-CDM) were centrifuged (4500 *g* for 10 min at 22 °C) and washed twice in 0.1 M Tris–HCl buffer, pH 7.5, for 15 min, centrifuged and frozen at −80 °C until cell lysis. Bacterial pellets were resuspended in 5 mL of 1 M Tris–HCl buffer, pH 7.5, and cells were broken by a single pass through a cell disrupter (Basic Z; Constant Systems Ltd., Daventry, UK) at 2.5×10^5 Pa. Unbroken cells and cell debris were removed by centrifugation at 4500 *g* for 15 min at 4 °C. Membrane vesicles were discarded from the solution by ultracentrifugation at 50,000 *g* for 30 min at 4 °C. Protein concentration was estimated using the Bradford method (Bradford, 1976) according to the manufacturer's instructions (Coomassie Protein Assay Reagent; Pierce Biotechnology, Rockford, IL). Aliquots of 350 µg of proteins were stored at −80 °C until isoelectrofocusing assay.

2.6. 2D electrophoresis

Sample preparation and 2D electrophoresis gels were carried out according to Sánchez et al. (2005). Isoelectrofocusing strips of pH 4.0 to 7.0 (BIORAD), were rehydrated for 12 h at 50 V using the Protean IsoElectric Focusing Cell II (BIORAD) and then focused at 60,000 V/h. The second dimension was performed by SDS-PAGE on gels containing 12.5% polyacrylamide and carried out with a BIORAD Protean II xi cell. Proteins were resolved overnight at a constant current of 10 mA/gel at 4 °C. Gels were stained with Biosafe colloidal Coomassie blue (BIORAD) and scanned with an Image Scanner (Amersham Biosciences, Piscataway, NJ,

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