Food Microbiology 28 (2011) 597–604

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

The kinetics of the arginine deiminase pathway in the meat starter culture *Lactobacillus sakei* CTC 494 are pH-dependent

T. Rimaux [a], G. Vrancken [a], V. Pothakos [a], D. Maes [b], L. De Vuyst [a], F. Leroy [a,*]

[a] Research Group of Industrial Microbiology and Food Biotechnology (IMDO), Faculty of Sciences and Bio-engineering Sciences, Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium
[b] Department of Macromolecular Structure, Flanders Institute for Biotechnology (VIB), Vrije Universiteit Brussel, Pleinlaan 2, B-1050 Brussels, Belgium

ARTICLE INFO

Article history:
Received 15 September 2010
Received in revised form
26 November 2010
Accepted 26 November 2010
Available online 2 December 2010

Keywords:
Lactobacillus sakei
Arginine deiminase pathway
Modelling
Fermented sausage
Stationary phase
Acid stress

ABSTRACT

Lactobacillus sakei is frequently present as the dominant lactic acid bacterium in spontaneously fermented meat products, demonstrating its competitiveness in and adaptation to the meat environment. Since meat is generally low in carbohydrate content, the ability to utilize other energy sources to generate ATP, such as arginine via the arginine deiminase (ADI) pathway, represents a competitive benefit. In this study, the kinetics of growth and arginine conversion capabilities of *Lb. sakei* CTC 494 were analyzed, and a model was set up to describe the influence of pH on growth and arginine conversion. A series of *in vitro* batch fermentations using reconstituted MRS medium at different constant pH values (pH 4.50–pH 7.75) was performed. Arginine conversion through the ADI pathway, which was activated from the stationary growth phase on, resulted in the production of both citrulline and ornithine for all pH conditions tested. However, the pattern and the ratio of the end-products of the ADI pathway were influenced by pH. For certain pH values (between pH 5.0 and 6.5), a further conversion of citrulline into ornithine was found when all arginine was depleted. Characterization of responses of the ADI pathway in *Lb. sakei* CTC 494 to environmental conditions will allow a better understanding and control of this important starter culture in meat fermentations.

1. Introduction

Lactobacillus sakei, a facultative heterofermentative species of lactic acid bacteria (LAB), is frequently used as a starter culture for meat fermentation (Champomier-Vergès et al., 2001). Together with *Lactobacillus curvatus*, it is the most prevalent LAB species encountered in spontaneously fermented dry sausages (Chaillou et al., 2009; Hammes et al., 1990), which demonstrates its competitiveness in and adaptation to the meat environment (Chaillou et al., 2005; Leroy et al., 2006). This is also reflected in the fact that *Lb. sakei*, like most other LAB, lacks genes encoding biosynthetic capabilities for most amino acids, naturally present in the meat, but contains abundant genes encoding several amino acid and peptide transporters (Chaillou et al., 2005). Furthermore, whole-genome analysis of *Lb. sakei* 23K has shed light on some metabolic properties and potential survival strategies that enable *Lb. sakei* to effectively compete in the raw-meat environment (Chaillou et al., 2005), as well as during technological processing in which this species is exposed to several stress conditions, such as cold temperatures and the presence of curing agent (Hüfner et al., 2007). An improved oxygen tolerance, numerous proteins involved in adhesion, several genes for coping with salt and cold stress, and a myriad of transport systems for sugars, amino acids, peptides, nucleosides, and nucleotides give this species an advantage over several other LAB species for colonization of the meat environment (Chaillou et al., 2005; De Angelis and Gobbetti, 2004). Also, some strains of *Lb. sakei* possess the ability to produce bacteriocins, antibacterial peptides active against various related bacterial species, which prevents the growth of undesirable background bacteria and enhances competitiveness (Leroy et al., 2005a, 2005b).

Unlike other facultative heterofermentative lactobacilli, *Lb. sakei* and some strains of *Lactobacillus plantarum* utilize arginine via the arginine deiminase (ADI) pathway (Spano et al., 2007; Zúñiga et al., 2002a). Since meat is generally poor in carbohydrate content, the ability of meat LAB to utilize other energy sources, such as arginine, represents a benefit (Montel and Champomier, 1987). The ADI pathway, which is widely present among Bacteria and Archaea (Cunin et al., 1986), comprises three cytoplasmatic enzymes: (I) arginine deiminase, converting arginine into citrulline and ammonia; (II)

* Corresponding author. Tel.: +32 2 6293612; fax: +32 2 6292720.
E-mail address: fleroy@vub.ac.be (F. Leroy).

0740-0020/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.fm.2010.11.016

catabolic ornithine carbamoyl transferase, converting citrulline into carbamoyl phosphate and ornithine; and (III) carbamate kinase, converting carbamoyl phosphate into ammonia and ATP (Zúñiga et al., 2002b). In addition, a fourth transmembrane arginine/ornithine antiporter, catalyzing the stoichiometric and electroneutral exchange between extracellular arginine and intracellular ornithine, is present (Poolman et al., 1987). Thus, the ADI pathway results in the conversion of 1 mol of arginine into 1 mol of ornithine, with the concomitant production of 2 mol of ammonia and 1 mol of ATP (Fig. 1a).

As it is apparent from the end-products, the ADI pathway can serve several physiological functions. Firstly, the ammonia produced results in an alkalization of the cytoplasm, thereby keeping intracellular processes operational in acid stress conditions (Cotter and Hill, 2003; Konings et al., 1989; Ryan et al., 2009). Secondly, the supply of the intermediate carbamoyl phosphate, is essential for *de novo* pyrimidine biosynthesis (Kilstrup et al., 2005). Thirdly, the ADI pathway provides additional energy, which favours the growth of certain LAB species (Arena et al., 1999a, 1999b) or contributes to an improved cell survival in the stationary growth phase, as has been shown for *Lb. sakei* (Champomier Vergès et al., 1999). Moreover, the enzymes of the ADI pathway are less sensitive to low pH than the enzymes involved in glycolysis (De Angelis et al., 2002). This is of particular interest for acidifying LAB, since acid stress conditions are typically encountered during carbohydrate starvation (De Angelis and Gobbetti, 2004).

Several investigations concerning the ADI pathway in LAB have been performed at the molecular level (Hüfner et al., 2007; Zúñiga et al., 2002a). Also, the corresponding enzymes have been purified and characterized (De Angelis et al., 2002; Hiraoka et al., 1986; Liu et al., 1995). However, information on the kinetics of the enzymatic conversions is scarce (De Angelis et al., 2002; Poolman et al., 1987). Recently, a mathematical model has been constructed to describe the kinetics of the ADI pathway in *Lactobacillus fermentum* IMDO 130101, a sourdough isolate (Vrancken et al., 2009a). A detailed kinetic analysis of the ADI pathway in meat-associated LAB is lacking. The aim of the present study was to perform such an analysis for *Lb. sakei* CTC 494, a natural isolate from Spanish fermented sausage (Hugas et al., 1993) used as starter culture in various fermented sausage productions (Ravyts et al., 2008), as to evaluate the impact of environmental pH on arginine conversion. Also, it was envisaged to know in which bacterial growth phase arginine conversion precisely occurs and hence may contribute to competitiveness and/or survival.

2. Materials and methods

2.1. Strain, media, and growth conditions

Lb. sakei CTC 494 was the meat-associated LAB strain used throughout this study. The strain was stored at −80 °C in de Man–Rogosa–Sharpe (MRS) medium (Oxoid Ltd., Basingstoke, UK), supplemented with 25% (vol/vol) glycerol as a cryoprotectant. Reconstituted MRS medium, *i.e.* MRS medium (de Man et al., 1960) supplemented with 3 g/L of arginine and lacking a carbohydrate source, was used for the fermentation experiments. Solid MRS medium was prepared by adding 1.5% (wt/vol) agar (Oxoid) to the liquid medium. All chemicals were purchased from VWR International (Darmstadt, Germany).

2.2. Fermentation conditions

Batch fermentations were carried out in 15-L Biostat® C fermentors (Sartorius, Göttingen, Germany), containing 10 L of reconstituted MRS medium. The pH of the medium was adjusted to the desired value prior to sterilisation. The fermentor was sterilized *in situ* at 121 °C and 2.1 bar for 20 min. The fermentation temperature was kept at 30 °C. During fermentation the pH of the medium was kept constant through automatic addition of a 10 M NaOH and a 5 M HCl solution to the fermentation medium. The stirring speed was fixed at 100 rpm to keep the medium homogeneous. Temperature, pH, and agitation were controlled on line (Micro MFCS for Windows NT software, Sartorius). The inoculum was prepared through three subcultures of the strain in reconstituted MRS medium for 12 h each. The first two subcultures were carried out in 10 mL of reconstituted MRS medium, the third subculture was carried out in 100 mL of reconstituted MRS medium.

2.3. Fermentation experiments

Firstly, two *Lb. sakei* CTC 494 fermentations without pH control were carried out, using reconstituted MRS medium, each with a different initial pH (one starting at pH 5.00 and one at pH 6.50), to assess the influence of pH on the ADI pathway. Secondly, to study the influence of the initial concentration of arginine on the kinetics of arginine conversion, fermentations in reconstituted MRS medium with 1, 2, and 3 g/L of added arginine were carried out at a constant pH of 6.50. Thirdly, to investigate the influence of pH on both growth and ADI activity of *Lb. sakei* CTC 494, a series of

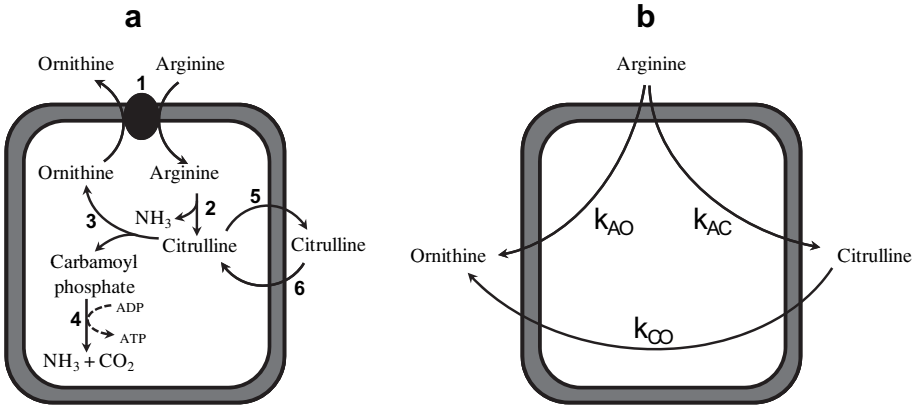

Fig. 1. (a) Metabolic scheme representing the steps involved in the ADI pathway (1: arginine/ornithine antiporter; 2: arginine deiminase; 3: ornithine transcarbamoylase; 4: carbamate kinase; 5: unknown mechanism for citrulline excretion; 6: unknown mechanism for uptake and further conversion of citrulline) and (b) conceptual model to simulate the operation of the ADI pathway in *Lactobacillus sakei* CTC 494 (k_{AO}: the rate of arginine conversion into ornithine; k_{AC}: the rate of arginine conversion into citrulline; k_{CO}: the rate of citrulline conversion into ornithine).