EXPLORING BACTERIAL METABOLISM OF NON-GLUCOSE ENERGY SOURCES IN FERMENTED MEATS

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Abstract – Several bacteria that are predominantly associated with meat fermentation processes are able to metabolize arginine and nucleosides, which are naturally occurring non-glucose energy sources in meat. All investigated *Lactobacillus sakei* strains in this study displayed arginine deiminase activity, although strain differences occurred with respect to the onset and intensity of the pathway. The capacity of coagulase-negative staphylococci to metabolize arginine was less uniformly present among species and strains. For all strains of *L. sakei* and for all coagulase-negative staphylococci, inosine and adenosine consumption was found, albeit with considerable strain-related differences in efficiency. Although arginine and nucleosides may contribute to the dominance of certain meat bacteria, the observed heterogeneity in the associated metabolisms is expected to play a role, for instance in the establishment of microbiota in spontaneously fermented products and in the selection of appropriate starter cultures.

Key Words – Arginine, Adenosine, Coagulase-negative staphylococci, Inosine, *Lactobacillus sakei*

I. INTRODUCTION

Whereas *Lactobacillus* sakei is frequently cited as the most competitive bacterium in fermented sausages, coagulase-negative staphylococci also may form an important subpopulation [1, 2]. The ability of *L. sakei* and some coagulase-negative staphylococci to use alternative energy sources in meat, such as nucleosides and arginine, could help to partially explain their typical occurrence and competitiveness in fermented sausages.

With respect to nucleosides, which can reach a concentration twice as high as the concentration of glucose in meat, conversion of the ribose moiety into mixed acids has been described for *L. sakei* [3]. With the exception of some insights into adenosine-related metabolism by the coagulase-positive *Staphylococcus aureus* [4], still little is known about the role of nucleosides in the competitive behaviour of staphylococci.

The use of arginine through the arginine deiminase pathway is an interesting property as well, as this pathway results in the production of extra ATP and ammonia, which increases the competitiveness and acid tolerance of the producing strain, respectively. Whereas the relevance of arginine deiminase activity by *L. sakei* in meat has been documented [5, 6], more information is needed on the situation in coagulase-negative staphylococci. Moreover, in addition to an arginine deiminase pathway encoded on the core genome, an additional arginine catabolic mobile element (ACME) may be present in staphylococci [7].

The aim of the present study was to explore the ability of major meat-associated bacteria to use alternative, non-glucose energy sources present in meat, in particular nucleosides and arginine.

II. MATERIALS AND METHODS

Microorganisms and media

Throughout this study, 15 strains of *L. sakei* from a meat or vegetal origin and 61 strains of coagulase-negative staphylococci isolated from fermented sausage, milk, or bovine teat apex skin, were used. For the screening and fermentation experiments, a previously described meat simulation medium (MSM) [8] was used, but without glucose. Depending on the experiment, the MSM was supplemented with 3 g/l of inosine (11.2 mM), adenosine (11.2 mM), or arginine (17.2 mM). Growth of coagulase-negative staphylcocci was followed on mannitol salt agar (MSA), whereas *L. sakei* growth was followed on de Man-Rogosa-Sharpe (MRS) medium.

Phenotypic screening and fermentations

All strains were subjected to screening experiments in 10 ml of MSM, as to investigate their ability to utilize alternative energy sources at 30°C for 48 h. The kinetics of nucleoside metabolism and of the arginine deiminase pathway were studied for selected strains in 10 liters of MSM at 30°C and at different constant pH values. Concentrations of inosine, adenosine, hypoxanthine, and adenine were determined using high-performance liquid chromatography (HPLC) with UV detection [3]. Concentrations of arginine, citrulline, and ornithine were determined through HPLC coupled to mass spectrometry (MS) [5].

Genotypic screening

DNA of the coagulase-negative staphylococci was isolated from overnight cultures in brain heart infusion at 30 °C. Primers targeting the native *arcA* gene were designed based on sequences of the native *arcA* gene of *S. carnosus* TM300, *S. epidermidis* RP62, and *S. haemolyticus* JCSC1435 (www.ncbi.nlm.nih.gov/gene). For the detection of the ACME-associated *arcA* gene in coagulase-negative staphylococci, previously designed primers were used [7]. The presence of PCR products and their sizes were controlled on a 1.0 -% (m/v) agarose gel.

III. RESULTS AND DISCUSSION

Nucleoside metabolism

A screening of 15 *L. sakei* strains revealed that inosine and adenosine could be used as energy sources by all strains (results not shown). This resulted in the production of a mixture of acetic acid, formic acid, and ethanol from ribose, while the nucleobase (hypoxanthine and adenine in the case of fermentations with inosine and adenosine, respectively) was excreted into the medium stoichiometrically. At pH 5.0, lactic acid was produced too. Hence, nucleoside catabolism occurred as a mixed-acid fermentation in a pH range relevant for sausage fermentation [3].

All of the 61 investigated coagulase-negative staphylococci possessed the capacity to metabolize adenosine and inosine, but considerable variations in efficiency were found between strains (Table 1).

Arginine metabolism

Arginine was metabolized into citrulline and ornithine by all 15 tested *L. sakei* strains (results not shown), supporting the fact that arginine deiminase is a common feature for this species [5]. A further pH-dependent conversion of citrulline into ornithine was found when all arginine was depleted, probably involving a citrulline/ornithine antiporter [9]. Moreover, the expression of the arginine deiminase pathway in *L. sakei* is known to be pH-dependent, but also depends on the growth phase in a strain-dependent manner [10]. Indeed, the highest relative gene expression level is to be found in the end exponential growth phase in the case of *L. sakei* CTC 494, but in the mid-exponential growth phase for *L. sakei* 23K.

Table 1 Conversion of inosine (11 mM) into hypoxanthine (Hyp; +, 1-7 mM; ++, > 7 mM) and of adenosine (11 mM) into adenine (Ade: +, 1-7 mM; ++, > 7 mM), and conversion of arginine (17 mM) into citrulline and ornithine (Orn: +, 5-10 mM; ++, > 10 mM) by coagulase-negative staphylococci in MSM after 48 h of incubation

	Conversion ability		
Species (strains)	Inosine	Adenosine	Arginine
S. arlettae (2)	++ (1), + (1)	++ (1), + (1)	++ (1), - (1)
S. auricularis (1)	+ (1)	+ (1)	+ (1)
S. capitis (1)	+ (1)	+ (1)	- (1)
S. carnosus (6)	++ (2), + (4)	+ (6)	++ (6)
S. chromogenes (2)	+ (2)	+ (2)	++ (1), - (1)
S. cohnii (3)	+ (3)	++ (1), + (2)	- (3)
S. devriesei (2)	+ (2)	++ (1), + (1)	- (2)
S. epidermidis (6)	+ (6)	++ (2), + (4)	++ (6)
S. equorum (2)	+ (2)	++ (1), + (1)	- (2)
S. fleuretti (1)	+ (1)	+ (1)	- (1)
S. haemolyticus (5)	++ (2), + (3)	+ (5)	++ (3), + (1), - (1)
S. pasteuri (2)	+ (2)	+ (2)	++ (2)
S. saprophyticus (8)	+ (8)	++ (7), + (1)	++ (2), + (1), - (5)
S. succinus (3)	+ (3)	+ (3)	- (3)
S. sciuri (3)	+ (3)	++ (1), + (2)	- (3)
S. warneri (3)	+ (3)	+ (3)	++ (2), - (1)
S. xylosus (11)	++ (2), + (9)	+ (11)	++ (2), + (1), - (8)

Within the group of coagulase-negative staphylococci, the capacity to catabolize arginine via the arginine deiminase pathway varied between species and strains (Table 1). Strains that belonged to *S. carnosus* and *S. epidermidis* all displayed arginine deiminase activity, which was confirmed by the common presence of an *arcA* gene. For only three strains (*S. epidermidis* ATCC 12228, *S. xylosus* 3PA6, and *S. chromogenes* G222), an ACME was found in addition to the native *arcA* gene on the core genome. However, arginine deiminase activity was not a common feature of all coagulase-negative staphylococci, for instance being absent in the tested strains of *S. equorum* and *S. succinus*. During monoculture fermentations in MSM, strains of *S. carnosus* catabolized arginine through more efficiently than strains of *S. xylosus* and *S. epidermidis* (results not shown). Accelerated arginine deiminase activity was found for an ACME-positive *S. epidermidis* strain (ATCC 12228), when compared to an ACME-negative strain of the same species (2S7-4) (Fig. 1).

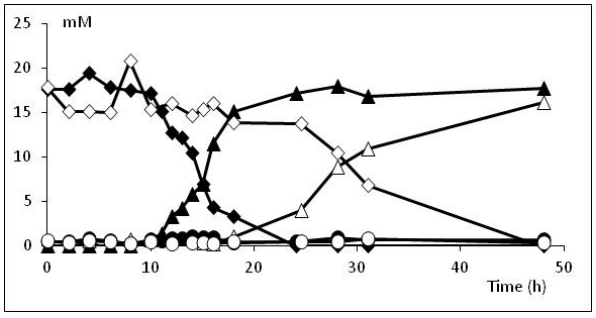

Figure 1. Extracellular concentrations (in mM) of arginine (diamonds), citrulline (circles), and ornithine (triangles) for *S. epidermidis* 2S7-4 (open symbols) and *S. epidermidis* ATCC 12228 (closed symbols) in modified MSM containing 17.2 mM of arginine at 30°C and a constant pH value of 5.8.

IV. CONCLUSION

The results obtained in the present study show that the ability of *L. sakei* and coagulase-negative staphylococci to use meat-associated, alternative energy sources, such as nucleosides and arginine, was rather common. This ability is hence expected to play a role in the adaptation to the ecological niche, *i.e.* the fermented meat matrix. However, the capacity of coagulase-negative staphylococci to catabolise arginine through the arginine deiminase pathway is a trait which can vary considerably on both species and strain level. This will have to be contrasted with the arginine utilization by *L. sakei*, also displaying strain variability. An improved understanding of the use of alternative energy sources by meat bacteria may provide an explanation for the dominance of some of the more prevailing species and even of particular strains during meat fermentations. This opens perspectives to better understand bacterial community dynamics in fermented meats and, possibly, to improve the selection of appropriate meat starter cultures.

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