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The use of nucleosides and arginine as alternative energy sources by coagulase-negative staphylococci in view of meat fermentation

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ABSTRACT

The ability of coagulase-negative staphylococci (CNS) to use alternative energy sources in meat may partially explain their occurrence in fermented meats. Of 61 CNS strains tested, all metabolized adenosine and inosine in a meat simulation medium (MSM). The ability to catabolize arginine via the arginine deiminase (ADI) pathway varied between strains. All tested strains of *Staphylococcus carnosus* and *Staphylococcus epidermidis* possessed an *arcA* gene and showed ADI activity, whereas other species, such as *Staphylococcus equorum* and *Staphylococcus succinus*, did not. Arginine catabolic mobile elements (ACME), as in the positive control *S. epidermidis* ATCC 12228, were uncommon and only found in *Staphylococcus xylosus* 3PA6 (sausage isolate) and *Staphylococcus chromogenes* G222 (teat apex isolate). Monoculture experiments were performed in MSM with *S. carnosus* 833 and SS3-4, *S. xylosus* G211, and *S. epidermidis* ATCC 12228 and 2S7-4. At all pH values tested (5.3, 5.8, and 6.5), the strains of *S. carnosus* catabolized arginine faster than the strains of *S. xylosus* and *S. epidermidis*. Only at pH 6.5 could a low ADI activity be found for *S. xylosus* G211. Increased ADI activity occurred in the case of the ACME-positive *S. epidermidis* ATCC 12228, when compared to the ACME-negative *S. epidermidis* 2S7-4.

1. Introduction

Traditional meat fermentations are usually driven by the spontaneous growth of a desirable indigenous microbiota, with lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS) as the most dominant bacterial groups (Ravyts et al., 2012). This is in contrast with industrial processing, where defined starter cultures of LAB and CNS are used (Leroy et al., 2006; Talon et al., 2007). Whereas the initial LAB communities are rapidly converging to a clear governance by mostly *Lactobacillus sakei*, the CNS species diversity is less predictable (Papamanoli et al., 2003; Comi et al., 2005; Leroy et al., 2006; Talon et al., 2007; Janssens et al., 2012; Ravyts et al., 2012). Although *Staphylococcus xylosus* is frequently dominant in traditionally fermented sausages (Cocolin et al., 2001; Blaiotta et al., 2004; Comi et al., 2005; Talon et al., 2007; Ravyts et al., 2010), mostly followed by *Staphylococcus equorum* and *Staphylococcus saprophyticus* (Blaiotta et al., 2004; Talon et al., 2007), many other species of staphylococci may occur. These include, for instance, *Staphylococcus carnosus*, *Staphylococcus cohnii*, *Staphylococcus epidermidis*, *Staphylococcus haemolyticus*, *Staphylococcus pasteuri*, *Staphylococcus sciuri*, *Staphylococcus succinus*, and *Staphylococcus warneri* (Blaiotta et al., 2004; Mauriello et al., 2004; Iacumin et al., 2006; Garriga and Aymerich, 2007; Lebert et al., 2007; Talon et al., 2007; Janssens et al., 2012; Ravyts et al., 2012). The establishment of a specific CNS community is nevertheless of technological importance. Indeed, the contribution to colour stabilization, antioxidant activity, and aroma generation during meat fermentation may differ between CNS species and even on strain level (Ravyts et al., 2010; Janssens et al., 2013). The overall variability in CNS species diversity between different meat fermentations may have to do with a variable CNS microbiota in the raw materials, differing per producer, batch, and type of meat (Janssens et al., 2012). In addition, species-dependent adaptation to different unfavourable environmental conditions may occur, including disparities in the tolerance to acid stress (Janssens et al., 2013). Also, the capability to use alternative energy sources present in meat, when carbohydrate levels are low, may play a role. In fermented meats, such potential sources include nucleosides and arginine, which are known to be metabolized by *L. sakei* (Chaillou et al., 2005; Rimaux et al., 2011a,b).

Nucleosides, such as adenosine and inosine, are formed due to the ATP breakdown during *post-mortem* reactions in muscle. Their pentose moiety may be metabolized when other carbon and energy sources are depleted (Tozzi et al., 2006; Beck and O'Donovan, 2008). Nucleosides first enter the cell through

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Table 1
Conversion of inosine (11 mM) into hypoxanthine, of adenosine (11 mM) into adenine, and of arginine (17 mM) into citrulline and ornithine in MSM after 48 h of incubation [+, intermediate conversion (2–7 mM of residual inosine or adenosine; 4–14 mM of residual arginine); ++, near depletion of the substrate (≤1 mM of residual inosine or adenosine; ≤3 mM of residual arginine)], extended with a genotypic screening with the appropriate PCR primers for the presence of a native *arc*A gene located on the core chromosome (n-*arc*A) and an *arc*A gene located on an arginine catabolic mobile element (ACME-*arc*A).

Species	Strain	Origin	Conversion ability			Gene presence	
			Inosine	Adenosine	Arginine	n-*arc*A	ACME-*arc*A
S. arlettae	G130	Teat apex skin	++	++	++	+	−
	G238	Teat apex skin	+	+	−	−	−
S. auricularis	G162	Teat apex skin	+	+	+	+	−
S. capitis	G265	Teat apex skin	+	+	−	−	−
S. carnosus	833	Fermented sausage	+	+	++	+	−
	10P1-3	Meat starter culture	+	+	++	+	−
	carnimp	Fermented sausage	+	+	++	+	−
	F4P1	Fermented sausage	++	+	++	+	−
	SS3-4	Fermented sausage	+	+	++	+	−
	SS7-2	Fermented sausage	++	+	++	+	−
S. chromogenes	G222	Teat apex skin	+	+	++	+	+
	i160	Teat apex skin	+	+	−	+	−
S. cohnii	2.63	Milk	+	+	−	−	−
	G166	Teat apex skin	+	+	−	−	−
	G207	Teat apex skin	+	+	−	−	−
S. devriesei	G194	Teat apex skin	+	++	+	+	−
	G246	Teat apex skin	+	+	−	−	−
S. epidermidis	2S7-4	Fermented sausage	+	+	++	+	−
	ATCC 12228	Unknown	+	+	++	+	+
	G153	Teat apex skin	+	++	++	+	−
	G262	Teat apex skin	+	++	++	+	−
	i162	Teat apex skin	+	+	+	+	−
	αSg3	Fermented sausage	+	+	++	+	−
S. equorum	2.55	Milk	+	++	−	−	−
	G101	Teat apex skin	+	+	−	−	−
S. fleuretti	2.05	Milk	+	+	−	−	−
S. haemolyticus	12S24-4	Fermented sausage	++	+	++	+	−
	G110	Teat apex skin	+	+	−	+	−
	G191	Teat apex skin	+	+	++	+	−
	SB1-6	Fermented sausage	++	+	++	+	−
	αS1-1	Fermented sausage	+	+	++	+	−
S. pasteuri	αS3-13	Fermented sausage	+	+	++	−	−
	αS3-14	Fermented sausage	+	+	++	+	−
S. saprophyticus	3.02	Milk	+	+	−	−	−
	DFL-12	Fermented sausage	+	+	−	−	−
	FPP1	Fermented sausage	+	+	++	+	−
	FPS1	Fermented sausage	+	+	+	−	−
	G089	Teat apex skin	+	+	++	+	−
	G131	Teat apex skin	+	+	−	−	−
	G243	Teat apex skin	+	++	−	−	−
	αSG1	Fermented sausage	+	+	++	−	−
S. succinus	3PA1	Fermented sausage	+	+	−	−	−
	4pb1	Fermented sausage	+	+	−	−	−
	SS0-7	Fermented sausage	+	+	−	−	−
S. sciuri	G160	Teat apex skin	+	+	−	+	−
	G173	Teat apex skin	+	++	−	−	−
	I20-1	Fermented sausage	+	+	−	−	−
S. warneri	2.06	Milk	+	+	++	+	−
	2.25	Milk	+	+	++	+	−
	i184	Teat apex skin	+	+	−	−	−
S. xylosus	10P1-1	Meat starter culture	+	+	−	−	−
	2.15	Milk	+	+	−	−	−
	2.17	Milk	+	+	−	−	−
	2S7-2	Fermented sausage	+	+	+	−	−
	3PA3	Fermented sausage	+	+	−	+	−
	3PA6	Fermented sausage	+	+	−	+	+
	G211	Teat apex skin	++	+	++	+	−
	G223	Teat apex skin	+	+	−	−	−
	TP4	Fermented sausage	+	+	−	−	−
	W1-1	Fermented sausage	++	+	+	−	−
	xylimp	Fermented sausage	+	+	−	−	−

specific transporters, after which they are cleaved by nucleoside phosphorylases or nucleoside hydrolases, into ribose 1-phosphate or ribose, respectively, and their concomitant nucleobase (adenine or hypoxanthine). Prior to this reaction, intracellular adenosine can first be converted into inosine and ammonia by an adenosine deaminase (Chaillou et al., 2005; Kilstrup et al., 2005; Rimaux et al., 2011b). During fermentations of nucleosides with *L. sakei* CTC 494 as meat starter culture, the produced nucleobases are stoichiometrically excreted into the medium (Rimaux et al., 2011b). Whether CNS are readily able to catabolize nucleosides and use the pentose moiety in a meat context is yet unclear.