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Technological and safety characterization of coagulase-negative staphylococci from traditionally fermented sausages of Basilicata region (Southern Italy)

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ABSTRACT

Thirty-seven strains of coagulase-negative staphylococci, isolated from traditional fermented sausages of Basilicata region, were screened on the basis of their technological properties (nitrate reductase, proteolytic, lipolytic, amino acid-decarboxylase and antimicrobial activities) and for their ability to grow in the presence of different salt concentrations, temperatures and pH values, to elucidate their possible role during meat fermentation process.

All strains grew at 20 °C and 30 °C in all conditions, while at 10 °C, only few isolates showed a good growth at pH values ranging from 6 to 5.2.

Staphylococci strains had different enzymatic profiles. A significant level of proteolytic activity was observed in 75.6% of the strains and 23 staphylococci hydrolysed sarcoplasmatic proteins. Nitrate was reduced by 62% of the strains, while most of isolates (80.9%) were able to decarboxylate at least one of the tested amino acids.

This is a preliminary study focused on the selection of autochthonous starter cultures to standardize the production of fermented sausages, to preserve their organoleptic and sensory properties and to improve the quality of final product.

1. Introduction

Naturally fermented sausages are traditional Mediterranean products with a large diversity within the different regions (Aymerich, Martin, Garriga, & Hugas, 2003). In Italy, several fermented sausages are manufactured using traditional technologies without the addition of starter cultures and the ripening is carried out in rooms with lesser (o lower) control of temperature and relative humidity (RH) compared to that used in industrial scale production (Lebert et al., 2007; Parente, Greco, & Crudele, 2001). Traditional process favours the growth of autochthonous microflora, also known as "house-flora", which in turn influences flavour, texture, nutritional properties and safety of meat products (Martin, Colin, Aranda, Benito, & Cordoba, 2007). Nevertheless, it is not possible to ensure that the number and the strains of microorganisms present in the raw material will always be the same and behave in the same way. The use of starter cultures guarantees products with repeatable hygienic and organoleptic properties in a shorter ripening time. However, commercial starter cultures not always are able to compete with house flora, resulting in a losses of desirable sensory properties. Therefore, appropriate starter cultures should be selected from indigenous microorganisms, which are well adapted to meat environment and more competitive because of their specific metabolic capabilities (Leroy, Verluyten, & De Vuyst, 2006).

Coagulase-negative staphylococci (CNS) and lactic acid bacteria (LAB) are the most important microorganisms used as starter cultures in meat fermentations. The organoleptic properties of fermented sausages, in fact, are due to the metabolic activities of these microorganisms together to the activity of endogenous meat enzymes (Martin et al., 2007). Several studies suggested that *Staphylococcus* species, rather than LAB, play an important role in the development of sensory properties (flavour, texture, color) of fermented sausages by reduction of nitrates, proteolytic and lipolytic activities (Mauriello, Casaburi, Blaiotta, & Villani, 2004; Olesen, Meyer, & Stahnke, 2004; Tjener, Stahnke, Andersen, & Martinussen, 2004). Additionally, the ability of CNS to produce antimicrobial compounds may improve safety and shelf-life of sausages (Martin et al., 2007; Simonova et al., 2006). Safety of meat products also depends on the content of biogenic amines (BA) which requires the presence of amino acid-decarboxylating microorganisms, which are usually detected in fermented sausages during the fermentation process (Simonova et al., 2006). Although LAB and enterobacteria are the most important aminogenic microorganisms, amino acid-decarboxylase activity has been described in CNS from fermented sausages (Martin et al., 2006; Martuscelli, Crudele, Gardini, & Suzzi, 2000).

The CNS more frequently used as commercial starter cultures in dry sausage processing are *Staphylococcus* (*S.*) *xylosus*, *S. carnosus*,

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S. saprophyticus and *Kocuria* (*K.*) *varians*. The selection of CNS strains from the indigenous population, based on the most important technological criteria as well as on their biogenic amines production and antimicrobial properties, would allow to obtain starter cultures better adapted to sausages that help to preserve the typical characteristics of these products (Martin et al., 2007; Mauriello et al., 2004).

Knowledge of safety and technological properties of indigenous microflora is of practical relevance for improvement of starter culture technology and production of meat fermented products, as already evaluated in other studies (Aymerich et al., 2003; Comi et al., 2005; Drosinos et al., 2005; Fontana, Cocconcelli, & Vignolo, 2005; Lebert et al., 2007; Mauriello et al., 2004; Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003; Rantsiou et al., 2005).

The aim of this study was to investigate the technological properties and the safety aspects of CNS of fermented sausages to better understand their role during the fermentation process as well as to identify particular properties that may be relevant to use of the organisms in starter cultures.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Thirty-seven strains of coagulase-negative staphylococci (CNS) isolated from traditional fermented sausages of Basilicata region were used in this study (Table 1). Thirty-five strains were previously identified by phenotypic tests and molecular analyses (Blaiotta, Casaburi, & Villani, 2005; Blaiotta, Ercolini, Mauriello, Salzano, & Villani, 2004a; Blaiotta, Pannacchia, Ercolini, Moschetti, & Villani, 2003a; Blaiotta, Pannacchia, Parente, & Villani, 2003b; Blaiotta et al., 2004b), while the remaining two strains were identified in this study by molecular analyses. All staphylococci strains were subjected to the technological and safety characterization.

Table 1
Origin, source and identification method to classify CNS strains used in this study.

Species	Strains	Origin[a]	Source[b]	Identification method[c]	Reference
S. caseolyticus	DBPZ0231	DBDBAF	SoB	A (AY126157), B and C	Blaiotta et al. (2005, 2004b)
S. caseolyticus	DSM20597	DSM			
S. equorum	DBPZ0223	DBDBAF	SaB	B and C	Blaiotta et al. (2004a)
S. equorum	DBPZ0237	DBDBAF	SaB	A (AY227265)	Blaiotta et al. (2004b)
S. equorum	DBPZ0246	DBDBAF	SaB	A (AY126184), B and C	Blaiotta et al. (2005, 2004b)
S. equorum	DBPZ0247	DBDBAF	SoB	B and C	Blaiotta et al. (2004a)
S. equorum	DBPZ0241	DBDBAF	SaB	A (AY126199), B and C	Blaiotta et al. (2004b)
S. equorum	DBPZ0044	DBDBAF	SoB	A (AY126198), B and C	Blaiotta et al. (2004b)
S. equorum	DBPZ0248	DBDBAF	SaB	A (AY126297), B and C	Blaiotta et al. (2004b)
S. equorum	DSM20674	DSM			
S .pasteuri	DBPZ0208	DBDBAF	SaB	A (AY227267)	Blaiotta et al. (2004b)
S .pasteuri	DBPZ0206	DBDBAF	SoB	A (AY126212), B and C	Blaiotta et al. (2005, 2004b)
S .pasteuri	DSM10656	DSM			
S. pulvereri/vitulus	DBPZ0235	DBDBAF	SaB	A (AY227271)	Blaiotta et al. (2004b)
S. pulvereri/vitulus	DBPZ0054	DBDBAF	SaB	A (AY126218), B and C	Blaiotta et al. (2004b)
S. pulvereri/vitulus	DBPZ0032	DBDBAF	SoB	A (AY227281)	Blaiotta et al. (2004b)
S. pulvereri/vitulus	DBPZ0253	DBDBAF		A (FJ627042) and B	In this study
S. pulvereri/vitulus	DSM9931	DSM			
S. saprophyticus	DBPZ0034	DBDBAF	SoB	A (AY227269) and E	Blaiotta et al. (2004b)
S. saprophyticus	DBPZ0229	DBDBAF	SaB	A (AY227268), B and E	Blaiotta et al. (2005, 2004b)
S. saprophyticus	DSM20229	DSM			
S. warneri	DBPZ0236	DBDBAF	SaB	A (AY126245), B and C	Blaiotta et al. (2005, 2004b)
S. warneri	DBPZ0207	DBDBAF		A (FJ627041) and B	In this study
S. warneri	DSM20316	DSM			
S. succinus	DBPZ0251	DBDBAF	SaB	A (AY126240), B and C	Blaiotta et al. (2005, 2004b)
S. succinus	DBPZ0218	DBDBAF	SoB	A (AY227270)	Blaiotta et al. (2004b)
S. succinus	DSM14617	DSM			
S. xylosus	DBPZ0203	DBDBAF	SaB	A (AY227260), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0200	DBDBAF	SaB	A (AY227259), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0250	DBDBAF	SaB	A (AY126262) B and C	Blaiotta et al. (2005, 2004b)
S. xylosus	DBPZ0240	DBDBAF	SaB	A (AY227254), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0219	DBDBAF	SaB	A (AY227273), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0215	DBDBAF	SoB	A (AY227253), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0220	DBDBAF	SoB	A (AY227256), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0225	DBDBAF	SoB	A (AY126258), B and C	Blaiotta et al. (2005, 2004b)
S. xylosus	DBPZ0245	DBDBAF	SoB	A (AY227263) and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0224	DBDBAF	SoB	A (AY227264), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0232	DBDBAF	SaB	A (AY227277), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0222	DBDBAF	SoB	A (AY227257), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0204	DBDBAF	SaB	A (AY227261), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0214	DBDBAF	SoB	A (AY227262), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0254	DBDBAF	SaB	A (AY227255), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0226	DBDBAF	SoB	A (AY227258), B, C and D	Blaiotta et al. (2004b)
S. xylosus	DBPZ0042	DBDBAF	SaB	A (AY227274)	Blaiotta et al. (2004b)
S. xylosus	DSM20266	DSM			

[a] DBDBAF: Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata; DSM: Deutsche Sammlung von Mikroorganismen und Zelkulturen, Braunschweig, Germany.

[b] SoB: "Soppressata" from Basilicata region; SaB: Sausage from Basilicata region.

[c] A: 16S rDNA partial sequencing (GenBank Accession numbers; http://www.ncbi.nlm.nih.gov, Altschul et al., 1997); B: ISR-PCR described by Blaiotta et al. (2003a); C: PCR-DGGE approach described by Blaiotta et al. (2003a); D: species-specific PCR assays described by Blaiotta et al. (2003b); E: species-specific PCR assays described by Martineau et al. (2000).