Meat Science 93 (2013) 387–396

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Characterization of coagulase-negative staphylococci isolated from Spanish dry cured meat products

G. Landeta [a], J.A. Curiel [a], A.V. Carrascosa [b], R. Muñoz [a], B. de las Rivas [a,*]

[a] Laboratorio de Biotecnología Bacteriana, Instituto de Ciencia y Tecnología de Alimentos y Nutrición, ICTAN-CSIC, Juan de la Cierva 3, 28006 Madrid, Spain
[b] Departamento de Biotecnología y Microbiología de los Alimentos, Instituto de Investigación en Ciencias de la Alimentación, CIAL-CSIC, Nicolás Cabrera, 9, Campus de la Universidad Autónoma de Madrid, 28040 Madrid, Spain

ARTICLE INFO

Article history:
Received 14 February 2012
Received in revised form 6 September 2012
Accepted 13 September 2012

Keywords:
Coagulase-negative staphylococci
Starter culture
Fermented meat products
Dry-cured meat products
Technological properties
Safety-related properties

ABSTRACT

Technological and safety-related properties were analyzed in a coagulase-negative staphylococci (CNS) collection isolated from Spanish dry-cured meat products in order to use them as starter cultures. The highest nitrate reductase and proteolytic activity was showed by *Staphylococcus carnosus* and *Staphylococcus equorum*. Only a few strains were able to form biofilms and the presence of the *ica* gene was analyzed on them. In relation to antibiotic resistance, all *S. carnosus* and most of the *S. equorum* strains were sensitive to the antibiotics tested and the presence of the *blaZ* gene in the β-lactamic resistant strains was studied. Biogenic amines were produced by 25% of the strains analyzed being all the *S. carnosus* strains tyramine producers. Taking into account the studied properties, two *S. equorum* strains could be selected as adequate and safe potential starter cultures for the elaboration of meat products.

1. Introduction

The role of the microbiota in fermented meat products is fundamental in the final characteristics of these products, influencing product quality. In these meat products the microorganisms isolated more frequently are lactic acid bacteria (LAB) and coagulase-negative staphylococci (CNS). LAB are responsible for the rapid fermentation of carbohydrates leading to a decrease in pH with a consequent loss of water in the meat; in addition, the presence of salt and other additives generates a very selective environment (Miralles, Flores, & Pérez-Martínez, 1996). On the other hand, CNS are one of the main microbial groups responsible of flavor (Casaburi et al., 2007). CNS participate in the development and stability of the red color through nitrate reductase activity that leads to the formation of nitrosomyoglobin. Furthermore, nitrate reduction produces nitrite that can limit lipid oxidation (Talon, Walter, Chartier, Barrier, & Montel, 1999). As the organoleptic properties of fermented and dry-cured meat products are influenced by the metabolic activities of these microorganisms their taxonomical identification at species level is of great interest. Identification methods based on biochemical test may sometimes be uncertain, complicated and time-consuming due to an increasing number of species that varied in few of the taxonomical characters. Moreover, new species of staphylococci are continually being described, making further identification tools necessary. In this sense, many molecular methods have been developed allowing the accurate identification of CNS from meat products (Blaiotta, Ercolini, Mauriello, Salzano, & Villani, 2004; Corbière Morot-Bizot, Talon, & Leroy, 2004; Landeta, Reverón, Carrascosa, de las Rivas, & Muñoz, 2011).

Traditional fermented and dry-cured meat processes favor the growth of autochthonous microbiota which in turn influences flavor, texture, nutritional properties and safety of the final products (Martín, 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; therefore, the use of starter cultures in fermented dry-cured meat elaboration ensures a fermentation and ripening process that can be carried out under controlled conditions. One of the main advantages of the use of starter cultures is that food poisoning and food spoilage microorganisms could be suppressed. Identification of technologically relevant bacteria is necessary to select strains to be employed as starter cultures. CNS are important microorganisms used as starter cultures in meat fermentations. In spite that nitrate reductase and catalase activity are considered the most important properties of CNS to make them eligible as starter cultures for fermented meat products (Mauriello, Casaburi, Blaiotta, & Villani, 2004), there are other relevant technological and safety properties that need to be analyzed.

The aims of this study were to taxonomically identify, and to analyze several activities relevant for their use as starter culture (such as nitrate reductase, catalase, proteolitic, and lipolitic activities, as well as the resistance to bile, biofilm formation, antibiotic susceptibility, and biogenic

* Corresponding author. Tel.: +34 91 5622900; fax: +34 91 5644853.
E-mail address: blanca.r@csic.es (B. de las Rivas).

0309-1740/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2012.09.019

amine formation), of a collection of CNS isolated from Spanish dry-cured meat products.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Seventy-one CNS strains were analyzed in this study. Fifty-one strains were isolated during the elaboration of Spanish dry-cured ham industrial processes (Carrascosa & Cornejo, 1991; Cornejo & Carrascosa, 1991) and were previously molecularly identified by sequencing their 16S rDNA (Landeta et al., 2011). The additional twenty CNS strains were isolated from dry-cured sausages (De las Rivas et al., 2008) and have been molecularly identified in this study by sequencing their 16S rDNA.

All the strains were grown in Brain Heart Infusion (BHI, Difco, France) medium at 37 °C and 150 rpm during 24 h under aerobic conditions. The strains were grown also on BHI agar plates (1.5%) at 37 °C under aerobic conditions.

2.2. DNA isolation

Bacterial chromosomal DNAs from CNS were isolated from overnight cultures using a protocol previously described (Sambrook, Fritsch, & Maniatis, 1989). DNA precipitates were resuspended in an appropriate volume of TE solution (10 mM Tris–HCl, pH 8.0; 1 mM EDTA).

2.3. Taxonomical identification of CNS strains

CNS strains were identified by PCR amplification and DNA sequencing of their 16S rDNA. The 16S rDNAs were PCR amplified using the eubacterial universal pair of primers 63f and 1387r previosly described by Marchesi et al. (1998) (Table 1). The 63f and 1387r primer combination generates an amplified product of 1.3 kb. PCR was performed in 25 μL amplification reaction mixture by using the following cycling parameters: 35 cycles of 1 min at 94 °C, 1 min at 50 °C and 1:30 min at 72 °C. Amplified products were resolved on 0.7% agarose gels. The amplification products were purified on QIAquick spin Columns (Quiagen, Germany) for direct sequencing. DNA sequencing was carried out by using an Abi Prism 377™ DNA sequencer (Applied Biosystems, USA). Sequence similarity searches were carried out by comparing to sequences from type strains included on the Ribosomal Database (http://rdp.cme.msu.edu).

2.4. Technological properties of the strains

2.4.1. Nitrate reductase assay

Nitrate reductase activity was determined as described previously (Miralles et al., 1996). The colorimetric assay performed was an adaptation of the method described by Smibert and Krieg (1994). Briefly, 1.5 mL of culture was grown during 24 h. After incubation, the culture was centrifuged and the cellular pellet was resuspended in induction buffer [Bactotryptone (Difco), 10 g/L; KNO_3, 1 g/L; cysteine, 1 g/L pH 7.0] to an $OD_{550} = 1$. A fraction of the cell suspension was used for the determination of the dry weight. Anaerobic induction of nitrate reductase activity was achieved by incubation for 2 h at 30 °C of 1 mL of the cell suspension in Eppendorf tubes covered with a layer of sterile mineral oil. As a control for the induction process, 1 mL of the cell suspension was kept on ice for 2 h. The cells were centrifuged and then permeabilized in 500 μL reaction buffer (50 mM KNO_3, 50 mM potassium phosphate, 100 mM NaCl, pH 7.0) by the addition of 30 μL of an acetone–toluene mixture (9:1, v/v). Then the tubes were vigorously shaken in a vortex for 3 min. The reaction was allowed to proceed for 30 min at 30 °C. A 100 μL aliquot of the samples was transferred to a new Eppendorf. Then, 2 mL water, 1 mL solution A [0.6 mg N-(1-Naphthyl)-ethylenediamine dihydrochloride in 100 mL 5 N acetic acid] and 1 mL solution B (0.8 g sulphanilic acid in 100 mL 5 N acetic acid) were added. Absorbance at 540 nm was measure against a control tube. Relative activity was calculated as the ratio: $OD_{540} \times mg^{-1}$ dry weight.

2.4.2. Catalase activity

The catalase activity was determined as described previously by Herrero, Mayo, González, and Suárez (1996). The strains were grown in BHI agar supplemented with 0.2% of glucose. The plates were incubated for 24 h at 30 °C; then, a drop of H_2O_2 was deposited over each colony. The appearance of bubbles indicated a positive response.

In this study a second assay was also used, the spectrophotometer assay previously described by Essid, Ben Ismail, Bel Hadj Ahmed, Ghedamsi, and Hassouna (2007). The strains were incubated in BHI to an $OD_{600} = 1$, and then 5 mL were centrifuged and the resulting pellet was mixed with 1.5 mL of 60 mM H_2O_2 in 20 mM phosphate buffer pH 7.0. Then, 200 μL were deposited in 96-well plates. Catalase activity was measured spectrophotometrically at 240 nm after 3 min of incubation at room temperature in a microplate reader (Synergy™HT, Biotek, EEUU). Results were expressed in arbitrary units (μmol of degraded H_2O_2/min/mL of cells with $OD_{600} = 1.0$).

Table 1
Primers used in this study.

Gene	Primer	Sequence[a]	Amplicon size (bp)	References
16S	63f	CAGGCCTAACACATGCAAGTC	1324	Marchesi et al. (1998)
	1387R	GGGCGGWGTGGTTACAAGGC		
ica	ica4f	TGGGATACTGAYAATGATTAC	568	Møretrø et al. (2003)
	ica2r	CCTCTGTCTGGGCTTGACCATG		
blaZ	blaZ-F	ACTTCAACACCTGCTGCTTTC	173	Resch, Nagel, and Hertel (2008)
	blaZ-R	TGACCACTTTTATCAGCAACC		
hdc	HIS1-F	GGNATNGTNWSNTAYGAYMGNGCNGA	372	De las Rivas, Marcobal, Carrascosa, and Muñoz (2006)
	HIS1-R	ATNGCDATNGCNSWCCANACNCCRTA		
tdc	TDC-F	TGGYTNGTNCCNCARACNAARCAYTA	825	De las Rivas et al. (2006)
	TDC-R	ACRTARTCNACCATRTTRAARTCNGG		
odc	PUT1-F	TWYMAYGCNGAYAARACNTAYTTYGT	1440	De las Rivas et al. (2006)
	PUT1-R	ACRCANAGNACNCCNGGNGGRTANGG		
	PUT2-F	ATHWGNTWYGGNAAYACNATHAARAA	624	De las Rivas et al. (2006)
	PUT2-R	GCNARNCCNCCRAAYTTNCCDATRTC		
ldc	CAD2-R	CAYRTNCCNGGNCAYAA	1185	De las Rivas et al. (2006)
	CAD2-F	GGDATNCCNGGNGGRTA		

[a] Y = C or T; R = A or G; W = A or T; S = C or G; M = A or C; D = A, G, or T; H = A, C, or T; B = C, G, ot T; N = A, C, G, or T.