Meat Science 95 (2013) 272–280

Contents lists available at SciVerse ScienceDirect

Meat Science

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

Technological and safety properties of lactic acid bacteria isolated from Spanish dry-cured sausages

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 6 February 2013
Received in revised form 10 May 2013
Accepted 14 May 2013

Keywords:
Lactic acid bacteria
Starter culture
Meat products
Biogenic amines
Biofilms
Antibiotic resistance

ABSTRACT

Technological and safety-related properties were analyzed in lactic acid bacteria isolated from Spanish dry-cured sausages in order to select them as starter cultures. In relation to technological properties, all the strains showed significative nitrate reductase activity; *Lactobacillus plantarum*, *Lactobacillus paracasei* and 52% of the *Enterococcus faecium* strains showed lipolytic activity and only *Lactobacillus sakei* strains (43%) were able to form biofilms. Related to safety aspects, *E. faecium* strains were the most resistant to antibiotics, whereas, *L. sakei* strains were the most sensitive. In relation to virulence factors, in the *E. faecium* strains analyzed, only the presence of *efaA* gene was detected. The analysis of biogenic amine production showed that most *E. faecium* strains and *L. sakei* Al-142 produced tyramine. In conclusion, *L. paracasei* Al-128 and *L. sakei* Al-143 strains possess the best properties to be selected as adequate and safe meat starter cultures.

1. Introduction

Elaboration of dry-cured sausages is a complex process characterized by deep changes on the main meat components, resulting in the production of specific taste and aroma. This process favors the growth of microorganisms which influence the sensorial and nutritional qualities, safety, and other characteristics of sausages. The fermentation of sausages involves the participation of mainly lactic acid bacteria (LAB), coagulase-negative staphylococci (CNS), and less importantly, yeast and molds (Ruiz-Moyano et al., 2009). The most frequent LAB species present in fermented sausage processes are *Lactobacillus sakei*, *Lactobacillus curvatus*, and *Lactobacillus plantarum*. However, in some instances, the contribution of enterococci seems to be also relevant (Comi et al., 2005).

LAB play an important role in the formation of lactic acid by fermenting carbohydrates, and hence could contribute to the safety of the process. Moreover, the lactic acid production contributes to the formation of the texture and in the acid taste. As a consequence of this process, the muscle protein coagulates, resulting in the slice ability, firmness and cohesiveness found in the final product. The development of curing color occurs also in acidic conditions when nitric oxide is produced from nitrite and can then react with myoglobin.

Today, modern meat industry has to ensure high quality, reduce variability and enhance organoleptic characteristics in sausages production, which are not feasible using spontaneous fermentation methods. Regarding such situations, the use of selected starter cultures is important to produce the desired flavor and aroma compounds and extend the shelf life of the final product. The indigenous LAB isolated from fermented meats are especially well adapted to the ecological conditions of specific meat fermentations, controlling the ripening processes and inhibiting the growth of spontaneous microorganisms. Autochthonous starter cultures are recommended to achieve the desired fermentation parameters specific for the product type. The identification of the autochthonous microbiota of traditional dry-fermented sausages is of great interest to standardize the fermentation process and for the selection of strains for their use as starter culture. LAB from the genus *Lactobacillus* in Europe, mainly *L. sakei*, *L. curvatus*, *L. plantarum*, and *Lactobacillus casei*, and from the genus *Pediococcus* in the USA represent the most common starter cultures used in the production of fermented sausages (Rantsiou et al., 2006).

Generally, LAB from fermented sausages have been traditionally identified based on simple physiological, biochemical, and chemotaxonomic methods. Although valuable from a practical point of view, results obtained by these methods are not always sufficient to characterize strains to species level, mainly within species from genera *Lactobacillus* (Ammor et al., 2005) and *Enterococcus* (Velasco et al., 2004).

Despite that the main role of LAB in fermented meat products is related with lactic acid production, LAB possess additional relevant characteristics that need to be taken into account in order to select them as starter cultures. The aims of the present study were

* 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 © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.meatsci.2013.05.019

to taxonomically identify a collection of LAB strains isolated from Spanish dry-cured sausages and analyze activities relevant for their use as starter cultures.

2. Materials and methods

2.1. Bacterial strains and growth conditions

Forty-six LAB strains were analyzed in this study. The strains were previously isolated from dry-cured sausages, and have been molecularly identified in this study by sequencing their 16S rDNA. Dry cured sausages were prepared in local meat factories using traditional techniques and without using microbial starter cultures. They were made with 70–80% of lean pork meat and 20–30% of pork back fat. Ten grams of each sausage sample was homogenized with 90 mL of a sterile solution of tryptone (0.3%) and NaCl (0.85%), for 2 min, in a Stomacher 400 Lab Blender (Seward Medical, London, UK). Ten fold dilutions were made in the same diluent.

All the strains were grown in MRS medium (Pronadisa, Spain) at 30 °C during 24 h under microaerobic conditions. The strains were grown also on MRS agar plates (1.5%) at 30 °C under microaerobic conditions.

2.2. DNA extraction

DNA extraction was carried out from overnight cultures as described by Sambrook, Fritsch, and 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 LAB strains

LAB 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 previously 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 using AmpliTaq Gold DNA polymerase (Roche) in 25 μL amplification reaction mixture by using the following cycling parameters: 10 min at 94 °C, 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 measured using a spectrophotometric method described previously (Miralles, Flores, & Pérez-Martínez, 1996), using MRS medium for bacterial growth. Briefly, an overnight culture (1.5 mL) was harvested by centrifugation and the pelleted cells were 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 incubating 1 mL of cell suspension in Eppendorf tubes covered with a layer of sterile

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		
ebpA	Ef1091F	CCGCTCGAGAACTAACAAAAATGATTCGGCTCCAG	1064	Cobo-Molinos, Hikmate, Ben-Omar, Lucas-López, and Galvez (2008)
	Ef1091R	CCGCTCGAGCCATCTCACGCATTTTATCTTCAACT		
tetM	tetM-F	GAYACNCCNGGNCAYRTNGAYTT	1513	Gervers, Danielsen, Huys, and Swings (2003)
	tetM-R	CACCGAGCAGGGATTTCTCCAC		
ace	Ace1	GGAATGACCGAGAACGATGGC	616	Creti et al. (2004)
	Ace2	GCTTGATGTTGGCCTGCTTCCG		
gelE	GEL11	AGTTCATGTCTATTTTCTTCAC	213	Vankerckhoven et al. (2004)
	GEL12	AGATGCACCCGAAATAATATA		
*Asa*1	ASA11	CACGCTATTACGAACTATGA	375	Vankerckhoven et al. (2004)
	ASA12	TAAGAAAGAACATCACCACGA		
efaA	efaA1	CGTGAGAAAGAAATGGAGGA	499	Mannu et al. (2003)
	efaA2	CTACTAACACGTCACGAATG		
esp	ESP 14 F	AGATTTCATCTTTGATTCTTGG	510	Vankerckhoven et al. (2004)
	ESP 12R	AATTGATTCTTTAGCATCTGG		
cylA	CYT I	ACTCGGGGATTGATAGGC	688	Vankerckhoven et al. (2004)
	CYT I1b	GCTGCTAAAGCTGCGCTT		
hyl	HYL n1	ACAGAAGAGCTGCAGGAAATG	276	Vankerckhoven et al. (2004)
	HYL n2	GACTGACGTCCAAGTTTCCAA		
hypR	hypR1	CGTGGCAAGAAGATTCCTTAC	465	Verneuil et al. (2004)
	hypR2	GCTAAATATTCTCCTTCAGGTG		
hdc	HIS1-F	GGNATNGTNWSNTAYGAYMGNGCNGA	372	De las Rivas, Marcobal, Carrascosa, and Muñoz (2006)
	HIS1-R	ATNGCDATNGCNSWCCANACNCCRTA		
tdc	TDC-F	TGGYTNGTNCCNCARACNAARCAYTA	825	De las Rivas, Marcobal, Carrascosa, and Muñoz (2006)
	TDC-R	ACRTARTCNACCATRTTRAARTCNGG		
odc	PUT1-F	TWYMAYGCNGAYAARACNTAYTTYGT	1440	De las Rivas, Marcobal, Carrascosa, and Muñoz (2006)
	PUT1-R	ACRCANAGNACNCCNGGNGGRTANGG		
	PUT2-F	ATHWGNTWYGGNAAYACNATHAARAA	624	De las Rivas, Marcobal, Carrascosa, and Muñoz (2006)
	PUT2-R	GCNARNCCNCCRAAYTTNCCDATRTC		
ldc	CAD2-R	CAYRTNCCNGGNCAYAA	1185	De las Rivas, Marcobal, Carrascosa, and Muñoz (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, or T; N = A, C, G, or T.