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*

* Corresponding author. Tel:+34-91-5622900; Fax: +34-91-5644853

e-mail address: blanca.r@csic.es (B. de las Rivas)

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.

Keywords

Coagulase-negative staphylococci, starter culture, fermented meat products, dry-cured meat products, technological properties, safety-related properties.

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 flavour (Casaburi, Aristoy, Cavella, di Monaco, Ercolini, Toldrá, & Villani, 2007). CNS participate in the development and stability of the red colour through nitrate reductase activity that leads to the formation of nitrosomyoglobin. Furthermore, nitrate reduction produces nitrite that can limit lipid oxidation (Talon, R., Walter, D., Chartier, S., Barrier, C., & Montel, M.C., 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, 2004a; Corbière Morot-Bizot, Talon, & Leroy, 2004; Landeta, Reverón, Carrascosa, de las Rivas, & Muñoz, 2011).

Traditional fermented and dry-cured meat processes favour the growth of autochthonous microbiota which in turn influences flavour, texture, nutritional properties and

105 safety of the final products (Martín, Colin, Aranda, Benito, & Cordoba, 2007). Nevertheless, it
106 is not possible to ensure that the number and the strains of microorganisms present in the raw
107 material will always be the same; therefore, the use of starter cultures in fermented dry-cured
108 meat elaboration ensures a fermentation and ripening process that can be carried out under
109 controlled conditions. One of the main advantages of the use of starter cultures is that food
110 poisoning and food spoilage microorganisms could be suppressed. Identification of
111 technologically relevant bacteria is necessary to select strains to be employed as starter
112 cultures. CNS are important microorganisms used as starter cultures in meat fermentations. In
113 spite that nitrate reductase and catalase activity are considered the most important properties of
114 CNS to make them eligible as starter cultures for fermented meat products (Mauriello,
115 Casaburi, Blaiotta, & Villani, 2004), there are other relevant technological and safety
116 properties that need to be analyzed.
117 The aims of this study were to taxonomically identify, and to analyze several activities
118 relevant for their use as starter culture (such as nitrate reductase, catalase, proteolitic, and
119 lipolitic activities, as well as the resistance to bile, biofilm formation, antibiotic susceptibility,
120 and biogenic amine formation), of a collection of CNS isolated from Spanish dry-cured meat
121 products.
122
123 **2. Materials and methods**
124
125 *2.1. Bacterial strains and growth conditions*
126
127 Seventy-one CNS strains were analyzed in this study. Fifty-one strains were isolated
128 during the elaboration of Spanish dry-cured ham industrial processes (Cornejo & Carrascosa,
129 1991; Carrascosa & Cornejo, 1991) and were previously molecularly identified by sequencing
130 their 16S rDNA (Landeta, Reverón, Carrascosa, de las Rivas, & Muñoz, 2011). The additional

131 twenty CNS strains were isolated from dry-cured sausages (De las Rivas, Ruiz-Capillas,
132 Carrascosa, Curiel, Jiménez-Colmenero, & Muñoz, 2008) and have been molecularly
133 identified in this study by sequencing their 16S rDNA .
134 All the strains were grown in Brain Heart Infusion (BHI, Difco, France) medium at 37
135 ºC and 150 rpm during 24 hours under aerobic conditions. The strains were grown also on BHI
136 agar plates (1.5 %) at 37 ºC under aerobic conditions.
137
138 2.2. *DNA isolation*
139 Bacterial chromosomal DNAs from CNS were isolated from overnight cultures using a
140 protocol previously described (Sambrook, Fritsch, & Maniatis, 1989). DNA precipitates were
141 resuspended in an appropriate volume of TE solution (10mM Tris-HCl, pH 8.0; 1 mM EDTA).
142
143 *2.3. Taxonomical identification of CNS strains*
144
145 CNS strains were identified by PCR amplification and DNA sequencing of their 16S
146 rDNA. The 16S rDNAs were PCR amplified using the eubacterial universal pair of primers 63f
147 and 1387r previosly described by Marchesi, Sato, Weghtman, Martin, Fry, Hion, & Wade,
148 (1998) (Table 1). The 63f and 1387r primer combination generates an amplified product of 1.3
149 kb. PCR was performed in 25 µL amplification reaction mixture by using the following
150 cycling parameters: 35 cycles of 1 min at 94ºC, 1 min at 50ºC and 1:30 min at 72ºC. Amplified
151 products were resolved on 0.7% agarose gels. The amplifications products were purified on
152 QIAquick spin Columns (Quiagen, Germany) for direct sequencing. DNA sequencing was
153 carried out by using an Abi Prism 377^{TM} DNA sequencer (Applied Biosystems, USA).
154 Sequence similarity searches were carried out by comparing to sequences from type strains
155 included on the Ribosomal Database (http://rdp.cme.msu.edu).
156

157 *2.3. Technological properties of the strains*

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159 *2.3.1. Nitrate reductase assay*

160

161 Nitrate reductase activity was determined as described previously (Miralles et al.,
162 1996). The colorimetric assay performed was an adaptation of the method described by
163 Smibert & Krieg (1994). Briefly, 1.5 mL of culture was grown during 24 h. After incubation,
164 the culture was centrifuged and the cellular pellet was resuspended in induction buffer
165 [Bactotryptone (Difco), 10 g/L; KNO_3, 1 g/L; cysteine, 1g/L pH 7.0] to an OD_{550}=1. A fraction
166 of the cell suspension was used for the determination of the dry weight. Anaerobic induction of
167 nitrate reductase activity was achieved by incubation for 2 h at 30 ºC of 1 mL of the cell
168 suspension in Eppendorf tubes covered with a layer of sterile mineral oil. As a control for the
169 induction process, 1mL of the cell suspension was kept on ice for 2 h. The cells were
170 centrifuged and then permeabilized in 500 µL reaction buffer (50 mM KNO_3, 50 mM
171 potassium phosphate, 100 mM NaCl, pH 7.0) by the addition of 30 µL of an acetone-toluene
172 mixture (9:1, v/v). Then the tubes were vigorously shaken in a vortex for 3 min. The reaction
173 was allowed to proceed for 30 min at 30ºC. A 100 µL aliquot of the samples was transferred to
174 a new Eppendorf. Then, 2 mL water, 1 mL solution A [0.6 mg N-(1-Naphthyl)-
175 ethylenediamine dihydrochloride in 100 mL 5 N acetic acid] and 1 mL solution B (0.8 g
176 sulphanilic acid in 100 mL 5N acetic acid) were added. Absorbance at 540 nm was measure
177 against a control tube. Relative activity was calculated as the ratio: OD_{540} x mg^{-1} dry weight.

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179 *2.3.2. Catalase activity*

180

181 The catalase activity was determined as described previously by Herrero, Mayo,
182 González, & Suárez, (1996). The strains were grown in BHI agar supplemented with 0.2% of

glucose. The plates were incubated for 24 h at 30 ºC; 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, & 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^{TM}HT, Biotek, EEUU). Results were expressed in arbitrary units (µmoles of degraded H_2O_2/min/mL of cells with OD_{600}=1.0).

2.3.3 Proteolytic activity

To study the proteolitic activity of the CNS strains, gelatinase (Cariolato, Andrighetto, & Lombardi, 2008) and calcium caseinate agar (Martín, Hugas, Bover-Cid, Veciana-Nogués, & Aymerich, 2006) assays were used. Production of gelatinase was tested on BHI agar plates containing 10 g/L peptone (Pronadisa, Spain) and 30 g/L gelatine (Pronadisa, Spain). After 16-18 h of incubation at 37 °C, the plates were placed at 4°C for 5 h before examination for a zone of turbidity around the colonies indicating hydrolysis of gelatine. In the second method, the proteolitic activity was tested in calcium caseinate agar plates (Pronadisa, Spain). The strains were grown 18-20 h in BHI broth and then 10 µL of each culture was placed in the calcium caseinate agar at 37 ºC during 3 days. The diameter of the halos formed by the proteolitic strains was measure in millimetres.

2.3.4. Lipolytic activity

The lipolytic activity was analyzed using two lipase assay procedures, "Spirit Blue Agar" (Difco) and BHI agar supplemented with Tween 80 or Tween 20 (Essid et al., 2007). The assay with Spirit Blue Agar plus Lipase Reagent (tributyrin plus polysorbate 80) was performed according to the recommendations of the supplier. Lipolytic microorganisms metabolize the lipids present in the medium and halos around the colonies appear.

In order to evaluate the lipolytic activity on BHI agar supplemented with 1% of Tween 80 or Tween 20, an overnight culture of each strain was centrifuged and the pellet was resuspended in 20 mM phosphate buffer, pH 7. Each cell suspension (10 µl) was inoculated on a spot at the surface of the BHI plates containing Tween 80 or Tween 20. After incubation at 37 ºC for 48h, the lipolytic activity was determined by the appearance of a clear halo surrounding the spots which diameter was measured in mm.

2.3.5. Resistance to bile

Resistance to bile was tested according to methods described previously (Maragkoudakis, Zoumpopoulou, Miaris, Kalantzopoulos, Pot, & Tsakalidou , 2006; Saavedra, Taranto, Sesma, & de Valdez, 2003). In the first assay the CNS cultures were grown in BHI broth supplemented with Oxgall (0.3%) (Difco, France) at 37 ºC during 4h. The resistance was determined by the number of viable colonies after incubation during 0 and 4 h, reflecting the time spent by the food in the small intestine.

In the second assay, the CNS strains were grown in LAPTg broth (g/L: peptone, 15; tryptone, 10; yeast extract, 10; glucose, 10; Tween 80, 0.1% v/v) and LAPTgO broth (LAPTg containing 0.3% Oxgall) at 37ºC. Every hour, for the first 8 h, and after 24 h of incubation, the A_{560} nm was determined using a microplate reader (Synergy^{TM}HT, Biotek. EEUU). The time required for each of them to increase the A_{560} by 0.3 units was recorded. The difference in time

234 (min) between the LAPTg and LAPTgO cultures was considered as the growth delay. Each
235 assay was performed in triplicate and repeated three times.
236
237 *2.4. Presence of relevant safety traits in the strains*
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239 *2.4.1. Biofilm formation*
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241 The ability of CNS to form a biofilm was evaluated by two methods described
242 previously (Toledo-Arana et al., 2001; Freeman, Falkiner, & Keane, 1989). In the assay
243 described by Toledo-Arana et al. (2001) the CNS cultures were grown overnight at 37 ºC in
244 BHI broth containing 0.25% glucose. The culture was diluted 1/20 in fresh BHI broth
245 supplemented with 0.25 % glucose; 200 µL of this suspension was used to inoculate sterile 96-
246 well polystyrene microtitre plates. After 24 h at 37ºC, wells were washed with PBS, dried in an
247 inverted position and stained with 1% crystal violet (Merk, Germany) for 15 min. The wells
248 were rinsed once more with PBS and the crystal violet was solubilised in methanol/acetone
249 (80:20, v/v). The A_{595} was determined using a microplate reader (Synergy^{TM}HT, Biotek.
250 EEUU). Biofilm formation was scored as follows: -, non-biofilm forming ($A_{595} \leq 1$); +, weak
251 ($1 < A_{595} \leq 2$); ++, moderate ($2 < A_{595} \leq 3$); +++, strong ($A_{595} > 3$). Each assay was performed in
252 triplicate and repeated three times.
253 The assay described by Freeman et al. (1989) used Congo Red Agar (CRA). The
254 constituents of the CRA media were BHI broth (37g/L), sucrose (0.8 g/L), agar-agar (10 g/L)
255 and Congo red stain (0.8 g/L). Congo red stain was prepared as a concentrated aqueous
256 solution, autoclaved separately and added to the media when the agar had cooled to 55 ºC.
257 Plates were incubated aerobically for 24 h at 37ºC. Biofilm positive strains produced black
258 colonies while biofilm negative strains were pink.

The biofilm formation ability was also determined by PCR amplification of the *icaA* gene. It has been described that this gene is involved in cellular aggregation and biofilm accumulation. For the PCR reaction the primers ica4f and ica2r previously described were used (Møretrø, Hermansen, Holck, Sidhu, Rudi, & Langsrud, 2003) (Table 1). PCR was performed in 25 μL amplification reaction mixture by using the following cycling parameters: 35 cycles of 1 min at 94ºC, 1 min at 50ºC and 1:30 min at 72ºC. Amplified products were resolved on 0.7% agarose gels. The DNA polymerase used was AmpliTaq Gold® (Applied Biosystem)

2.4.2. Antibiotic susceptibility testing

Susceptibility testing was assayed by the agar overlay disc diffusion test recommended by the National Committee for Clinical Laboratory Standard (NCCLS, 2002). SensiDisc BBL discs (Oxoid, England) were placed onto Mueller-Hinton agar (Difco, France) plates and overlayed with 3 mL of BHI soft agar broth (0.7% agar, p/v) inoculated with 150 μL of the cultures with a cell concentration corresponding to 0.5 MacFarland turbidity standard. After incubation at 37 ºC for 24 h the diameter of inhibition halos around the colonies was measured. Susceptibility or resistance was determined according to the recommendation of NCCLS. The antibiotics used were: ampicillin (10 μg), chloramphenicol (30 μg), clindamycin (2 μg), erythromycin (15 μg), gentamicin (10 μg), kanamicin (30 μg), penicillin G (10 U), tetracycline (30 μg), vancomycin (30 μg), streptomycin (10 μg), and cephalotin (30 μg).

In β-lactamic resistant strains the presence of the *blaZ* gene, which codified a protein involved in this resistance, was determined. The primers used were blaZ-F and blaZ-R (Resch, Nagel, & Hertel, 2008) (Table 1).

2.4.3. Production of biogenic amines

285 Biogenic amine production was tested by TLC (for the detection of the biogenic amine
286 produced) and PCR (for the detection of the genes encoding the enzymes responsible of their
287 biosynthesis).
288 The TLC method was previously described by García-Moruno, Carrascosa, & Muñoz,
289 (2005). Briefly, the cultures were incubated at 37 ºC under aerobic conditions for 7 days in
290 BHI broth containing 0.5% of the corresponding amino acid precursor: L-histidine
291 monohydrochloride (Merk, Germany), L-tyrosine disodium salt (Merk, Germany), L-ornithine
292 hydrochloride (Sigma-Aldrich, Germany), L-lysine monohydrochloride (Merk, Germany). As
293 control, a stock standard solution of each amine (histamine, tyrosine, putrescine and
294 cadaverine) was made by preparing a 2% solution (5% in the case of histamine) in 40%
295 ethanol. After incubation, the supernatant, containing the corresponding biogenic amine, was
296 collected. Amines were converted into their fluorescent dansyl derivatives and were incubated
297 in the dark at 55º C during 1h. Amine derivative extracts were applied in TLC plates (silica gel
298 60 $F_{24,}$ Merk, Germany). The dansylated compounds were separated using
299 chloroform:triethylamine (4:1). The fluorescent dansyl derivative spots were visualized using a
300 ChemiDoc XRS^{+} (Bio Rad) under UV light exposure.
301 The detection of the genes encoding the decarboxylase enzymes involved in the
302 production of biogenic amines was performed by PCR using primers previously described:
303 HIS1-F/HIS1-R to amplify a fragment of the histidine decarboxylase gene from Gram-positive
304 bacteria; TDC-F/TDC-R to amplify a fragment of the tyrosine decarboxylase-encoding gene;
305 PUT1-F/PUT1-R and PUT2-F/PUT2-R to amplify the two groups of ornithine decarboxylase
306 genes, and finaly CAD2-F/CAD2-R to amplify a fragment of the lysine decarboxylase gene
307 present in Gram-positive bacteria (De las Rivas, Marcobal, Carrascosa, & Muñoz, 2006)
308 (Table 1).
309
310 **3. Results and discussion**

311

312 *3.1. Identification of CNS strains*

313 Seventy one CNS strains previously isolated from Spanish dry-cured meat products
314 (Carracosa & Cornejo 1991; Cornejo & Carrascosa, 1991; de las Rivas et al., 2008) were
315 analyzed in this study (Table 2). Fifty-one strains isolated from dry cured ham were identified
316 by Landeta et al. (2011) as *S. equorum* (36 strains), *S. vitulinus* (5 strains), *S. caprae* (1 strain),
317 *S. capitis* (1 strain), *S. aureus* (2 strains), *S. warneri* (3 strains), *S. epidermidis* (1 strain), *S.*
318 *hominis* (1 strain), and *S. lugdunensis* (1 strain). Twenty CNS strains were isolated from dry-
319 cured sausages and some of them were previously identified (de las Rivas et al., 2008).

320 Using phenotypic methods the CNS species most commonly identified in traditional
321 fermented sausages are *S. xylosus, S. equorum, S. succinus* and *S. saprophyticus*, being often *S.*
322 *xylosus* predominant (Mauriello et al., 2004; Corbiere Morot-Bizot et al., 2004). Sondergaad &
323 Stahnke (2002) and Landeta et al. (2011) reported that some strains isolated from fermented
324 meat products with high probability to be *S. xylosus* (by using API-STAPH system) were
325 indeed identified by molecular methods as *S. equorum*. Therefore, it is probable that the
326 presence of *S. equorum* in fermented meat products has been under-estimated for a long time
327 because of its confusion with *S. xylosus* by traditional microbiological methods based on
328 biochemical test.

329 As the characterization of CNS using traditional methods may sometimes be uncertain,
330 in this study the strains have been identified by sequencing its 16S rDNA. The CNS species
331 more frequently isolated from dry-cured sausages was *Staphylococcus carnosus* (80%),
332 whereas from dry-cured ham the higher incidence was for *S. equorum* strains (71%). In spite
333 that *S. carnosus* and *S. xylosus* are the most recommended staphylococcal starter culture for
334 dry sausage production in Europe (Samelis, Metaxopoulos, Vlassi, & Aristea, 1998), among
335 our isolates only one strain was identified as *S. xylosus*. Similarly to this study, the high

incidence of *S. equorum* has been also described (Mauriello, et al. 2004, Cordero & Zumalacárregui, 2000). Marty, Buchs, Eugster-Meier, Lacroix, & Meile, (2012) reported that in spontaneously fermented Swiss meat product *S. equorum* was prevalent in frequency and cell counts during maturation and in the end products (20.3 %) followed by *S. warneri*, *S. saprophyticus*, *S. epidermidis* and *S. xylosus*. In Slovak traditional sausages, Simonová, Strompfvá, Marcináková, Lauková, Vesterlund, Latorre Moratalla, Bover-Cid, & Vidal-Carou, (2006) identified using species-specific PCR, *S. xylosus* (63.6%) and *S. carnosus* (10.7%) as the predominant species. Martin et al. (2006) using molecular methods to identify the species present in slightly fermented sausages found a 80% of *S. xylosus* and only a 4.6% of *S. carnosus* but none of them was identified as *S. equorum*.

3.2. Selection of CNS based on their technological properties

The technological and safety related properties are important in order to select a strain eligible as starter culture for fermented meat products. In this study, seventy-one strains of CNS were screened for nitrate reductase, catalase, proteolitic, lipolytic, bile resistance, biofilm formation, antibiotic susceptibility, and biogenic amine production, because these activities are important in the development of the aroma, colour flavour, texture and in the safety of the final fermented meat products. The technological and safety-related properties analyzed in this study are reported in Table 2.

The nitrate reductase activity is the responsible for the red colour in the fermented meat products. The ability to reduce nitrate is one of the first criteria in the selection of strains to be used as starter cultures in these products. In our study, the CNS strains showed variable nitrate reductase activity (Table 2). The results varied from 0.04 (*S. equorum* IFIJ 39, 44, and 51) to 33.06 (*S. aureus* IFIJ 32) mM nitrate reduced to nitrite per milligram of dry weight. This variability was observed also amongst strains even belonging to the same species, e.g. in *S.*

362 *carnosus* strains the valour obtained ranged from 3.45 to 23.42, and in *S. equorum* from 0.04 to
363 15.26. This variability has been observed by the use of different methods to measure nitrate
364 reductase activity (Mauriello et al., 2004; Cassaburi et al., 2007; Martín et al., 2007; García-
365 Varona, Santos, Jaime, & Rovira, 2000; Papamanoli, Kotzekidou, Tzanetakis, & Litopolou-
366 Tzanetaki, 2002). Essid et al. (2007) used an agar plate method and a spectrophotometric
367 method to measure nitrate reductase activity, and concluded that the spectrophotometric
368 method appears to be more suitable for the detection of low nitrate reductase activity. In our
369 study a strain of *S. aureus* has the highest capacity to reduce nitrate (33.06) however since this
370 strain belonged to an opportunistic pathogenic species (O´Gara, 2007), it is not recommended
371 to be used as meat starter. Nevertheless, some strains, such as *S. carnosus* C-120 or *S. capitis*
372 IFIJ 12, have also a high nitrate reductase capacity and they could be used as starter cultures.
373 Hydrogen and organic peroxides are frequently formed as products of the microbial
374 metabolism. Hence, the presence of catalase-producing species would improve the appearance
375 and safety of fermented meat products. Catalase activity could help to prevent off-flavours
376 produced by lipid oxidation during product ripening. Peroxide radicals, involved in rancidity
377 development, are detoxified by superoxide dismutase activity with consequent production of
378 hydrogen peroxide, which is destroyed by catalase activity. In this study, the catalase activity
379 was analyzed using a direct method (bubbles production with H_2O_2) and a spectrophotometric
380 assay measuring the μmoles of degraded H_2O_2. All the strains analyzed were catalase positive
381 by the direct method (Table 2). On the other hand, the results showed high variability ranging
382 from 0.98 (*S. equorum* IFIJ 25) to 26.7 (*S. vitulinus* IFIJ 4) by using the spectrophotometric
383 method. Similarly to the nitrate reductase activity, amongst strains from the same species there
384 is a great variability, e. g., in *S. carnosus* strains catalase activity ranged from 1.3 to 14.1
385 (Table 2). Essid et al. (2007), using the same method, reported a high variability in *S. xylosus*
386 strains isolated from Tunisian traditional salted meat.

Various aromatic substances and organic acids are released by protease and lipase activity of CNS. Proteolysis and lipolysis influence both texture and flavour development due to the formation of low molecular weight compounds (peptides, aldehydes, free fatty acids, etc). In order to know the proteolitic activity of the CNS strains analyzed in this study, two different assays were used, proteolitic activity in gelatine (Cariolato et al., 2008) and in calcium caseinate agar (Martín et al. 2006). The obtained results showed great variability depending on the method applied (Table 2). Using the gelatinase activity assay only one strain, *S. caprae* IFIJ 10, showed a positive response. This strain also was positive by using calcium caseinate agar as substrate. Most of the positive strains by the caseinate method (*S. aureus* Al-84, *S. carnosus* C-120, *S. epidermidis* IFIJ 24, *S. equorum* IFIJ 39 and IFIJ 45, and *S. vitulinus* IFIJ 4, IFIJ 31, IFIJ 36, IFIJ 38, and IFIJ 41) were negative by the gelatinase assay. It is remarkable that all strains of *S. vitulinus* analyzed showed high proteolytic activity by the calcium caseinate agar (Table 2).

Related to lipolytic activity, the agar plate assays used in this work showed that none strain could hydrolyze Tween 20 or Tween 80. None of the strains showed lipolytic activity in "Blue Spirit" except a low activity presented by the *S. epidermidis* strains, and *S. warneri* IFIJ 52 (Table 2). Martín et al. (2006) reported that among CNS species, *S. xylosus* was the species showing the highest lipolytic activity on tributyrin agar; however, Papamaloni et al. (2002) found that only 30% of *S. xylosus* strains isolated from fermented sausages were able to hydrolyze tributyrin. Essid et al. (2007) showed that 76% of the *S. xylosus* strains could hydrolyze Tween 20, 33.3% tributyrin and only 10% hydrolyzed Tween 80. These apparently contradictory results could be explained since meat substrates (muscle proteins, sarcoplasmic, or pork fat) could be more suitable for the detection of lipolytic and proteolitic activities of potential meat starters than other substrates such as powdered milk, gelatine, tributyrin or Tween 80 and Tween 20 (Mauriello et al., 2004).

412 The survival ability of isolates in the presence of oxgall bile is an important
413 characteristic for the selection of potentially probiotic strains. Thirty strains analyzed in this
414 study showed resistance to bile salt using this method (Table 2). The results obtained with the
415 spectrophotometric method showed that, in general, *S. equorum* and *S. vitulinus* strains were
416 very susceptible to the presence of bile salts. Although studies about this property in
417 enterococci and lactic acid bacteria isolated from milk and meat origin have been published
418 (Vinderola & Reinheimer, 2003), information about CNS are limited. In *S. xylosus* strains
419 isolated from Slovak meat products, Simonova et al. (2006) found that the capacity to survive
420 in broth containing 1% oxgall varied between 54 and 99%. As adhesion to the intestinal mucus
421 is one of the main selection criteria for probiotics, Simonova et al. (2006) also reported that in
422 *S. xylosus* the adhesion ability to mucus was found to be strain-dependent.
423 Taking into account the activities analyzed so far among the strains analyzed in this
424 study, the CNS species possessing the best technological characteristics are *S. aureus, S.*
425 *carnosus,* and *S. equorum,* species as they possess high nitrate reductase and catalase activity.
426 However, as safety aspects are also relevant, the *S. aureus* strains are not eligible as potential
427 starter cultures. In this sense, it is important the study of safety related characteristics, such
428 antibiotic susceptibility and biogenic amine production, in order to get a safe meat product.
429
430 *3.3 Selection of CNS based in their safety-related properties*
431
432 CNS in fermented meat have a long tradition in food use but have not received the QPS
433 (Qualified Presumption of Safety) status from EFSA (European Food Safety Authority). They
434 require, therefore, thorough assessment with regard to toxigenicity and pathogenicity
435 determinants as well as presence of transferable antibiotic resistance (Hammes, 2012).
436 The survival of staphylococci in food processing environments could be associated to
437 their capacity to colonize abiotic surfaces (Møretrø et al., 2003). Biofilm formation is a two-

438 step process; first it involves the attachment of cells to a solid surface and, second, the
439 accumulation and aggregation of cells sticking together by intercellular adhesion (Christensen,
440 G. D., Simpson, W.A., Younger, J.J., Baddour, L.M., Barret, F.F., Melton, & Beachey, 1985).
441 In food industry, biofilm formation is undesirable for hygienic and safety reasons due to the
442 possible attachment of food spoilage or pathogenic microorganisms to food or food surfaces.
443 Nevertheless, several authors consider that in food systems, colonization of surfaces by
444 bacteria used as starters could be desirable to inhibit the colonization by pathogenic or spoilage
445 bacteria (Leriche & Carpentier, 2000). In this study (Table 2), biofilm formation only was
446 detected in four strains when the Congo red method was used (*S. equorum* IFIJ 29 and IFIJ 39,
447 *S. vitulinus* IFIJ 38, and *S warneri* IFIJ 15). By spectrophotometric method in spite that several
448 strains seem to produce biofilms, only two strains were clearly positives, *S. caprae* IFIJ 10 and
449 *S. caprae* Al-145. The results reflect a high variability depending on the method used, as
450 reported previously by Jain & Agarwal (2009). In addition Møretrø et al. (2003) described the
451 dependence of external factors in biofilm formation, such as stress conditions.
452 Among CNS biofilm formation has been studied in *S. aureus* (Jain & Agarwal, 2009), *S.*
453 *epidermidis* (Jain & Agarwal, 2009), *S. hominis* (Kotilainem, 1990), *S. sciuri* (Leriche &
454 Carpentier, 2000) and *S. equorum* (Leroy, Lebert, Charcornac, Chavant, Bernardi, & Talon,
455 2009). These studies concluded that, in general, biofilm formation is a strain-dependent
456 characteristic. However, it have been described that *S. carnosus* strains are not able to form
457 biofilm and this could explain why this microorganism is rarely isolated in meat processing
458 environments (Planchon, Gaillard-Martinie, Leroy, Bellon-Fontaine, Fadda, & Talon, 2007).
459 Planchon, Gaillard-Martinie, Dordet-Frisoni, Bellon-Fontaine, Leroy, Labadie, Hébraud, &
460 Talon, (2006) studied the ability of *S. xylosus* strains to form biofilm and found that the
461 majority of the strains analyzed could form biofilm preferentially on hydrophilic supports. In
462 addition, in the positive *S. xylosus* strains the presence of the *bap* and *ica4* genes, important for
463 biofilm formation in some staphylococci, was analyzed and all the biofilm-positive strains

were *bap* positive but *ica4* negative. In this sense, in the present study the presence of the *ica* gene have been checked (Figure 1). However, the presence of the *ica* gene was positive only in some strains that previously were positive using Congo red and/or spectrophotometric methods, therefore, this result indicates the existence of other genes involved in biofilm formation (e.g the presence *bap* gene.

Due to the intensive use of antibiotics in public health and animal husbandry, studies showed that resistance to antibiotics could occur in strains from *S. carnosus* and *S. xylosus* species (Martín et al., 2006). Antibiotic resistance determinants contained in starter organisms or naturally occurring CNS may thus be transferred to commensals or pathogenic bacteria as horizontal transfer of resistance genes occurs in food (Teuber, 1999). In this study, all the strains analyzed were sensitive to gentamicin (except *S. caprae* Al 145), kanamycin and cephalotin (Table 2). Regarding to β-lactamic antibiotics, all the ampicillin resistant strains (7 strains) were also resistant to penicillin G. In relation to vancomycin resistance, only 9 strains were resistant, being among them all the *S. aureus* and *S. warneri* strains. As soon as 1998, Holley & Blaszyk reported the isolation of vancomycin-resistant *S. carnosus* strains which grown in presence of 250 μg/mL vancomycin (*S. aureus* MIC is 32 μg/mL). However, later studies showed that none of the CNS analyzed exhibited resistance against vancomycin (Mauriello, Moschetti, Villani, Blaiotta, & Coppola, 2000; Martin et al., 2006; Resch et al., 2008).

Among the CNS strains analyzed, the strain possessing resistance to the high number of antibiotic were *S. epidermidis* IFIJ 24 (which was resistant to ampicillin, chloramphenicol, clindamycin, erythromycin, and penicillin G) and *S. hominis* IFIJ 26 (resistant to ampicillin, clindamycin, erythromycin and penicillin G). Contrarily, all the *S. carnosus* strains analyzed were sensitive to all the antibiotics assayed. Resch et al. (2008) reported that food-associated CNS were sensitive to the clinically important antibiotics chloramphenicol, clindamycin, cotrimoxazol, gentamicin, kanamycin, linezolid, neomycin, streptomycin, synercid and

vancomycin. However, other studies showed that strains of *S. carnosus* and *S. xylosus* could be resistant to gentamicin, kanamycin, neomycin and clindamycin (Mauriello et al., 2000).

The β-lactamic resistance also was evaluated by a molecular method based on the *blaZ* gene amplification by PCR (Figure 2). All the penicillin G resistant strains, by the disc diffusion test, from the *S. aureus, S. epidermidis, S. hominis, S. lugdunensis,* and *S. warneri* species were able to amplify the *blaZ* gene. Nevertheless, the *S. equorum* and *S. xylosus* β-lactamic resistant strains were *blaZ*-negative by PCR. A possible explanation for this resistance in absence of the *blaZ* gene could be due to the presence of differences in the penicillin binding proteins which could have less affinity by β-lactamic antibiotics (Chambers, 1988).

The presence of biogenic amines is a relevant food issue in meat products. In spite that biogenic amine production by some strains used in this study was previously published (de las Rivas et al., 2008; Landeta, Carrascosa, de las Rivas, & Muñoz, 2007), in this work, the study of biogenic amine production have been completed. Biogenic amine production was analyzed by two different methods, by TLC (Garcia-Moruno et al., 2005) and by PCR (de las Rivas et al., 2006). The obtained results are showed in Table 2. Most of the strains analyzed were not able to produce biogenic amines (75 % using the TLC method and 94.5% using the PCR method). By the TLC method, strains producing histamine, tyramine, putrescine, and cadaverine were found. In agreement with previous results only *S. capitis* IFIJ 12 strain produced histamine (Landeta et al 2007). All the *S. carnosus* strains produced tyramine (Figure 3) and two strains, *S. epidermidis* Al-190 and *S. lugdunensis* IFIJ 47, produced simultaneously two different amines, putrescine and cadaverine (data not shown). Previously, it have been reported that *S. carnosus* was able to produce biogenic amines in contrast with *S. xylosus* and *S. equorum* that were non-producer species [Seitter (née Resch), Geng, & Christian, 2011a]. In *S. carnosus*, 2-phenylethylamine, tryptamine, and/or tyramine were often detected whereas, cadaverine and putrescine were only rarely detected, and histamine was not produced. Other

516 authors reported similar results and concluded that amino acid decarboxylase activity is a
517 strain dependent characteristic in staphylococci (Ansorena, Montel, Rokka, Talon, Eerola,
518 Rizzo, Raemaekers, & Demeyer, 2002; Even et al., 2010).
519 In order to correlate the production of biogenic amines with the presence of the
520 corresponding decarboxylase genes, PCR assays for the detection of these genes was
521 performed. The results obtained by PCR are in agreement with the results obtained by TLC
522 except in the tyramine-producer *S. carnosus* strains on which the PCR was negative. The
523 production of this amine by *S. carnosus* strains was studied by Seitter (née Resch), Nerz,
524 Rosenstein, Götz & Christian, (2011b) and reported that, in the production of tyramine and 2-
525 phenylethylamine by *S. carnosus* strains, discrepancies between the phenotype and genotype
526 were observed, probably due that the corresponding genes are not still identified.
527 In conclusion, in this study the technological and safety characterization of CNS
528 allowed to select several strains, such as *S. equorum* IFIJ 23 and IFIJ 30 strains, possessing
529 interesting profiles to be used as starter cultures for fermented meat products. In general, *S.*
530 *equorum* strains were good candidates to be selected in spite that some of them were bile salt
531 sensitive and six strains (IFIJ 5, IFIJ 34, IFIJ 42, IFIJ 43, IFIJ 46, and IFIJ 53) presented
532 several antibiotic resistances. In this study the *S. equorum* strains were isolated from dry-cured
533 ham but this species is also present in sausages. Different studies have shown that *S. equorum*
534 species represents 49% of the staphylococcal isolates from French naturally fermented
535 sausages (Corbiere_Morot-Bizot, Leroy, Talon, 2006), 10 to 40% from southern Italian
536 sausages (Blaiotta , Pennacchia, Villani, Ricciardi, Tofalo, &. Parente, 2004b; Mauriello et al.
537 2004) and fresh sausages (Rantsiou , Iacumin, Cantoni, Comi, & Cocolin, 2005). The *S.*
538 *carnosus* strains analyzed, despite this species is one of the most used in meat starter cultures,
539 produce tyramine, and for this reason do not be selected anyone. On the other hand, several
540 strains showing good technological characteristics (such as *S. caprae* IFIJ 10, and *S. vitulinus*

IFIJ 31, IFIJ 36 and IFIJ 38) could not be selected due that strains from these species present some virulence factors and pathogenicity (Moodley, & Guardabassi, 2009).

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779 **Figure captions**
780
781 Fig. 1. PCR amplification of the *icaA* gene involved in cellular aggregation and biofilm
782 accumulation by using primers ica4f and ica2r that give an amplicon of 568 pb. PCR was
783 performed by using DNA from *S. caprae* Al-145 (lane 1), *S. caprae* IFIJ 10 (lane 2), *S.*
784 *equorum* IFIJ 5 (lane 3), *S. carnosus* C-9 (lane 4), *S. carnosus* C-120 (lane 5), *S. epidermidis*
785 Al-90 (lane 6) and *S. lugdunensis* IFIJ 47 (lane 7). A 100-pb ladder marker was included in the
786 right of the gel.
787
788 Fig. 2. PCR amplification of the *blaZ* gene involved in β-lactamic resistance by using primers
789 blaZ-F and blZ-R that give an amplicon of 173 pb. PCR was performed by using DNA from *S.*
790 *aureus* IFIJ 13 (lane 1), *S. epidermidis* IFIJ 24 (lane 2), *S. hominis* IFIJ 26 (lane 3), *S. aureus*
791 IFJ32 (lane 4), *S. equorum* IFIJ 42 (lane 5) and, *S. equorum* IFIJ 43 (lane 6). Some of the sizes
792 of the 100-pb ladder marker are indicated on the right.
793
794 Fig.3. Biogenic amine production by CNS strains. (A) TLC detection of tyramine production
795 by *S. carnosus* strains. The strains were grown in BHI containing tyrosine and the tyramine

796 produced was converted into its fluorescent dansyl derivative and detected. The analyzed
797 strains were *E. faecium* RM 58 (positive control) (lane 1); *S. equorum* IFIJ 6 as negative
798 control (lane 2); *S. carnosus* C-23 (lane 3); *S. carnosus* C-47 (lane 4); *S. carnosus* C-120 (lane
799 5). Tyramine standard solution (lane T). (B) PCR amplification of the tyrosine decarboxylase
800 encoding gene. Oligonucleotides TDC-F and TDC-R were used to amplify a 825-bp internal
801 fragment of the tyrosine decarboxylase from *S. carnosus* C-6 (lane 1); *S. carnosus* C-9 (lane
802 2); *S. carnosus* C-23 (lane 3); *S. carnosus* C-42 (lane 4); *S. carnosus* C-47 (lane 5); *S. carnosus*
803 C-48 (lane 7); *S. carnosus* C-67 (lane 8), *S. carnosus* C-76 (lane 9); *S. carnosus* C-93 (lane
804 10); *S. carnosus* C-10 (lane 11). *E. faecium* RM 58 (lane 6) was included as positive control. A
805 DNA marker standard (EcoRI/HindIII digested λ DNA) was included in the gel and some of
806 their fragments indicated on the right of the figure.
807
808

Figure 1

Figure 1 (Landeta et al.)

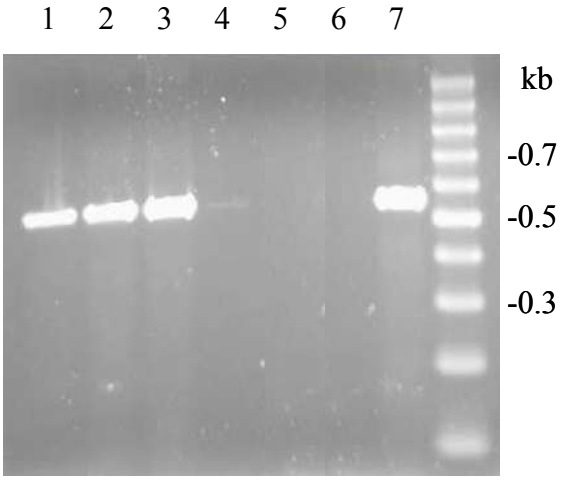

Figure 2

Figure 2 (Landeta et al.)

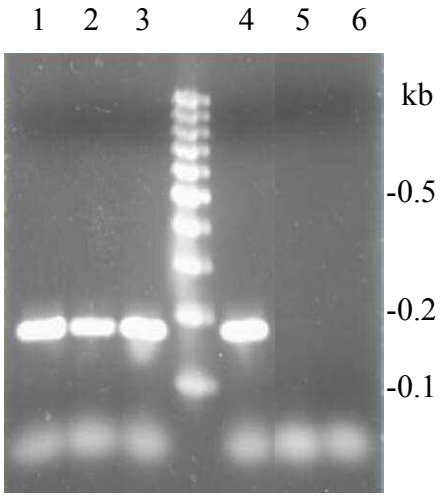

Figure 3

Figure 3 (Landeta et al.)

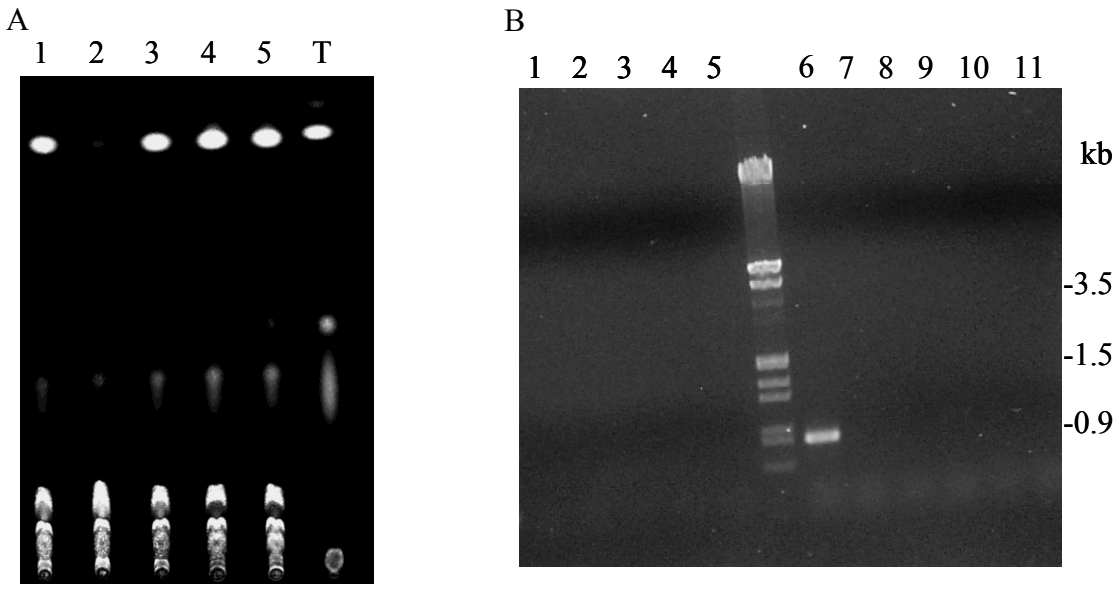

Table 1

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	Moretro et al., 2003
	ica2r	CCTCTGTCTGGGCTTGACCATG		
blaZ	blaZ-F	ACTTCAACACCTGCTGCTTTC	173	Resch et al., 2008
	blaZ-R	TGACCACTTTTATCAGCAACC		
hdc	HIS1-F	GGNATNGTNWSNTAYGAYMGNGCNGA	372	De las Rivas et al., 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.

Table 2

Table 2. Characteristics of analyzed strains.

Species	Strain	Source	NR[a]	Catalase[b]		Proteolytic[c]		Lipolytic[d]		Biofilm[e]			Bile salt[f]		Biogenic amines[g]								Antibiotic[h]											
															TLC				PCR															
				DM	EM	Gel	CCA	BS	T20/T80	RC	EM	icaA	Rt	DM	H	T	P	C	H	T	P	C	Am	Clo	Cli	Ery	Gen	Kan	PeG	Tet	Van	Est	Cef	*blaZ*[i]
S. aureus	IFIJ 13	Dry-cured Ham	12.93	+	16.5	-	-	-	-	-	-	ND	9	R	-	-	-	-	-	-	-	-	R	S	I	I	S	S	R	S	R	R	S	+
	IFIJ 32	Dry-cured Ham	33.06	+	14.4	-	-	-	-	-	-	ND	2	R	-	-	-	-	-	-	-	-	R	S	S	I	S	S	R	S	R	I	S	+
	AI-84	Dry-cured sausage	2.79	+	1.5	-	+	-	-	-	-	ND	0	R	-	-	-	-	-	-	-	-	S	S	S	I	S	S	S	S	R	I	S	ND
S. capitis	IFIJ 12	Dry-cured ham	23.39	+	0.99	-	-	-	-	-	-	ND	12	R	+	-	-	-	+	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
S. carnosus	C-6	Dry-cured sausage	3.98	+	9.6	-	-	-	-	-	-	ND	2	R	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-9	Dry-cured sausage	11.68	+	9.3	-	-	-	-	-	+	-	14	R	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-23	Dry-cured sausage	7.92	+	12	-	-	-	-	-	-	ND	9	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-42	Dry-cured sausage	11.43	+	14.1	-	-	-	-	-	-	ND	20	R	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-47	Dry-cured sausage	7.65	+	11.7	-	-	-	-	-	-	ND	4	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-48	Dry-cured sausage	7.31	+	12.9	-	-	-	-	-	-	ND	8	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-67	Dry-cured sausage	7.93	+	11.7	-	-	-	-	-	-	ND	6	S	-	+	-	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	C-76	Dry-cured sausage	7.92	+	13.5	-	-	-	-	-	-	ND	1	S	-	+	-	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	C-93	Dry-cured sausage	3.45	+	11.4	-	-	-	-	-	-	ND	2	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-106	Dry-cured sausage	11.39	+	12.3	-	-	-	-	-	-	ND	4	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-120	Dry-cured sausage	23.42	+	5.1	-	++++	-	-	-	+	-	6	R	-	+	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	C-129	Dry-cured sausage	18.13	+	4.2	-	-	-	-	-	-	ND	0	S	-	+	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	C-159	Dry-cured sausage	3.48	+	2.7	-	-	-	-	-	-	ND	0	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-177	Dry-cured sausage	17.34	+	3.3	-	-	-	-	-	-	ND	6	S	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-180	Dry-cured sausage	5.11	+	1.3	-	-	-	-	-	-	ND	0	R	-	+	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	C-190	Dry-cured sausage	4.81	+	10.2	-	-	-	-	-	-	ND	0	R	-	+	-	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
S. caprae	IFIJ 10	Dry-cured ham	9.62	+	18.6	+	+++	-	-	-	+++	+	59	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	AI-145	Dry-cured sausage	11.80	+	16.2	-	-	-	-	-	+++	+	0	S	-	-	-	-	-	-	-	-	S	S	I	I	I	I	S	I	R	R	S	ND
S. epidermidis	IFIJ 24	Dry-cured ham	7.87	+	9.9	-	++	+	-	-	-	ND	50	S	-	-	-	-	-	-	-	-	R	R	R	R	S	S	R	I	S	I	S	+
	AI-90	Dry-cured sausage	2.19	+	13.5	-	-	+	-	-	+	-	6	S	-	-	+	+	-	-	+	+	S	S	I	I	S	S	R	S	S	I	S	+
S. equorum	IFIJ 1	Dry-cured ham	3.42	+	6.9	-	-	-	-	-	-	ND	3571	I	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 2	Dry-cured ham	1.80	+	21.3	-	-	-	-	-	-	ND	1603	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 3	Dry-cured ham	3.42	+	6.6	-	-	-	-	-	-	ND	2826	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 5	Dry-cured ham	8.10	+	18	-	-	-	-	-	+	+	309	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	R	S	S	S	ND
	IFIJ 6	Dry-cured ham	1.42	+	14.4	-	-	-	-	-	-	ND	2497	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 7	Dry-cured ham	1.69	+	1.5	-	-	-	-	-	-	ND	1065	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 11	Dry-cured ham	1.37	+	22.2	-	-	-	-	-	-	ND	941	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 14	Dry-cured ham	1.63	+	1.5	-	-	-	-	-	-	ND	798	I	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 16	Dry-cured ham	3.20	+	21.9	-	-	-	-	-	-	ND	0	I	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 19	Dry-cured ham	0.82	+	16.5	-	-	-	-	-	-	ND	449	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 20	Dry-cured ham	13.42	+	21.3	-	-	-	-	-	-	ND	39	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 21	Dry-cured ham	4.30	+	10.8	-	-	-	-	-	-	ND	0	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 22	Dry-cured ham	1.47	+	8.4	-	-	-	-	-	-	ND	3123	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 23	Dry-cured ham	8.58	+	12.9	-	-	-	-	-	-	ND	766	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 25	Dry-cured ham	6.76	+	0.98	-	-	-	-	-	-	ND	1112	R	-	-	-	-	-	-	-	-	S	S	I	I	S	S	S	S	S	S	S	ND
	IFIJ 27	Dry-cured ham	8.07	+	22.5	-	-	-	-	-	-	ND	2294	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 28	Dry-cured ham	7.38	+	22.2	-	-	-	-	-	-	ND	959	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 29	Dry-cured ham	3.01	+	19.8	-	-	-	-	+	-	-	857	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 30	Dry-cured ham	15.26	+	10.5	-	-	-	-	-	-	ND	482	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 33	Dry-cured ham	1.22	+	16.5	-	-	-	-	-	-	ND	1156	I	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 34	Dry-cured ham	5.32	+	12.6	-	-	-	-	-	-	ND	566	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	R	S	S	ND
	IFIJ 35	Dry-cured ham	0.29	+	17.4	-	-	-	-	-	-	ND	1089	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND

(Cont.)

	IFIJ 37	Dry-cured ham	2.35	+	9.3	-	-	-	-	-	-	ND	1393	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 39	Dry-cured ham	0.04	+	0.99	-	+++	-	-	+	-	-	1935	R	-	-	-	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	IFIJ 42	Dry-cured ham	3.11	+	5.1	-	-	-	-	-	-	ND	1681	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	R	S	S	S	S	-
	IFIJ 43	Dry-cured ham	4.76	+	6.3	-	-	-	-	-	+	-	1950	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	R	S	S	S	S	-
	IFIJ 44	Dry-cured ham	0.04	+	1.2	-	-	-	-	-	+	-	4543	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 45	Dry-cured ham	0.07	+	4.8	-	++++	-	-	-	-	ND	1937	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 46	Dry-cured ham	6.13	+	5.6	-	-	-	-	-	++	-	3782	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	R	R	S	S	S	-
	IFIJ 48	Dry-cured ham	0.09	+	1.5	-	-	-	-	-	-	ND	2158	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 49	Dry-cured ham	0.59	+	16.8	-	-	-	-	-	++	-	1432	I	-	-	-	-	-	-	-	-	S	S	S	I	S	S	S	S	S	S	S	ND
	IFIJ 50	Dry-cured ham	1.48	+	18.6	-	-	-	-	-	++	-	1216	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 51	Dry-cured ham	0.04	+	19.8	-	-	-	-	-	++	-	2473	I	-	-	-	-	-	-	-	-	S	S	S	R	S	S	S	S	S	S	S	ND
	IFIJ 53	Dry-cured ham	0.88	+	10.5	-	-	-	-	-	+	-	981	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	R	S	S	S	ND
	IFIJ 55	Dry-cured ham	3.77	+	18.3	-	-	-	-	-	+	-	2182	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 56	Dry-cured ham	0.09	+	13.5	-	-	-	-	-	+	-	963	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
S. hominis	IFIJ 26	Dry-cured ham	1.40	+	21.9	-	-	-	-	-	-	ND	862	I	-	-	-	-	-	-	-	-	R	S	R	R	S	S	R	S	S	S	S	+
S. lugdunensis	IFIJ 47	Dry-cured ham	15.25	+	11.1	-	-	-	-	-	++	+	92	R	-	-	+	+	-	-	+	+	R	S	S	S	S	S	R	S	S	S	S	+
S. vitulinus	IFIJ 4	Dry-cured ham	9.48	+	26.7	-	++	-	-	-	+	-	450	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	R	S	S	ND
	IFIJ 31	Dry-cured ham	0.93	+	16.2	-	++++	-	-	-	-	ND	137	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	R	S	S	S	ND
	IFIJ 36	Dry-cured ham	0.16	+	12.9	-	++	-	-	-	-	ND	343	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 38	Dry-cured ham	3.59	+	6.9	-	++	-	-	+	-	-	243	S	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
	IFIJ 41	Dry-cured ham	1.52	+	15.9	-	++	-	-	-	-	ND	1712	I	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	S	S	S	ND
S. warneri	IFIJ 15	Dry-cured ham	7.46	+	25.8	-	-	-	-	+	-	-	4	R	-	-	-	-	-	-	-	-	S	S	S	S	S	S	S	S	R	S	S	ND
	IFIJ 18	Dry-cured ham	9.83	+	24	-	-	-	-	-	+	-	6	R	-	-	-	-	-	-	-	-	S	S	S	I	S	S	S	S	R	I	S	ND
	IFIJ 52	Dry-cured ham	3.75	+	10.5	-	-	+	-	-	-	ND	0	R	-	-	-	-	-	-	-	-	R	S	S	S	S	S	R	S	R	S	S	+
S. xylosus	AI-126	Dry-cured sausage	2.02	+	1.03	-	-	-	-	-	-	ND	15	R	-	-	-	-	-	-	-	-	R	S	S	S	S	S	R	S	S	I	S	-

[a] Nitrate reductase activity.

[b] Catalase activity. (DM) direct method, (EM) spectrophotometric method

[c] Proteolytic activity. (Gel) gelatinase agar, (CCA) calcium caseinate agar: (-) negative; (+) 0.1-2 mm; (++) 2-6 mm; (+++) 6-10 mm; (++++) >10 mm

[d] Lipolytic activity. BS, "Blue Spirit" agar; 20T, Tween 20; 80T, Tween 80

[e] Biofilm production. (RC) red Congo agar method, (EM) spectrophotometric method: (-) $DO_{595}\leq1$; (+) $OD_{595}\leq2$; (++) $OD_{595}\leq3$; (++++) $OD_{595}>3$. icaA: *icaA* gene-PCR amplification. ND: not determined.

[f] Bile salt resistance. (Rt) retardation in min. (DM) direct method. The number of colonies increases after bile salt treatment: resistant(R) ; the number of colonies keep constant after bile salt treatment: intermedium resistance (I); the number of colonies decrease after bile salt treatment: sensitive (S)

[g] Biogenic amine production. (H) histamine; (T) tyramine; (P) putrescine; (C) cadaverine by TLC or PCR

[h] Antibiotic resistance: (Am) ampicillin; (Clo) chloramphenicol; (Cli) clindamycin; (Ery) erythromycin; (Gen) gentamicin; (Kan) kanamycin; (PeG) penicillin G; (Tet) tetracycline; (Van) vancomycin; (Est) streptomycin; (Cef) cephalotin. R: resistant; I: intermedium resistance; S: sensitive-(NCCLS)

[i] PCR amplification of *blaZ* gene: (+) positive (-) negative (ND) not determined