Available online at www.sciencedirect.com
SCIENCE DIRECT
Meat Science 73 (2006) 559–564
MEAT SCIENCE
www.elsevier.com/locate/meatsci

Characterization of *Staphylococcus xylosus* and *Staphylococcus carnosus* isolated from Slovak meat products

Monika Simonová [a,*], Viola Strompfová [a], Miroslava Marciňáková [a], Andrea Lauková [a], Satu Vesterlund [b], Mariluz Latorre Moratalla [c], Sara Bover-Cid [c], Carmen Vidal-Carou [c]

[a] *Institute of Animal Physiology, Slovak Academy of Sciences, Šoltésovej 4-6, Košice, Slovakia*
[b] *Functional Foods Forum, Pharmacity 5 Krs., Itäinen Pitkäkatu 4A, 20520 Turku, Finland*
[c] *Department of Nutrition and Food Science-CeRTA, Faculty of Pharmacy, University of Barcelona, Av. Joan XXIII 8/n, E-08028 Barcelona, Spain*

Received 18 September 2005; received in revised form 12 December 2005; accepted 8 February 2006

Abstract

The aims of this study were to isolate, identify and characterize the population of coagulase-negative staphylococci in different types of Slovak traditional sausages and to determine the metabolic properties of selected *Staphylococcus xylosus* and *S. carnosus* strains for the selection of potential starter cultures to use in the processing of sausages. The strains were tested for lactic acid production, survival in the presence of bile and sensitivity to antibiotics. Bacteriocin production, adhesion ability as well as biogenic amine (BA) production by isolates were also analysed. Most of the isolates were identified as *S. xylosus* and *S. carnosus*. Lactic acid values ranged from 0.40 to 1.03 mmol/l and strains survived in the presence of 1% bile. Most of the strains studied were sensitive to all antibiotics. Two strains, *S. xylosus* SO3/1M/1/2 and *S. carnosus* SO2/F/2/5 inhibited *Listeria innocua* and *Pseudomonas* sp. *S. xylosus* strains did not produce any BA, while *S. carnosus* SO2/F/2/5 did. *S. xylosus* SO3/1M/1/2 and *S. carnosus* SO2/F/2/5 appeared as the most adhesive strains. *S. xylosus* SO3/1M/1/2 with antimicrobial activity against *Enterococcus avium* EA5, *L. innocua* LMG13568 and *Pseudomonas* sp. SO1/1M/1/4, adhesion ability and free BA production could be used as starter culture in sausage manufacture.

Keywords: *Staphylococcus xylosus*; *S. carnosus*; Fermented sausage; Bacteriocin

1. Introduction

Fermented meat products have been produced for 2000 years. Many countries possess traditional meat products and wish to keep their traditionality. However, the raw materials (meat) can be a source of different microorganisms; which can contaminate the final products. Many microorganisms induce changes in flavour, nutritional quality, texture, safety and other characteristics, depending on their activity. On the other hand, addition of specific microorganisms – starter cultures to meat products is well known and necessary in special types of sausages. The most frequently used starter cultures in meat products are lactic acid bacteria (LAB) in combination with coagulase-negative staphylococci (CNS), such as *Staphylococcus xylosus* and *S. carnosus*. While LAB ensure the safety of products by reducing the pH through fermentation of sugars, CNS influence other technological properties of fermented meat products (Lücke, 1998). Staphylococci play important role in the development of aroma as well as flavour and colour of fermented products (Jessen, 1995). Their ability to reduce nitrate in nitrite, leads to the production of nitrosylmyoglobin; which is important for the characteristic red colour of such products (Skibsted, 1992). Also, catalase activity is important to decompose hydrogen peroxide and to prevent lipid oxidation (Barriére et al., 2001). The production of lipases is a general property of staphylococci and they play a role in flavour development of fermented meat products (Lücke, 1986; Nychas & Arkoudelos, 1990; Talon &

* Corresponding author. Tel.: +421 55 633 6251; fax: +421 55 7287 842.
E-mail address: simonova@saske.sk (M. Simonová).

0309-1740/$ - see front matter © 2006 Elsevier Ltd. All rights reserved.
doi:10.1016/j.meatsci.2006.02.004

Montel, 1997). *S. xylosus* and *S. carnosus* are commonly used lipolytic starter cultures for fermented sausage (Jessen, 1995). Organoleptic quality of meat products is also dependent on the proteolytic activity of the starter cultures. Ability of CNS to produce bacteriocins is also well known (Lauková & Mareková, 1993); this property may be important for the safety of sausages. On the other hand, safety of these products for consumers also depends on the content of biogenic amines (BA), such as histamine, tyramine, phenyletylamine and tryptamine, which might represent a food poisoning hazard (Halász, Baráth, Simon-Sarkadi, & Holzapfel, 1994). The production of BA requires the presence of amino acid-decarboxylating microorganisms, which are usually detected in dry fermented sausages during the fermentation process (Bover-Cid, Schoppen, Izquierdo-Pulido, & Vidal-Carou, 1999).

The purpose of this study was to isolate the strains of *S. xylosus* and *S. carnosus* from Slovak traditional meat products and characterize their metabolic properties: antibiotic sensitivity, tolerance to oxgall bile, lactic acid production, adhesion and amino acid-decarboxylase activity as well as their ability to produce bacteriocins with the aim to select a new optimal starter culture.

2. Materials and methods

2.1. Isolation, bacterial counts and identification of bacteria

Staphylococci were isolated from four traditional Slovak meat products. The strains were selected by standard microbiological methods using appropriate dilutions in Buffered Peptone Water (Biomark, India). Dilutions were plated onto Mannitol Salt agar plates (MSA, Becton & Dickinson, Cockeysville, USA) and incubated at 37 °C for 24 h. After incubation, the colony forming units (cfu) were counted. Then, 187 colonies of staphylococci (including all samples) were randomly picked and maintained on MSA agar for further identification and testing. For DNA preparation, the strains were cultivated on MSA agar at 37 °C for 24 h and checked for purity. DNA from each strain was obtained by the following procedure: one loopfull of bacterial colony (10 μl) was resuspended in 30 μl H_2O and vortexed for 10 min. Then, the supernatants were used as template DNA. One μl of the template was added to 24 μl of the reagent mixture which contained 0.5 μM of each primer, 0.2 mM of each deoxynucleotide (dATP, dTTP, dCTP, dGTP – dNTPs, Invitrogen), 2.5 mM $MgCl_2$ (Invitrogen), 10*x*PCR buffer (Invitrogen), 1.25 U Taq polymerase (Invitrogen), and water to a total volume of 25 μL. The sequences of the primer pairs used for PCR-amplification of staphylococci were as follows: for *S. xylosus* 5′-AAGTCGGTTGAAAACCTAAA-3′ and 5′-CATTGACATATTGTATTCAG-3′, for *S. carnosus* 5′-GAACCGCATGGTTCTGCAA-3′ and 5′-CCGTCAAGGTGCGCATAGT-3′ (Aymerich, Martín, Garriga, & Hugas, 2003). The amplification protocol was as follows: initial denaturation at 96 °C for 3 min, 35 cycles of 95 °C for 30 s, 58 °C for 30 s, 72 °C for 30 s, 72 °C for 5 min. A Techgene KRD thermocycler (Techne, UK) was used for all samples. The PCR products (10 μL of each) were separated by electrophoresis in 0.8% agarose gels (SIGMA, Germany) buffered with 1*x*TAE (Merck, Germany) containing 1 μg/mL ethidium bromide (SIGMA). The molecular mass standard (Promega, USA) was used according to the manufacturer's instructions.

2.2. Sensitivity to oxgall bile, lactic acid production and antibiotic profil

Resistance to bile was tested according to Gilliland and Walker (1990). Brain Heart Infusion broth (BHI, Becton & Dickinson) was prepared by the addition of 1% (w/v) oxgall (Becton & Dickinson). The volume 50 μl of an 18 h culture of each strain was added to 5 ml of BHI broth with oxgall. After incubation at 37 °C for 24 h, the bacterial growth of strains was measured using a spectrophotometer (Spekol 11, Jena, Germany) at 600 nm. Numbers of viable cells were estimated at 0 h and after 24 h of incubation on MSA agar.

The ability to produce lactic acid was measured accordig to Pryce (1969) and expressed in mmol/l.

Antibiotic resistance of isolated staphylococci was tested by the agar disc diffusion method on Columbia agar (Becton & Dickinson) with 10% of defibrinated sheep blood. The following antibiotic discs (Becton & Dickinson) were used: clindamycin (2 μg), erythromycin, methicilin, neomycin (5 μg), ampicillin, tobramycin, lincomycin (10 μg), gentamycin, chloramphenicol, novobiocin, rifampicin, tetracycline, vancomycin and amoxicillin (30 μg). After incubation at 37 °C for 18 h, the strains were classified as resistant or sensitive (by comparing the size of the inhibitory zones in mm).

2.3. Bacteriocin and amino acid-decarboxylase activity of selected isolates

Bacteriocin activity was tested by the agar spot test (De Vuyst, Callevart, & Crabbe, 1996). A cell-free supernatant was prepared by centrifuging 1 mL of a 18 h culture (BHI, Becton & Dickinson) of tested strains (10,000*g* for 30 min). Generally, Brain Heart Infusion supplemented with 1.5% agar (for Gram-positive indicator bacteria) and Trypticase Soy agar (for Gram-negatives, Becton & Dickinson) were used for the bottom agar layer. For overlay, the same types of media (0.7% concentration) of soft agar were prepared. Plates were incubated overnight at 37 °C. Principal indicator bacteria *Enterococcus avium* EA5 (our isolate from piglets), *S. aureus* SA5 (our isolate from cow milk), *Listeria innocua* LMG13568 (supplied by Prof. L. DeVuyst, University of Brussel, Belgium), *L. monocytogenes* CCM4699 (Czech Collection of Microorganisms, Brno, Czech Republic), *Pseudomonas* sp. SO1/1M/1/4 and *Escherichia coli* (our isolates from fermented meat products) were used for bacteriocin activity determination. Brain Heart