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ScienceDirect

Meat Science 77 (2007) 204–212

MEAT SCIENCE

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Characterization and technological properties of *Staphylococcus xylosus* strains isolated from a Tunisian traditional salted meat

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Received 28 November 2006; received in revised form 3 March 2007; accepted 4 March 2007

Abstract

The technological properties of strains of *Staphylococcus xylosus* were studied to select the most suitable for use as starter cultures for the production of dried fermented meat products. Strains of *S. xylosus* were isolated from traditional salted Tunisian meat and were identified by biochemical and molecular methods. Thirty strains of *S. xylosus* were studied to evaluate their catalase, nitrate reductase, lipolytic, proteolytic and antibacterial activities as well as growth ability at different temperatures, pH's and NaCl concentrations. All strains of *S. xylosus* had catalase activity and were able to reduce nitrates to nitrites. The nitrate reductase activity increased when the strains were kept under anaerobic conditions. Proteolytic activity on milk and on gelatin agar was demonstrated for 100% and 83.3% of the *S. xylosus* isolates, respectively. However extracellular proteolytic activity as assessed by the azocasein method was poor in all the strains. Lipolytic activity as assessed by the agar method showed that 76.6% of strains of *S. xylosus* could hydrolyze Tween 20 against 33.3% that could hydrolyze tributyrin. Tween 80 was hydrolyzed by only 10% of strains. Strains of *S. xylosus* hydrolyzed pork fat better than beef and lamb fat. The majority of strains had antibacterial activity against *Salmonella arizonae*, *Staphylococcus aureus*, *Pseudomonas aeuroginosa*, *Escherichia coli* and *Enterococcus faecalis*.

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Keywords: Traditional salted meat; *Staphylococcus xylosus*; Enzymatic activities; Technological properties

1. Introduction

Meat conservation has used many methods including drying, salting, curing, heating and fermentation. In the past, fermentation of meat was carried out spontaneously by the natural microflora of the raw meat and environment. Many countries have traditional meat products and want to keep their characteristics; however, uncontrolled fermentation may produce products which are either inferior or even unsafe for consumption.

Thus the use of starter cultures for the production of fermented foods is becoming necessary to guarantee safety and standardize properties. In fact, starter cultures offer the possibility of controlling the fermentation process and inhibiting undesirable microorganisms (Spyropoulou, Chorianopoulus, Skandamis, & Nychas, 2001). The most commonly used starter cultures in fermented meats are the lactic acid bacteria (LAB) which are responsible for the rapid fermentation of carbohydrates added to the mixture, leading to a decrease in pH and thus prevention of spoilage. This acidification below the isoelectric point of

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doi:10.1016/j.meatsci.2007.03.003

muscle proteins causes reduced water-binding-capacity (Miralles, Flores, & Perez-Martinez, 1996). In addition with LAB, coagulase-negative staphylococci (CNS) such as *Staphylococcus xylosus* and *Staphylococcus carnosus* may be used to improve the flavor of the fermented meat. Berdagué, Monteil, Montel, and Talon (1993) suggested that CNS, rather than lactic acid bacteria, could have the predominant effect on dry sausage aroma. *S. xylosus* is the dominating CNS species isolated in many Italian sausages (Cocolin, Manzano, Aggio, Cantoni, & Comi, 2001; Rossi, Tofalo, Torriani, & Suzzi, 2001) and in Spanish chorizo (Garcia-Varona, Santos, Jaime, & Rovira, 2000) whereas, *S. carnosus* is the dominating species in traditional Greek sausages (Papamaloni, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002).

CNS plays an important role in the development of aroma as well as flavor and color of fermented meat products (Jessen, 1995). They participate in the development and stability of a desirable red color because of their nitrate reductase activity that leads to the formation of nitrosomomyoglobin. In addition, nitrate reduction produces nitrites that limit lipid oxidation by (i) binding heme and preventing release of catalytic iron, (ii) binding non-heme iron thus inhibiting catalysis, (iii) and/or stabilizing olefinic lipids against oxidation (Talon, Walter, Chartier, Barière, & Montel, 1999). Also the catalase and superoxide dismutase activities of CNS are important in decomposing hydrogen peroxide and thus preventing lipid oxidation (Barriére et al., 2001). Various aromatic substances and organic acids are released by the protease and lipase activity of CNS which have considerable roles in the development of the flavor of fermented meat due to the formation of low molecular weight compounds, including peptides, amino acids, aldehydes, amines and free fatty acids (Simonovà et al., 2006). Bacteriocins released by CNS also have some activity against pathogenic bacteria, such as enterohemorrhagic *Escherichia coli*, *Salmonella* (Papamaloni et al., 2002).

The aim of this work was to isolate strains of *S. xylosus* from a Tunisian traditional salted meat and to study some of their technological and safety properties in order to select the most suitable strains for use as starters in the production of a dry fermented meat product.

2. Materials and methods

2.1. Isolation of strains

Staphylococci strains were isolated from 10 Tunisian traditional salted dried meat samples "Kadid". These samples were taken from 10 different regions of Tunisia. "Kadid" is produced by cutting bovine meat in little slices, then adding ingredients (salt, garlic, olive oil) followed by drying of the sample in the sun for seven days.

Strains of staphylococci were isolated on Mannitol Salt Agar (Pronadisa, Madrid, Spain) after incubation at 30 °C for 48 h. Morphology, Gram-staining and coagulase tests were carried out to isolate only Gram-positive and coagulase-negative cocci. Staphylococci and micrococci were differentiated by lysostaphine, bacitracine and lysozym tests (Kloos & Bannerman, 1995). CNS were then identified by the API ID 32 STAPH system (BioMérieux, France).

2.2. Identification of S. xylosus isolates by a species-specific PCR

2.2.1. DNA extraction

DNA extraction was carried out as described by Soumet, Ermel, Fach, and Colin (1994): strains were grown in Tryptone Soy Broth (TSB) (Pronadisa, Madrid, Spain) for 24 h at 37 °C, the cells were harvested by centrifugation at 12,000*g* for 5 min. The pellets were suspended in 200 μl doubly distilled sterile water. After heating at 100 °C for 15 min, the tubes were placed on ice for 5 min, then centrifuged at 12,000*g* for 10 min. Finally the supernatant (DNA extract) was stored at −20 °C.

Strains of *S. xylosus*, specified by biochemical methods (API ID 32 STAPH system), were identified by means of species-specific PCR as described by Rantisou, Iacumin, Cantoni, Comi, and Cocolin (2005).

The sequences of the primers for the specific amplification of the *S. xylosus* strains were: *geh3* (5′-GTA GAA AAA GCG AAT GAA CAA C-3′) and *geh4* (5′-CCT GGT TGC CAA TCT TTA TAT AC-3′) designed on the basis of the *gehM* gene coding for the lipase of *S. xylosus* (accession number AF208229). PCR was performed in a final volume of 50 μl containing 2.5 μl of PCR buffer, 200 μM of each dNTP, 0.4 μM of each primer, 1 U of TAQ polymerase (Invitrogen, USA). The mixture was subjected to 30 cycles of amplification (GeneAmp 2700 Thermocycler, Applied Biosystems, Foster City, CA, USA) with the following parameters: initial denaturation at 95 °C for 1 min, 30 cycles of denaturation at 94 °C for 1 min, annealing at 55 °C for 1 min and extension at 72 °C for 1 min; ending with a final extension at 72 °C for 5 min. The size and the quality of DNA was checked by 1% agarose gel electrophoresis in TBE buffer (40 mM Tris-borate, 89 mM boric acid, 2 mM EDTA; pH 8) with a 100 bp DNA ladder (Invitrogen, USA). The gel was stained with ethidium-bromide and the bands were visualized under UV light.

2.3. Effect of temperature, pH and salt concentrations on microbial growth

Strains of *S. xylosus* were tested for growth ability at different temperatures, pHs and NaCl concentrations. Growth was evaluated at 10, 15 and 20 °C in TSB broth. The effect of pH on microbial growth was evaluated in TSB broth adjusted to pH values of 4, 5 and 5.5 by addition of HCl (0.1 N). Finally the effect of NaCl was determined in TSB broth supplemented with 10%, 15% and 20% NaCl. 1 ml of an overnight culture of each strain ($OD_{600} = 1$) was inoculated in 10 ml of the different media described above and growth was evaluated by the difference between the OD_{600} at time 0 and after 24 h incubation at 37 °C.

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