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Technological activities of *Staphylococcus carnosus* and *Staphylococcus simulans* strains isolated from fermented sausages

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Abstract

The aim of this study was to determine the technological properties of 2 strains of *Staphylococcus simulans* (Ssm12, Ssm21) and 4 strains of *S. carnosus* (SC28, SC31, SC54 and SC55) for the selection of a potential starter cultures to employ in the processing of dry fermented sausages. The strains were studied to evaluate nitrate reductase, proteolytic, lipolytic, decarboxylase and antioxidant activities as well as growth ability at different temperatures, pH and NaCl concentrations. Nitrate reductase activity was determined at 15, 20 and 30 °C. By spectrophotometric method all the strains were able to reduce nitrate to nitrite at the different temperatures but these results were not confirmed by the agar plate method. Antioxidant and lipolytic activities were evaluated by spectrophotometric assay. All the strains showed antioxidative enzymes superoxide dismutase (SOD) and catalase whereas all appeared unable to hydrolyse pork fat. Proteolytic activity was determined by agar plate method, spectrophotometric assay (OPA) and sodium dodecyl sulphate gel-electrophoresis (SDS–PAGE) and all strains appeared to be able to hydrolyse sarcoplasmic proteins but not myofibrillar proteins. Finally, all the strains grew at 15 and 20 °C, in presence of 10%, 15% and 20% of NaCl and at pH 5.0 and 5.5 and were unable to produce histamine, cadaverine and putrescine. The results showed that all strains studied possess useful technological activities that would make them eligible as a good starter cultures for fermented sausages.

Keywords: *Staphylococcus carnosus*; *Staphylococcus simulans*; Technological properties; Dry fermented sausages

1. Introduction

Coagulase negative staphylococci (CNS) are commonly found as natural flora of fermented meat products, in relatively high number also in dry fermented sausages produced without addition of starter cultures (Miralles, Flores, & Perez-Martinez, 1996).

CNS play a major role in the development of sensory properties of fermented sausages, by reduction of nitrates to nitrite and then to nitrous oxide, by preventing rancidity through peroxide decomposition, and by producing flavour and aroma compounds through proteolysis and lipolysis (Hammes & Hertel, 1998; Sondergaard & Stahnke, 2002).

Staphylococcus xylosus is the dominating CNS species in many Italian sausages (Cocolin, Manzano, Aggio, Cantoni, & Comi, 2001; Rossi, Tofalo, Torriani, & Suzzi, 2001) and in the Spanish sausage Chorizo (García-Varona, Santos, Jaime, & Rovira, 2000). *S. saprophyticus* and *S. carnosus* the dominating species in traditional Greek sausages (Papamanoli, Kotzekidou, Tzanetakis, & Litopoulou-Tzanetaki, 2002; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998) and in Naples type salami (Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000). Many other CNS species (*S. haemolyticus*, *S. warneri*, *S. equorum*, *S. cohnii*, *S. epidermidis*,

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S. hominis, *S. capitis*, *S. intermedius*) have been reported to occur in one or more sausage types.

Staphylococcus species are commonly used as starters cultures for fermented sausages. Actually Italian law permits the use of *S. xylosus*, *S. carnosus* and *S. simulans* as starter for fermented sausage production (Repubblica Italiana, 1995).

Among starters, only few data are available in the literature on technological properties of *S. simulans* and *S. carnosus* and little attention has been paid to their contribution in sausages fermentation (Sondergaard & Stahnke, 2002; Stahnke, Holck, Jensen, Nilsen, & Zanardi, 2001). Moreover, as some CNS can possess decarboxylase activities and consequently deteriorate the hygienic quality and safety of the meat product, the absence of biogenic amines formation represent an essential selection criterion for starter cultures (Straub, Kicherer, Schilcher, & Hammes, 1995).

The aim of this work was to study some of the technological and safety properties of 2 *S. simulans* and 4 *S. carnosus* strains in order to select the most suitable as starters for fermented dry sausage production.

2. Materials and methods

2.1. Bacterial strains and culture conditions

Four strains of *S. carnosus* and two strains of *S. simulans* (Table 1) isolated from traditional fermented sausages from Vallo Diano (Campania region–Italy) were used in this study. The strains were isolated and identified as reported previously (Mauriello, Casaburi, Blaiotta, & Villani, 2004) Briefly, Micrococcaceae present in dry fermented sausages were enumerated on Mannitol Salt Agar (MSA, Oxoid) after 48 h at 30 °C. Colonies from countable plates were initially tested for morphology, Gram-stain and catalase production. Gram-positive and catalase-positive cocci were purified by streaking on MSA and maintained on P-agar (Phillips & Nash, 1985) slants stored at 4 °C. They were subjected to the oxidation/fermentation test in OF medium and to the anaerobic growth in semisolid thioglycollate medium (Evans & Kloos, 1972). Sensitivity to furazolidone, bacitracin and lysostaphin was determined as described by Kloos and Bannerman (1995). Production of pigment was observed on P-agar. Staphylococci were assayed for coagulase activity using the tube test with coagulase plasma (Becton, Dickinson & Company, NJ, USA) and for novobiocin sensitivity (Kloos, Tornabene, & Schleifer, 1974). Other biochemical properties were studied using API Staph identification strips and API LAB Plus software according to the manufacturer's instructions (API, Biomérieux System).

Species-specific PCR assays were used to confirm the species identifications as reported in previous study (Blaiotta, Casaburi, & Villani, 2005). Briefly, DNA extraction was carried out from a single colony by using an Insta-Gene Matrix (Bio-Rad Laboratories, Hercules, CA) and 5 μl (about 25 ng) were used for PCR amplifications.

Primers couples simF–simR (ATCCTTTCACCTAACTCTGAAGAG and GTAATTGGGTTGTCTTGGTTTGCT) and carF–carR (CTTCTAACACCTAATTCTGAAGA and GATTGGATTGTCTTGGTTAGGA) were used to amplify specific-*sod* A gene fragments of *S. simulans* and *S. carnosus*, respectively. Moreover, specificity of the amplified fragments were further verified by restriction endonucleases analysis of the PCR products by *Eco*RI and *Dra*I restriction enzymes, respectively. Working cultures were grown overnight at 30 °C in Tryptone Soya Broth (TSB Oxoid Ltd, London, England) and were maintained on P-agar (Phillips & Nash, 1985) slants stored at 4 °C.

2.2. Detection of nitrate reductase activity

2.2.1. Agar plate method

Nitrate reductase activity was determined as described by Miralles et al. (1996) on YTA (1.0% tryptone, 0.5% yeast extract, 1.5% agar, pH 7.0) supplemented with 1 g l^{-1} KNO_3. The cell pellet of an overnight culture was resuspended in 10 ml of 50 mM phosphate buffer pH 7.0 and 30 μl loaded into wells (6 mm diameter) bored in YT agar plates. After incubation at 30 °C for 7 h and at 20 and 15 °C for 24 and 72 h, respectively, the plates were flooded with 1 ml of a 1:1 solution of NIT1 (0.8 g of sulphanilic acid in 100 ml of 5 N acetic acid) and NIT 2 (0.6 g of *N*-*N*-dimethyl-1-Naphthylamine in 100 ml of 5 N acetic acid) for the detection of nitrite. The appearance of red haloes surrounding the wells indicate the presence of nitrate reductase activity.

2.2.2. Spectrophotometric assay

Ten millilitres of YT-broth (YTA without agar) supplemented with 250 ppm of KNO_3 were inoculated with 100 μl of an overnight culture of each strain. After incubation at 30 and 20 °C for 24 h and at 15 °C for 72 h a fraction of overnight cultures was used for the determination of dry weight while another fraction was used to detect nitrate reductase activity by spectrophotometric assay according to Gerhardt, Murry, Willis, and Krieg (1994). One hundred microlitres of each overnight culture were added to 250 μl of Griess I (0.5 g of sulphanilic acid in 150 ml of 5 N acetic acid), 250 μl of Griess II (0.5 g of Naphthylamine in 50 ml of distilled water and 100 ml of 5 N acetic acid) and 2 ml of distilled water and incubated at room temperature for 15 min after shaking in a vortex for 1 min. Nitrite production from nitrate was determined by reading optical density (OD) at 540 nm. Relative activity was calculated as the rate: $OD_{540\ nm}$/mg dry weight.