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Molecular and physiological characterization of natural microbial communities isolated from a traditional Southern Italian processed sausage

Federico Baruzzi *, Alessandra Matarante, Leonardo Caputo, Maria Morea

Institute of Sciences of Food Production, Italian National Research Council (CNR), Via G. Amendola 122/o, 70126 Bari, Italy Received 9 January 2005; received in revised form 24 June 2005; accepted 22 July 2005

Abstract

The development of the natural microbial populations during traditional processing of the "Salame di Senise", a typical little known sausage produced in the South of Italy, was investigated by using molecular and physiological techniques for taxonomic identification and technological characterization of strains.

The application of RAPD-PCR over more than 90 colonies made it possible to isolate 18 bacterial and two yeast biotypes identified by partial rDNA sequencing as belonging mainly to three species of *Bacillus*, three species of *Lactobacillus*, three species of *Staphylococcus* and *Debaryomyces hansenii*. The physiological analyses revealed that the isolates belonging to *Lactobacillus* genus were the most acidifying, whereas *Staphylococcus* strains did not develop significant proteolytic and lipolytic activities. Interestingly, some *Bacillus* strains produced the highest values of proteolytic and lipolytic activities. The results for the technological properties of *Bacillus* strains isolated from this Southern Italian sausage, made without a selected starter, suggest that *Bacillus* strains, always present in meat curing, could play a role in the development of texture and organoleptic characteristics of the sausages. © 2005 Elsevier Ltd. All rights reserved.

Keywords: Natural microbial community; Cured sausage; Proteolytic-lipolytic activities; Bacillus; Lactobacillus; Staphylococcus; Debaryomyces hansenii

1. Introduction

The data regarding dry cured sausages show that Germany, Italy, Spain and France rank as the biggest producers and consumers of sausage (Lücke, 1998). In Italy, the meat-based processing sector is made up of more than 3500 companies, including those turning out artisanal products which continue to hold their ground in the production of traditional Italian foods (Istituto per la Valorizzazione dei Salumi Italiani).

In recent years, the microflora of many Southern European traditional sausages have been characterized. The fermenting microflora of "Fuets" and "Chorizos", two low-acid cured Spanish sausages, is constituted by Lactobacillus sakei, L. curvatus, L. plantarum, Staphylococcus xylosus, S. carnosus, and S. epidermidis depending on the sausage type (Aymerich, Martín, Garriga, & Hugas, 2003). Also two types of naturally fermented Greek dry sausages showed a large presence of L. sakei, L. curvatus, L. plantarum, S. saprophyticus, and S. xylosus strains, but sporadic isolates of Weissella viridescens, Enterococcus faecium, E. faecalis, Leuconostoc pseudomesenteroides, and Pediococcus sp. were also detected (Papamanoli, Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003; Samelis, Metaxopoulos, Vlassi, & Pappa, 1998; Tzanetakis, Litopoulou-Tzanetaki, & Kotzekidou, 2003).

Abbreviations: PCR, polymerase chain reaction; RAPD, randomly amplified polymorphic DNA; LAB, lactic acid bacteria.

^c Corresponding author. Tel.: +39 80 5929319; fax: +39 80 5929374. *E-mail address:* federico.baruzzi@ispa.cnr.it (F. Baruzzi).

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In Italy, many fermented meat products are still made with traditional technologies without selected starters, resulting in a wide range of sausages with different flavours, consistencies, and microbiological quality (Coppola, Mauriello, Aponte, Moschetti, & Villani, 2000; Zambonelli, Papa, Romano, Suzzi, & Grazia, 1992).

The microflora present in some Southern Italian traditional fermented sausages are dominated by *L. sakei*, *L. bavaricus, Micrococcus kristinae, S. xylosus* and *S. saprophyticus* (Blaiotta et al., 2004; Coppola, Giagnacovo, Iorizzo, & Grazia, 1998; Coppola, Iorizzo, Saotta, Sorrentino, & Grazia, 1997; Coppola et al., 2000).

In Basilicata, a region in Southern Italy, different kinds of traditional fermented sausages are produced without microbial starters (Amato, Di Gennaro, Lanorte, Caruso, & Suzzi, 1997; Parente, Di Matteo, Spagna Musso, & Crudele, 1994; Parente, Greco, & Crudele, 2001; Parente, Ricciardi, & Crudele, 1993). These products are obtained from pigs bred locally; minced fresh meat is mixed with other ingredients, such as NaCl, dried vegetables and additives (nitrate, nitrite, and spices).

In the recent past, in the heart of Basilicata pigs browsed in the Mediterranean maquis feeding off fruits and tubers of the underbrush. A particular sausage named "Pezzente della Montagna Materana" (Beggar of the Mountains of Matera) was obtained from the native breed pig, Black of Lucania, a dying species; this sausage was produced using less noble parts of pork like throat, harder muscles, stomach and residual fats from previous processings. Powder of the pepper of Senise, wild fennel, garlic and salt were added to the minced meat. Today the use of discarded parts of pork for sausage processing is no longer carried out (Sardo, Milano, & Ponzio, 2004).

"Salame di Senise" is a traditional Southern Italian dry sausage manufactured in some small-scale sausage factories throughout the Sinni Valley in the Basilicata region. The processing is based on the experience and skill of local manufacturers, rather than on scientific and technological know-how. The main characteristic of this cured sausage is the addition of powder from a locally produced pepper, named "Peperone di Senise". The "Peperone di Senise" (Capsicum annuum cv. Senise) is a bell-shaped, thin-skinned red pepper with a low water content and a distinctive mild taste. This vegetable grows only in the Sinni and Agri valleys and was added to the list of products with protection of geographical indications, PGI, (EC Regulation No. 1263/96). The absence of a selected starter and the addition of significant amounts of un-pasteurized spices, lead us to suppose that the contribution of naturally occurring microflora is essential to ensure safe, palatable products. Distribution of this premium food product outside the Italian borders is hampered by the difficulties inherent in controlling the fermentation and curing processes. During the last 30 years, much attention has been focused on the use of starter cultures to guarantee safety and standardize product properties, including consistent flavour and colour and shorter ripening time. A wide variety of microorganisms, mainly lactic acid bacteria (LAB) and *Staphylococcus* and *Kocuria* spp., have already been isolated from sausage fermentations and have been selected for metabolic activities especially suited for fermentation in meat ecosystems (Coppola et al., 2000; del Carmen de la Rosa, Mohino, Mohino, & Mosso, 1990; Hugas, Garriga, Aymerich, & Monfort, 1993), improving the quality and safety of the final product (Metaxopoulos, Genigeorgis, Fanelli, Franz, & Cosma, 1981; Nychas & Arkoudelos, 1990).

Recently, various molecular typing methods such as restriction fragment length polymorphism (RFLP), pulsed-field gel electrophoresis (PFGE), ribotyping and PCR-derived techniques such as repetitive extragenic palindromic (REP-) and enterobacterial repetitive intergenic consensus (ERIC-) PCR have been used to distinguish between isolated bacterial biotypes. Moreover, RAPD analysis has been used to estimate the diversity among several genera of bacteria such as *Lactobacillus*, *Bacillus*, and *Staphylococcus* isolated from many sources and 16S rDNA sequence analysis has made the taxonomic identification of biotypes feasible (Morea, Baruzzi, Cappa, & Cocconcelli, 1998; Morea, Baruzzi, & Cocconcelli, 1999; Rebecchi, Crivori, Sarra, & Cocconcelli, 1998).

The increasing interest in preserving the biodiversity of the microorganisms involved in the production of traditionally fermented food products led us to investigate the natural microbial association that brings about the curing of "Salame di Senise".

Randomly amplified polymorphic DNA (RAPD), partial rDNA sequence analysis, and physiological assays were used to characterize the growth and the changes of dominant microbial populations during "Salame di Senise" curing. The physiological characterization of the strains contained in this typical sausage such as acid production, proteolytic and lipolytic activities and nitrate reduction was carried out to gain an insight into the role played by microbial strains belonging to different genera in meat fermentation and curing.

2. Materials and methods

2.1. Production technology and sampling

The sausages were produced by a local craft plant in Tursi according to the traditional method (without the adjunct of starter cultures). The typical protocol for production entails the addition of NaCl (28 g kg⁻¹), seeds of wild fennel (*Foeniculum sylvestre*) harvested from wild plants (1–3 g kg⁻¹) and powder from the local pepper cultivar "Peperone di Senise" (8–12 g kg⁻¹) to the minced

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