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Review

# Biodiversity and dynamics of meat fermentations: The contribution of molecular methods for a better comprehension of a complex ecosystem

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ABSTRACT

The ecology of fermented sausages is complex and includes different species and strains of bacteria, yeasts and molds. The developments in the field of molecular biology, allowed for new methods to become available, which could be applied to better understand dynamics and diversity of the microorganisms involved in the production of sausages. Methods, such as denaturing gradient gel electrophoresis (DGGE), employed as a culture-independent approach, allow to define the microbial dynamics during the fermentation and ripening. Such approach has highlighted that two main species of lactic acid bacteria, namely *Lactobacillus sakei* and *Lb. curvatus*, are involved in the transformation process and that they are accompanied by *Staphylococcus xylosus*, as representative of the coagulase-negative cocci. These findings were repeatedly confirmed in different regions of the world, mainly in the Mediterranean countries where dry fermented sausages have a long tradition and history. The application of molecular methods for the identification and characterization of isolated strains from fermentations highlighted a high degree of diversity within the species mentioned above, underlining the need to better follow strain dynamics during the transformation process. While there is an important number of papers dealing with bacterial ecology by using molecular methods, studies on mycobiota of fermented sausages are just a few. This review reports on how the application of molecular methods made possible a better comprehension of the sausage fermentations, opening up new fields of research that in the near future will allow to unravel the connection between sensory properties and co-presence of multiple strains of the same species.

**Contents**

## 1. Introduction

Meat fermentations are challenging microbial ecosystems in which bacteria, yeasts and molds coexist. A great diversity can be revealed, including not only several species belonging to different genera, but also strains of the same species, that participate in the fermentation process. Through fermentation, perishable raw materials, such as meat and fat, supplemented with sodium chloride and subjected to a drying process, are transformed in microbiologically stable final products, characterized by a defined sensory profile.

In meat fermentations, different groups of microorganisms, possessing different biochemical potentials, contribute for the formation of the sensory profile of the final product. The acidification process, in which sugars, such as glucose, lactose and sucrose, are transformed in lactic acid, thereby reducing the pH of the meat and creating a hostile environment for pathogenic bacteria, is the main activity of lactic acid bacteria (LAB) (Ammor & Mayo, 2007). These microorganisms may also be able to produce proteinaceous compounds, called bacteriocins (De Vuyst & Leroy, 2007), active against closely related bacteria, including pathogens such as *Listeria monocytogenes*, thereby increasing the competitiveness of the producer cells and the safety of the final products.

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Other bacteria relevant for the transformation process are the coagulase-negative cocci (CNC). They participate in the development and stability of a generally appreciated red color through nitrate reductase activity that eventually leads to the formation of nitrosomyoglobin. Furthermore, nitrate reduction produces nitrite that can limit lipid oxidation (Talon, Walter, Chartier, Barriere, & Montel, 1999).

LAB and CNC are also responsible for flavor generation through proteolysis and lipolysis, although it should be pointed out that the process involved in aroma formation is very complex and it has been recently reviewed in great detail elsewere (Toldrà, 2008).

Lypolytic and proteolytic activities are also associated to yeasts and molds. In fermented sausages, yeasts are preferably found internally, while molds are only present on the surface where there is availability of oxygen. Yeasts have been described as powerful proteolytic agents (Santos et al., 2001), and their contribution to the volatile compound production in salchichón was recently reported (Andrade, Córdoba, Casado, Córdoba, & Rodríguez, 2010). Molds participate in the transformation process thanks to their ability to produce lipases and proteases, thereby enhancing the final organoleptic characteristics. Moreover due to their capability to create micro-pores on the casing, they facilitate the dehydration process. Lastly, growing as an homogeneous layer on the surface of the sausage, they also protect lipids from oxidation in the presence of light (Incze, 2004).

The microbial activity and interaction are a key factor for the final quality characteristics of fermented sausages. As described by Lücke (2000), LAB are the main responsible for the production of semi-dried products sold after less than 2 weeks ripening, which are characterized by an acid flavor. When longer ripening times are employed, there is a greater diversity and activity of microorganisms, which lead to higher levels of volatile compounds with low sensory thresholds, resulting in products with more rich organoleptic profile.

In this review, the most recent advancements in the identification, characterization and dynamics of the microorganisms involved in the fermentation of sausages, as unraveled by molecular methods, will be presented. Understanding the microbial biodiversity and ecology can allow a better control of the transformation process, resulting in products with high quality and safety and unique sensory characteristics.

## 2. Molecular approaches in sausage fermentation

In the last 20 years the advancement in molecular biology has revolutionized the way research is carried out. Today it is possible to detect, identify and quantify microorganisms by targeting their nucleic acids. Moreover, there are methods that can be implemented in research laboratories, able to differentiate strains belonging to the same species, opening up new area of interest in which specific strain dynamics during fermentation can be studied. This last aspect is receiving much attention in the field of food fermentations because it allows to understand if a strain inoculated as starter culture is able to dominate the transformation process. In the case of naturally fermented sausages, strain(s) capable to drive the fermentation can be identified, therefore facilitating development of autochthonous starters.

Nucleic acids can be analyzed either from isolates obtained from the food matrix by traditional microbiological methods, or more recently their direct extraction from the food sample has been proposed. In the first case, the approach is considered culture-dependent while the second case is culture-independent. There is a scientific consensus on the fact that culture-dependent methods are not able to properly describe the diversity of complex ecosystems (Hugenholtz, Goebel, & Pace, 1998): populations that are present in low numbers or that are in a stressed or injured state will most probably be unintentionally excluded from consideration if traditional microbiological methods are used. Moreover, cells that are in a viable but not culturable (VNBC) state will not be detected, because of their incapability to form colonies on microbiological media (Cocolin, Dolci, & Rantsiou, 2008). The use of culture-dependent methods, relying on the cultivation of the microorganisms can produce a picture of the ecology of fermented sausages, which is not totally correct, because of the biases inherent to the different capabilities of the microorganisms to grow on the synthetic media used in the laboratory. An example of the use of culture-dependent and -independent molecular methods in the study of the microbiota of fermented sausages is presented in Fig. 1. After the homogenization of the fermented sausage sample, researchers can either approach the study of its microbiota by applying traditional microbiological methods, that often include cultivation on synthetic media and after growth, isolation of individual colonies, or a direct extraction of the nucleic acids. It should be pointed out that also culture-dependent methods can exploit molecular approaches, however these are used to characterize isolates that have grown on agar media. In the last 10 years, a number of evidences have been produced highlighting that often, there are significant differences between the results obtained with culture-independent and -dependent methods. It is now accepted that such

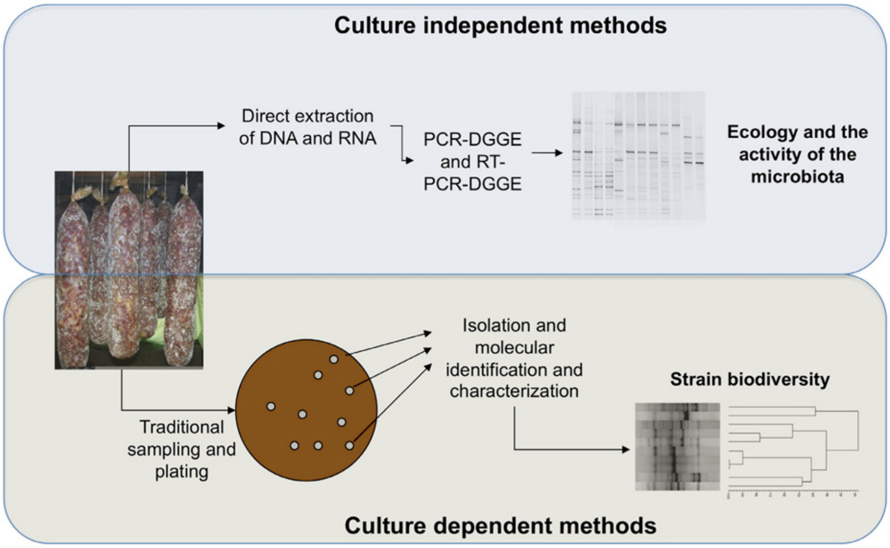

**Fig. 1.** Schematic representation of the molecular approaches used in the study of the ecology of fermented sausages.