

## IDENTIFICATION OF MICROFLORA ISOLATED FROM PORTUGUESE DRY SAUSAGE - "SALPICÃO"

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### Summary

Twenty strains of lactic acid bacteria (LAB) and twenty strains of *Micrococcaceae* were isolated from a portuguese traditional sausage "salpicão". The strains of LAB were identified by carbohydrate fermentation tests, configuration of lactic acid, production of gas from glucose, growth in acetate agar, citrulline production from arginine and detection of *meso*-Diaminopimelic acid. The strains of *Micrococcaceae* were identified by using API ID 32 Staph, by determining their resistance to lysostaphin and lysozyme. The strains of LAB were identified as *Pediococcus pentosaceus*, *Lactobacillus plantarum*, *L. curvatus*, *L. sake*, *L. pseudopantarum* and one group of heterofermentative rods belonging to *L. brevis/buchneri* group. The strains of *Micrococcaceae* were identified as *Micrococcus kristinae*, *M. luteus*, *M. roseus*, *M. varians*, *Staphylococcus xylosum*, *S. warneri*, *S. saprophyticus*, *S. auricularis* and two other strains belonging to *S. epidermidis* or *S. hominis*.

### Introduction

Portugal has a large variety of traditional meat products, still made on the basis of tradition and empirical experience. Those products are traditionally kept without refrigeration, due to its reduced water activity (aw). Only in a few products, might the pH have a

synergistic effect with the aw, to achieve stability (Martins *et al.*, 1987; Martins and Patarata, 1991). Among the various types of meat products, there is the "Salpicão", which is not fermented, as are the north Europe fermented sausages, since no sugar or starters are added. However, it presented low values of pH ( $5.28 \pm 0.28$ ), and a high counting of lactic acid bacteria (LAB) ( $8.7 \pm 1.1$  log c.f.u./g) and *Micrococcaceae* strains ( $3.9 \pm 2.7$  log c.f.u./g) (Martins and Patarata, 1991).

According to Leistner (1990), excellent fermented sausages can be manufactured without starters, if the ripening conditions are suitable for maintenance of the LAB and *Micrococcaceae* strains present in the raw material. The manufacturing process of "salpicão" appears to be favorable to the microflora, as the mixture is matured at a low temperature for a few days (5 to 10) before stuffing. Afterwards, the sausage is subject to the drying effect of the smoke, at the room temperature; finally, it is kept at a low temperature for a few months.

The microflora naturally present in "salpicão", might have an important role on its organoleptic characteristics, and contribute to increase its stability, shelf life and assurance of the consumer (Bacus, 1986). However, the use of selected starters is generally beneficial for the quality of fermented sausages with respect to the standardization and stability of the product (Leistner, 1987).

The aim of the present study was to establish the nature of the LAB and *Micrococcaceae* isolated from salpicão, so that, information concerning their possible effect in the manufacturing of salpicão, and their ability to be used in a starter, could be obtained in subsequent studies.

## Material and methods

**Bacterial strains:** 298 strains were isolated on MRS medium (Oxoid) modified according to Ribeiro *et al.* (1967) and 169 strains were isolated on mannitol salt agar (Difco), from 35 samples of "Salpicão". They were kept at  $-20^{\circ}\text{C}$  in MRS broth and in tryptone meat

broth (tryptone 0.05%, meat extract 0.1%) with 15% glycerol. 20 strains of each group were randomly drawn, in order to identify them on the basis of physiological and biochemical tests

### **Physiological and biochemical characteristics**

**LAB:** Production of gas from glucose was performed by the method of Sperber & Swan (1976); Growth in acetate agar was evaluated on Bacto Rogosa SL agar (Difco) cultivated with a loop of cells solution in 0.85% NaCl (Mac Farland 2) incubated for 48 h; The configuration of lactic acid was determined spectrophotometrically in the supernatant fluids of 24 h cultures by an enzymatic method using (D) or (L) lactate dehydrogenase (Boehringer, Mannheim); Citrulline production from arginine was determined with Niven' medium with two concentrations of glucose (0.03% and 2%), according to Montel and Champomier (1987); *meso*-Diaminopimelic acid (mDAP) was detected in whole cell hydrolysates by thin layer chromatography as described by Bousfield *et al.* (1985); The acid production from carbohydrates was tested using the API 50 CH System (API, Biomerieux), according to the manufacturer's instructions.

***Micrococcaceae:*** Resistance to lysostaphin and lysozyme was performed as described by Schleifer and Kloos (1975); The configuration of lactic acid was determined as described for LAB; The pigment production was detected in P agar medium (Kloos *et al.*, 1974) and observed after 4 days of incubation; The API ID32 Staph System (API, Biomerieux), was used according to the manufacturer's instructions.

### **Computer analysis**

The data were examined with STAT.I.T.C.F. software by Hierarchical Ascendent Classification by measuring Euclidian distance and using pondered distance means as aggregation criteria. The strains of LAB were compared with the reference strains from API CH (Biomerieux, France), completed with characters from Bergey's Manual of Systematic and Determinative Microbiology (1986). The strains of *Micrococcaceae* were compared with the reference strains from API ID 32 STAPH, completed with characters from Bergey's Manual of Systematic and Determinative Microbiology (1986), and Kloos *et al.*(1974); Kloos and Schleifer (1975); Montel *et al.* (1992).

## Results

**Lactic acid bacteria:** Six groups were formed, and identified as: *Pediococcus pentosaceus* (six strains); *Lactobacillus plantarum* (five strains); *Lactobacillus curvatus* (two strains); one group of heterofermentative rods belonging to *Lactobacillus .brevis/buchneri* group. Only two strains were identified as *Lactobacillus sake* and *Lactobacillus pseudoplanarum*, respectively. Two strains remained unidentified. The characteristics of the strains are presented on the table 1

The majority of the identified facultatively homofermentative strains, belong to the species usually used as starter cultures. However, some heterofermentative strains were found, which are often responsible for defaults on fermented sausages (Lucke, 1985). In this particular product, the heterofermentative strains could not have a role of deterioration, as the product has no added sugar, the excessive production of gas is reduced.

**Micrococccaceae:** This group showed a high heterogeneity, forming nine groups. Two strain were identified as *Micrococcus kristinae* and one strain as *M. varians*. Two strains were close to *M. luteus* and *M. roseus*, however, it was possible to distinguish them for the colour of the pigment The *Staphylococcus* strains were identified as *S. xylosus* (one strain); *S. warneri* (six strains); *S. saprophyticus* (three strains); *S. auricularis* (one strain) and two belonging to the group *S. epidermidis/S.hominis*. Two strains (M18 and M69) remained unidentified. The characteristics of the strains are presented on the table 2

## Conclusion

Craftsmen of this region have designed formulations and ripening conditions which favour the desired microorganisms so strongly that the products are safe and palatable. A high counting of lactic acid bacteria and *Micrococccaceae* was found in "salpicão" The strains of

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