IDENTIFICATION OF *STAPHYLOCOCCUS* SPECIES ISOLATED FROM A TRADITIONAL WORKSHOP

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Background

In the meat sector, the recent BSE crisis, but also the recurring food poisoning cases, have undermined public confidence on intensive or industrial meat producing systems. Consumers are, therefore, turning to "traditional" products such as fermented dry sausages. Traditional dry sausages rely on natural contamination by environmental flora. Each workshop has a specific house flora, composed of useful micro organisms for the fermentation and flavour of sausages, but also spoilage and pathogenic flora. Few sporadic studies have been conducted on traditional meat products and have shown that hygienic shortcomings can lead up to 25% of product loss with high economic consequences and may undermine consumer confidence for traditional products. It is crucial, therefore, to give traditional producers the means to produce safe and standardised products.

Objectives

One way to improve safety of traditional dry sausages while preserving their typical sensory quality is to develop specific starters for each producer. This study has been carried out to identify the staphylococci contaminating the entire workshop and the products in order to select starters well adapted to the process.

Methods

Strains: staphylococci were numerated from 17 samples from the environment of a traditional curing workshop, the raw materials and the products in MSA media (Biokar) supplemented with nalidixic acid (40 mg/l) and an inhibitor of moulds (devocid, 200 mg/l). From these samples a collection of staphylococci was constituted. A set of 37 strains was identified by phenotypic and molecular methods. 32 reference strains of coagulase-negative *Staphylococcus* were also studied.
***Phenotypic method*:** strains of staphylococci were identified by using API Staph strips (Bio Mérieux).
Molecular methods : the oligonucleotide probes CARNO 440, WAR 180 and SAPRO 157 [1] for dot blot hybridisation were labelled at 3'ends with digoxygenin (MWG-Biotech). The hybridisation was performed according to the manufacturer protocol (Roche). For RAPD assays, the primer PH5 was used according to Wieser *et al.* [2]

Results and discussion

Colonisation of the workshop and the product by staphylococci: staphylococci were numerated in the 17 samples studied (Figure1). In the environment of the workshop, the highest contamination was found in the chopping block, the cold room, the drying room and the stuffing machine, the level varied between 10^3 to 3.5 10^4 CFU/cm_2. They were found in low level in the batter and they multiplied during the process to reach a high level after one week (2.6 10^5 CFU/g) and in the finish product (2.0 10^6 CFU/g), which is very close to levels found in industrial inoculated sausages [3].

Identification of staphylococci by phenotypic method: by using API Staph strips, some misclassifications occurred with the reference strains: *S. equorum* was identified to *S. xylosus* and *S. haemolyticus* could not be identified. On the 37 strains isolated from the workshop, 20 were identified to *S. xylosus*, 8 to *S. sciuri*, 5 to *S. saprophyticus,* 2 to *S. lentus*, 1 to *S. hominis* and 1 to *S. epidermidis* (Table 1). *S. xylosus* seemed to be the dominant species : 54% of the flora and *S. sciuri* the second one with 22%. These two species were already found dominant in the raw meat pork [4,5] and in the sausage [5]. If we considered the origin of the strains, the dominant species *S. xylosus* seemed to colonise the entire workshop and was present in the raw material and in the sausage (Table 1). *S. sciuri* was essentially found in the raw material, the sausage and the drying room. *S. saprophyticus* colonised the cutting tables and the mincing machine.

Identification of staphylococci by molecular methods: dot blots of staphylococcal DNA was hybridised with three probes. The probe CARNO 440 specific of *S. carnosus* confirmed that no strains belonged to this species (data not shown). The probe WAR 180 described to hybridise with *S. warneri* and *S. auricularis* [1] was found to hybridise with *S. warneri* and *S. auricularis* but also with *S. saprophyticus, S. simulans, S. carnosus* and 8 strains of the workshop in our conditions (Table 1). So it was not possible to conclude on the identification of these 8 strains. The probe SAPRO 157 was found specific and hybridised with all the strains identified as *S. saprophyticus* with the API Staph strips (Table 1). By RAPD, the primer PH5 had allow us to establish 10 RAPD types described in table 1. Dendrograms (data not shown) constructed with these RAPD types showed that the major strains couldn't been clearly identified in comparison with the RAPD types obtained from the reference strains. Nevertheless, some strains belonging to *S.saprophyticus* species were well-distinguished with API strips, dot-blots and RAPD techniques.

Conclusion

It is not possible to identify strains of staphylococci isolated from curing environments by using only API Staph strips. So it is necessary to develop molecular tools such primers or probes that will allow rapid and specific identification.

Pertinent literature

[1] Gory, L.; Millet, L.; Godon, J. J., and Montel, M. C. (1999). Identification of *Staphylococcus carnosus* and *Staphylococcus warneri* isolated from meat by fluorescent *in situ* hybridization with 16S rRNA-targeted oligonucleotide probes. *Systematic and Applied Microbiology*, **22,** 225-228.
[2] Wieser, M. and Busse, H.J (2000). Rapid identification of *Staphylococcus epidermidis*. International Journal of Systematic and Evolutionary Microbiology, **50**, 1087-1093.
[3] Nychas, G.J.E and Arkoudelos, J.S (1990). Staphylococci : their role in fermented sausages. *Journal of Applied Bacteriology* (symposium supplement), 167-188.
[4] Ramon, D., Molina, I. and Flores, J. (1992). Development of microbial cultures as starters for spanish dry-cured meat products. *New technologies for meat products,* 53-65.
[5] Rebecchi, A., Crivori, S, Sarra, P.G and Cocconcelli, P.S (1998). Physiologycal and molecular techniques for the study of bacterial community development in sausage fermentation. *Journal of Applied Microbiology*, **84**, 1043-1049.

Figure 1. Repartition of staphylococci in the workshop and the products

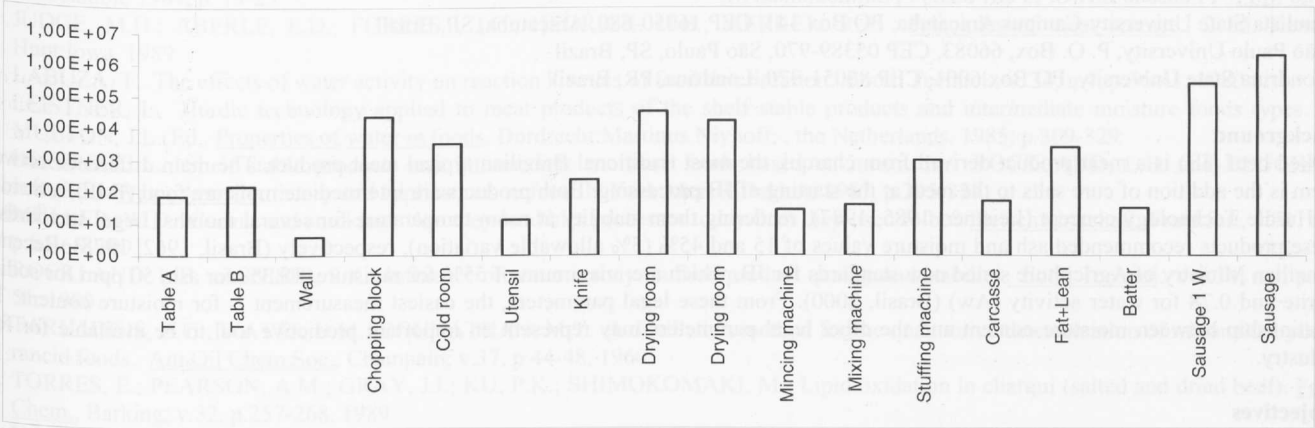

Table 1: Identification of the staphylococci by phenotypic and molecular methods

Strains n°	Origin	Galerie API Staph	War 180	Sapro 157	PH5 *
S1	Table A	*Staphylococcus saprophyticus*	-	+	P0
S2	Table A	*Staphylococcus saprophyticus*	-	+	P0
S3	Table A	*Staphylococcus saprophyticus*	-	+	P0
S4	Table B	*Staphylococcus xylosus*	-	-	P1
S5	Table B	*Staphylococcus xylosus*	-	-	P1
S6	Table B	*Staphylococcus saprophyticus*	-	+	P0
S9	Cold room	*Staphylococcus xylosus*	-	-	P7
S10	Knife	*Staphylococcus xylosus*	-	-	P1
S11	Drying room	*Staphylococcus sciuri*	-	-	P1
S12	Drying room	*Staphylococcus xylosus*	+	-	P7
S13	Drying room	*Staphylococcus sciuri*	-	-	P1
S14	Drying room	*Staphylococcus xylosus*	+	-	P7
S15	Mincing machine	*Staphylococcus saprophyticus*	-	+	P6
S16	Mincing machine	*Staphylococcus xylosus*	+	-	P4
S18	Stuffing machine	*Staphylococcus xylosus*	-	-	P5
S19	Stuffing machine	*Staphylococcus xylosus*	-	-	P3
S20	Stuffing machine	*Staphylococcus xylosus*	+	-	P7
S21	Stuffing machine	*Staphylococcus xylosus*	-	-	P3
S22	Fat + lean	*Staphylococcus hominis*	+	-	P7
S23	Fat + lean	*Staphylococcus sciuri*	-	-	P1
S24	Fat + lean	*Staphylococcus sciuri*	-	-	P1
S25	Fat + lean	*Staphylococcus xylosus*	-	-	P1
S26	Fat + lean	*Staphylococcus lentus*	-	-	P1
S27	Batter	*Staphylococcus xylosus*	-	-	P1
S28	Sausage 1 week	*Staphylococcus epidermidis*	-	-	P2
S29	Sausage 1 week	*Staphylococcus sciuri*	-	-	P1
S30	Sausage 1 week	*Staphylococcus xylosus*	+	-	P8
S31	Sausage 1 week	*Staphylococcus xylosus*	+	-	P8
S32	Sausage 1 week	*Staphylococcus xylosus*	+	-	P9
S33	Sausage final	*Staphylococcus xylosus*	-	-	P7
S34	Sausage final	*Staphylococcus xylosus*	-	-	P1
S35	Sausage final	*Staphylococcus sciuri*	-	-	P1
S36	Sausage final	*Staphylococcus xylosus*	-	-	P1
S38	Chopping block	*Staphylococcus sciuri*	-	-	P1
S39	Chopping block	*Staphylococcus sciuri*	-	-	P1
S40	Chopping block	*Staphylococcus lentus*	-	-	P1
S41	Chopping block	*Staphylococcus xylosus*	-	-	P1

*The different RAPD types obtained with PH5 primer are P0 (no amplification obtained), P1 (1 band of 800-bp), P2 (1 band of 1400-bp), P3 (1 band of 1650-bp), P4 (2 bands of 800 and 550-bp), P5 (2 bands of 1500 and 1000-bp), P6 (2 bands of 1600 and 500-bp), P7 (2 bands of 1650 and 1400-bp), P8 (3 bands of 1400, 900 and 550-bp) and P9 (3 bands of 1650, 1450 and 550-bp).