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Selection of staphylococci strains isolated from a Portuguese traditional fermented/dry sausage for potential use as starter cultures

L. Carvalho*, M.J. Fernandes*, H. Fernandes*, T. Semedo-Lemsaddek*, M. Elias**, A.S. Barreto* and M.J. Fraqueza*¹

*Faculty of Veterinary Medicine, CIISA, TULisbon, Av. da Universidade Técnica, Polo Univeristário, Alto da Ajuda, 1300-477 Lisbon (Portugal)

**Universidade de Évora, Instituto de Ciências Agrárias e Ambientais Mediterrânicas, Évora (Portugal)

¹email:mjoaofraqueza@fmv.utl.pt

Abstract. In order to evaluate its potential use as starter cultures, 104 isolates of *Staphylococcus* spp. were obtained from sausages and different sampling environment points in workshops A and B, at southern region of Portugal. PCR amplification was carried out to confirm genus and species allocation. From workshop A, 47 isolates were staphylococci, the majority belonging to the specie *S. equorum*, while from workshop B, of the 57 isolates identified as staphylococci, most were *S. xylosus*. The genetic profiles of isolates were further compared using PCR fingerprinting analysis, guiding to the selection of 43 representative strains subsequently characterized for their nitrate reductase, lipolytic and proteolytic activities. Among these strains, 30% revealed proteolytic ability while 42% had lipolytic activity. 65% of the strains reduced nitrate. Subsequently, 10 strains (representatives of different fingerprinting groups) were evaluated for their ability to grow at different temperatures, pH and NaCl concentrations. *S. xylosus* and *S. equorum* showed to grow under all the studied conditions being more effective at 15°C, 20°C and pH 5.5 at 20°C, and NaCl concentrations of 10% and 15%; therefore can be guaranteed their application in technological processes with varying temperatures. In workshop A, strains P05-58 *S. carnosus* and P05-74 *S. equorum* and, from workshop B, P06-01 and P06-26 *S. xylosus* presented the most interesting features.

Keywords. *Staphylococcus* coagulase negative – Fermented meat products – Starter culture – Proteolytic activity – Lipolytic activity – Nitrate reductase activity.

Sélection de souches de staphylocoques isolées à partir de produits carnés traditionnels portugais fermentés pour leur utilisation potentielle comme culture starter

Résumé. Afin d'évaluer leur potentielle utilisation comme culture starter, 104 isolats de *Staphylococcus* ont été obtenus à partir de "chouriços" et de différents points de l'environnement des unités A et B, au sud du Portugal. La confirmation génétique du genre et de l'espèce a été réalisée par PCR en identifiant 47 isolats de staphylocoques dans l'unité A, dont la plupart ont été *S. equorum*, tandis que dans l'unité B les 57 isolats étaient en majorité *S. xylosus*. Par PCR fingerprinting on a sélectionné 43 souches, qui ont été caractérisées concernant les activités nitrate réductase, lipolytique et protéolytique. De ces souches, 30% ont montré une activité protéolytique, 42% avaient une activité lipolytique, et 65% avaient la capacité de réduire les nitrates. Par la suite, 10 souches de différents groupes de fingerprinting ont été évaluées pour leur capacité de croissance à différentes températures, pH et % de NaCl. *S. xylosus* et *S. equorum* ont montré une croissance dans les conditions étudiées, étant plus efficaces à 15°C et 20°C, et dans un pH de 5,5 à 20°C, et dans des conditions de NaCl de 10% et 15%; ainsi on peut garantir leur application dans des procédés technologiques avec des températures variables. Les souches P05-58 *S. carnosus* et P05-74 *S. equorum* de l'unité A, autant que les *S. xylosus* P06-01 et P06-26, de l'unité B, ont démontré être les plus intéressantes.

Mots-clés. *Staphylococcus* coagulase négative – Produits carnés fermentés – Culture starter – Activité protéolytique – Activité lipolytique – Activité nitrate réductase.

I – Introduction

In Portugal there is a wide variety of traditional fermented/dry meat products, produced in small scale units being characterized by having in their manufacture a spontaneous fermentation by means of the so-called "house flora". Such products suffer a slight acidification and sensory characteristics are highly appreciated by consumers. Chorizo is a sausage made with pork, slightly fermented, smoked and dried, with a low pH and a decreased water activity, its production is characterized by a close relationship with the fermentative flora naturally present in the industrial environment. This type of flora is introduced in the meat during slaughter and increased in its concentration during manufacture of the fermented product. Each processing unit has a specific environmental flora, composed of useful microorganisms for the fermentation and the development of sensory characteristics in traditional sausages. The role of *Staphylococcus* as technological culture is well defined and the selection of the most interesting strains is a challenge to improve the hygienic and sensory characteristics of traditional sausages.

This study aimed to characterize the technological flora of *Staphylococcus* spp. isolated from dry fermented sausages and the traditional manufacture environments, in order to assess their potential use as starter cultures.

II – Material and methods

1. Origin of *Staphylococcus* isolates

The experimental work was carried out using 104 staphylococci isolated from the manufacturing environment, samples of traditional fermented and smoked sausage (chorizo) and secondary ingredients (garlic, pimento paste or bell pepper paste and natural casing) at two traditional meat industries (Plants A and B) located in Alentejo. The isolates were previously identified by biochemical miniaturized tests APIStaph (Biomerieux, France) by Fraqueza *et al.* (2006). At each manufacturing plant, samples were taken from the work surfaces (table, knife, grinder, mixer or stuffing machines) and from the fermented/smoked sausages in three different stages of manufacture (product after filling, product with half time smoking period and the final product) and secondary ingredients.

2. Extraction of DNA

The isolates were grown in tryptose salt agar to obtain fresh culture by 24 h incubation at 37°C, and the extraction of genomic DNA was performed using the QIAamp DNA Mini Kit 02/2003" from Quiagen (Germany).

3. Identification

The isolates were identified as belonging to the species *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Staphylococcus xylosus* and *Staphylococcus saprophyticus* using a multiplex PCR method described by Morote-Bizot *et al.*, (2004). *Staphylococcus carnosus* and *Staphylococcus simulans* were identified by PCR performed according to the methodology described by Blaiotta *et al.*, (2004) while the identification of *Staphylococcus equorum* was based on the protocol described by Blaiotta, *et al.*, (2004).

4. Genetic characterization and strain selection

The selection of isolates to assess for technological interest was based on their genetic profiles using the PCR fingerprinting methodology according to Tenreiro (2007). Representative strains were selected by comparison of the fingerprinting profiles obtained.

5. Phenotypic characterization of technological functions

The nitrate reductase activity was determined as described by Miralles *et al* (1996), while proteolytic activity was performed by sowing among PCA with gelatin, peptone and milk powder with subsequent flooding of the plate with a solution of mercuric chloride after incubation. To evaluate the lipolytic activity we used the Spirit Blue Agar medium (France).

Selected representatives ($n = 10$) groups with different genetic profiles were characterized for their ability to grow in different conditions of temperature, pH values and sodium chloride concentrations in order to evaluate their potential use as starter cultures. We investigated 3 incubation temperatures (10°C, 15°C and 20°C), two pH values (5.0 and 5.5), the temperatures of 15 and 20°C and two different concentrations of sodium chloride (10 and 15%).

6. Statistical analysis

Descriptive analysis of the results and their graphical presentation was obtained using Microsoft Office Excel 2007. The fingerprinting profiles obtained for each isolate were introduced in the Bionumerics software (version 4.61 [Applied Maths, Kortrijk, Belgium]) and dendrograms were constructed using the Pearson correlation coefficient and clustering method based on the unweighted average distance (UPGMA – unweighted pair group method with arithmetic average).

III – Results and conclusions

In Plant A, forty-seven *Staphylococcus* isolates were identified as members of the species *S. equorum* while in Plant B, fifty-seven isolates belong to the species *S. xylosus*. The genetic profiles of the isolates were compared by PCR fingerprinting (Figs 1 and 2) and led to the selection of 43 staphylococci which were subsequently characterized for their nitrate reductase, lipolytic and proteolytic activities.

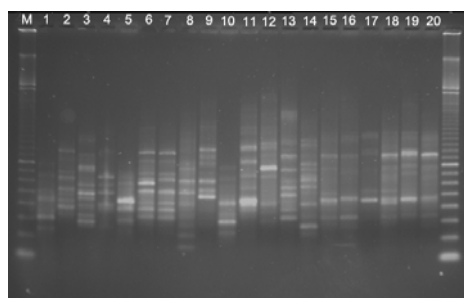


Fig. 1. PCR fingerprinting profiles of *Staphylococcus* strains.

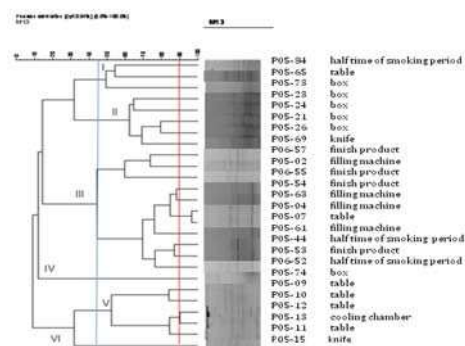


Fig. 2. Dendrogram analysis of the genetic profile of strains of *Staphylococcus equorum*.

By analyzing Fig. 2 we can see that the genetic profiles clustered the isolates in three main groups, with similarities below 45%. Strains P05-04 and P05-07 harbor profiles with a genetic similarity above 90%, being considered identical or highly related.

It was found that 30% of the strains showed proteolytic activity, while 42% showed lipolytic activity. As for the nitrate reductase activity, it was observed that about 65% of the strains reduced nitrate.

Strains of *S. xylosus* and *S. equorum* (n = 10) grew in all the conditions studied, but their development was more effective at 15°C and 20°C, pH 5.5 at 20°C and 10% and 15% concentration of chloride sodium, thus suggesting features that can guaranteed their application in technological processes with varying temperatures (Table 1)

Table 1 . Evaluation of proteolytic, lipolytic and nitrate reductase activity in strains of *Staphylococcus xylosus* and *Staphylococcus equorum*

	<i>S. xylosus</i> (n=10)		<i>S. equorum</i> (n=8)	
	Positive	Negative	Positive	Negative
Proteolytic Act. (n=18)	3	7	3	5
Lipolytic Act (n=18)	10	-	1	7
Nitrate reductase Act. (n=18)				
30°C/7 h (n=18)	5	5	-	8
20°C/24 h (n=18)	6	4	7	1
15°C/72h (n=18)	2	8	7	1

IV –Conclusions

Strains of *S. carnosus* P05-58 and *S. equorum* P05-74 from Plant A and strains of *S. xylosus* P06-01 and P06-26 from Plant B, demonstrated by their characteristics to be the most interesting from the technological point of view. These results suggest their potential usage as starter cultures.

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References

- Blaiotta G., Ercolini D., Mauriello G., Salzano G. and Villani F., 2004. Raid and reliable identification of *Staphylococcus equorum* by a Species-Specific PCR Assay targeting the *sda* gene, In: *Systematic and Applied Microbiology*, 27: 696-702
- Fraqueza M.J., Elias M., Talon R., Aymerich M.T., Ferreira M.C. and Barreto A.S., 2006. Microbiota of traditional dry sausage workshops from south of Portugal. In: *Actas del Congreso Internacional de Ciencia y Tecnología de los Alimentos*, Cordoba, Argentina. pp. 197.
- Miralles M.C., Flores J. and Perez-Martinez G., 1996. Biochemical tests for the selection of *Staphylococcus* strains as potencial meat starter cultures. In: *Food Microbiology*, 13: 1-10
- Morot Bizot C., Talon R. and Leroy S., 2004. Development of a multiplex PCR for the identification of *Saphylococcus* genus and four staphylococcal species isolated from food, In: *Journal of Applied Microbiology*, 97: 1087-1094
- Tenreiro R., 2007. 'Curso Teórico e Prático de Diagnóstico e Diferenciação Molecular em Microbiologia-Microbiologia on the road'; Organização: Rogério Tenreiro. Co-organização: Manuela Pintado 'Escola Superior de Biotecnologia da Universidade Católica Portuguesa'; Local: Escola Superior de Biotecnologia da Universidade Católica Portuguesa-Porto.