CHARACTERIZATION AND IDENTIFICATION OF LACTIC ACID BACTERIA IN "MORCILLA DE BURGOS"

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Background

"Morcilla de Burgos" is a typical cooked meat product very popular in Spain. It consists of a mixture of onion, rice, animal fat, blood, different spices and salt stuffed in a natural casing. The product is cooked for one hour at 94-95°C, air cooled to 8-10°C and finally chilled stored at 4°C. Since the product is subjected to high-temperature cooking during processing, the initial microbiological population of the finished product is very low and the surfaces of the cooked products can be considered sterile. The product is, however, manipulated after cooking which leads to post-cooking contamination. If the product is packaged under vacuum or modified atmosphere, the dominant spoilage flora will consist of lactic acid bacteria (LAB).

The increasing use of vacuum and modified atmosphere packaging of "morcilla" has resulted in problems caused by spoilage LAB. The typical sensory changes occurring are blowing of the packs, development of drip, slime formation and souring of the product. In some packages blowing has been very pronounced. The self-life of the product packaged under low permeability films has varied from 2 weeks to 6 weeks depending on the initial level of LAB and the presence of particularly active spoilage strains.
No data is available in literature about LAB species growing in this kind of a blood sausage.

Objetive

The aim of this work was to characterise the LAB strains isolated from "morcilla" produced in Burgos region. Phenotypic and biochemical characteristics were used in order to identify the species responsible for the spoilage of vacuum-packaged "morcilla".

Material and methods

Origin of the strains: a total of 176 strains were characterised. 99 strains of LAB were isolated from unspoiled samples of "morcilla" packaged under air, vacuum and modified atmosphere and elaborated in the region of Burgos. 59 strains were isolated from spoiled "morcilla" stored aerobically, under vacuum or modified atmosphere packages at 4°C. The product was considered spoiled according to the results from sensory evaluation performed by 5 expert panellists. 18 strains were isolated from vacuum-packaged "morcilla" which had been subjected to a mild pasteurisation.

Strain characterisation: Identification of the isolates was done by comparing the phenotypic and biochemical characteristics of the strains with the previously published data (Schillinger and Lücke, 1987; Shaw and Harding, 1989; Collins et al., 1993; Villani et al., 1997). Phase contrast microscopy was used for examining the cell morphology. Growth at 8 and 15°C was tested according to Schillinger and Lücke (1987) in tubes containing MRS broth and growth on Rogosa agar was tested on Rogosa agar plates (Oxoid, Basingstoke, UK) having the pH adjusted to 5.5 with glacial acetic acid (Panreac, Badalona, Spain). Fermentation of carbohydrates was determined according to the method described by Schillinger and Lücke (1987). Gas production from glucose, dextran production from saccharose and hydrolysis of arginine were tested using the methods described by Schillinger and Lücke (1987) with the exception of adding glucose to the final concentration of 0.3 g/l to test NH_3 production from arginine. Production of acetoin was detected by the Voges Proskauer test (Reuter, 1970). The configuration of lactic acid isomers was determined enzymatically (Roche Molecular Biochemicals, Mannheim, Germany) using supernatant from growth cultures incubated for 24h.

Results and discussion

According to the schema by Schillinger and Lücke (1987), Shaw and Harding (1989) and Collins et al. (1993), 165 strains from the total of lactic isolates were identified and 11 isolates with an uncertain identity were classified as *Lactobacillus spp.* The strains identified were grouped as it is shown in table 1. Heterofermentative bacteria (93.2%) were found to be the predominating LAB in "morcilla de Burgos". Only 12 strains of the total isolates were homofermentative and they were included in groups VI and VII. All bacteria grew at 8 and 15°C and only six strains (3.4%) produced acetoin. Most of the isolates grew on Rogosa agar except 9 strains of group I and 4 isolates of group VII. The high presence of heterofermentative bacteria can be considered to be responsible for the abundant blowing of the packs observed in the case of "morcilla" packed in vacuum or modified atmosphere. The proportion of heterofermentative LAB is clearly higher in "morcilla" compared to the LAB found by other authors in meat and meat products

The different lactic flora described in "morcilla" can be attributed to the different raw materials employed in its elaboration as onion, rice and blood and the absence of curing salts which might favour the development of heterofermenters as contrasted with the species habitually found in emulsion sausages. In this way raw material could be thought the source of spoilage LAB which recontaminates the product during handling after cooking step. However more information about contamination sources are necessary to confirm this hypothesis.

Table 1. Characteristics of the LAB species identified[a].

LAB groups	N° of strains	Percentage	Gas production	NH_3 from arginige	Dextran formation	Voges Proskauer	Lactic acid isomer	Growth			Acid produced from										
								At 8°C	At 15°C	On Rogosa agar	Cellobiose	Galactose	Inulin	Maltose	Mannitol	Melezitose	Melibiose	Ribose	Salicin	Trehalose	Xylose
W. viridescens	74	42.0	+	–	7	–	DL	+	+	(+)	5	–	–	+	–	–	4	85	–	88	4
Leuconostoc	42	23.9	+	–	71	–	D	+	+	79	50	(50)	–	60	–	–	55	(90)	50	98	50
mesenteroides	20	11.4	+	–	+	–	D	+	+	+	75	+	–	+	–	–	+	+	95	+	+
carnosum	17	9.7	+	–	47	–	D	+	+	47	24	–	–	–	–	–	–	(+)	–	+	–
spp.	5	2.8	+	–	40	–	D	+	+	+	40	(20)	–	+	–	–	60	(20)	40	80	20
W. confusa	20	11.4	+	+	+	–	DL	+	+	+	+	85	–	+	–	–	–	(10)	+	–	(+)
Lb. fructosus	10	5.7	+	–	–	–	DL	+	+	+	10	–	–	–	–	–	–	–	–	+	–
Lb. sanfrancisco	7	4.0	+	–	–	–	DL	+	+	+	71	+	–	+	–	–	+	71	71	+	–
Homoferment. strains	7	4.0	–	71	–	43	L	+	+	+	86	+	–	43	–	–	71	+	+	86	–
C. piscicola	5	2.8	–	+	–	60	L	+	+	20	+	60	40	+	+	40	–	+	+	+	40

[a]Symbols: +: all strains positive; –: all strains negative; 7: 7% strains positive; ( ): some strains weak reaction.

The flora in unspoiled "morcilla" was more diverse than in spoiled and pasteurised product since bacteria from all groups were detected (Table 2). W. *viridescens* predominated in the unspoiled and pasteurised product and the percentage decreased in spoiled "morcilla" whereas the proportion of leuconostocs increased. The fact that *W. viridescens* was the main group of bacteria that survived to the pasteurisation treatment confirms the findings made by other authors in cooked meat products which consider this species as a heat resistant microorganism.

Table 2. Distribution of the strains in according to the origin of the isolates. Percentages are in brackets.

	Weissella viridescens	*Leuc. mesenteroides*	*Leuc. carnosum*	*Leuc. spp.*	*Weissella confusa*	*Lactob. fructosus*	*Lactob. sanfrancisco*	Homofermt strains	*Carnob. piscicola*	Unidentified
Unspoiled "morcilla"	44 (44)	7 (7)	6 (6)	5 (5)	10 (10)	8(8)	5 (5)	6 (6)	5 (5)	3 (3)
Spoiled "morcilla"	19 (32)	10 (17)	11 (19)	–	9 (15)	1 (2)	2 (3)	–	–	7 (12)
Pasteurised "morcilla"	11 (61)	3 (17)	–	–	1 (6)	1 (6)	–	1 (6)	–	1 (6)

Conclusion

It can be concluded that *W. viridescens*, *Leuc mesenteroides*, *Leuc. carnosum* and *W. confusa* are the main members of the lactic flora of "morcilla de Burgos". During cold storage development of *Leuconostoc* species is favoured while *W. viridescens* is predominant when the product is pasteurised after packing. Phenotypic characterizacion based on sugar fermentation pattern and conventional phenotypic properties may not always provide sufficient basis for the reliable identification of LAB though it is an useful tool for presumptive classification. Newer technologies based on molecular techniques have been proved to be efficient in the classification of this kind of bacteria and should be the next step to the present work.

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