ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM TRADITIONAL SPANISH DRY FERMENTED SAUSAGE "CHORIZO"

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Background: "chorizo" is a very traditional and popular dry fermented sausage in Spain. In this kind of products, microorganisms play a crucial role. The conditions prevailing in sausage fermentation strongly favour the growth of lactic acid bacteria (LAB), which decrease the pH of the product due to the production of lactic acid.

Most of the available information about isolation and characterization of LAB in meat has been focussed on different types of products: vacuum packed meat (Hitchener et al., 1982; Shaw and Harding, 1984), meat and meat products (Morishita and Shiromizu, 1986; Schillinger and Lücke, 1987), vacuum-packed cooked ring sausages (Korkeala and Mäkelä, 1989) and Greek dry salami (Samelis et al., 1994). Less information is available on the isolation and characterization of LAB from Spanish dry fermented sausages, and specially concerning to "chorizo" (Sanz et al., 1988; Sanz Gómez et al., 1992; Hugas et al., 1993).

Objetives: the aim of this study is the identification and characterization of LAB present in different kinds of "chorizo": "Cantimpalos" (Segovia), "ibérico" (Salamanca) and "chorizo" from Burgos, for the further selection of strains which could be used as starter cultures in this product.

Methods: strains were isolated by the following procedure: 16 strains for sample were randomly selected from high dilution MRS agar plates, purified by streaking on MRS agar and kept in MRS broth for the further characterization. Strains were tested for Gram reaction and catalase production. Only Gram positive and catalase negative strains were further characterizated.

Biochemical tests: Fermentation of carbohydrates and arginine hydrolysis were determined according to the method described by Schillinger and Lucke (1987), using the miniplate method described by Jayne and Williams (1976) but with bromocresol purple as indicator. Gas and dextran production from glucose were tested according to Schillinger and Lucke (1987). Production of H_2S was determined according to Shay and Egan (1981). Production of H_2O_2 was determined as described by Whittenbury (1964). Production of acetoin was tested by Voges Proskauer test. The configuration of lactic acid formed was determined enzymatically (Boehringer Mannheim, GmbH, Germany).

Physiological tests: Growth of LAB was followed at different conditions in MRS broth: pH 3.9 ajusted with HCl, temperatures of 8°C and 15°C, and in the presence of 7% and 10% NaCl (miniplate method).

Results and discussion: identification scheme of Schillinger and Lucke (1987) was applied to classify 516 strains isolated from "chorizo". 355 of the strains (68.8%) were identified as *Lactobacillus sake*, 85 strains (16.5%) as *Lactobacillus curvatus*, 32 strains (8.5%) belonged to *Pediococcus* genus and 44 strains were grouped as *Lactobacillus sp* (Table 1).

In agreement with other works (Schillinger and Lucke, 1987; Hugas et al., 1993; Samelis et al., 1994), *L. sake* (S) and *L. curvatus* (C) (atypical streptobacteria) were the dominant species. It was impossible to use the morphology to distinguish *L. sake* from *L. curvatus* because curved forms were found frequently in both species. However, these strains were divided into four technological groups according to the utilization of different sugars, maltose and lactose, usually employed by the industry in the formulation of "chorizo".

L. sake was the predominant species (68.8%) as other authors have reported for different meat products (Schillinger and Lucke, 1987; Sanz et al., 1988; Hugas et al., 1993) unlike works of Sanz Gómez et al. (1992) and Samelis et al. (1994) where *L. curvatus* was the most important species. The characteristics of the *L. sake* species isolated in this study are reflected in table 1. This species resembled cluster II (Shaw and Harding, 1984) and *L. sake* described by Schillinger and Lucke (1987) and Hugas et al. (1993), although only 5% and 9% strains produced dextran and H_2O_2 respectively, and none of them produced H_2S. They were also good halotolerants because they grew in the presence of 7% and 10% NaCl.

The main group within the *L. sake* species was S1 (*L. sake* maltose, lactose negative) and most of them showed the following carbohydrate fermentation pattern: glucose, ribose, galactose, sucrose, melibiose and trehalose. Fermentation pattern of this group was very similar to the pattern found for group 2 of Hitchener et al. (1982), being that group L-arabinose negative, and quite similar to groups 3 and 4 described by Samelis et al. (1994), although those groups showed a high rate in arginine hydrolysis.

The percentage of *L. curvatus* (16.5%) found in this study was slightly lower than the obtained by Schillinger and Lucke (1987), Hugas et al. (1993) and Samelis et al. (1994), probably due to they analysed different kinds of meat products. Common characteristics with preceding authors were found, since all strains were mannitol, melezitose, melibiose, raffinose, rhamnose, sorbitol and xylose negative.

but it was more difficult to establish a comparison with them. Again, only 13% produced H_2O_2 and no strain produced H_2S, in contrast to the results described by Schillinger and Lücke (1987) in which 77% and 61% strains produced H_2S and H_2O_2, respectively.

Although, most strains belonging to *Pediococcus* genus (6.2%) were isolated from one manufacturer of "Cantimpalos chorizo", high initial total counts suggest that a starter culture with a strain belonging to that genus had been used in the formulation of that product.

Table 1. Biochemical and physiological characteristics of the LAB investigated.

			CARBOHYDRATE FERMENTATION														GROWTH					PRODUCTION					
	Number of strains	(%)	glucose	ribose	galactose	sucrose	maltose	lactose	melibiose	arabinose	cellobiose	trehalose	raffinose	rhamnose	xylose	inulin	8°C	15°C	pH 3.9	7% NaCl	10% NaCl	GAS	DEXTRAN	H_2S	H_2O_2	ACETOIN	NH_3 from arginine
L. sake	**355**	**68.8**	+	+	+	+	**23**	**23**	**95**	**45**	**31**	**92**	**3**	**1**	**2**	**1**	+	+	**(61)**	+	**98**	-	**5**	-	**9**	**57**	**(46)**
S1 (M-L-)	204	39.5	+	+	+	+	-	-	94	39	28	88	2	1	1	-	+	+	(61)	+	98	-	6	-	12	47	(39)
S2 (M+)	69	13.4	+	+	+	+	+	-	+	70	39	+	3	-	7	3	+	+	(78)	+	97	-	4	-	7	77	(54)
S3 (L+)	68	13.2	+	+	99	+	-	+	91	29	25	96	3	1	-	-	+	+	(37)	+	+	-	3	-	3	63	(57)
S4 (M+L+)	14	2.7	+	+	+	+	+	+	+	93	71	+	21	14	-	-	+	+	(86)	+	93	-	14	-	7	64	(50)
L. curvatus	**85**	**16.5**	+	+	+	**42**	**61**	**25**	-	**38**	**64**	**79**	-	-	-	**6**	+	+	**(76)**	+	+	-	-	-	**13**	**33**	**(22)**
C1 (M-L-)	24	4.6	+	+	+	17	-	-	-	79	71	63	-	-	-	-	+	+	(79)	+	+	-	-	-	4	38	(21)
C2 (M+)	40	7.7	+	+	+	53	+	-	-	23	63	88	-	-	-	3	+	+	(83)	+	+	-	-	-	13	35	(23)
C3 (L+)	9	1.7	+	+	+	33	-	+	-	44	44	56	-	-	-	22	+	+	(56)	+	+	-	-	-	11	-	(33)
C4 (M+L+)	12	2.3	+	+	+	67	+	+	-	-	67	+	-	-	-	17	+	+	(67)	+	+	-	-	-	33	42	17
Pediococcus	**32**	**6.2**	+	+	+	**84**	+	**6**	**81**	+	+	+	**81**	**9**	**16**	-	+	+	+	+	+	-	-	-	-	**94**	**(38)**
Lactobacillus sp.	**44**	**8.5**																									

Symbols: +: all strains positive; -: all strains negative; 36: 36% of strains positive; (36): weak reaction in some strains.

Conclusions: *L. sake* maltose, lactose negative was the major group of LAB found in this study. Most strains of this group had the following carbohydrate fermentation pattern: glucose, ribose, galactose, sucrose, melibiose and trehalose. In general, the majority of strains did not produce dextran, H_2O_2 and H_2S, which is an important factor to consider for selection of a strain as starter culture. It has been difficult to compare our results with those obtained by other authors due to the different classification criteria adopted by them.

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