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Comparative study of lactic acid bacteria house flora isolated in different varieties of 'chorizo'

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Abstract

A total of 516 strains of lactic acid bacteria isolated from 'chorizo' (a Spanish dry fermented sausage) were identified. The 'chorizo' was from three zones of Castilla and León in Spain: Burgos, Segovia and Salamanca. Two factories were chosen in each zone and the samples were taken at three stages of ripening. *L. sake* was the most predominant species present (68.8%) followed by *L. curvatus* (16.47%) and *Pediococcus* sp. (8.52%). Different strains that do not belong to the above species were grouped as *Lactobacillus* sp. Group S1 comprising maltose and lactose negative *L. sake* was the main group present in all factories except in a factory in Segovia where group S3 comprising lactose positive *L. sake* and pediococci were the predominant ones. Group S1 increased during the ripening process in all six factories and it dominated in the ripened 'chorizo' except in the mentioned factory in Segovia. In general strains of *L. sake* and *L. curvatus* which fermented maltose but not lactose were more dominant at the beginning and in the middle of the process, whereas, *L. sake* and *L. curvatus* which could ferment lactose, or lactose and maltose occurred in higher numbers in semi-ripened 'chorizo' and in the final product. This indicates that strains which could ferment lactose were more competitive towards the end of the process. Strains from group S1 were the microorganisms responsible for the pH drop in most of the factories, giving the correct texture. As a result it would appear that a strain from this group would be most suitable for use as starter culture. © 1998 Elsevier Science B.V.

Keywords: 'Chorizo'; Lactic acid bacteria; Dry fermented sausage; Starter culture

1. Introduction

'Chorizo' is a popular dry fermented sausage in Spain. It is made of minced meat, mixed with fat, spices and different additives, stuffed into natural or artificial casings and ripened at low temperature and relative humidity.

The shelf-life is determined by a low water activity (A_w) and a pH level close to 5.0 which prevents growth of pathogenic and spoilage microorganisms. Lactic acid produced by lactic acid bacteria (LAB) is responsible for the low pH. The pH decrease leads to the coagulation of the soluble

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proteins which produces a compact structure and also favours lipolytic and proteolytic reactions (González-Fernández et al., 1997). In addition, the low pH is responsible for the colour development, inducing the reduction of nitrites to nitric oxide and giving the light acidic taste, typical of this kind of product. Finally, lactobacilli have a low lipolytic activity on low molecular weight fatty acid triglycerides (Samelis et al., 1993).

Although the use of starter cultures is on the increase in large scale factories, small manufacturers are continuing to use the traditional method without starter cultures. In this way, the LAB present in these products come from the meat itself or the environment i.e. the so-called 'house flora'.

Different studies on the identification and characterisation of these 'house flora' have been carried out on different types of products (Hitchener et al., 1982; Shaw and Harding, 1984; Morishita and Shiromizu, 1986; Schillinger and Lücke, 1987; Korkeala and Mäkelä, 1989; Samelis et al., 1994a,b). Little information is available on the isolation and characterisation of LAB, from Spanish dry fermented sausages (Sanz et al., 1988; Sanz-Gómez et al., 1992; Hugas et al., 1993; Armengol et al., 1994). Some lactobacilli have been isolated at different stages of ripening (Sanz et al., 1988; Hugas et al., 1993; Samelis et al., 1994a,b; Armengol et al., 1994) but the evolution of different species during the ripening process has been largely ignored.

The aim of this project was the isolation and characterisation of the LAB present in the traditional 'chorizo' from three different zones in Castilla and León in Spain in order to select a suitable strain to be used as a starter culture in such products. Samples were taken at three different stages of ripening to determine the species present and the succession of these species during the process. In addition, the 'house flora' from different factories within the same zone and between different zones were compared.

2. Material and methods

Samples studied for this project were 'chorizos' from three zones in Castilla and León: Burgos, Segovia and Salamanca. The 'chorizos' from Burgos and Segovia were produced with meat and fat from Landrace white pig, while the 'chorizos' from Salamanca with meat and fat from Iberian pig. The 'chorizos' from Burgos (35 mm in diameter) were fermented for 24–48 h at 23°C and 90% of relative humidity (RH) and then ripened at 12–18°C for 17–18 days at lower RH (60–80%). The 'chorizos' from Segovia had 45 mm diameter and they were fermented for 30 h at 25°C and 90% RH and then ripened for 20 days at 14–18°C and 70% RH. Finally, the 'chorizos' from Salamanca zone (80 mm in diameter) were ripened for 2–3 months at 10–12°C and 80–90% RH without an initial fermentation period at higher temperatures. Two manufacturers from each zone were chosen, and two series of samples were taken at three different stages of ripening: the early minced meat stage, the semi-ripened stage and the ripened stage, according to Table 1. Sixteen strains from each sample were randomly selected from high dilution MRS agar plates, purified by streaking on MRS agar (Oxoid, Basingstoke, UK) and kept in MRS broth (Oxoid) for characterisation. Only Gram positive and catalase negative strains were further identified. The strains were preserved frozen at −80°C in MRS broth (Oxoid) with 20% glycerol (Difco).

Fermentation of carbohydrates, and arginine hydrolysis was determined according to the method described by Schillinger and Lücke (1987) using the miniplate method prescribed by Jayne-Williams (1976) but with bromocresol purple used as an indicator. Gas and dextran production from glucose were tested according to Schillinger and Lücke (1987). Production of H_2S was determined according to Shay and Egan (1981). Production of H_2O_2 was

Table 1
Sampling time (days) for 'chorizos' produced in different zones

	Burgos	Segovia	Salamanca
Minced meat stage	0	0	0
Semi-ripened stage	8–9	9–11	30–35
Ripened stage	17–18	20–22	60–75

determined as described by Whittenbury (1964). Production of acetoin was tested by the Voges Proskauer test (Reuter, 1970). The configuration of lactic acid formed was determined enzymatically (Boehringer Mannheim, Germany). Growth of the LAB in different conditions was studied in MRS broth: pH 3.9 adjusted with HCl, temperatures of 8°C and 15°C, and in the presence of 7% and 10% NaCl (w/v). The miniplate method was employed to study the growth at different temperatures and different salt concentrations.

3. Results and discussion

The factories chosen in Burgos were labelled 1 and 2, those in Segovia, 3 and 4 and those in Salamanca, 5 and 6. The identification scheme described by Schillinger and Lücke (1987) was applied for the identification of 516 strains from the 576 strains isolated. The number of strains identified in each factory varied between 73 and 93. Overall 355 (68.8%) were identified as *Lactobacillus sake*, 85 (16.47%) as *Lactobacillus curvatus*, 32 (6.20%) belonged to genus *Pediococcus* and 44 strains (8.52%) were classified as *Lactobacillus* sp. Reported studies on traditional European sausages (Lücke, 1985, 1988; Hammes et al., 1990) have also identified *L. sake* and *L. curvatus* as the predominant species present. Sugars in fermented sausages are usually added in the form of maltodextrins or skim milk powder in addition to glucose and sucrose. For this reason, four groups were established for *L. sake* and *L. curvatus* according to fermentation of maltose and lactose as indicated in the explanatory notes of Table 2. Ten groups (S1, S2, S3, S4, C1, C2, C3, C4, *Pediococcus* sp. and *Lactobacillus* sp.) were determined where S represents *L. sake*, C represents *L. curvatus* and *Lactobacillus* sp. lactobacilli not grouped in the those species named above.

In agreement with the physiological and biochemical characteristics of these groups described by Santos et al. (1997), all *L. sake*, *L. curvatus* and *Pediococcus* strains grew at 8°C and 15°C in the presence of 7% NaCl (w/v) while most of them also grew in the presence of 10% NaCl (w/v). None of them produced gas or H_2S. Only 5% of the total strains identified were dextran positive and these strains were only found in the *L. sake* species.

Table 2
Percentages of strains of different groups[a] in each factories

	Burgos		Segovia		Salamanca	
	1	2	3	4	5	6
L. sake S1	46.6	47.8	12.9	61.9	40.5	31.5
L. sake S2	26	24.4	8.6	9.5	5.9	7.6
L. sake S3	4.1	5.6	29	15.5	14.3	8.7
L. sake S4	2.7	–	6.5	2.4	2.4	2.2
L. curvatus C1	1.4	6.7	10.8	1.2	1.2	5.4
L. curvatus C2	9.6	4.4	4	–	11.9	16.3
L. curvatus C3	–	–	–	2.4	2.4	5.4
L. curvatus C4	–	–	–	–	2.4	10.9
Pediococcus sp.	8.2	–	27.9	–	–	–
Lactobacillus sp.	1.4	11.1	–	7.1	19.4	11.9

[a] 1, maltose negative, lactose negative; 2, maltose positive, lactose negative; 3, maltose negative, lactose positive; 4, maltose positive, lactose positive.

Although most of the groups showed strains that produced acetoin, this production was particularly high in the strains from factory 1 (83.6%). None of the strains isolated in the Burgos zone produced H_2O_2 whereas some strains of *L. sake* which produced this compound were found in the 'chorizos' from Segovia (8.6% in factory 3 and 6.0% in factory 4). In the case of Salamanca H_2O_2 producing strains belonged to *L. sake* and *L. curvatus* species (9.5% in factory 5 and 23.9% in factory 6). Although H_2O_2 inhibits the growth of other microorganisms (Whittenbury, 1964), peroxide producing LAB are undesirable in dry sausage (Lücke, 1988). While other authors found high percentages of H_2O_2- and H_2S-producing strains (Schillinger and Lücke, 1987; Samelis et al., 1994b), our results showed that, in general the majority of strains did not produce dextran, H_2O_2 or H_2S which is an important factor to consider when selecting a strain as starter culture (Hammes et al., 1990; Buckenhüskes, 1993).

Table 2 shows the distribution of the strains in the ten groups for the six factories. A similarity between factories is seen for the zones of Burgos and Salamanca, whereas, factories 3 and 4 from Segovia showed a different distribution. Factory 4 showed the highest percentage of group S1 (*L. sake* maltose lactose negative) and factory 3 the lowest of the six factories. Among the LAB *L. sake* was the predominant species in all six factories. This is in

accordance with the results obtained by Schillinger and Lücke (1987); Sanz et al. (1988); Hugas et al. (1993). *L. curvatus* was present in lower percentages in the factories than *L. sake*. In the Salamanca zone all four groups of *L. curvatus* strains were found in both factories.

In factory 3 (Table 2) S3 and *Pediococcus* sp. were dominant at levels of nearly 30% each. Most strains from group S1 presented the following carbohydrate fermentation pattern: glucose, ribose, galactose, sucrose, melibiose and threalose positive (results not shown).

The evolution of the different groups during the ripening process in each factory is shown in Tables 3–5. As the tables indicate group S1 progressively increased during the ripening process in all six

Table 3
Percentages of different lactic acid bacteria groups[a] during the ripening period in Burgos

	Factory 1			Factory 2		
	Minced meat	Semi-ripened 'chorizo'	Ripened 'chorizo'	Minced meat	Semi-ripened 'chorizo'	Ripened 'chorizo'
L. sake S1	–	43.8	62.5	43.8	46.9	43.8
L. sake S2	3.1	40.6	15.6	15.6	15.6	37.5
L. sake S3	–	3.1	6.3	–	9.4	6.3
L. sake S4	–	–	6.3	–	–	–
L. curvatus C1	3.1	–	–	9.4	6.3	3.1
L. curvatus C2	3.1	12.5	6.3	6.3	–	6.3
L. curvatus C3	–	–	–	–	–	–
L. curvatus C4	–	–	–	–	–	–
Pediococcus sp.	18.75	–	–	–	–	–
Lactobacillus sp.	–	–	3.1	6.3	21.9	3.1

[a] 1, maltose negative, lactose negative; 2, maltose positive, lactose negative; 3, maltose negative, lactose positive; 4, maltose positive, lactose positive.

Table 4
Percentages of different lactic acid bacteria groups[a] during the ripening period in Segovia

	Factory 3			Factory 4		
	Minced meat	Semi-ripened chorizo	Ripened chorizo	Minced meat	Semi-ripened chorizo	Ripened chorizo
L. sake S1	15.6	6.3	15.6	25.0	71.9	67.7
L. sake S2	12.5	12.5	–	12.5	9.4	3.2
L. sake S3	3.1	31.3	50.0	6.3	12.5	22.6
L. sake S4	3.1	6.3	9.4	3.1	3.1	–
L. curvatus C1	–	12.5	18.8	–	–	3.2
L. curvatus C2	9.4	–	3.1	–	–	–
L. curvatus C3	–	–	–	–	3.1	3.2
L. curvatus C4	–	–	–	–	–	–
Pediococcus sp.	46.9	31.3	3.1	–	–	–
Lactobacillus sp.	–	–	–	18.8	–	–

[a] 1, maltose negative, lactose negative; 2, maltose positive, lactose negative; 3, maltose negative, lactose positive; 4, maltose positive, lactose positive.

Table 5
Percentages of different lactic acid bacteria groups[a] during the ripening period in Salamanca

	Factory 5			Factory 6		
	Minced meat	Semi − ripened chorizo	Ripened chorizo	Minced meat	Semi-ripened chorizo	Ripened chorizo
L. sake S1	3.1	40.6	62.5	15.6	40.6	34.4
L. sake S2	–	12.5	3.1	6.3	–	15.6
L. sake S3	9.4	3.1	25.0	6.3	3.1	15.6
L. sake S4	–	6.3	–	–	–	6.3
L. curvatus C1	3.1	–	–	–	12.5	3.1
L. curvatus C2	–	21.9	9.4	25.0	15.6	6.3
L. curvatus C3	–	6.3	–	–	6.3	9.4
L. curvatus C4	–	6.3	–	3.1	18.8	9.4
Pediococcus sp.	–	–	–	–	–	–
Lactobacillus sp.	46.9	3.1	–	31.3	3.1	–

[a] 1, maltose negative, lactose negative; 2, maltose positive, lactose negative; 3, maltose negative, lactose positive; 4, maltose positive, lactose positive.

factories and it dominated the LAB flora in all final products, except in those of factory 3 (Table 4).

Group S2 was present in 24.4–26.0% (Table 2) in the Burgos zone and 5.9–9.5% in the other four zones. In general *L. sake* strains belonging to this group, that is those which could ferment maltose but not lactose, were more plentiful at the beginning and in the middle of the process, except in factory 2 and 6 (Tables 3 and 5). The same may be said for *L. curvatus* C2 as it followed a similar pattern in all factories as group S2. Perhaps the low level of maltose positive strains in the ripened 'chorizo' was due to the fact that maltose levels were not very high to begin with and they were quickly consumed during the first stages of ripening. In the case of factory 2, maltodextrins were added during production which allowed the development of maltose positive strains, especially group S2.

Group S3 was present at higher percentages than group S2 in the Salamanca and Segovia zones (Table 2). Group S3 was the dominant group in factory 3 (Table 4) with a tendency towards increasing percentages along the ripening period in the rest of the factories. In general, group S4 was present in the highest numbers in the semi-ripened 'chorizo' and in the final product. *L. curvatus* was present mainly at the semi-ripened stage in both factories in the Salamanca zone and also in the ripened 'chorizo' in factory 6 (Table 5). Groups of *L. curvatus* lactose positive (C3 and C4) became more important with time. These facts indicate that strains of *L. sake* and *L. curvatus* which could ferment lactose were more competitive and they increased in numbers at the end of the ripening process when the sugars commonly fermented by most of the LAB strains such as glucose, sucrose or maltose had almost disappeared.

Pediococci appeared only in the minced meat in factory 1 (Table 3) and during the entire process in factory 3 (Table 4), but declined as the process evolved. This fact might be indicative of inability of pediococci to compete effectively with *L. sake* and *L. curvatus*. The same occurred for the group *Lactobacillus sp*. In the Salamanca zone where this group seemed to play a role (Table 2). In factory 2, most strains from this group belonged to the *L. alimentarius* species.

It appears that group S1 in general contain the microorganisms responsible for decreasing pH. The use of strains from this group in starter cultures for 'chorizo' should therefore be more effective than any other LAB, since a quick decrease of pH during the first days of the ripening process gives the correct texture to the product and enhances its preservation (González-Fernández et al., 1997). However, further studies are necessary in order to determine the relationships between the different groups, their

succession and the effect of these factors on the sensory properties of the final product.

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