BIOCHEMICAL CHARACTERIZATION OF LACTOBACILLI FROM DRY FERMENTED SAUSAGES

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SUMMARY

254 strains were isolated from fermented dry sausages from 15 processors at different stages of processing . The species identified were L.sake, L.curvatus, L.bavaricus and L.plantarum. Deamination of arginine occurred to be positive in L.sake and negative in 92% of L.curvatus strains. Different methods were tested. In two L.sake strains arginine catabolism was dependent on culture media and in two other on the presence of oxygen.

INTRODUCTION

The species of lactobacilli most commonly found in meat and meat products including dry sausages processed with different technologies are L.sake, L.curvatus and L.plantarum (Hastings and Holzapfel, 1984; Morishita and Shiromizu, 1986; Schillinger and Lücke, 1987; being L.sake the species most frequently isolated.

In Spain, lactobacilli in dry sausages were not characterized to species level before this study.

The aim of this study is to identify the lactobacilli population present in dry sausages for the further selection of several strains which could be potentially used as starter cultures in the production of dry sausages.

MATERIALS AND METHODS

For every origin, 5 different stages of ripening/curing were sampled: 0h, 48h, 72h, 7 days, 15 days and 30 days.

10 g of sausage were homogenized in an Stomacher, diluted and plated in MRS agar (Difco) with the double layer technique. The plates were incubated at 30°C for 72 h. After incubation, individual isolates and selected on the basis of cell morphology, Gram and catalase reaction.

Carbohydrate fermentation was determined as cited from Schillinger and Lücke (1987).

Gas production from glucose after Schillinger and Lücke (1987).

Salt tolerance of the strains (5% and 8% sodium chloride), resistance to different concentrations of sodium nitrite (150, 450, 750 ppm), potassium nitrate (300, 900, 1500 ppm) and sodium nitrite plus potassium nitrate (150+150, 450+450, 750+750 ppm) and growth at different temperatures (8°C, 15°C, 45°C) was observed after three and seven days of incubation.

Deamination of arginine was determined anaerobically according to Schillinger and Lücke (1987) and according to Niven et al. (1942) modified by Hitchener et al. (1982). Citrulline production was measured in the supernatants after Montel (pers. comun.). The configuration of lactic acid was determined enzymatically.

Acetoin production was determined by the Voges-Proskauer test (Reuter, 1970).

The presence of meso-diaminopimelic acid (mDpm) in the cell wall was detected after Abo-Elnaga

and Kandler (1965) modified (Lücke, pers. comm.).

Growth on agar acetate was detected using Rogosa's medium (Merck).

RESULTS AND DISCUSSION

254 lactobacilli strains were isolated from MRS agar plates. In 86% of isolates a typical morphology could be observed formed by short chains of curved rods, horseshoe cells and circles of three or more curved cells as described for L.curvatus strains (Kandler and Weiss, 1986). This morphology was observed in all the four species isolated.

The isolates were distributed in 4 groups (Table 1).Group A was constituted by the largest number of isolates (55%) and was assigned to species L.sake. The strains assigned to group A, represent a very homogeneous group whose major characteristics are their lack of mDpm acid in the cell walls, the production of DL lactic acid, the hydrolysis of arginine, the unability to ferment mannitol, raffinose, rhamnose and melezitose and the fermentation of ribose, melibiose and saccharose.

Group B with 66 isolates (26%) is similar to Group A except for the deamination of arginine and the fermentation of melibiose respectively . This group was assigned to L.curvatus.

The characteristics above mentioned were reported from Champomier et al. (1987) and Schillinger and Lücke (1987) as a means to differentiate L.sake from L.curvatus.

L.bavaricus with 27 strains (11%) isolated constitutes the group C and it is the third species in number found in dry sausages. These isolates were basically identified as L.bavaricus for being mDmp negative, producing L(+) lactic acid without fermenting mannitol.

Niemand and Holzapfel (1984) where the first to describe this species in meat environment. In their study they found 10.5% of isolates belonging to this species in irradiated meat. According to Kandler and Weiss (1986) this species may be regarded as a racemase-free subspecies of L.sake and L.curvatus because of the high DNA-DNA homology shown by some isolates.

Group D of strains assigned to L.plantarum (22 strains, 8% of isolates) was the group best defined concerning carbohydrate fermentation, since in 9 out of 12 sugars a homogenous behaviour was observed. Nonetheless, four isolates allotted to this species do not ferment ribose, which is fermented by all other strains isolated in the whole study. Inspite of this fact, we believe they constitute representatives of the species L.plantarum because of the consistency of the other tests performed like the presence of mDmp in cell walls, the production of DL lactic acid and their lack of enzymes for the arginine catabolism.

The data obtained agree with previous studies published pointing out that L.sake constitutes the main species isolated in meat and meat products followed by L.curvatus, although the percentages of these species vary (Niemand and Holzapfel, 1984; Hastings and Holzapfel, 1987; Schillinger and Lücke, 1987; Montel et al., 1989).

The total amount of lactic acid produced by the isolates varies from strain to strain, however in groups A, B and C, 72%, 67% and 41% of strains respectively produce from 8 to 10 grs/l of lactic acid; while 82% of strains in group D produce more than 11 grs/l. From our results L.curvatus and L.sake isolates produce more L(+) lactic acid than D(-), in L.plantarum strains the D(-) isomer exceeds L(+) lactic acid.

L.sake was the predominant species throughout indicating its supremacy all over the manufacturing process.

The studies of Montel and Champomier (1987) have shown that L.sake can hydrolize arginine via the arginine deiminase pathway. Kandler and Weiss (1986) in the 9th Edition of Bergey's Manual described L.sake as arginine negative. Other authors (Niemand and Holzapfel, 1984 and Morishita and Shiromizu, 1986) found all meat isolates being arginine negative.

In this study, the isolates belonging to group A, assigned to L.sake by other tests than the degradation of arginine can produce NH_3 from this amino acid. In the same way, our results agree with those of Montel et al (1990) showing L.curvatus to be unable to hydrolize arginine, except for 8% of strains which hydrolize arginine and produce citrulline.

In seventy strains the arginine degradation was determined by two different methods, detecting ammonia as the final catabolic product and measuring citrulline which is the product of enzyme arginine deiminase. The citrulline production ranged from 0 to 8.1 mmols/ ml.

In 94% of isolates both methods agreed. In four L.sake strains there was an inconsistency of the results obtained using the methods described by Schillinger and Lücke (1987) and by Niven et al. (1942). The measurement of citrulline as well as the detection of ammonia in the aerobic cultures with 0.03% of glucose was negative although Nessler's reaction was positive in the cultures without glucose.

When these strains were grown anaerobically in the medium of Niven, ammonia was detected in the supernatant of two strains L.sake CTC445 and CTC 292. In the other two strains L.sake CTC 344 and CTC 347, the deamination of arginine only was possible in a medium supplemented with sodium citrate and sodium acetate followed by anaerobic incubation.

Therefore, there is evidence that in some L.sake strains the arginine deiminase pathway is induced only at a low axygen level. In Bacillus licheniformis this type of induction was reported by Broman et al (1975).

CONCLUSSIONS

From all the results reported it is shown that the species of lactobacilli predominant in Spanish fermented products are the same as in other European countries. When testing L.sake strains for the deamination of arginine it is suggested: supplement the medium of Niven with sodium citrate and sodium acetate and to incubate the cultures anaerobically.

REFERENCES

HASTINGS, J.W. AND HOLZAPFEL, W.H. (1987) Conventional taxonomy of lactobacilli surviving radurization of meat. J. of Appl. Bacteriol. 62, 209-216.
HITCHENER, B.J.; EGAN, A.F. AND ROGERS P.J. (1982) Characteristics of lactic acid bacteria isolated from vacuum packed beef. J. of Appl. Bacteriol. 52, 31-37.
KANDLER, O. AND WEISS, N. (1986) Regular non-sporing Gram positive rods. In: Bergey's Manual of Systematic Bacteriology. Williams & Wilkins. Baltimore. pp. 1208-1234.
MONTEL, M.C. AND CHAMPOMIER, M.C. (1987) Arginine catabolism in Lactobacillus sake isolated from meat. Appl. and Environm. microb. 53, 2683-2685.
MONTEL, M.C., TALON, R. AND CHAMPOMIER, M.C. (1989) Identification of some lactic acid bacteria from meat. Proceed. 35th Int. Congress of Meat Science and Technology. Copenhagen.
MORISHITA, Y. AND SHIROMIZU, K. (1986) Characterization of lactobacilli isolated from meats and meat products. Int. J. of Food Microb. 3, 19-29.
NIEMAND, J.E. AND HOLZAPFEL, W.H. (1984) Characteristics oflactobacilli isolated from radurised meat. Int. J. of Food Microb. 1, 99-110.
NIVEN, C.F. JR., SMILEY, K.L. AND SHERMAN, J.M. (1942) The hydrolysis of arginine by streptococci. J. of Bacteriol. 43, 651-660.
REUTER, G. (1975) Classification problems, ecology and some biochemical properties of lactobacilli of meat products. In: J.G. Carr, J.G. Cutting and C.V. Whitting (Eds), **Lactic Acid Bacteria in Beverages and Foods** Academic Press, London, pp 221-229.
SCHILLINGER, U. AND LUCKE, F.K. (1987) Identification oflactobacilli from meat and meat products. Food. Microb. 4, 199-208.

TABLE I
Phenotypic traits of the lactobacilli investigated

Groups	A	B	C	D
Species	L.sake	L.curvatus	L.bavaricus	L.plantarum
Total strains	139	66	27	22
Fermentation of...				
cellobiose	34	74	67	100
lactose	31	53	74	100
maltose	28	79	67	100
manitol	0	0	0	100
melibiose	99	0	44	100
melezitose	6	0	7	100
raffinose	0	0	0	77
rhamnose	0	0	0	41
ribose	100	100	100	77
saccharose	99	59	44	100
trehalose	96	55	100	100
NH_3 from arginine	100	8	41	0
Growth				
45 C	20	33	55	64
8% NaCl	70	74	78	100
Voges-Proskauer	17	14	22	86
Lactic acid isomer(s)	DL	DL	L	DL
Mesodiaminop.acid	-	-	-	+
pH_{24h}	<3.9	0	0	68
Agar acetate	100	94	100	100

All the groups fermented glucose, did not produce gas from glucose and grew at 8°C, 15°C and in 5% NaCl. The values are in percentages.

TABLE II

Species isolated from different processors

Species	Processor															
	A	C	E	F	H	L	N	O	P	R	S	T	U	X	Z	TOT.STRAINS
L.sake	0	9	17	12	13	12	14	12	10	7	15	5	5	7	1	139
L.curvatus	3	15	2	4	1	3	1	3	1	8	1	2	14	1	7	66
L.bavaricus	0	2	2	0	3	3	2	2	0	1	1	0	0	0	11	27
L.plantarum	0	4	12	0	0	0	2	0	0	1	0	1	0	2	0	22
Number of strains	**3**	**30**	**33**	**16**	**17**	**18**	**19**	**17**	**11**	**17**	**17**	**8**	**19**	**10**	**19**	**254**