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CHARACTERIZATION OF LACTOBACILLI ISOLATED FROM 'ADIN' - A SMOKE-DRIED MEAT PRODUCT

R.N.Borpuzari and T. Borpuzari
Department of Animal Production & Management, Faculty of Veterinary Science, Assam Agricultural University, P.O. Box No.13, Khanapara, Guwahati - 781 022, Assam (INDIA). Tel: 91-361-561 303; Fax: 91-361-563 633

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Background: Lactobacilli are essential components of the bacterial flora of meat and meat products and they exert considerable influence on the quality of the product. The processing conditions and preservation temperature effect the demographic pattern of the different groups of lactobacilli. Of the various groups of lactobacilli, streptobacteria and betabacteria have been reported to be present in large numbers in low temperature preserved meat products (Enfors et al., 1979; Patterson and Gibbs, 1977; Sutherland et al., 1975) and their biochemical characteristics have been studied (Egan, 1983; Holzapfel and Gerber, 1983; Mol et al., 1971); whereas, studies on thermobacteria are comparatively fewer. Adin - a traditional smoke-dried meat product of Arunachal Pradesh, the north eastern most state of India is held over firewood smoke for a prolonged period after its brief preliminary processing. The processing conditions of adin is described elsewhere (Borpuzari et al., 1996). As the product is held at high temperature of about 50-60 °C, it is expected that the population pattern of the different groups of lactobacilli will have a characteristic feature. This report describes the biochemical characteristics of the different groups of lactobacilli and their distribution in adin at different stages of its production.

Materials and methods:

Isolation of organisms: Samples of adin were collected aseptically during its various stages of production, viz., raw mithun meat (**Stage I**), after long strips of meat were being stitched with bamboo sticks (**Stage II**), 5th d (**Stage III**), 15th d (**Stage IV**) and 35th d of smoking (**Stage V**) for bacteriological examinations. Decimal dilutions of the samples were made in 1% peptone water and were inoculated on to double layered MRS agar plates (de Mann et al., 1960) and incubated at 15°C for 7d, 30°C for 3d and 37°C for 2d. About 20% (Ordonez, 1979) randomly selected typical spindle shaped colonies showing Gram +ve reactions were isolated for further studies. After purification, these were maintained on MRS agar slants at refrigeration temperature.

Biochemical test: For biochemical examinations, active broth cultures of the isolates were used. Most of the isolates were grown in MRS broth at 30°C and the thermophilic strains at 37°C. The test medium was inoculated with 0.1ml of the active culture (24h old) and incubated at 30°C.

Gas production from glucose was determined in MRS broth and acid production from carbohydrates was determined in MRS fermentation medium containing 2% of the respective sugar. Tubes were incubated at 30°C upto 7d and were examined at 24h interval.

Hydrolysis of arginine was determined by inoculating the cultures in MRS broth containing 0.3% arginine as per method described by Sharpe (1962). Growth at 7.5% NaCl was determined in MRS broth containing 7.5% NaCl and growth at 15 and 45°C was examined in MRS broth.

Results and discussion: A total of 149 isolates were examined out of which 70 strains belonged to thermobacteria, 37 betabacteria, 34 streptobacteria and the remaining 8 strains could not be identified due to their erratic biochemical reactions.

The differential characteristics of lactobacilli isolates belonging to the 3 groups are presented in **Table 1**. Of the thermobacterial strains, T1 and T2 showed similar biochemical characteristics excepting that 11-89% of the strains of T1 showed positive reaction for melibiose, raffinose and trehalose and the strains under T2 were negative for all these three sugars. T3 demonstrated similar biochemical characteristics to those of T1 and T2; however, T3 were positive for trehalose and negative for cellobiose and esculin and were doubtful fructose fermenters. T4 showed identical fermentation pattern to those of T1 and T2 excepting that this group could utilise melibiose and raffinose. Only 11-89% strains of T1 could produce acid from melibiose and raffinose and thus showed that they were doubtful fermenters for these sugars. From the differential characteristics depicted in Table 1, groups T1, T2, T3 and T4 were identified to be identical to Lactobacillus acidophilus, L. gasseri, L. helveticus and L. vitulinus, respectively.Most of the strains of thermobacteria were isolated at 37°C indicating this to be the optimum temperature for their isolation from meat and meat products. Morishita and Shiromizu (1986) also noted similar observations.

All the isolates of the betabacterial group exhibited positive reaction for fructose and galactose and negative for mannitol and melezitose. Groups B1, B3 and B4 showed identical patterns in fermentation of cellobiose, melibiose and salicin. Only B1 could not utilise lactose, raffinose and sucrose. B3 and B4 differentiated from B1 and B2 by their ability to grow well at 45°C. B1 was identical to L. brevis. This group grew well in presence of 7.5% NaCl. Occurrence of L. brevis in meat and meat proucts was reported by many workers (Reuter, 1970; Schillinger and Lucke, 1987). B2 showed biochemical and morphological characteristics akin to L. confuscus (Sharpe et al., 1972) and B3 to L. fermentum. Distinguishing characteristics of B4 guided that this group might belong to L. reuteri (Kandler and Weiss, 1986).

All the isolates of the streptobacterial group could ferment fructose and galactose. They were negative for arabinose with the exception of S5 and to some extent by S4. S2 could be differentiated from other streptobacterial groups as it was the only group which showed positive reaction for melezitose while S1 was positive for rhamnose and negative for cellobiose, ribose and doubtful for esculin and salicin. All the other groups showed positive reaction for esculin and salicin. S1 and S2 showed similar fermentation pattern excepting that S1 was doubtful for melibiose and raffinose but S2 was negative for these two sugars. S3 could be differentiated from S1, S2 and S4 by its inability to produce acid from mannitol and from S5 as the latter group could not ferment sucrose and trehalose. From these mutually differentiating biochemical characteristics, the 5 groups of streptobacteria, namely, S1, S2, S3, S4 and S5 were found to be identical to L.coryneformis, L. casei, L. curvatus, L. plantarum and L. sake, respectively. Hiu et al.(1984), Kagermeier (1981), Schillinger (1985) and Schillinger and Lucke (1987) have also reported that these species are important components of the lactobacilli in meat and meat products.

Table 1. Differential characteristics of lactobacilli isolated from adin

Group	Growth at					Fermentation of														
	15°C	45°C	7.5% NaCl	Gas from glucose	Hydrolysis of arginine	Arabinose	Cellobiose	Esculin	Fructose	Galactose	Lactose	Mannitol	Mannose	Melezitose	Melibiose	Raffinose	Ribose	Salicin	Sucrose	Trehalose	Xylose
T1	–	+	–	–	–	–	+	+	+	+	+	–	+	–	d	d	–	+	+	d	–
T2	–	+	–	–	–	–	+	+	+	+	+	–	+	–	–	–	–	+	+	–	–
T3	–	+	–	–	–	–	–	–	d	+	+	–	+	–	–	–	–	+	+	+	–
T4	–	+	–	–	–	–	+	+	+	+	+	–	+	–	+	+	–	+	+	d	–
B1	–	–	+	+	+	+	–	–	+	+	–	–	–	–	+	–	+	–	–	–	+
B2	–	–	–	+	+	–	+	+	+	+	d	–	+	–	–	–	–	+	+	–	+
B3	–	+	–	+	+	d	–	–	+	+	+	–	W	–	+	+	d	–	+	d	d
B4	–	+	–	+	+	+	–	O	+	+	+	–	–	–	+	+	+	–	+	–	–
S1	+	–	+	–	–	–	–	d	+	+	d	+	+	–	d	d	–	d	+	–	–
S2	+	–	+	–	–	–	+	+	+	+	d	+	+	+	–	–	+	+	+	+	–
S3	+	–	+	–	–	–	+	+	+	+	d	–	+	–	–	–	+	+	+	–	–
S4	+	–	+	–	–	d	+	+	+	+	+	+	+	d	+	+	+	+	+	+	d
S5	+	–	+	–	–	+	+	+	+	+	+	–	–	–	–	–	+	W	+	+	–

All the groups fermented maltose and were rhamnose negative with the exception of S1 which fermented rhamnose. +, positive for 90% or more strains; –, negative for 90% or more strains; d, positive for 11-89% strains; O, not determined; W, weak reaction.

The distribution of the different groups of lactobacilli during the various stages of production of adin (**Table 2**) showed that the thermobacterial group was the dominating lactobacilli followed by betabacteria and streptobacteria. Of the 70 thermobacterial strains, 35 could be isolated from Stage III. Amongst the thermobacterial organisms, L. acidophilus was predominant followed by L. vitulinus. The betabacterial organisms were also isolated in maximum numbers in Stage III and L. fermentum was the predominant species of lactobacilli amongst the betabacteria. This is an indicator of the poor sanitary conditions in slaughtering of the animal and subsequent processing of the meat for preparation of adin. Blickstad et al.(1981), Morishita and Shiromizu (1986) and Reuter (1970) reported occurrence of this species in meat and meat products.

Table 2. Distribution of groups of lactobacilli in adin at various stages of its production

Stages of adin production	Groups of lactobacilli													Total
	Thermobacteria				Betabacteria				Streptobacteria					
	T1	T2	T3	T4	B1	B2	B3	B4	S1	S2	S3	S4	S5	
Stage I	3[a]	0	2	1	2	3	1	2	0	3	1	4	3	25
Stage II	4	1	5	2	3	3	4	3	2	3	2	3	4	39
Stage III	17	3	5	10	2	1	8	3	2	4	1	0	2	58
Stage IV	5	2	3	2	0	0	2	0	0	0	0	0	0	14
Stage V	2	1	0	2	0	0	0	0	0	0	0	0	0	5
Total	**31**	**7**	**15**	**17**	**7**	**7**	**15**	**8**	**4**	**10**	**4**	**7**	**9**	**141**

a, No. of strains

L. casei closely followed by L. sake were the predominant species of the streptobacterial group of lactobacilli isolated from adin. While L. sake could be isolated in maximum numbers from Stage II, L. casei could be isolated in highest numbers in Stage III.

Conclusion: Unlike in cold stored and fermented meat products, where either streptobacteria or betabacteria are reported to be predominant groups of lactobacilli, in adin - a smoke-dried meat product, thermobacteria was the predominant group. This indicated that the temperature of preservation and processing conditions influenced the distribution of lactobacilli in meat products. Occurrence of L. fermentum and L. brevis in large numbers suggested that there is scope to improve the sanitary condition in preparation of the product.

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