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CHARACTERIZATION OF LACTIC ACID BACTERIA ISOLATED FROM SOME POPULAR DAHI SAMPLES

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Abstract

One hundred and forty lactic acid bacteria were isolated from seven popular dahi products collected from Dhaka, Mymensingh, Tangail, Comilla Barisal and Bogra. The organisms were identified on the basis of their morphological, cultural and biochemical characteristics. The organisms were treated with different physical and chemical agents to determine their tolerance. One hundred per cent species of *Lactobacillus* and *Streptococcus* were found to grow at 37°C and at pH 4.5 to 5.5 and 7.5, respectively. Seventy isolates of *Streptococcus* and *Lactobacillus* were identified as *Streptococcus thermophilus*, *S. lactis, S. cremoris, S. faecalis, and Lactobacillus bulgaricus, L. helveticus, L. acidophilus* and *L. plantarum,* respectively. Of these *S. thermophilus* and *L. bulgaricus* were frequently found in all dahi samples. The latter organisms also exhibited longer viability than the other species.

Introduction

Although the art of preparing dahi in Indian subcontinent has been practiced for ages, their science and technology as practiced today are still not of modern origin. The traditional dahi fermentation processes involved unpredictable change in milk because they are effected by mixed or unknown cultures of bacteria.(1) As dahi is made in many regions of Bangladesh, the taste and other organoleptic characteristics differ from locality to locality.(2) The differences in organoleptic characters of the traditional products depend mainly on the characteristics of the lactic starter cultures. In our country, dahi is traditionally manufactured and is not made commercially on a huge scale. Therefore, its microbiology is varied and accordingly the products are also variable with its taste and appearance. The dahi isolates, therefore, need to be identified, because of the variation in the organoleptic characters found in the traditional products. Identification of the isolates requires thorough characterization with a view to achieving desired manufacturing practice. Advances in science and microbial technology have led the way for the isolation and identification of more

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specific cultures and for the development of newer and a wider variety of cultured dairy products.[3] Therefore, modern technologies invariably help in the development and use of 'pure' or known cultures with predictable performance potential.

A huge number of research publications on the yogurt microbiology are available from different countries of the world.[4-7] Except some fragmentary reports[2,8-10] from Bangladesh no scientific research work on dahi microbiology are known so far. This present work concerns detailed characterization of microorganisms of dahi derived from traditional dahi of our country.

Materials and Methods

Seven well-known dahi samples were collected from Mymensingh (Daya Moni and Krishna Cabin), Comilla (Matri Bhander), Tangail (Tangail Sweets), Bogra (Akboria Sweets), Dhaka (Bikrampur Sweets) and Barisal (Gournadi). All samples were transported to the laboratory at the shortest possible time. Techniques for the isolation of lactic acid bacteria were described previously. [2] Lactic acid bacteria were identified according to the Bergey's Manual of Determinative Bacteriology.[11] Tests for bacterial tolerance to different temperature and pH and different concentrations of NaCl were performed in YGL broth and MRS broth media, while methylene blue tolerance was performed using fluid milk.

For viability test different species of *Streptococcus* and *Lactobacillus* were grown in YGL broth and Rogosa broth, respectively. The cells were harvested by centrifugation, suspended in Ringers solution[12] and mixed with measured amount of fluid and powder milk. The organisms were then stored at room temperature up to 106 days. The cultures were then tested for cell viability by standard plate count [13] using YGA and Rogosa agar media.

Results and Discussion

Cultural characteristics of streptococci and lactobacilli isolated from dahi samples were examined on YGL agar and MRS agar media, respectively and in the respective broth media. Ten well-spaced morphologically distinct colonies from each sample were chosen for further study. Pure cultures were obtained after a couple of subculturings on the respective agar plates. Colonies on YGL agar plate were small, about 1 - 2 mm in diameter, and discrete. Most of the organisms produced raised pinpointed colonies with entire margin. The colony colour varied from white to gray. In broth cultures, the organisms produced turbidity and sediment. On MRS agar, most of the *Lactobacillus* isolates produced small rough colonies of about 1 - 3 mm in diameter with entire margin. The isolates were non-pigmented and produced white to

light gray colonies. Some isolates produced larger (3 - 4 mm in diameter), smooth, round colonies; they were all pigmented and formed yellowish colonies.

Suspected streptococci and lactobacilli were Gram positive. The organisms grown on YGL agar medium were non-motile, non-endospore former cocci. Most of them were ovoid and were arranged predominantly in short chains and occasionally in pairs. Cells of some isolates were spherical and arranged predominantly in short chains. A few formed long chains, occasionally in pairs and their cells were spherical to ovoid. Colonies isolated from MRS agar plates were all regular rods with rounded end. Most of the isolates predominantly existed in single rods, but pairs and short chains were also observed. Other isolates occurred as single and in chain but not in pair.

Differential biochemical characteristics of streptococci and lactobacilli were studied and the results are summarized in Table 1. All streptococci isolates were able to ferment galactose, glucose and lactose, but the results were varied in other biochemical tests. Streptococci and lactobacilli isolates exhibited no catalase activity and were unable to liquefy gelatin. Results of other biochemical tests were stated in Table 1.

Table 2 shows the results of the ability of lactic bacteria to grow at various temperatures and pH, their tolerance to NaCl and methylene blue. All lactic acid organisms grew best at 37°C. At higher temperatures (45 - 50°C) ca. 63 - 67% streptococci and ca. 84 - 96% lactobacilli were able to grow. Lactobacilli showed better growth in acidic condition (pH 5.5 or below) than streptococci. At pH 5.5, only ca. 39% streptococci were found to grow, while all lactobacilli showed good growth at pH 4.5 - 5.5. Salt and methylene blue tolerance tests were performed only for the streptococcal isolates.

Table 3 represents the list of *Streptococcus* and *Lactobacillus* isolates identified on the basis of the conventional taxonomic criteria. Four species of *Streptococcus*, *viz*., *S. thermophilus*, *S. lactis*, *S. cremoris* and *S. faecalis*, and four species of *Lactobacillus*, *viz*., *L. bulgaricus*, *L. helveticus*, *L. acidophilus* and *L. plantarum*, were isolated and identified from the dahi samples in this study. The most predominant species of *Streptococcus* in all dahi samples was *S. thermophilus*, which represented ca. 63% of the total streptococcal isolates. The most predominant species of *Lactobacillus* in this study was *L. bulgaricus* (67%), which was found in highest number in all dahi samples. Other *Lactobacillus* species were identified as *L. helveticus*, *L. acidophilus* and *L. plantarum*.

The organisms isolated from dahi samples in this study were commonly found in the starter cultures used for preparation of dahi except *S. faecalis*.[14] The most prevalent dahi producing lactics were *S. thermophilus* and *L. bulgaricus*, which are

also used for commercial production of yogurt in a 1 : 1 proportion.[14,15] The mixed culture of the two organisms results in lactic acid production at a greater level than that produced by either when growing alone. More acetaldehyde and the chief volatile flavour component of yogurt or dahi, are produced by *L. bulgaricus* when growing in association with *S. thermophilus*.[7] Banerjee[16] suggested that a mixed culture of *S. lactis*, *S. thermophilus* and *L. cremoris* (*L. citrovorum*) could be used for the preparation of sweet dahi and a mixed culture of *S. thermophilus* and *L. bulgaricus* could be used for preparation of dahi of high acidity. Sharma and Jain[17] also reported that a mixed culture of *S. thermophilus* and *L. bulgaricus* produced greater amounts of acid than mixed cultures of *S. thermophilus* and *S. lactis* subspecies *diacetylactis* and *S. lactis*.

Table 1. Differential biochemical characteristics of lactic acid bacteria isolated from dahi samples.

Biochemical properties	No. (in percentage) positive	
	Lactic Streptococci (n = 70)	Lactic Lactobacilli (n = 70)
Acid production from:		
Arabinose	15 (21.4)	1 (1.4)
Fructose	65 (92.9)	58 (82.9)
Galactose	70 (100)	70 (100)
Glucose	70 (100)	70 (100)
Lactose	70 (100)	70 (100)
Maltose	19 (27.1)	23 (32.9)
Mannitol	19 (27.1)	3 (4.3)
Mannose	4 (5.7)	11 (15.7)
Ribose	0 (0)	3 (4.3)
Salicin	21 (30.0)	11 (15.7)
Sorbitol	4 (5.7)	3 (4.3)
Sucrose	63 (90.0)	11 (15.7)
Xylose	15 (21.4)	1 (1.4)
Catalase activity	0 (0)	0 (0)
Gelatin liquefaction	0 (0)	0 (0)
NH_3 formation from arginine	18 (25.7)	0 (0)
Nitrate reduction	ND	0 (0)
Haemolysis on blood agar:		ND
α-reaction	55 (78.6)	
β-reaction	0	
γ-reaction	21 (30.0)	

ND = Not done.

For long-term preservation of different species of *Streptococcus* and *Lactobacillus*, the bacterial suspension was mixed with sterilized fluid and powder milk. Viable bacterial counts were performed at time intervals. Viability of the predominant lactic acid bacteria, namely *S. thermophilus* and *L. bulgaricus* is depicted in Figs. 1 and 2,

respectively. Counts reduced considerably after one week but the cell viability remained same after subsequent storage for up to 30 days, and thereafter, the viable cell counts reduced to 2.19 x 10^5 CFU/g of fluid milk and 4.27 x 10^5 CFU/g of powder milk. After 106 days of storage, the viable cell counts dropped down to 2.4 x 10^3 CFU/g and 1.99 x 10^3 CFU/g of milk and of powder milk, respectively (Fig. 1). The initial *S. lactis* counts were about 8 x 10^8 CFU/g and after 7 days of storage, the viable counts reduced to 2.6 x 10^5 CFU/g. Afterwards, the viability lost very slowly,

Table 2. Tolerance of lactic acid bacteria isolated from dahi products to physical and chemical agents.

Physical/chemical agents	No. (in percentage) positive	
	Lactic Streptococci (n = 70)	Lactic Lactobacilli (n = 70)
Growth in broth at temperature (°C):		
10	26 (37.1)	3 (4.3)
37	70 (100)	70 (100)
45	47 (67.1)	67 (95.7)
50	44 (62.8)	59 (84.3)
Growth in broth at initial pH:		
4.5	ND	70 (100)
5.5	27 (38.6)	70 (100)
6.5	ND	62 (88.6)
7.5	70 (100)	ND
8.5	50 (71.43)	0 (0)
9.2	46 (65.7)	ND
9.6	43 (61.4)	ND
Growth in broth containing:		ND
2.0 % NaCl	24 (34.2)	
4.0 % NaCl	18 (25.7)	
6.5 % NaCl	3 (4.3)	
Growth in milk containing:		ND
0.01 % methylene blue	26 (37.1)	
0.1 % methylene blue	26 (37.1)	
0.3 % methylene blue	18 (25.7)	

ND = Not done.

but after 106 days of storage, only a few hundred cells survived. *S. cremoris* exhibited a different pattern of survival curve. The viable cell counts decreased progressively with the increase of storage period. After 106 days, only a few cells survived. *S. faecalis* showed greater viability than the other *Streptococcus* species. The viable counts decreased slowly with the storage period. The viable cell counts after 106 days storage were 7.1 × 10^4 CFU/g of milk and 8.8 × 10^4 CFU/g of dried milk (Figure is not shown).

Table 3. Prevalence of different lactic acid bacteria in different dahi samples.

Dahi samples	Prevalence			
	Streptococcus	Frequency (%)	*Lactobacillus*	Frequency (%)
Daya Moni dahi (Mymensingh)	*S. thermophilus*	60	*L. bulgaricus*	70
	S. lactis	20	*L. helveticus*	30
	S. faecalis	20		
Krishna Kabin dahi (Mymensingh)	*S. thermophilus*	50	*L. bulgaricus*	60
	S. lactis	30	*L. helveticus*	20
	S. cremoris	20	*L. acidophilus*	20
Matri Bhander dahi (Comilla)	*S. thermophilus*	80	*L. bulgaricus*	60
	S. cremoris	20	*L. helveticus*	40
Tangail dahi (Tangail)	*S. thermophilus*	70	*L. bulgaricus*	60
	S. lactis	20	*L. acidophilus*	20
	S. faecalis	10	*L. plantarum*	20
Akboria dahi (Bogra)	*S. thermophilus*	60	*L. bulgaricus*	80
	S. lactis	20	*L. helveticus*	20
	S. cremoris	20		
Bikrampur dahi (Dhaka)	*S. thermophilus*	60	*L. bulgaricus*	80
	S. lactis	40	*L. helveticus*	10
			L. plantarum	10
Gournadi dahi (Barisal)	*S. thermophilus*	60	*L. bulgaricus*	60
	S. lactis	20	*L. acidophilus*	40
	S. cremoris	20		
Total	*S. thermophilus*	63	*L. bulgaricus*	67
	S. lactis	21	*L. helveticus*	17
	S. cremoris	12	*L. acidophilus*	12
	S. faecalis	4	*L. plantarum*	4

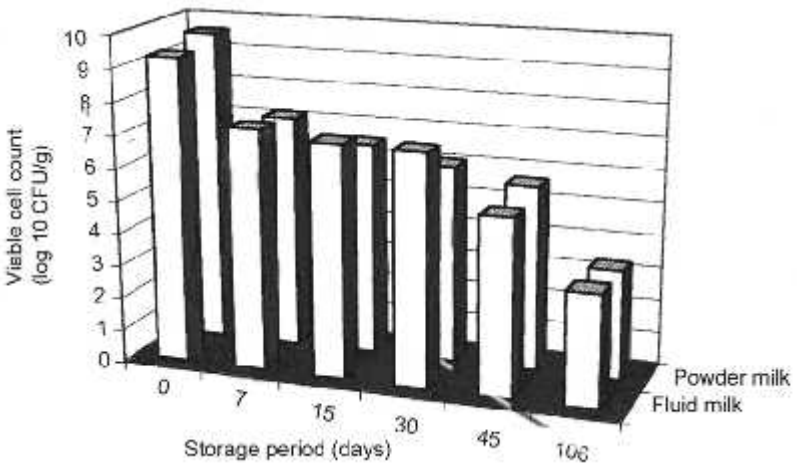

Fig. 1. Viability of *Streptococcus thermophilus* during storage.

Lactobacillus bulgaricus showed greater viability than all other lactic acid bacteria tested (Fig. 2). It lost its viability slowly and after 106 days of storage, the viable cell counts were above 10^4 CFU/g of both media. *L. helveticus* counts were remarkably reduced within 7 days of storage, and after 106 days only tens of cells remained viable. *L. acidophilus* and *L. plantarum* were least stable in storage condition. The viable counts of these organisms dropped down considerably within 7 days. Only a few cells were able to develop into colonies after 106 days of storage.

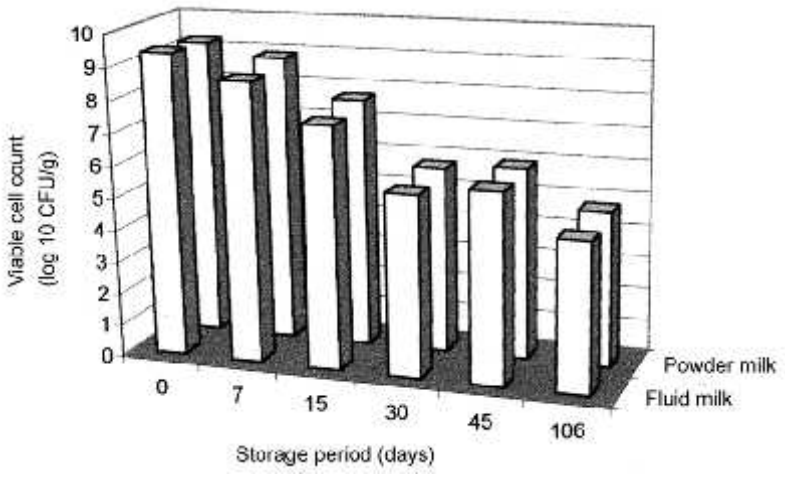

Fig. 2. Viability of *Lactobacillus bulgaricus* during storage.

Conclusion

It is well documented that the maintenance of starter culture of dahi for long time is very difficult without lyophilyzation. Traditionally dahi is prepared using small quantity of previously prepared dahi (natural starter culture). The frequent use of this culture for dahi preparation does not conserve the consistency of dahi. Sometimes the culture is contaminated with other non-lactic acid bacteria, which may change the taste and aroma of dahi that consumers do not like. From the experimental findings it was apparent that the pure starter culture of dahi could be preserved for long time in liquid and powder milk. This study needs more extensive work for better achievement and to produce instant dahi.

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