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Research article ***Short Communication***

Isolation of exopolysaccharide producing *Streptococcus thermophilus* organism of yoghurt

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ABSTRACT

The present study was conducted to isolate the exo-polysaccharide producing *Streptococcus thermophilus* organism from the available yoghurt in Chittagong areas of Bangladesh and to develop pure culture in laboratory. A total of 100 samples (25 of each brand) were collected from four different brands namely, A, B, C and D (Food plaza, Banoful, Fulkoli and Genuine, respectively). All the samples were subsequently cultured in nutrient agar for detecting live bacterial growth followed by deMan Rogosa and Sharpe agar to isolate *Streptococcus thermophilus* and finally Ruthenium red milk plate for the isolation of exopolysaccharide producing *Streptococcus thermophilus*. Then fermented product, yoghurt was prepared using this isolated organisms and sensory evaluation was done by a panel of judges. The study reveals 36% of the Brand C dahi were *Streptococcus thermophilus* positive. 44.44% cases were exopolysaccharide producing amongst the positive cases.16% of the Brand D yoghurt were *Streptococcus thermophilus* positive and 20% for positive case of the Brand B were exopolysaccharide producing. In sensory evaluation, the yoghurt was made by using the isolated organism was well acceptable regarding taste, appearance, acidity, flavor. It achieved on average 87% acceptability regarding the selected criteria. In conclusion, the isolated *Streptococcus thermophiles* might be a good seed for preparing yoghurt having excellent probiotic capability.

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1. INTRODUCTION

Polysaccharides contribute in foods as viscosifying agents, stabilizers, emulsifiers, gelling agents, or water-binding agents. Most of these are plant and algae derived polysaccharides and their use is strongly restricted for food applications (Freitus et al. 2011). Commercially available microbial EPS are xanthan and gellan which are produced by *Xanthomonas campestris* and *Pseudomonas elodea*, respectively. Lactic acid bacteria (LAB), are bacteria with health benefits, considered as probiotics, associated with many fermented milk products; particularly curd, yoghurt and cheese etc. LAB possess generally regarded as safe (GRAS) status which allows them to be incorporated in food without labelling. Most of the LAB producing EPS belongs to the genera *Streptococcus*, *Lactobacillus*, *Lactococcus*, Leuconostoc, and *Pediococcus*. Production of EPS is also reported from some non-starter LAB like Bifidobacteria. LAB are able to produce mainly two types of polysaccharides according to their location in the cell, intracellular polysaccharides and extracellular polysaccharides. Some bacteria produce only capsular EPS, some produce only slime (ropy) form, whereas, in some cases, bacteria can produce both forms of EPS. EPS-producing LAB has a greater ability to withstand technological stresses and survive the passage through the gastrointestinal tract compared to their non-producing bacteria. EPS impart highly desirable rheological changes in the food matrix such as increased viscosity, improved texture and reduced syneresis. Further, EPS may induce positive physiological responses including lower cholesterol levels, reduced formation of pathogenic biofilms, modulation of adhesion to epithelial cells and increased levels of bifidobacteria showing a prebiotic

potential. Hence, the choice of EPS-producing starter cultures seems to give several advantages over non-producing ones. *Streptococcus thermophilus* is a potent probiotic, may be found in the fermented milk products like dahi (fermented dairy product popular in Indian subcontinent), an EPS producer in the products. Purified EPS from same strain of *Streptococcus thermophilus* was found to be effective for preventing chronic gastritis. EPS-protein interaction might be responsible for the observed gastroprotective effect (Rodriguez, 2008).

Over 80% of the people in Bangladesh are suffering from gastritis. It is of utmost importance to introduce something in diet to prevent gastritis. Dahi, cultured with exopolysaccharide producing *Streptococcus thermophilus* can meet the gap. For this reason, the study was conducted to isolate the EPS producing *Streptococcus thermophilus* from the available yoghurt in Chittagong areas of Bangladesh and to develop pure culture in the laboratory.

2. MATERIALS AND METHODS

A cross sectional study was conducted and the culturing and isolation of EPS producing *Streptococcus thermophilus* from yoghurt samples were done in the Dairy Science Laboratory of Chittagong Veterinary and Animal Sciences University during a period from December 2014 to May 2015.

2.1. Collection of samples

A total of 100 samples were collected from four different brands in Chittagong namely, Food plaza, Banoful, Fulkoli and Genuine following the standard procedure of sample collection. Twenty five samples were collected from each of the brand. Samples were collected at 03 days interval for each brand.

2.2. Procedure of isolation

Yoghurt samples were inoculated in nutrient agar to be sure for live organism. The successful colonies were inoculated in MRS agar to isolate the *Streptococcus thermophilus* and *Lactobacillus sp* (Figure 1). The colonies grown in MRS agar were stained with Grams staining procedure and observed under microscope in order to determine the colonies with *Streptococcus thermophilus* (Figure 2). The *Streptococcus* colonies were then inoculated in Ruthenium Red milk plate to isolate the exopolysaccharide producing *Streptococcus thremophilus*. The pure developed cultures were used for the development of fermented dairy products (yoghurt) with EPS producing *Streptococcus thermophilus.*

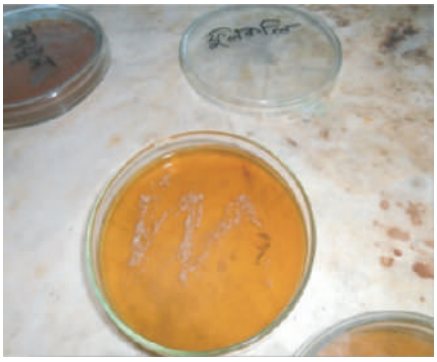


Figure 1: Colony formation on agar

Figure 2: *Streptococcus thermophilus* under microscope

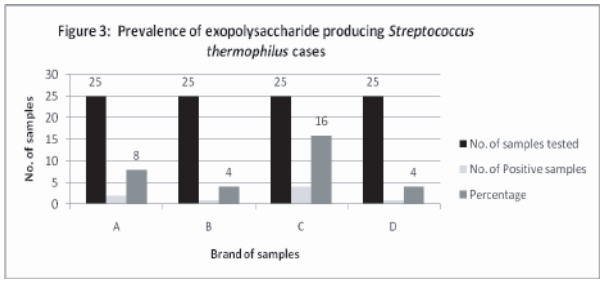
2.3. Sensory evaluation

Then the newly fermented product (Yoghurt) was prepared using the isolated organism and sensory evaluation was done by a panel of judges regarding taste, appearance, acidity, flavor etc.

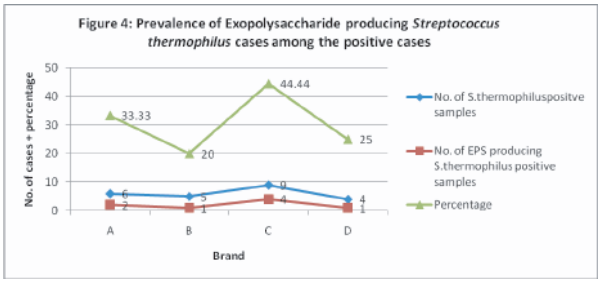
3. RESULTS

Among the four brands the prevalence of *Streptococcus thermophiles* was highest in the Brand C (36%), while the Brand D had the lowest percentage of 16. The samples from Brand A and B had the percentage of 24 and 20 respectively.

The prevalence of EPS producing *Streptococcus thermophilus* was also highest in the Brand C (16%), while the prevalence of EPS producing *Streptococcus thermophiles* in Brand A, B and D yoghurt were 8%, 4% and 4% respectively (Figure 3).



The percentage of EPS producing *Streptococcus thermophilus* among the positive case was highest in the Brand C (44.44%). The other brand yoghurt (A, B, D) had this percentage as 33.33, 20 and 25 respectively (Figure 4).



Among the positive cases, the exopolysaccharide producing *Streptococcus thermophilus* were highest in brand C yoghurt and lowest in brand B yoghurt.

The panel of judge scored the Acidity as the highest acceptable parameter (about 91) and the lowest acceptable was the colour (about 83). The taste was scored about 90 whereas the appearance was about 85 in the acceptability score of maximum 100 (Table 1).

Table 1: Sensory evaluation of yoghurt made by using isolated culture

Parameters	Mean score (Acceptability)	Overall
Appearance	84.6	87.13
Taste	89.8	
Colour	82.7	
Acidity	91.4	

4. DISCUSSION

The variation in positive percentage of EPS producing *Streptococcus* might occur due to the use of different undefined cultures in different brands of yoghurt. Wong et al., (1983) examined that yogurt is produced by lactic acid fermentation of milk by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*. Both Lactobacillus bulgaricus and *Streptococcus thermophilus* exhibited a protocoorperation association to produce lactic acid at a greater rate. Symbiotically growing, *Lactobacillus bulgaricus* provides *Streptococcus thermophilus* with formic acid, which provides better growth, while *Streptococcus thermophilus* releases amino acids, mainly valine to accelerate *Lactobacillus bulgaricus* growth. The test variables were consisted of milk, yogurt, milk fermented individually by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*, and milk which both cultures has been added. The microorganism isolated were *Lactobacillus acidophilus* (14%), *Lactobacillus casei* (20%), *Lactobacillus* helveticus (34%), *Lactobacillus bulgaricus* (86%), *Streptococcus thermophillus* (80%), *Streptococcus lactis* (74%), and *Streptococcus cremoris* (30%).Brand C showed highest percentage of presence *Streptococcus spp*. and exopolysaccharide producing *Streptococcus spp*. Neither, brand B had third highest of presence of *Streptococcus spp*. nor brand C had lowest exopolysaccharide producing *Streptococcus spp*. Iyer et al., (2011) studied the bioprospecting of folate producing strains of *Streptococcus thermophilus* isolated from milk and different fermented milkproducts of Indian origin. From a total of 500 randomly selected colonies isolatedfrom 209 different samples, 117 isolates were identified as *Streptococcus thermophilus* by classical biochemical and molecular characterization. Frequency of incidence of *Streptococcus thermophilus* in the different samples of milk and milk products was variable with the highest in the dahi followed by yogurt and lassi and a very low incidence in case of milk and cheese. On screening for folate using a microbiological assay with a trienzyme extraction, about 15% of strains was found to produce folate in the range of 40-50µg/L, 35% in the range of 20-30 µg/L, and the remainder in the range of4-16µg/L. *Streptococcus spp* presenting brand A, C and D yoghurt was more exopolysaccharide producing than *Lactobacillus spp* among the positive cases. But in brand B showed different circumstances. *Lactobacillus spp* were more exopolysaccharide producing then *Streptococcus spp* among the positive cases. Vuyst et al., 2003 studied with five interesting heteropolysaccharide producing strains have been tested. Both *Streptococcus thermophilus* LY03 and *Streptococcus thermophilus* CH101 produced the highest amounts of EPS and also displayed the highest apparent viscosities in fermented milk. *Streptococcus thermophilus* ST 111 and *Streptococcus thermophilus* STD differed considerably in EPS yields, but not in apparent viscosities of fermented milk. *Streptococcus spp* presenting yoghurt showed highest panel score in taste and acidity compared to *Lactobacillus spp* presenting yoghurt. But almost equal panel score showed in color criteria. Slightly rising panel score noticed in appearance criteria of *Lactobacillus spp* presenting yoghurt compared to *Streptococcus spp* presenting yoghurt. Rahman et al., 2009 studied five samples of camel milk fermented for 6h at 43ºC by selected starter cultures were prepared and sensory evaluated by 10 untrained panelists for color, smell, consistency, taste and overall acceptability. The mean scores value for color of the all fermented samples ranged from 7.9 to 8.1 (good). The results showed that there were no significant differences ($p > 0.05$) in color of the five fermented products. The mean score for

smell of camel milk fermented by yogurt culture (*Lactobacillus bulgaricus* and *Streptococcus thermophilus*,1:1) was significantly higher ($p < 0.05$) than mean scores for other fermented milk products by other starter cultures, indicating that camel milk fermented by yogurt culture (7.5) was the most acceptable followed by those fermented by *Lactobacillus bulgaricus* (6.4), *Streptococcus thermophilus* (6.2) and *Lactobacillus acidophilus* (6.0) while the least acceptable was that fermented by *Lactococcus lactis* (5.1).In general, the panelists gave lower sensory scores for consistency for all fermented cow milk but that one fermented by yogurt culture was slightly better in consistency score (4.3) than those fermented by other starter cultures. The panelists preferred fermented camel milk made by yogurt starter culture followed by *Lactobacillus bulgaricus*, *Lactobacillus acidophilus*, *Streptococcus thermophilus* and *Lactobacillus lactis*. The overall acceptability scores of the sensory evaluation revealed that the camel milk fermented by yogurt starter culture was the most accepted, while that fermented by *Lactobacillus lactis* was the least. Cow milk fermented by yogurtculture had significantly higher rating for smell, consistency, taste and acceptability compared with other cultures.

5. CONCLUSION

In a nutshell, the Brand C was the best quality yoghurt from the probiotic aspects. The newly developed fermented dairy product was found well acceptable as the score overall 87 out of 100. The pure culture of the EPS producing *Streptococcus thermophilus* developed can be used for yoghurt production which will be beneficial for both yoghurt marketing

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