International Journal of Natural Sciences (2017), 6(3):123-127

pISSN : 2221-1012
eISSN : 2221-1020

www.ijns.net

Isolation of exopolysaccharide producing *Lactobacillus bulgaricus* organism from yoghurt

Bari MS[1*], Sen AR[1], Mokbul SB[1], Rahman MK[2], Nath BK[1], Faruk MSA[3] and Kober AKMH[1]

[1]Department of Dairy and Poultry Science, [2]Department of Medicine and Surgery, [3]Department of Physiology, Biochemistry and Pharmacology, Faculty of Veterinary Medicine, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong – 4225, Bangladesh

[Received: February 05, 2017; Accepted: April 18, 2017]

ABSTRACT

The present study was conducted to isolate the exo-polysaccharide producing *Lactobacillus bulgaricus* 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 Food plaza (A), Banoful (B), Fulkoli (C) and Genuine (D). All the samples were subsequently cultured in nutrient agar for detecting live bacterial growth followed by de Man Rogosa and Sharpe (MRS) agar to isolate *Lactobacillus bulgaricus* and finally Ruthenium red milk plate for the isolation of exopolysaccharide producing *Lactobacillus bulgaricus.* Then fermented product, yoghurt was prepared using this isolated organisms and sensory evaluation was done by a panel of judges. The study reveals 20% of the Brand B yoghurt was *Lactobacillus bulgaricus* positive. About 40% cases were exopolysaccharide producing amongst the positive cases. Almost 32% of the Brand D yoghurt were *Lactobacillus bulgaricus* positive and 50% for positive case of the Brand D were exopolysaccharide producing. In sensory evaluation, the yoghurt was made by using the isolated organism. The product was well acceptable regarding taste, appearance, acidity, flavor etc. and it achieved on average 84% acceptability regarding the selected criteria. In conclusion, the isolated *Lactobacillus bulgaricus* might be a good seed for preparing yoghurt having excellent probiotic capability.
Key words: Isolation, exopolysaccharide, yoghurt, pure culture

INTRODUCTION

Yoghurt is increasingly being used as a carrier of probiotic bacteria for their potential health benefits. This requires a working method for selective enumeration of these probiotic bacteria and lactic acid bacteria in yoghurt such as *Streptococcus thermophilus, Lactobacillus delbrueckii subsp. bulgaricus, Lactobacillus acidophilus*, *Lactobacillus casei* and *Bifidobacterium* (Ashraf & Shah, 2011). 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 (Freitas *et al*., 2011). Exopolysaccharides (EPS) from lactic acid bacteria (LAB) contribute to specific rheology and texture of fermented milk products such as yoghurt and finds applications even in non-dairy foods and in therapeutics (Ismail & Nampoothiri, 2010). LAB is 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 (Patel *et al.,* 2013).

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 (Patel *et al.,* 2013). *Lactobacillus sp* is important probiotics that mostly found in dahi (the most popular dairy product) and produce exopolysaccharide which is very much effective to prevent chronic gastritis. EPS-protein interaction might be responsible for prevent gastritis. Released exopolysaccharide produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic *Escherichia coli*. To control microbial biofilm, exopolysaccharide become the novel food-grade adjuncts which naturally found in Lactobacillus of yoghurt (Kim *et al.,* 2009).

Most of the people in Bangladesh are suffering from gastritis. It is of utmost importance to introduce something in diet to prevent gastritis. Yoghurt, cultured with exopolysaccharide producing

* Corresponding author: drmsb09@gmail.com

Lactobacillus can meet the gap. For this reason, the study was conducted to isolate the EPS producing *Lactobacillus bulgaricus* from the available yoghurt in Chittagong areas of Bangladesh and to develop pure culture in the laboratory.

MATERIALS AND METHODS

A cross sectional study was conducted for isolation of exopolysaccharide producing *Lactobacillus bulgaricus* organism of yoghurt. The culturing and isolation of EPS producing *Lactobacillus spp* from yoghurt samples were done in the Dairy Science Laboratory of Chittagong Veterinary and Animal Sciences University during a period from December 2015 to May 2016.

Collection of samples:

A total of 100 samples were collected from four different brands in Chittagong namely, A, B, C and D for Food plaza, Banoful, Fulkoli and Genuine, respectively following the standard procedure of sample collection. The compositions of the yoghurt from different brands were exhibited somewhat different as these were made according to the direction of each company. Twenty five samples were collected from each of the brand. Samples were collected at 03 days interval for each brand.

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 *Lactobacillus spp.* The colonies grown in MRS agar were stained with Grams staining procedure and observed under microscope in order to determine the colonies with *Lactobacillus spp.* The *Lactobacillus* colonies were then inoculated in Ruthenium Red milk plate to isolate the exopolysaccharide producing *Lactobacillus spp.* The developed pure cultures were used for the development of fermented dairy products (yoghurt) with EPS producing *Lactobacillus spp.*

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.

RESULTS

Prevalence of *Lactobacillus* spp

Amalgamation of twenty five (n=25) samples for each four type's brands were observed for the presence of *Lactobacillus spp* (Table 1). Brand D exhibits highest percentage (32%) of *Lactobacillus spp* whereas brand B showed lowest percentage (20%). Seven (7) and six (6) samples of the Brand A and Brand C, respectively, were positive with *Lactobacillus spp.*, where the percentage of exopolysaccharide producing *Lactobacillus* were 28% and 24%, respectively (Table 1).

Prevalence of exopolysaccharide producing *Lactobacillus* spp

Table 2 shows the prevalence of exopolysaccharide producing *Lactobacillus spp* in 25 samples of each brand. As like as presence of highest percentage of *Lactobacillus* spp also having maximum amount of exopolysaccharide producing *Lactobacillus* spp in brand D (16%) yoghurt. Brand A exhibited inferior amount of exopolysaccharide producing *Lactobacillus* spp (4%). Both the brand B and C had the similar amount (8%) of exopolysaccharide producing *Lactobacillus spp* as stated in table 2.

Among four brands of yoghurt, brand D had the highest percentage of *Lactobacillus spp* positive cases and brand A had the second highest percentage of *lactobacillus spp* positive cases. But in case of the percentage of exopolysaccharide producing *lactobacillus spp* positive case, brand D was highest and brand A was the lowest while brand B and C remain almost similar in presence of *lactobacillus spp* and exopolysaccharide producing *lactobacillus spp* as shown in table 2.

Prevalence of exopolysaccharide producing *Lactobacillus* spp containing yoghurt

Table 3 shows the prevalence of exopolysaccharide producing *Lactobacillus spp* cases among the positive cases. In this examination brand D (50%) were offered greatest amount of percentage but brand B (40%) were showed superior production of exopolysaccharide producing *Lactobacillus spp* than brand A (14.29%) and C (33.33%). Fig 1 represents the percentages of *lactobacillus* spp positive in yoghurt and exopolysaccharide producing *lactobacillus spp* in yoghurt.

Sensory evaluation of yoghurt made by using isolated culture

The yoghurt was distributed among the panel of judge who gave the score based on some determined parameter (color, taste, appearance, acidity) (Table 4). The panel scored peak acceptable level at appearance (85.33%) and lowest at color (82.52%) which was also in acceptable level of parameter. Taste (83.67%) and acidity (84.74%) were also in agreeable level of parameter as stated in table 4).

DISCUSSION

Among twenty five (n=25) samples for each four type's brands observed presence of *Lactobacillus spp*. But brand D exhibit highest percentage (32%) in whereas brand B were showed lowest percentage (20%). Hoque *et al.*, 2010 stated that *Lactobacillus spp*. were isolated from two regional yoghurts in Bangladesh, which were identified on the basis of their colony morphologies and some biochemical tests. 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 thermophiles* exhibited a proto coorperation 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 thermophiles release amino acids, mainly valine to accelerate *Lactobacillus bulgaricus* growth. Masud *et al.*, 1991 observed in fifty samples of indigenous dahi were collected from Rawalpindi, Islamabad to determine incidence of Lactic acid bacteria. The microorganism isolated were *Lactobacillus acidophilus* (14%), *Lactobacillus casei* (20%), *Lactobacillus helveticus* (34%), *Lactobacillus bulgaricus* (86%), *Streptococcus thermophillus* (80%), *Streptococcus lactis* (74%), and

Table 1: Prevalence of *Lactobacillus* spp positive cases

Brand	N	No. of Positive samples	Percentage
A	25	7	28
B	25	5	20
C	25	6	24
D	25	8	32

Table 2: Prevalence of exopolysaccharide producing *Lactobacillus* spp cases

Brand	N	No. of Positive samples	Percentage
A	25	1	4
B	25	2	8
C	25	2	8
D	25	4	16

Table 3: Prevalence of exopolysaccharide producing *Lactobacillus* spp cases among the positive cases

Brand	N	No. of Positive samples	Percentage
A	7	1	14.29
B	5	2	40
C	6	2	33.33
D	8	4	50

Table 4: Sensory evaluation of yoghurt made by using isolated culture

Parameters	Mean score (Acceptability)	Overall
Appearance	85.33	
Taste	83.67	84.065
Colour	82.52	
Acidity	84.74	

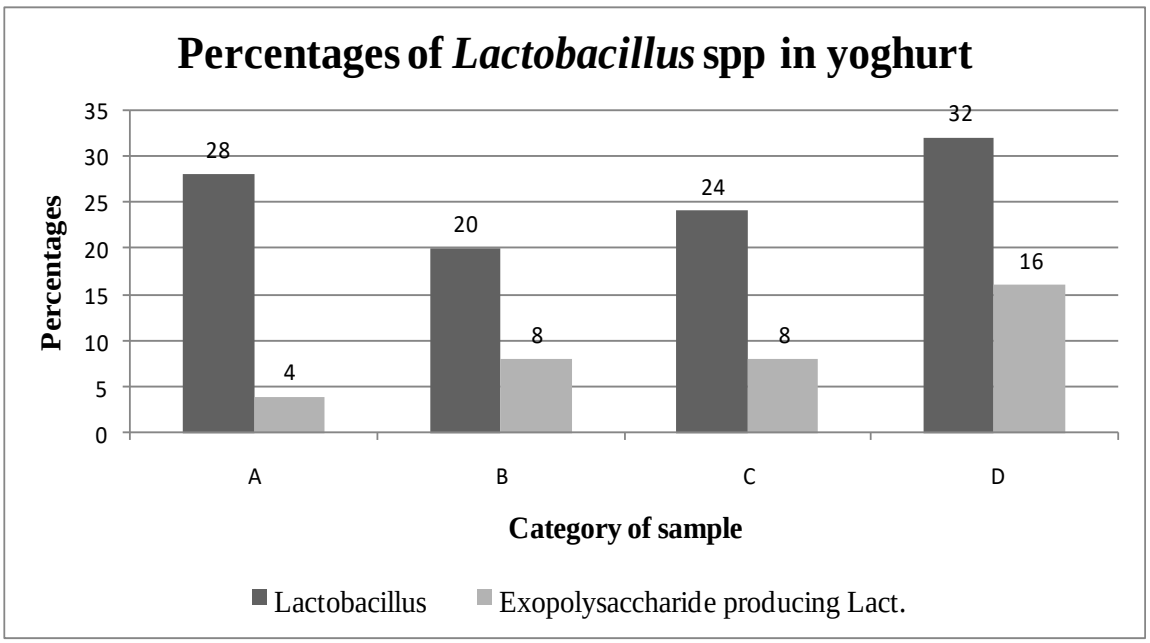

Fig 1: The graph shows the percentages of *lactobacillus spp* positive in yoghurt and exopolysaccharide producing *lactobacillus spp* in yoghurt. The horizontal axis presents the brand name and the vertical axis shows percent. The graph indicates that percent of exopolysaccharide producing *lactobacillus spp* in yoghurt increased as the percent of *Lactobacillus spp* increased in yoghurt.

Streptococcus cremoris (30%). Maximum amount of exopolysaccharide producing *Lactobacillus spp* in brand D (16%) yoghurt. Brand A (4%) exhibited inferior amount of exopolysaccharide producing *Lactobacillus spp*. Gürsoy *et al.,* 2010 stated that the yoghurt sample produced with commercial culture only had the highest level of acidity. Tyrosine content of the yoghurt produced by wild strains was higher than that of others, whereas acetaldehyde and EPS levels were lower. The domestic strains did not influence the viscosity of yoghurt in greater extent. On contrary, the gel firmness of yoghurts produced with *Lactobacillus delbrueckii spp. bulgaricus* (B3) was lower than the other samples.

In this study brand D (50%) were offered greatest amount of percentage but brand B (40%) showed superior production of exopolysaccharide producing *Lactobacillus spp* among the positive cases than brand A (14.29%) and C (33.33%). Tabibloghmany *et al.*, 2014 stated that exopolysaccharides (EPS) synthesized by lactic acid bacteria (LAB) play a major role in the manufacturing of fermented dairy products such as yoghurt, drinking yoghurt, cheese, fermented cream, milk based desserts. The demand of consumers for natural dairy products with a smooth and creamy texture, low in fat and sugars, can be satisfied by a judicious use of LAB producing EPS. One of the major sensory attributes important for consumer preference of dairy products is firmness and creaminess. EPS's may act both as texturizers and stabilizers, firstly increasing the viscosity of a final product, and secondly by binding hydration water and interacting with other milk constituents, such as proteins and micelles, to strengthen the rigidity of the casein network. As a consequence EPS can decrease syneresis and improve product stability. The panel scored peak acceptable level at appearance (85.33%) and lowest at colour (82.52%) which was in acceptable level of parameter. Taste (83.67%) and acidity (84.74%) was also in agreeable level of parameter. Olugbuyiro *et al.*, 2011 observed the results of the sensory evaluation revealed that flavor with respect to taste and smell had greater influence on overall acceptability of yogurt product. 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 panelists preferred fermented camel milk made by yogurt starter culture followed by *Lactobacillus bulgaricus, Lactobacillus acidophilus, Streptococcus thermophiles* 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.

CONCLUSION

In conclusion, 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 *Lactobacillus bulgaricus* was developed. This can be used for yoghurt production after further investigation and this could be beneficial for both human health and good quality yoghurt production.

ACKNOWLEDGEMENT

The authors would like to take the privilege to acknowledge University Grants Commission (UGC), Bangladesh and Chittagong Veterinary and Animal Sciences University (CVASU) for funding and necessary supports for the research.

REFERENCES

1. Ashraf R and Shah NP (2011). Selective and differential enumerations of Lactobacillus delbrueckii subsp. bulgaricus, Streptococcus thermophilus, Lactobacillus acidophilus, Lactobacillus casei and Bifidobacterium spp. in yoghurt - A review. *Int. J. Food Microb*. 149(3): 194 – 208.
2. Freitas F, Alves VD and Reis MAM (2011). Advances in bacterial exopolysaccharides: From production to biotechnological applications. *Tren. Biotech.* 29 (8): 388 – 398.
3. Ismail B and Nampoothiri KM (2010). Production, purification and structural characterization of an exopolysaccharide produced by a probiotic Lactobacillus plantarum MTCC 9510. *Arch. Microb.* 192(12), 1049–1057.
4. Kim Y, Oh S and Kim SH (2009). Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic Escherichia coli O157:H7. *Biochem. Biophy. Res. Commun.* 379(2): 324–329.
5. Hoque MZ, Akter F, Hossain KM, Rahman MSM, Billah MM and Islam KMD (2010). Isolation, Identification and Analysis of Probiotic Properties of Lactobacillus Spp. From Selective Regional Yoghurts. *World J. Dairy Food Sci.* 5(1): 39-46.
6. Gursoy A, Durlu-ozkaya F, Yildiz F and Aslim B (2010). Set Type Yoghurt Production by Exopolysaccharide Producing Turkish Origin Domestic Strains of *Streptococcus thermophilus* (W22) and *Lactobacillus delbrueckii sp. bulgaricus* (B3). *Kaf. Üniv. Vet. Fakül. Derg.* 16: 81 – 86.
7. Tabibloghmany FS, and Ehsandoost E (2014). An Overview of Healthy and Functionality of Exopolysaccharides Produced by Lactic Acid

Bacteria in the Dairy Industry. *Euro. J. Nutri. Food. Saf.* 4(2): 63-86.
8. Joseph AO, Olugbuyiro and Joy EO (2011). Physico-chemical and Sensory Evaluation of Market Yoghurt in Nigeria. *Pak. J. Nutri.* 10(10): 914-918.
9. Wong NP, McDonough FE and Hitchins AD (1983). Contribution of *Streptococcus thermophilus* to Growth-Stimulating Effect of Yogurt on Rats. *J. Dairy Sci.* 66: 3.
10. Rahman IEA, Dirar HA and Osman MA (2009). Microbiological and biochemical changes and sensory evaluation of camel milk fermented by selected bacterial starter cultures. *Afri. J. Food Sci.* 3(12):398-405.
11. Patel A and Prajapati JB (2013). Food and Health Applications of Exopolysaccharides produced by Lactic acid Bacteria. *Adv. Dairy Res.* 1: 107.
12. Masud T, Sultana K and Shah MA (1991). Incidence of lactic acid bacteria isolated from indigenous dahi. *Asian-Austra. J. Ani. Sci.* 4(4): 329-331.