

## **ACKNOWLEDGEMENT**

*I first praise to my creator, the almighty Allah, the omnipresent, omnipotent whose blessings have enabled the author in successful materialization and fulfillment of this study.*

*I feel great pleasure to express my deepest sense of gratitude and humble respect to reverend teacher and internship supervisor Dr. A K M Humayun kober, Professor, Department of Dairy & Poultry Science, Chittagong Veterinary and Animal Sciences University for his scholastic guidance, kind help, valuable suggestions, simultaneous inspiration, constructive criticism and who was involved with this study through its inception.*

*I would like to express my deepest sense of respect and heartfelt appreciations to DR. Md. Saiful Bari, Assistant professor, Department of Dairy & poultry science, Chittagong Veterinary and Animal Sciences University, for his advice and providing laboratory facilities during this study.*

*I also express my gratitude and deep sense of respect to all of my friends, Lab technicians and well wishers for their support, encouragement, help and inspiration throughout the study period and for preparing this report*

***The Author***

# ABSTRACT

The present study was conducted to isolate the exopolysaccharide (EPS) producing *Streptococcus sp* organism from the available dahi in Chittagong area and development of pure culture in laboratory. A total of 100 sample (25 of each brand) were collected from 4 different brands namely A, B, C, D (Food plaza, Banoful, Fulkoli, Genuine respectively). All the samples were subsequently cultured in nutrient agar for detecting live bacterial growth followed by MRS agar to isolate *streptococcus sp* and finally Ruthenium red milk plate for the isolation of exopolysaccharide producing *Streptococcus sp*. After that a new fermented product (yoghurt) was prepared using the isolated organism and sensory evaluation was done by a panel of judges. The study reveals 36% of the Brand C dahi were *Streptococcus sp* positive where 44.44% cases were exopolysaccharide (EPS ) producing while 16% cases of the Brand D dahi were *Streptococcus sp* positive and 20% of the positive case of the Brand B were exopolysaccharide (EPS) producing. In sensory evaluation, the newly fermented dairy product (yogurt) made by using the isolated organism was well acceptable regarding taste, appearance, acidity, flavor etc. It achieved on average 87% acceptability regarding the selected criteria. In conclusion, the isolated *Streptococcus sp* might be a good seed for preparing yoghurt having excellent probiotic capability.

**Key words:** Isolation, exopolysaccharide, MRS, dahi, cultu

# CHAPTER - I

## INTRODUCTION

Polysaccharides may function in foods as viscosifying agents, stabilizers, emulsifiers, gelling agents, or water-binding agents(Badel S et al,2011). Most of them are plant and algae derived polysaccharides and their use is strongly restricted for food applications. Commercially available microbial EPS are xanthan and gellan which are produced by *Xanthomonas campestris* and *Pseudomonas elodea*, respectively . But these bacteria are plant pathogen (Becker ,1998). Lactic acid bacteria (LAB) are bacteria with health benefits, considered as probiotics, associated with many fermented milk products; particularly products such as curd, yoghurt 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*(Marshall,1999). 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(Reis MA et al,2011).

EPS-producing LAB have a greater ability to withstand technological stresses and survive the passage through the gastrointestinal tract compared to their non-producing bacteria(Patel A et al,2013). 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(Mory Y,2005). Hence, the choice of EPS-producing starter cultures seems to give several advantages over non- producing ones. *Streptococcus sp* is a potent probiotic, may be found in the fermented milk products like dahi, an EPS producer in the products.Purified EPS from *Streptococcus sp* CRL 1190 was found to be effective for

preventing chronic gastritis. EPS-protein interaction might be responsible for the observed gastroprotective effect (Rodriguez C 2008).

Over 80% of the people in BD are suffering from gastritis. It is of utmost important to introduce something in diet to prevent gastritis. Dahi, cultured with exopolysaccharide producing *Streptococcus sp* can meet the gap here. For this reason, the study was conducted to isolate the EPS producing *Streptococcus sp* from the available dahi in Chittagong area and development of pure culture from it.

**Specific objectives:**

- To develop pure culture of EPS producing *Streptococcus sp*.
- To use the strain in production of fermented dairy products.
- Sensory evaluation of newly developed fermented dairy products (yoghurt).

## **CHAPTER - II**

### **METERILS AND METHODS**

The culturing and isolation of EPS producing *Streptococcus sp* from dahi samples were done in the Dairy science laboratory Chittagong Veterinary and Animal Sciences University during a period from January 2015 to May 2015. 100 samples of dahi were collected from 4 different brands (25 of each) available in Chittagong area. They were named as A, B, C and D (Food plaza, Banoful, Fulkoli, Genuine respectively).

#### **Collection of samples:**

A total of 100 samples were collected from 4 different brands in Chittagong namely Food plaza, Banoful, Fulkoli and Genuine. 25 samples were collected from each of the brand. Samples were collected at 3 days interval for each brand.

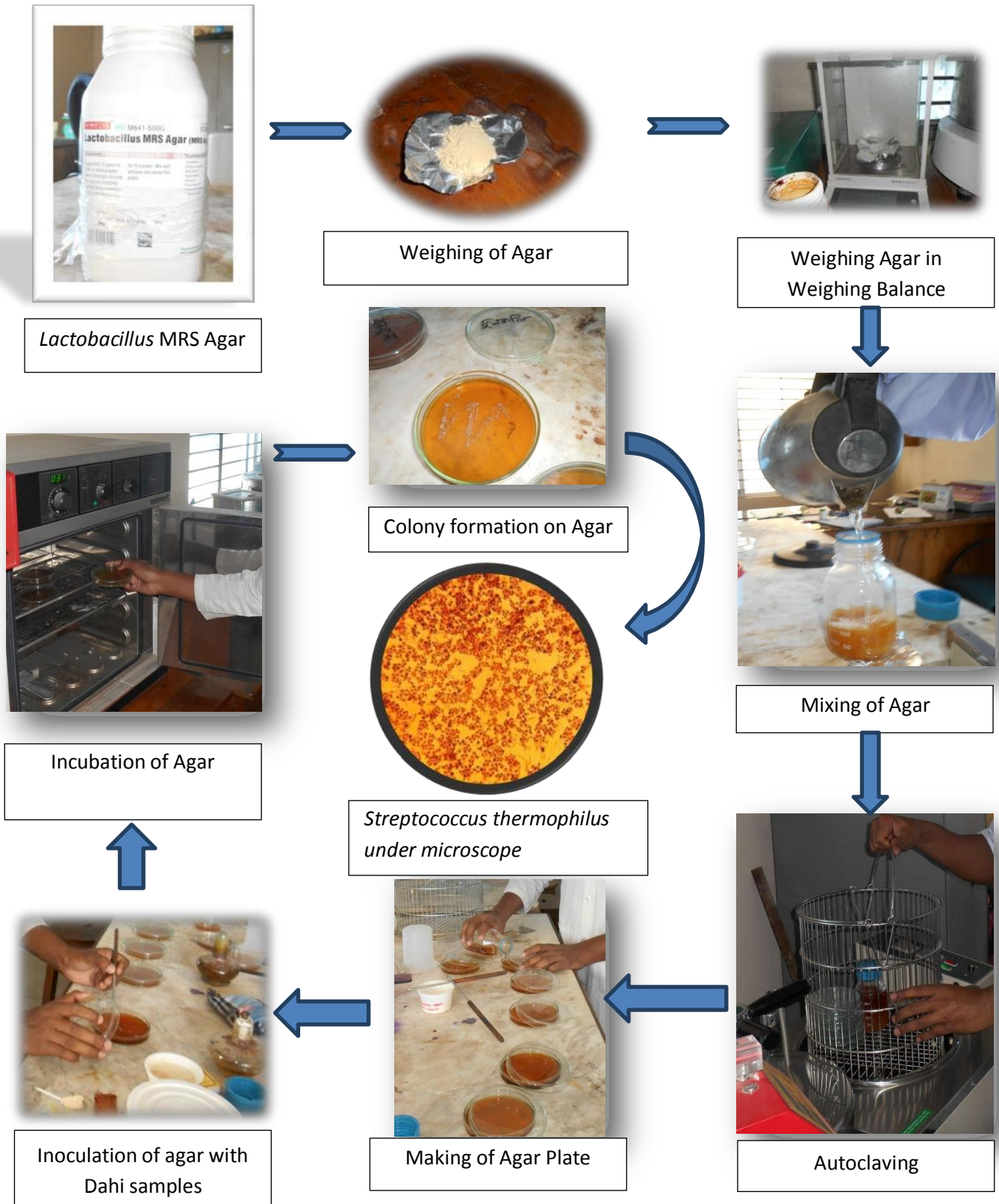
#### **Procedure of isolation:**

The Dahi samples were inoculated in nutrient agar to be sure for live organism. The successful colonies were inoculated in MRS agar to isolate the *Streptococcus sp* and *Lactobacillus sp*. The colonies grown in MRS agar were stained with Grams stain and observed under microscopy to determine the colonies with *Streptococcus sp*. The *Streptococcus* colonies were then inoculated Ruthenium Red milk plate to isolate the exopolysaccharide producing *Streptococcus sp*. The pure developed cultures were used to produce fermented dairy products (yoghurt) with EPS producing *Streptococcus sp*. (Procedure was followed as previously described by K. A Paari al 2011 )

#### **Sensory evaluation:**

After that a new 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( Procedure was followed as previously described by P Behare et al 2009 )

**Pictorial presentation of methodology:**



**Figure: Procedure of Isolation of Bacteria**

## CHAPTER – III

# RESULTS

**Table 1: Prevalence of *Streptococcus sp* positive cases**

Brand	N	No. of Positive samples	Percentage
A	25	6	24
B	25	5	20
C	25	9	36
D	25	4	16

Among the four brands the prevalence of *Streptococcus sp* 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 % of 24 and 20 respectively. This variation might occur due to the use of different undefined cultures in different brands of dahi.

**Table 2: Prevalence of exopolysaccharide producing *Streptococcus sp* cases**

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

The prevalence of EPS producing *Streptococcus sp* was also highest in the Brand C (16%), while the other Brand Dahi (A, B and D) had a % of 8, 4 and 4 respectively.

**Table 3: Prevalence of Exopolysaccharide producing *Streptococcus sp* cases among the positive cases**

<b>Brand</b>	<b>N</b>	<b>No. of positive samples</b>	<b>Percentage</b>
A	6	2	33
B	5	1	20
C	9	4	45
D	4	1	25

The percentage of producing *Streptococcus sp* among the positive case was highest in the Brand C (45%) . The other brand ( A , B , C ) had this percentage as 33 , 20 and 25 respectively. Among the positive cases the expolysaccharide producing *Streptococcus sp* were highest in brand C dahi and lowest in brand B dahi.

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

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

The panel of 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.



## CHAPTER - IV

# DISCUSSION

Among the four brands the prevalence of *Streptococcus* spp 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 % of 24 and 20 respectively. This variation 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 *Streptococcus* sp . *Streptococcus* sp exhibited a protocoooperation association to produce lactic acid at a greater rate. A study was done in rats to identify the source of contribution to growth stimulating effect in yogurt containing active lactic acid bacteria *Streptococcus* sp. The test variables were consisted of milk, yogurt, milk fermented individually by *Streptococcus* sp, and milk which both cultures has been added. Masud *et al.*, observed in fifty samples of indigenous dahi were collected from Rawalpindi, Islamabad to determine incidence of Lactic acid bacteria. The microorganism isolated were *Streptococcus* sp (60%). Brand C showed highest percentage of presence *streptococcus* sp and exopolysaccharide producing *Streptococcus* sp. Neither, brand B had third highest of presence of *Streptococcus* sp nor brand C had lowest exopolysaccharide producing *Streptococcus* sp. Iyer *et al.*, 2011 studied the bioprospecting of folate producing strains of *Streptococcus* sp isolated from milk and different fermented milk products of Indian origin. *Streptococcus* spp presenting brand A, C and D yoghurt was more exopolysaccharide producing than among the positive cases. But in brand B showed different circumstances where *Streptococcus* sp was less exopolysaccharide producing among the positive cases. Vuyst *et al.*, 2003 studied with five interesting heteropolysaccharide producing strains have been tested. *Streptococcus* sp LY03 produced the highest amounts of EPS and also displayed the highest apparent viscosities in fermented milk. *Streptococcus* sp presenting yoghurt showed highest panel score in taste and acidity .Rahman *et al.*, 2009 studied five samples of camel milk fermented for 6h at 43°C 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 ( *Streptococcus* sp. 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 was the most acceptable followed by those fermented by *Streptococcus sp* while the least acceptable was that fermented by *Streptococcus sp*. In general, the panelists gave lower sensory scores for consistency for all fermented camel milk but that one fermented by yogurt culture was slightly better in consistency score than those fermented by other starter cultures. The panelists preferred fermented camel milk made by yogurt starter culture followed by *Streptococcus sp*. The overall acceptability scores of the sensory evaluation revealed that the camel milk fermented by yogurt starter culture was the most accepted. Camel milk fermented by yogurt culture had significantly ( $P < 0.05$ ) higher rating for smell, consistency, taste and acceptability compared with other cultures.

## CHAPTER – V

# CONCLUSION

The study was performed aiming to isolation of exopolysaccharide producing *Streptococcus sp* organism in Dahi. From the above discussion it can be concluded that the Brand C was the best quality dahi from the probiotic aspects. The newly developed fermented dairy product was found well acceptable at the score overall 87 out of 100 . The pure culture of the EPS producing *Streptococcus sp* developed can be used for dahi production which will be beneficial for both dahi marketing and human health at consumers level.

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## **BIOGRAPHY**

I'm Shahjada Mohammad Julqarnain from Banshkhali, Chittagong .My father's name is S. Aktar Ahmad Chowdhury and mother's name is Nilufa Akther . I completed my SSC under Chittagong board in 2006 with GPA – 5.00 and HSC under Chittagong board in 2008 with GPA – 4.90 at scale of 5.00 . Now I 'm a internship student of DVM (Doctor of Veterinary Medicine) of Chittagong Veterinary and Animal Sciences University . In future I want to work as a researcher . I 'm interested to work in Dairy sector . My aim is to establish a Veterinary health complex in field level to serve the farmer and also create new techniques in Veterinary profession especially Dairy.