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SUITABILITY OF EXOPOLYSACCHARIDE PRODUCING LACTIC ACID BACTERIAL CULTURES IN FERMENTED DAIRY PRODUCT

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ABSTRACT

In this study, lactic acid bacteria (LAB) having the ability to produce Exopolysaccharides (EPS) was screened from the available culture collection (previously isolated from natural sources) at the Department of Dairy Science. Based on the primary and secondary identification tests, 10 LAB isolates were obtained, of which, four were EPS producing isolates. The EPS producing LAB appeared as mucoid colonies in the solid medium. Suitability of these EPS producing cultures in the preparation of curd was analysed by physico-chemical, rheological and sensory parameters of the product. There was no significant difference in overall sensory scores obtained among different curd samples prepared using EPS producing LAB isolates. However, the curd samples IS-3 and IS-4 showed better viscosity, acidity and textural properties. The culture isolates IS-3 and IS-4 were selected as the best LAB isolates among the screened samples.

Key Words: Exopolysaccharides, Curd, Lactic acid bacteria

INTRODUCTION

Exopolysaccharides are long-chain polysaccharides produced extracellularly mainly by bacteria and it consist of branched, repeating units of sugars or sugar derivatives. Most of the polysaccharides used currently by the food industry as bio-thickeners are of plant or animal origin. The quest to find bio-thickener from food grade organisms has encouraged interest in EPS from LAB. The EPS which serve as viscosifying, stabilizing, emulsifying and gelling agents, control crystallization, inhibit syneresis and film formation in food products. The LAB strains capable of production of texture-improving EPS for the manufacture of dairy products are a novel concept which needs to be explored.

LAB from natural sources, under optimized growth conditions have the potential to produce quality EPS to meet the demand. With these concepts, the present study was designed to screen LAB isolates and to evaluate the suitability for fermented dairy product.

MATERIALS AND METHODS

Procurement of LAB and identification

The LAB cultures were procured from the culture collection available in the Department of Dairy Science, College of Veterinary and Animal Sciences, Mannuthy. After preliminary screening, those isolates tentatively identified as LAB, were further propagated in sterilized skim milk and stored at 4^0C. Identification was done based on their morphological, cultural and biochemical characteristics as detailed in Bergey's manual of determinative bacteriology (1974) followed by Collins and Lyne (1980).

Screening of LAB for EPS production

The LAB cultures which showed mucoid colonies streaked on MRS agar supplemented with 5 per cent w/v sucrose were considered to possess the capability of EPS production (Patil *et al.,* 2015). EPS production was further confirmed as per the method suggested by Paulo *et al.* (2012). Discs of sterile filter paper were inoculated with five µl of culture and placed in petri dishes containing the culture medium. After incubation at 37^0C for 48 hours, mucoidness of the colonies was checked. Formation of mucoid colonies indicated EPS production. Precipitate formation on mixing of this mucoid substance with 2 ml absolute ethanol established the EPS production by the isolates.

Preparation and quality evaluation of fermented dairy product

To technologically characterize the EPS producing cultures, curd was prepared by inoculating milk with the culture (2%) and incubating at 28^oC for 16 hrs. Later on, its quality was evaluated. The physico-chemical properties like pH, titratable acidity (IS, 1973), texture (texture analyser, TX HD PLUS, Stable Micro Systems), viscosity (Brookfield Viscometer, D445/446), syneresis (Rodarte *et al.*, 1993) and water holding capacity (Sodini *et al.*, 2002) of the curd samples were determined, using instruments or procedures mentioned in bracket. Sensory evaluation of curd samples was done as per the method recommended by American Dairy Science Association (ADSA) using a score card. EPS+ *Lactobacillus delbrueckii* subsp. *bulgaricus* NCDC 285 served as control.

Statistical analysis

Data obtained from this study (six replications) was statistically analysed using SPSS version 21.

RESULTS AND DISCUSSION

Identification of EPS producing Lactic acid bacteria

In the MRS medium, colonies that appeared in different morphological forms such as (1) pin head creamy white (Streptococci) (2) large glistening soft colonies (Lactobacilli) and (3) triangular or spindle shaped colony (Lactococci) (Fig.1) were tentatively identified as LAB colonies. Pure single colonies were aseptically picked up from the agar plate and transferred to MRS broth for carrying out the identification tests. Goyal *et al.* (2012), isolated *Lactobacillus* strains from different samples of curd and all the LAB colonies revealed circular, small/large and creamy white characteristics in the solid agar medium.

The genus of the LAB isolates was identified by various primary and secondary identification tests (Tables 1 and 2). Based on formation of mucoid colony in the agar medium (Fig. 2) four LAB isolates were found to be EPS producing *viz.* IS-3, IS-4, IS-5 and IS-6. Isolate 3 (IS-3) belonged to genus Streptococcus, while IS-4 and IS-6 were Lactobacilli. IS-5 was identified as Lactococcus. EPS producing cultures were utilized for the preparation of curd and its quality was evaluated.

Physico-chemical analysis of curd samples

The results of quality evaluation of curd samples are given in table 3.

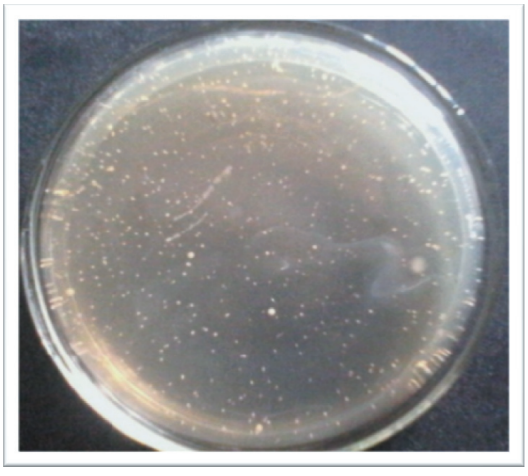

Fig. 1. Appearance of Lactic acid Bacteria in MRS agar

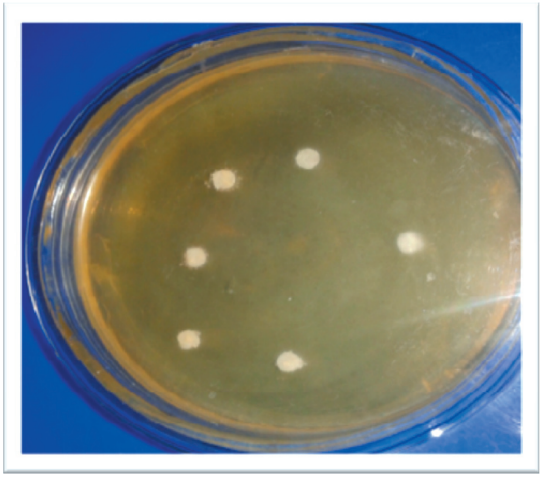

Fig. 2. Mucoid colony of LAB

Table 1. Test results of identification of LAB

LAB Isolates	Morphology	Temperatures of incubation			Ammonia from Arginine
		15^0C	37^0C	45^0C	
IS-3	Cocci in chain	+ve	+ve	-ve	+ve
IS-4	Rod	-ve	+ve	+ve	-ve
IS-5	Cocci	+ve	+ve	-ve	+ve
IS-6	Rod	+ve	+ve	-ve	+ve

Table 2. Results of Sugar utilization tests

LAB isolates	Sugars												Genus
	Lactose	Xylose	Maltose	Fructose	Dextrose	Galactose	raffinose	trehalose	melibiose	sucrose	l-arabinose	mannose	
IS-3	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	Streptococci
IS-4	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	Lactobacilli
IS-5	+ve	-ve	+ve	+ve	+ve	+ve	-ve	+ve	-ve	-ve	-ve	+ve	Lactococci
IS-6	+ve	-ve	-ve	+ve	+ve	+ve	-ve	+ve	-ve	+ve	-ve	+ve	Lactobacilli

Table 3. Results of physico chemical analysis of curd samples

Curd sample (LAB isolate no.)	Acidity (% Lactic acid)	pH	Syneresis per 100 ml of curd	WHC (g/kg of curd sample)
IS-3	$0.84^{a} \pm 0.01$	$4.035^{ns} \pm 0.174$	$2.62^{ns} \pm 0.123$	$475.67^{ns} \pm 243$
IS-4	$0.83^{a} \pm 0.01$	$4.12^{ns} \pm 0.183$	$2.52^{ns} \pm 0.092$	$496^{ns} \pm 18.55$
IS-5	$0.75^{b} \pm 0.02$	$4.20^{ns} \pm 0.188$	$2.74^{ns} \pm 0.087$	$451.67^{ns} \pm 17.48$
IS- 6	$0.79^{a,b} \pm 0.02$	$4.01^{ns} \pm 0.129$	$2.63^{ns} \pm 0.110$	$496.67^{ns} \pm 21.53$
Control	$0.84^{a} \pm 0.01$	$4.02^{ns} \pm 0.180$	$2.52^{ns} \pm 0.080$	$494.17^{ns} \pm 11.49$

Each value is a mean of six observations with SE
Means having different superscript differs significantly within a column ($p<0.01$)
ns – no significant difference within a column

Acidity

The acidity of the curd samples varied from 0.75 to 0.84 per cent of lactic acid. Our findings were in accordance with the FSSA (2006) standard. Curd samples with isolates IS-3 and IS-4 showed a significantly higher acidity when compared to IS-5 and IS-6.

Table 4. Results of rheological analysis of curd samples

Curd sample (LAB isolate no.)	Texture profile			Viscosity (cP)
	Hardness (Nsec)	Gumminess (Nsec)	Fracturability (Nsec)	
IS-3	567.62^{a} ± 80.33	348.49^{a} ± 33.64	277.40^{ns} ± 21.53	1000.50^{a} ± 66.63
IS-4	540.47^{a} ± 61.54	353.17^{a} ± 40.04	297.60^{ns} ± 30.35	1155^{a} ± 63.07
IS-5	319.96^{b} ± 32.22	177.63 ^{b}± 35.72	306.10^{ns} ± 27.92	601.83^{b} ± 59.39
IS-6	387.03^{a,b} ±66.84	167.76^{b} ±21.84	298.08^{ns} ± 30.92	772.50^{b} ± 48.34
Control	552.52^{a} ± 60.54	345.17^{a} ± 38.03	283.60 ^{ns}± 33.45	1058^{a} ± 43.08

Each value is a mean of six observations with SE
Means having different superscript differs significantly within a column ($p<0.05$)
ns – no significant difference within a column

Table 5. Sensory scores of curd samples

Curd sample (Lab isolate no.)	Flavour	Body and texture	Colour and appearance	Container	Total score
IS-3	39.17^{a}±1.93	26.33^{ns}±2.02	8.67^{ab}±0.55	4.67^{ns}±0.33	85.83^{ns}±4.75
IS-4	41.17^{a,b}±1.39	27.67^{ns}±1.30	9.00^{b}±0.36	4.67^{ns}±0.33	90.33^{ns}±3.403
IS-5	39.50^{a,b}±1.38	26.17^{ns}±1.53	7.83^{a}±0.70	4.67^{ns}±0.33	85.55^{ns}±3.96
IS-6	42.50^{b}±0.67	26.33^{ns}±1.56	9.33^{b}±0.21	4.67^{ns}±0.33	91.50^{ns}±2.99
Control	41.00^{a,b}±1.21	25.20ns±1.56	8.41^{ab}±0.51	4.67^{ns}±0.33	89.31^{ns}±2.40

Each value is a mean of six observations with SE
Means having different superscript differs significantly within a column ($p<0.05$)
ns – no significant difference within a column

pH

There was no significant difference in pH of curd samples prepared using four different LAB isolates and it ranged from 4.01 to 4.20. The result is in accordance with the observation of Dassanayake *et al.* (1994) who conducted a field survey on curd and reported that the pH ranged from 3.04 to 6.022 among the screened samples. Maximum recommended pH was 4.5.

Syneresis and water holding capacity (WHC)

The syneresis of curd samples varied from 2.52 to 2.74 ml per 100 ml of sample and the differences between

the curd samples including control were non significant. The mean water holding capacity of the curd samples also didn't show any significant differences and it varied from 451.6 to 496.6 g/kg of the sample. Marshall and Rawson (1999) observed that higher content of EPS would contribute to the lower whey separation which gives higher water holding capacity for the curd samples.

Rheological analysis of curd samples

The rheological factors like, texture (hardness, gumminess) and viscosity of curd samples were analysed and the results shown in table 4.

Upon texture analysis of curd samples, sample having isolate IS-5 was found to have significantly lower hardness when compared to other curd samples. Samples IS-3 and IS-4 showed significantly higher gumminess when compared to others. These findings are in agreement with Ayana and Ibrahim (2015). According to them, hardness was the lowest, while gumminess and fracturability were the highest due to the water absorbed by EPS produced by LAB. In the present study, better textural characteristics were evident in products prepared with EPS producers.

Viscosity analysis of curd samples revealed that, samples having isolates IS-3 and IS-4 had comparatively higher viscosity than other samples but was non significantly different from control. Our observations were in accordance with the findings of Hassan *et al.* (2003). EPS produced, prevent whey separation and increase viscosity which gives the curd samples a firm consistency.

Sensory evaluation of curd samples

Sensory scores obtained for the curd samples are given in table 5. Results revealed that there was a significant difference among the different curd samples in the parameters *viz.,* flavour, colour and appearance. IS-6 showed a significantly higher flavor score than other curd samples. However, the overall score differences remained non-significant between the test samples. The present findings are in agreement with a study conducted by Ayana and Ibrahim (2015), who prepared yogurt using EPS producing and non-producing LAB. They reported a score of 58/60 based on appearance, consistency and flavour for yogurt with EPS producing organism. In this study also, the products prepared using EPS producing isolates were quite appealing because of their uniform body with smooth texture and glossy appearance.

SUMMARY

In our study, four EPS producing LAB could be identified from culture collection of Department of Dairy science.

The application of EPS producing LAB in the production of fermented dairy foods is dependent on its ability to bind water and increase the viscosity of the product. An attempt was made to study the impact of the identified EPS producing LAB cultures on physico - chemical, rheological and sensory properties of curd. Curd samples prepared using isolates IS-3 and IS-4 showed better viscosity, acidity and textural properties. Although curd sample with isolate IS-6 scored maximum (91.50±2.99) total score on sensory evaluation, significant differences were not evident between different test samples. Hence, IS-3 and IS-4 were selected to be better for product preparation. The four EPS producing strains evaluated in this study has the potential to be used as starter culture for small scale and commercial production of fermented foods.

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