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Exopolysaccharide production and lipolytic activity of promising Lactic acid bacteria isolated from traditional artisan curds of Karnataka for quality yoghurt preparation

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Abstract

As many as 77 lactic acid bacterial strains were isolated from various natural sources and artisan curd samples of Karnataka. They were further subjected for exopolysaccharide (EPS) production and lipolytic activity isolate numbers BC19 and RSC64 produced the highest EPS, 1.65 mm each. Isolate SCR59 produced the highest lipolytic activity of 16.5mm zone of solubilization, diameter. The promising ten isolates were selected and three starter culture consortia were prepared. Based on organoleptic evaluation, starter culture consortia no. I was found to be the best treatment with the overall acceptance of 8.31.

Keywords: Exopolysaccharide, lipolytic activity, lactic acid bacteria, isolates, artisan curds

Introduction

Lactic acid bacteria (LAB) are Gram positive, non-spore forming, Catalase negative, acid tolerant, fastidious, non-motile and facultative anaerobic friendly gut bacteria. They are constitute heterogeneous group of industrially important bacteria. In food industries, they used as preservatives, acidulant and flavouring agents by the virtue of their probiotic traits. They are also used as starter cultures in food fermentations such as beverages, yoghurt, vegetables, cereals, meat, cocoa beans etc. They are also utilized in the development of functional foods and more specifically, their application as vaccines, pro and prebiotics, neutraceuticals has attracted new research arena for food scientists and health professionals (Mozzi *et al.*, 2010 [6]; De Vuyst and Leroy, 2004) [3].

LAB producing exopolysaccharides (EPS) have received much attention of scientific community in the recent years, due to their useful role in the improvement of physical, rheological and sensory properties of fermented milk (Behare *et al.*, 2013) [1]. EPS are long-chain polysaccharides produced extracellularly mainly by bacteria and microalgae. EPS consist of branched, repeating units of sugars or sugar derivatives. These sugar units are mainly glucose, galactose, mannose, N-acetyglucosamine, N-acetyl galactosamine and rhamnose, in variable ratios.

To boost the taste of yoghurt, different fruit pulps can be added (either individually or in combination) which also confer flavor and color to yoghurt to attract the consumers. Such attempts can further augment rheological and sensory attributes of the yoghurt. They are supplemented as probiotics that are live microbial food supplements which beneficially affect the host by improving the intestinal microbial balance. Hence, as many as 77 isolates of lactic acid bacteria were obtained from various sources such as traditional artisan curds, fermented vegetables, Dosa and Idli batter, etc. out of which 10 promising were selected based on EPS production and lipolytic activity.

Thus, selection of efficient LAB strains isolated from diverse natural environments and traditional artisan curds serve as efficient starter cultures for producing yoghurt with improved nutritional, rheological and sensory properties. Hence, in order to obtain productive LAB strains which impart health benefits for exploring their application in developing yoghurt is the objective of the present study.

Materials and methods

Isolation of LAB strains, media and culture conditions

As many as 77 LAB strains were isolated from various sources such as traditional fermented arisan curds of Karnataka such as Kohlar, Banashankari, Almatti areas, fermented vegetables

etc. by the method of serial dilution and plate count (Table.1). The 48 h grown cultures were further purified and maintained on de Mann Rogosa Sharpe broth (MRS) (De Man *et al.*, 1960 [2]) at -80^0 C by DMSO (cryoprotectant) method.
EPS production by the isolates was estimated according to the method given by Rimida and Abraham (2003). The isolates were streaked on MRS agar and incubated at 30^o C for 48 h. The sticky aspect of the colonies was determined by testing them for slime formation using the inoculation loop method. The mucoidness and ropyness was observed and measured in mm using inoculation loop method. The isolates were considered positively slimy producer if the length of slime was above 1.5 mm.

Table 1: Sources of the samples used for isolation of lactic acid bacteria

Isolate No	Isolate Code No.	Source of the isolate
1	KC1	Kolhar Curd
2	KC2	Kolhar Curd
3	KC3	Kolhar Curd
4	KC4	Kolhar Curd
5	KC5	Kolhar Curd
6	KC6	Kolhar Curd
7	KC7	Kolhar Curd
8	KC8	Kolhar Curd
9	KC9	Kolhar Curd
10	KC10	Kolhar Curd
11	BC11	Badami Curd
12	BC12	Badami Curd
13	BC13	Badami Curd
14	BC14	Badami Curd
15	BC15	Badami Curd
16	BC16	Badami Curd
17	BC17	Badami Curd
18	BC18	Badami Curd
19	BC19	Badami Curd
20	BC20	Badami Curd
21	BC21	Badami Curd
22	BC22	Badami Curd
23	BC23	Badami Curd
24	BJC24	Bijjaragi Curd
25	BJC25	Bijjaragi Curd
26	BJC26	Bijjaragi Curd
27	BJC27	Bijjaragi Curd
28	BJC28	Bijjaragi Curd
29	BJC29	Bijjaragi Curd
30	BJC30	Bijjaragi Curd
31	BJC31	Bijjaragi Curd
32	BJC32	Bijjaragi Curd
33	BJC33	Bijjaragi Curd
34	BJC34	Bijjaragi Curd
35	BJC35	Bijjaragi Curd
36	BJC36	Bijjaragi Curd
37	BJC37	Bijjaragi Curd
38	BJC38	Bijjaragi Curd
39	BJC39	Bijjaragi Curd
40	BJC40	Bijjaragi Curd
41	BJC41	Bijjaragi Curd
42	BJC42	Bijjaragi Curd
43	BJC43	Bijjaragi Curd
44	BJC44	Bijjaragi Curd
45	BJC45	Bijjaragi Curd
46	SPG46	Sprouted Grains
47	SPG47	Sprouted Grains
48	SPG48	Sprouted Grains
49	SPG49	Sprouted Grains
50	SPG50	Sprouted Grains
51	SPG51	Sprouted Grains
52	TCW52	Tender Coconut Water
53	TCW53	Tender Coconut Water
54	TCW54	Tender Coconut Water
55	TCW55	Tender Coconut Water
56	TCW56	Tender Coconut Water
57	SCR57	Sugarcane Rhizosphere Soil
58	SCR58	Sugarcane Rhizosphere Soil
59	SCR59	Sugarcane Rhizosphere Soil
60	CRS60	Corn Rhizosphere Soil
61	CRS61	Corn Rhizosphere Soil
62	CRS62	Corn Rhizosphere Soil
63	CRS63	Corn Rhizosphere Soil
64	RSC64	Rhizosphere Soil Chilli
65	RSC65	Rhizosphere Soil Chilli
66	RSC66	Rhizosphere Soil Chilli
67	ORS67	Orchard Rhizosphere Soil
68	ORS68	Orchard Rhizosphere Soil
69	ORS69	Orchard Rhizosphere Soil
70	ORS70	Orchard Rhizosphere Soil
71	CoRS71	Cocoa Rhizosphere Soil
72	CoRS72	Cocoa Rhizosphere Soil
73	CoRS73	Cocoa Rhizosphere Soil
74	AC74	Almatti Artisan Curd
75	AC75	Almatti Artisan Curd
76	AC76	Almatti Artisan Curd
77	AC77	Almatti Artisan Curd

Lipolytic activity
LAB isolates were tested for their lipolytic activity as described by Katz *et al.* (2002) [5].

Starter culture consortia
Based on EPS production and lipolytic activity, 10 efficient LAB isolates were selected and consortia were prepared. The details of the treatments are given below.

Treatment combinations formulated based on potential probiotic and functional characteristics

Treatment Details
T1: Curd prepared using KMF curd culture
T2: Yoghurt prepared using Standard reference cultures
T3: Yoghurt prepared using Starter culture consortium- I BJC41+RSC64+BJC35+BJC42+BJC37I)
T4: Yoghurt prepared using Starter culture consortium- I (BJC41+ RSC64+SPG49+ BJC42+ BJC37)
T5: Yoghurt prepared using Starter culture consortium-III (BJC41+ RSC64+BJC40+BJC37+KC6)

Results and discussion
Exopolysaccharide is an important functional trait of LAB. Exopolysaccharide (EPS) produced by LAB with GRAS (generally regarded as safe) status is an important source of natural alternatives. Recently, EPS produced by LAB have gained considerable attention in the fermented dairy industry because of their potential as viscosifiers, texturizers and emulsifying agents. It has been reported that EPS produced yoghurt starter cultures could affect the texture of yoghurt and improve sensory characters such as mouthful, shinyness, clean, cut, ropyness and creamyness. Hence, the LAB collection was subjected to production of EPS. It was observed that many isolates produced EPS (Table. 2). Isolate BC19 and RSC64 produced the highest amount of EPS of 16.5 each and hold promise to be used in the development of starter consortium.
As many as 50 isolates showed lipolytic activity. The highest activity was observed in isolate No. 69 with a zone of

solubilization of 21.5 mm dia. followed by isolate No. 59 (16.5mm dia.) whereas the least activity was recorded in the isolate No. 9 (6.00 mm dia.).

Based on EPS production and lipolytic activity, finally, 10 LAB isolates were selected. Using these 10 isolates, three starter consortia were made and yogurt prepared and organoleptic evaluation of the different yoghurts was done in comparison with the yogurt prepared using the reference yogurt cultures.

Form Table. 4, out of three starter culture consortia, T3 was found to be the best starter consortium (BJC41+RSC64+ BJC35+BJC42+ BJC37) which produced a yoghurt with good flavour, acceptable color, appearance and texture with the highest overall acceptability of 8.31.

Table 2: Production of Exopolysaccharide from lactic acid bacteria isolates (measured in terms of ropyness, mm)

LAB isolates	Production of EPS (mm)	LAB isolates	Production of EPS (mm)
KC1	0.70	BJC41	1.10
KC2	0.50	BJC42	1.10
KC3	0.35	BJC43	0.80
KC4	0.50	BJC44	0.65
KC5	0.60	BJC45	0.70
KC6	1.45	SPG46	0.70
KC7	0.45	SPG47	0.35
KC8	0.95	SPG48	0.40
KC9	0.65	SPG49	0.95
KC10	0.60	SPG50	1.50
BC11	0.70	SPG51	0.50
BC12	0.25	TCW52	0.70
BC13	0.30	TCW53	0.95
BC14	0.50	TCW54	0.70
BC15	0.55	TCW55	0.50
BC16	0.50	TCW56	0.35
BC17	0.50	SCR57	0.50
BC18	0.55	SCR58	0.60
BC19	1.65	SCR59	0.65
BC20	0.00	CRS60	0.60
BC21	0.65	CRS61	1.00
BC22	0.00	CRS62	1.00
BC23	0.50	CRS63	0.55
BJC24	0.30	RSC64	1.65
BJC25	0.00	RSC65	0.65
BJC26	0.00	RSC66	0.55
BJC27	0.00	ORS67	0.50
BJC28	0.55	ORS68	0.45
BJC29	0.65	ORS69	0.60
BJC30	0.55	ORS70	0.35
BJC31	0.30	CoRS71	0.50
BJC32	0.50	CoRS72	0.40
BJC33	0.80	CoRS73	0.35
BJC34	0.45	AC74	0.45
BJC35	1.30	AC75	0.50
BJC36	0.30	AC76	0.00
BJC37	1.45	AC77	0.35
BJC38	0.55	SEM ±	0.11
BJC39	1.15	CD @ 0.01%	0.41
BJC40	0.55		

Table 3: Lipolytic activity of LAB isolates measured in mm (Zone of solubilisation, mm=dia)

LAB isolates	Lipolytic activity	LAB isolates	Lipolytic activity
KC1	10.50	BJC41	10.00
KC2	0.00	BJC42	6.50
KC3	0.00	BJC43	8.50
KC4	9.00	BJC44	0.00
KC5	0.00	BJC45	11.50
KC6	0.00	SPG46	0.00
KC7	0.00	SPG47	12.50
KC8	6.50	SPG48	0.00
KC9	6.00	SPG49	0.00
KC10	0.00	SPG50	0.00
BC11	0.00	SPG51	0.00
BC12	9.00	TCW52	0.00
BC13	7.00	TCW53	6.50
BC14	0.00	TCW54	13.00
BC15	0.00	TCW55	0.00
BC16	13.50	TCW56	9.00
BC17	0.00	SCR57	0.00
BC18	0.00	SCR58	0.00
BC19	10.00	SCR59	16.50
BC20	0.00	CRS60	0.00
BC21	8.50	CRS61	0.00
BC22	0.00	CRS62	0.00
BC23	12.00	CRS63	0.00
BJC24	8.50	RSC64	0.00
BJC25	0.00	RSC65	0.00
BJC26	0.00	RSC66	0.00
BJC27	0.00	ORS67	0.00
BJC28	0.00	ORS68	0.00
BJC29	8.50	ORS69	21.50
BJC30	8.00	ORS70	0.00
BJC31	7.00	CoRS71	0.00
BJC32	0.00	CoRS72	0.00
BJC33	0.00	CoRS73	0.00
BJC34	8.50	AC74	0.00
BJC35	0.00	AC75	0.00
BJC36	10.50	AC76	0.00
BJC37	9.00	AC77	0.00
BJC38	0.00	SEM ±	0.59
BJC39	0.00	CD @ 0.01%	2.19
BJC40	0.00		

Table 4: Organoleptic evaluation of the yoghurt

Treatments	Hedonic scale values				
	Flavor	Color	Appearance	Texture	Overall acceptance
T1: Curd prepared using KMF curd culture	6.17	6.67	7.63	7.33	7.30
T2: Yoghurt prepared using Standard Reference cultures	8.33	8.13	7.67	8.33	8.25
T3: Yoghurt prepared using starter culture consortium-I (BJC41+RSC64+BJC35+BJC42+BJC37)	7.97	8.00	7.33	7.80	8.31
T4: Yoghurt prepared using starter culture consortium-II (BJC41+ RSC64+SPG49+ BJC42+ BJC37)	6.33	6.00	6.67	6.00	6.50
T5: Yoghurt prepared using Starter culture consortium-III (BJC41+ RSC64+BJC40+ BJC37+KC6)	7.00	6.67	6.33	6.67	6.73
S. Em±	0.28	0.34	0.42	0.39	0.34
CD@0.01	1.21	1.45	1.80	1.67	1.5

Conclusion

In conclusion, most of the 77 LAB isolates used in the study showed EPS production and lipolytic activity. Using the selected isolates, different consortia were made and the yoghurt prepared by using consortia no. I exhibited the highest quality and the overall acceptance as revealed by organoleptic evaluation. These isolates have a great potential and prospective candidates as one of the probiotic traits besides good overall acceptability to carry forward for further study in the preparation of yoghurt.

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