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Screening of Lactic Acid Bacteria Isolated from Fermented Food for Bio-molecules Production

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Abstract

Production of bio-molecules is an important factor in assuring the proper consistency and texture of fermented foods. Lactic acid bacteria (LAB) isolated from fermented food were screened for lactic acid, diacetyl, hydrogen peroxide, pH development and Exopolysaccharide (EPS) production. Thirty-five strains of LAB were isolated and characterized from fermented dairy and non-dairy foods. The LAB species identified include: Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus cellobiosus, Lactobacillus delbruekii, Lactobacillus coryniformis, Lactobacillus casei, *and* Leuconostoc messenteroides. *The most predominant species was* Lactobacillus plantarum *(34.29%). All the isolates were screened for lactic acid, hydrogen peroxide, diacetyl and pH and EPS production. Lactic acid production ranges within 0.11-1.96 mg/l in which the highest was produced by* L. plantarum *LPF2.* L. plantarum *LPF2 also produced the largest amount of diacetyl (1.92 mg/l). Hydrogen peroxide produce by the isolates ranges within 0.0002-.35 mg/l and* L. fermentum *LFBO1 produced the highest. The pH ranged within 3.2-6.5 in which* L. plantarum *LPF2 had the least.* L. plantarum *LPW7 and LPBO9,* Leu. messenteroides *LMWO2 and LMW4 bring the reduction of the pH of the fermentation medium to 3.8 at 36 hours. All the isolates were screened for EPS production on solid medium. The isolates were all creamy; four were highly mucoid, eight were mucoid while twenty-three were slightly mucoid. All the isolates are EPS producers, EPS production ranged within 120-1,390 mg/l in which the highest was produced by* L. fermentum *LF6.*

Keywords: *Lactic acid bacteria, bio-molecules, lactic acid, H_2O_2, diacetyl.*

Introduction

Lactic Acid Bacteria (LAB) are Gram positive, fastidious, acid tolerant, generally non-sporulating, catalase negative, devoid of cytochrome, and non-respiring rod or cocci that are associated by their common metabolic and physiological characteristics that produce lactic acid as a major or sole product of fermentative metabolism (Fooks *et al*. 1999; Holzapfel *et al*. 2001). Lactic acid bacteria (LAB) have been used for the fermentation of food and feed products since ancient days and today their major applications are still in the food and feed industry as starter cultures (Desmons *et al*. 1998; van Casteren *et al.* 1998; Boonmee *et al*. 2003).

Lactic acid, one of the metabolites produced by LAB, has various industrial applications such as a preservative, acidulant, and flavor in food, textile, and pharmaceutical industries. It can also be used in the production of lactate-esters, propylene glycol, propylene oxide, acrylic acid, 2,3-pentanedione, propanoic acidacetaldehyde, and dilactide (Åkerberg and Zacchi 2000; Varadarajan and Miller (1999).

LAB produce variety of antimicrobial compounds such as ethanol, formic acid, acetone, hydrogen peroxide, diacetyl and bacteriocins which confer preservative ability on them as a natural competitive means to overcome other microorganisms sharing the same niche (Oliveira *et al.* 2008). Recently, there has been a great demand for lactic acid as it can be used as a monomer for the production

of the biodegradable polymer polylactic acid (PLA), which is an alternative to synthetic polymers derived from petroleum resources (Datta *et al.* 1995).

EPS formation by lactic acid bacteria during the production of fermented milk products either acts as a viscosifying, emulsifying agent or imparts favourable rheological properties. Nevertheless, it has been reported that EPS from food grade organisms, particularly lactic acid bacteria, have potential as food additives and functional food ingredients with both health and economic benefits (Welman and Maddox 2003). It is therefore essential to isolate LAB species as well as knowing the best optimum cultural condition for quality EPS production and biomass polysaccharide polymer growth in large quantity in order to meet the demand of EPS production in industries.

Lactic acid bacteria are food grade organisms, possessing the generally-recognized-as-safe (GRAS) status, and can secret exopolysaccharide (EPS). LAB EPS is economically important because it can impart functional effect to foods and confer beneficial health effects to the consumer (Welman and Maddox 2003; Tallon *et al.* 2003). EPS produced by LAB is the subject of an increasing number of studies, since EPS-producing LAB have become an alternative way of improving the texture and stability of fermented dairy and non-dairy products. It is therefore essential to isolate LAB species as well as conduct more research into the metabolites produced by them in order to get overproducing strains and to meet the demand of EPS production in industries. This research aimed at isolating lactic acid bacteria from fermented food and screening them for bio-molecules production.

Materials and Methods

Collection of Samples

The lactic acid bacteria isolates were obtained from fermented dairy products (Yoghurt, "Nunu", "Fura" "Fura da nono" and "wara") and non-dairy traditionally prepared "fufu" from cassava and "ogi" made from white maize (*Zea mays*) and red guinea corn (*Sorghum bicolor*) from various locations in Nigeria: Bodija and Sabo markets in Ibadan, Oyo State. Samples were taken to the laboratory for microbiological analysis.

Isolation and Identification of Lactic Acid Bacteria

Ten grams of each sample were aseptically added into 90 ml of sterile 0.9% NaCl solution. Homogenized and serially diluted, 1 ml of the diluents was pour-plated on de Man, Rogosa and Sharpe (MRS) agar, respectively. Plates were incubated for 24 hrs at 35^{o}C. Total of 35 representative colonies were randomly picked and sub-cultured to obtained pure culture. The isolates were maintained on MRS agar plates (Oxoid No. CM361) containing 50 mg/l of nystatin (Sigma, Australia) kept at 4^{o}C under anaerobic conditions. The stock cultures were stored at -4^{o}C for subsequent use and sub-cultured for 4-week interval.

The bacteria were characterized by microscopic morphological examination and by conventional biochemical and physiological tests. Gram staining, catalase activity, gas production from glucose, growth in NaCl (2-6.5%), growth at different temperature (10-45^{o}C), and production of amino acid from arginine were determined according to the methods of Harrigan and McCance (1976) and Roissart and Luguet (1994). The identification work was done according to the methods described in Bergey's Manual (Sneath *et al.* 1986). All the strains were maintained by weekly sub-culturing from 48-hour MRS agar cultures.

Inoculums Preparation

The working cultures were prepared by transferring 0.5 ml of the stock frozen culture to 10 ml of MRS broth and incubated for 16 hrs at 30^{o}C. The resulting culture was transferred (2% ^{v}/v) to modified exopolysaccharide selection medium (mESM) (van den Berg *et al.* 1993) containing 5% (^{w}/v) skim milk (Oxoid), 0.35% yeast extracts (Oxoid), 0.35% peptone (Difco), and 5% glucose (BDH) and incubated

at 30°C for 16 hrs. 10 ml inocula of the 16-hour old culture containing 2.5×10^6 cfu/ml were used to inoculate larger volume of the fermentation medium.

Production of Bio-molecules by the LAB Strains Using mESM Medium

The identified isolates were cultivated in exopolysaccharides selection medium (mESM) (van den Berg *et al.* 1997). A loopful of each of the working cultures was transferred into 100-ml conical flasks containing 10 ml of mESM broth and the broths were incubated anaerobically for 24 hrs at 30°C. 10 ml inocula were transferred into 200-ml conical flasks containing 90 ml of mESM broth and incubated at 30°C for 36 hrs. Samples were taken and analyzed for lactic acid, diacetyl, hydrogen peroxide, pH development, growth and EPS production.

Determination of Lactic Acid

The production of lactic acid was determined by titrating 10 ml of the homogenized sample against 0.25 mol/l NaOH using 1 ml of phenolphthalein indicator (0.5% in 50% alcohol). The titratable acidity was calculated as percentage lactic acid (v/v). Each millilitre of 1 N NaOH is equivalent to 9.008 mg of lactic acid (AOAC 1990).

Quantitative Estimation of Hydrogen Peroxide Production

Twenty-five millilitres of the fermenting samples and 20 ml of diluted H_2SO_4 were titrated against 0.1 N potassium permanganate (AOAC 1990). 1 ml is equivalent to 1.70 mg of H_2O_2.

Quantitative Estimation of Diacetyl Production

The amount of diacetyl produced during the fermentation of the samples was also determined by titration: 25 ml of the fermented sample and 7.5 ml hydroxylamine solution were titrated against 0.1 M HCl according to a standard procedure (AOAC 1990). The equivalent factor of HCl to diacetyl was taken as 21.5 mg.

pH Determination

The pH change of the fermenting samples was monitored using a Kent pH meter (Kent Ind. Measurements Ltd. Survey) model 7020 equipped with a glass electrode. The pH probes were sanitized by swabbing with 96% ethanol prior to placing it in the fermenting samples. Duplicate determination was made in all cases.

Measurement of Growth

Growth of the test organisms was determined by taking the optical density reading at 650 nm after appropriate dilution of the samples.

Isolation, Purification and Quantification of EPS Produced by the LAB Isolates

The exopolysaccharides were isolated according to the method of Garcia-Garibay and Marshall (1997). The lactic acid culture was treated with 17% (^{w}/v) of 80% trichloroacetic acid solution and centrifuged at 16,000-× g at 4°C for 30 min. The clarified supernatant was concentrated 5 times by evaporation using a rotavap evaporator. The exopolysaccharides were precipitated by adding 3 volumes of cold absolute ethanol, and stored overnight at 4°C. Finally, the recovered precipitates were re-dissolved with distilled water and dialyzed against the same solution for 24 hrs at 4°C. The polysaccharides were freeze-dried and stored at 4°C. The total amount of carbohydrates in the polysaccharides was determined by the phenol-sulfuric acid method described by DuBois *et al.* (1956). The exopolysaccharides production is expressed in mg/l.

Total Sugar Determination

The total sugar concentration was determined by phenol-sulfuric acid method using glucose as a standard (Chaplin 1986). The results are expressed in milligrams of glucose per litre.

Results and Discussion

Thirty-five lactic acid bacteria were obtained from different fermented dairy products (Yoghurt, "Nunu", "Fura", "Fura da Nono" and "Wara") and fermented foods ("fufu", white and brown "ogi"). The isolates were initially differentiated on the basis of their cultural and morphological studies after which they were subjected to various physiological and biochemical tests. The LAB isolates were: *Lactobacillus fermentum, Lactobacillus plantarum, Lactobacillus brevis, Lactobacillus cellobiosus, Lactobacillus delbruekii, Lactobacillus lactis, L. casei,* and *Leu. mesemteroides.* The cell studies revealed medium short rods to relatively long rods. The isolates were Gram positive, non-sporing, non-motile, Catalase, Oxidase, methyl red, voges-proskauer and indole negative. They cannot produce H_2S gas and cannot hydrolyse starch. Fermentation tests reveal the isolates possessing the ability to ferment almost all sugars used exception of *L. delbruekii* which was able to ferment few sugars.

Different types of LAB isolated from various fermented food samples are shown in Table 1 while Fig. 1 shows the percentage frequency of occurrence of the LAB isolates from various fermented food samples. *L. plantarum* had the highest frequency of occurrence (34.29%) while *L. lactis, L. casei, L. cellobiosus, and L. delbruekii* had the least (5.71%), respectively. The lactic acid bacteria constitute an important group of organisms, particularly in the food processing industry. All the bacteria isolated from the fermented foods fit the classification of LAB as Gram positive, catalase negative and oxidase negative.

Table 1. LAB strains associated with the fermented food samples.

Isolates	Food Samples	Occurrence
Lactobacillus plantarum	"Fufu"	2
Lactobacillus plantarum	White "ogi"	2
Lactobacillus plantarum	Brown "ogi"	5
Lactobacillus plantarum	"Nono"	1
Lactobacillus plantarum	"Fura danono"	1
Lactobacillus plantarum	"Wara"	1
Lactobacillus delbrueckii	White "ogi"	2
Lactobacillus delbrueckii	"Fura"	1
Lactobacillus fermentum	Brown "ogi"	2
Lactobacillus fermentum	White "ogi"	2
Lactobacillus fermentum	"Fura"	1
Lactobacillus fermentum	"Nono"	2
Lactobacillus lactis	White "ogi"	1
Lactobacillus lactis	"Fura"	1
Leuconostoc messenteroides	White "ogi"	2
Leuconostoc messenteroides	Brown "ogi"	1
Leuconostoc messenteroides	"Wara"	2
Lactobacillus casei	"Fura"	1
Lactobacillus casei	"Fura da nono"	1
Lactobacillus cellobiosus	"Wara"	2
Lactobacillus brevis	White "ogi"	2
Total		35

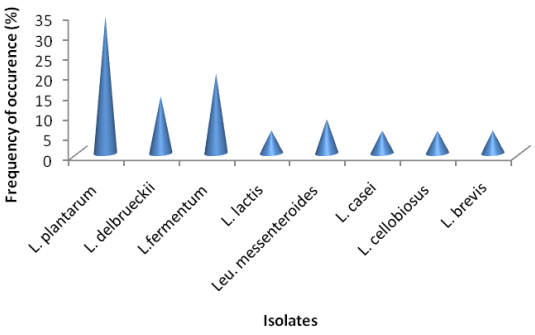

Fig. 1. Frequency of occurrence (%) of LAB isolated from various fermented food samples.

Generally, the cultural and biochemical properties of the isolates agreed with the description of Kandler and Weiss (1986) and confirmed with Bergey's Manual of systematic bacteriology (Sneath *et al.* 1986). Among the isolated lactic acid bacteria, *Lactobacillus plantarum* has the highest frequency of occurrence; this has being reported by various workers (Olukoya *et al.* 1993; Steinkraus 1983; Cooke *et al.* 1987; Adebayo-Tayo and Onilude 2008).

Table 2 shows the lactic acid produced by the LAB strains. It ranged within 0.11-1.96 mg/l in which *L. plantarum* LPF2 had the highest at 36 hrs after incubation. Reasonable quantity of lactic acid was produced by the isolates agreed with the report of Pinthong *et al.* (1980) that lactic acid bacteria could also lead to products with sufficient acidity (low pH) for good keeping properties. The production of reasonable level of acidity by LAB will also help improve the flavour of the product. Other workers have obtained similar results (Adda *et al.* 1982; Prentice and Brown 1983). Lactic acid bacteria are present in fermented foods because they are able to survive under high acidic conditions and also have the ability to produce a high level of lactic acid. Reasonable amount of lactic acid was produced as a major end product of fermentation of carbohydrate by the screened isolates. This gives the fermented product more shelf-stable quality with characteristic aroma and flavors which is in line with the work of Axelsson (1998). The fermented dairy produce relies for its manufacture on the growth of relatively high population of *lactobacilli* whose immediate function is to convert lactose to lactic acid (Fox 1982). It has been reported that approximately 90% of the total lactic acid produced worldwide is by bacterial fermentation. Lactic acid is used as a substrate in the manufacture of polylactic acid (PLA), which could be a good substitute for synthetic plastic derived from petroleum feedstock (Zhou *et al.* 2006).

Table 3 shows the hydrogen peroxide produce by the LAB strains, the highest (0.35) was produced by *L. fermentum* LFBO1 at 36 hrs after incubation.

Table 4 shows the diacetyl produced by the isolates, it ranged within 0.91-1.92 (*L. plantarum* LPF2). The highest diacetyl was produced at 36 hrs of incubation. Reasonable quantity of dicaetyl was produced by the screened isolates. Diacetyl has a strong, buttery flavor and is essential at low concentrations in many dairy products, such as butter, buttermilk and fresh cheese.

Lactic acid bacteria give fermented milk the slightly sharp and sour taste. Additional characteristic flavor and aroma are often the result of other products of LAB. For example, acetaldehyde is known to provide the characteristic aroma of yoghurt while diacetyl imparts a buttery taste to other fermented milks.

Inhibition activity of LAB has been reported to be due to a combination of many factors such as production of lactic acid which brings about reduction of pH of the fermentation medium (Adebayo-Tayo and Onilude 2008) and production of inhibitory bioactive compounds such as hydrogen peroxide and bacteriocins which are responsible for most antimicrobial activity (Ogunbanwo 2005). Lactic acid bacteria (LAB) play a major part in most fermentation processes, not only because of their ability to improve the flavour and aroma but especially for their preservative effects on food.

The pH development during fermentation by the LAB isolates is shown in Table 5. The pH ranged within 3.2-6.5 in which *L. plantarum* LPF2 had the least. *L. plantarum* LPW7 *and* LPBO9, *Leu. messenteroides* LMWO2 and LMW4 had the ability to reduce the pH of the fermentation medium to 3.8, respectively, at 36 hrs after incubation. Reduction in pH during fermentation is due to the fermentative transformation of carbohydrates to lactic acid

and acetic acid by the isolates. The ability of LAB to lower the pH of the fermented food leads to an inhibition of food spoilage and thus an increase in its shelf life. In addition to lowering the pH and acid production (acetic, lactic and carbonic), LAB contribute to preservation by the production of a vast array of antimicrobial compounds and proteins (Ray and Daeschel 1992; Elliason and Tatini 1999).

The result of the screening of the isolates for EPS production on solid agar is shown in Table 6. It was observed that all the isolates were creamy, four were highly mucoid, eight were mucoid, and twenty-three were slightly mucoid.

Table 7 shows the EPS produced by the isolates. The EPS production ranged within 120-1,390 mg/l in which *L. fermentum* LF6 gave the highest.

Among thirty-five LAB isolates screened during this study, all were found to be potential EPS producers. This result is in contrast to the work of van Geel-Schutten *et al*. (1998) in which 60 lactobacillus strains were active producers of EPS among 82 isolates screened. This work is also in contrast with the work of Adebayo-Tayo and Onilude (2008) in which out of 119 isolates screened, only 103 isolates had EPS-producing potential. *L. fermentum* was found to be the best EPS producer. This is in contrast with the report of Ludbrook *et al.* (1997) having best EPS production by *L. plantarum* isolated from Hahndorf Mettwurst.

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Table 2. Lactic acid production by the LAB isolates at different incubation time.

S/N	Isolate Codes	Lactic Acid Production (mg/g)					
		Incubation Time (hrs)					
		6	12	18	24	30	36
1	*L. plantarum* LPF1	0.29	0.34	0.47	0.44	0.56	0.79
2	*L. plantarum* LPF2	0.36	1.29	1.52	1.62	1.69	1.96
3	*L. plantarum* LPWO2	0.17	0.30	0.41	0.55	0.62	0.67
4	*L. plantarum* LPWO4	0.31	0.62	0.67	0.72	0.72	0.81
5	*L. plantarum* LPN5	0.26	0.14	0.27	0.32	0.47	0.56
6	*L. plantarum* LPFDN6	0.16	0.22	0.23	0.28	0.33	0.36
7	*L. plantarum* LPW7	0.14	1.18	0.24	0.28	1.31	0.34
8	*L. plantarum* LPBO8	0.27	0.31	0.36	0.39	0.40	0.47
9	*L. plantarum* LPBO9	0.16	0.22	0.23	0.28	0.33	0.36
10	*L. plantarum* LPBO10	0.14	1.17	1.21	1.35	0.47	0.55
11	*L. plantarum* LPBO11	0.31	0.32	0.41	0.42	0.44	0.53
12	*L. plantarum* LPBO12	0.27	0.35	0.38	0.40	0.42	0.67
13	*L. delbruekii* LDF1	0.47	1.28	1.32	1.71	1.41	0.47
14	*L. delbruekii* LDWO2	0.27	0.45	1.48	1.02	1.05	1.17
15	*L. delbruekii* LDWO3	0.12	0.16	0.32	0.53	0.41	0.52
16	*L. fermentum* LFBO1	0.14	1.18	0.24	0.28	1.31	0.34
17	*L. fermentum* LFBO2	0.31	0.32	0.41	0.42	0.44	0.53
18	*L. fermentum* LFBO3	0.11	0.14	0.24	0.28	0.35	0.38
19	*L. fermentum* LFWO4	0.18	0.21	0.28	0.31	0.33	0.48
20	*L. fermentum* LFWO5	0.14	1.17	1.21	1.35	0.47	0.55
21	*L. fermentum* LF6	0.14	0.28	0.35	0.42	0.44	0.54
22	*L. fermentum* LFN7	0.41	0.73	1.43	1.71	1.80	1.91
23	*L. lactis* LLWO1	0.14	0.32	0.35	0.43	1.44	0.47
24	*L. lactis* LLWO2	0.17	0.30	0.41	0.42	0.43	0.47
25	*Leu. messenteroides* WO1	0.34	1.08	1.34	1.42	1.39	1.40
26	*Leu. messenteroides* WO2	0.22	0.26	0.32	1.03	1.11	1.12
27	*Leu. messenteroides* BO3	0.14	0.18	0.24	0.32	0.39	0.44
28	*Leu. messenteroides* W4	0.31	0.62	0.67	0.72	0.72	0.81
29	*Leu. messenteroides* W5	0.17	0.30	0.41	0.42	0.43	0.47
30	*L. casei* LCF1	0.31	0.54	0.69	1.44	1.54	1.61
31	*L. casei* LCFDN2	0.47	1.41	1.53	1.59	1.68	1.87
32	*L. cellobiosus* LCEW1	0.52	0.76	1.28	1.58	1.43	1.52
33	*L. cellobiosus* LCEW2	0.26	0.29	0.32	0.35	0.39	0.43
34	*L. brevis* LBWO1	0.27	0.35	0.38	0.40	0.42	0.67
35	*L. brevis* LBWO2	0.14	0.28	0.35	0.42	0.44	0.54

Table 3. Hydrogen peroxide production by the LAB isolates in mESM at different incubation time.

S/N	Isolate Codes	Hydrogen Peroxide Production (mg/l)					
		Incubation Time (hrs)					
		6	12	18	24	30	36
1	*L. plantarum* LPF1	0.0025	0.0027	0.0031	0.0035	0.0044	0.0047
2	*L. plantarum* LPF2	0.00067	0.0079	0.081	0.0087	0.0095	0.0097
3	*L. plantarum* LPWO2	0.0003	0.0005	0.0015	0.0019	0.0023	0.0028
4	*L. plantarum* LPWO4	0.0013	0.0015	0.0027	0.0029	0.00032	0.0035
5	*L. plantarum* LPN5	0.0003	0.0004	0.0009	0.0014	0.0019	0.00245
6	*L. plantarum* LPFDN6	0.0013	0.0024	0.0027	0.0033	0.0035	0.0049
7	*L. plantarum* LPW7	0.0014	0.0016	0.0019	0.0027	0.0034	0.0037
8	*L. plantarum* LPBO8	0.0013	0.0025	0.0028	0.0035	0.0038	0.0041
9	*L. plantarum* LPBO9	0.0013	0.0024	0.0027	0.0033	0.0035	0.0049
10	*L. plantarum* LPBO10	0.0023	0.0024	0.0027	0.0024	0.0033	0.0035
11	*L. plantarum* LPBO11	0.0028	0.0029	0.0032	0.0039	0.0025	0.0040
12	*L. plantarum* LPBO12	0.0022	0.0044	0.0047	0.0053	0.0062	0.0067
13	*L. delbruekii* LDF1	0.0019	0.0021	0.0026	0.0029	0.0035	0.0039
14	*L. delbruekii* LDWO2	0.0052	0.0056	0.0062	0.0071	0.0079	0.0083
15	*L. delbruekii* LDWO3	0.0013	0.0027	0.0029	0.0034	0.0037	0.0040
16	*L. fermentum* LFBO1	0.0013	0.0022	0.0030	0.0033	0.0035	0.35
17	*L. fermentum* LFBO2	0.0028	0.0029	0.0032	0.0039	0.0025	0.0040
18	*L. fermentum* LFBO3	0.0011	0.0013	0.0026	0.0023	0.0031	0.0035
19	*L. fermentum* LFWO4	0.0012	0.0023	0.0027	0.0034	0.0037	0.0043
20	*L. fermentum* LFWO5	0.0023	0.0024	0.0027	0.0024	0.0033	0.035
21	*L. fermentum* LF6	0.0022	0.0024	0.0037	0.0035	0.0042	0.0047
22	*L. fermentum* LFN7	0.0044	0.057	0.0061	0.0066	0.0073	0.0076
23	*L. lactis* LLWO1	0.0013	0.0015	0.0018	0.0023	0.0027	0.0035
24	*L. lactis* LLWO2	0.0011	0.0013	0.0015	0.0023	0.0031	0.0036
25	*Leu. messenteroides* WO1	0.0003	0.0017	0.0029	0.0031	0.0033	0.0038
26	*Leu. messenteroides* WO2	0.0005	0.0008	0.0011	0.0029	0.00035	0.0042
27	*Leu. messenteroides* BO3	0.0034	0.0035	0.0027	0.0033	0.0041	0.0045
28	*Leu. messenteroides* W4	0.0013	0.0015	0.0018	0.0023	0.0027	0.0035
29	*Leu. messenteroides* W5	0.0011	0.0013	0.0015	0.0023	0.0031	0.0036
30	*L. casei* LCF1	0.0003	0.0007	0.0011	0.0017	0.0023	0.0028
31	*L. casei* LCFDN2	0.0045	0.0058	0.00064	0.0068	0.0083	0.0086
32	*L. cellobiosus* LCEW1	0.002	0.0003	0.0005	0.00013	0.0021	0.00275
33	*L. cellobiosus* LCEW2	0.0012	0.0023	0.0026	0.0033	0.0036	0.0043
34	*L. brevis* LBWO1	0.0022	0.0024	0.0037	0.0035	0.0042	0.0047
35	*L. brevis* LBWO2	0.0022	0.0044	0.0047	0.0053	0.0062	0.0067

Table 4. Diacetyl production by the LAB isolates in mESM at different incubation time.

S/N	Isolate Codes	Diacetyl Production (g/l)					
		Incubation Time (hrs)					
		6	12	18	24	30	36
1	*L. plantarum* LPF1	0.19	0.32	0.32	0.34	0.47	0.64
2	*L. plantarum* LPF2	0.82	0.96	1.51	1.60	1.77	1.92
3	*L. plantarum* LPWO2	0.30	0.28	0.42	0.57	0.67	0.78
4	*L. plantarum* LPWO4	0.19	0.32	0.49	0.49	0.57	0.61
5	*L. plantarum* LPN5	0.30	0.32	0.43	0.60	0.77	0.74
6	*L. plantarum* LPFDN6	0.30	0.33	0.36	0.49	0.57	0.62
7	*L. plantarum* LPW7	0.32	0.42	0.48	0.57	0.60	0.64
8	*L. plantarum* LPBO8	0.24	0.30	0.44	0.43	0.57	0.63
9	*L. plantarum* LPBO9	0.30	0.33	0.36	0.49	0.57	0.62
10	*L. plantarum* LPBO10	0.20	0.31	0.38	0.44	0.49	0.54
11	*L. plantarum* LPBO11	0.22	0.28	0.37	0.35	0.41	0.46
12	*L. plantarum* LPBO12	0.30	0.37	0.44	0.57	0.62	0.65
13	*L. delbruekii* LDF1	0.20	0.29	0.37	0.37	0.43	0.54
14	*L. delbruekii* LDWO2	0.64	0.40	0.53	0.79	0.85	0.89
15	*L. delbruekii* LDWO3	0.26	0.36	0.36	0.39	0.42	0.43
16	*L. fermentum* LFBO1	0.32	0.42	0.48	0.57	0.60	0.64
17	*L. fermentum* LFBO2	0.22	0.28	0.37	0.35	0.41	0.46
18	*L. fermentum* LFBO3	0.30	0.31	0.36	0.49	0.54	0.61
19	*L. fermentum* LFWO4	0.28	0.34	0.40	0.47	0.49	0.53
20	*L. fermentum* LFWO5	0.20	0.31	0.34	0.44	0.49	0.54
21	*L. fermentum* LF6	0.32	0.38	0.42	0.57	0.57	0.62
22	*L. fermentum* LFN7	0.67	0.79	0.83	0.85	0.94	1.04
23	*L. lactis* LLWO1	0.30	0.36	0.42	0.53	0.60	0.63
24	*L. lactis* LLWO2	0.30	0.36	0.40	0.47	0.53	0.57
25	*Leu. messenteroides* WO1	0.27	0.30	0.37	0.49	0.53	0.61
26	*Leu. messenteroides* WO2	0.28	0.20	0.43	0.77	0.82	0.86
27	*Leu. messenteroides* BO3	0.30	0.35	0.41	0.47	0.52	0.57
28	*Leu. messenteroides* W4	0.32	0.38	0.42	0.57	0.57	0.62
29	*Leu. messenteroides* W5	0.30	0.36	0.42	0.47	0.53	057
30	*L. casei* LCF1	0.24	0.30	0.42	0.53	0.67	0.72
31	*L. casei* LCFDN2	0.73	0.86	0.92	1.07	1.24	1.47
32	*L. cellobiosus* LCEW1	0.36	0.42	0.51	0.77	0.85	0.67
33	*L. cellobiosus* LCEW2	0.24	0.31	0.36	0.46	0.49	0.53
34	*L. brevis* LBWO1	0.30	0.37	0.44	0.57	0.62	0.65
35	*L. brevis LBWO2*	0.32	0.38	0.42	0.57	0.57	0.62

Table 5. pH development by the LAB isolates in mESM at different incubation time.

S/N	Isolate Codes	pH Development					
		Incubation Time (hrs)					
		6	12	18	24	30	36
1	*L. plantarum* LPF1	6.2	6.0	5.7	4.8	4.3	4.0
2	*L. plantarum* LPF2	5.4	4.9	4.3	3.7	3.5	3.2
3	*L. plantarum* LPWO2	6.0	5.8	5.4	4.7	4.3	4.1
4	*L. plantarum* LPWO4	5.9	5.7	5.2	4.7	4.1	3.9
5	*L. plantarum* LPN5	5.9	5.7	5.3	5.0	4.9	4.4
6	*L. plantarum* LPFDN6	6.0	5.6	5.9	4.3	4.1	3.9
7	*L. plantarum* LPW7	6.0	5.8	5.1	4.6	4.2	3.8
8	*L. plantarum* LPBO8	6.4	6.0	5.8	5.4	4.1	4.0
9	*L. plantarum* LPBO9	5.9	5.7	5.1	4.5	4.1	3.8
10	*L. plantarum* LPBO10	6.1	5.4	5.1	4.9	4.3	3.9
11	*L. plantarum* LPBO11	5.6	5.0	4.9	4.4	4.4	3.9
12	*L. plantarum* LPBO12	5.9	5.7	5.3	5.0	4.9	4.4
13	*L. delbruekii* LDF1	6.0	5.8	5.4	4.3	4.0	3.9
14	*L. delbruekii* LDWO2	6.0	5.8	5.3	4.8	4.2	3.5
15	*L. delbruekii* LDWO3	5.9	5.7	5.3	4.7	4.6	4.1
16	*L. fermentum* LFBO1	6.0	5.8	5.5	4.8	4.6	4.4
17	*L. fermentum* LFBO2	5.7	5.4	4.9	4.4	4.1	3.9
18	*L. fermentum* LFBO3	6.5	6.2	5.9	5.3	4.9	4.2
19	*L. fermentum* LFWO4	6.1	5.8	5.4	4.9	4.6	4.2
20	*L. fermentum* LFWO5	6.1	5.4	5.1	4.9	4.3	3.9
21	*L. fermentum* LF6	5.7	5.4	4.9	4.4	4.1	3.9
22	*L. fermentum* LFN7	5.8	5.3	4.8	4.5	4.1	3.3
23	*L. lactis* LLWO1	6.3	6.4	5.7	5.3	5.0	4.6
24	*L. lactis* LLWO2	6.3	5.8	5.3	4.8	4.4	4.1
25	*Leu. messenteroides* WO1	6.0	5.6	5.1	4.9	4.5	4.0
26	*Leu. messenteroides* WO2	5.9	5.7	5.1	4.5	4.1	3.8
27	*Leu. messenteroides* BO3	6.2	5.9	5.5	5.1	4.8	4.4
28	*Leu. messenteroides* W4	5.9	5.7	5.1	4.5	4.1	3.8
29	*Leu. messenteroides* W5	6.3	5.8	5.3	4.8	4.4	4.1
30	*L. casei* LCF1	6.1	5.7	5.3	4.1	3.9	3.7
31	*L. casei* LCFDN2	5.4	5.1	4.8	4.5	4.2	3.9
32	*L. cellobiosus* LCEW1	6.4	6.2	5.7	5.4	4.7	4.2
33	*L. cellobiosus* LCEW2	6.0	5.7	5.3	5.1	4.7	4.5
34	*L. brevis* LBWO1	5.9	5.7	5.3	5.0	4.9	4.4
35	*L. brevis* LBWO2	5.7	5.4	4.9	4.4	4.1	3.9

Table 6. Screening of the LAB isolates for EPS production on solid agar.

S/N	Isolate Codes	EPS Production on Solid Agar	
		Appearance on the Agar Plate	
1	*L. plantarum* LPF1	creamy	Slightly mucoid
2	*L. plantarum* LPF2	creamy	Highly mucoid
3	*L. plantarum* LPWO2	creamy	Slightly mucoid
4	*L. plantarum* LPWO4	creamy	Slightly mucoid
5	*L. plantarum* LPN5	creamy	Mucoid
6	*L. plantarum* LPFDN6	creamy	Mucoid
7	*L. plantarum* LPW7	creamy	Mucoid
8	*L. plantarum* LPBO8	creamy	Mucoid
9	*L. plantarum* LPBO9	creamy	Slightly mucoid
10	*L. plantarum* LPBO10	creamy	Slightly mucoid
11	*L. plantarum* LPBO11	creamy	Slightly mucoid
12	*L. plantarum* LPBO12	creamy	Slightly mucoid
13	*L. delbruekii* LDF1	creamy	Slightly mucoid
14	*L. delbruekii* LDWO2	creamy	Mucoid
15	*L. delbruekii* LDWO3	creamy	Slightly mucoid
16	*L. fermentum* LFBO1	creamy	Slightly mucoid
17	*L. fermentum* LFBO2	creamy	Slightly mucoid
18	*L. fermentum* LFBO3	creamy	Mucoid
19	*L. fermentum* LFWO4	creamy	Slightly mucoid
20	*L. fermentum* LFWO5	creamy	Slightly mucoid
21	*L. fermentum* LF6	creamy	Mucoid
22	*L. fermentum* LFN7	creamy	Highly mucoid
23	*L. lactis* LLWO1	creamy	Mucoid
24	*L. lactis* LLWO2	creamy	Slightly mucoid
25	*Leu. Messenteroides* WO1	creamy	Highly mucoid
26	*Leu. Messenteroides* WO2	creamy	Slightly mucoid
27	*Leu. Messenteroides* BO3	creamy	Slightly mucoid
28	*Leu. messenteroides* W4	creamy	Slightly mucoid
29	*Leu. messenteroides* W5	creamy	Slightly mucoid
30	*L. casei* LCF1	creamy	Slightly mucoid
31	*L. casei* LCFDN2	creamy	Highly mucoid
32	*L. cellobiosus* LCEW1	creamy	Slightly mucoid
33	*L. cellobiosus* LCEW2	creamy	Slightly mucoid
34	*L. brevis* LBWO1	creamy	Slightly mucoid
35	*L. brevis* LBWO2	creamy	Slightly mucoid

Table 7. EPS production by the LAB isolates in mESM at different incubation time.

S/N	Isolate Codes	EPS Production (mg/l)					
		Incubation Time (hrs)					
		6	12	18	24	30	36
1	*L. plantarum* LPF1	480	390	350	509	610	674
2	*L. plantarum* LPF2	328	470	510	520	630	630
3	*L. plantarum* LPWO2	384	420	350	386	550	620
4	*L. plantarum* LPWO4	239	370	410	320	380	610
5	*L. plantarum* LPN5	449	120	269	470	569	329
6	*L. plantarum* LPFDN6	256	376	370	420	468	531
7	*L. plantarum* LPW7	439	562	590	610	780	440
8	*L. plantarum* LPBO8	439	562	590	610	780	440
9	*L. plantarum* LPBO9	420	448	566	691	730	590
10	*L. plantarum* LPBO10	323	148	496	592	600	390
11	*L. plantarum* LPBO11	420	448	566	691	730	590
12	*L. plantarum* LPBO12	390	436	546	792	209	484
13	*L. delbruekii* LDF1	550	630	757	985	980	680
14	*L. delbruekii* LDWO2	449	120	269	170	569	329
15	*L. delbruekii* LDWO3	420	448	566	691	730	590
16	*L. fermentum* LFBO1	450	630	757	685	719	680
17	*L. fermentum* LFBO2	320	180	280	160	330	110
18	*L. fermentum* LFBO3	347	391	437	489	562	379
19	*L. fermentum* LFWO4	310	420	478	539	410	384
20	*L. fermentum* LFWO5	428	498	510	540	629	406
21	*L. fermentum* LF6	550	730	990	1,390	1,040	950
22	*L. fermentum* LFN7	450	562	575	1,100	549	410
23	*L. lactis LLWO1*	410	230	660	710	490	569
24	*L. lactis* LLWO2	270	358	395	369	310	440
25	*Leu..messenteroides* LLWO1	489	585	390	860	990	660
26	*Leu. messenteroides* WO2	411	455	593	420	635	770
27	*Leu. messenteroides* WO3	480	390	400	560	598	620
28	*Leu. messenteroides* BO4	347	391	437	489	562	379
29	*Leu. messenteroides* W5	480	390	350	509	610	674
30	*L. casei* LCF1	431	320	390	494	570	590
31	*L. casei* LCFDN2	550	680	870	1,070	960	835
32	*L. cellobiosus* LCEW1	510	539	670	330	379	450
33	*L. cellobiosus* LCEW2	120	210	259	330	375	440
34	*L. brevis* LBWO1	270	359	397	369	310	440
35	*L. brevis* LBWO2	120	210	259	330	375	440