EFFECT OF SOME FACTORS ON EXOPOLYSACCHARIDE PRODUCTION OF Lactobacillus fermentum TC21 ISOLATED FROM ‘TOM CHUA’ IN HUE, VIETNAM

Tran Thi Ai Luyen*, Do Thi Bich Thuy2, Tran Bao Khanh2, Nguyen Quoc Khanh2

1 College of Sciences, Hue University
2 College of Agriculture and Forestry, Hue University

Abstract: Exopolysaccharide (EPS) biosynthesis ability of four Lactobacillus fermentum strains isolated from ‘Tom Chua’, a Hue traditional fermented shrimp was investigated. When compared with other strains, Lactobacillus fermentum TC21 produced the highest EPS amounts. The EPS yield produced by this strain was 177.151 µg/mL in the MRS broth with the addition of 1 % glucose and 2 % lactose after 48 h incubation at 37 °C. Therefore, the effects of sugar composition, nitrogen sources, and the culture conditions (initial pH, temperature, initial cell density and time course of incubation) on EPS production by Lactobacillus fermentum TC21 were studied. With the presence of 4 % sugar added into MRS broth, the EPS yields were maximal compared with other concentrations. The stimulation of EPS synthesis of Lactobacillus fermentum TC21 by lactose was the best (179.752 µg/mL). The use of coconut milk containing 20 % sugar instead of distilled water in MRS broth significantly affected the EPS production. When 50 % of distilled water was replaced by coconut milk, the EPS yield was the highest (182.638 µg/mL). The effect of nitrogen sources such as peptone, beef extract and yeast extract) on the EPS biosynthesis of this strain was also investigated with their supplements into MRS containing 4 % lactose. All of the samples were incubated at 37 °C for 48 h. The EPS amount was 301.988 µg/mL when 0.8 % (w/v) beef extract was added into the medium. The results indicated that the medium for EPS production by Lactobacillus fermentum TC21 containing 0.8 % (w/v) of beef extract and 4 % lactose was optimal. With this composition, the EPS yield was the highest at 405.728 mg/l after 36 h of incubation with initial pH 6.0 and initial cell density of 10^6 cfu/mL at 35 °C.

Keywords: coconut milk, cultural conditions, exopolysaccharide, Lactobacillus fermentum, sugar

1 Introduction

In recent years, several bacteria have been known to produce the exopolysaccharides (EPSs), especially lactic acid bacteria (LABs). These bacteria are used in many fermented foods, particularly fermented dairy products. Alternatively, these products were also a source that gave diversified kinds of LABs. Some LABs secrete a polysaccharide polymer and this EPS is economically important. EPSs produced by LABs play a major role in the food industry as viscosifiers, texturizers, thickeners, stabilizers, emulsifiers, binders, gelling agents, etc. [7, 8, 15]. Especially, since LABs strains are generally food-grade microorganisms with a GRAS (Generally Recognised As Safe) status, the use of EPS-producing LABs in food fermentation could result in safe and natural products with improved stability [5, 20]. Recently, EPS produced by LABs have received increasing attention among researchers because of their health benefits to the consumers [19, 24]. Some studies indicated that these EPSs may have
immunoreactivity [10], antitumor activities [11], or cholesterol-lowering activity [17], antioxidant and antibacterial activities [13, 23], and prebiotic effects [3].

Although EPSs of LABs have important function roles, their commercial exploitation is still limited. The low yield and high production cost are the two main reasons for their weak commercialization [5, 7, 19]. EPS characteristics and amounts can be influenced by several factors such as the composition of the medium (carbon and nitrogen sources), as well as incubation conditions (temperature, pH, time, etc.) [5, 20, 24].

For these reasons, a number of studies on EPS production by LABs were performed with regard to the factors affecting the production, properties of EPS, structure, synthesis mechanism, etc. [8, 9, 12].

There are a variety of traditional fermented products in Viet Nam such as “Tom chua”-fermented shrimp, “nem chua”-fermented lean meat, fermented vegetables, etc. They are abundant sources of LABs. Therefore, studying the EPSs biosynthesis by LABs isolated from these products is very necessary.

In the present study, the effect of the medium compositions (carbon and nitrogen sources) on the EPS production of *Lactobacillus fermentum* TC 21 strain isolated from ‘Tom Chua’, a Hue traditional fermented shrimp was investigated. The effect of culture conditions on the EPS biosynthesis by this strain was also studied.

### 2 Materials and methods

#### 2.1 Microorganisms

*Lactobacillus fermentum* (TC20, TC21, TC22, TC23) strains were isolated from ‘Tom Chua’. The strains were stored at -20 °C in an MRS broth containing 30 % glycerol until required. To prepare the inoculum, the frozen culture was plated on MRS (Man Rogosa Sharpe) agar and grown in MRS broth at 37 °C for 24 h. The cells were harvested, washed and dissolved again with peptone 1 % and NaCl 0.85 %.

#### 2.2 Methods

**The EPS production of *L. fermentum* (TC20, TC21, TC22, TC23) strains**

To incubate the different strains in an MRS medium with the supplement of glucose 1 % and lactose 2 % at 37 °C, the initial pH 6.0 – 6.2 for 48 h. After incubation, the cells were removed with a centrifuge (10000 ×g for 10 min at 4 °C) twice. The crude EPS released in cultures was determined with the phenol-sulfuric method [6], using glucose as a standard.

**Cultivation conditions**

*The effect of carbon sources on EPS production:* The effect of carbon sources on EPS production was studied by adding different sugars (sucrose, lactose, glucose) at various concentrations (1, 2, 3,
4, 5, 6 %) and reducing sugar in coconut milk (0.5, 1.0, 1.5, 2.0 %) to the MRS broth. The coconut milk containing about 20 g/L reducing sugar was also used as a source of carbon.

The effect of nitrogen sources on EPS production: The effect of nitrogen sources and their concentrations on the EPS production with L. fermentum TC21 was studied in a number of medium containing nitrogen, such as peptone (0.2, 0.4, 0.6, 0.8, 1.0 % (w/v)), beef extract (0.2, 0.4, 0.6, 0.8, 1.0 % (w/v)), yeast extract (0.1, 0.2, 0.3, 0.4, 0.5 % (w/v)), and a suitable carbon source was also tested.

The cultivation conditions for the EPS: The effect of the initial cell density on EPS production with L. fermentum TC21 was studied when the strain was grown at 37 °C, pH 6.0 – 7.2 in the medium containing the replacing carbon and nitrogen for 48 h. The various initial cell densities include 10⁴, 10⁵, 10⁶, 10⁷, 10⁸ cfu/mL. The initial pH of the medium in the study was at 4.0, 4.5, 5.0, 5.5, 6.0 and 6.5; the pH value was adjusted with 0.1 M NaOH and 0.1 M HCl solutions. The effect of temperature and incubation time was studied at 30, 35, 40 and 45 °C and 12, 24, 36, 48, 60, 72 h, respectively.

After the cultivation, the samples were taken for determination of EPS yield. The total EPS yield was determined with the phenol-sulfuric method [6], using glucose as a standard.

Isolation of EPS [20, 24]
EPS was isolated from the fermented sample using a modified procedure previously described by Yang et al. (1999). Briefly, the sample culture was heated and kept at 100 °C for 15 min to inactivate the enzymes that can potentially cause polymer degradation. Then, it was cooled and added with trichloroacetic acid to a final concentration of 30 % (w/v). After centrifugating (13000 ×g for 10 min at 4 °C) twice to remove the precipitated proteins and bacteria again, the supernatant containing EPS was mixed with a double volume of cold ethanol and then stored at 4 °C for 24 h. The precipitated EPS was separated by centrifugating (10000 ×g for 10 min at 4 °C) (repeat two times), collected and dissolved in a suitable deionized water.

2.3 Statistical analysis
Each test was carried out in triplicate. Data from each test were subjected to SPSS 22.0 for the analysis of variance. Duncan’s multiple range test was used to determine the significant difference (p < 0.05) among treatments.

3 Result and discussion
3.1 The EPS production of L. fermentum (TC20, TC21, TC22, TC23) strains
In comparison with other strains, Lactobacillus fermentum TC21 grew strongly and produced the highest EPS amounts (177.151 µg/mL) in the MRS broth with the supplement of 1 % glucose and 2 % lactose at 37 °C for 48 h incubation (Fig 1).
Figure 1 also shows that the growth of *L. fermentum* (TC20, TC22, TC23) strains at this cultivation condition was very poor, especially those of *L. fermentum* TC20 and *L. fermentum* TC23. These strains produced much less EPS at 92.923 µg/mL for TC22, 55.606 µg/mL for TC23, and 49.630 µg/mL for TC20.

The quantity of exopolysaccharides produced varies with different bacteria species and the EPS synthesis in different growth phases and under a variety of conditions depending on the organism studied (Cerning, 1995). Furthermore, the physicochemical factors playing a crucial role in the yield of these EPS include pH, temperature, incubation time (laboratory conditions), and medium composition (carbon, nitrogen and cation sources) [16].

Based on these results, *L. fermentum* TC21 strain was chosen for the further investigation.

**Fig. 1.** The EPS production of *L. fermentum* (TC20, TC21, TC22, TC23) strains

### 3.2 Effect of carbon sources on EPS production

Both the carbon sources and their concentration have a stimulating effect on EPS biosynthesis ability. In this study, the effect of the supplementation of different carbon sources (glucose, lactose, sucrose and coconut milk) in various concentrations on the EPS biosynthesis by *L. fermentum* TC21 in the MRS broth was tested at 37 °C with initial pH of 6.0 – 6.2 for 48 h. The EPS production was improved significantly when the cultivation took place in the medium containing these carbon sources (Table 1). Sugar solutions of concentrations from 1 % to 4 % stimulated the increase of the EPS production. The maximum EPS amounts were achieved using lactose, followed by glucose and sucrose. The results revealed that the optimal concentrations of tested carbon sources appeared to be 4 % (w/v) for these sugars added to the MRS broth, and the EPS yield was 179.752 µg/mL, 168.492 µg/mL, and 129.752 µg/mL, respectively. At higher concentrations (5 %, 6 %), the EPS yield remained stable or decreased.

The influence of reducing sugar in coconut milk on the production of EPS by the strain TC21 is very clear. Table 1 shows that if a half of distilled water in MRS was replaced with coconut milk (approximately 1 % reducing sugar), this strain produced the highest amount of EPS (175.890 µg/mL). However, this yield was much less than that obtained in the medium containing 4 % lactose.
The effect of carbon sources on the growth and EPS production by LABs was reported to be dependent on the specific strain, the type of sugar, and the properties of the cell carbon metabolism [15]. Zhang et al. (2011) reported that glucose was more effective than fructose, lactose and galactose for the optimal production of EPS with \textit{L. fermentum} F6 [28]. While sucrose influenced the yield of EPS by \textit{Streptococcus thermophilus} ST1 in skim milk medium the most among the carbon sources tested [27], glucose, lactose and galactose were found to be suitable carbon sources for EPS synthesis by \textit{L. helveticus} ATCC 15807 in a chemically defined medium [21].

These results indicated that LABs tend to grow on a wide range of carbon sources. Thus, lactose was recognized as a good carbon source for \textit{L. fermentum} TC21 to enhance the EPS biosynthesis. Therefore, lactose with a concentration of 4 % was chosen for the following experiments.

\textbf{Table 1. Effect of carbon sources on EPS production by \textit{L. Fermentum} TC21}

| Carbon sources | Supplement concentrations (%) | Yield of EPS (µg/mL) | Carbon sources | Supplement concentrations (%) | Yield of EPS (µg/mL) |
|----------------|-------------------------------|----------------------|----------------|-------------------------------|----------------------|
| Glucose        | 0                             | 97.882               | Sucrose        | 0                             | 96.622               |
|                | 2                             | 137.029              |                | 2                             | 107.882              |
|                | 3                             | 132.842              |                | 3                             | 117.923              |
|                | 4                             | 168.492              |                | 4                             | 129.752              |
|                | 5                             | 156.378              |                | 5                             | 129.345              |
|                | 6                             | 150.159              |                | 6                             | 88.980               |
| Lactose        | 0                             | 96.988               | Reducing sugar in coconut milk               | 0                             | 100.281             |
|                | 2                             | 151.744              |                | 0.5                           | 166.906              |
|                | 3                             | 157.435              |                | 1                             | 175.890              |
|                | 4                             | 179.752              |                | 1.5                           | 152.273              |
|                | 5                             | 176.581              |                | 2                             | 125.484              |
|                | 6                             | 171.622              |                |                               |                      |

3.3 \textbf{Effect of nitrogen sources on EPS production in the medium containing 4 % lactose}

Peptone, beef extract and yeast extract are widely used nutrients for the cultivation of bacteria. The effect of these nitrogen sources on the growth and EPS production by \textit{L. fermentum} TC21 in the medium containing 4 % lactose was also examined.

The supplementation of nitrogen sources with various concentrations was found to influence the EPS synthesis by \textit{L. fermentum} TC21. Table 2 shows that all the tested concentrations stimulated the growth of this strain very well. \textit{L. fermentum} TC21 can produce a higher amount of EPS than the lactose optimal medium, and the most efficient nitrogen source was beef extract.

For beef extract, in comparison with other concentrations, the EPS amount was maximized (301.988 µg/mL) in the medium supplemented with 0.8 % (w/v). Besides, with the addition of peptone and yeast extract, the highest EPS yields were 0.6 % (283.695 µg/mL) and
0.4 % (281.378 µg/mL), respectively. Perhaps, those sources have the stimulating affect the EPS production because they may contain vitamins and minerals necessary for the growth of bacteria.

**Table 2.** Effect of adding of nitrogen sources on the EPS production by *L. Fermentum* TC21

| Peptone          | Beef extract | Yeast extract |
|------------------|--------------|---------------|
| Supplement       | Yield of EPS | Supplement     | Yield of EPS | Supplement | Yield of EPS |
| concentration (%)| (µg/L)       | concentration (%)| (µg/L)       | concentration (%)| (µg/L)       |
| 0.2              | 195.890      | 0.2           | 203.573      | 0.1        | 221.622      |
| 0.4              | 214.549      | 0.4           | 253.085      | 0.2        | 257.998      |
| 0.6              | 283.695      | 0.6           | 265.768      | 0.3        | 262.842      |
| 0.8              | 278.085      | 0.8           | 301.988      | 0.4        | 281.378      |
| 1.0              | 256.500      | 1.0           | 291.622      | 0.5        | 211.256      |

It can be seen from Table 2, high concentrations of nitrogen sources in MRS plus 4 % lactose did not affect the EPS biosynthesis by *L.fermentum* TC21. This result was the same as that of other studies on the strains producing EPS [20]. In short, the growth of all microorganisms requires many factors, such as physical, chemical conditions to support their bioactivity. However, each bacteria have to differ from the various substance. On the other hand, the growth of bacteria was dependent on the alternative carbon source in the cultural medium. Seesuriyachan et al. (2011) reported that using a mod-MRS-CW medium in which the de-ionized water was replaced with 100 % coconut milk, and the supplements of 20 g/l crystalline sucrose and reduced quantity (50 %) of the three expensive supplements (5 g/l of peptone, 2.5 g/l of beef extract and 2.5 g/l of yeast extract) were provided, gave the highest yield of EPS [20]. Zhang et al. (2011) revealed that under the optimal cultural conditions (pH 6.5, 42 °C with 2 % sucrose and 0.5 % WPC) *Streptococcus thermophilus* ST1 produced the highest amount of EPS [27]. Wu et al., (2007) indicated that yeast peptone powder was the nitrogen source most affecting the EPS production by *P. citrinopileatus* in the medium containing 4 % fructose as a carbon source [25].

In summary, the beef extract was recognized as the most suitable nitrogen source for *L. fermentum* TC21 to impulse the development of the EPS biosynthesis, and its most suitable concentration was 0.8 %. Therefore, this beef extract concentration was chosen for investigating the cultural conditions.

### 3.4 Effect of cultural conditions on EPS production by *L. fermentum* TC21 in the medium containing 4 % lactose and 0.8 % beef extract

**Effect of the initial cell density**

The EPS production by *L. fermentum* TC21 was studied when the strain was cultivated in the medium, containing 4 % lactose, 0.8 % beef extract at 37 °C, and at the initial pH of 6.0 – 6.2 for 48 h with the different initial cell densities (10^4, 10^5, 10^6, 10^7, 10^8 cfu/mL). The cell density levels did not result in the same growth and EPS yields. The maximum EPS production was 304.508
µg/mL with the initial cell density at $10^6$ cfu/mL (fig 2). The EPS yield was very low with the initial cell density of $10^8$ cfu/mL (196.663 cfu/mL).

The data revealed that three initial cell density levels, namely $10^4$, $10^5$, $10^7$ cfu/mL were not significantly different ($p < 0.05$) and did not affect the EPS amounts. The yields of EPS were only in the range of 222.150 – 229.142 µg/mL. This result implies that high or low levels of initial cell density in cultivation did not affect the EPS productivity of the TC21. However, when the initial cell density dropped to $10^8$ cfu/mL, the yield of EPS became poor due to the low growth of bacteria. Hence, the initial cell density of $10^6$ cfu/mL was chosen for further study in the next experiment.

**Fig. 2. Effect of initial cell density on the growth of L fermentum TC21**

(The data with different letters in the figure are significant at $p < 0.05$.)

**Effect of the initial pH**

The effect of the initial pH of the medium on the bacterial growth and EPS synthesis by *L. fermentum* TC21 was studied at pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5. When this strain was grown at 37 °C in the medium supplemented with 4 % lactose, 0.8 % beef extract, the EPS synthesis took place at all pH levels tested. However, the maximal amount of EPS of 328.654 µg/mL was obtained at pH 6.0 (Fig 3).

The EPS production slightly increased at pH 4.5, 5.0, 5.5 with the amount of 199.224 µg/mL, 209.061 µg/mL, 244.102 µg/mL, respectively and then decreased at pH 6.5 (299.793 µg/mL). At pH 4.0, the bacterial growth was poor with low EPS yield of only 178.289 µg/mL. This could be because high acidity of the medium might cause stress to the cells [22].

**Fig. 3. Effect of initial pH on the yield of EPS**
Although there were results of various optimal pHs for different bacterial strains, this value is usually around 6.0 [4]. The initial pH 6.5 was the most suitable for the growth and EPS production by strains *L. fermentum* F6 [28], *Streptococcus thermophilus* ST1 [27]. *L. confusus* TISTR 1498 gave high EPS yield in the medium in which de-ionized water was replaced with coconut milk at pH 5.5 [20]. Meanwhile, the EPS production by *Lactobacillus plantarum* YW11 was found to be higher in the MRS broth where whey was added at pH 6.3 [26].

**Effect of incubation temperature**

The effect of temperature on EPS production by *L. fermentum* TC21 was investigated at different values of 30, 35, 40 and 45 °C (Figure 4). The results showed that the optimal temperature for the bacterial growth was 35 °C. At this temperature, *L. fermentum* TC21 produced EPS with the maximal yield of 376.134 µg/mL after 48 h incubation. The strain produced much less EPS at 30, 40 and 45 °C with the yields of 274.264 µg/mL, 337.394 µg/mL and 294.223 µg/mL, respectively.

The optimal conditions (35 °C and pH 6.0) for the growth of *L. fermentum* TC21 were also favourable for its EPS production. However, there were other reports showing that optimal conditions for EPS production by some LAB strains were different from those for their optimal growth. The production of EPS by *S. thermophilus* BN1 reached the maximum value at 37 °C in skimmed milk with an amount of 548 PDM mg/l, followed by the amounts of 375 PDM mg/l and 325 PDM mg/l in whey and whole milk, respectively [18]. The temperature of 37 °C and pH 6.5 were also favourable for both the growth and the EPS production by *Lactobacillus fermentum* F6 [28]. Zang *et al.* (2011) showed that the highest EPS production by *Streptococcus thermophilus* ST1 took place at 42 °C and pH 6.5, and these conditions were also optimal for the bacterial growth [27]. Mozzi *et al.* (1995) demonstrated an increased EPS production by *S. thermophilus* and *Lb bulgaricus* at the incubation temperature of 32 or 37 °C [14]. The EPS production by mesophillic lactic-acid bacteria is almost 50 % higher when the organisms are grown at 25 °C instead of 32 °C [1].

![Fig. 4. Influence of different temperature on the EPS production by *L. fermentum* TC21](image)

**Effect of incubation time**

The influence of different incubation time on the growth and EPS production by *L. fermentum* TC21 is shown in Fig. 5.
Fig. 5. Effect of the cultivation time on the EPS production by *L. fermentum* TC21

It can be seen from the picture that the EPS production increased with the incubation time. During the first twelve hours from 12 to 24 h, this value rose slightly by around 15 units from 278.81 to 294.183 µg/mL. In the next 12 hours the EPS yield increased rapidly, reaching a maximum value of 405.728 µg/mL after 36 h. With further increase of incubation time, the amount of EPS produced went down first to around 382 µg/mL after 48 and 60 h and finally to the lowest value of 341.175 µg/mL after 72 h, but still higher than the value after 12 h of incubation. The decreased EPS biosynthesis after 48 h could be due to the depletion of the nutrients. Additionally, during their metabolism, the microorganisms secreted substances (e.g. acid, carbon dioxide, etc.) that inhibited their growth, causing the EPS biosynthesis to decline.

In summary, the growth and EPS production of different kinds were dependent on the initial incubation conditions, namely the incubation temperature and time, the initial pH of culture medium, and the initial cell density. Seesuriyachan et al. (2011) reported that good EPS biosynthesis by *L. confusus* TISTR 1498 in the optimal conditions (pH 5.5, 35 °C) was obtained after 24 h of growth [20]. Meanwhile, at 24 h, the maximal amount of EPS produced by *S. thermophilus* ST1 was attained with the supplementation of WPC [27]. The EPS production by *L. fermentum* F6 in skimmed milk supplemented with 2 % glucose and 0.5 % whey protein concentrate was the highest after 32 h when this strain was cultivated at 37 °C and at initial pH 6.5 [28]. These results indicated that the growth and EPS production by each bacterium were different. In this research, the most suitable incubation time for the EPS production by *L. fermentum* TC21 was 36 hours.

4 Conclusion

Like other EPS-producing LAB strains reported earlier, the EPS production by *L. fermentum* TC21 was dependent on the cultural conditions, namely the growth temperature, the initial pH of growth medium, and the composition of the medium (including various carbon and nitrogen sources and their concentrations). Under the cultural conditions used in this study, the optimal medium for EPS production by *Lactobacillus fermentum* TC21 contains 0.8 % (w/v) beef extract and 4 % lactose. Under the optimal medium, the EPS yield was the highest (405.728 mg/l) after 36 h of incubation with initial pH 6.0 and initial cell density of 10^6 cfu/mL at 35 °C. These results also revealed that the
original amount of sugar in the coconut milk could not clearly stimulate the enhancement of the EPS biosynthesis by Lactobacillus fermentum TC21.

References

1. Cerning J, Bouillanne C, Landon M, Desmazeaud M (1992), Isolation and characterization of exopolysaccharides from slime-forming mesophilic lactic acid bacteria. J Dairy Sci 75, 692-699.
2. Cerning J, (1995), Production of exopolysaccharides by lactic acid bacteria and dairy propionibacteria. Lait 75, 463-472.
3. Dal Bello FD, Walter J, Hertel C, and Hammes WP (2001), In vitro study of prebiotic properties of levan-type exopolysaccharides from lactobacilli and non-digestible carbons using denaturing gradient gel electrophoresis. Syst. Appl. Microbiol. 24, 232–237.
4. De Vuyst L, Vanderveken F, Van De Ven S, Degeest B (1998), Production by and isolation of exopolysaccharides from Streptococcus thermophilus grown in a milk medium and evidence for their growth-associated biosynthesis. J. Appl. Microbiol, 84, 1059-1068.
5. De Vuyst, L; Degeest, B (1999), Heteropolysaccharides from lactic acid bacteria. FEMS. Microbiol. Rev. 23,153–177.
6. Dubois M, Gilles KA, Hamilton JK, Rebers PA, and Smith F, (1956), Colorimetric method for determination of sugars and related substances. Analytical Chemistry, 28, 350-356.
7. Freitas F, Alves VD and Reis MAM (2011), Advances in bacterial exopolysaccharides: from production to biotechnological applications. Trends in Biotechnology, Vol. 29, No. 8, 388-399.
8. Fukuda K, Shi T, Nagami K, Leo F, Nakamura T, Yasuda K, Senda A, Motoshima H, Urashima T, (2010), Effects of carbon source on physicochemical properties of the exopolysaccharide produced by Lactobacillus fermentum TDS030603 in a chemically defined medium. Carbon Polymers, 79, 1040–1045.
9. Górska S, Jachymek W, Rybka J, Strus M, Heczko PB, Gamian A, (2010), Structural and immunochemical studies of neutral exopolysaccharide produced by Lactobacillus johnsonii 142. Carbon Research, 345, 108–114.
10. Górska-Fra˛czek S., Sandstrom C, Kenne L, Pas´ciak M, Brzozowska E, Strus M,Heczko P, Gamian A, (2013), The structure and immunoreactivity of exopolysaccharide isolated from Lactobacillus johnsonii strain 151. Carbon Research, 378, 148–153.
11. Kitazawa, H, Harata T, Uemura J, Saito T, Kaneko T, and Itoh T (1998), Phosphate group requirement for mitogenic activation of lymphocytes by an extracellular phosphopolysaccharide from Lactobacillus delbrueckii spp. bulgaricus. Int. J. Food Microbiol. 40, 169–175.
12. Laws A, Gu Y, Marshall V (2001), Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. Biotechnology Advances, 19, 597–625.
13. Li S, Huang R, Shah NP, Tao X, Xiong Y, and Wei H (2014), Antioxidant and antibacterial activities of exopolysaccharides from Bifidobacterium bifidum WBIN03 and Lactobacillus plantarum R315. J. Dairy Sci. 97, 1–10.
14. Mozzi F, Oliver G, De Giori GS, De Valdez GF (1995), Influence temperature on the production of exopolysaccharides by thermophilic lactic acid bacteria. Milchwissenschaft 50, 80-82.
15. Nicolaus B; Schiano MV; Lama. L; Poli. A; Gambacorta A (2004), Polysaccharides from extremophilic microorganisms. Origins. Life. Evol. Biospheres, 34, 159–169.
16. Nwodo UU, Green E and Okoh A I (2012), Bacterial Exopolysaccharides: Functionality and Prospects. *Int. J. Mol. Sci.*, 13, 14002-14015.

17. Pigeon RM, Cuesta EP, and Gilliland S E (2002), Binding of free bile acids by cells of yoghurt starter culture bacteria. *J. Dairy Sci.* 85, 2705–2710.

18. Rabha B, Victor L, Miguel A, Maria F and Ahmed B, (2011), Effect of fermentation conditions (culture media and incubation temperature) on exopolysaccharide production by *Streptococcus thermophilus* BNI, *IPCBEE* vol.24, 1-5.

19. Ruas-Madiedo P, De Los Reyes-Gavilán CG (2005), Methods for the screening, isolation and characterization of exopolysaccharides produced by lactic acid bacteria. *J. Dairy Sci.* 88, 843-856.

20. Seesuriyachan P, Kuntiya A, Hanmoungai P and Techapun C, (2011), Exopolysaccharide production by *Lactobacillus confusus* TISTR 1498 using coconut milk as an alternative carbon source: the effect of peptone, yeast extract and beef extract. *Songklanakarin J. Sci. Technol.*, 33(4), 379-387.

21. Torino MI, Mozzi F and Font de Valdez G (2005), Exopolysaccharide biosynthesis by *Lactobacillus helveticus* ATCC 15807. *Appl Microbiol Biotechnol*, 68, 259-265.

22. Vaningelgem F., Zamfir M., Adriany T.and De Vuyst L. (2004), Fermentation conditions affecting the bacterial growth and exopolysaccharide production by *Streptococcus thermophilus* ST 111 in milk-based medium, *Journal of Applied Microbiology*, 97, 1257–1273.

23. Wang J, Zhao X, Tian Z, Yang Y, Yang Z, (2015), Characterization of an exopolysaccharide produced by *Lactobacillus plantarum* YW11 isolated from Tibet Kefir. *Carbon Polymers*, 125, 16–25.

24. Welman AD and Maddox IS, (2003), Exopolysaccharides from lactic acid bacteria: perspectives and challenges. *Trends in Biotechnology*, 21, 269-274.

25. Wu C, Liang Z, Lu C, Wu S, (2008), Effect of carbon and nitrogen sources on the production and carbon composition of exopolysaccharide by submerged culture of *Pleurotus citrinopileatus*. *Journal of Food and Drug Analysis*, Vol. 16, No. 2, 61-67.

26. Zhang L, Chunhong Liu C, Li D, Zhao Y, Zhang X, Zeng X, Yang Z, Li S (2013), Antioxidant activity of an exopolysaccharide isolated from *Lactobacillus plantarum* C88. *International Journal of Biological Macromolecules* 54, 270-275.

27. Zhang T, Zhang C, Li S, Zhang Y, Yang Z, (2011), Growth and exopolysaccharide production by *Streptococcus thermophilus* ST1 in skim milk. *Brazilian Journal of Microbiology*, 42, 1470-1478.

28. Zhang Y, Li S, Zhang C, Luo Y, Zhang H, Yang Z (2011), Growth and exopolysaccharide production by *Lactobacillus fermentum* F6 in skim milk. *African Journal of Biotechnology*, 10(11), 2080-2091.