



Original Contribution

COMPARATIVE INFLUENCE OF IMMOBILIZATION MEDIUM AND MUTATION ON EPS –PRODUCTION BY *L. PLANTARUM* MK O2 ISOLATED FROM FERMENTED MILK

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ABSTRACT

PURPOSE: Comparative effect of mutation and immobilization on EPS-production *L. plantarum* MKO2 was investigated. **METHODS:** *L. plantarum* strain and EPS produced by wild, mutants, immobilized and unimmobilized strains was characterized. **RESULTS:** The EPS production by the Wild type *L. plantarum* MK O2 and the Mutant *L. plantarum* Muv 11 and Muv 12 ranged from 209.89 – 268.19 mg/l in which the highest value was produced by Mutant *L. plantarum* Muv 12. EPS production on immobilization in sodium alginate ranged from 225.00 – 263.44mg/l. Mutant *L. plantarum* Muv 12 had the highest while Wild type *L. plantarum* MK O2 had the least. EPS – production by un –immobilized Wild type *L. plantarum* MK O2 and Mutant *L. plantarum* Muv 11 and Muv 12 ranged from 215.00 – 255.00 mg/l. Immobilization in sodium alginate favoured EPS-production by wild type *L. plantarum* MK O2 (225.00 mg/l) and Mutant *L. plantarum* Muv 12 (272.00 mg/l) while immobilization in agar matrix favoured EPS –production by Mutant *L. plantarum* Muv 11 (265.67 mg/l). FT-IR spectroscopy of the EPS showed the presence of the varying degrees of functional groups which are usually associated with polysaccharides, thus confirming the EPS. **CONCLUSION:** Mutation and immobilization increased EPS production by the *L. plantarum* strains.

Key words: EPS - production, Mutation, Immobilization, Sodium alginate, Agar matrix.

INTRODUCTION

Microbial exopolysaccharides (EPS) are a broad group of polymers secreted by microorganisms, which may be associated with the cell surface as capsular polysaccharides, or excreted into the surroundings of the cell as extracellular slime (1-2). Lactic acid bacteria (LAB) are a very important group of bacteria normally utilized in the food industry. They are involved in the natural fermentation of a variety of foods including milk, vegetables, beverages and meat. The activities of LAB in these foods result in the production of finished products with improved properties such as enhanced aroma and flavour, extended shelf life and improved functional properties. These properties are ensured through the production of useful metabolites such as

lactic acid, diacetyl, hydrogen peroxide and bacteriocins. In addition to these metabolites, some LAB also redirect a small proportion of fermentable sugars in their environment towards the biosynthesis of exopolysaccharides (3). A wide variety of EPS are known to be produced by several genera of LAB, including *Lactobacillus*, *Leuconostoc*, *Pediococcus* and *Streptococcus* (4). Approximately thirty species of lactobacilli are described as EPS producers. Among them, the best known are *L. casei*, *L. acidophilus*, *L. brevis*, *L. curvatus*, *L. delbrueckii*, *L. helveticus*, *L. rhamnosus*, *L. plantarum* and *L. johnsonii* (5). Dextran (synthesized by certain LAB such as *Leuconostoc mesenteroides*) was the first microbial polysaccharide to be commercially exploited and to receive authorization for use in foods (6).

Two major groups of EPS have been identified: homopolysaccharides and heteropolysaccharides. Homopolysaccharides are made up of a

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single type of monosaccharide, usually glucose or fructose which occurs as repeated units of three to eight monosaccharides. Thus, they are referred to as α -glucans (dextrans) or fructans. Heteropolysaccharides consist of at least two different sugars out of D-glucose, D-galactose and L-rhamnose in different ratios. Some other residues such as n-glycerol-3-phosphate, N-acetyl-amino sugars, phosphate and acetyl groups may also be found in EPS produced by LAB (7, 4). EPS produced by LAB have been are mostly composed of repeated units of a certain number of various sugar residues or sugar derivatives (8-9) joined by α - and β -linkages (10). The carbohydrate composition of EPS is unique to different strains of bacteria and may vary depending on the growth conditions; however, they usually consist of similar monomer subunits, with D-galactose, D-glucose and L-rhamnose occurring almost always, though in different ratios (11-14).

LABs are currently used in the production of many fermented dairy products due to their contribution to the textural characteristics of the food product through the production of EPS. For example, several strains of *Lactobacillus helveticus* used for mozzarella production produce EPS and contribute to water retention in the product (12-15). EPS may act both as a texturizer by improving the rheology (viscosity and elasticity) of a product, and as physical stabilizers by binding hydration water and interacting with other milk constituents (ions and proteins) thus limiting syneresis (16). EPS also contribute to human health as prebiotics or due to antitumor, immunomodulating or cholesterol-lowering activities (4). It helps probiotics to survive the gastric acid and bile salts in the gastrointestinal tract (17). The increased viscosity of foods containing EPS is thought to increase the residence time of ingested fermented milk in the gastrointestinal tract, thereby supporting transient colonization by probiotic bacteria (18).

Random mutagenesis, a classical method of strain improvement, has been widely used in the food industry to improve microbial strains with desirable qualities (19 – 20). Since the genome of an organism controls its potential productivity, consequently, the genome may be modified to increase the potential yield. Ultraviolet (UV) light has been shown to be lethal and mutagenic in a variety of organisms, including bacteria. Mutagenic lesions are formed in the DNA as a result of exposure of cells to

UV radiation. Two of the most frequent lesions are the cyclobutane pyrimidine dimers and the 6-4 pyrimidine-pyrimidone photoproducts formed at adjacent pyrimidines (21). Other types of DNA lesions include pyrimidine hydrates, purine photoproducts, strand breaks, and DNA cross-links. These however occur at a much lower frequency (22).

Immobilisation involves restricting the mobility of microbial cells, enzymes or other proteins inside or on the surface of a carrier such that their catalytic activity is preserved (23). Currently, different immobilization techniques have found wide applications not only in the field of biotechnology, but also in pharmaceutical, environmental, food and biosensor industries (24). It is an important means of increasing the performance of microbial cultures (25) and it has been used to carry out high cell density fermentations for both cell and metabolite production. The main advantage of immobilization is easy separation of biological material from the reaction medium containing the desired product. This significantly accelerates the production process of a variety of compounds (26). Another advantage offered by immobilization is cost-effectiveness of the reaction. Immobilized cell biomass may be re-used several times without necessarily re-culturing them. Other advantages offered by immobilization cell technology are protection against shear damage, high biological stability during long-term continuous fermentation (27 – 29) improved resistance to contamination and bacteriophage attack, enhancement of plasmid stability and prevention from washing-out during continuous cultures (30). This research aimed at investigating the comparative influence of immobilization medium on EPS – production by *L. plantarum* MK O2 isolated from fermented milk.

MATERIALS AND METHODS

Bacterial Strains

The *Lactobacillus plantarum* strain used in this study was isolated from milk obtained from Sabo market in Ibadan, Oyo State, Nigeria.

Isolation of Organisms

The milk sample was allowed to ferment in the laboratory for 48 hours after which isolation of the LAB was carried out according to the method of Sobrun et al (30). Serial dilution of the sample was carried out and it was plated on de Man, Rogosa and Sharpe (MRS) agar supplemented with sucrose (2% w/v), sodium azide (0.02% w/v) and bromocresol purple

(0.012% w/v). The cultures were incubated at 35°C for 48 hours under anaerobic conditions. Mucoid and ropy colonies exhibiting yellow zones were selected and re-streaked on MRS agar repeatedly to obtain pure cultures. The isolates were maintained on MRS agar slants 4°C and in a maintenance medium consisting of MRS broth with 20% glycerol.

Quantification of EPS produced by the strains

The amount of EPS produced was determined according to the method of Dubois et al. (31) using the exopolysaccharide selection medium (ESM) described by Van den Berg (32) and modified by Ludbrook et al. (33). The sterile ESM was inoculated with 24 hour broth culture of the LAB isolates and incubated at 35°C for 24 hours. The cultures were heated in a water bath at 80°C for 15 minutes and left to cool at room temperature. They were then treated with 5% (w/v) trichloroacetic acid (TCA) and left at 4°C overnight followed by centrifugation at 15000g for 20 minutes at 4°C to remove cells and precipitated proteins. The crude EPS was precipitated out by the addition of two volumes of chilled absolute ethanol and kept at 4°C overnight. The samples were centrifuged again at 15000g for 10 minutes and the resulting precipitate re-dissolved in 10ml of water. Phenol (1ml, 5% w/v) was added to 1ml of the dissolved precipitate in a test tube and allowed to stand for 1 minute. 5ml of concentrated sulphuric acid was added and allowed to stand for 30 minutes. The absorbance was measured using a spectrophotometer at 490 nm against a blank (distilled water) and compared to a graph generated from the results obtained for a series of D-glucose standards to determine the sugar concentration. The *L. plantarum* strain used in this study was selected because of its high EPS production capacity.

Identification of Isolate

Identification of the isolate was carried out using morphological, physiological and biochemical characteristics such as gram staining, casein hydrolysis, starch hydrolysis, gelatin hydrolysis, oxidase test, homofermentative and heterofermentative test and carbohydrate fermentation.

Confirmation of the identity was done using molecular characterization. DNA extraction was carried out according to the method of De et al. (34). The isolated DNA was then used as a template for selective amplification by the

Polymerase Chain reaction (PCR). DNA sample from the isolate obtained by this protocol were assayed by restriction enzyme digestion. Sequencing of the fragments was carried out using the automatic Big Dye (dideoxy chain terminator) sequencer ABIPRISM 3730xl. For identification of the closest relatives, newly determined sequences were compared to those available in the V2-V3 region of the 16S rRNA sequences using the GenBank DNA databases and the standard nucleotide-nucleotide BLAST algorithm.

Molecular Identification of the Highest EPS-Producing Strains

The highest EPS –producing *L. plantarum* (MK 02) was identified using molecular technique based on the 16SrRNA gene amplification by polymerase chain reaction (PCR). Purification of the PCR products and the determination of sequences were performed by MacroGen USA (9700 Great Seneca Highway, Rockville, MD 20850, USA). The amplified gene was sequence and the sequence compared with the NCBI database (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Mutagenesis

Ultraviolet radiation was used to induce mutagenesis in the *L. plantarum* strain. Dilutions of the bacterial culture were plated on MRS agar and irradiated with UV light of wavelength 254 nm at a distance of 30 cm for 5, 10, 15, 20, 25, 30, 60, 90 and 120 seconds. Cultures which were not exposed to UV radiation served as control. The cultures were wrapped in aluminium foil to prevent the penetration of light which induces photoreactivation and incubated at 35°C for 24 hours. Colony count of all the cultures was carried out and colonies were selected from cultures having 2% survival rate.

Immobilisation of Cells

In Sodium alginate: The entrapment method was used to immobilise the cells in sodium alginate according to the method of Pasha et al. (35). Two gram (2g) of sodium alginate was dissolved in 100 ml 0.9% NaCl. The solution was then autoclaved at 121°C for 15 min and 2 ml inoculums of wild type whole cells and mutants each were added to 10 ml of the solution prepared. The sodium alginate-cell solution was then added drop wise with stirring to a 0.1 M CaCl₂ solution. The gel beads formed were left in solution for 1 hour before being filtered off. The beads were then washed in a 0.9 % NaCl solution for 20 min.

In Agar: The method of Manohar and Karegoudar (36) was used to immobilize both wild type and mutant cells in agar matrix. The LAB strains were cultured in MRS broth for 12 hours and centrifuged at 5000 rpm for 10 minutes to obtain cell pellets. The cell pellets were suspended in sterile saline solution and mixed with sterile 4% (w/v) agar-saline solution, allowed to set and cut with a sterile cork borer to obtain cell-entrapped beads. The agar beads were washed successively with distilled water and saline.

Production of EPS by Un-immobilized Wild type and Mutant Strains

The bacterial cells were grown in MRS broth at 35°C for 18 hours after which the broth cultures were used to inoculate the exopolysaccharide selection medium (10% v/v) and incubated at 35°C for 48 hours. The EPS produced was then isolated and purified according to the method of Cerning et al. (37). Quantification of the EPS was carried out as described by Dubois et al. (31).

Production of EPS by Immobilised Wild type and Mutant Strains

The exopolysaccharide selection medium was inoculated with 5% (w/v) of the immobilised cells according to the method of Karigar et al. (38) and incubated at 35°C for 48 hours. The EPS produced was isolated and purified according to the method of Cerning et al. (39) while its quantification was carried out using the method described by Dubois et al. (31).

Fourier Transform Infrared Spectroscopy (FT-IR) of EPS

The EPS were prepared for infrared analysis by grinding a mixture of 2 mg of the EPS with 200 mg dry KBr, followed by pressing the mixture into a 16 mm diameter mold. The Fourier transform-infrared (FT-IR) spectra were recorded on a Shimadzu IR Affinity 1S instrument with a resolution of 4 cm⁻¹ in the 4000-400 cm⁻¹ region.

RESULTS AND DISCUSSION

The isolate was characterized genotypically and the EPS produced by the strains was characterized. A BLAST (Basic Local Alignment Search Tool) analyses of the 16SrRNA gene nucleotide sequence of strain MKO2 amplified product showed a 97% similarity to *L. plantarum*. A phylogenetic tree was constructed between it and similar sequences found in GenBank.

Molecular identification of the LAB strain MK 02 as *Lactobacillus plantarum* follows the trend of previous works which have identified this organism as a potent EPS producer. Talon et al. (40) and Francois et al. (41) reported the production of both capsular and extracellular EPS by *L. plantarum* strains. Remus et al. (42) reported the presence of four clusters of genes in the genome of *L. plantarum* which are associated with surface polysaccharide production, indicating the capacity of this strain for EPS production.

The wild type *L. plantarum* strain (MK 02) used in this study produced an EPS concentration of 218.67 mg/l after 18 hours of fermentation in a medium containing skim milk. EPS produced by wild type *L. plantarum* MK 02 is shown in **Figure 1**.



Figure 1. EPS produced by wild type *L. plantarum* MK 02

This study was carried out with the aim of increasing the EPS production capacity of the isolate using mutagenesis and immobilization techniques.

Induction of Mutation in the Parental Strain

The parental wild type strain (MK 02) was exposed to ultraviolet (UV) radiation which is a known physical mutagen. The number of colony forming units (CFU) observed after UV exposure at various time intervals ranging from 0 to 120 seconds is shown in **Table 1**. Exposure time zero (0) represents the control which was not exposed to UV radiation. The control

produced 2040 CFU at the 10^{-4} dilution, which corresponds to 100% survival. The CFU reduced to 1836, 1836, 408, 40, 16 and 10 after UV exposure for 5s, 10s, 20s, 30s, 60s and 120s respectively at the 10^{-4} dilution. At the 10^{-5} dilution, the total CFU decreased from 1252 in the control to 1189, 62, 6, 25, 37 and 6 CFU after UV irradiation for 5s, 10s, 20s, 30s, 60s and 120s respectively. The 10^{-6} dilution resulted in a count of 968 CFU in the control while the count reduced to 484, 242, 96, 9, 4 and 19 CFU after 5s, 10s, 20s, 30s, 60s and 120s of UV irradiation respectively.

Table 1. Effect of UV radiation on the colony forming unit and survivability of cell

Exposure time (s)	Dilution	CFU	Survival rate (%)	Average survival rate (%)
0	10^{-4}	2040	100	100
	10^{-5}	1252	100	
	10^{-6}	968	100	
5	10^{-4}	1836	90	78
	10^{-5}	1189	95	
	10^{-6}	484	50	
10	10^{-4}	1836	90	40
	10^{-5}	62	5	
	10^{-6}	242	25	
15	10^{-4}	40	20	9
	10^{-5}	62	5	
	10^{-6}	193	2	
20	10^{-4}	408	20	10
	10^{-5}	6	0.5	
	10^{-6}	96	10	
25	10^{-4}	10	0.5	4
	10^{-5}	12	1	
	10^{-6}	96	10	
30	10^{-4}	40	2	2
	10^{-5}	25	2	
	10^{-6}	9	1	
60	10^{-4}	16	0.8	1
	10^{-5}	37	3	
	10^{-6}	4	0.5	
90	10^{-4}	40	2	1
	10^{-5}	12	1	
	10^{-6}	9	1	
120	10^{-4}	10	0.5	1
	10^{-5}	6	0.5	
	10^{-6}	19	2	

The survival rate after UV exposure as seen in **Figure 2** shows that after 5s of UV irradiation, 78% of the cells were found to have survived, 10% survived 20s of UV irradiation while only

1% were able to grow after 120s of radiation. In general, the percentage of organisms which survived the UV irradiation reduced as the exposure time increased.

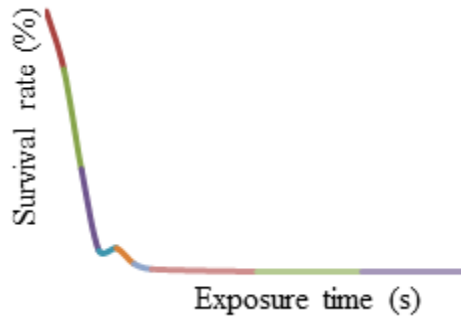


Figure 2. Survival rate of *L. plantarum* cells after exposure to UV radiation

Following the mutagenesis procedure, 12 mutants were selected and further screened for EPS production. The EPS production rate of the mutant strains (Muv 1- Muv 12) as compared to the wild type (MK 02) is shown in **Figure 3**. The wild type *L. plantarum* MK 02 and the selected mutants have the ability to produce EPS. The EPS-production ranged from 98.00 –

240.00mg/l. Four mutants (Muv7, Muv 10, Muv 11 and Muv 12) produced higher EPS than the wild type while the rest 8 mutants produced EPS lower than the wild type *L. plantarum* MK 02. The two highest EPS-producing mutant *L. plantarum* Muv 11 and Muv 12 were selected for further study.

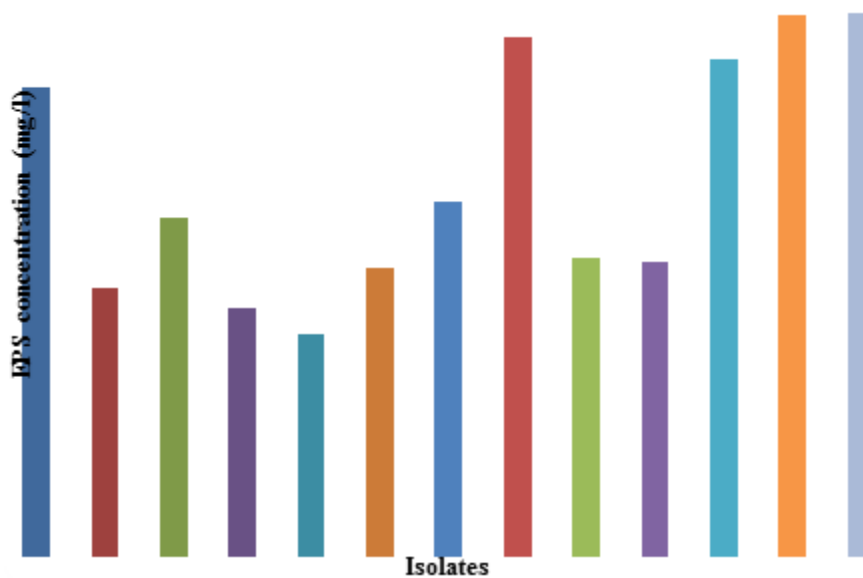


Figure 3. Comparison of EPS production by wild type *L. plantarum* MK 02 and Mutant *L. plantarum* Muv 11 and Muv 12

The results of ultraviolet mutagenesis in the *L. plantarum* strain (MK 02) indicate that the rate of survival of the organism decreased as the time of exposure to UV radiation increased. The number of cells which survived UV irradiation reduced drastically from 2.04×10^3 CFU to about 10 CFU after 120s of UV exposure, thereby achieving a survival rate of 1%

at 120s of UV irradiation. Four, out of the twelve mutants isolated showed more yields of EPS than the wild type strain. This implies that the genetic modifications that occurred in the LAB as a result of random mutagenesis had led to the development of strains with improved characteristics. Increase in EPS production from 218.67 mg/l in the wild type strain MK 02 to 252.0 mg/l and 253.22 mg/l in its mutant strains

Muv 11 and Muv 12 respectively represent about 14% increase in EPS production. Previous studies have shown UV radiation to be a potent mutagenic agent and it has been used as a strain improvement technique for the development of microbial strains capable of producing higher yields of desired metabolites. Increased lactic acid production was observed by Sobrun *et al.* (30) in a mutant strain of LAB which produced a maximum concentration of 29.0 g/l, compared to the wild type strain which produced about 19.0 g/l of lactic acid. Pasha (43) also reported higher yields of glutamic acid from UV-induced mutants of *Corynebacterium glutamicum*.

Immobilization of wild type *L. plantarum* MK O2 and the mutant *L. plantarum* Muv 11 and Muv 12 on agar matrix and sodium alginate is shown in **Figure 4**. The EPS production by the wild type *L. plantarum* MK O2 and the Mutant *L. plantarum* Muv 11 and Muv 12 when immobilized on agar matrix ranged from 209.89 – 268.19mg/l in which the highest value was produced by Mutant *L. plantarum* Muv 12 and the least was produced by wild type *L. plantarum* MK O2.

The EPS – production by wild type *L. plantarum* MK O2 and the Mutant *L. plantarum* Muv 11 and Muv 12 when immobilized on sodium alginate ranged from 225.00 – 263.44mg/l.

Cell Immobilization

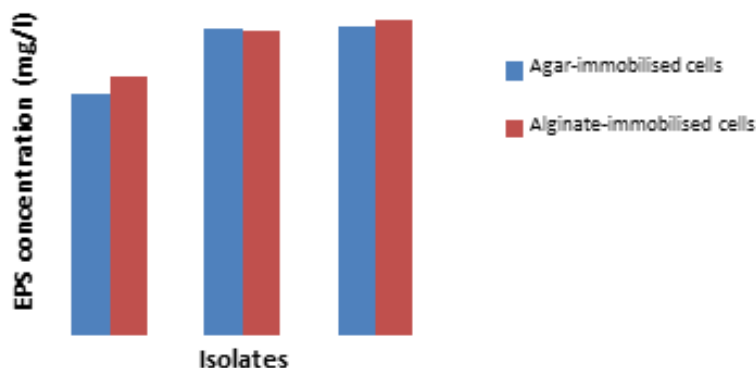


Figure 4. EPS production by wild type *L. plantarum* MK O2 and Mutant *L. plantarum* Muv 11 and Muv 12 immobilized in agar and sodium alginate.

Mutant *L. plantarum* Muv 12 had the highest value while wild type *L. plantarum* MK O2 had the least.

Immobilization in agar matrix favoured EPS – production by Mutant *L. plantarum* Muv 11 (265.67mg/l).

Increased EPS production resulted from immobilization of both wild type and mutant strains. Immobilization in sodium alginate favoured EPS-production by wild type *L. plantarum* MK O2 (225.00mg/l) and Mutant *L. plantarum* Muv 12 (272.00mg/l).

Figure 5 shows EPS production by the un-immobilized cells. EPS – production by un – immobilized wild type *L. plantarum* MK O2 and mutant *L. plantarum* Muv 11 and Muv 12 ranged from 215.00 – 255.00 mg/l. Mutant *L. plantarum* Muv 12 had the least production.

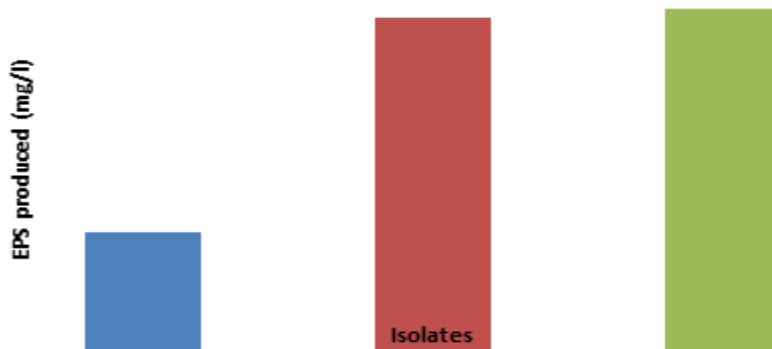


Figure 5. EPS production by un-immobilized wild type *L. plantarum* MK O2 and Mutant *L. plantarum* Muv 11 and Muv 12

Immobilization of wild type and mutant strains of LAB was carried out to improve the EPS production capacity of the strains. Immobilized cell technology has been applied in a wide variety of research applications. For instance, the high potential of *Lactobacillus rhamnosus* RW-9595M for EPS production and the importance of immobilized cell technology were emphasized in the studies of Bergmaier et al. (44) and Bergmaier et al. (45).

From this study, higher EPS concentrations were obtained from immobilized cells as compared to un-immobilized cells, while sodium alginate was a more favourable matrix for immobilizing cells for enhanced EPS production. From these investigations, immobilization in sodium alginate produced up to 7% increase in EPS production in the mutant Muv 12 while agar immobilization produced about 5% increase in EPS yield as observed in Muv 11. The increase in EPS production by immobilized cells could be attributed to the fact that immobilization enables the concentration of high density of cells in the immobilization matrix, thus making more cells available for EPS production. Competition between cells may be reduced when immobilized than when they are free in the medium as fewer cells are encapsulated within each unit of the immobilization matrix. Also, prevention of feedback inhibition could account for higher yields of EPS by immobilized cells because as the EPS is being produced, the immobilization matrix separates the cells from the products formed. Thus, inhibition of further EPS synthesis is prevented. These observations are in agreement with the work of El-Gizawy et al. (46) where the production of EPS was found to have been enhanced by microencapsulation due to an increase in the viable count of the used strain of *Lactobacillus bulgaricus*. Ismail and

Nampothiri (47) also studied EPS production by encapsulated *L. bulgaricus* and found that encapsulated cells gave higher EPS production than free cells.

Characterization of the EPS produced by the strains using Fourier Transform Infrared (FT-IR) Spectroscopy

The EPS produced by the bacterial strains was analysed using FT-IR to determine the functional groups present in them. The spectra produced by the EPS of both wild type (**Figure 6a**) and mutant strains (**Figure 6b**, **Figure 6c**) showed similarities in having absorption peaks around 3000cm^{-1} (characteristic of carbon- and hydrogen-containing species), 2928 cm^{-1} (corresponding to methyne C-H stretch), 2070cm^{-1} (CO group), 1654 cm^{-1} (indicating the presence of olefinic un-saturation C=C), $1456\text{-}1236\text{cm}^{-1}$ (representing the phenol or tertiary alcohol bend), $1153\text{-}1101\text{ cm}^{-1}$ (tertiary alcohol C-O stretch), 1051cm^{-1} (C-H out-of-plane bending) and $908\text{-}455\text{ cm}^{-1}$ (alcohol OH out-of-plane bend). Absorptions in the region of 3300cm^{-1} indicate that the compound is likely to be unsaturated or aromatic while peaks at 1654cm^{-1} further highlight the presence of un-saturation in the EPS. The absorption peak at 2063.90cm^{-1} shows the presence of nitrogen compounds having multiple bonds such as cyanides (nitriles), cyanates, isocyanates, thiocyanates and diazo compounds (48). Also, the presence of multiply-bonded CO group is indicated by absorption bands around 2000cm^{-1} . However, the EPS produced by both mutants differ from that of the wild type strain in having one peak less in the regions $1456.30\text{-}1236.41\text{cm}^{-1}$ and $908.50\text{-}455.22\text{ cm}^{-1}$. This implies lesser OH bonding in the mutants' EPS as these two regions are characteristic of alcohol bends.

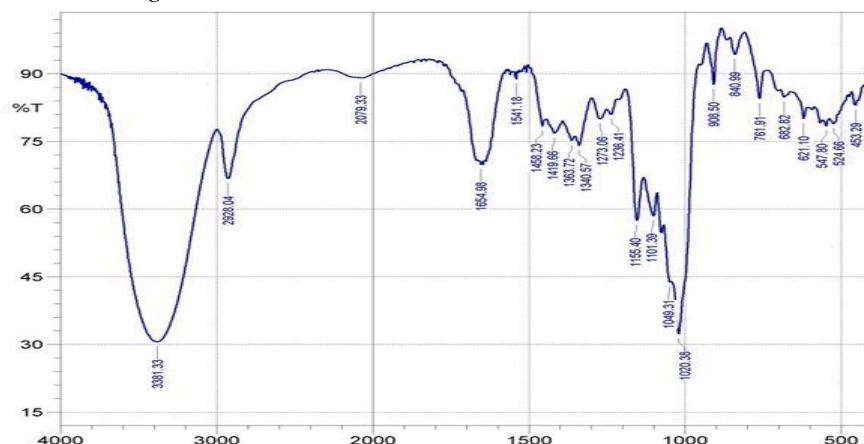


Figure 6a. FTIR Spectrum of EPS from wild type *L. plantarum* MK O2

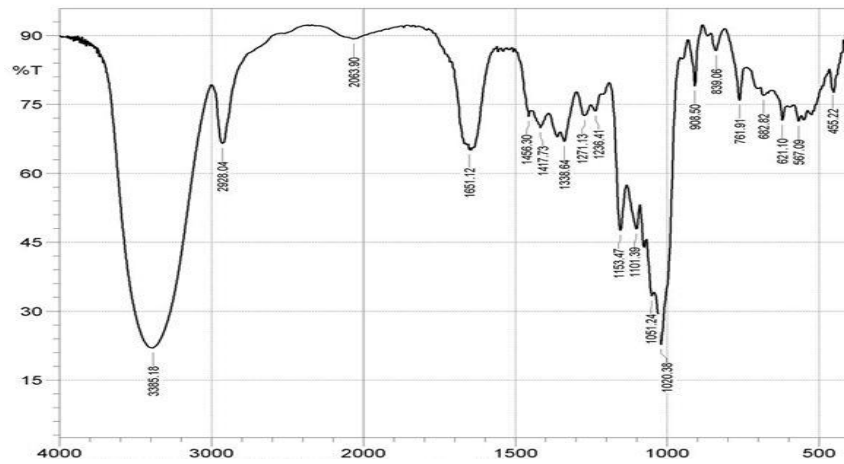


Figure 6b. FTIR Spectrum of EPS from Mutant *L. plantarum* Muv 11

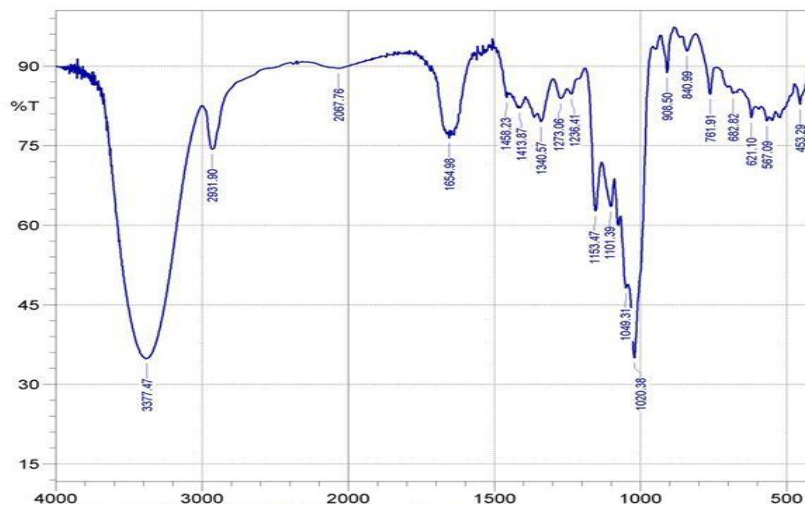


Figure 6c. FTIR Spectrum of EPS from Mutant *L. plantarum* Muv 12

Characterization of the EPS produced by the isolates using Fourier Transform Infrared Spectroscopy (FT-IR) revealed the presence of functional groups such as the hydroxyl, methyl and methylene groups as well as stretches of C-C, C-O-C and C-O of alcohol groups in carbohydrates in all of the EPS. The presence of these bands in a substance indicates that the substance is a polysaccharide (49 – 50).

In conclusion, UV radiation induces mutations in LAB which enables them to produce higher amounts of EPS. Immobilization studies also revealed that cell immobilization is a useful technology for the production of increased yields of EPS. Alginate-immobilized cells produced more yields of EPS than agar-immobilized cells. As the structure of EPS is related to their function, FT-IR analysis showed the presence of functional groups which

confirmed the EPS as polysaccharides, thus their functions can be elucidated from the functional groups present.

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