

PRODUCTION, CHARACTERIZATION, AND APPLICATION OF BIOSURFACTANT FROM *LACTOBACILLUS PLANTARUM* OG8 ISOLATED FROM FERMENTING MAIZE (*ZEA MAYS*) SLURRY

– Research paper –

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Abstract: Biosurfactants have wide applications in several industries. However, high production costs and safety concerns have limited their comprehensive use. Twenty-five strains of lactic acid bacteria, isolated from fermenting maize slurry, were screened for biosurfactant production using the emulsification activity (E_{24}) assay. The selected bacterium was identified molecularly using the 16S rRNA gene sequencing as *Lactobacillus plantarum* OG8. The effect of some cultural factors on biosurfactant production from the bacterium, using pineapple peel as a low-cost substrate, was investigated. The optimum yield of biosurfactant occurred at a 48 h incubation period, using glucose and peptone as carbon and nitrogen sources, respectively. The biosurfactant was characterized to possess mostly carbohydrates, followed by protein and lipid contents. Optima pH 10.0 and temperature 60 °C were the best for the biosurfactant activity. The biosurfactant exhibited antimicrobial activity against bacterial pathogens *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, and *Klebsiella pneumoniae*, at a concentration of 5.0 mg/mL. The use of pineapple peel as a low-cost substrate for biosurfactant production from *Lactobacillus plantarum* OG8 will serve for cost-effective production. The biosurfactant produced exhibited promising properties such as thermostability and antimicrobial activity against food spoilage and pathogens that could make it suitable for food processing and preservation.

Keywords: Biosurfactant, *Lactobacillus plantarum*, Emulsifying activity, Fermented food, Optimization, Pineapple peel

INTRODUCTION

Surfactants are amphiphilic molecules with hydrophobic and hydrophilic moieties which distribute themselves between two interfaces. They reduce surface and interfacial tensions thereby causing non polar compounds to be stable in polar solvents (Udoh and Vinograd, 2019). Emulsifying, detergency, lubrication, solubilization, stabilizing, and foaming capacities are exhibited by surfactants (Sobrinho et al., 2013; Pradhan & Bhattacharyya, 2018). Surfactants could be produced chemically or biologically with the biologically-derived ones being referred to as biosurfactants (Fenibo et al., 2019).

Biosurfactants are surface-active biomolecules produced by different groups of microorganisms such as bacteria, yeasts, and filamentous fungi (Grusha et al., 2014). Thus, they are a structurally diverse and heterogeneous groups of metabolites

(Banat et al., 2010). The hydrophilic moiety of each biosurfactant may be an acid, peptide, cation, anion, mono-, di- or polysaccharide; while the hydrophobic moiety may be saturated or unsaturated hydrocarbon chains, or fatty acids. These compounds are classified mainly on the basis of their chemical nature, molecular weight, or microbial origin. They can be broadly divided into low molecular weight molecules such as glycolipids, lipopeptides, and flavolipids and, high molecular weight molecules such as polysaccharides, proteins, lipopolysaccharides, and lipoproteins (Fracchia et al., 2010; Mnif and Ghribi, 2016; Sharma and Saharan, 2016).

The amphiphilic nature of biosurfactants enables them to accumulate at interfaces such as oil-water and air-water (Gudina et al., 2013). The surface and interfacial tensions between these surfaces are thereby reduced. These properties play important roles and enable them to find applications in several industrial fields such as bioremediation, medical, food processing, pharmaceutical, cosmetic, and detergent (Rodrigues et al., 2006; Fracchia et al., 2014; Franzetti et al., 2014). Biosurfactants are molecules of increasing interest because of their diversity, environmental-

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friendly nature, biological safety, the possibility of their production through fermentation, and their application in various industrial fields (Franzetti et al., 2014). The use of biosurfactants has several advantages over chemical surfactants such as better environmental compatibility, higher biodegradability, lower toxicity, higher selectivity and effectiveness at high temperatures, different salinity levels, and low pH (Kaur et al., 2015; Souza et al., 2017; Dong et al. 2020).

Despite these obvious advantages, the problem of low quality and yield limits their industrial applications (Dong et al., 2020). Several approaches have been proposed for economical biosurfactant production including improvement in fermentation technology, microbial strain selection, and the use of low-cost renewable substrates (Helmy et al., 2011; Marchant and Banat, 2012). Biosurfactants must be produced at the optimum capacity of the microorganism. The production of biosurfactants by microorganisms can be affected by varieties of factors such as culture media, inoculum size, pH, temperature, carbon sources, nitrogen sources, metallic ions, and agitation speed (Satpute et al., 2010).

The use of low-cost substrates in the production medium contributes significantly to the overall economics of the biosurfactant production process as they account for about 50% of the final production cost (Kamalijeet and Sokhon, 2014). Therefore, to reduce costs, it is desirable to use low-cost raw materials such as agro-industrial wastes (Satpute et al., 2017). Agro-industrial wastes are sustainable sources of organic bio-materials because of their ready availability. They include cassava wastewater, palm oil effluent, groundnut waste, soybean waste, fruit peel waste, potato peel, corn oil, molasses, whey, and sugarcane bagasse (Santos et al., 2013; Banat et al., 2014). The use of such waste materials does not only produce biosurfactants but also reduces waste disposal problems (Makkar et al., 2011). Pineapple (*Ananas*

comosus) peel is produced in large quantities as a waste product of the pineapple juice processing industry. Due to disposal problems, it is usually discarded into the environment where it constitutes pollution hazards. Utilization of this abundant waste material, on a large scale, would be profitable from both economic and environmental points of view.

A diversity of biosurfactant compounds with distinct structural characteristics and physical properties could be obtained with the use of various renewable substrates, different microbial species, and modification of cultural parameters (Gudina et al., 2015). Therefore, in terms of efficiency, biosurfactants are better than synthetic surfactants. *Lactobacillus* species is one of the most important genera of lactic acid bacteria (LAB) (Vernoux et al., 2003). They are widely implicated in fermented food production such as in the traditional fermentation of maize (*Zea mays*) grains and cassava (*Lycopersicum esculenta* L.) root tubers for the production of *ogi* and *fufu*, respectively, which are two Nigerian staple fermented food products (Oyedemi et al., 2013). Also, they are considered 'generally regarded as safe (GRAS)' organisms and can be safely used in various applications such as food processing and health care (Hoque et al., 2010; Masci, 2013). Recently, attention has been directed towards biosurfactant production using various probiotic bacteria including LAB, due to their non-pathogenicity (Sharma et al., 2015; Dong et al., 2019). The biosurfactants produced by LAB exhibit antimicrobial as well as anti-adhesive activity to inhibit some pathogenic microorganisms (Gudina et al., 2010; Sambanthamoorthy et al., 2014).

This study was designed to optimize biosurfactant production from lactic acid bacteria isolates from fermenting maize slurry, using pineapple peel as a cheap substrate. The biochemical characteristics of the produced biosurfactant as well as its antimicrobial potential were then determined.

MATERIALS AND METHODS

Collection and fermentation of maize grains

Maize (*Zea mays*) grains were procured from the Central Market, Ile-Ife, Nigeria (7° 31' 14.76" North, 4° 31' 49.13" East) and brought to the laboratory where they were thoroughly washed. The maize grains were soaked in water for 48 h during which time they softened and undergo fermentation. The grains were then wet-milled and sieved and the filtrate was allowed to undergo further fermentation for 48 h (Oyedemi et al., 2013).

Isolation of lactic acid bacteria and culture maintenance

Samples collected during the second fermentation of the filtrate were serially diluted and appropriate dilutions inoculated onto sterile De Man, Rogosa and Sharpe (MRS) agar plates which were then incubated anaerobically for 48 h at 37 °C. Distinct colonies were randomly picked and sub-cultured on fresh MRS agar plates until pure cultures were

obtained. Thereafter, they were maintained on MRS agar slants at 4 °C.

Screening of lactic acid bacteria for biosurfactant production

The emulsification index and oil displacement assays were used to screen the lactic acid bacteria isolates for their relative ability for biosurfactant production.

Emulsification index test

Eighteen hour-old colonies of the bacterial isolates were grown in MRS broth at 37 °C for 24 h. The broth culture was centrifuged at 3,000 rpm for 15 min and the supernatant was decanted. Equal volumes (2 mL) of cell-free supernatant and motor engine oil (5W-30 Sea Gold Super D) were vigorously mixed using a vortex mixer in a glass test tube for 2 min. It was then left to stand, undisturbed, for 24 h. The height of the emulsion layer that formed after 24 h of standing was measured using a metre rule and the emulsification index (E_{24}) was calculated as the height of the layer, divided by the total height, multiplied by 100 (Cooper and Goldenberg, 1987).

Oil displacement assay

Eighteen hour-old colonies of the isolates were grown in MRS broth at 37 °C for 24 h. The broth culture was centrifuged at 3,000 rpm for 15 min and the supernatant was collected. One millimeter of motor oil was added to the surface of 20 mL distilled water in a Petri dish to form a thin oil layer. Twenty microlitres of culture supernatant was gently dropped at the centre of the oil layer for 1 min. The diameters of cleared oil displacement zones were measured (Chandran and Das, 2010).

Characterization and identification of selected lactic acid bacterium

The selected LAB was characterized and tentatively identified by using phenotypic methods (morphological, biochemical, and physiological characterizations) (Harrigan and McCance, 1976). Thereafter, the identity of the bacterium was confirmed by molecular characterization. Deoxyribonucleic acid (DNA) extraction was by the modified method of Trinidade et al. (2007). The identification of the selected study isolate was confirmed by polymerase chain reaction (PCR) and 16S rDNA sequence analysis method. Forward primer sequence (CCAGCAGCCGCGGTAATACG) and reverse primer sequence (ATCGGCTACCTTGTTACGACTTC) were used and the amplification conditions were those recommended by Barghouthi (2011). The amplified

PCR fragment was sequenced and aligned with the most similar bacterial species found in the GenBank (<https://www.ncbi.nlm.nih.gov/Blast.cgi>).

Collection and preparation of agro-wastes

The agro-wastes (pineapple and plantain peels) were collected from the fruit stall, rinsed with distilled water, and then oven-dried at a temperature of 60 °C for 2 weeks. The peels were then milled into 0.5 mm particle sizes using a milling machine (Rocklabs RM1000 Batch Pulverizer). The powdered peels were stored in airtight containers.

Screening of agro-wastes as substrates for biosurfactant production

Two millimeters of the inoculum from 24 h-old MRS broth culture was introduced into 50 mL of composed MRS broth with glucose being replaced with each of 4.5 g/L of agro-waste (Peptone 10.0 g/L; meat extract 8.0 g/L, Yeast extract 4.0 g/L; Pineapple peel 4.5 g/L or Plantain peel 4.5 g/L; $K_2HPO_4 \cdot 3H_2O$ 2.0 g/L; Sodium acetate trihydrate 5.0 g/L; Triammonium citrate 2.0 g/L; $MgSO_4 \cdot 7H_2O$, $MnSO_4 \cdot 4H_2O$). Incubation was carried out at 37 °C for 48 h. The turbidity was determined using UV spectrophotometer (Single cell holder Jenway model). The broth culture was centrifuged at 3,000 rpm for 20 min. The supernatant from each tube was decanted into a clean test tube. Emulsification index (E_{24}) activity was determined using the standard method. The peel which produced higher emulsification index activity of the study isolate was selected as the better substrate for biosurfactant production.

Proximate analysis of pineapple peel

Pineapple peel was selected as the better substrate for biosurfactant production. The pineapple was chemically and nutritionally analyzed. The total carbohydrate, crude protein, crude fibre, moisture and ash contents were determined using the method described by AOAC (2005).

Biosurfactant production

The production of biosurfactant from the selected bacterium was carried out in Erlenmeyer flasks containing composed mineral salt medium (K_2HPO_4 1.6 g/L; KH_2PO_4 0.4 g/L; NaCl 0.1 g/L; $MgSO_4$ 0.1 g/L; $CaCl_2$ 0.02 g/L, Peptone 8.0 g/L Yeast extract 5.0 g/L, Pineapple peel 10.0 g/L and trace elements ($CuSO_4 \cdot 5H_2O$ 0.5 mg/mL, $MnSO_4 \cdot 5H_2O$ 0.5 mg/mL, $ZnSO_4 \cdot 7H_2O$ 0.7 mg/mL, Boric acid 1.0 mg/mL and Molybdate 1.0 mg/mL). The medium was inoculated with 1.0 mL of the 18-24 h old bacterial inoculum ($OD_{600nm} = 1$) and incubated at 37 °C on a closed rotary shaker at 150

rpm for 48 h (Rodrigues et al., 2006). The medium was centrifuged (Scilogex MicroCentrifuge D3024) at 7,000 rpm for 20 min and the supernatant was collected and assayed for biosurfactant production using the emulsification index (E_{24}) assay, as previously described.

Extraction of biosurfactant

Biosurfactant was extracted using the method of Saravanan and Ijayakumar (2012). The culture supernatant obtained after centrifugation was subjected to acidification to pH 2.0 using 6 N HCl and left overnight at 4 °C. The precipitated biosurfactant was collected by centrifugation (10,000 rpm for 5 min at 4 °C) and the pH adjusted to 7.0 in phosphate buffered saline.

Effect of cultural parameters on biosurfactant production

The effect of different nutritional and physical parameters on biosurfactant production from the lactic acid bacterium was determined.

Effect of incubation period on biosurfactant production

The bacterial isolate was inoculated into 50 mL of the production medium and incubation was carried out at 37 °C for different periods 24 h, 48 h, 72 h and 96 h in a rotary shaker at 150 rpm. Samples were collected at 24 h intervals and observed for biosurfactant production using the emulsification index (E_{24}) assay.

Effect of pH on biosurfactant production

Fifty milliliters of the production medium in different Erlenmeyer flasks were adjusted to different pH 4.0, 4.5, 5.0, 5.5, 6.0, 6.5 and 7.0. They were inoculated with the bacterial isolate and incubated at 37 °C on a rotary shaker at 150 rpm. Emulsification index (E_{24}) assay was carried out to evaluate for biosurfactant production.

Effect of temperature on biosurfactant production

The production medium inoculated with bacterium was incubated at different temperature conditions 25 °C, 30 °C, 35 °C, 40 °C, 45 °C and 50 °C on a rotary shaker, at 150 rpm for 48 h. The cultures were analyzed for biosurfactant production using the emulsification index (E_{24}) activity assay.

Effect of carbon sources on biosurfactant production

The effect of different carbon sources on biosurfactant production was studied by inoculating bacterium into the production medium supplemented with 2% (w/v) of each of the carbon

sources (glucose, glycerol, pineapple peel, plantain peel and starch). Emulsification index (E_{24}) activity assay was carried out to evaluate biosurfactant production.

Effect of nitrogen sources on biosurfactant production

Biosurfactant production medium each supplemented with 0.3% (w/v) of different nitrogen sources peptone, ammonium nitrate, urea and yeast extract were inoculated with bacterium and incubated at 37 °C for 48 h with agitation at 150 rpm. Emulsification index (E_{24}) activity assay was carried out to evaluate biosurfactant production.

Characterization of biosurfactant

The optimized parameters were used for the bulk production of biosurfactant in the production medium with pineapple peel as the sole source of carbon. Incubation was carried out at 37 °C on a rotary shaker at 150 rpm for 48 h. Afterwards, the cell-free supernatant was collected and biosurfactant extraction was carried out as described previously.

Characterization of the biosurfactant was carried out by determining its chemical composition and the effect of physicochemical factors on its activity.

The chemical composition of biosurfactant was determined by analyzing for the carbohydrate, protein and lipid content. The carbohydrate content was determined by using the phenol-mercuric method of Gerchakov and Hatcher (1972) with D-glucose as standard. The protein content was determined by using the method of Bradford (1976) with bovine serum albumin being used as a standard while the method of Folch et al. (1957) was used to determine the lipid content of the biosurfactant.

To study the effect of temperature on biosurfactant activity, the biosurfactant solution (1.0% w/v) was prepared in 0.1 M phosphate buffer solution (pH 7.0). Test tubes containing biosurfactant solutions were incubated at different temperatures (20, 40, 60, 80, 100, and 121 °C) for 1 h. The emulsification index (E_{24}) activity was evaluated as described previously.

The effect of pH on biosurfactant activity was evaluated by adjusting the pH of the 1% biosurfactant solution to pH 2.0, 4.0, 6.0, 8.0, 10.0, and 12.0, with HCl or NaOH solutions. Test tubes containing biosurfactant solution were incubated for 1 h. Thereafter, the emulsification index (E_{24}) was determined.

The effect of salt concentrations on biosurfactant activity was evaluated by adding different salt concentrations (0, 2.0, 4.0, 6.0, 8.0 and 10.0% w/v) to the 1% biosurfactant solution. Incubation was

carried out for 1 h after which the emulsification index (E_{24}) activity was evaluated.

Application of biosurfactant to inhibition of selected bacterial pathogens

The extracted biosurfactant was used as an antimicrobial agent against some selected bacterial pathogens (*Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli*, *Klebsiella pneumoniae*), obtained from the Culture Collection of the Microbiology laboratory, Obafemi Awolowo University, Ile-Ife, Osun State, Nigeria.

To assess the antimicrobial activity of the biosurfactant against several bacterial strains, the agar well diffusion assay as described by Ennahar et al. (1999) was used. The selected pathogenic strains were sub-cultured on trypticase soy agar and incubated at 37 °C for 24 h. Then, the different isolates were suspended in normal saline and

standardized to 0.1 McFarland Turbidity Standards. Sterile Mueller Hinton agar was poured into Petri dishes and allowed to solidify. Then, one hundred microliters of each bacterial suspension were spread-plated on the surface of the medium and left to stand for one hour during which it was absorbed into the medium. A well of 6 mm diameter was made on each of the set plates and thirty microlitres of the biosurfactant were pipetted into it. The plates were stored at 4 °C for 4 h before being incubated at 37 °C for 24 h. After incubation, the diameter of the inhibition zone was measured.

Statistical Analysis

Data were obtained by carrying out triplicate experimental determinations and these were expressed as means $\pm$ standard deviation. Graphical representation of results of the study was by the use of the software Microsoft excel.

RESULTS AND DISCUSSION

Isolation and screening of lactic acid bacteria for biosurfactant production

A total of 25 morphologically distinct lactic acid bacteria were isolated from fermented maize slurry. The isolated strains of lactic acid bacteria were screened for their relative biosurfactant-producing abilities by using methods of emulsification index ($E_{24}\%$) and oil displacement tests. All the LAB isolates exhibited emulsification activity and were positive to the oil displacement test. However, *Lactobacillus plantarum* (OG8), gave the highest emulsification index activity and was therefore selected as the study isolate (Table 1). Lactic acid bacteria and most especially the genus *Lactobacillus* species are reported to be the dominant organisms in a wide variety of traditional food and beverage fermentations (Oyedemi et al. 2013; Ng et al., 2015; Adesulu-Dahunsi et al., 2018). Several methods are available for the screening of microorganisms for biosurfactant production including haemolytic activity, oil displacement and emulsification index assays (Garcia-Reyes and Yanez-Ocampo, 2016). Satpute et al. (2010) recommended a combination of methods since a single method may not be adequate. However, the emulsification index ($E_{24}\%$) assay has been reported to be a more reliable method for the detection and quantification of the soluble biosurfactant in a liquid medium (Abdalsadiq Nagea and Hassan, 2018). Several species of the genus *Lactobacillus* isolated from different fermented food products have been reported to exhibit appreciable potential for biosurfactant

production (Kaur et al., 2015; Cornea et al., 2016; Moukala et al. 2019). In this study, *Lactobacillus plantarum* OG8 isolated from fermenting maize slurry can be regarded as a good biosurfactant-producing lactic acid bacterium.

Characterization and identification of the biosurfactant-producing lactic acid bacterium

The phenotypic methods of morphological, biochemical, and physiological characterization were used for the identification of the biosurfactant-producing lactic acid bacterium (Table 2). The cells were Gram-positive long rods and non-spore-forming. It did not produce catalase, endospore, and oxidase nor did it utilize citrate and indole. It grew at 15 °C and 45 °C and, at pH 3.6 and 9.6. It was Methyl red positive but Voges Proskauer negative. It was able to utilize carbohydrates, including glucose, sucrose, sorbitol, maltose, lactose, fructose, galactose, mannitol, and mannose but not, raffinose, as the sole source of carbon. From these various tests, the isolate was tentatively identified as *Lactobacillus* sp. Thereafter, the identity of the bacterium was confirmed by molecular characterization, based on 16S rRNA nucleotide sequencing and comparison to the sequences in the NCBI database. The strain was labeled as *Lactobacillus plantarum* OG8 with a similarity identity of 95.95% with *Lactobacillus plantarum* (Accession No. MH899312.1). The relationship of this bacterial strain to other strains of *Lactobacillus plantarum* in the Genbank is presented in the established phylogenetic tree (Figure 1).

Table 1. Screening of lactic acid bacteria isolates for biosurfactant production.

Isolate code	Emulsification index ($E_{24}\%$)	Oil displacement (mm)
OG1	36.91 $\pm$ 0.39	31.31 $\pm$ 0.31
OG2	34.20 $\pm$ 0.38	42.86 $\pm$ 0.40
OG3	36.37 $\pm$ 0.26	37.66 $\pm$ 0.26
OG4	36.56 $\pm$ 0.37	40.98 $\pm$ 0.29
OG5	40.15 $\pm$ 0.81	37.51 $\pm$ 0.46
OG6	33.37 $\pm$ 0.36	32.32 $\pm$ 0.60
OG7	36.48 $\pm$ 0.42	48.40 $\pm$ 0.31
OG8	50.61 $\pm$ 0.24	50.13 $\pm$ 0.59
OG9	47.55 $\pm$ 0.28	39.08 $\pm$ 0.29
IG1	42.16 $\pm$ 0.42	38.47 $\pm$ 0.26
IG2	41.37 $\pm$ 0.28	42.83 $\pm$ 0.22
IG3	41.40 $\pm$ 0.33	34.20 $\pm$ 0.39
IG4	41.76 $\pm$ 0.35	46.82 $\pm$ 0.25
IG5	33.91 $\pm$ 0.31	35.22 $\pm$ 0.37
IG6	44.76 $\pm$ 0.30	36.02 $\pm$ 0.22
IG7	43.18 $\pm$ 0.25	26.40 $\pm$ 0.35
IG8	42.84 $\pm$ 0.54	44.46 $\pm$ 0.27
IG9	33.16 $\pm$ 0.33	34.31 $\pm$ 0.23
GA1	37.33 $\pm$ 0.42	37.46 $\pm$ 0.17
GA2	37.08 $\pm$ 0.30	36.00 $\pm$ 0.22
GA3	38.49 $\pm$ 0.31	47.81 $\pm$ 0.23
GA4	47.58 $\pm$ 0.24	48.57 $\pm$ 0.24
GA6	31.27 $\pm$ 0.52	33.46 $\pm$ 0.33
GA7	31.30 $\pm$ 0.32	48.94 $\pm$ 0.33
GA8	34.96 $\pm$ 0.45	33.44 $\pm$ 0.25

Values are mean of three replicates $\pm$ standard deviation

Table 2: Morphological, biochemical and physiological characteristics of *Lactobacillus plantarum* OG8

Characteristics	Lactic acid bacterium
Gram's reaction	+
Morphology	Long rods
Catalase test	-
Spore formation	-
Starch hydrolysis	+
Citrate utilization	-
Indole utilization	-
Methyl red	+
Voges Proskauer	-
Nitrate reduction	+
H ₂ S production	-
Growth at 15 °C	+
Growth at 45 °C	+
4% NaCl	+
Growth at pH 3.6	+
Growth at pH 9.6	+
Glucose	+
Sorbitol	+
Sucrose	+
Raffinose	-
Maltose	+
Mannitol	+
Xylose	+
Lactose	+
Mannose	+
Trehalose	+
Fructose	+
Galactose	+
Presumptive identity	<i>Lactobacillus</i> sp.

Keys: + = Positive, - = Negative

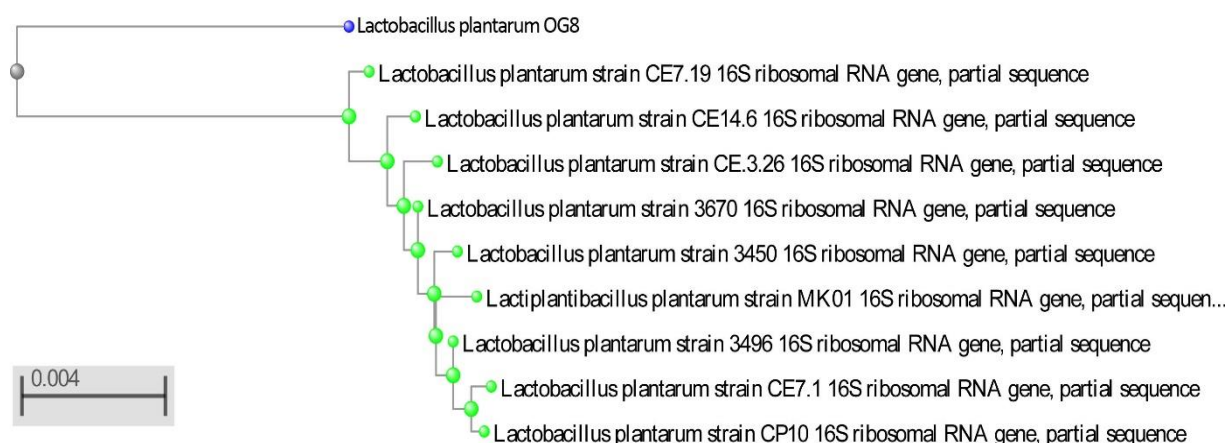


Figure 1: Phylogenetic tree constructed from 16S rRNA gene sequences of *Lactobacillus plantarum* OG8 elucidating its closeness to other *Lactobacillus plantarum* strains.

Screening of agro-wastes as substrates for biosurfactant production from *Lactobacillus plantarum* OG8

Screening of the agro-wastes pineapple and plantain peels, as substrates, for biosurfactant production from *L. plantarum* OG8 was carried out. Pineapple

peel acted as the better substrate for biosurfactant production with an emulsification index (E_{24}) of $49.0 \pm 1.0\%$, while the use of plantain peel produced lesser biosurfactant with an emulsification index (E_{24}) $35.4 \pm 1.04\%$ (Table 3).

Table 3: Screening of agro-wastes as substrates for biosurfactant production

Agro-wastes	Emulsification index (E ₂₄ %)
Pineapple peel	49.5 ± 0.54
Plantain peel	35.39 ± 0.40

Values are means of three replicates ± standard deviation

Biosurfactants possess numerous advantages over chemical surfactants owing to their lower toxicity, biodegradability, better environmental compatibility and sustainability (Kaur et al. 2015). However, the major challenges limiting the comprehensive application of biosurfactants in industries are the low yield of biosurfactants and the high cost of raw materials or substrates needed to run large-scale fermentations for biosurfactant production. The use of cheaper, renewable substrates has been reported by several researchers for the production of biosurfactants as a possible strategy of improving the economics of production in reducing costs (Makkar et al., 2011; Adekilekun et al., 2019). Agricultural residues or wastes are abundant and renewable substrates that are suitable carbon sources for biosurfactant production. Pineapple peel and plantain peel were screened as substrates for biosurfactant production from *L. plantarum* OG8 in this study and pineapple peel was observed to be the more suitable substrate and hence was selected for the fermentation process in this study. This could be as a result of the fact that the component nutrients in the pineapple peel are in forms that allow easier metabolism by the bacterium for the synthesis of biosurfactants. Several species *Lactobacillus* species have been reported to produce biosurfactants in fermentation processes utilizing various agro-wastes as alternative and cheaper substrates. These include *Lactobacillus delbrueckii* using peanut oil cake (Thavasi et al., 2011), *Lactobacillus paracasei* using vineyard pruning waste (Vecino et al., 2017) and *Lactobacillus rhamnosus* using date syrup (Ghasemi et al., 2018).

Proximate analysis of pineapple peel

The result of the proximate analysis of the agro-waste pineapple peel is presented in Table 4. The pineapple peel contained 11.17 ± 0.35% moisture, 4.98 ± 0.40% ash, 8.68 ± 0.19% crude fibre, 1.85 ± 0.19% ether extract, 6.58 ± 0.20% crude protein,

1.10 ± 0.17% nitrogen and 75.87 ± 0.44% total carbohydrate. Pineapple peels contain abundant carbon, nitrogen, and mineral sources, and are cheap, making them suitable substrates for use in microbial fermentations for the production of biotechnologically-important molecules such as biosurfactants (Selvankumar et al., 2011; Siti Roha et al., 2013). Also, the utilization of such residues helps to mitigate the pollution hazards associated with their accumulation in the environment.

Effect of cultural parameters on biosurfactant production

Biosurfactant production can be affected by various parameters such as the incubation period, temperature, pH, carbon sources, and nitrogen sources (Vandana and Singh, 2018).

Effect of incubation period on biosurfactant production

Biosurfactant production from *L. plantarum* increased with the incubation period until it reached the maximum at 48 h with an emulsification index (E₂₄%) of 58.1 ± 0.42% (Figure 2). Beyond this period, biosurfactant production declined. This report is similar to that of Nwaguma et al. (2016), who reported 48 h as the optimum period for biosurfactant production from a strain of *Klebsiella pneumoniae* with an emulsification index of 20%. The emulsification index at the optimum incubation period observed in this study was higher. Thus, *L. plantarum* OG8 isolated in this study had higher biosurfactant production at the optimum incubation period than that reported by Nwaguma et al. (2016).

Effect of temperature on biosurfactant production

Biosurfactant production by *L. plantarum* OG8 increased until it reached the maximum temperature at 37°C with an emulsification index of 63.35 ± 0.64%. Beyond this temperature, biosurfactant production gradually declined (Figure 3). Temperature is an important factor influencing the culture growth and biosurfactant production. The optimum temperature for biosurfactant production from the bacterium was observed to be 37 °C. This is similar to the report of Morais et al. (2017) who reported the optimum temperature for biosurfactant production from *L. jensenii* P6A and *L. gasserii* P65 to be 37 °C, respectively. However, Nwaguma et al.

Table 4: Proximate analysis of pineapple peel

Moisture (%)	Ash (%)	Crude Fibre (%)	Ether extract (%)	Crude Protein (%)	Nitrogen (%)	Total carbohydrate (%)
11.17 ± 0.35	4.98 ± 0.40	8.68 ± 0.19	1.85 ± 0.19	6.58 ± 0.20	1.10 ± 0.17	75.87 ± 0.44

Values are means of three replicates ± standard deviation

(2016) stated that biosurfactant production was lower at temperatures higher than 30 °C. According to Youssef et al. (2004), temperature changes can cause alterations in the composition of biosurfactants. Chander et al. (2012) reported that different bacterial species produce biosurfactants at different temperatures with most bacterial species

producing the biosurfactant at temperature range 30 °C-37 °C.

Effect of pH on biosurfactant production

Production of biosurfactant from *L. plantarum* increased until it reached its maximum production at pH 7.0 (emulsification index of $71.25 \pm 0.21\%$) (Figure 4).

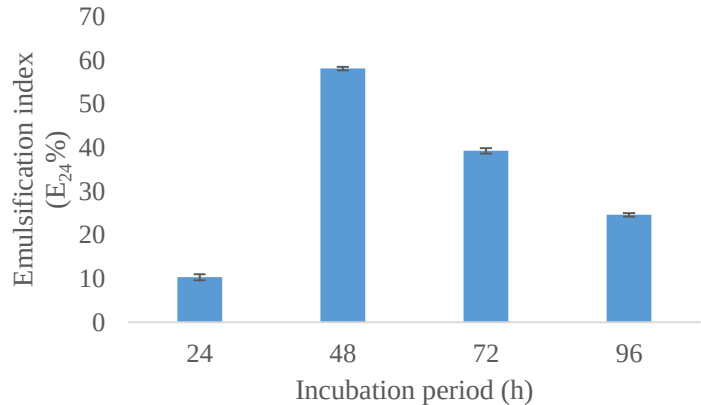


Figure 2: Effect of incubation period on biosurfactant production from *Lactobacillus plantarum* OG8. Values are means of three replicate determinations

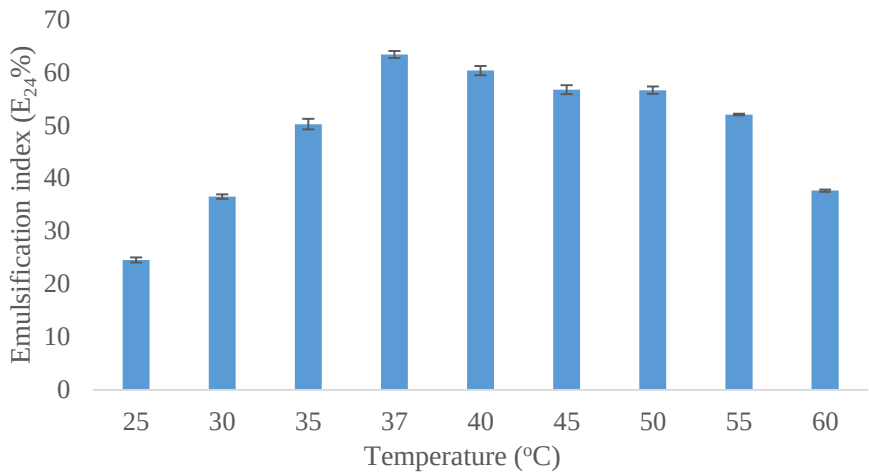


Figure 3: Effect of temperature on biosurfactant production from *Lactobacillus plantarum* OG8. Values are means of three replicate determinations

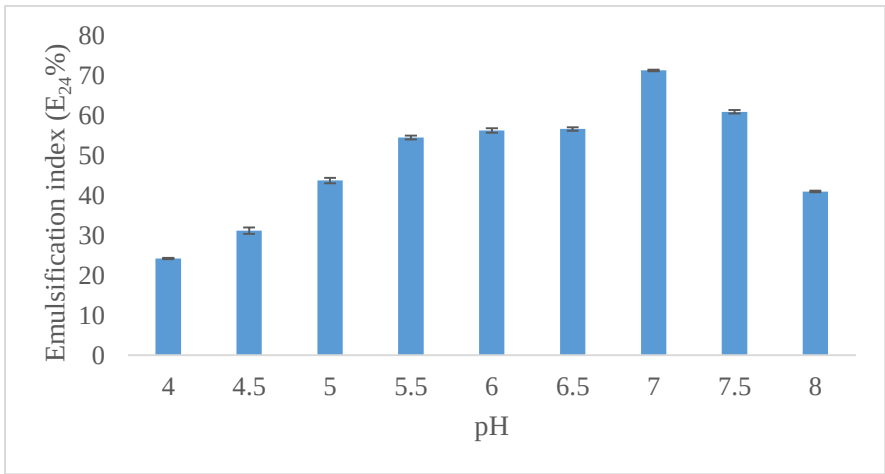


Figure 4: Effect of pH on biosurfactant production from *Lactobacillus plantarum* OG8. Values are means of three replicate determinations

There was a decrease in the production when the pH was increased beyond this pH level. A similar optimum pH of 7.0 was reported for biosurfactant production from *Lactobacillus jensenii* P_{6A} and *Lactobacillus gasserii* (Morais et al., 2017). Hussain and Shawakat (2019) reported the optimum pH for biosurfactant production from *Saccharomyces cerevisiae* to be pH 5.0 while Zinjarde and Pant (2002) reported the optimum pH 8.0 for the production of a biosurfactant from *Yarrowia lipolytica*. These results imply differences in the optima pH values for biosurfactant production from different microbial species.

Effect of carbon sources on biosurfactant production

Biosurfactant production from *Lactobacillus plantarum* OG8 increased when glucose and pineapple peel were used as carbon sources. Emulsification index activity produced by the use of glucose was $52.05 \pm 0.21\%$ while that produced by pineapple peel was $49.0 \pm 0.28\%$. The other carbon

sources used elicited low biosurfactant production from the bacterium (Figure 5). Other substrates such as cheese whey and sugarcane molasses have been used as carbon sources for biosurfactant production by previous researchers (Rodrigues et al. 2006; Gudina et al., 2015). Joshi et al. (2008) reported the use of molasses alongside cheese whey as substrates for biosurfactant production from *Bacillus* sp.

Effect of nitrogen sources on biosurfactant production

Biosurfactant production from *Lactobacillus plantarum* OG8 reached its maximum with the use of peptone as the nitrogen source (emulsification index $56.2 \pm 0.57\%$) followed by the use of yeast extract emulsification index $48.65 \pm 0.78\%$) (Figure 6). The isolate exhibited a relatively low production with other nitrogen sources. Nitrogen is essential for the microbial production of surface-active compounds (Mercade et al., 1996; Nwaguma et al., 2016).

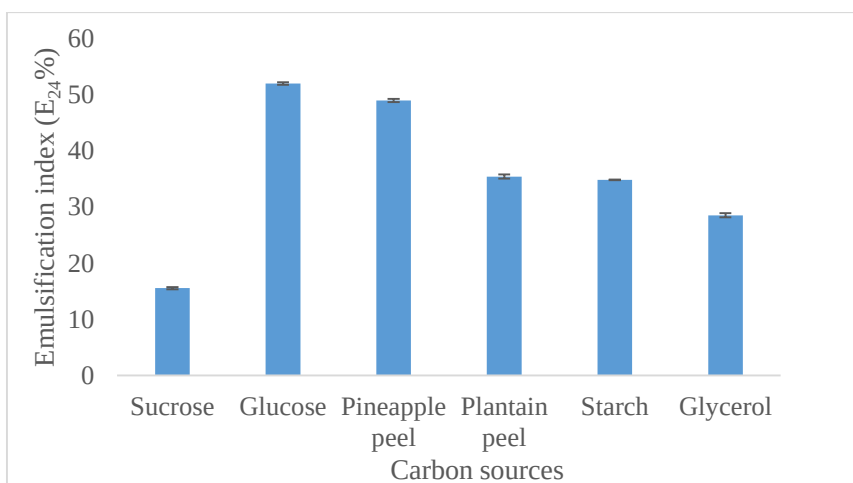


Figure 5: Effect of carbon sources on biosurfactant production from *Lactobacillus plantarum* OG8. Values are means of three replicate determinations

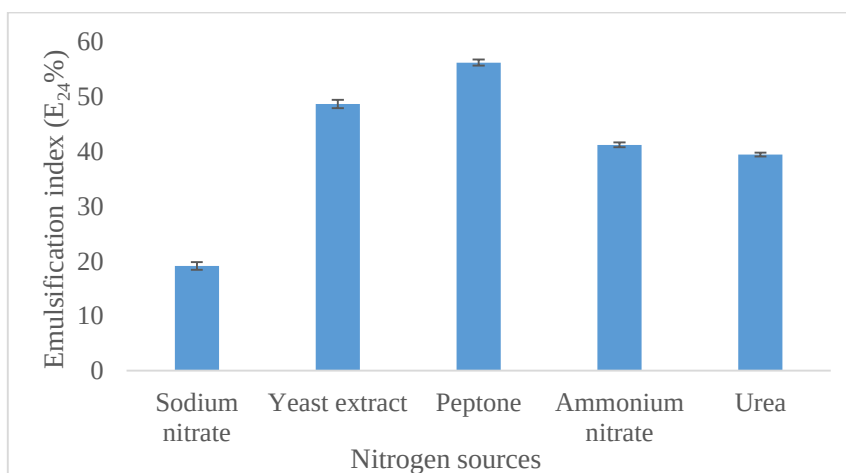


Figure 6: Effect of nitrogen sources on biosurfactant production from *Lactobacillus plantarum* OG8. Values are means of three replicate determinations

The type of nitrogen sources used for biosurfactant production is dependent on the type of organism. Biosurfactant production from *Arthrobacter paraffineus* and *Ps. aeruginosa* was at an optimum level with the use of ammonium salts and nitrates, respectively (Adamczak and Bednarski, 2000). However, Gudina et al. (2015) reported peptone to be crucial for biosurfactant synthesis from *Lactobacillus agilis* CCUG31450.

Characterization of Biosurfactant

Chemical nature of biosurfactant

The carbohydrate content of the biosurfactant was $49.84 \pm 0.11\%$, protein content was $27.75 \pm 0.26\%$, while the lipid content was $22.09 \pm 0.25\%$ (Table 5). These results show that the biosurfactant had a higher carbohydrate content than the protein and the lipid contents. This report is similar to that of Morais et al. (2017) who reported the chemical composition of biosurfactant from *Lactobacillus jensenii* P_{6A} and *Lactobacillus gasseri* P₆₅ as having a high concentration of carbohydrates and lipid concentrations but a lower concentration of protein. Glycoproteins consisting of glucose, rhamnose, fructose, and mannose make up the biosurfactants from *Lactococcus lactis* 53 (Rodrigues et al., 2006). These results indicate variation in the chemical nature of the biosurfactant produced.

Table 5: Chemical nature of biosurfactant from *Lactobacillus plantarum* OG8

Chemical composition	Percentage (%)
Carbohydrate	49.84 ± 0.11
Protein	27.8 ± 0.26
Lipid	22.0 ± 0.25
Others	1.4 ± 0.17

Values are means of three replicate determinations

Effect of temperature on biosurfactant activity

Figure 7 presents the result of the effect of temperature on the activity of the biosurfactant produced from *L. plantarum* OG8. The biosurfactant had good activity between a wide range of temperature (20°C-121°C) with the optimum being observed at 60°C (emulsification index activity $48.9 \pm 0.14\%$). At higher temperatures, the emulsification index reduced drastically. The biosurfactant was very stable at 60 °C. This result indicates the thermostability of the produced biosurfactant and its usefulness in applications requiring heating or high temperature. This report is similar to that of Ndigbe et al. (2018) who reported that the emulsification index ($E_{24}\%$) decreased when temperature increased to 100°C. Biosurfactant produced from *Lactobacillus* strains were stable at 25°C, 37°C and 60°C (Eduardo et al., 2011).

Effect of pH on biosurfactant activity

The optimum biosurfactant activity was at the alkaline condition (pH 10.0) with the emulsification index $51.6 \pm 0.28\%$ while it was $50.6 \pm 0.85\%$ at pH 8.0 (Figure 8). At pH 12.0, the activity decreased to an emulsification index of 43.5%. The activity of the biosurfactant produced from *L. plantarum* OG8 was greatly affected by changes in pH. At extreme pH values like 2.0 and 12.0, the activities were low. At pH 6.0 – pH 10.0, the activity of the biosurfactant was relatively stable. This result is similar to that reported by Mbawala et al. (2013) who stated that biosurfactants produced by strains of *Lactobacillus* sp. were stable at pH values of 6 to 12. The emulsification index increasing at pH 6.0 – pH 10.0 is due to the negative charge of the polar head which lead to an increased solubility for biosurfactant (Abdel-Mawgoud et al., 2009). This result is similar to that of UmmulKhair and Ainon (2016) who reported that the activity of biosurfactant produced from *Bacillus subtilis* was low at extreme pH values of 2.0, 4.0, and 10.0 but had a stable activity at pH 6.0. Also, the biosurfactant produced from *Pediococcus dextrinicus* SHU1593 was more stable at alkaline conditions (Ghasemi et al., 2019). Felix et al. (2019) reported that for pH values lower than 6.0, the samples became turbid which may be a result of the biosurfactant partial precipitation.

Effect of salt concentration on biosurfactant activity

Figure 9 presents the effect of salt concentrations on the biosurfactant activity. The salt concentration was varied from 2.0% to 10.0%. The biosurfactant had good stability at salt concentration of 2.0% to 8.0%, with emulsification index ranging from 42.25 to 44.55%. The maximum activity was observed at the salt concentration 8.0%. Thereafter, the activity of the biosurfactant decreased when the concentration was increased to 10% with emulsification index 36.85%. Mbawala et al. (2013) reported the stability of biosurfactant at salt concentrations of 5-15%. Salt concentrations below 15% tend to give for stable biosurfactant activity and this can be due to the presence of phosphate groups preventing protein denaturation (Rodrigues et al. 2006). Also, Joshi et al. (2008) reported that the biosurfactant activity produced from *Bacillus subtilis* remained stable at salt concentration of 7%. The observed biosurfactant activity at extreme salinity conditions as in this study, indicates the feasibility of their application in food processing and oil-related systems, most of which occur in highly saline conditions (Shavandi et al., 2011).

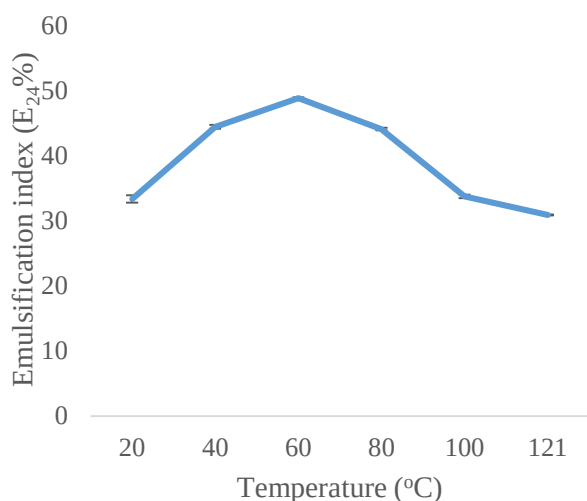


Figure 7: Effect of temperature on biosurfactant activity. Values are means of three replicate determinations.

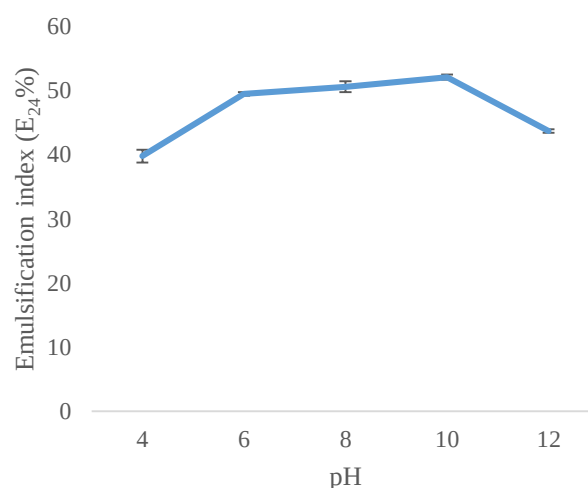


Figure 8: Effect of pH on biosurfactant activity. Values are means of three replicate determinations

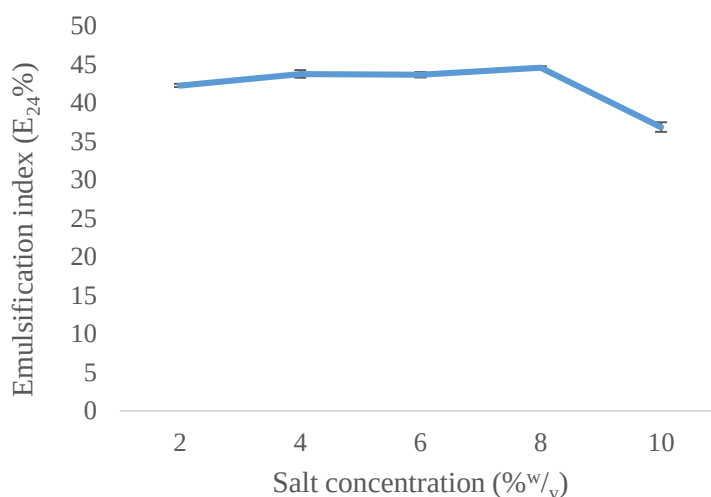


Figure 9: Effect of salt concentration on biosurfactant activity. Values are means of three replicate determinations.

Application of Biosurfactant to Inhibition of Selected Bacterial Pathogens

The antimicrobial effect of the biosurfactant derived from *Lactobacillus plantarum* OG8 was observed against *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Klebsiella pneumoniae*, at a concentration of 5.0 mg/mL. The biosurfactant from the bacterium was inhibitory to all the selected bacterial pathogens. The zone of clearance observed around each of the selected bacteria pathogens were *Staphylococcus aureus* (24.7 ± 0.14 mm), *Bacillus subtilis* (28.55 ± 0.35 mm), *Klebsiella pneumoniae* (13.7 ± 0.14 mm) and *Escherichia coli* (13.95 ± 0.21 mm). The biosurfactant was observed to be more inhibitory against the Gram-positive relative to the Gram-negative organisms (Table 6). Glycolipids are reported to exhibit higher inhibitory activity against Gram positive pathogens such as *B. cereus* and *S. aureus* (deFreitas Ferreira et al., 2019). This result is similar to that of Gudina et al. (2015) who

reported that biosurfactant derived from *Lactobacillus agilis* CCUG31450 exhibited antimicrobial activity against *Staphylococcus aureus*. Also, Sambanthamoorthy et al. (2014) reported that biosurfactants derived from *L. jensenii* and *L. rhamonus* exhibited antimicrobial activity against *Staphylococcus aureus*, *Escherichia coli* and *Acinetobacter baumannii*. Biosurfactant molecules tend to self-associate and create pores inside the microbial cell membrane structure leading to microbial inhibition (Deleu et al., 2008). Due to the antimicrobial property of biosurfactants, they can be utilized as potential therapeutic molecules for numerous microbial infections (Sharma and Saharan, 2016). Therefore, biosurfactants may be appropriate alternatives to conventional antibiotics as a result of their safety and efficiency.

Table 6: Antimicrobial activities of biosurfactant against selected bacterial pathogens

Bacterial pathogens	Zone of inhibition (mm)
<i>Staphylococcus aureus</i>	24.7 ± 0.14
<i>Bacillus subtilis</i>	28.6 ± 0.35
<i>Escherichia coli</i>	14.0 ± 0.21
<i>Klebsiella pneumoniae</i>	13.7 ± 0.14

Values are mean of three replicates ± standard deviation

CONCLUSIONS

It can be concluded that the use of pineapple peel as a cheap substrate for the cost-effective production of biosurfactant from *Lactobacillus plantarum* OG8, isolated from fermenting maize slurry, is feasible. Enhanced production of the biosurfactant was achieved after 48 h incubation period and, at the pH of 7.0 and temperature of 37 °C, respectively. Glucose and peptone were the best carbon and nitrogen sources for optimum biosurfactant production. The partially purified biosurfactant of this bacterium exhibited its optimum activity at a temperature of 60 °C and pH of 10.0. A preliminary

structural characterization indicates the presence of mostly carbohydrates followed by protein and lipid content of the biosurfactant. The produced biosurfactant was observed to exhibit an antimicrobial effect on selected bacterial food pathogens, especially Gram-positive bacteria. Thus, the biosurfactant produced from *L. plantarum* OG8 exhibited physicochemical and antimicrobial properties making it suitable for industrial, biotechnological, and medical applications.

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Conflict of interest

The authors declare no conflict of interest.

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