Life Science Informatics Publications

Research Journal of Life Sciences, Bioinformatics, Pharmaceutical and Chemical Sciences

Journal Home page http://www.rjlbpcs.com/

RJLBPCS

ISSN 2454-6348

Original Research Article **DOI: 10.26479/2018.0404.21**

IDENTIFICATION OF BIOSURFACTANT PRODUCED BY *LACTOBACILLUS SPP.* USING MASS SPECTROMETRY

Nagea Abdalsadiq[1]*, Zaiton Hassan[1], Mohd Lani[2], Bulgasem Bulgasem[1]

1.Department of Microbiology, Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM), 71800 Nilai, Negeri Sembilan, Malaysia.

2. School of Food Science and Technology, Universiti Malaysia Terengganu (UMT), 21030 Kuala Terengganu, Terengganu, Malaysia.

ABSTRACT: Biosurfactants produced by *Lactobacillus* strains have an anti-adhesive activity and decreases the amount of biofilm formed by many pathogens. The quantity and structure of the biosurfactant produced vary according to the microorganism type and its growth conditions. The purpose of this study was to extract the biosurfactants from *L. acidophilus* and *L. pentosus* isolated from dairy products under the optimised condition and to characterise them using column chromatography, TLC, LC-MS and ESIMS/MS. Results of TLC and ESIMS/MS showed that *L. acidophilus* products produced lipopeptide compound composed of a mixture of protein and lipid when it was grown with olive oil as a carbon source while *L. pentosus* produced glycolipid compound composed of a mixture of lipid and sugar when it was grown with palm oil as a carbon source.

KEYWORDS: Biosurfactant, *Lactobacillus,* Mass spectrometry, Lipopeptide, Glycolipid.

Corresponding Author: Dr. Nagea Abdalsadiq* Ph.D.

Department of Microbiology, Faculty of Science and Technology, Universiti Sains Islam Malaysia (USIM), 71800 Nilai, Negeri Sembilan, Malaysia.

Email Address: nageyaa@gmail.com

1. INTRODUCTION

Microbial biosurfactants comprise various groups of surface active agents that are the products of a broad range of microorganisms, mainly bacteria, actinomycetes, yeast, and filamentous fungi from varied environmental locations which either attach to cell surface or are produced

extracellularly [1]. Microbial surfactants are amphiphilic molecules, mainly glycolipids, phospholipids, lipopeptides, and polymeric compounds [2]. Biosurfactants possess diverse chemical compositions, arrangements, and an extensively applied in dairy, food, beverage, cosmetics, detergent, petroleum, and pharmaceutical industries [3]. *Bacillus, Pseudomonas*, and other genus of soil inhabitant microorganisms are commonly reported for biosurfactant production. Several researches have indicated the potential of *lactobacilli* as a biosurfactant [1]. LAB-derived biosurfactants have been reported as complex mixtures of different composition including proteins, glycolipids and polysaccharides, which could comprise phosphate groups [4]. Reference [5, 6] characterised biosurfactant from *L. paracasei* and *L. lactis* as a mixtures compound comprises protein, polysaccharide and the phosphate group. Several LAB strains such as *L. fermentum* RC-14 was reported to possess collagen-binding proteins that induce anti-biofilm activity against *E. faecalis* [7]. Purified biosurfactant isolated from *L. lactis* was shown to be a crystalline white mixture and naturally anionic [8]. Component of fatty acids was determined employing Gas chromatography-Mass spectroscopy of the biosurfactant derived from *L. lactis*, which verified the occurrence of peaks for xylopyranoside sugar and fatty acid such as octadecanoic acid. The compound of xylosepyranoside a relationship with glycolipids existing in rhamnolipid obtained from *P. aeruginosa*. Analysis of gas chromatography and NMR approved that the biosurfactant LAB is a glycolipid identified as xylolipid [8]. Information of chemical composition and structure complexity of biosurfactants derived from LAB is inadequate or limited to fewer reports [9]. The limitation of commercial production due to the lack of structural and molecular information. Hence, the purpose of this study was to extract the biosurfactants produced by *L. acidophilus* and *L. pentosus* under the optimised condition and to characterise them uses column chromatography, TLC, LC-MS and ESIMS/MS.

2. MATERIALS AND METHODS

L. acidophilus and *L. pentosus* respectively were grown in medium under optimum conditions (olive oil and palm oil as carbone source, peptone as nitrogen source, pH 7 and 6, temperature 37 ^{o}C and 36 ^{o}C, agitation 150 rpm and salt concentration 2% for 24 h). The cells were eliminated by centrifugation at 10,000 rpm for 15 min at 4ºC. The CFS was found and pH of the CFS was set to 2, using 1 N HCl and kept at 4ºC overnight. Following this biosurfactant was gathered by centrifugation at 12,000 rpm for 15 min at 4ºC. The resulting dry pellet was lyophilised by freeze-drying (Freeze dryer FD-550), stored at −20°C. Extracts were concentrated and kept at -20°C until characterisation [10].

Purification Of Biosurfactant By Silica Gel Chromatography

Biosurfactant mixture was purifying using a 60 silica gel column (Sigma-Aldrich). *L. acidophilus* biosurfactant was eluted with chloroform: methanol: water (60:20:1, v/v) gradient ranging as the mobile phase, collecting three fractions while *L. pentosus* biosurfactant was eluted with

chloroform: methanol (95:5, v/v) gradient ranging as the mobile phase, collecting three fractions as well. 5 mL of chloroform: methanol (2:1) was used to dissolve the crude extract followed by filtration through a 0.22 μm pore filter syringe. Separation of crude biosurfactants was carried out using preparative layer chromatography column (4.0cm by 30.0cm). The column was filtrated and the fraction was withdrawn (fractions A, B, and C). Three reactants were identified by different colours by *L. acidophilus* and *L. pentosus* as well. This was followed by the application of 10 mL of the mobile phase for the purpose of washing off impurities. Then the elution and pooling of factions were done and evaporated the solvents. The fractions weight was expressed as dry weight by mg/mL. Next was the drying of fractions under nitrogen stream followed by MS/MS analysis to establish the structure and molecular weight of the biosurfactant derived from *L. acidophilus* and *L. pentosus* [11].

Thin Layer Chromatography (TLC)

Concentrated residue of biosurfactant was spotted on TLC plates (Merck, Germany). Chloroform: methanol: water (60:20:1, v/v) was used as mobile phase for *L. acidophilus* and chloroform: methanol (95:5, v/v) for *L. pentosus*. The silica containing the spot was scraped from the plate and extracted with chloroform. The fraction A from *L. acidophilus* and *L. pentosus* demonstrated the highest surface activity compared to other fractions. Ninhydrin, iodine and sulfuric acid solution, were used to detected the materials on the plate, then air-dried and heating the plate for 5 min at 100°C to detect the carbohydrates, lipid, and protein respectively [11].

Tandem Mass Spectrometry

The pseudomolecular compound was frequently employed in tandem with mass spectrometry of the parent ion, to provide further structural information. The isolated fractions eluted from the silica gel column were analysed by mass spectrometry. HPLC system (Finnigan MAT, San Jose, CA, USA) coupled to ion Trap mass spectrometer equipped with an ESI source was used. Separations were performed on an analytical Luna 5 μmC18 (2), 150×4.6mm. The mobile phase components of the lipopeptide fraction were: water, 1% formic acid, acetonitrile; the lipopeptide fractions were eluted following the linear gradient: Solvent A (v/v): 0.1% formic acid in water (99:1), solvent B (v/v): acetonitrile/ water/ 0.1% formic acid (80:20:1). The mobile phase components of the glycolipid fraction were: water, acetonitrile; the glycolipid fraction was eluted following the linear gradient: Solvent A: water (30%), solvent B: acetonitrile (70%). The experiments were conducted on a mass spectrometer using electrospray in positive ion modes. LC/ESI-MS/MS modalities were applied to the selected precursor ions [12].

3. RESULTS AND DISCUSSION

Purification Of Biosurfactant By Silica Gel Chromatography

Lipopeptides such as surfactin and glycolipids such as rhamnolipids are the best known class of biosurfactants with antimicrobial effects [13]. The crude biosurfactant obtained from the liquid

culture of *L. acidophilus* and *L. pentosus* was purified after solvent extraction by adsorption chromatography silica gel. The biosurfactant compositions of the derived from *L. acidophilus* and *L. pentosus* were successfully divided into three main fractions. The bioactive fractions were concentrated and the compound was analysed to determine the structural details of the biosurfactant. The crude biosurfactant contained these three fractions, named A, B and C. Among the fractionated components of *L. acidophilus*, fraction A was identified as lipopeptide in the crude biosurfactant, fraction B was lipid. There was no compound detected in fraction C (figure-1).

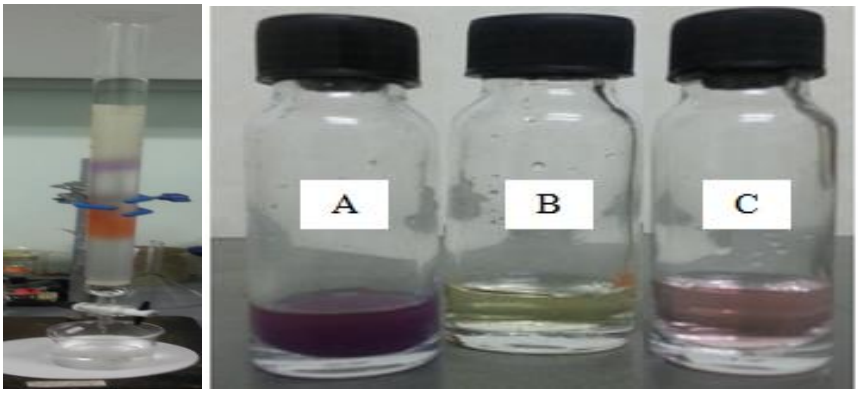

Figure 1: Fractions of biosurfactant produced by *L. acidophilus*. (a) lipopeptide, (b) lipid and (c). No compound detected

However, the fractionated components of *L. pentosus*, fraction A was identified as glycolipid in the crude biosurfactant, fraction B was carbohydrate and fraction C was lipid fraction (figure-2).

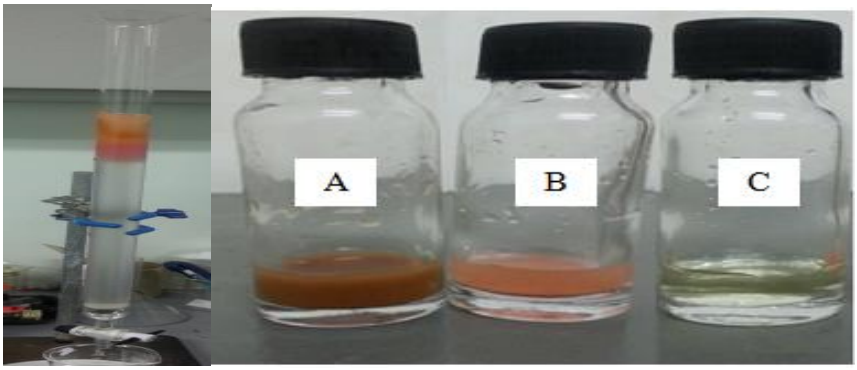

Figure 2: Fractions of biosurfactant produced by *L. pentosus*. (a) glycolipid, (b) carbohydrate (c) Lipid

Analysis Of Biosurfactant Fractions By TLC

TLC showed that *L. acidophilus* biosurfactant were identified as lipopeptide (figure-3). *L. acidophilus* fractions, proteins were identified by ninhydrin, indicating the existence of amino acids which emerged as a purple zone on the TLC plate, lipids were identified by iodine reagent, showing the existence of fatty acids which emerged as a light brown zone on the TLC plate. This study is in agreement with the study of biosurfactant from *L. lactis* by [14] which confirmed that biosurfactant produced by *L. lactis* was identified by adsorption chromatography silica gel and TLC as a lipopeptide with olive oil as carbon source. Another study by [15] reported that biosurfactant from *L. acidophilus* was identified as glycoprotein. A study of biosurfactant produced

by *lactobacillus* spp. identified it as surlactin which has a glycoproteinaceus [16].

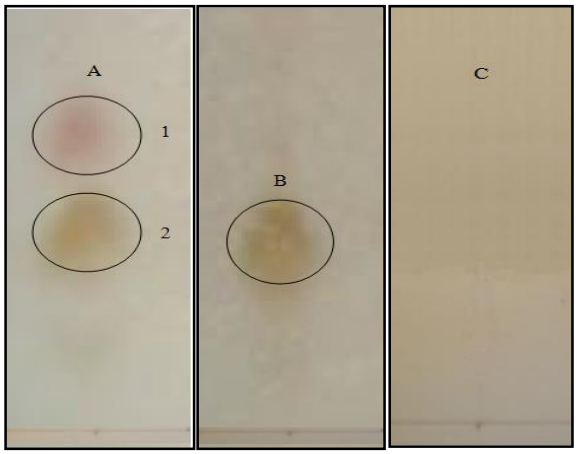

Figure: 3 TLC of *L. acidophilus* (a) lipopeptide (1-protein and 2-lipid)(b) lipid (c).
No compound detected in fraction C

TLC of biosurfactant fractions from *L. pentosus* were identified as glycolipid (figure-4). The dark brown spot which appeared on the plate of TLC related to the presence of carbohydrates which revealed the presence of sugars and the light brown spot which appeared on the plate of TLC related to the presence lipids which revealed the presence of fatty acids. Similarly, a study by [17] of biosurfactant from *E. faecium* and [18] of biosurfactant from *L. helveticus* reported that the structural characterisation of biosurfactant by TLC and column chromatography showed its glycolipidic nature. TLC plate when sprayed with Sulfate reagent to see sugar moieties as a dark red spot. The plate spraying with ammonium molybdate to see of lipid moiety as a dark blue spot. No spots were appearance when the plate was staining with Ninhydrin reagent which reflects no amino acid was found. In general glycolipids are long-chain aliphatic or hydroxyl aliphatic acids with carbohydrates [19]. The crude biosurfactant of *L. helveticus* was at first characterised by TLC as glycolipid which appear as a single spot when viewed under UV light. Staining the plate with iodine produced yellow spot related to lipid compound [18].

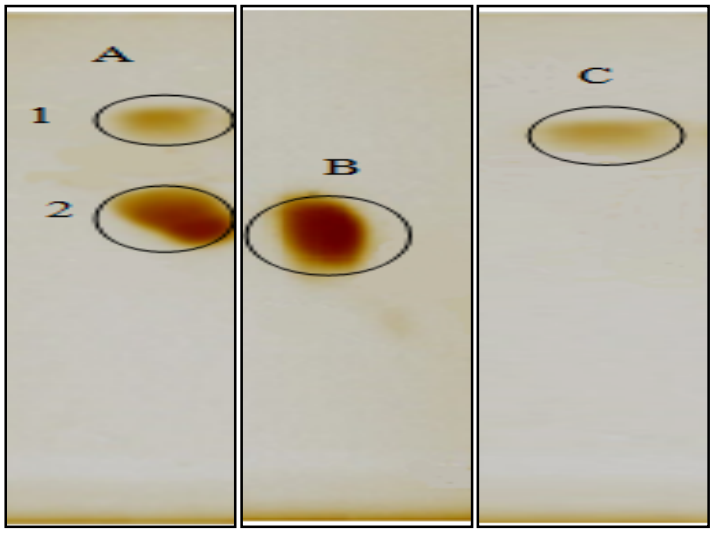

Figure: 4 TLC of *L. pentosus* (a) 1- glycolipid (lipid and 2-carbohydrate) (b) carbohydrate (c) lipid

Analysis of biosurfactant fraction from *L. aciophillus* by liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS)

The structures of the fractions isolated from L. aciophillus and showed surface activity was purified by the commercial TLC plate and submitted to LC–MS and LC-MS/MS. The mass spectrum and chemical structure of the fractionation components are indicated in the figures below.

Analysis of biosurfactant fraction from *L. aciophillus* by liquid chromatography–mass spectrometry (LC–MS)

Results of LC-MS and ESIMS/MS show that bisurfactant fraction from *L. acidophilus* is a lipopeptide compound containing nine amino acids and C12–C17$^{\beta}$ -hydroxy fatty acids with the appearance of sodium and potassium adduct**.** LC–MS for active fraction showed molecular ions at m/z = 1445.56 [M+K]+= [1406.0 + 39] with corresponding structure ([M+K]+ C17, CH3-(CH2)12-CH2-CHO-CH2-CO-E-Trp-Tyr-L/I- L/I -V-D- L/I - L/I -C=O),] with corresponding structure ([M+Na]+C17,CH3-(CH2)12-CH2-CHO-CH2-CO-E-Trp-Tyr-L/I- L/I -V-D- L/I-L/I-C=O),1415.38[M+Na]+=[1392.50+23] with corresponding structure (([M+Na]+C16,CH3-(CH2)11-CH2-CHO-CH2-CO-E-Trp-Tyr-L/I- L/I -V-D- L/I - L/I -C=O), 1401.31[M+Na] +=[1378.17+23] with corresponding structure ([M+Na]+C15, CH3-(CH2)10-CH2-CHO-CH2-CO-E-Trp-Tyr-L/I- L/I -V-D- L/I - L/I -C=O) and 1392.50 with corresponding structure ([M] C16, CH3-(CH2)11-CH2-CHO-CH2-CO-E-Trp-Tyr-L/I- L/I -V-D- L/I - L/I -C=O (figure-5) Similarly *B. subtilis* produce biosurfactant (Surfactin) contain hydrophobic fatty acid tail and seven amino acids which have the affinity for divalent and monovalent cations ([M+Na] + C12, CH3-(CH2)7-CH2-CHO-CH2-CO-E- L/I- L/I -V-D- L/I - L/I -C=O) [20].

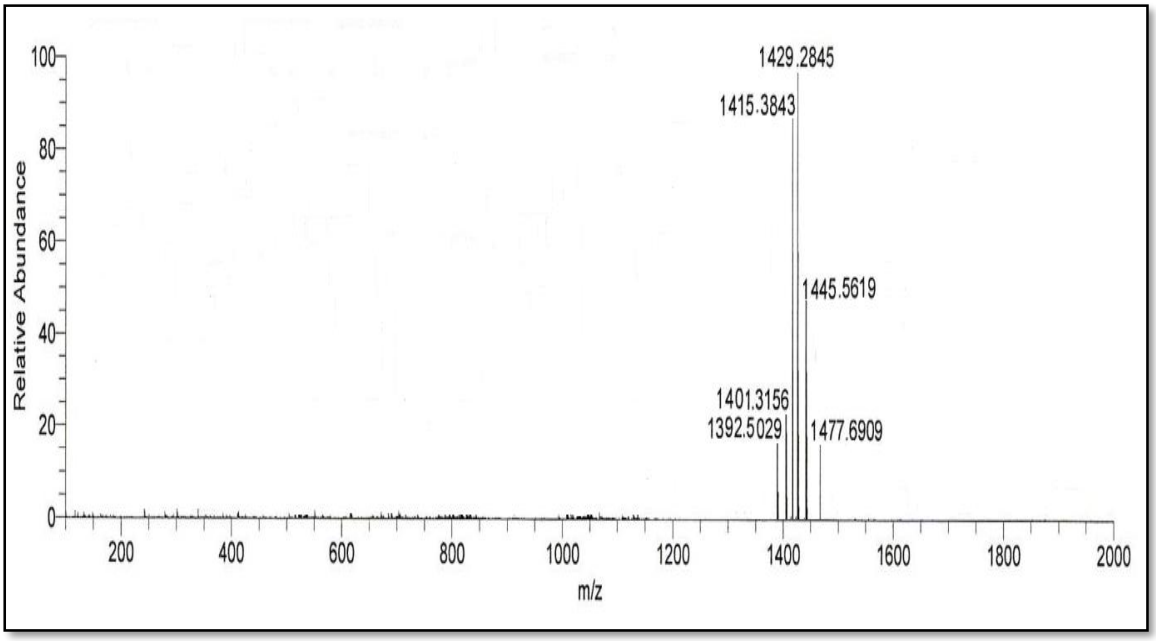

Figure: 5 LC-MS spectrum, positive ion mode of *L. acidophilus* lipopeptide fraction

Analysis of biosurfactant fraction from *L. aciophillus* of [M+Na] ion at m/z = 1415.38 by liquid chromatography tandem mass spectrometry (LC-MS/MS)

The molecular ion (HRMS m/z = 1415.38 with 1392.50Da mass (13992.50 + 23) for the structure [C71H104N10O18+Na] +. Figure-6 shows the fragment ions in the spectrum of m/z 1415.38. The peak at m/z 481.26 in m/z 1415.38 spectrum refers to a fragment ion [Val6-Asp7-Leu8-Leu9 (OH) +H+Na. Another peaks at 594.34 and m/z 707.44 refer to peptide fragment ions [Leu5-Val6-Asp7-Leu8Leu9(OH)+H+Na]+ and [Leu4-Leu5-Val6-Asp7-Leu8-Leu9 (OH) +H+Na] +, respectively. Im addition, two peaks at 1056.64 and m/z 870.42 which reflect peptide fragment ions [Trp2-Tyr3-Leu4-Leu5-Val6-Asp7-Leu8 Leu9 (OH) +H+Na] + and [Tyr3 -Leu4-Leu5-Val6-Asp7-Leu8-Leu9 (OH) +H+Na]+, respectively. This study concluded that out of five fragments patterns of lipopeptide of 1415.38 (13992.50 + 23) molecular ion, three fragments (m/z 481.26, 594.34, and 707.44) were similar to the fragmentation patterns of lipopeptide in the surfactin produced by *B. sabtilis*. Depending on the calculation of the fragments molecular weights it seems to be the sequence of amino acids in the lipopeptide fraction produced by *L. acidophilus* are similar to that found in surfactin with two additional amino acids (tryptophan and tyrosine in positions 2 and 3. respectively) [21]. Similarly, chemical characterization showed that the biosurfactant derived from *L. acidopilus* were classified as a glycoprotein which are utilised in cosmetic industry [15].

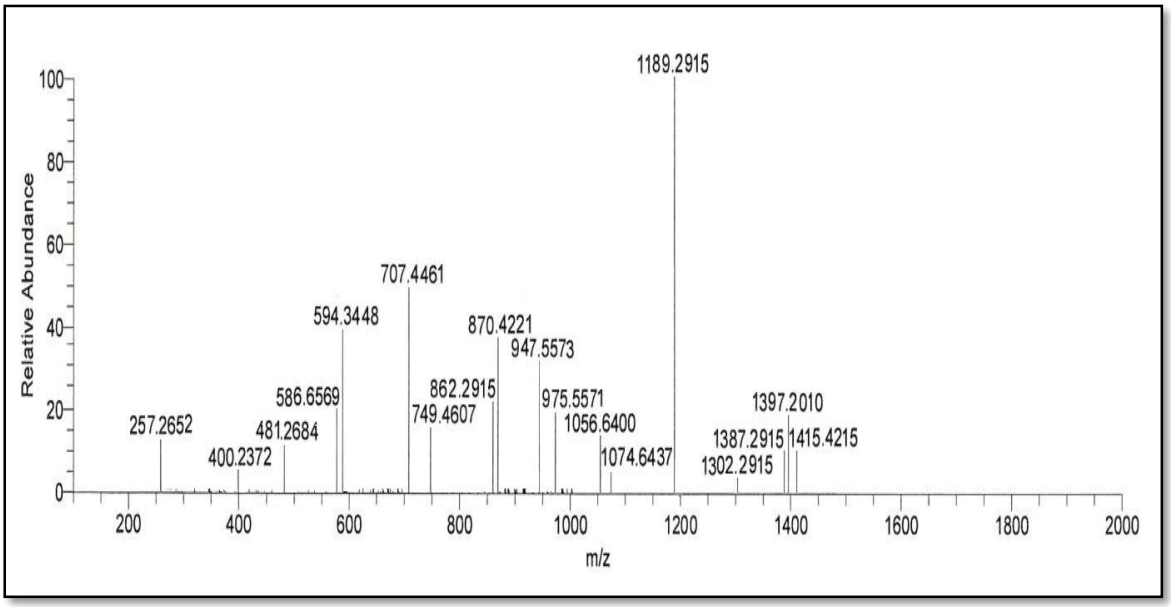

Figure: 6 LC-MS/MS spectrum, positive ion mode of *L. acidophilus* lipopeptide fraction of [M+Na] ion at m/z =1415.38

Analysis of biosurfactant fraction from *L. aciophillus* of [M+K] Ion at m/z = 1445.56 by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Analysis of the molecular ion 1445.56 with 1406.0Da mass (1406.0 + 39) and molecular structure [C72H106N10O18+K] + display several fragment ions (figure-7).The fragment ions [Leu5-Val6-

Asp7-Leu8- Leu9 (OH) +H+K] + and Leu4-Leu5-Val6-Asp7-Leu8-Leu9 (OH) +H+K] + refer to 610.43 and m/z 723.26, respectively. Another ion at m/z 495.25 reflect [Val6-Asp7-Leu8-Leu9(OH+H+K+. The next fragment ions[Trp2-Tyr3-Leu4-Leu5-Val6-Asp7-Leu8-Leu9 (OH) +H+K] + and [Tyr3-Leu4-Leu5-Val6-Asp7-Leu8-Leu9 (OH) +H+K] + appear in the spectrum with molecular weight 1072.53 and m/z 886.69 respectively. The last fragment ion at m/z 495.25 with the sequence [Val6-Asp7-Leu8-Leu9 (OH) +H+K]+. In accordance with the TLC and ESI MS/MS calculation for [C71H104N10O18+Na] + = 1415.38 and [C72H106N10O18+K]+ = 1445.56 and compared with the literature, this study observed that biosurfactant fraction from *L. acidophillus* was lipopeptide consisting of protein and lipid. In addition result of ESIMS/MS showed that three fragments (m/z 723.26, 610.43 and 495.25) of 1445.56 (1406.0+39) with the molecular structure [C72H106N10O18+K] +) ions from five fragments, were similar to the fragmentation patterns of lipopeptide in the surfactin produced by *B. sabtilis*. Biosurfactant contain hydrophilic and hydrophobic chain which comprises proteins and lipids, respectively [21].

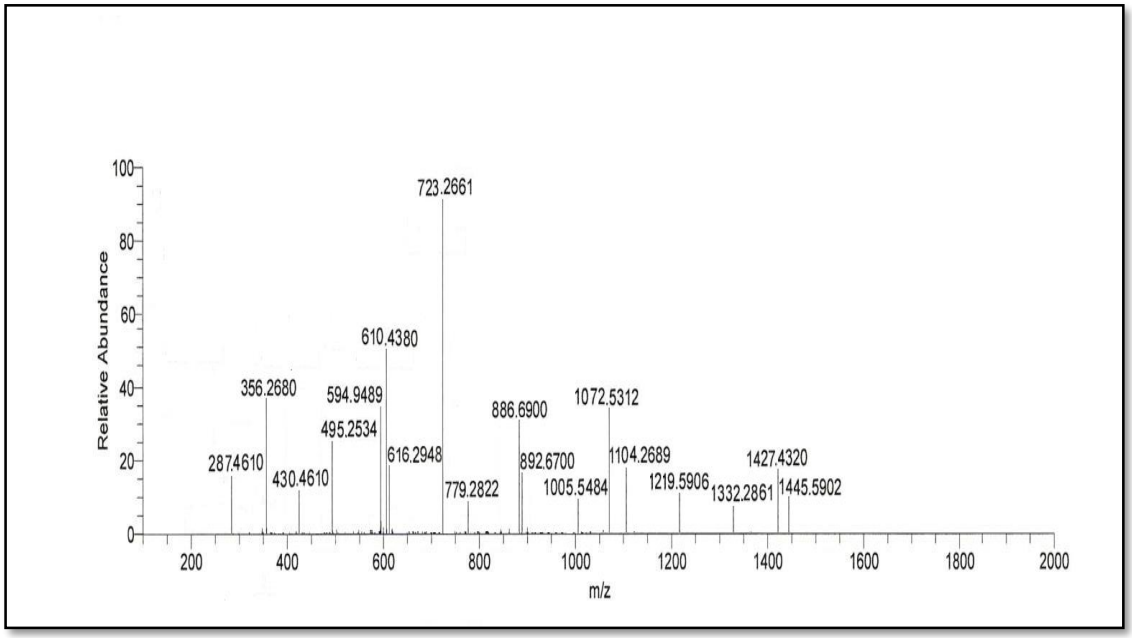

Figure: 7 LC-MS/MS spectrum, positive ion mode of *L. acidophilus* lipopeptide fraction of [M+K] ion at m/z = 1445.56

Analysis of biosurfactant fraction from *L. pentosus* by liquid chromatography–mass spectrometry (LC–MS) and liquid chromatography tandem mass spectrometry (LC-MS/MS)

Analysis of biosurfactant fractions by LC–MS and LC-MS/MS were used to validate the previous results and to identify the structure of the total active fractions with various corresponding molecular weights. The compositions of the isolated product purified by plate of TLC were further identified by mass spectrometry.

Analysis of biosurfactant fraction from *L. pentosus* by liquid chromatography–mass spectrometry (LC–MS)

Analysis of biosurfactant fraction from *L. pentosus* using ESI-LC–MS ESI-MS/MS spectroscopy showed that it is a mixture of lipid and carbohydrates. Results of LC–MS indicated molecular ions refer to predicted compounds. The spectrum of LC–MS biosurfactant fraction showed a molecular ion at m/z = 1084.67 [M+Na] += [1061 + 23], which corresponds to the molecular formula of [C64H117O11+Na] +). The other peaks were produced by the loss two groups of methylene (CH2 = 14) (1056) and (986), loss one group of methylene (CH2 = 14) (1042, 1028 and 1014), loss ten groups of methylene (CH2 = 14) (846) and groups nine groups of methylene (CH2 = 14) (720, 594, 468, 342 and 216) (figure-8). The molecular structure of glycolipid was validated using mass spectrometry with positive ion.

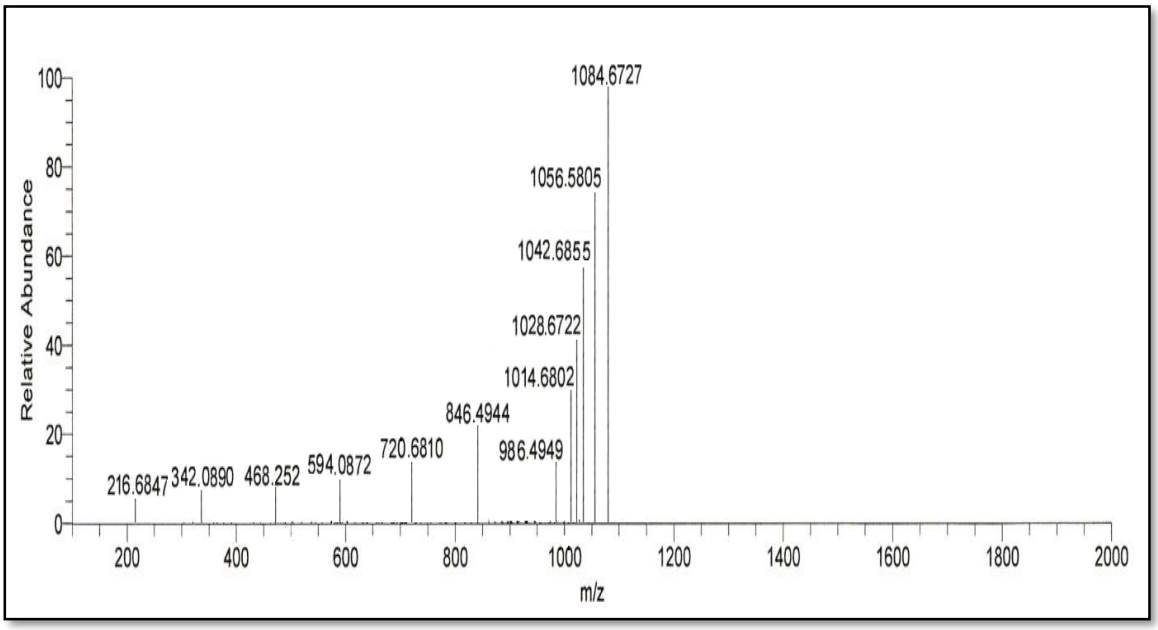

Figure: 8 LC-MS spectrum, positive ion mode of *L. pentosus* glycolipid fraction

Analysis of biosurfactant fraction from *L. pentosus* by liquid chromatography tandem mass spectrometry (LC-MS/MS)

Analysis of the molecular ion 1084.67 with molecular structure [C64H117O11+Na] +) indicated several fragment ions. Initial loss of dihydrosterculic acid (C19H35O2- m*/z* = 295.487) and oleic acid (C18H34O2 *m/z* = 282.468) to produce m/z = 788.51 and 802.69 (the base peak), respectively (figure-9). Thus, the big fragment amount of the *m/z* = 788.51 suggests that the dihydrosterculic acid in the glyceride esterified at the position of *sn*-1 therefore, the ion become more stable due to the loss of the fatty acid at this position. Another fragment ion of *m/z* 375.70 was due to the loss of a monoacylated-containing oleic acid-monosaccharide from *m/z* 788.51. The fragment obtained for the monoacylated-containing oleic acid-monosaccharide itself (*m/z* = 450.41) was also clearly seen. Therefore result of calculation of the fragments molecular weights in this study showed that the glycolipid was identified as 6-O-acyl-α-D-glucopyrano

syldiglyceride, a glycoglycerolipid. In accordance with the TLC and ESI MS/MS calculation for [C64H117O11+Na] +) = 1084.67 and compared with the literature, this indicated that biosurfactant fraction from *L. pentosus* was glycolipid composed of lipid and carbohydrate This result is similar to the result of the study by [9] in the first fraction of glycolipid isolated from *L. plantarum* where were both of them α-D-glucopyranosyldiglyceride compound but the fraction from *L. pentosus* in this study contains an extra group (6-O-acyl). On the other hand, *L. pentosus* biosurfactant fraction from this study is different from the third fraction of glycolipid isolated from *L. plantarum* in case of glucose molecule numbers where were only one molecule of glucose in the fraction from this study while there were three molecules in the third fraction of *L. plantrum* biosurfactant from the previous study. This study is the first to report the production of biosurfactant type as 6-O-acyl-α-D-glucopyranosyl diglyceride as a novel class of promising biosurfactants from *L. pentosus.*

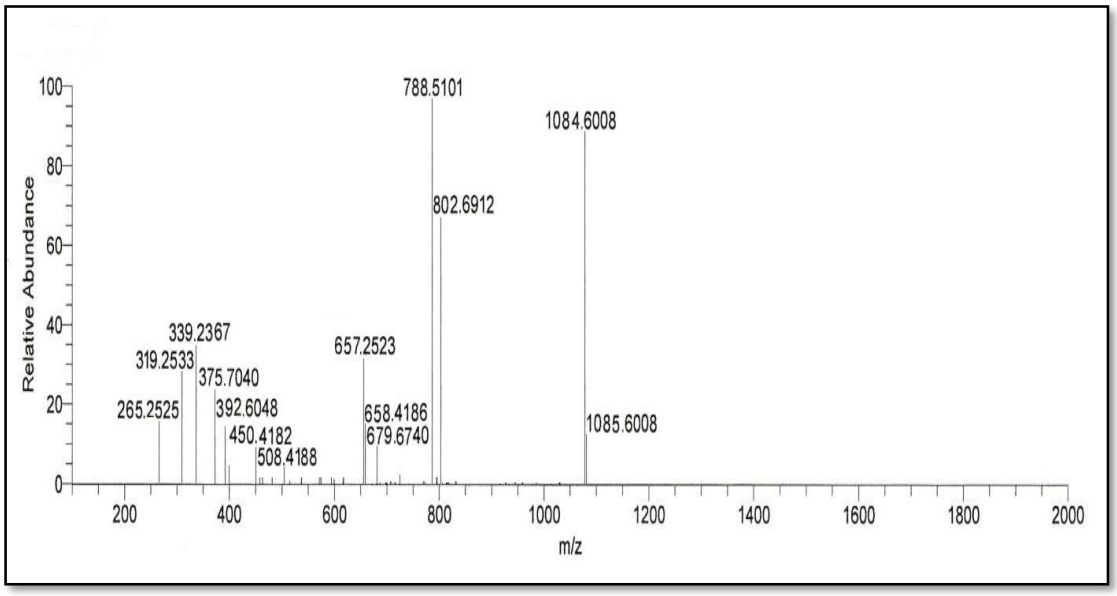

Figure: 9 LC-MS/MS spectrum, positive ion mode of *L. pentosus* glycolipid fraction of [M+Na] ion at m/z = 1084.60

4. CONCLUSION

Microorganisims such as *L. acidophilus* and *L. pentosus* require special growth conditions to produce a special type of biosurfactant. Results of TLC and ESIMS/MS showed that biosurfactant from *L. acidophilus* was a lipopeptide compound composed of a protein and lipid while biosurfactant from *L. pentosus* was a glycolipid compound consisting of a sugar and lipid.

5. CONFLICT OF INTEREST

None

6. ACKNOWLEDGEMENT

The authors would like to thank the Faculty of Science and Technology, Universiti Sains Islam Malaysia for their support.

REFERENCES

1. Saharan B. S Grewal A Kumar, P. Biotechnological production of polyhydroxyalkanoates: a review on trends and latest developments. *Chinese Journal of Biology*. 2014; 1–18.
2. Perfumo A, Smyth TJP, Marchant R, Banat IM. Production and roles of biosurfactants and bioemulsifiers in accessing hydrophobic substrates. In: Timmis, Kenneth N. (Ed.). In: *Handbook of Hydrocarbon and Lipid Microbiology*. Springer, UK. 2010; 501–1512.
3. Nitschke, M, Costa, SG. Biosurfactants in food industry. *Trends Food Sci. Technol*. 2007; 18: 252–259.
4. Rodrigues, LR, Teixeira J, Van der Mei HC, Oliveira R. Physicochemical and functional characterization of a biosurfactant produced by *Lactococcus lactis* 53. *Colloids Surf B Biointerfaces*. 2006; 49: 79-86.
5. Gudina EJ, Teixeira JA, Rodrigues LR. Isolation and functional characterization of a biosurfactant produced by *Lactobacillus paracasei. Colloids Surf B Biointerfaces*. 2010; 76:298-304.
6. Velraeds MMC,Van der Mei HC, Reid G, Busscher HJ. Physicochemical and biochemical characterization of biosurfactants released by *Lactobacillus* strains. *Colloids Surf B Biointerfaces.* 1996; 8: 51-61.
7. Heinemann C, Hylckama V J, Janssen D B, Busscher H J, van der Mei H C, Reid G, Purification and characterization of a surface-binding protein from *Lactobacillus fermentum* RC-14 that inhibits adhesion of *Enterococcus faecalis* 1131. *FEMS Microbiol Lett*; 2000; 190:177– 80.
8. Saravanakumari P, Mani K. Structural characterization of a novel xylolipid biosurfactant from *Lactococcus lactis* and analysis of antibacterial activity against multi-drug resistant pathogens. *Bioresource Technol*. 2010; 101(22): 8851–8854
9. Sauvageau J, Ryan J, Lagutin K, Sims IM, Stocker BL, Timmer MS. Isolation and structural characterization of the major glycolipids from *Lactobacillus plantarum. Carbohydrate Res*. 2012; 357: 151–156.
10. Anandaraj B, Thivakaran P. Isolation and production of biosurfactant producing organism from oil spilled soil. *J Biosci Tech*. 2010; 1 (3):120-126.
11. Suzuki T, Tanaka H, Itoh S. Sucrose lipids of Arthrobacter, Corynebacteria and Nocardia grown on sucrose. *Agric. Biol. Chem*. 1974; 38: 557-563.
12. Pornsunthorntawee O, Wongpanit P, Chavadej S, Rujiravanit Abe MR. Structural and physicochemical characterization of crude biosurfactant produced by *Pseudomonas aeruginosa* SP4 isolated from petroleum-contaminated soil. *Bioresource Technol*. 2008; 99(6): 1589–1595.

13. Kulakovskaya T, Shashkov A, Kulakovskaya E, GolubevW, Zinin A, Tsvetkov Y, Nifantiev N. Extracellular cellobiose lipid from yeast and their analogues: structures and fungicidal activities. *J Oleo Sci*. 2009; 58 :(3). 133–40.
14. Machado TR, Mohideen RAH, Saravanakumari M, Prabhavathi P. Anti adhesive, antimicrobial and biodegradability assay of a lipopeptide biosurfactant from *Lactococcus lactis. IJSID*. 2013; 3: 478–483.
15. Tahmourespour A, Salehi R, Kermanshahi RK, Eslami G. The anti-biofouling effect of *Lactobacillus fermentum*-derived biosurfactant against *Streptococcus* mutans. *Biofouling*. 2011; 27 (4): 385–392.
16. Gołek P, Bednarski W, Lewandowska M. Characteristics of adhesive properties of *Lactobacillus* strains synthesizing biosurfactants. *Pol. J. Natur. Sc*. 2007; 22: 333-342.
17. Sharma D, Saharan BS, Chauhan N, Procha S. Lal S. Isolation and functional characterization of novel biosurfactant produced by *Enterococcus faecium*. *Springer Plus*. 2015; 4 (1): 1-14.
18. Sharma D, Saharan B S. Functional characterization of biomedical potential of biosurfactant produced by Lactobacillus helveticus. *Biotechnology Reports*. 2016; 11: 27 35.
19. Lang S. Biological amphiphiles (microbial biosurfactants). *Curr Opinion Colloid and Interface Sci.* 2002; 7(1): 12–20.
20. Iroakasi O I. Characterisation, optimisation and environmental application of selected biosurfactant producers, PhD. thesis, University of Aberdeen., Scotland, 2012.
21 Pathak KV, Keharia H. Identification of surfactins and iturins produced by potent fungal antagonist, *Bacillus subtilis* K1 isolated from aerial roots of banyan (Ficus benghalensis) tree using mass spectrometry. *Biotech*. 2014; 4:283–295.