Malaysian Journal of Microbiology, Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

Malaysian Journal of Microbiology
Published by Malaysian Society for Microbiology
(In SCOPUS since 2011)

Optimization of *Bifidobacterium pseudocatenulatum* KAKii cultivation conditions for exopolysaccharide production by using response surface methodology

Aliza Mahdi[1], Maslinda Musa[1], Siti Aqlima Ahmad [2] and Khalilah Abdul Khalil[1]*

[1]Department of Biomolecular Science, School of Biology, Faculty of Applied Sciences, Universiti Teknologi MARA, 40450 Shah Alam, Selangor, Malaysia.
[2]Faculty of Biotechnology and Molecular Sciences, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.
Email: khali552@salam.uitm.edu.my

Received 3 May 2018; Received in revised form 16 August 2018; Accepted 29 April 2019

ABSTRACT

Aims: Bifidobacteria is a non-motile, Gram-positive, strictly anaerobic and non-spore-forming bacteria that can produce exopolysaccharide (EPS). EPS is a polymer of sugars, long chained polysaccharide which have been shown to give benefit towards human health. The optimum conditions for EPS production by *Bifidobacterium* are still scarce. Therefore, a study was conducted to optimize the growth conditions (pH, temperature and cultivation time) for a better improvement of EPS production.
Methodology and results: Three *Bifidobacterium* strains were cultured and the highest EPS producing strain was selected for optimization. Response Surface Methodology (RSM) was used to optimize the growth conditions for a maximum EPS production. Subsequently, EPS was characterized by using FT-IR and GC-MS. Based on the result obtained, *B. pseudocatenulatum* KAKii had the highest EPS production compared to the other two strains namely *B. pseudocatenulatum* ATCC 27919 and *B. animalis*. Meanwhile, the optimization of the three factors towards selected strain found that EPS produced crucially depends on time of cultivation (23.59 h) other than pH (5.0) and temperature (34.75 °C). The validation showed that the predicted and experimental values were not significantly different ($P > 0.05$), indicating that the developed model is fitted well for the optimization. Meanwhile, FT-IR and GC-MS results showed that the EPS was composed of D-glucose, mannose, galactose, maltose and acetic acid as by-product.
Conclusion, significance and impact of study: This result showed that the EPS produced by *B. pseudocatenulatum* KAKii is from hetero-exopolysaccharide group with acetic acid as by-product made them a possible anticancer agent in future.

Keywords: Exopolysaccharide, bifidobacteria, optimization, response surface methodology

INTRODUCTION

Bifidobacteria belongs to the phylum of Actinobacteria, which are non-motile, Gram-positive, strictly anaerobic and non-spore-forming bacteria (Ishibashi *et al.*, 1997; Fabio *et al.*, 2013; Khalilah *et al.*, 2014). It is one of the first bacteria to be considered as probiotics where the strains of several *Bifidobacterium* species have been proved to stimulate health promoting activities such as enhancement of resistance against pathogens, reduction of blood cholesterol levels, immunomodulation and others (Gill and Rutherfurd, 2001; Shu and Gill, 2002; Rosenfeldt *et al.*, 2002; Jones *et al.*, 2004; Seema *et al.*, 2012).

Besides of their granted status as a GRAS (Generally Recognised as Safe) in food, which is associated with LAB (Lactic Acid Bacteria), these living microorganisms have also been discovered to be the suitable candidates to produce functional EPS (exopolysaccharide) (Laws *et al.*, 2001; Seema *et al.*, 2012). Bifidobacteria can be found in human gut and also known as commensal bacteria, with a population of around 3% to 7% of microbiota in adults and up to 91% in newborns according to some reports (Miyashita *et al.*, 2012; Fabio *et al.*, 2013).

EPS are high molecular weight and biodegradable polymers synthesized by a wide range of bacteria including LAB and also bifidobacteria (Pinar and Gürcü, 2016). Salma *et al.* (2017) stated that EPS has gained attention due to its ability and properties as an anticancer agent. Total EPS produced by LAB were reported to be influenced by many factors such as strains, composition of growth medium (carbon and nitrogen sources, vitamins, minerals and etc) and cultivation parameters (pH, incubation temperature, cultivation time and etc). Most of the study carried out involved different strains and optimization process were done considering the carbon

*Corresponding author

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

as well as nitrogen source as factors for better EPS production (Seema *et al.*, 2012; Pinar and Gürcü, 2016). Cultivation parameters are one of the critical points to achieve maximal yield of EPS production. Therefore, the optimization of cultivation conditions using RSM was performed to identify the optimum condition for the production of EPS by *B. pseudocatenulatum* KAKii. Characterization of EPS was conducted using Fourier Transform-Infrared (FT-IR) and Gas Chromatography-Mass Spectrometry (GC-MS) subsequently.

MATERIALS AND METHODS

Medium preparation

Medium was first prepared for the growth of the bacteria from the stock culture without controlling any cultivation conditions. Then, another medium was prepared for the bacterial growth following optimized pH (5.0, 6.0, 7.0). MRS [de Man, Rogosa and Sharpe (Merck, Germany)] medium was also prepared for a morphological analysis (Figure 1) and cultivation of all three bifidobacteria strains used in the study.

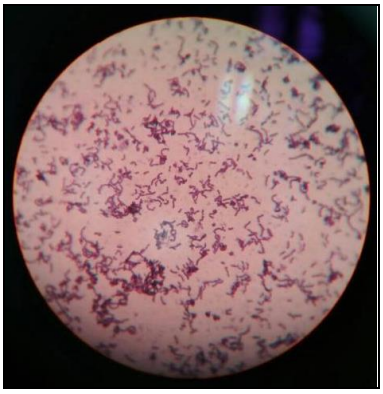

Figure 1: *Bifidobacterium pseudocatenulatum* KAKii under 1000× total magnification.

Cultivation of Bifidobacteria strain for EPS production

In this study, *B. pseudocatenulatum* KAKii was selected due to its highest EPS production. Approximately, 1% (v/v) of all bifidobacteria used in this study was placed individually into 10 mL sterile MRS broth supplemented with L-cysteine at 0.05% (w/v) at 37 °C for 24 h under anaerobic condition. After 24 h of incubation, the cell cultures were centrifuged at 7900 rpm for 15 min and 9 mL new sterilized MRS broth with 0.05% (w/v) L-cysteine was added and further incubated for another 24 h prior to inoculum dose determination. Subsequently, 10^7 CFU/mL of inoculum dose, 10% (v/v) of the cell culture was further incubated in new MRS broth with 0.05% (w/v) L-cysteine and 2% (w/v) lactose addition under different range of pH, temperature and cultivation time condition as shown in Table 1.

Table 1: Cultivation conditions range suggested by CCD-RSM.

Conditions	Minimum	Middle	Maximum
pH	5.0	6.0	7.0
Incubation Temperature (°C)	20	30	40
Cultivation time (h)	6	21	36

The range of pH, incubation temperature and cultivation time were used based on suggestion by CCD-RSM (Central Composite Design-Response Surface Methodology) approach. Approximately, 10 mL of samples were taken for EPS extraction and quantification after 24 h of cultivation period for strain selection. Meanwhile, 10 mL of samples were also taken for identification of EPS production following cultivation time suggested by CCD-RSM.

EPS extraction

Extraction of EPS was carried out according to Venkataraman *et al.* (2016) with some modifications. EPS was extracted by using cold ethanol method. The suspension was subsequently heated at 100 °C for 15 min in order to denature the EPS-degrading enzymes. Then, the cultures were kept at room temperature for 15 min and followed by Trichloroacetic Acid (TCA) addition to a final concentration of 10%. The mixtures were then incubated at 4 °C for 3 h and the mixture was centrifuged at 7900 rpm for 15 min. Supernatant was collected while the pellet was discarded. Ice cold ethanol was added in a 2:1 ratio to the supernatant before incubated at 4 °C overnight. EPS, which formed as precipitate, were collected after another centrifugation. EPS was purified by repeatedly dissolving it in distilled water and extraction with cold ethanol for another 24 h. The purified EPS was dialyzed, dried through a freeze dryer and used in further studies.

Characterization of EPS by FT-IR

Characterization of EPS by FT-IR was carried out according to Venkataraman *et al.* (2016) with some modifications. EPS extracted was dried through a freeze dryer and subjected to FT-IR spectroscopy using the KBr pellet method. About 20 mg of EPS grinded with 200 mg KBr powder and pressed into 1 mm pellets for FT-IR (Zhihong *et al.*, 2016). The pellet was analyzed for FT-IR spectrum using a FT-IR spectrometer in the range of 400-4500 cm^{-1}.

Characterization of EPS by GC-MS

Characterization step using GC-MS was carried out according to Amit (2013). The EPS were hydrolyzed in 2 M Trifluoroacetic Acid (TFA) at 120 °C for 2 h, and resulting sugars were derivatized to its corresponding alditol acetates by reduction with $NaBH_4$, and acetylation with pyridine and acetic anhydride. The derivatization

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

process is crucial in order to convert the EPS into a more specific sugars for further detection. The sample was analyzed by GC-MS (Varian 45 GC and MS 240, USA) using a DB-5 MS column (30 m × 0.25 mm × 0.25 µm). Helium was used as carrier gas at a flow rate of 1 mL/min. Oven temperature was held at 40 °C for 2 min, which was increased to 130 °C at the rate of 25 °C/min, and then again increased to 180 °C at a rate of 12 °C/min. It was finally heated to 280 °C at 3 °C/min and held for 7 min. Injector and ion trap temperatures were set at 270 °C and 240 °C, respectively. The acquisition mass range was set from 50 to 1000 Da. A 1 µL sample was injected into GC and fragmentation pattern was observed.

RESULTS AND DISCUSSION

Selection of *Bifidobacterium* strains

These bacteria can be found in irregular rod-shaped bacteria that often resemble Y or V shape especially when it undergoes mature phase (Khalilah *et al.,* 2014; Tortora *et al.*, 2015). As a Gram-positive bacteria, it consists of thick cell wall of peptidoglycan which retained crystal violet colour during staining procedure and appeared purple or violet colour under microscope (Figure 1). *Bifidobacterium pseudocatenulatum* ATCC 27919 showed the lowest EPS concentration obtained with 5.65 mg/100mL, followed by *B. animalis* with 13.62 mg/100mL. While the highest EPS yield was obtained from *Bifidobacterium pseudocatenulatum* KAKii with 15.63 mg/100mL (Table 2). The EPS yield from the three strains was not under any optimized condition (pH, temperature, cultivation time). The medium used was the same, including the cultivation time and incubated temperature set before the extraction phase. *B. pseudocatenulatum* KAKii which showed the highest EPS yield was selected and further used in this study. The cells were grown under an optimized condition as suggested by the response surface methodology.

Table 2: *Bifidobacterium* strains selection.

Bifidobacterium strain	EPS (mg/100mL)
B. pseudocatenulatum ATCC 27919	5.65±0.15
B. pseudocatenulatum KAKii	15.63±0.23
B. animalis	13.62±0.23

Optimization of EPS production using central composite design-response

Surface mehtodology (CCD-RSM)

The effect of pH (A), temperature (B) and cultivation time (C) on EPS production during the cultivation of *B. pseudocatenulatum* KAKii using 2^3 central composite design was shown in Table 3. Nineteen experiments were performed in duplicates with the selected pH, temperature and cultivation time. There was a great variation in the amount of EPS, which ranged from 66.29 mg/100mL to 121.99 mg/100mL. The maximum EPS production (121.99 mg/100mL) was obtained at 21 h of cultivation, pH 6.0 and a temperature at 30 °C. The analysis of variance (ANOVA) results were presented in Table 4.

Table 4 shows ANOVA result of the second-order response surface model. The F-value of 7.58 implied that the model was significant and there was only 0.29% chance that a "Model F-value" this large could occur due to noise. The R^2 (coefficient of determination) gave sample variation value of 0.8834 for EPS production (y) and this indicated that about 0.1166 of total variation was not explained by the model. Therefore, it indicated a good agreement between the experimental and predicted values for the EPS production. Meanwhile the lack of fit was used to measure the failure of the model to represent the data in the experimental domain at points (Gao and Gu, 2007). The lack of fit value of 0.50 ($P > 0.05$) suggested that it was not significant in relative to the pure error, which implied that the model was fitted well to the data in the experimental region.

The optimal values for the tested factors were pH (5.0); temperature (34.75 °C); and cultivation time (23.61 h). Based on the result obtained, the maximum predicted EPS production was 115.89 mg/100mL while the mean of actual experimental value was 115.73 mg/100mL. Based on Figure 2, EPS production was observed to be least affected by both pH (A) and temperature (B). pH ranging from 5.0 to pH 7.0 with temperature ranging from 20 °C to 40 °C had a less significant effect on the response (EPS production). However, minimum EPS production was still be observed at a temperature of 20 °C and 40 °C and at pH 7.0. A slight increment of EPS production was observed at the central point of temperature 35 °C and pH 5.0.

Based on Figure 3, an increasing of cultivation time (C) had increased the EPS production. However, the production was decreased as the cultivation time continued. By comparing the cultivation time to the *B. pseudocatenulatum* KAKii growth curve, the three-dimensional graph showed that the EPS production had increased during the exponential phase and stationary phase then start to decrease as the stationary phase continued. This might be due to nutrients deprivation under prolong cultivation period. Therefore, the production of EPS by the bacterial strain started to cease after 30 h. The graph also showed that EPS production was optimal in pH 5.0 media rather than pH 6.0 and pH 7.0. This pattern supported the observation as shown in Figure 1.

Meanwhile, Figure 4 showed the temperature ranging from 20 °C to 40 °C had a less significant effect towards the response (EPS production). However, minor changes in an increment trend for EPS production can be observed around 35 °C, confirming the outcomes for optimal condition for maximum EPS production at a temperature of 34.75 °C. Additionally, the same pattern for EPS production affected by cultivation time was also observed in Figure 3. It shows that EPS production by *B. pseudocatenulatum* KAKii was essentially depending on the cultivation time. It showed that the optimal cultivation

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

time was ranged from 21.0 to 28.50 h, supporting the predicted EPS production under optimal condition as suggested by response surface methodology which was 115.89 mg/100mL during 23.61 h of cultivation.

Validation of the models

In order to validate the model equations, the experiments were done in triplicates and were carried out under selected suggested conditions. Selected combinations of the three conditions were predicted to yield the maximum EPS production of 115.89 mg/100mL. The design matrix of the factors which were pH, temperature and cultivation time was suggested along with predicted values for EPS production (Table 5). The experiments were conducted under the predicted optimal conditions. It was found that the experimental values fitted well to the predicted results with no significance difference ($P > 0.05$). The result was encountered during the validation of response surface methodology optimization process where the experimental data were closed to the predicted value.

Table 3: The matrix of CCD experiment and corresponding experimental data of *B. pseudocatennulatum* KAKii.

Standard	Run	Factor 1 A: pH	Factor 2 B: Temperature (°C)	Factor 3 C: Time (hours)	Response 1 EPS (mg/100mL)
3	1	5.0	40	6	97.58
19	2	6.0	30	21	121.99
13	3	6.0	30	6	86.35
1	4	5.0	20	6	85.32
12	5	6.0	40	21	106.20
7	6	5.0	40	36	99.64
16	7	6.0	30	21	105.61
9	8	5.0	30	21	118.14
8	9	7.0	40	36	81.15
18	10	6.0	30	21	104.31
4	11	7.0	40	6	66.29
17	12	6.0	30	21	105.72
6	13	7.0	20	36	102.08
5a	14	5.0	20	36	103.11
2	15	7.0	20	6	92.32
15	16	6.0	30	21	113.69
11	17	6.0	20	21	106.86
10	18	7.0	30	21	97.53
14	19	6.0	30	36	103.98

Table 4: Analysis of Variance (ANOVA) for response surface quadratic model for the yield of EPS production in *B. pseudocatenulatum* KAKii in RSM based approach.

Source	Sum of Square	Mean Square	F value	Prob > F	
Model	2793.88	310.43	7.58	0.0029	significant
A	414.99	414.99	10.13	0.0111	
B	150.78	150.78	3.68	0.0872	
C	385.64	385.64	9.42	0.0134	
A^2	8.00	8.00	0.20	0.6689	
B^2	24.86	24.86	0.61	0.4559	
C^2	565.11	565.11	13.80	0.0048	
AB	388.51	388.51	9.49	0.0131	
AC	2.84	2.84	0.069	0.7981	
BC	14.12	14.12	0.34	0.5715	
Residual	368.60	40.96			
Lack of Fit	141.61	28.32	0.50	0.7677	not significant
Pure Error	226.99	56.75			
Cor Total	3162.48				

R-Squared = 0.8834; Adj R-Squared = 0.7669; Pred R-Squared = 0.2867; Adeq Precision = 10.039.

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

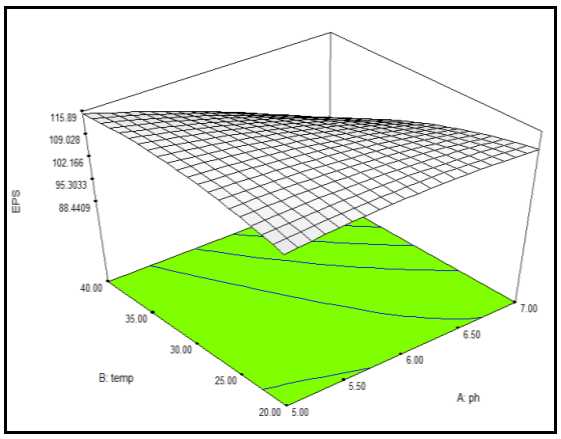

Figure 2: Response surface plot of the effect of pH (A) and temperature (B) on EPS yield.

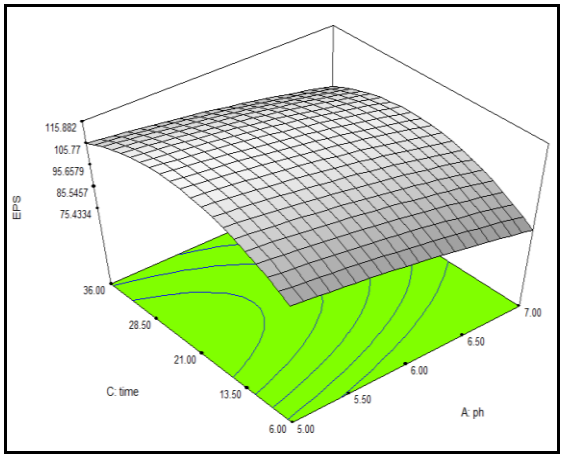

Figure 3: Response surface plot of the effect of pH (A) and time (C) on EPS yield.

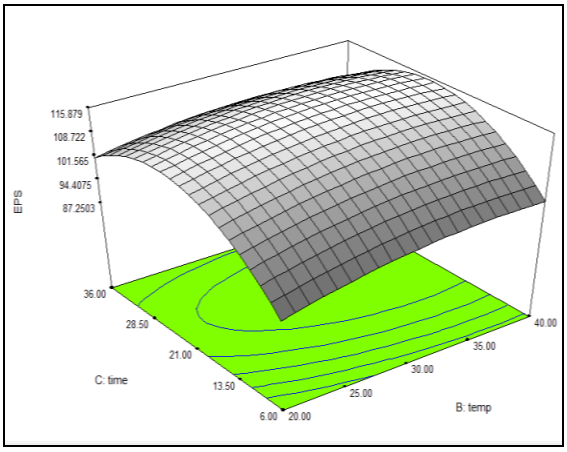

Figure 4: Response surface plot of the effect of temperature(B) and time (C) on EPS yield.

Table 5: Model validation experiments for *B. pseudocatenulatum* KAKii.

pH	Temperature (°C)	Time (h)	EPS production (mg/100mL)	
			Predicted	Experimental (triplicate)
5.0	34.75	23.61	115.893	116.01 115.50 115.70

FT-IR characterization of EPS

FT-IR spectra for the purified EPS samples were presented in Figure 5. The broad peak at 3435.58 cm^{-1} could be attributed to the stretching vibration of hydroxyl groups (Zhihong *et al.*, 2016; Venketaraman *et al.*, 2016). This group is important as an anticancer agent and can be recommended that EPS from the studied strain can be further explore on the effects towards the cancer cell line. The broad peak at 2088.70 cm^{-1}, could be attributed as C-H stretching vibration of methyl and methylene groups. Meanwhile the peak at 1645.74 cm^{-1} was assigned to the stretching of C=O and carboxyl group (Zhihong *et al.*, 2016). The weak peak appearing at 712.59 cm^{-1} was mainly attributed to the stretching vibration of C-O, and may attribute for the mannose sugar characteristic absorption (Vidya *et al.*, 2009). These chemical analyses supported a common pattern of FTIR spectra of EPS extracted from other probiotic.

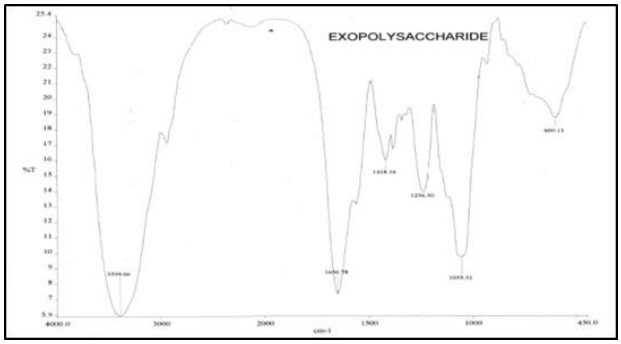

Figure 5: FTIR spectrum from EPS obtained.

GC-MS characterization of EPS

Based on the results obtained, it clearly showed that EPS extracted from *B. pseudocatenulatum* KAKii was composed of D-glucose, galactose, mannose and maltose and a strong presence of acetic acid as a by-product of EPS synthesis in the cell (Figure 6). These results indicated that extracted EPS from this specific *B. pseudocatenulatum* KAKii was came from hetero-exopolysaccharide group. EPS composed of identified sugars were also had a potential as an anticancer agents (Salma *et al.*, 2017). Besides that, the results obtained can be regarded as an initiatory steps towards the utilization and modification of EPS as future cheap

ISSN (print): 1823-8262, ISSN (online): 2231-7538

Malays. J. Microbiol. Vol 15(6) 2019, pp. 432-438
DOI: http://dx.doi.org/10.21161/mjm.180217

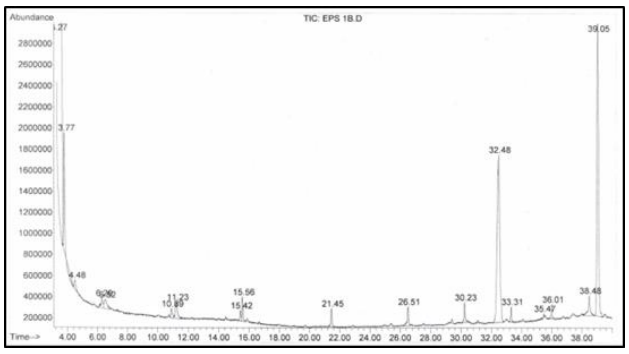

Figure 6: GCMS chromatograph for extracted EPS.

sources for production of valuable drugs for antioxidant and anticancer properties.

CONCLUSION

This study had demonstrated that the optimization of pH, temperature and time using response surface methodology (RSM) can greatly increase the EPS production from selected strain, *B. pseudocatenulatum* KAKii. The optimal condition consisted of pH 5.0 media, a temperature of 34.75 °C and cultivation time of 23.61 h could predicted EPS production of 115.89 mg/100mL. The triplicate experimental values fitted well with the predicted values with no significance difference ($P > 0.05$). The application of RSM improved the EPS production from this strain under certain pH, temperature and cultivation time. FT-IR spectra of the extracted EPS also showed similar pattern as other EPS extracted from other probiotic, indicating that a successful extraction of EPS was achieved and the presence of hydroxyl group and type of sugars (D-glucose, galactose, mannose and maltose) in it made it a potential anticancer agent in future. The result also showed that sugars identified from the extracted EPS indicated that the EPS from the strain was from hetero-exopolysaccharide group with a by-product which was acetic acid made it a promising anticancer agent.

ACKNOWLEDGEMENTS

This study was conducted at Universiti Teknologi MARA, UiTM Shah Alam. *Bifidobacterium* strains were obtained from Institute of Science, Universiti Teknologi MARA, UiTM Shah Alam.

REFERENCES

Amit, B. (2013). Characterization and optimization of production of exopolysaccharide from *Chlamydomonas reinhardtii. Carbohydrate Polymers* **95, 746-752.**

Fabio, A., Eduardo, M. B., Attilio, C., Paul, D., Ricardo, P. (2013). Bacteriocin production by *Bifidobacterium* spp. *Biotechnology Advances* **31, 482-488.**

Gao, H. and Gu, W. Y. (2007). Optimization of polysaccharide and ergosterol production from *Agaricus brasiliensis* by fermentation process. *Biochemical Engineering Journal* **33(3), 202-210.**

Gill, H. S. and Rutherfurd, K. J. (2001). Viability and dose-response on the effects of the immunoenhancing lactic acid bacterium *Lactobacillus rhamnosus* in mice. *Journal of Nutritional* **86, 285-289.**

Ishibashi, N., Yaeshima, T. and Hayasawa, H. (1997). Bifidobacteria: Their significance in human intestinal health. *Malays Journal Nutrition* **3, 149-159.**

Jones, M. L., Chen, H., Ouyang, W., Metz, T. and Prakash, S. (2004). Microencapsulated genetically engineered *Lactobacillus plantarum* 80 (pCBH1) for bile acid deconjugation and its implication in lowering cholesterol. *Journal of Biomedical Biotechnology* **2004, 61-69.**

Khalilah, A. K., Shuhaimi, M., Rosfarizan, M., Arbakariya, A., Yamin, S., Yazid, A. M., Siti, A. A. and Farrah, A. D. (2014). Optimization of milk-based medium for efficient cultivation of *Bifidobacterrium pseudocatenulatum* G4 using face-centered central composite-response surface methodology. *Biomedical Research International* **2014, 787989.**

Laws, A., Gu, Y. and Marshall, V. (2001). Biosynthesis, characterisation, and design of bacterial exopolysaccharides from lactic acid bacteria. *Biotechnology Advance Journal* **19, 597–625.**

Miyashita, M., Yukphan, P., Chaipitakchonlatarn, W., Malimas, T., Sugimoto, M., Yoshino, M., Potacharoen, W., Tanasupawat, S., Nakagawa, Y., Kirtikara, K. and Tanticharoen, M. (2012). 16S rRNA gene sequence analysis of lactic acid bacteria isolated from fermented foods in Thailand. *Journal of Microbiology* **28, 1-9.**

Pinar, S. and Gürcü, A. C. (2016). Exopolysaccharides production by lactic acid bacteria. *Applied Microbiology* **2(2), 1-5.**

Rosenfieldt, V., Michaelsen, K. F., Jakobsen, M., Larsen, C. N., Moller, P. L., Tvede, M., Weyrehter, H., Valerius, N. H. and Paerregaard, A. (2002). Effect of probiotic *Lactobacillus* strains on acute diarrhea in a cohor nonhospitalized chidren attending day-care centers. *Pediatric Infection Disease Journal* **21, 417-419.**

Salma, M. A., Shaymaa, M. M. Y., Wafaa, F. Mohsen, M. S. A., Hala, M. A. S., Manal, G. M. and Magdy, A. G. (2017). Antitumor exopolysaccharides derived from novel marine *Bacillus*: Isolation, characterization aspect and biological activity. *Asian Pacific Journal of Cancer Prevention* **18, 1847-1854.**

Seema, P., Avishek, M. and Arun, G. (2012). Potentials of exopolysaccharides from lactic acid bacteria. *Indian Journal Microbiology* **52(1), 3-12.**

Shu. Q. and Gill, H. S. (2002). Immune protection mediated by the probiotic *Lactobacillus rhamnosus* HN001 (DR20) against *Escherichia coli* Q157: H7 infection in mice. *FEMS Immunology Medical Microbiology Journal* **34, 59-64.**

 ISSN (print): 1823-8262, ISSN (online): 2231-7538

Tortora, G. J., Funke, B. R., Case, C. L. (2015). Microbiology: An Introduction. 12th Edn. Pearson. Global Edition Harlow, United Kingdom. ISBN-13: 978-0321929150. **pp. 120-126.**

Venkataraman, D., Sureshbabu, R. K. P., Shiva, D. S., Hariharan, N. and Krishnan, S. (2016). Optimization of anticancer exopolysaccharide production from probiotic *Lactobacillus acidophilu*s by response surface methodology. *Preparative Biochemistry and Biotechnology* **46(3), 288-297.**

Vidya, P. K., Subhasish, D. and Ramkrishna, S. (2009). An exopolysaccharide from a probiotic: Biosynthesis dynamics, composition and emulsifying activity. *Food Research International* **42(5-6), 695-699.**

Zhihong, Z., Zhengqi, L., Xueying, T. and Hua, W. (2016). Characterization and sulfated modification of an exopolysaccharide from *Lactobacillus plantarum* ZDY2013 and its biological activities. *Carbohydrate Polymers* **153, 25-33.**