



BIOCHEMICAL CHARACTERIZATION OF EXOPOLYSACCHARIDES AND IT ACT AS THE PREBIOTIC

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ABSTRACT **BACKGROUND INFORMATION** : In recent years, micro and macro algae are very much attracted by researchers, due to their global demand. The algae are the source of various types of secondary metabolites, which possess various biological activities. **METHODS:** Exopolysaccharide extracted from microalgae *S.platensis* by alcoholic precipitation method and analyzed. The chemical composition of the extract and its prebiotic potential were analyzed. EPS acts as the prebiotic substances and they enhanced the growth of beneficial microorganisms in the gut microbiome. **RESULTS:** The EPS production was optimized by different concentrations of sodium chloride, sodium nitrate, and various pH conditions. The carbohydrate, protein, phenol, sulfate and uronic acid content were analyzed for all the optimized samples. The prebiotic characteristics of extracted EPS were also analyzed. According to the results, this EPS enhanced the growth of *Lactobacillus* sp. The extracted EPS has enhanced the growth of *Lactobacillus* sp.

KEYWORDS

Prebiotics, Eps, *S.platensis*

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INTRODUCTION:

Microalgae are autotrophic microorganisms and they synthesized the bioactive compounds which are high nutritional value and therapeutic functions by using the light energy and inorganic nutrients (Markou and Nerantzis, 2013). Polysaccharides and oligosaccharides from microalgae are promising compounds with potential health benefits and is used as prebiotics (Wells *et al.*, 2017). *Arthrospira*, *Chlorella*, and *Nannochloropsis* are a very good source of proteins and also the important sources of polysaccharides or oligosaccharides, being proposed as potential prebiotic (Roposo *et al.*, 2016). The secondary metabolites from *Arthrospira* sp. can stimulate the growth of required probiotic bacteria in yogurts and fermented milk, increasing the viability of the probiotics (Moreno *et al.*, 2017)

Prebiotics are nondigestible food constituent that affects the host by motivating the growth of valuable bacteria in the colon, and thus improves host health" (Gibson and Roberfroid, 1995). The characteristics of potential prebiotic are resistance to digestion in the upper sections of the alimentary tract, easily fermented by intestinal microflora, produced some beneficial effect on host's health, to stimulate the growth of probiotics and it is stable in various food processing conditions (Ze *et al.*, 2012. Angelakis, 2017)

Prebiotic substances that stimulate the growth of the microorganisms present in the intestinal ecosystem of the host. Stimulation of the intestinal microbiota by prebiotics that enhances their fermentation activity, at the same time it influenced the SCFA level, which confers a health benefit on the host (Van Den Abbeele, 2013)

The carbohydrates used as prebiotics, that resist digestion and can be easily metabolized by certain gut bacteria has attracted most of this attention (Rastall, 2010).

Prebiotics possess several valuable effects, enhance the growth of beneficial bacteria, stimulation of intestinal peristalsis, production of short-chain fatty acids, and a shortened orofecal transit time (Cummings *et al.*, 2001).

Spirulina platensis is a planktonic, filamentous blue green algae found in freshwater environments. It contains 78% of proteins, 13.6 % of carbohydrates, vitamins, 4-7 % of lipids, and some natural pigments. This algae uptake nitrogen from the medium and released extracellular polysaccharides from the surrounding environment. These extracellular products promote the growth of three probiotic bacteria such as *L.lactis*, *B.longum*, and *L.bulgaricus*. (Parada *et al.*, 1998). The main purpose of this study was to analyze and evaluate the chemical composition and the prebiotic characteristics of exopolysaccharide from the microalgae *S. platensis*.

MATERIALS AND METHODS:

The culture *S. platensis* was grown in standard Zarrouk medium (Zarrouk, 1966) and maintained pH at 9.5-10.5 at 37°C. The culture flasks were aerated with 1ml/min of air provided by aeration pump and constantly irradiated with a light intensity of 2500 lux for 9 days.

Precipitation of EPS by using ice-cold ethanol:

The *Spirulina* culture was centrifuged at 15,000 rpm for 20 min at 4°C. The supernatant was pressure-filtered through a rotary vacuum evaporator and concentrated. The EPS was precipitated after the addition of three volumes of ice-cold ethanol and the solution was chilled to 4°C overnight. Excess water was removed under vacuum evaporator before lyophilization. The lyophilized exopolysaccharide powder was stored at room temperature. The extracted EPS was characterized by FTIR.

The EPS production was optimized by different concentrations of NaCl [1g (T1), 2g (T2), 3g (T3), 4g (T4), 5g (T5) and 6g (T6)], NaNO_3 [0.125g (T1), 0.25g (T2), 0.375g (T3), 0.55g (T4), 0.675g (T5)], and various pH conditions (pH-7, pH-8, pH-9, pH-10, pH-11, pH-12). The salt tolerance and nitrogen concentration induce the metabolic and structural changes in cyanobacteria.

Bio chemical characterization of EPS:

The carbohydrate content of *Spirulina* EPS was determined by the phenol sulfuric acid method (Dubois *et al.*, 1956). The protein content

was estimated by the Lowry method (Lowry *et al.*,1951), the phenolic content was calculated by the Folin-Ciocalteu method, the sulfate content was analyzed by Barium chloride gelatin assay method (Dodgson and Price, 1962) and the uronic acid content was calculated by Carbazole method using galactouronic acid as a standard (Bitter and Muir,1962). DPPH radical scavenging assay was calculated by using 1,1-diphenyl-2-picrylhydrazyl (Li *et al.*,2006)

Prebiotic characteristics of extracted EPS:

Prebiotic score:

A prebiotic score is used to estimate the health effects of prebiotics in humans or animals. It is used to study the ability of the extracted exopolysaccharide to support the growth of the probiotic organisms (Anprung and Sangthawan,2012). De Mann Rogosa Sharpe agar (MRS) is the selective media for probiotics. MRS broth was prepared with EPS as a carbon source, instead of glucose used as a control. *Lactobacillus* sp., *Bifidobacterium* sp., *Fusobacterium* sp., were used as the representative probiotic cultures.

Resistance towards Acid Digestibility:

Non-digestibility of exopolysaccharides is defined as the ability to resist digestive processes which include resistance towards the gastric acidity, hydrolysis by mammalian enzymes, and gastrointestinal absorption.

Prebiotic efficiency:

The monosaccharides as prebiotic additives were empirically chosen to stimulate the growth of probiotic microorganisms. The exopolysaccharide from *S.platensis* was incorporated into the MRS medium. The probiotic organisms were inoculated. The prebiotic efficiency was calculated by the growth of probiotics was measured by using a spectrophotometer at different time intervals (0th, 1st, 2nd, 3rd, 4th and 5th hours)

RESULTS:

The present study mainly emphasizes the optimization of EPS production and their biochemical characterization and biological activities. All the triplicate readings were tabulated and the graphs were performed by Origin 8.5 data analysis and graphing Software. In this study, the EPS production was optimized by different concentrations of sodium chloride, sodium nitrate, and various pH conditions. The carbohydrate, protein, sulfate, phenol and uronic acid contents were analyzed.

Fig 1. Optimization of EPS production at different concentration of Sodium chloride

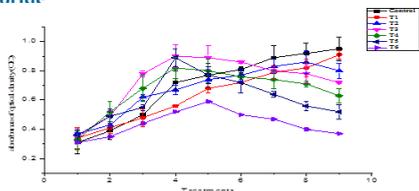


Fig .2. Optimization of EPS production at various pH conditions

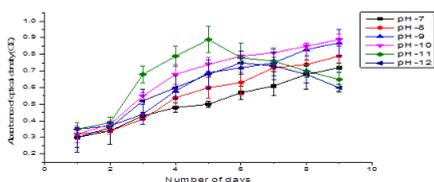


Fig 3. Optimization of EPS production at different concentration of sodium nitrate

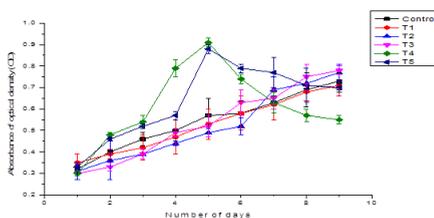


Fig 4. Bio chemical characterization of EPS from different concentration of sodium chloride

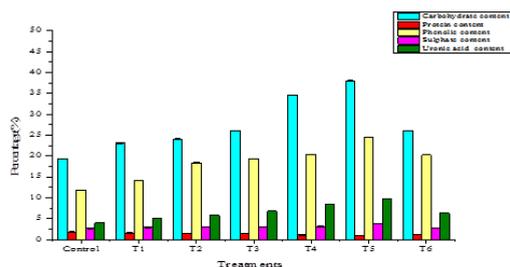


Fig.5.Bio chemical characterization of EPS from various pH conditions

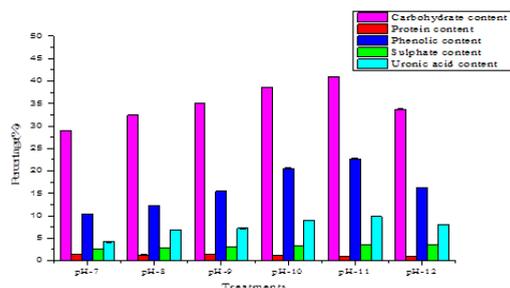


Fig.6. Bio chemical characterization of EPS from different concentration of sodium nitrate

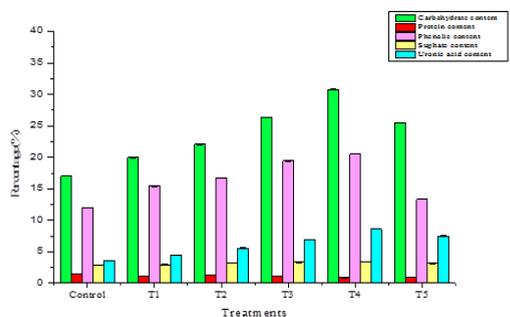


Fig.7 Prebiotic score:

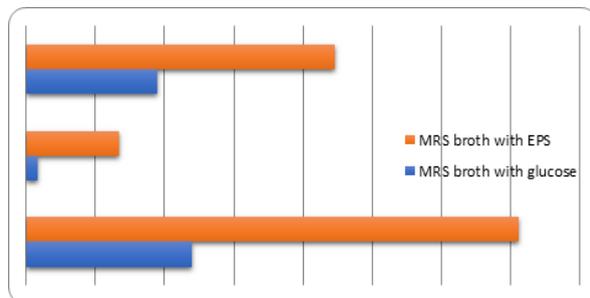


Fig.8 Prebiotic efficiency

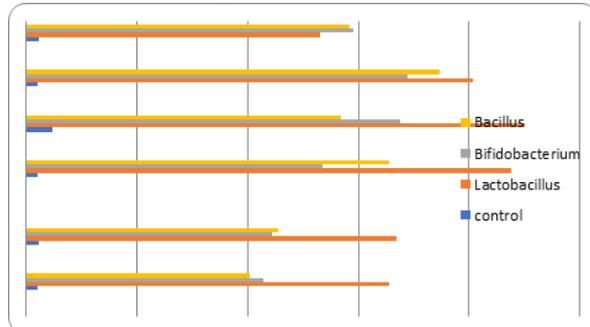
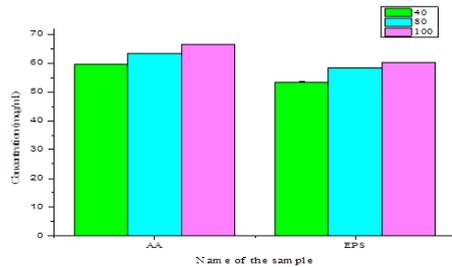


Fig9. DPPH Assay of EPS



DISCUSSION:

Chentir *et al.*, (2017) investigated that the algae *Spirulina* was cultivated in two-step cultivation processes such as a higher salinity condition (NaCl concentration 40g/L) and the light intensity was low ($10\mu\text{mol photons m}^{-2}\text{s}^{-1}$) enhanced the production of EPS. He also reported the extract with EPS contains 67.3 % of carbohydrate, 5.14 % of proteins, 2.42 % of sulfate.

Angelis *et al.*, 2012 extracted the EPS using the Co-Culture method (used cyanobacteria and macromycetes). The EPS obtained from a pure culture of *S.platensis* contained 22.1% of carbohydrate and co-culture of (*S.platensis* + *A.blazei*) and (*S.platensis* + *T.versicolor*) contained 17.3% and 27.3% respectively.

Lee *et al.*, (2011) proposed that *Spirulina* was cultivated in a two-stage culture method under 0.75mol/L of NaCl concentration and $196\mu\text{mol photons m}^{-2}\text{s}^{-1}$ for the production of EPS. The total amount of carbohydrate content was 17.6 %.

The Zarrouk medium with 5g (Fig.1) of NaCl contained 38% of carbohydrate, 1.01% of protein, 24.4% of phenol, 3.33% of sulfate, and 9.7% of uronic acid (Fig.4). The results of the present study agreed with the report of Chen *et al.*, (2006) salt tolerance ability enhanced the carbohydrate metabolism.

In the present work, *Spirulina* was cultivated for EPS production in the Zarrouk medium at various pH conditions. (Fig.2). The EPS extracted from Zarrouk medium with pH 11 (Fig.5) possess the high carbohydrate content (40.84%), protein content (0.93%), phenol content (16.3 %) sulphate content (3.56%) and the uronic acid content (9.0%).

The *Spirulina* was cultivated under different concentrations of sodium nitrate. EPS from the medium containing 0.55g of sodium nitrate have high carbohydrate content (30.8%), protein content (0.88%), phenol content (20.5%), sulphate content (3.46%) and the uronic acid content (8.6%) (Fig.6).

De Philippis *et al.*, (2001), Behera and Varma, (2016) reported that carbohydrates extracted from algal species, it provided the extra source of nutrients for the renovation to the energy required for the growth and metabolic activities of probiotics, resulting in stimulation of probiotic bacterial growth.

Al-Sheraji *et al.*, (2013) proposed that any carbohydrate that enters the cecum it act as the substrate for fermentation. The probiotic bacteria fermented this substrate to produce short-chain fatty acids, they are organic fatty acids with 1 to 6 carbon atoms. SCF improves bacterial survival and enhances the absorption of Ca, Mg, and Fe, and also it must able to keep away from cancer and destroy any pathogens in the large intestines.

Bhowmik *et al.*, (2009), proposed that the growth of lactic acid bacteria was improved in the presence of *S. platensis*. Parada *et al.*, (1998) explained that *S.platensis* biomass consumed the nitrogen in the growth medium and released the extracellular carbohydrates and other growth substances which, may be accountable for stimulating the growth of *Lactobacilli* (and other lactic acid-producing strains). Therefore *S. platensis* extracellular carbohydrate can alternatively substitute for inulin (from chicory) as a prebiotic

Rolfe *et al.*, (2012) explained that the algal extract was obtained by using different solvents contained the oligo, di, and polysaccharide with a longer grade of polymerization, slower fermentation rate to facilitate the formation of by-products and to generate a new

environment that is more favorable for the probiotic bacteria to use all the types of carbohydrates in the algal extract, thus enhancing the growth of beneficial bacterial in the intestine.

In our study, The EPS has substituted the carbon source in the MRS medium. The prebiotic score and prebiotic efficiency (Fig..8) were calculated. The EPS from *Spirulina* enhanced the growth of *Lactobacillus* sp prebiotic score 1.423 (Fig..7) and prebiotic efficiency 2.25 (Fig.8). These results were similar to the report of Haider *et al.*, (2016). He demonstrated that different types of algal aqueous extracts were collected and tested for prebiotic potential. This *S. platensis* extract was significantly increasing the growth of three probiotic bacteria at 48 h, and the other algae such as *D. salina*, *S. magus*, *Chlorococcum*, and *Chorella* on *L. lactis*; *D. salina*, *S. magus*, *Chlorococcum*, and algal extract no. 48 enhanced the growth of *L. bulgaricus*. These aqueous algal extracts significantly augmented the growth of probiotic bacteria when compared to inulin, and were regarded as exhibiting prebiotic activity.

Add El -Baky *et al.*, (2009) reported that DPPH scavenging activity based on the phenolic content, methods used for extraction and the media composition. In this study the different concentration of EPS samples (40,80,100 g/ml) were analysed for DPPH assay. The highest scavenging activity (70.18%) was found at the 100 g/ml of EPS (Fig.9).

CONCLUSION:

This study demonstrated the extraction of extracellular polysaccharide from *S.platensis* by alcoholic precipitation method. The EPS production was optimized by different concentrations of sodium chloride, sodium nitrate, and various pH conditions. The carbohydrate, protein, phenol, sulfate and uronic acid content were analyzed for all the optimized samples. The prebiotic characteristics of extracted EPS were also analyzed. According to the results, this EPS enhanced the growth of *Lactobacillus* sp.

REFERENCES

- Parada JL, Zulpa De Cariae G, Zaccaro De Mule MAC, Storni De Cano MM (1998). Lactic acid bacteria growth promoters from *Spirulina platensis*. *Int J Food Microbiol* 45(3):225- 228
- Gibson GR, Roberfroid MB (1995). Dietary modulation of the human colonic microbiota: introducing the concept of prebiotics. *J Nutr* 125: 1401-1412.
- Ze, X.; Duncan, S.H.; Louis, P; Flint, H.J. (2012). Ruminococcus bromii is a keystone species for the degradation of resistant starch in the human colon. *ISMEJ*, 6, 1535-1543.
- Angelakis, E. Weight gain by gut microbiota manipulation in productive animals. *Microb. Pathog.* 2017, 106, 162-170.
- Van Den Abbeele, P; Venema, K.; van de Wiele, T; Verstraete, W; Possemiers, S. (2013). Different human gut models reveal the distinct fermentation patterns of arabinoxyylan versus inulin. *J. Agric. Food Chem.* 61, 9819-9827
- Cummings, J.H., G.T. Macfarlane, H.N. Englyst. (2001). Prebiotic digestion and fermentation. *Am. J. Clin. Nutr.* 73:S415-S420.
- De Philippis R, Sili C, Paperi R, Vincenzini M. (2001). Exopolysaccharide-producing cyanobacteria and their possible exploitation: a review. *J. Appl. Phycol.* 13, 293-299.
- Dodgson KS, Price RG (1962). A note on the determination of the ester sulphate content of sulphated polysaccharides. *Biochem J* 84:106-110.
- Dubois M, Gillis, KA, Hamilton, JK, Rebers PA, and Smith F (1956). Colorimetric method for determination of sugars and related substances. *Analytical chemistry*, 28, 350-356.
- Zarrouk, C. Contribution à l'étude d'une cyanophycée: influence de divers facteurs physiques et chimiques. B.K. & varma, A. 2016. Microbial resources for sustainable energy. Springer. miques sur la croissance et photosynthèse de *Spirulina maxima* Geitler. 1966. Thesis (Ph.D.) - University of Paris, Paris, 1966.
- Al-sheraji, s. H., ismail, a., manap, m. Y., mustafa, s., yusof, r. M. & hassan, f. A. (2013). Prebiotics as functional foods: a review. *Journal of functional foods*, 5, 1542-1553.
- Bhowmik, d., Dubey, j. & Mehra, s. (2009). Probiotic efficiency of *Spirulina platensis*- stimulating growth of lactic acid bacteria. *World journal of dairy food science*, 42, 160- 163.
- Rolfe, m.d., Rice, c.j., Lucchini, s., Pin, c., Thompson, a., Cameron, a.d., Aston, m., Stringer, m.f., Betts, r.p., Baranyi, j. & Peck, m.w. (2012). Lag phase is a distinct growth phase that prepares bacteria for exponential growth and involves transient metal accumulation. *Journal of bacteriology*, 194, 686- 701

14. Behera, b.k. and varma, a. 2016. Microbial resources for sustainable energy, springer.
15. Angelis. S., Novak. A.C., Sydney. E.B. Soccol. V.T., Carvalho. J.C., Pandey. A., Nosedá. M. D.,
16. Tholozan. J.L., Lorqun. J., Scoccol. C.R. (2012). Co-Culture of Microalgae, cyanobacteria and Macromycetes for exopolysaccharide production: Process preliminary optimization and partial characterization. *Appl Biochem Biotechnol* 167:1093-1106
17. Bitter T, Muir HM (1962). A modified uronic acid carbazole reaction. *Anal Biochem* 4:330-334
18. Wells ML, Potin P, Craigie JS, Raven JA, Merchant SS, Helliwell KE, Algae as nutritional and functional food sources: revisiting our understanding. *J Appl Phycol.* (2017) 29:949-82.
19. Raposo MFJ, Bernardo de Morais AMM, Costa de Morais RMS. Emergent sources of prebiotics: seaweeds and microalgae. *Mar Drugs* (2016) 14:E27.
20. Moreno JF, Corzo N, Montilla A, Villamiel M, Olano A. (2017). Current state and latest advances in the concept, production and functionality of prebiotic oligosaccharides. *Curr Opin Food Sci.* 13:50-5.
21. Anprung P, Sangthawan S. (2012). Prebiotic activity and bioactive compounds of the enzymatically depolymerized Thailand-grown mangosteen aril. *J Food Res*;1:268-276.
22. Chentir I, Hamdi M, Doumandji A, HadjSadok A, Ouada HB, Nasri M, Jridi M. (2017). Enhancement of Extracellular Polymeric Substances (EPS) Production in *Spirulina* *Arthrospira* Sp. by Two-Step Cultivation Process and Partial Characterization of Their polysaccharidic Moiety. *Int J Biol Macromol.* :1412-1420.
23. Lee Meng-Chou, Yean-Chang Chen, Tzu-Chien Peng, (2011). Two stage culture method for optimized polysaccharide production in *Spirulina platensis*. *J Sci Food Agric*. 92:1562-1569
24. Giorgos Markou, Elias Nerantzis (2013). Microalgae for high value compounds and biofuels production: Review with focus on cultivation under stress conditions. *Biotechnol Adv.* 1853-42
25. Hadebe, Nontando (2016). Isolation and characterization of prebiotic oligosaccharide from algal extracts and their effects on gut microflora. URI: <http://hdl.handle.net/10321/1754>.
26. Lowry, H.O., Rosenbrough, N. J., Farr, A. L., and Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* 193, 265-275.
27. Chen L-Z., Li D-H, Song L-R, C-X, Wang G-H, Liu Y-D (2006) Effects of salt on carbohydrate metabolism in desert soil alga *Micrococcus vaginatus* Gom. *J Integr Plant Biol* 48:914-919.
28. Li X, Zhou A, Han Y. *Carbohydrate polymers* 66 (2006) 408-416
29. Abd EI-Baky, F.K. EI Baz, G.S. EI-Baroty, African journal of Biotechnology (2009) 7059-7067.