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Exposition of antitumour activity of a chemically characterized exopolysaccharide from a probiotic *Lactobacillus plantarum* MTCC 9510*

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Abstract: As majority of chemical compounds identified as anti-cancerous are toxic to normal cells, the discovery and identification of new safe drugs is a necessity in the biomedical field. The antioxidant, antitumour and immunomodulating properties of an exopolysaccharide of sequence -α-D-glucose, α-D-mannose and β-D-glucose-, purified from a probiotic *Lactobacillus plantarum* was studied. The immunostimulation of the compound in human lymphocytes was seen at a concentration of 0.1 mg/mL with 20% proliferation rate. The antitumour studies by morphological apoptosis determination and 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide assay on breast adenocarcinoma cell line (MCF-7) exhibited an IC_{50} of 10 mg/mL for the compound.

Key words: antitumour; cytotoxicity; exopolysaccharide; immunomodulation; probiotic.

Abbreviations: AO, acridine orange; COSY, correlation spectroscopy; DMEM, Dulbecco's modified eagle medium; EB, ethidium bromide; EPS, exopolysaccharide; FBS, foetal bovine serum; HMQC, hetero-nuclear multiple quantum coherence; HSQC, hetero-nuclear single quantum coherence; IC, inhibitory concentration; MCF-7, Michigan Cancer Foundation-7; MTT, 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide; NOESY, nuclear Overhauser effect spectroscopy.

Introduction

Exopolysaccharides (EPSs) exhibit wide spectrum applications in pharmaceutical, biomedical and food industries. EPSs possess the potential to contribute to human health as prebiotics or due to its antitumour, antiulcer, immunomodulating or cholesterol-lowering activities (Ruas-Madiedo et al. 2002). Natural or biopolysaccharides derived from microbial and plant sources were reported to have antioxidant activities (Kong et al. 2010) and antitumour activity (Leung et al. 2006). The major biotechnological advantages of microbial polysaccharides are short fermentation process and easily formed and stable emulsions. These polysaccharides usually have low cytotoxicity and side effects, which make them good candidates for immunotherapy against cancer and as anti-oxidants (Yu et al. 2001).

Reactive oxygen species are known to be involved in various biological processes resulting in the development or progression of several diseases. EPSs are found to participate in the removal of free radicals, thereby functioning as potent anti-oxidants. Two main bioactivities exhibited by EPS from lactic acid bacteria are the anticancer and immunomodulatory effects. The potential broad spectrum bioactivity of this class of compounds as anti-cancer adjuvants is highlighted by the biological mechanisms, such as apoptotic and anti-angiogenic effects including their effects on the c-Myc, c-Fos and vascular endothelial growth factor expression (Yang et al. 2005). EPS produced by *Lactococcus lactis* ssp. *cremoris* KVS20 exhibits bioactivity, such as lymphocyte mitogenicity (Kitazawa et al. 1993), macrophage cytostaticity, and cytokine (IFN-γ and IL-1β) production in macrophages (Kitazawa et al. 1996). Mechanism of antitumour action of polysaccharides consists in the stimulation of certain components of the immune system, mainly T- and B-lymphocytes, macrophages, and induction of interleukin release by NK cells (Wasser 2002; Lin & Zhang 2004). Polysaccharides are most often administered parenterally, sometimes orally, when the presence of peptide fragment allows for such route. Method of administration of these compounds, resulting mostly from their chemical structure, is not burdensome to patients, which is the undoubted advantage of these compounds.

An important feature concerned with the bioac-

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tivity of immunomodulatory polysaccharides is their structure-function relationship. Molecular weight, tertiary structure or conformation and composition can affect the biological activity of polysaccharides. In general, polysaccharides in a configuration with β-1,3, β-1,4, or β-1,6 branch chains are necessary for activity; and complex branch-chained polysaccharides with anionic structures and higher molecular weights have greater immuno-stimulating activities (Cleary et al. 1999). β-Glucans (with the 1,3-linkage having substitution at C-2) producer lactic acid bacteria belonging to the *Lactobacillus* and *Pediococcus* genera are able to immunomodulate macrophages *in vitro* (Fernandez de Palencia et al. 2009; Garai-Ibabe et al. 2010). Differences in bioactivity may be due to differences in receptor affinity or receptor-ligand interaction on the cell surface (Mueller et al. 2000). The present study unveils the therapeutic properties of the EPS produced by *Lactobacillus plantarum* MTCC 9510 and its relation to the elucidated structure.

Material and methods

Bacterial strain and media

Lactobacillus plantarum MTCC 9510 having vital probiotic features is a facultative anaerobe isolated from curd (Aswathy et al. 2008). The organism was subcultured in de Man Rogosa-Sharpe medium (Himedia, Mumbai) and was maintained in de Man Rogosa-Sharpe agar at 4 ℃ for immediate use and prepared 20% (w/v) glycerol stocks for long time preservation at –80 ℃.

Cell lines and maintenance

The human breast adenocarcinoma cell line (Michigan Cancer Foundation-7; MCF-7) for antitumour studies and normal cell line L929 (American Type Culture Collection; Manassas, VA, USA) in Dulbecco's Modified Eagle Medium (DMEM; Gibco, Invitrogen, Grand Island, NY, USA) supplemented with 10% (v/v) foetal bovine serum (FBS; Gibco), 100 IU/mL penicillin and 100 µg/mL streptomycin, were maintained at 37 ℃ in a 5% CO_2 humidified incubator. Lymphocytes were maintained in RPMI 1640 Medium (Invitrogen, Grand Island, NY, USA) with 10% (v/v) FBS (Gemini Bio-Products, Inc., CA, USA) at 37 ℃ in 5% CO_2 incubator.

EPS production, extraction and purification

Eighteen-hour old inoculum (10^9 CFU/mL) was prepared in de Man Rogosa-Sharpe medium by incubating in static condition at 37 ℃. EPS production was achieved in EPS production medium comprising lactose, yeast extract, ammonium sulphate, di-potassium hydrogen phosphate, sodium acetate, magnesium sulphate, manganese sulphate and Tween 80 (Ismail & Nampoothiri 2010) incubated under the same conditions for 72 h. The culture was centrifuged at 11,500×g for 15 min at 4 ℃ to remove cell pellet. Double volume-chilled ethanol was used to precipitate the supernatant and incubated overnight at 4 ℃. The mixture was centrifuged at 2,500×g for 20 min and the pellet collected was dissolved in de-mineralised water, and again precipitated using double-volume cold ethanol. It was further centrifuged at 2,500×g for 20 min to collect the pellet. The total carbohydrate present in the pellet was estimated by phenol-sulphuric acid method (Dubois et al. 1956). The pellet was dialysed using 5 kDa membrane against distilled water for 24 h with two changes of water and lyophilized. The lyophilized crude EPS was purified as per our previous report (Ismail & Nampoothiri 2010).

Monosaccharide sequence and linkage confirmation of EPS by two-dimensional NMR techniques

A combination of homo-nuclear and hetero-nuclear NMR experiments was carried out at 500 MHz using pure EPS from *L. plantarum*. A set of homo-nuclear experiments like correlation spectroscopy (COSY) and nuclear Overhauser effect spectroscopy (NOESY) were performed to assign the ^{1}H resonances and sequence confirmation. ^{13}C resonances were assigned with the help of hetero-nuclear experiments like hetero-nuclear single quantum coherence (HSQC) and hetero-nuclear multiple quantum coherence (HMQC). The two-dimensional NMR was recorded at room temperature using a Bruker Avance II-500 spectrometer. The polysaccharide was dissolved and analyzed in 99.96% D_2O yielding clear solutions at 30 mg/500 µL, spectra were referenced to internal trimethylsilylpropanoic acid. For COSY NMR spectra the sample was submitted to a delay (D1) and acquisition time (AQ) of 1.4 s and 0.2 s, respectively, whereas for NOESY NMR, the D1 and AQ were 1.8 s and 0.3 s, respectively. Chemical shifts were expressed in parts per million (ppm). In all experiments the number of scans was optimized to achieve a signal-to-noise ratio higher than 150 in order to minimize the experimental uncertainty due to the noise level (below 5%).

In vitro assay for anti-oxidant activity

Anti-oxidant activity of crude and pure EPS was investigated by reducing power assay (Oyaizu 1986). The concentration of crude and pure EPS selected for the studies were in the range of 0.05-1 mg/mL. Reaction was carried out in a mixture containing 2.5 mL of sample (0.05-1 mg/mL), 2.5 mL of 0.2 M sodium phosphate buffer (pH 6.6) and 2.5 mL of $K_3Fe(CN)_6$ (1%, w/v) by incubating at 50 ℃ for 20 min. Trichloroacetic acid (2.5 mL, 10%, w/v) was added to the reaction mixture and was centrifuged at 5,000 rpm for 10 min. The upper layer (5 mL) was mixed with 0.5 mL of fresh $FeCl_3$ (0.1%, w/v), and the absorbance was measured at 700 nm against a blank. A higher absorbance indicates a higher reducing power. De-ionized water and ascorbic acid were used as blank and control, respectively.

In vitro assay for cytotoxicity

Cytotoxicity of *L. plantarum* EPS in normal cells was checked using L929 fibroblast cell line. Approximately 5×10^3 cells in 100 µL of DMEM [10% v/v FBS] were seeded per well in 96 well plates. The cells were incubated overnight at 37 ℃ in a humidified incubator of 5% CO_2. The cells were then treated with EPS of different concentrations 1×10^{-6}–50 mg/mL. The cells were incubated for 24, 48 and 72 h at 37 ℃ under the same conditions. 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay was performed (van de Loosdrecht et al. 1994) to see the inhibitory effect of the polysaccharide in normal cells. Growth inhibition rate (GIR) was calculated based on the equation: GIR = 100 – Proliferation Rate (PR), where PR = (A_{570} of treated cells/A_{570} of control (untreated) cells) × 100.

Antitumour activity of L. plantarum EPS

Morphological apoptosis determination

Human breast adenocarcinoma cells (MCF-7) treated with *L. plantarum* EPS (1×10^{-6}–25 mg/mL) were observed morphologically by fluorescent microscopy for apoptosis. Two

different morphological staining techniques employing fluorescent DNA binding dyes, Hoechst, ethidium bromide and acridine orange (EB/AO) were made to use for the purpose. For Hoechst staining, approximately 10^6 cells were placed in a test tube and centrifuged at $300\times g$ for 5 min. Supernatant was removed and 500 µL of DMEM were added and pre-warmed to 37℃. The mixture was mixed gently and 5µL of Hoechst 33342 stock solution (1 mg/mL) was added and mixed again. The solution was incubated at 37℃ for 45 min. After treatment, the cells were washed three-times with 1X PBS and viewed under microscope. EB/AO staining was performed by adding 25 µL of 1 X EB/AO to the treated cells and viewed under microscope (Ribble et al. 2005).

In vitro assay for antitumour activity

The human breast adenocarcinoma cells (MCF-7) were seeded in 96 well plates at a concentration of approximately 5×10^3 cells per well in 100 µL of DMEM (10% v/v FBS). The cells were incubated overnight at 37℃ in a humidified incubator of 5% CO_2 and then treated with EPS of varying concentrations (1×10^{-6}–25 mg/mL). The antitumour property of the polysaccharide was studied by cellular viability assessment employing MTT assay (van de Loosdrecht et al. 1994) and was compared with an anticancer drug doxorubicin in the same concentrations as the polysaccharide.

In vitro assay for lymphocyte proliferation

Lymphocytes were isolated from heparinised blood of a healthy, adult male volunteer by Ficoll-Hypaque^{TM} centrifugation method of Boyum (1968). Cells were washed twice in 0.5 mL RPMI 1640 with 10% v/v FBS, by centrifugation at 3,500 rpm for 2 min. The final pellet was suspended in 10 mL PBS. Approximately 2×10^4 lymphocytes in RPMI 1640 (10 % v/v FBS) medium were seeded per well in 96-well plates and incubated with *L. plantarum* EPS of various concentrations: 1×10^{-6}–25 mg/mL. Cells were incubated at 37℃ for 72 h in a humidified incubator which maintained a constant atmosphere of 5% CO_2. Finally, the lymphocyte proliferation was checked by MTT assay. The optical density at 570 nm (A_{570}) of the cells was measured in an ELISA reader (BIO-RAD 550, USA).

Experimental statistics

All treatments and assays were performed in triplicates and the results represented by their mean ± SD (standard deviation).

Results and discussion

Monosaccharide sequence and linkage confirmation of EPS by two-dimensional NMR techniques

The two-dimensional NMR COSY spectrum profiled the chemical shifts of each proton in the monosaccharide units of the EPS. The profile of the spectrum coincided with the one-dimensional ^{1}H NMR spectrum. By correlating the proton shifts with the carbon shifts obtained from ^{13}C-^{1}H HMQC and HSQC, the resonances of the anomeric and the non-anomeric carbons were assigned. The signals from NOESY confirmed the signals from COSY and the sequence of monosaccharides present in the EPS backbone. The proton-proton interactions were obtained from COSY and NOESY spectra. The interaction of the anomeric protons of the three residues with the adjacent protons were assigned from COSY and NOESY spectra. The one dimensional ^{1}H NMR showed a singlet, doublet and a triplet signal corresponding to three prominent hexoses in the anomeric region (Ismail & Nampoothiri 2010); the correlation of this anomeric proton resonance with the adjacent proton resonance was assigned from the COSY experiment. The additional information of spatial interaction between the residues was obtained from NOESY, which could lead to monosaccharide sequence determination and linkage between the residues.

Table 1. Summary of ^{1}H-^{1}H COSY NMR, ^{1}H-^{13}C HMQC and HSQC of *Lactobacillus plantarum* EPS.[a]

H-H signals/ C-H signals	Monosaccharide units		
	α-D-Glucose	α-D-Mannose	β-D-Glucose
H1/H2	5.0/4.0	5.2/4.1	4.8/4.4
H1/C1	5.0/100.78	5.2/103.09	4.8/104.7
H2/H3	4.0/3.8	4.1/3.9	4.4/3.4
H2/C2	4.0/68.87	4.1/78.77	4.4/69.40
H3/H4	3.8/3.6	3.9/3.8	3.4/3.3
H3/C3	3.8/78.42	3.9/81.23	3.4/80.97
H4/H5	3.6/3.4	3.8/3.7	3.3/3.2
H4/C4	3.6/75.63	3.8/59.40	3.3/68.87
H5/H6	3.4/3.2	3.7/3.5	3.2/3.0
H5/C5	3.4/76.03	3.7/73.32	3.2/74.94
H6/C6	3.2/74.94	3.5/69.61	3.0/–

[a] A standard deviation of less than5 % was observed. H-H and C-H signal are proton-proton and carbon-proton signals, respectively.

The cross peaks of the anomeric protons with the non-anomeric protons were clearly obtained from ^{1}H-^{1}H COSY spectrum. The cross peaks δ5.0/δ4.0, δ5.2/δ4.1 and δ4.8/δ4.4 were detected in ^{1}H-^{1}H COSY, since δ5.2, δ5.02 and δ4.8 resonances corresponded to anomeric protons; the other resonances were assigned to H-2 of the three residues. Likewise, the resonances of the rest of the protons of all the three residues, H-3 to H-6 were assigned. Based on the resonances from the COSY experiment (Table 1), the resonances of the anomeric and non-anomeric carbons (Table 1) were obtained from ^{1}H-^{13}C HMQC and HSQC spectra except the C-6 of β-D-glucose. The signals in ^{1}H-^{13}C HSQC and HMQC represent a proton that is bound to a carbon atom. The anomeric proton resonances δ5.2, δ5.02 and δ4.8 of α-D-mannose, α-D-glucose and β-D-glucose obtained on comparison with standards, correlated to anomeric carbon signals at δ103.09, δ100.78 and δ104.7 in the HSQC and HMQC experiments. These three signals in the anomeric region are indicative of the three monosaccharide residues, α-D-mannose, α-D-glucose and β-D-glucose. The signals at δ81.23, δ78.42 and δ80.97 confirm the carbons involved in linkage. As the particular signals are of third carbon atoms, it can be concluded that linkage present in the polysaccharide is 1,3-linkage. Carbons linked to other sugars are found between δC 75 and 85 ppm, and non-substituted ring carbons usually have the chemical shifts between δC 65 and 75 ppm. Along with the carbon and proton resonances obtained, some inter-residue and intra-residue

NOE contacts were obtained with NOESY spectrum, which could lead to the conclusion of the monosaccharide sequence present in the carbohydrate backbone. The inter-glycosidic NOE resonances between the protons δ5.0/δ3.9, δ5.0/δ3.7, δ5.2/δ3.4, δ5.2/δ3.2, δ4.8/δ3.8, δ3.9/δ3.6, δ4.4/δ4.1, δ4.4/δ3.5, δ4.4/δ3.7 indicated that the sequence of carbohydrate backbone is -α-D-glucose-α-D-mannose-β-D-glucose- linked by α-1,3-linkages and a possibility of β-1,3-linkage at the terminal. The anomeric proton of α-D-glucose connected to non-anomeric protons of α-D-mannose and α-D-mannose connected to β-D-glucose non-anomeric protons through NOE inter-glycosidic resonances. The inter-residue correlations obtained between the monosaccharide units were of the anomeric protons of each unit with the third and fifth protons of the nearby unit due to the 1,3-linkage between the units. Linkages through C1 were obtained for all the three sugars from the NOESY spectrum. The data obtained from the two-dimensional NMR techniques reveal the complexity of the EPS structure.

In vitro assay for anti-oxidant activity

Reducing power of a compound can serve as a significant indicator of antioxidant activity. Antioxidants are able to reduce $Fe^{3+/}$ ferricyanide complex to its ferrous form, which is monitored by measuring the formation of Perl's prussian blue at 700 nm (Chung et al. 2002). Experiment with crude and pure EPS showed that the EPS possessed relatively low level of anti-oxidant activity (Fig. 1). In comparison with ascorbic acid, the antioxidant activity of both crude and pure EPS was observed to be around 3%. The antioxidant activity of crude and pure EPS was found to be increasing with increasing concentration; a comparatively higher antioxidant activity was observed with crude EPS and could be due to contaminant proteins if any. The particular chemical nature of the EPS could be the reason for its relatively low level of antioxidant property. Liu et al. (2011) showed *Lactobacillus* EPS to have potent antioxidant activity. Chemical modification of the polysaccharide, such as alkylation, acylation, reduction, oxidation, sulphonation, etc., could improve the property. It has been reported by Du et al (2010) that the sulphated derivative of a polysaccharide from *Tremella aurantialba* fruit bodies presented an intense increase in the biological activity in comparison with the native polysaccharide.

In vitro assay for cytotoxicity

The cytotoxicity of EPS in normal fibroblast cells (L929) was measured in terms of cellular viability by MTT assay. The *L. plantarum* EPS exhibited cytotoxicity in normal cells only at 50 mg/mL concentration, while all other concentrations for all incubation periods were found to be safe (Fig. 2). The experiment proved that this EPS will be selective if chosen as an anticancer agent. The solubility of the compound in water is another selective factor for its anticancer studies as most of the anticancer drugs are not water soluble (Gollahon et al. 2011) and are administered with vehicles, which bring out serious side effects.

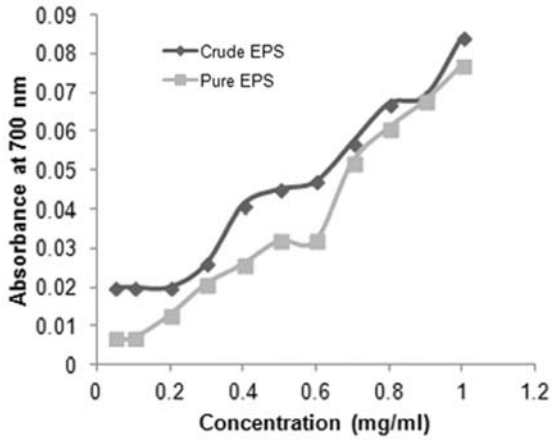

Fig. 1. Reducing power of crude and pure *L. plantarum* EPS. A higher absorbance indicates a higher reducing power.

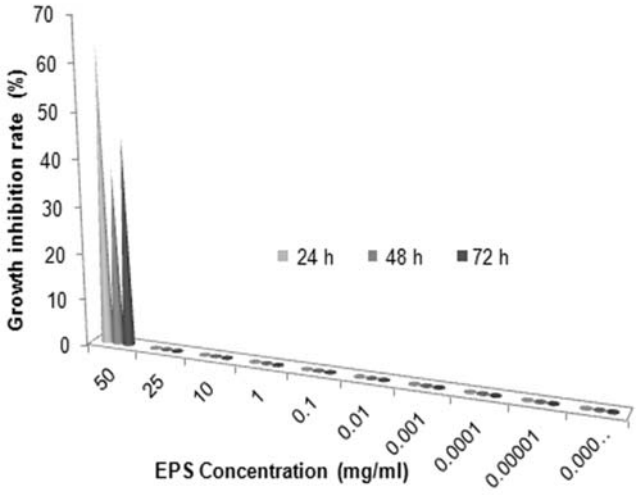

Fig. 2. Cytotoxicity of *L. plantarum* EPS in L929 cells.

Antitumour activity of L. plantarum EPS

Morphological apoptosis determination

The MCF-7 breast cancer cell line is among the most used and well characterized. These cells are ER^+, PR^+ and $HER2/neu^+$ (Creighton et al. 2008). Expression levels of the oestrogen, progesterone and HER2/neu receptors, which characterize clinically distinct breast tumours, have been shown to change during disease progression and in response to systemic therapies.

Hoechst 33342 is a permeable DNA dye that binds preferentially to A-T base-pairs. In Hoechst staining, the nucleus of live and dead cells is stained blue. The difference is in the intensity of the colour, which will be more in dead cells due to chromatin condensation. Figure 3 shows the control cells and treated cells stained with Hoechst and EB/AO. It was observed during the experiment that the *L. plantarum* EPS was showing apoptotic activity in breast adenocarcinoma cells (MCF-7). Hoechst-stained apoptotic cells were blue in

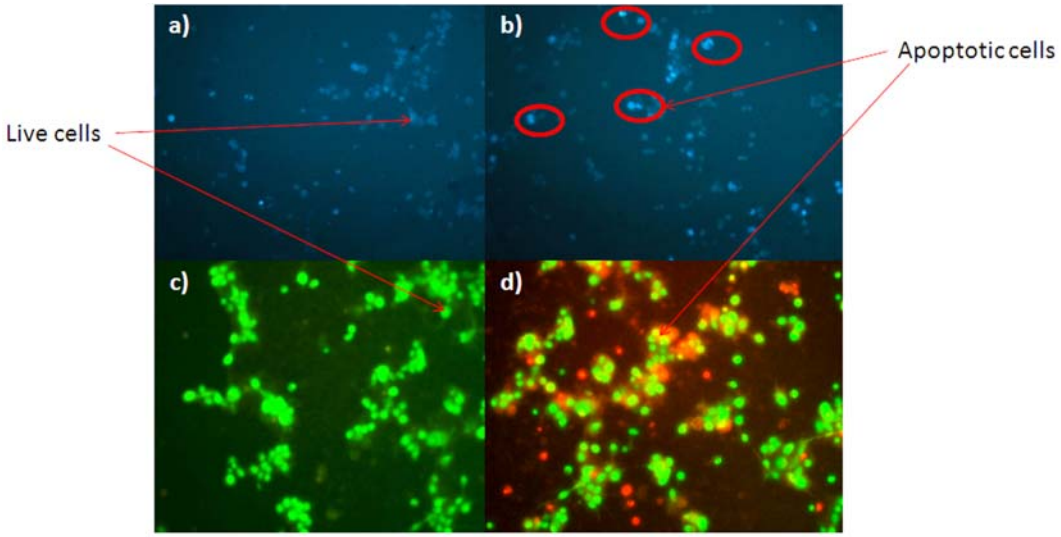

Fig. 3. Hoechst staining: (a) MCF-7 control cells without treatment; (b) MCF-7 cells treated with EPS. EB/AO staining: (c) MCF-7 control cells without treatment; (d) MCF-7 cells treated with EPS. The cells were treated with EPS of concentration in the range 1×10^{-6}–25 mg/mL.

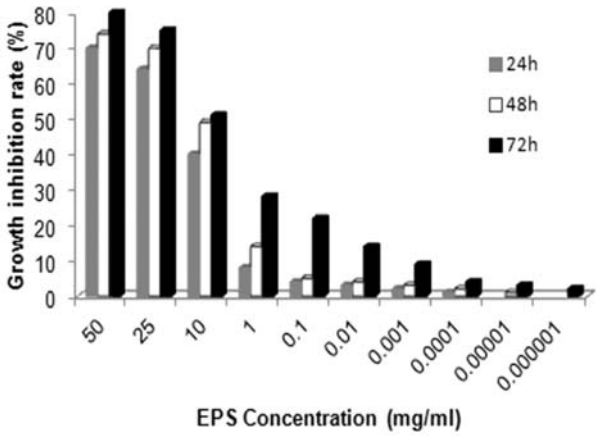

Fig. 4. Anti-tumour activity of *L. plantarum* EPS by MTT assay.

colour with condensed chromatin. This was further confirmed by the EB/AO staining, which could distinguish orange apoptotic cells from green live cells. The two fluorescent dyes ethidium bromide and acridine orange allow rapid and easy recognition of live and dead cells when visualized by fluorescence microscopy. Acridine orange stains live cells green, while ethidium bromide stains dead cells red-to-orange, depending on the filter employed in the microscope. The excitation and emission maxima for acridine orange are 500 and 530 nm, while for ethidium bromide 510 and 595 nm, respectively. Early-stage apoptotic cells are stained green as they take up acridine orange but not ethidium bromide, whereas nonviable cells take up both dyes and are stained orange. The microscopic images (Fig. 3) showed a higher percentage of apoptotic cells in the EPS treated cells.

In vitro assay for antitumour activity

Antitumour activity of *L. plantarum* EPS was quantitatively assessed by MTT assay in MCF-7 adenocarcinoma cells (Fig. 4). Even though the IC_{50} for the compound was attained at 10 mg/mL on comparison to the control, doxorubicin of IC_{50} 0.1 mg/mL, the absence of any side effects in normal cells under the *in vitro* conditions could make the polysaccharide a good candidate for future studies. The particular antitumour property of the polysaccharide could be mainly attributed by the 1,3-linkages in the EPS (Ismail & Nampoothiri 2010; present study). It has already been reported that EPSs with β-1,3-linkages exhibit antitumour properties initiated by the binding of glucans to β-glucan receptor, such as dectin-1 (Brown & Gordon 2001; Taylor et al. 2002), of immune cells. Dectin-1 cooperates with Toll-like receptors and many other surface receptors for the recognition of different microbial products, such as fungal cell wall, lipopolysaccaharide, lipoprotein, flagellin, and bacterial DNA (Underhill 2003).

The antitumour effects of cell-bound EPS from *Lactobacillus acidophilus* 606 on colon cancer cells was studied by Kim et al (2010). They found that the EPS from *L. acidophilus* developed antitumourigenic property against HT-29 colon cancer cells due to the activation of autophagic cell death directly by the induction of Beclin-1 and GRP78, as well as indirectly through the induction of Bcl-2 and Bak. Chemical modifications of the polysaccharide by oxidation, reduction, transglycosylation or sulphonation can decrease the IC_{50} and improve the antitumour properties. Eventhough the mode of action of the EPS is through apoptosis, further *in vitro* and *in vivo* studies are required to reveal the detailed mechanism behind the action.

In vitro assay for lymphocyte proliferation

The immunologic action of polysaccharides, generally considered as a kind of biological response modifiers, may begin with activating effector cells, such as lymphocytes, macrophages, natural killer cells. It exerts

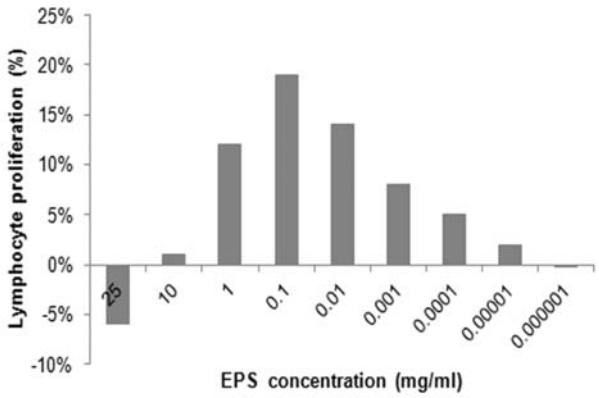

Fig. 5. Lymphocyte proliferation of *L. plantarum* EPS.

a nonspecific action on the body and supports vital systems, including nervous, hormonal and immune systems. The EPS from *L. plantarum* exhibited lymphocyte proliferation property in a concentration range 1×10^{-5}–10 mg/mL (Fig. 5). The other two concentrations (1×10^{-6} and 25 mg/mL), which were tried, were found to be inhibitory. The concentration of 0.1 mg/mL was found as the best in lymphocyte proliferation with approximately 20% proliferation rate.

The stimulated immune pathway of EPSs is different from that of lipopolysaccharides. Mainly, mannose-rich polysaccharides collaborate with Toll-like receptors for the activation of immunity. There is a possibility of the particular polysaccharide to act with Toll-like receptors as it comprises glucose and mannose as its subunits (Shao et al. 2004). Kim et al. (2007) reported that macrophage activation by lactic acid bacteria EPS resulted in the release of TNF-α. The experiment by Liu et al. (2011) proved that the EPS isolated from lactic acid bacteria can promote macrophage growth and induce production of pro-inflammatory responses in murine macrophage cell lines. The understanding of this complex process requires further *in vitro* and *in vivo* experiments.

Conclusion

The structural study of EPSs from lactic acid bacteria becomes important in industry as it unveils the various functional roles played by the polysaccharide due to the particular structure. EPS obtained from the probiotic *L. plantarum* can be an excellent candidate for making functional foods with improved health properties, texture and rheology. Two-dimensional NMR spectroscopic techniques played a prominent role in sequence determination of the hetero-polysaccharide, from *Lactobacillus plantarum* MTCC 9510, comprising glucose and mannose. A detailed structural study of EPS from *Lactobacillus plantarum* has not been reported till now especially from a probiotic strain, although many other species of *Lactobacillus* has been studied. The findings showed that the EPS could be used as an anticancer agent taking into consideration its non-toxicity towards normal cells as well as its water solubility. The study also implies that its structure of glucose and mannose linked by α- and β-1,3-linkages can activate immune system, such as lymphocyte proliferation. However, the relatively higher IC_{50} of the compound needs further attention and could be explored in future since several chemical modification of the polysaccharide could bring down the IC_{50}.

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