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Short Communication

Preparation, characterization and immunomodulatory activity of selenium-enriched exopolysaccharide produced by bacterium *Enterobacter cloacae* Z0206

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ABSTRACT

The tolerant-selenium exopolysaccharide-producing bacterial strain *Enterobacter cloacae* Z0206 was batch cultured in PDA medium containing optimal concentration of sodium selenite. Selenium was accumulated efficiently in *Enterobacter cloacae* Z0206 during cultivation with selenium. Inorganic selenite could be transformed into organic forms. Selenium-enriched exopolysaccharide (Se-ECZ-EPS-1) was purified from the fermentation liquid. Selenium content of Se-ECZ-EPS-1 was 12.962 μg/g. Se-ECZ-EPS-1 with Mw of 29,300 Ka was composed of Glc, Gal and Mann with molar ratio of 8.530:0.061:0.706. Administration of Se-ECZ-EPS-1 to cyclophosphamide (CP)-exposed animals resulted in improvement of cellular and humoral immune responses. These findings indicated that Se-ECZ-EPS-1 may act as potent immunomodulatory agents.

1. Introduction

Microorganisms are now considered as efficient producers of biologically active and/or chemically novel compounds. And no "supply issue" will appear in the process of industrializations of the microbial products since scaled-up productions can be achieved through bioreactors of any capacity that can be designed nowadays (Jensen and Fenical, 1994). Polysaccharides from microorganism may prove to be one of the useful candidates in the search for effective, non-toxic substances with immunomodulatory, antitumor, antioxidant activity, etc. (Wang et al., 2007). Bacterial exopolysaccharides were claimed to have a wide range of health benefits (Adriana et al., 2005; Chen et al., 2008). The immunostimulatory activity was regarded as one of the most important biological activities of polysaccharide (Zhou et al., 2004).

Selenium (Se) is an essential micronutrient (Kaur and Sandhu, 2008). Because of the health problems induced by many environmental pollutants, many efforts have been undertaken in evaluating the biological activities of organoselenium compounds (Zeng et al., 2008). Microorganism fermentation with selenium technique provides a feasible and economic approach for production of organic selenium compounds and becomes the focus in recent years (Zhang et al., 2008).

However, little is known about selenium enriched culture technique. There is also lack of knowledge concerning the structure–function relationship and exact pharmacological effects of selenium exopolysaccharide derived from bacterium, which would allow a better understanding of the functional effects described for them, and be beneficial to explore new more bioresources. Therefore, the current study was designed to prepare organoselenium compound-Se-ECZ-EPS-1 through bacterium *Enterobacter cloacae* Z0206 fermentation with selenium and investigate its physicochemical properties and immunomodulatory activity on CP-induced immunosuppressed mice.

2. Methods

2.1. Microorganism culture

The tolerant-selenium producing exopolysaccharide bacterial strain *Enterobacter cloacae* Z0206 was identified and kept in our laboratory. Exopolysaccharide production was carried out in a 10 dm^3 bioreactor (Shanghai Biotech Ltd., China) in 7 dm^3 growth volume with stirring rate of 200 rpm at 30 °C for 2 days. Growth medium consisted of 1000 ml/l potato juice (200 g potato); 3 g/l bacto-peptone; 3 g/l yeast extract; 20 g/l sucrose. Concentration and time of adding selenium into culture were optimized through experiments. Aeration rate (1 vvm), growth temperature, foam

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level, dissolved oxygen tension (DOT) and pH were measured and/or controlled by the bioreactor control unit.

2.2. Isolation and purification of the selenium exopolysaccharide

The fermentation liquid was collected and centrifuged at 4500×g for 20 min. The supernatant was concentrated and precipitated with chilled 95% EtOH followed by being kept at 4 °C overnight. The precipitate was collected by centrifugation and freeze-dried to give yellow powder, crude Se-ECZ-EPS. After deproteinization and decolouring, purified Se-ECZ-EPS was subjected to a DEAE-52 column (2.6 × 50 cm) eluting with distilled water and a linear gradient of 0–0.5 M NaCl respectively at a flow rate of 30 ml/h. The collected major peak was concentrated and fractionated over a Sephadex G-100 column (1.6 × 50 cm) eluting with distilled water at a flow rate of 12 ml/h. The main peak fractions were dialyzed and lyophilized to give white powder, Se-ECZ-EPS-1. Selenium contents were determined spectrophotometrically by using a modified method of Kessi et al. (1999) with 850 fluorimeter (HITACHI), where λex = 378.1 nm, λem = 518 nm, EX = 5 nm, EM = 5 nm. The general properties were determined by HPLC, atomic force microscopy and FTIR analysis.

2.3. Immunomodulatory activity evaluation of Se-ECZ-EPS-1

40 ICR male mice (18 ± 2 g) were randomly allocated to four groups of 10 each. Three immunosuppressed groups were administered by gavage once daily with Se-ECZ-EPS-1 (0, 200 and 400 mg/kg body weight (B.W.)) for 14 days, and CP was given intraperitoneally at 50 mg/kg B.W. on the 12th day. Control mice received same volume of 0.9% normal saline. Animals of all the groups were challenged with 0.2 ml of 0.1% SRBC, i.p. on the 10th day. The animals were sacrificed by cervical dislocation after the last dose. Relative organ weight (organ weight/100 g of body weight) of spleen and thymus were determined for each animal. Spleen lymphocytes were prepared in usual way and adjusted to 5×10^6/ml with RPMI 1640 media, then incubated in 96-well plates with 100 μl/well, adding with either 100μl of concanavalin A (ConA; 2.5 μg/ml, SIGMA), lipopolysaccharide (LPS; 10μg/ml, SIGMA) or complete medium (controls). After 44-h incubation at 37°C with 5% CO_2, 20 μl MTT (5 mg/ml, SIGMA) were added into each well incubating for another 4-h and then 100μl of DMSO were added into each well to dissolve the precipitation completely. The light absorbance was measured at 570 nm with Enzyme-linked Immunosorbent Assay Reader (Model BIO-RAD-550, USA).

2.4. Statistical analysis

Data were analyzed by one-way analysis of variance (ANOVA), followed by Dunnett's *t*-test. Results were presented as mean ± S.D. Values of $p < 0.05$ were considered to be a statistically significant finding.

3. Results

3.1. Biotransformation of selenite and red-Se phenomenon

Increasing the selenite concentration in the culture medium (e.g. higher than 20 μg/ml) led to significantly suppressive effect on the growth of *Enterobacter cloacae* Z0206 (data not shown). In particular, cultures were found to turn red due to the occurrence of Se (0) under higher selenite stress and the color would increase with selenite concentration. We chose 20 μg/ml as optimal concentration of adding selenium to fermentation medium. Take fermentation period and biotransformation efficiency into account according to the growth curve of selenium-tolerant strain *Enterobacter cloacae* Z0206, we chose sixth hour as the ideal time of adding selenite.

3.2. Isolation, purification and general properties of Se-ECZ-EPS-1

Se-ECZ-EPS was separated into two fractions on DEAE-52 (Fig. 1). The main peak fraction (Se-ECZ-EPS-1) was sequentially purified through Sephadex G-100, which giving a single elution peak. The yield of Se-ECZ-EPS-1 was about 76.2% from Se-ECZ-EPS and appeared as a white powder. The GPC profile showed that Se-ECZ-EPS-1 was homogeneous polysaccharide with Mw of 29,300 Da. Se-ECZ-EPS-1 was composed of 91.75% Glucose, 0.66% Galactose and 7.59% Mannose with more branches, α-configuration and pyranoside (data not shown). The content of selenium in Se-ECZ-EPS-1 was 12.962 mg/kg.

3.3. Immune activity of Se-ECZ-EPS-1

CP treatment caused a significant reduction in the spleen and thymus weight compared with control animals ($p < 0.05$) (Table 1). A significant increase of relative spleen and thymus weight in the Se-ECZ-EPS-1 treatment group mice was observed compared with model control groups. The proliferative responses of lymphocytes to ConA and LPS were reduced markedly in CP-treated mice compared with the control lymphocyte proliferation

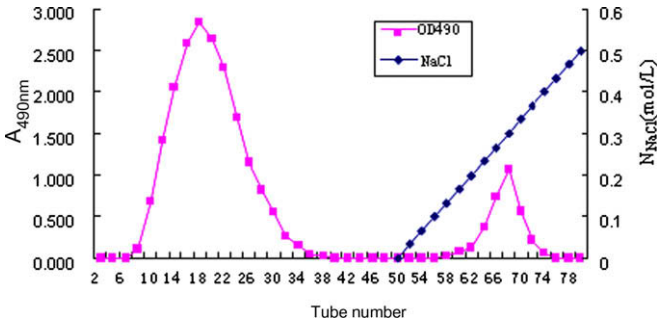

Fig. 1. Elution profile of Se-ECZ-EPS on DEAE-52 column (2.6 × 50) eluting with distilled water and a linear gradient of 0–0.5 M NaCl, respectively at a flow rate of 30 ml/h.

Table 1
Effect of Se-ECZ-EPS-1 on immune organ index.

Group	Dose/mg kg^{-1}	Spleen index	Thymus index
Control	–	0.462 ± 0.039^a	0.338 ± 0.018^a
CP	50	0.326 ± 0.006^b	0.178 ± 0.010^b
CP+Se-ECZ-EPS-1	200	0.377 ± 0.012^c	0.191 ± 0.011^b
CP+Se-ECZ-EPS-1	400	0.382 ± 0.022^c	0.222 ± 0.010^c

Values are the mean ± S.D. of six animals. Means within a column with different letters (a, b, c) differ significantly ($p < 0.05$).

Table 2
Effect of Se-ECZ-EPS-1 on spleen lymphocyte proliferation.

Group	Dose/mg kg^{-1}	A_{570nm}	
		ConA	LPS
Control	–	0.538 ± 0.017^a	0.475 ± 0.020^a
CP	50	0.365 ± 0.035^b	0.269 ± 0.013^b
CP+Se-ECZ-EPS-1	200	0.369 ± 0.025^b	0.269 ± 0.005^b
CP+Se-ECZ-EPS-1	400	0.412 ± 0.013^c	0.305 ± 0.012^c

Values are the mean ± S.D. of six animals. Means within a column with different letters (a, b, c) differ significantly ($p < 0.05$).

(Table 2). Se-ECZ-EPS-1 (400 mg/kg B.W.) promoted recovery of spleen lymphocyte proliferation responses induced by ConA and LPS significantly compared with CP-treated animals alone. Moreover, Se-ECZ-EPS-1 showed significant recovery in the serum hemolysin concentration ($p < 0.05$) and in the QHS assay compared with CP-treated animals alone (data not shown).

4. Discussion

The tolerant-selenium bacterial strain *Enterobacter cloacae* Z0206 produced a large quality of exopolysaccharide. Selenium is an essential trace element at low concentrations, as well as a toxicant at high concentrations, to *Enterobacter cloacae* Z0206. The current results suggested that, at 30 °C, a sodium selenite concentration of 20 mg/l is more suitable for selenium enriched culture of *Enterobacter cloacae* Z0206. Wong and Luis (1991) have suggested that energy-transduction systems may be severely affected by selenium toxicity, which may lead to substantial decreases or even elimination of storage products and major reductions in growth. Previously it has been demonstrated that some microorganisms are able to reduce selenite to Se (0) or other forms (Li et al., 2003). Obviously, *Enterobacter cloacae* Z0206 could bioaccumulate Se efficiently during the culture. The exact structural characteristics including conjoint form of Se was investigating in our laboratory.

Modulation of the immune responses to alleviate the diseases has been of interest for many years. In the experiments, we made an immunosuppressive animal model to evaluate the immunostimulating activities of Se-ECZ-EPS-1. The relative spleen and thymus weight were important index for nonspecific immunity. Immunopotentiator could increase spleen and thymus weight. The results indicated that Se-ECZ-EPS-1 had protective effect on CP-induced reduction of immune organ weight. Lymphocyte proliferation is a crucial event in the activation cascade of both cellular and humoral immune responses (Zhao et al., 2006). Humoral immunity, via antibody response, is involved in antibody production and immunization, which always determined by hemolytic activity and antibody concentration. T cells, including natural kill cells, specially mediated cell immunity (Gan et al., 2004). The current results indicated that Se-ECZ-EPS-1 had potent effect on the cellular and humoral immune response.

This is the first report on the immunostimulating exopolysaccharide from the *Enterobacter cloacae* Z0206. In the past decade, bacterial exopolysaccharides have been widely used as biological response modifiers (Leung et al., 2006). It is currently unclear how exopolysaccharides affect the intracellular immune system. Monosaccharide component, molecular weight and branching, chain conformation, and water-solubility may affect their activities (Zjawiony, 2004). Se-ECZ-EPS-1 is composed of glucose, mannose and galactose with α-configuration, pyranoside and more branches, which may be related to its immunomodulatory activity. The polysaccharide may contain biological information since the polysaccharide contains types of essential sugars (e.g. glucose, mannose and xylose) that predominate in human glycoproteins and glycoprotein receptors (Murray, 2003). Further investigation of biological activity and the mechanisms of Se-ECZ-EPS-1 action are wanted.

5. Conclusion

We, for the first time, prepared the selenium-enriched exopolysaccharide derived from bacterium *Enterobacter cloacae* Z0206 and confirmed that Se-ECZ-EPS-1 may be explored as a potential natural immunomodulator. These suggest that a selenium enriched health food, feed additive or therapeutic agents with notable market value may be produced efficiently as selenium enriched protein, polysaccharides and other components.

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