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Microbial Exopolysaccharides: Biosynthesis and Potential Applications

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ABSTRACT

Many bacteria synthesize extracellular polysaccharides (EPSs) with commercially significant physiological and therapeutic activities. Microbial polysaccharides have also been reported to have potential therapeutic applications. Recently, much attention has been devoted to the microbial exopolysaccharides (EPSs) due to their numerous health beneûts.EPSs from lactic acid bacteria are reported to possess antitumor effects, immunostimulatory activity, and the ability to lower blood cholesterol. EPSs also over an alternative class of biothickeners that are widely used in the food and dairy industries and have been proven to provide strong emulsifying activity, which is important in many food formulations. It is also important to understand the mechanism of microbial biosynthesis of EPSs in order to enhance their production by genetic alterations. The potential applications and the mode of microbial biosynthesis of the EPSs have been presented in this article.

Key words: Exopolysaccharide; Biosynthesis; Potential applications.

INTRODUCTION

The probiotic bacteria play beneficial role in the ecosystem of the human and animal gastrointestinal tract. The spectrum of the beneficial effects can be divided into nutritional, physiological and therapeutic effects. The mechanism of action of these beneficial effects relies on their metabolic end products termed as “probio-active substances” (Naidu *et al*., 1998). Exopolysaccharides (EPSs) are one of the important class of potential probioactive molecules. The EPSs are economically important because they can impart functional effects to foods and confer beneficial health effects on the host. These molecules have been proved to have antitumor, immunostimulatory, antioxidant, antiulcer and to lower blood cholesterol activities (Welman and Maddox, 2003). EPSs play a major role in the texture, mouth-feel, taste perception and stability of the dairy products and have been widely used

in the production of fermented dairy products in Northern Europe, Eastern Europe and Asia (Arena *et al*., 20056; Kodali and Sen, 2009). Polysaccharides derived from diverse microbial genera that include *Saccharomyces cerivisiae*, *Ganoderma applanatum, Cordyceps sinensis* have shown very good promise in the treatment of infectious diseases. The fungal and bacterial polysaccharides have been reported to modulate key components of the immune system (Schepetkin *et al*., 2008) It has been postulated that the polysaccharides with significant antioxidant activities stimulate macrophages. This is an important positive immunomodulatory property of the polysaccharides (Toklu *et al*., 2006).

Biosynthesis, genetics and bioengineering of EPSs

The biosynthesis of EPSs occurs in different growth phases, depends on environmental conditions and the organism used for the production (Cerning, 1995). However, very little is known on biosynthesis of EPSs and extensive study is required to understand the mechanism involved. The HoPSs are synthesized by extracellular enzymes which are secreted from the bacteria or located on the cell surface where as the synthesis of HePSs is complex (Welman and Maddox, 2003). They are produced by utilizing precursors formed intracellularly at the cytoplasmic membrane. The synthesis of EPSs involves a larger number of genes coding for enzymes and regulatory proteins which are not unique to EPS production and secretion. Nucleotide diphosphates serve as precursors for EPS biosynthesis which are intermediates of the central carbon metabolism that starts from the transport of the sugar from surrounding medium (Boels *et al*., 2001). In Gram negative bacteria, undecaprenyl phosphate is a lipid carrier for EPS assembly. The assembly of basic repeating unit occurs at the cytoplasmic membrane and involves sequential transfer of sugar nucleotide diphospho precursors to an isoprenoid lipid carrier, undecaprenyl phosphate (Sutherland, 2001). Once the basic repeating unit is assembled, the lipid-linked intermediates are usually translocated across the membrane and polymerized outside of the cell. Then, the EPS may be covalently linked to the cell surface to form a capsule, or released into the medium as slime. The undecaprenyl phosphate plays a significant role in the EPS biosynthesis in Gram positive bacteria also. The biosynthetic pathway is divided into four separate reaction sequences. These are the sugar transport into the cytoplasm, the synthesis of sugar-1- phosphates, activation of and coupling of sugars, and the processes involved in the export of the EPS (De Vust & Degeest, 1999).

Sugar transport into the cytoplasm

The monosaccharides and disaccharides are the principal carbon sources of microorganisms. The movement of these carbon sources into the cytoplasm from surrounding medium is tightly regulated by a number of different control proteins. The transport of sugar molecules from the surrounding medium into the cytoplasm is basically carried out by three different transport systems. In the primary transport systems, ATP hydrolysis is directly coupled to sugar translocation via sugar transport ATPase. In secondary transport systems, the import is coupled to transport of ions and other solutes. Phosphoenolpyruvate (PEP)-sugar phosphotransferase system (PTS) is the third and most important sugar transport machinery in microorganisms. The PEP–PTS system contains a group of proteins that are responsible for binding, transmembrane transport, and phosphorylation of a variety of sugar substrates. In this system, a phosphate group is released from the conversion of PEP into pyruvate catalyzed by pyruvate kinase. In the case of lactose metabolism, there are two possible pathways exist. The lactose may be either cleaved by β-galactosidase to generate galactose and glucose or hydrolysis of lactose-6-phosphate by phospho-β-galactosidase to yield galactose-6-phosphate and glucose. The glucose is broken down by glycolytic pathway or phosphoketolase pathway (Jolly *et al*., 2002; Welman and Maddox, 2003; De Vust & Degeest, 1999) The galactose-6-phosphate is fermented by the tagatose-6-phosphate pathway, galactose is either metabolized via the Leloir pathway by enzymes encoded within one or more gal gene clusters (Grossiord *et al*., 1998) or in some species, like Streptococcus thermophilus or *Lactobacillus delbrueckii* subsp. *bulgaricus*, secreted into the medium. (Hutkins *et al*., 1985).

Synthesis of sugar-1-phosphates

The majority of the sugar molecules entered into the cytoplasm of bacteria are phosphorylated into sugar-6-phosphates and degraded through glycolysis. Few of the sugar-6-phosphates are converted into

sugar-1-phosphates by phosphoglucomutases. These sugar-1-phosphates are the central metabolites for the formation of sugar nucleotides such as UDP–glucose and dTDP–glucose via the action of UDP–glucose pyrophosphorylase and dTDP–glucose pyrophosphorylase, respectively. The galactose is converted into glucose-1-phosphate via galactose-1-phosphate by Leloir pathway. The second central intermediate in sugar nucleotides biosynthesis is the glycolysis intermediate fructose-6-phosphate from which UDP-GlcNAc and UDP-GalNAc are formed via aminosugars metabolism. GDP-fucose is formed from fructose-6-phosphate via fructose-mannose metabolism (Jolly *et al*., 2002; van Kranenburg *et al*., 1999)

Synthesis of sugar nucleotides and polymerisation into the EPS repeat unit

The genes involved in EPS biosynthesis are classified into 2 groups. Those genes required for the synthesis of sugar nucleotides belong to first group and the second group genes are those which are specific to EPS. The genes belong to first group are called house keeping genes because these genes required for the biosynthesis of EPS and not specific for EPS. It should be noted that the sugar nucleotides these sugar nucleotides are essential for various metabolic pathways including sugar, fatty acid and nucleotide metabolisms. The majority of EPSs are synthesized from UDP-glucose, UDP-galactose, and dTDP-rhamnose precursors. All these precursor sugar nucleotides are synthesized from glucose-1-phosphate by a sequence of enzymes encoded by galU, galE, rfbA, rfbB, rfbC, and rfbD genes. 21 genes (epsCBAKLDEFGHIJMNOPQRSTU) were predicted to participate in methanolan synthesis on the basis of the features of the primary sequence.
The exopolysaccharide (EPS) biosynthesis gene clusters of four *Lactobacillus rhamnosus* strains consist of chromosomal DNA regions of 185 kb encoding 17 ORFs that are highly similar among the strains. Six potential glycosyltransferase genes were identified that account for the assembly of the heptasaccharide repeat unit containing an unusually high proportion of rhamnose. Four genes involved in the biosynthesis of the sugar nucleotide precursor dTDP-L-rhamnose were identified in the EPS biosynthesis locus, which is unusual for lactic acid bacteria. It was demonstrated that the activity of the precursor-producing enzyme UDP-*N*-acetylglucosamine 4-epimerase, converting UDP-*N*-acetylglucosamine into UDP-*N*-acetylgalactosamine, is responsible for the presence of *N*-acetylgalactosamine in the EPS repeating units of both strains. The activity of UDP-*N*-acetylglucosamine 4-epimerase was higher in both *S. thermophilus* strains than in a non-EPS-producing control strain.

The EPS specific gene cluster coding for EPS production and secretion was first identified and characterized in *S. thermophilus Sfi 6(Stingele et al., 1996) and L. lactis* NIZO B40 (van Kranenburg *et al*., 1997). In *S. thermophilus Sfi 6,* the size EPS specific gene cluster is 14.5 kb, contains 13 genes and located on the chromosome where as in *L. lactis* NIZO B40 the size is 12 kb, contains 14 genes and located on the plasmid.

The organisation of the gene clusters is similar for both organisms and consists of four separate domains. Stingele *et al*., (1999) have demonstrated that the gene cluster contains EPS-specific enzymes by insertion of the cluster into the non-EPS-producing heterologous host *L. lactis* MG1363 and demonstrating EPS synthesis. van Kranenburg *et al*., (1997) proved by in vitro experiments using [14C]-labelled sugar nucleotides, that the monosaccharide repeat unit is assembled on a isoprenoid C55 lipid carrier which is attached to cytoplasmic membrane. EPS biosynthesis is energy consuming process. One ATP is required for the conversion of sugar to sugar phosphate, another is needed for synthesis of each nucleotide and one more is required for phosphorylation of isoprenoid C55 lipid carrier

EPS polymerization and export to the surrounding medium

Highly specific sugar transferases affect the transfer of the monosaccharides and acyl groups to the isoprenoid lipid acceptor molecule (bactoprenol, C55-isoprenoid lipid) located in the cytoplasmic membrane.The biosynthesis of polysaccharide backbone in *L. lactis* NIZO B40 is initiated by the linkage of a glucose from UDP-glucose to lipid carrier by the glucosyl transferase EpsD (van Kranenburg *et al*., 1997). Subsequently, the combined activity of EpsE and EpsF link glucose to the lipid-linked glucose generating a lipid-linked cellobiose. Finally,

EpsG links galactose to the lipid-linked cellobiose to complete the backbone of B40-EPS repeating unit. Oba *et al*. (1999) proved that the repeating unit is organism specific by analyzing the sugar moieties obtained from mild hydrolysis of lipid extracts from *L.lactis* SBT 0495 and *L. lactis* NIZO B40. The polymerization takes place on the cytoplasmic face of the lipid carrier and the details of the mechanism is not understood. It has been assumed that the polymerization and export of EPS requires the action of a flippase to translocate the lipid-bound repeat units, a polymerase to catalyze the coupling of repeat units and finally an enzyme to catalyze the detachment of the lipid-bound polymer and that will control chain length.

Gonzalez *et al*., 1998 showed that the gene product from ExoQ catalyzes the polymerization of EPS and showed the sequence and topological similarities with the O antigen polymerase gene. Becker *et al*. (1995) have suggested that the ExoP product of *R. meliloti* is involved in chain length determination. The complete study of EPS biosynthesis and secretion is unclear and need further study.

Engineering of EPS production

The overproduction of EPSs from food grade bacteria is required to reach the market demand as effective thickeners and food ingredients. We need to understand the physiology and genetic organization of EPS producing bacteria to overproduce the EPS by genetic modification. The physical properties of EPSs can be changed by changing the repeating unit. The viscosity of EPSs can be increased or decreased by targeting the specific genes that are involved acetylation and/or pyruvylation.

The increasing number of glycosyltransferase genes characterised could be applied in engineering approaches by introducing new or exchanging existing glycosyltransferase genes in EPS-producing bacteria to produce polymers with different sugar compositions.

Looijesteijn *et al*., 1999 showed that fructose-1, 6- diphospahatase palys a cental role in the EPS overproduction by *L. lactis* NIZO B40 when grown on fructose containing medium instead of medium containing glucose. Relevant metabolic engineering strategies have been aimed to increase the EPS formation pathways.

A key consideration of LAB metabolism is the large proportion of carbon that flows to lactate; it has been suggested that if EPS production were coupled to growth of the cell then a reduction in the formation of lactate, which is known to inhibit growth, could elevate EPS formation. More carbon could be diverted away from glycolysis and into EPS formation. This strategy has been used to obtain overproduction of metabolic end products such as alanine by overexpression of the *Bacillus sphaericus* alaD gene for an alanine dehydrogenase in an LDH-deficient strain of *L. lactis*. The conversion of glucose-6-phosphate to glucose-1- phosphate by phosphoglucomutase and the subsequent formation of UDP-glucose, which is catalysed by UDP– glucose pyrophosphorylase, have been proposed as potential controlling points in the production of EPS. Overexpression of the pgm gene (for phosphoglucomutase) and the galU gene (for UDP–glucose pyrophosphorylase) result in an accumulation of UDP–glucose and UDP– galactose, respectively, in *L. lactis.* Another approach to enhancing the production of EPS exists at the level of biosynthesis of the EPS polymer, and in particular, by raising the activity of glycosyltransferases associated with this process. An example of this is the small increase in EPS production obtained because of overexpression of the priming glycosyl transferase epsD gene in *L. lactis.* Heterologous expression of glycosyltransferases could also allow to add other sugars at strategic positions to generate EPSs with new properties. The *epsD* gene, encoding a priming glucosyltransferase, and part of the plasmid-located operon was placed under control of the nisin-controlled expression (NICE) system. After induction with nisin A, EPS production of a transformed *Lactococcus lactis* strain was higher than that of a wild-type (van Kranenburg *et al*. 1999). Intensive studies are being done to increase the yield of EPS by engineering at molecular level. The modeling of carbon-metabolism and experimental studies of the regulation of the glycolytic flux in *L.lactis* are currently in progress (Hugenholtz. & Kleerebezem, 1999)

Application of EPSs

Commercial applications

BioFill is a microbially derived cellulose

material from *Acetobacter xylinum* can be used as an implantable material in general and plastic surgery (U. S. Pat. No. 6599518). The product 'BioFill' also has a number of potential uses and is manufactured in the form of wound dressings for patients with burns, chronic skin ulcers (Sutherland, 1998). *Agrobacterium* and *Rhizobium* species produce an important EPS curdlan, which has been widely used in food industry (Table 1). The food-grade curdlan is used to improve the texture of foods such as bean curd (tofu), bean jelly and fish pastes in Japan. Sulphated curdlan possesses antithrombotic activity and other biomedical applications (Sutherland, 2001). Gellan, isolated from *Sphingomonas paucimobilis* marketed as Kelcogel® or Gelrite® which is approved in the USA and the EU as gelling, stabilizing and suspending agent in wide range of foods.

Xanthan, from *Xanthomonas campestris*, is a major commercial biopolymer (Sutherland, 1998). The polysaccharide is incorporated into foods to alter the rheological properties of the water present, and has found applications that take advantage of many of its physical properties (Cerning, 1995, Welman & Maddox, 2003)

Table 1: Commercial applications of EPSs

EPS name	Source	Application	Reference
Kelcogel® or Gelrite®	*Sphingomonas paucimobilis*	As gelling, stabilizing and suspending agent in foods	Sutherland, 2001
Xanthan	*Xanthomonas compestris*	As viscosifying agent in various foods, oil recovery	Cerning, 1995
Emulsan	*Pseudomonas fluorescence*	Emulsifying agent in various foods	Neu, 1996.
Dextran	*Leuconostoc mesenteroides, Streptococcus mutans*	Used to purify different molecules (Sephadex)	Sutherland, 1998
Curdlan	*Agrobacterium* and *Rhizobium* spp	Antithrombotic activity and other biomedical applications	Sutherland, 2001
BioFill®	*Acetobacter xylinum*	Implantable material in general plastic surgery	U.S. Pat. No. 6599518 (2003)

Table 2: Exopolysaccharides of bacteria and their health benefits

S. No	Source of EPS	Health benefit	Reference
1.	*L. casei*	Activated mouse acrophages	Nomoto *et al*., 1989
2.	*Lactobacillus rhamnosus*	Stimulated mouse lymphocytes	Chebot *et al*., 1992
3.	*Bifidobacterium bifidum*	Antiuler activity	Nagaoka *et al*., 1994
4.	*L. delbrueckii* ssp. *bulgaricus*	Stimulated murine splenocytes.	Kitazawa *et al*., 1998
5.	*Bifidobacterium lactis* Bb12	Proliferated mouse lymphocyte	Amrouche *et al*., 2005
6.	*Bacillus licheniformis*	Antiviral and immunostimulatory activities	Arena *et al*., 2006
7.	*Lactobacillus kefiranofaciens*	Increased gut mucosal immunity,	Vendarola, 2006
8.	*Pantoea agglomerans*	Free radicals-scavenging activity.	Wang *et al*., 2006
9.	*L. plantarum*	Antimutagenic activity	Tsuda *et al* 2007
10.	*Bacillus coagulans* RK–02	Antioxidant and antihyperglycemic activities	Kodali & Sen, 2008; Kodali and Sen (Patent Appl. No. 594/ KOL/03-04-09

Dairy industry

EPSs play a major role in the texture, mouth-feel, taste perception and stability of the dairy products and have been widely used in the production of fermented dairy products in Northern Europe, Eastern Europe and Asia. EPS producing bacteria are widely used in the production of fermented milk (De Vuyst *et al*., 2001, Ruas-Madiedo, 2002 & 2003).

Nordic ropy milk is the generic name for fermented milks with mesophilic cocci which produce slime The major LAB used are *Lactococcus lactis* subsp. *cremoris*, *lactis* and *lactis* biovar diacetylactis, *Leuconostoc mesenteroides* subsp. *cremoris* and *dextranicum* for the mesophilic bacteria, *S. thermophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus* and *L. helveticus* for the thermophilic bacteria. Viili is a fermentedmilk consumed in Finland. The process consists of fermenting milk with *L. lactis* subsp. *lactis* biovar *diacetylactis*, *Leuconostoc mesenteroides* subsp. *cremoris* and the mould *G. candidum*. Kefir is traditional self-carbonated slightly alcoholic fermented milk from Eastern Europe(De Vuyst & Degeest, 1999; Brodbent *et al*., 2003). *L. lactis* subsp. *lactis* and *cremoris*, *L. mesenteroides* subsp. *dextranicum* and *S. thermophilus*, yeasts of the genera *Saccharomyces, Kluyveromyces, Candida, Mycotorula, Torulopsis, Cryptococcus*, *Torulaspora, Pichia*, and the acetic acid bacteria *Acetobacter aceti* and *Acetobacter racens.*

Dairy starter cultures that contain slime-forming LAB strains are also commercially available in other parts of the EU and the USA. Ropy, thermophilic LAB starter cultures for yoghurt production are largely used in some countries of the EU because the addition ofstabi lizers is prohibited in yoghurts (De Vuyst & Degeest, 1999).

An HePS-producing *S. thermophilus* strain was also responsible for an increased moisture level in low-fat mozzarella cheese (Perry *et al*., 1997; Perry *et al*., 1998; Low *et al*., 1998). Incorporation into a dough of a sufficient amount of EPS may also result in an improved texture build-up by softening the gluten content of the dough, and extending the shelf life and increasing the specific volume of the resultant bakery product.

EPSs can also be used as texturizer in various food products like yoghurt (Table 1). The microstructure of yoghurt consists of a matrix of aggregated casein particles. Fat globules are embedded in this matrix. The cavities of the gels are filled with serum and bacterial cells (Toba, Nakajima, Tobitani, & Adachi, 1990). An envelope of EPS is observed surrounding the bacterial starter strains, by which ropy cells attach to the protein matrix via a web of filaments (Kalab, Allan-Wojtas, & Phipps-Todd, 1983; Teggatz & Morris, 1990). Differences were observed in the microstructure of yoghurt products manufactured with ropy (i.e. with EPS producing) and non-ropy strains where the protein gels obtained with the ropy strains showed an homogenous structure with randomly distributed small cavities. By contrast, gels obtained with non-ropy strains showed larger cavities filled with bacteria and serum.

Health benefits of EPSs

EPSs have been proved to show important health benefits like antioxidant (Kodali & Sen, 2008) cholesterol lowering (Welman & Mddox, 2003), antitumor (Hosono *et al*, 1997), antiviral and immunomodulatory activities (Arena *et al*., 2006). The reactive oxygen species (ROS) like) hydroxyl (OHÏ), superoxide ($O\ddot{I}_2$), nitric oxide (NOÏ) etc. lead to various serious diseases including Parkinson's disease, atherosclerosis, cancer, rheumatoid arthritis. EPSs have been proved to have antioxidant and free radical scavenging properties. The free radical scavenging property of EPSs can also be used to inhibit oxidation of vegetable oils. Kishk *et al*., (2007) showed that the antioxidant and free radical scavenging activities of exopolysaccharide (RPS) isolated from *Rhizobium meliloti* and also showed the ability of RPS to inhibit lipid oxidation in sunflower oil emulsions during holding time for 50 h at 60°C. EPSs from probiotic bacteria show antiulcer activity. Nagaoka *et al*., (1994) showed the anti-ulcer effects of bifidobacteria, lactobacilli and streptococci were examined using the acetic acid-induced gastric ulcer and ethanol-induced erosion models in rats. *Bifidobacterium breve* YIT4014 and 4043, and *Bifidobacterium bifidum* YIT4007 were administered orally, and anti-ulcer effects were confirmed for not only these organisms but also their polysaccharide fractions (PSFs). The major component of these anti-ulcer polysaccharides was rhamnose. In particular, polysaccharides in which the rhamnose content exceeded 60% were

more effective in healing gastric ulcers. It has been found that the EPSs stimulate the immune system and shown anticancer effects. Hosono *et al*., (1997) showed that polysaccharide from *Bifidobacterium adolescentis* M101-4 and showed that it is effective for stimulating the proliferation of murine splenocytes. Chabot *et al* (2001) isolated EPS from *Lactobacillus rhamnosus* RW- 9595M and tested in vitro for their possible modulating properties on mouse splenocytes from the C57Bl/6 and BALB/c strains, on the murine RAW 264.7 macrophage-like cell line and on human Peripheral Blood Mononuclear Cells (PBMC) and found that EPSs stimulate to synthesize TNF, IL-6 and IL-12 from the above cells.

Several studies have suggested that LAB and fermented dairy products have anticarcinogenic activity (Table 2). Kitazawa *et al*. (1991) found that the intraperitoneal injection of lyophilised L. lactis subsp. cremoris KVS 20 cells resultedin the growth inhibition of Sarcoma-180 tumors in mice, but the LAB strain did not exhibit cytotoxicity in "in vitro" studies against S-180 tumor cells. This suggests that the effectiveness of this strain in preventing tumor proliferation was mediated through immune activity. These authors postulate that the slime material produced by L. lactis subsp. cremoris KVS 20 may be the principal component in the antitumoral effect. A later study (Kitazawa, Yamaguchi, & Itoh, 1992) showeda significant increase of the B-celldependent mitogenic activity induced by the slime material products from L. lactis subsp. cremoris KVS 20. Also Nakajima, Toba, andToyod a (1995) found that EPS of L. lactis subsp. cremoris SBT 0495 administered intraperitoneally enhanced the production of specific antibodies in mice, indicating that this EPS may act as adjuvant. The yoghurt starter Lb. delbrueckii subsp. bulgaricus OLL 1073R-1, which produces an EPS, has been reportedto exert a host-mediated antitumor activity (Kitazawa *et al*., 1998). The isolated EPS was fractionatedinto two components: a neutral polysaccharide and an acidic polysaccharide (APS). It was foundthat the APS-1073 fraction was a phosphopolysaccharide with effective mitogenic activity mainly for murine B-lymphocytes andprovedthat the phosphate group actedas a trigger of the mitogenic induction of this EPS. Extracellular polysaccharides produced by LAB enhance other immunological functions such as proliferation of T-lymphocytes (Forsen, Heiska, Herva, & Arvilommi, 1987), macrophage activation and induction of cytokine (interferon-g andinterleuk in-1a) production (Kitazawa, Itoh, Tomioka, Mizugaki, & Yamaguchi, 1996). All the studies referred to have been done "in vitro" or by injecting the EPS material into mice. But very scarce experiments have been done "in vivo" by oral administration. The water-soluble EPS (KGF-C) from kefir grains was shown to have the property of retarding tumor growth when administrated orally. Oral immune enhancement by this EPS is induced probably through T-cell and not through B cell participation (Zubillaga *et al*., 2001). Further research is necessary in this topic as prerequisite to employ the EPS or EPS-producing LAB in functional foods. Arena *et al*., (2007) EPS-1 is a novel extracellular polysaccharide produced by a strain of thermotolerant *Bacillus licheniformis*, isolated from a shallow marine hot spring of Vulcano Island (Italy). In this paper, antiviral and immunomodulatory effects of EPS-1 were evaluated. It was found that EPS-1 treatment impaired HSV-2 replication in human peripheral blood mononuclear cells (PBMC) but not in WISH cells. EPS-1 induced IL-12, IFN-g, IFN-a, TNF-a and IL-18, but not IL-4 (Table 2). Thus, the antiviral effect of EPS-1 on PBMC seems to be related to the pattern of cytokines induced. The EPSs have also been proved to have the cholesterol lowering activity (Nakajima *et al*., 1992), antiulcer and antidabetic activity (Naidu *et al*., 1999) but the detailed mechanism have to be studied.

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