Microbial Exo-Polysaccharides for Biomedical Applications

I.-L. Shih*

*Department of Environmental Engineering, Da-Yeh University, Chang-Hwa, Taiwan

Abstract: The productions and applications of various microbial exopolysaccharides have been under intensive researches over the past few decades. Some of these exopolysaccharides are commercial available and some are currently under intensive development; they include ionic heteropolysaccharide and neutral homopolysaccharide. These extracellular polymers constitute a structurally diverse class of biological macromolecules with a wide range of physiochemical properties which are the basis for the different applications in the broad fields of pharmacy and medicine. They have found applications in such diverse biomedical fields as ophthalmology, orthopedic surgery, tissue engineering, implantation of medical devices and artificial organs, prostheses, dentistry, bone repair and drug delivery.

Keywords: Microbial exopolysaccharides; ionic heteropolysaccharide; neutral homopolysaccharide; fermentation; biomedicine.

INTRODUCTION

Polysaccharides are composed of many monosaccharide units that are joined one to the other by a glycosidic linkage to give a long chain. Many natural polysaccharides and oligosaccharides participate in variety of biochemical reactions *in vivo*. Traditionally, polysaccharides are extracted from plant seeds, plant exudates, marine algae and from animals [1]. Over the past few decades, the number of polysaccharides produced by microbial fermentation has been gradually increasing. In recent years, significant progress has been made in discovering and developing new microbial polysaccharides that possess novel and highly functional properties; especially, there are many microbial polysaccharides that are of medical or pharmaceutical importance (Table **1**).

Most of polysaccharides derived from microorganisms are of the exo-polysaccharide (EPS) type [2,3]; they form regular-shaped capsules adhering to the surface of the bacterial cell or amorphous slime loosely bound to the outer surface of microbial cells, which were released into the medium upon autoclaving or upon aging and autolysis of the cells. The microbial exo-polysaccharides can be divided into homo-polysaccharides which are composed of a single monosaccharide unit, and hetero-polysaccharides in which regular repeat units are formed from two to eight monosaccharides [4]; the chemical structures of some of the common exo-polysaccharides are shown in Table **2**. These exo-polysaccharides are generally of uniform structure and fairly limited poly-dispersity [5]; they are usually water soluble, biodegradable, biocompatible, edible and nontoxic toward humans and the environment. In addition, they possess a wide range of physical and rheological properties in that they disperse in water to give a thickening or a viscosity building effect [6]; they showed properties of stabilization, suspension of particulate, control of crystallization, inhibition of synaeresis, encapsulation and formation of film [7]; they synergized with many other biopolymers [8,9].

The application of polysaccharide materials and their derivatives for medical purposes is growing very fast; some are employed because of their unique and superior physical properties relative to other biomaterials. Their applications are diverse ranging from laboratory through clinical to tabletting; they have found applications in such diverse bio-medical fields as ophthalmology, orthopedic surgery, tissue engineering, implantation of medical devices and artificial organs, prostheses, dentistry, bone repair and many other medical fields [10]. In addition, they have therapeutic and pharmaceutical usages in that they enable controlled slow release of drugs into the body. They also make possible targeting of drugs into sites of inflammation or tumors for disease treatment and they can be used for skin rejuvenation and wound healing [11,12]. Several of these microbial polysaccharides are commercially industrial products, while others are in various stages of developments. Results of some of the recently conducted investigations of these subjects are discussed in the present review.

Hyaluronic Acid (HA)

Hyaluronic acid (HA) is a high-molecular-weight linear polysaccharide composed of repeating units of D-glucuronic acid (GlcUA) and *N*-acetylglucosamine (GlcNAc) joined alternately by β-(1-3) and β-(1-4) glycosidic bonds: [β-1,4-glucuronic acid-β-1,3-N-acetyl glucosamine-]$_n$ [13,14]. It is found in all vertebrates as a major constituent of the extracellular matrices. In animals, it is found in the vitreous body of the eye, the synovial fluid of articular joints, and the intercellular space of the epidermis [15]. Although traditionally extracted from connective tissue, it is now well established that the polymer can be produced by microbial fermentation. Reports have been made about the production of this mucopolysaccharide by *Streptococcus A* [16] and by *Pseudomonas aeruginosa* [17]. However, *Streptococcus zooepidemicus* remains the most preferred bacteria for the production of hyaluronic acid, and more attention has been paid to bacterial fermentation by group C *Streptococci*, in

*Address correspondence to this author at the Department of Environmental Engineering, Da-Yeh University, 168, University Rd., Dacun, Changhua, Taiwan 51591; Tel: 886-4-8511344; Fax: 886-4-8511344;
E-mail: ils@mail.dyu.edu.tw

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Table 1. Potential Biomedical Applications of Microbial Exopolysaccharides

Polymers/	Microorganisms	Applications	References
Hyaluronic acid	*Streptococcus zooepidemicus*	Osteoarthritis treatment Ophthalmic surgery Adhesion prevention Wound healing, Disease indicator Drug delivery	[10, 25, 36] [10, 25] [38] [10, 34, 37] [33] [35]
Levan	*Bacillus subtilis;* *Bacillus polymyxa*	Anti-cancer treatment Anti-AIDS agents Controlled-release formulations Plasma volume extender Immuno-modulator	[54, 55, 58] [65,66] [60] [55, 56] [61, 63]
Pullulan	*Aureobasidium pullulans*	Sustained-release formulations & coating Oral care Wound-healing Vaccine and drug carrier	[79, 80, 81] [82] [84] [85-87, 91]
Cellulose	*Acetobacter xylinum*	Wound dressing & skin substitute Dental surgery Artificial blood vessels Bio-sensor coating	[96, 100, 101, 118] [119] [120] [121]
Dextran	*Leuconostoc mesenteroides*	Blood plasma substitute Blood volume expander Anaemic therapy Magnetic resonance imaging (MRI) Antiviral agent	[127] [125, 127] [130, 131] [132] [133]
Gellan	*Sphingomonas paucimobilis*	Drug delivery and sustained release of drugs	[139, 140]
Alginate	*Pseudomonas species*	Wound healing Cell immobilization and tissue engineering	[142] [141, 142]
Xanthan	*Xanthomonas compestris*	controlled-release agent	[145]

Table 2. Chemical Structures of Some Exopolysaccharides

Exopolysaccharides	Structures (Repeating Sequence)
Hyaluronic acid	→3)-β-D-GlcNAc-(1→4)-β-D-GlcA-(1→
Levan	[→6)-β-D-Fruf-(2→] n (branched via β-(2→1) linkages)
Pullulan	→6)-α-[D-Glc-(1→4)]$_2$-α-D-Glc-(1→6)-α-[D-Glc-(1→4)]$_3$-α-D-Glc-(1→
Cellulose	→4)-β-D-Glc-(1→4)-β→D-Glc-(1→
Dextran	→6)-α-D-Glc-(1→ (low level ~5% α-(1→3) branch linkages)
Gellan	→3)-β-D-Glc-(1→4)-β-D-GlcA-(1→4)-β-D-Glc-(1→4)-α-L-Rha-(1→
Alginate[a]	→4)-β-D-ManA-(1→4)-[β-D-ManA-(1→4)]$_n$-β-D-ManA-(1→4)-α-L-Gul-(1→ ↑ (OAc)$_m$
Xanthan	→4)-β-D-Glc-(1→4)-β-D-Glc-(1→ 3 ↑ 1 β-D-Man-(1→4)-β-D-GlcA-(1→2)-α-D-Man-6-OAc 4 ╲╱ 6 HO_2C CH_3

[a] Linear polymers of irregular structure composed of D-Mannuronic acid and L-guluronic acids with only D-mannuronosyl residues carry O-acetyl groups

Abbreviations: GlcNAc = ; N-Acetyl Glucosamine; GlcA = Glucuronic acid; Fruf= Fructose; Glc= Glucose; Rha= Rhaminose; ManA =Mannuronic acid; GulA= Guluronic; Man= Mannose; OAc= O-Acetyl-

particular *S. equi subsp. equi* and *S. equi subsp. zooepidemicus.* The fermentation biotechnology for the production of HA was successfully developed by Holmstrom, Johns *et al.* [18,19]. There have been several studies investigating into the nutritional requirements in bacterial culture [20] and the optimization of fermentation process [21-24] for improving HA production; these have been thoroughly reviewed [25,26]. Currently, a concentration of 5–10g/l HA, beyond which the high viscosity makes the fermentation not practical, is readily obtained using wild-type strains in batch mode [25]. Given the high price of HA product (Medical-grade HA sells at US $40,000–60,000/kg) [25], it is the quality rather than quantity that has been the focus of the researches on strain and process development for HA production. Efforts have been paid to improve the quality (e.g. molecular weight distribution and purity) of HA products in the following way: to screen and isolate nonhemolytic, hyalurondase-negative and high-molecular weight mutants [21-23]; to use chemically defined medium (CDM) [27] and aeration control [28,29]; to apply continuous cultures to avoid contamination by cell wall proteins and pathogens [30,31]. Batch culture is the most frequently reported HA culture method producing a polymer with the weight-average molecular weight (Mw) typically in the range of 1 x 10^6 to 3.5 x 10^6, and polydispersity (P) between 1.8 and 2.5; the molecular weight and polydispersity of *S. zooepidemicus* varies significantly, which is affected by variables such as temperature, aeration, and initial glucose concentration [32]. The culture conditions, productivities and molecular weights of HA and various exopolysaccharides are shown in Table **3**.

Hyaluronic acid plays an important role in many developmental and regulatory processes of the body in that it interacts with cell receptors and proteins such as CD44, RHAMM (Receptor for Hyaluronan-Mediated Mobility) and fibrinogen. Thus, it influence many biological processes such

Table 3. Various Exopolysaccharide Producing Strains and their Culture Conditions

(Exopolysaccharide) Strains	Major Nutrient		Cultivated conditions	Productivity (Molecular weight, kDa)	References
	Components	(g/l)			
(Hyaluronic acid) *Streptococcus zooepidemicus*	Glucose Yeast Extract	20-60 10	37 4 days	5-6 g/L (1.0 -3.5x10^3)	[32]
(Levan) *Bacillus subtilis*	Sucrose	200	33, pH 7.0 21h	40-50 g/L (1,794 , 11)	[45]
(Levan) *Zymomonas mobilis*	Yeast extract Sucrose	2.0-5.0 250	20℃, pH 6.5 72h	14.67 g/L ------ [a]	[47]
(Levan) *Bacillus polymyxa*	Sucrose Peptone Yeast Extract $(NH_4)_2SO_4$	150 2 2 2	30℃, pH 7.0 240h	36g/L (2 x 10^3)	[52]
(Pullulan) *Aureobasidium pullulans*	Sucrose Yeast Extract $(NH_4)_2SO_4$	50 0.4 0.6	28℃, pH 7.5 120h	31 g/L (1.0 -3.0x10^3)	[74]
(Cellulose) *Acetobacter xylinum*	Glucose Yeast extract Bactopetone Ethanol	20 5.0 5.0 10	30℃, pH 5.7 7 days	4.2 g/L (Dry) [b] (10-700)	[43]
(Dextran) *Leuconostoc mesenteroides*	Sucrose Yeast extract Peptone	100 5 5	26℃, pH 7.5 18h	4.8 g/L (Dry) (2 x 10^3)	[128]
(Gellan) *Sphingomonas paucimobilis*	Soluble starch Yeast extract	40 2.5	30℃, pH 7.0 48h	43.6 g/L (~500)	[139]
(Alginate) *Pseudomonas species*	Glucose $(NH_4)_2HPO_4$ Yeast extract	50 1.25 0.2	30℃, pH 7.0 45h	20.0 g/L (~100)	[142]
(Xanthan) *Xanthomonas compestris*	Glucose Citric acid NH_4Cl	50 2.2 2.0	30-33℃, pH 7-8 30h	15-30 g/L (2.0 -20.0x10^3)	[143]

[a] Molecular weight is not determined; [b] Dry cellulose was obtained

as angiogenesis, cancer, cell motility, wound healing, and cell adhesion [26]. It involved in morphogenesis and differentiation. In addition, it aids tissue formation and repairs, and provides a protective matrix for reproductive cells; it also serves as a regulator in the lymphatic system and acts as a lubricating fluid in joints. HA have significant structural, rheological, physiological, and biological functions in the body [10]. Its distinctive viscoelastic properties, coupled with its lack of immunogenicity and toxicity, have led to a wide range of applications in biomedical fields including osteoarthritis treatment, ophthalmic surgery, adhesion prevention after abdominal surgery, wound healing, disease indicator and drug delivery [33,35]. Various biomedical application for HA are listed in Table **4**. There are several hyaluronan-based products in the market for various medical applications. Healon® from Pharmacia [now Pfizer (New York, N.Y.)] was the first product on the market, which is a viscous gel that is used as a surgical aid in cataract extraction, *intraocular lens* (IOL) implantation, corneal transplantation, glaucoma filtration, and retinal attachment surgery. A number of other viscosurgical aids are now marketed by competing firms in the ophthalmic market, such as Viscoat® and Provisc syringe® (Alcon), Amvisc syringe® (Chiron) and AMO vitrax syringe® (Allergen). These products accounts for a worldwide market share of US $140 million [10,26]. The next major application of HA was in viscosupplementation in arthritic joints. Hyalurona gel (Seikagaku, Japan), Hyalgan® (Sanofii Pharmaceuticals), Orthovisc® (Anika Therapeutics) and SynVisc® (Biomatrix, now Genzyme) are the major brand products in the market, which accounts for a worldwide market share of US $700 million. These products are used to relieve pain and improve joint mobility in the treatment of osteoarthritis *via* intra-articular injections, they have also been proposed as an alternative to traditional steroid therapy for several degenerative joint diseases [10,25,36]. For wound healing and scarring, a line of hyaluronan-based product has been developed to foster the healing process; HYAFF™ (ConvaTec) is being used in Europe and US for burn and chronic ulcer patients [10,37]. In adhesion prevention, hyaluronan preparations such as Seprafilm from Genzyme can reduce undesired connective tissue adhesion in surgical procedures and thus improve surgical outcome [38]. In recent years, microbial HA has replaced collagen and other tissue fillers in cosmetic and reconstructive surgery in fear of disease-causing contaminations in animal-derived materials. In addition, there are many on-going researches focus on the development of hyaluronan and derivatives for topical and intravenous drug delivery [35]. A comprehensive book regarding the practical aspects of hyaluronan-based medical products has been published recently [39].

Microbial Levan

Levan is a polymer of fructose linked by β-(2→6) fructofuranosidic bond present in many plants and microbial products [40]. The microbial levans are produced from sucrose-based substrate by transfructosylation reaction of levansucrase (beta-2, 6 fructan:D-glucose-fructosyl trans-ferase, EC 2.4.1.10) by a variety of microorganisms [41]: *Bacillus subtilis* [42-45] *Bacillus polymyxa* [46], *Zymomonas mobilis* [47], *Aerobacter levanicum* [48], *Streptococcus sp*.

Table 4. Some Commercially Available HA or HA Derived Biomedical Products[a]

Applications	Products (Manufacturers)
Ophthalmic surgery (a surgical aid in cataract extraction, Iol implantation, corneal transplantation, Glaucoma filtration and retinal attachment Surgery)	Healon® (Pharmacia, now Pfizer); Amvisc syringe, Amvisic plus syringe (Chiron); Provisic syringe and Viscoat syringe (Alcon); Amo vitrax syringe (Allergan)
Osteoarthritis treatment (an intra-articular injection for relieve Pain and improve joint mobility)	Hyalgan® (Sanofi); Orthovisc® , Hyvisc® (Anika); Synvisc® (Biomatrix, now Genzyme); Hyalurona gel (Seikagaku); Hyalgen (Fidia) Crossed linked Ha (Q-med); Bacterial high M.W. Ha (Biotechnology General Corp)
Wound healing and scaring (stimulates tissue repair for burn Or ulcer patient etc.)	Hyaff™(Convatec) Hyalgen (Fidia)
Adhesion prevention (anti-adhesion) (prevention of undesired tissue damage and undesired connective tissue bridges In abdominal, cardiac, arthroscopic, spinal and nasal/sinus surgeries)	Separafilm® (Genzyme)
Cosmetic and reconstructive surgery (replacement of collagen and other tissue fillers in aesthetic plastic surgery)	Halosol™, Ha-Quat™, Halogel™ (Clear Solution Biotechnology) Hylucare® (Genzyme) (Q-med)
Drug delivery (carrier of drugs)	Hazomes-B2™, Cancept-Ha™ (Clear Solution Biotechnology) Hylumed® (Genzyme) Hyanalgese™ (Hyal Pharm.)
Tissue engineering (scaffold materials)	HA-Bed™ (Clear Solution Biotechnology)

[a]Reported by Prehm (2002), Chong (2005) and Prestiwich and Vercruysse (1998)

[49], *Pseudomonas sp.*[50] and *Corynebacterium laevaniformans* [51]. At present, a strain of *Bacillus polymyxa* (NRRL B-18475) and *Bacillus subtilis* (Natto) Takahashi were shown to produce a high yield of polysaccharide when grown on sucrose solution. *Bacillus subtilis* (natto) Takahashi, a commercial natto starter for preparing fermented soybeans, produced 40-50 g/ l of levan in medium containing 20% (w/w) sucrose (about 40-50 % yield on an available fructose, where 10.52 g fructose are available from 20 g sucrose) after cultivation for 21 h (Table **3**). The product consisted of two fractions with different molecular weight (1, 794 kDa and 11 kDa), which were easily separated by fractionation using an ethanol gradient. The products were well characterized by GPC, ^{13}C-NMR and ^{1}H-NMR [44,45]. In contrast, *B. polymyxa* produced about 36 g/l of levan in a growth medium containing 15% sucrose) after cultivation for 10 d. Hydrolysis and subsequent analysis showed the product to consist entirely of D-fructose. ^{13}C-NMR and methylation analyses indicated the product to be a β(2→6)-linked polymer of fructose, with 12% branching. The polysaccharide has a molecular weight of about 2 million and is readily soluble in water [40,52,53]. Currently, a Korean start-up company, Real-Biotech Co., Ltd (BRT), is the first and only company worldwide to produce levan on a commercial basis (http:///www.realbio.com).

Water-soluble levan can be used in a wide variety of applications in the pharmaceutical industry. The viscosity of levan varies with its degree of polymerization (DP) and degree of branching, which relates to the number of side fructose chains attached to one fructose unit in the main fructose chain, and in this respect levan can be used in pharmaceutical formulations in various ways. The potential applications of the high and low molecular weight levans are well documented in the literature [54-57]. Levans with molecular-weight higher than 10^7 kDa were effective for a direct effect on tumor cells that is related to a modification in the cell membrane, including changes in cell permeability [54,55]; the effect was lost when the polymer degraded. Levan is similar to bacterial dextran in physicochemical properties in that it is not toxic and it is not antigenic in small molecular weight. In addition, it is slowly eliminated from the body when injected into the bloodstream [55,58]. Therefore, levan with molecular weight below 100 kDa has potential as a blood plasma volume extender [55,59]. In the pharmaceutical applications, it is known that the low molecular-weight, less branched levan usually provides a low viscosity, and can be used as a tablet binder in immediate-release dosage forms, while levans of medium- and high-viscosity grade are used in controlled-release matrix formulations [60]. It is undoubted that the levans with different molecular weights are needed for different purposes. In addition, levan has anti-inflammatory, hypocholesterolemic properties as well as radioprotective and antibacterial activities [61-64]. Levan derivatives have also been suggested as inhibitors of smooth muscle cell proliferation and as agents to transit water into gels. Sulfated, phosphated and acetylated levans have also been suggested as anti-AIDS agents [65,66].

Microbial Pullulan

Pullulan is a neutral extracellular polysaccharide produced from starch or sugar by strains of the polymorphic fungus *Aureobasidium pullulans* (*A. pullulans*). It is a linear mixed linkage α-D-glucan consisting of either linear chain of glucopyranose units with regular alteration of two α-(1–4) and one α-(1–6) linkages or a linear polymer of maltotriose units connected by α-(1–6) linkages [67,68]. Excellent reviews on the production and industrial application has been published [69-72].

The production of pullulan from well defined chemical media by *A. pullulans* using batch and continuous culture has been studied. Much of the published research on pullulan in recent years has been concerned with improving the economics of production, mainly by identifying even less expensive feedstocks (e.g. agro-industrial wastes like whey, molasses, peat hydrolyzate etc.), isolating improved production strains or developing alternative fermentation schemes [70]. On an industrial scale, pullulan is produced only by a few commercial firms (e.g. Hyashibara Biochemical Laboratories, Inc, Okayama, Japan) despite the large number of fermentation studies of *A. pullulans* reported in the literature. For more than 2 decades, the Hayashibara Company remains the principle commercial source of pullulan today; it produces approximately 300 metric tons per year and sells two different pullulan grades: Food grade pullulan (PF-20) at approximately US $20/kg, and pharmaceutical grade (deionized) pullulan (PI-20) at approximately US $25/kg [71]. Hayashibara Company has used a simple batch-wise fermentation process for commercial production of pullulan [73]. *A. pullulans* is cultivated on medium containing starch hydrolysates of dextrose equivalent 40–50, at 10–15% concentration in addition to peptone, phosphate and basal salts. After cultivation at pH 6.5, 30°C with aeration for 100 h, optimal pullulan yields are obtained in that yields of greater than 70% of initial substrate are claimed. The production of pigment-free pullulan by *Aureobasidium pullulans* in batch and fed-batch culture was investigated [74]. Batch culture was proved to be a better fermentation system for the production of pullulan than the fed-batch culture system. A maximum polysaccharide concentration (31 g/L), polysaccharide productivity (4.5 g/L/day), and sugar utilization (100%) were obtained in batch culture, in which the bacteria were cultivated at pH 7.5, 28 ℃ with shaking at 200 rpm for 120 h in a medium containing sucrose 50.0 (g/L), $(NH_4)_2SO_4$ 0.6 g/L), yeast extract 0.4 (g/L), K_2HPO_4 5.0 (g/L), $MgSO_4 \cdot 7H_2O$ 0.2 (g/L), and NaCl 1.0 (g/L) (Table **3**). Pullulan is recovered and purified by precipitation with alcohols after the filtration of culture broth to remove cells and treatment with activated charcoal to remove pigment. Pullulan of pharmaceutical grade may be further purified through the use of ultrafiltration and ion exchange resins. The molecular homogeneity of the alcohol-precipitated polysaccharides from the fermentation broths as well as the structural features of pullulan were generally confirmed by ^{13}C-NMR and pullulanase treatments followed by gel filtration chromatography of the debranched digests [74]. The average molecular weight (M_W) and poly-dispersity (M_W/M_n), which can be determined by using size exclusion chromatography

coupled with multi-angle laser-light scattering (SEC/ MALLS), varies in the range of $1x10^3$-$3x10^6$ Da and 1.4-2.4 (Table **3**), respectively, depending on the strains and cultivation conditions employed [67,75,76].

Pullulan is non-toxic, non-mutagenic, odorless, tasteless, and edible [77,78]. Dry pullulan powders are white and non-hygroscopic and dissolve readily in hot or cold water. It is water-soluble and forms and edible film which is transparent, oil and grease resistant, impermeable to oxygen and heat-sealable. Pullulan and its derivatives have many potential pharmaceutical, clinical and health care uses; it can be used in pharmaceutical coatings, including sustained-release formulations [79-81]; it can be used as a denture adhesive, a binder and stabilizer; thus, oral care products based on pullulan films have recently been commercialized [82]. Pullulan and its derivatives exhibit adhesive properties [83] and can be used in wound-healing compositions [84], they also are promising as non-toxic conjugates for vaccines [85,86] and interferon [87].

Pullulan-based hydrogels and nanoparticles have been studied and may have a variety of uses [88,89]. Pollulan modified by attachment of certain number of cholesteryl groups (PCh) self aggregates to form stable mono-dispersed nanoparticles of a hydrogel [90], which spontaneously complex such soluble proteins as bovine serum albumin, α-chemotrypsin, and insulin as well as other bioactive, water soluble molecules [91,92]. Such complexes are of great interest from the point of view of application in medicine because thermal and colloidal stability of the complexed proteins have been enhanced greatly as a result of incorporation into hydrogel nanoparticles; such a device is thought to sufficiently enhance an insulin delivery system which required thermal and bio-colloidal stability in solution. The supramolecular assembly of proteins with amphiphilic polymers represents a new methodology for functionalization of polymers that have potential for biomedical application [93].

Bacterial Cellulose

Cellulose (ß-1,4-glucan), composed of linear polymer chains of ß-1,4-linked glucose residues, is one of the most abundant polymers in nature. Bacterial cellulose (BC) has unique physicochemical properties, which differ from those of plant cellulose with respect to its size, crystallinity and purity [94,95]; it has therefore attracted much attention as a new functional material [95]. Recently there has been interest in new fields of application and development of new methods for its mass production, which resulted in a considerable increase in interdisciplinary cellulose research and product development over the past decade. Several excellent reviews concerning the productions and applications of cellulose in various areas have been published recently [96-100].

Bacterial extracellular cellulose is synthesized by bacteria belonging to the genera *Acetobacter*, *Rhizobium*, *Agrobacterium* and *Sarcina* [101]. Among these, Gram-negative *Acetobacter xylinum* is claimed to be the most effective cellulose-producing bacterium and is widely used [102]. To achieve high productivity and yields of BC and to reduce cost of its production, special emphasis has been placed on several areas: to select high BC producing strains by screening or genetic engineering methods; to optimize culture broth compositions (carbon and nitrogen sources and stimulating compounds) and process conditions (pH, temperature, agitation and aeration); to optimize culture conditions through fermenter design. Several fermentation techniques for BC production, such as stationary culture (in plastic trays), agitated culture (jar fermentors), cultivation in horizontal fermentors or cultivation in internal loop airlift reactors have been reported [95,103-109], some of which have potential for economic and commercial BC production. While static cultivation has been widely investigated and applied for production of some successful commercial cellulose products, the development of cultivation methods for improvements of BC productivity demonstrated that BC production by *A. xylinum* was higher in stirred tank reactors and in airlift reactors than in a conventional static cultivation; it was shown that agitated culture with high oxygen supply and high volumetric agitation power is required for increase of BC productivity [108,110-112]. Indeed, by improving the culture conditions in a stirred tank fermenter, a productivity of 0.115 $gl^{-1}h^{-1}$ was obtained. Analysis of the influence of pH and temperature on cellulose yields and properties by *A. xylinum* indicates that the optimum temperature for *A. xylinum* growth ranges from 25°C to 30°C, and optimum pH varies between 4.0 and 7.0, depending on the strain used. The variation of pH and temperature cause changes of cellulose DP and water-binding capacity in addition to affect the BC yields [96,110,113]. Although glucose is a good carbon source for BC production, two sugar alcohols (arabitol and mannitol) have led to the production of 6.2 and 3.8 times more BC respectively than was achieved with glucose [114]. Yeast extract, peptone and casamino acids are good complex nitrogen sources for BC production by *A. xylinum*; however, one of the cheapest complex nitrogen sources, corn steep liquor, has been used successfully in BC production; a high BC productivity (0.62 $gl^{-1}h^{-1}$) could be achieved in continuous fermentation using a corn steep liquorfructose-based medium [115]. *A. xylinum* produces cellulose on the surface of liquid and solid culture media; a cellulose network formed as a sheet floating on the medium surface has been proved to have high tensile strength, elasticity, resilience, durability, shape-retention, and high water-binding capacity, and is nontoxic and non-allergen [116]. In addition, it resists heat up to 100 °C for at least 3 hours [117]. However, cellulose ultra-structure and its physical and mechanical properties are strictly influenced by the culture method. In stationary culture conditions, a thick, gelatinous membrane of BC is accumulated on the surface of a culture medium, whereas under agitated culture conditions, cellulose can be produced in the form of a fibrous suspension, irregular masses, pellets or spheres [95,96]. The choice of a cultivation method will determine both forms and properties of BC, which have to be tailored to the further polymer application [113].

Bacterial cellulose is a natural polymer whose properties are similar to the hydrogels produced from synthetic polymers; it displays high water content (98-99%), good sorption of liquid; it is non-allergenic and can be safely sterilized without any change to its characteristics. In

addition, it is biocompatible, porous, elastic, easy to handle and store; it adsorbs exudation, protects from secondary infection and mechanical injury, prevent sticking to the newly grown tissue. These features make bacterial cellulose an excellent skin substitute and dressing material for treating different kinds of wounds, burns and ulcers. Several commercial preparation of *A. xylinum* cellulose have been tested in clinical trials with satisfactory results; Cel ™ produced by Xylos Corp. is a wound dressing to heal ulcers [101], Biofill® is ideal as a temporary human skin substitute and is used as a bandage that can be applied to cases of second and third degree burns and ulcers [96,100,118]. Recently, modifying bacterial cellulose with chitosan during its biosynthesis results in a composite material with glucosamine and N-acetylglucosamine units incorporated into the cellulose chain, which is characterized by a number of valuable features as good mechanical properties, high moisture-keeping properties in addition to bacteriostatic activity against Gram (-) and Gram (+) bacteria and bactericidal activity against Gram (+) bacteria. The application of wound dressing material of this cellulose/ chitosan composite, which possess good antibacterial and barrier properties against microorganisms, will prevent infection caused by microorganisms present in the environment during healing process. Another preparation, Gengiflex®, has intended applications within the dental industry; it was developed to aid periodontal tissue recovery [119] with benefits including the re-establishment of aesthetics and function of the mouth and that a reduced number of surgical steps were required. The use of BC hollow fiber as artificial blood vessels and ureters are promising; a product called BASYC® (BActerial SYnthesised Cellulose), which was designed tubularly directly during the cultivation with the aim to develop biomaterials for medical application, was developed by a team of chemists, biologists and surgeons to be used as a replacement blood vessel. BASYC® tube, a tube of 1 mm inner diameter and 5mm length, was applicated as covers in experimental micro-nerve surgery because it has high mechanical strength in wet state; in addition, it has enormous water retention values, low roughness of the inner surface which was smoother than other synthetic materials used for similar purposes. After BASYC® tube was inserted as an endo-prosthesis into a white rat, a histological exam showed that the micro-vessel had become covered with well orientated endogenous cells indicating a regular vascular wall had formed inside the cellulose wall [120]; this demonstrate the high potential of BASYC® as an artificial blood vessel in microsurgery. Another biomedical application of BC was its use as an additional membrane to protect immobilized glucose oxidase in biosensors used for assay of blood glucose level. A classical amperometric glucose sensor covered with a bacterial cellulose membrane exhibited a long-term stability of more than 200 h. In undiluted blood, it was stable for about 24 h, which is about 6-7 times longer than the stability of the classical Cuprophan (Cup; AKZO England) membrane-covered sensor [121].

Dextran

Dextran is one polysaccharide that has a very old history of application in the medical sphere. It is a high molecular mass homopolysaccharide of D-glucose connected by consecutive α-(1→6) linkages with various degree of branching in the 1→4 and 1→3 positions [122]. The production of dextran was first reported by Bijerinck in 1912 [123]. Later, dextran was obtained in cell-free extracts from *Leuconostoc mesenteroides* [124]. Currently, it is commercially produced by the fermentation of sucrose based media by *Leuconostoc mesenteroides* and its annual production well exceeds 2000 metric tons and is mainly for clinical application [125,126]. Methods and conditions for dextran fermentation have been detailed [125,127,128]. Dextran can be synthesized by using either large-scale industrial fermenters or enzymatic filtration methods [127, 129]. The latter approach is generally favored since it enhanced dextran yield and a uniform product quality, which allows the product to be readily purified. In addition, it also offers the benefit of obtained fructose as a valuable co-product.

Dextran polymers have a number of medical applications. It is mainly used as a blood plasma substitute and is marketed as 6 % (w/v) solution in saline that is used as a non blood expander [125,127], it has also been used for wound coverage in surgical sultures, as blood volume expander to improve blood flow in capillaries in the treatment of vascular occlusion and in treatment involving the supply of iron in anaemic patients [130,131]. When used as blood expanders, the molecular mass range of dextran must be withn 75,000 ± 25,000 Da in that products of lower molecular mass are eliminated too rapidly from circulation to be of therapeutic value and larger polymers interfere with the coagulation of blood [12]. Special iron/dextran preparation also has been developed to enhance magnetic resonance imaging (MRI) techniques [132]. Chemically modified dextrans such as dextran sulfate have both antiulcer and anticoagulant properties; they have been used as a substitute for heparin in anticoagulant therapy. Furthermore, it is being studied as an antiviral agent, particularly in the treatment of human immuno-deficiency virus (HIV) infection [133].

Other Potential Exopolysaccharide for Biomedical Applications

In addition to the exopolysaccharide discussed above, several other exopolysaccharides have been in the development for biomedical applications; xanthan from *Xanthomonas compestris*, gellan and a range of structurally related polysaccharides from the strain of *Sphingomonas paucimobilis*, bacterial alginates secreted by *Pseudomonas species, Azotobacter vinelandii* and *Azotobacter chrococcum*, have also found medical and pharmaceutical applications [1,2,130,134-136].

Gellan is a high molecular mass anionic heteropolysaccharide from *Sphingomonas paucimobilis*, which is composed of a linear repeating tetrasaccharide of D-glucose (glcp), D-glucuronic acid (glcp A) and L-rhamnose(rhap) in the ratio of 2:1:1. The viscous nature of gellan gum places it as a major possible vehicle for ophthalmic drugs [137,138]. Chemical derivatives of gellan, such as micro-spheres of benzyl esters [139] and co-crosslinked hydrogels [140] have found applications in the sustained release of drugs.

Bacterial alginate secreted by *Pseudomonas species* is a polyanionic heteropolysaccharide, it is composed of D-manuronic acid and L-guluronic acid. Bacterial alginate is used for the production of calcium alginate fibers which are non-irritant and haemostatic and can be manufactured to wound dressing which absorb fluid from wound exudates; the dressing can finally be removed easily and painlessly without damaging the scar tissues and causing further trauma. Bacterial alginate has been tested for potential use either as immunostimulants or as gel-forming agents for the immobilization of cells (e.g. in tissue engineering). Immobilized cells might be used for a variety of biotechnological production process, or in medical transplantation technologies [141,142].

Xanthan, from *Xanthomonas compestris*, is currently considered the most generally approved microbial gum for industrial usages; the production from several commercial sources probably exceeds 20,000 metric tons per annum. It is also a heteropolysaccharide composed of alternate glucose residues of a cellulose back-bone carrying the side-chains of D-mannose and D-glucuronic acid [143,144]. Xanthan gum can be useful as controlled-release agent for pharmaceuticals [145], with microspheres of gellified xanthan encapsulated active ingredients.

CONCLUSION

This review contains the most recent information on the various microbial exopolysaccharides with the practical applications in medicine and pharmacy. The usefulness of the microbial exopolysaccharides in medicine (wound, burn and ulcer dressing materials, component of implants, carriers of drug delivery) is no longer questioned. Indeed, a number of important products have already reached the market, and the introduction of many devices and drugs derived from microbial exopolysaccharides is eagerly anticipated during the next decades.

Although commercial production of exopolysaccharides *via* fermentation methods has been achieved, the production in high amounts and in a standardized quality for them to be practical in biomedical applications needs to be continually improved. This requires increasing knowledge about chemistry, biochemistry, molecular biology and biotechnology of microorganisms and exopolysaccharides. Deciphering of all the riddles regarding exopolysaccharide biosynthesis, optimizing of the parameters for the fermentation and recovery process, establishing of analytical methods to control suitable quality will lead to improvement and tailoring of noble structures and properties. As a consequence, novel concepts for both its inexpensive production and for its bulk and special application will be developed. In addition, alteration of the chemical properties of the original exopolysaccharides will also greatly enhance their values and extend their range of applications. The elaboration of either biotechnological or technical procedures for the production of polymers of diverse structures (e. g. varied stereochemical composition or molecular sizes) and ultimate product functions (e. g. varied biodegradability, water-solubility and physico activity) to meet special demand of practical application are being launched and will soon provide a broad spectrum of new polymers.

In recent years, the notion to prevent use of products of animal origin that might be contaminated by pathogen of animal contagious diseases is intensified. The interest in the enhancement or potentiation of host immune system's innate ability to combat disease without harming the host itself is rekindled. In addition, the concept toward “greener” products and technologies that are more environmentally friendly is prevailing. Thus, the usage of microbial exopolysaccharides prepared from renewable resources in the biomedical fields will undoubtedly increase in the future. The technological contents described above provide an interesting starting basis for further development of these polysaccharides in biotechnological applications.

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