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Biopolymer Production by Natural And Engineered Microbes

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ABSTRACT
Biopolymers are materials that are created by biological agents, using enzymes to join together building ingredients such as amino acids, hydroxyl fatty acids and carbohydrates to create molecules with large molecular weight. Numerous biopolymers such as polyesters (hydroxyl fatty acids linked by ester bonds), polyphosphates (inorganic phosphates linked by anhydride bonds), polyamides (amino acids linked by peptide bonds) and polysaccharides (sugars or sugar acids linked by glycosidic bonds) are produced by bacteria. Bacteria produce biopolymers for proliferation, survival, adhesion, energy storage and protection, preserving cellular behaviour and reliant on external inputs. Bacteria create extracellular polymeric substance (EPS) consisting of DNA, polysaccharides, proteins, lipids and enzymes that encapsulate cells, providing nutrition and structural stability through biofilm formation. Microbial biopolymer synthesis methods have been enhanced by metabolic engineering and synthetic biology approaches, providing sustainable substitutes for natural sources. Constant efforts are made to modify the physiological pathways and gene expression machinery of the host strains in order to produce tailored biopolymers for various applications. There are still many obstacles to overcome, particularly in getting designed cell factories to produce biopolymers at their peak efficiency during fermentation in bioreactors with the right monomer composition and physical characteristics. The present review highlights the tremendous potentiality of natural and engineered microbes in the production of different biopolymers.

Keywords: *Extracellular polymeric substance; microbial biopolymer; metabolic engineering; synthetic biology; cell factories*

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INTRODUCTION

With emerging new techniques, microorganisms are endowed with capabilities to produce various chemicals and metabolites, many of which are important for our daily life [1]. In this new era of material science, biopolymers, which as the name suggests, derived from natural sources and synthesized by both natural and engineered microbes, present a promising area for reducing our dependence on fossil fuels and mitigating the environmental impact of traditional polymer production processes. There are studies suggesting the production of sustainable biodegradable plastic from biopolymers such as poly-β-hydroxybutyrate (PHB) which can be potent substitute for non-degradable petroleum based plastics [2, 3]. The artificial scaffolds for nerve regeneration have been produced using a variety of polymers [4]. PHB is a natural polyester that can be easily processed and offer several advantages; hence, one of the purposes of this review is to provide a better understanding of the efficacy of therapeutic approaches involving PHB scaffolds in promoting peripheral nerve regeneration following nerve dissection in animal models [5]. All the biopolymers are synthesized by enzymatic processes in the cytoplasm of the specific microorganism, in the various compartments or organelles of the cell, at the cytoplasmic membrane or in the cell wall components, at the surface of cells or even extracellularly, which even makes it easier to extract by downstream processing steps to obtain the biopolymers in a purified state. Many biopolymers occur abundantly in nature and are isolated from algae, which grow in natural environment, viz., agar and alginates are isolated from red algae [6]. Fermentative production of biopolymers is also used in industries like the polysaccharides. Microbial intervention and its application of thermoplastic polymers has led to a massive improvement in the performance of industrial and consumer goods bringing about societal and economic benefits. Thermoplastics have become one of the most disruptive innovations spurred by their high versatility, light weight and toughness in many different sectors such as packaging [7], infrastructure and electronic devices.

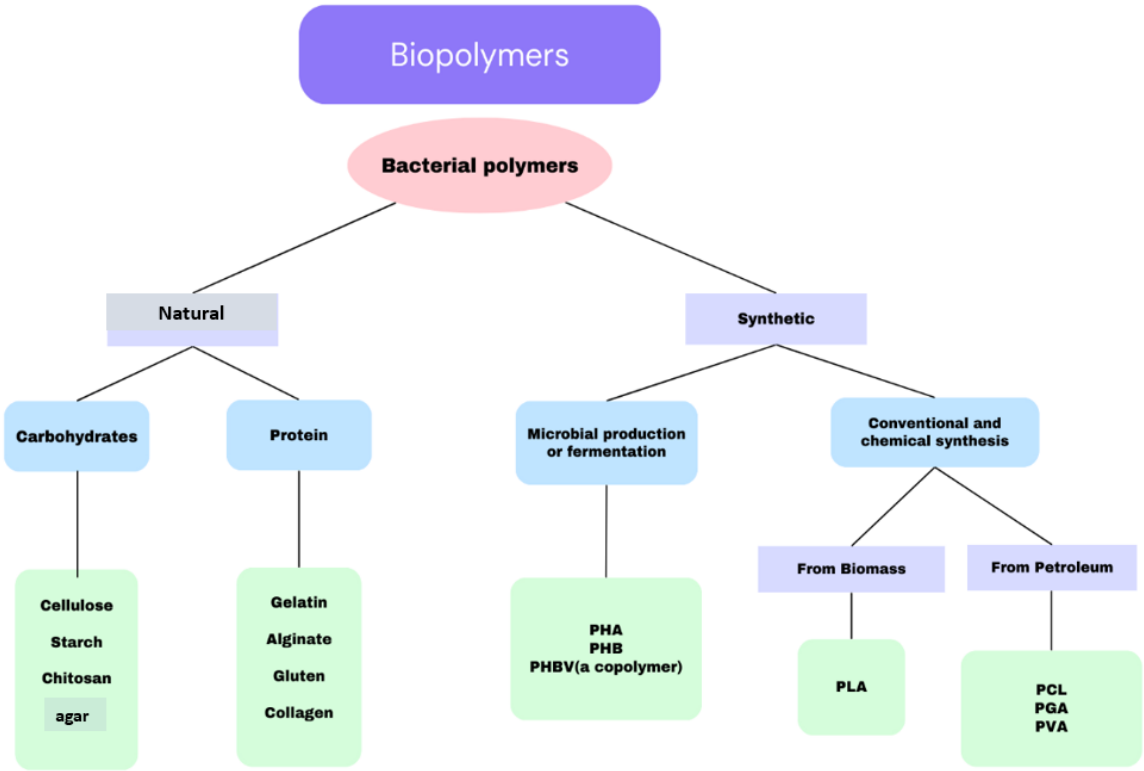

Fig. 1.1**:** A schematic representation of natural and synthetic biopolymer (Modified from Biorender)

PROPERTIES AND PRODUCTION OF BIOPOLYMERS

Biopolymers are different compounds used in our day-to-day life for creating a stable green earth; they are made from living organisms which can include plants as well as various microorganisms. These compounds are composed of repeated units of the same or similar structures (monomers) linked together. Biopolymers are a diverse and remarkably versatile class of materials that are either produced by biological systems or by living organisms such as bacteria, yeast and mold species involved in biopolymer production. The word biopolymer indicates those substances that are bio-degradable and produced from renewable resources [7]. As an alternative, biopolymers can be modified or engineered to improve their biodegradability or to perform precisely the same functions as traditional synthetic polymers. However, it is not that all the biopolymers can be considered as biodegradable, and also not all the biodegradable polymers are considered as biopolymers. For instance, petroleum-based polymers such as polybutylene adipate or terephthalate are biodegradable, but due to the production method, they are not considered bio-based polymers. PLA, PHA and cellulosic material are all bio-based and biodegradable; however, PLA has no biodegradable properties similar to PHA [8, 9], and biomass-derived feedstock such as starch, cellulose and lignin could also be utilized for the production of biopolymers in the greener manner (Fig. 1.1) [1]. As bioplastics become more extensively utilized, a few groups of research have been conducted on their properties, applications, sustainability and biodegradability [10]. The study of biodegradation in diverse environment, such as soil, water and compost has been performed using isolated strains of bacteria. Due to microbial enzymatic activities, biopolymer degradation can occur rapidly in the environment. During the breakdown of organic matter like carbon dioxide, methane and humus, various other naturally occurring chemicals are released into the surrounding environment [11]. Different bacterial strains can synthesize biopolymers by converting waste materials to valuable intracellular (e.g., polyhydroxyalkanoates) and extracellular (e.g., exopolysaccharide EPS) bioproducts, which are useful in biochemical production. For waste management and reducing pollutant emissions, technologies have been developed that convert organic waste into bioenergy and biomaterials [12]. Being eco-friendly, such technology can reasonably help in the replacement of fossil fuels, reducing greenhouse gas emissions into the atmosphere. The increase of carbon dioxide emissions due to fossil fuel or industrial emissions causes the greenhouse effect and increases the ozone hole every year. Chemolithotrophic bacteria perform a significant role in sequestering this carbon dioxide and other greenhouse gases, thereby generating products including lipids, bioplastics, exopolysaccharides and fatty acids [13]. The use of biopolymers minimizes carbon dioxide emissions, municipal solid waste, and reliance on petroleum-based resources.

The advantages of using biopolymers compared to chemically synthesized polymers are the high structural and chemical diversity, bioactivity, bioavailability and presence of definite functional groups defined by the stereo- and enantio-selectivity, biostability under similar environmental conditions, biodegradability and average molecular weight, and more biocompatibility [14]. In vivo methodologies such as molecular biology, genetic engineering, and metabolic engineering were used to obtain modified biopolymers of novel properties and physicochemical characteristics. Many types of polyhydroxyalkanoates are produced by engineered microbes like *Ralstonia eutropha*, *Bacillus megaterium* and several other bacteria. Polylactic acid is another bioplastic produced by engineered *Bacillus coagulans*. The use of metabolic engineering and other molecular tools for enhanced biopolymer production is limited to a few microorganisms, such as *Escherichia coli*, *Pseudomonas spp*., *Pichia pastoris* and some *Bacillus spp*., but in general, most developments and studies were conducted at laboratory scale for academic purposes. In recent studies, a better yield of production of a biopolymer, viz., glucaric acid was derived by a direct synthetic pathway. This was done using *E.coli* as a host by introducing *myo*-inositol-1-phosphate synthase from *Saccharomyces cerevisiae*, *myo*-inositol oxygenase from mouse and urinate dehydrogenase from *Pseudomonas syringae*. The biosynthetic production of glucaric acid was found to be higher than the same production via the pentose phosphate pathway in mammalian cells [14]. The production of lactic acid was done successfully, using *E. coli* knockout mutant strain, with a production of 138 g/L of lactic acid, yield of 0.99 g lactic acid/g glucose, and an overall production of 3.45 g/L/h [3, 15].

Biocomposites are a category of biopolymers that are compatible and eco-friendly and the fillers in them are the biomass, derived from living organisms [15]. There are certain species of bacteria where pure cellulose, secreted in the form of semicrystalline fibrils, are deposited in the extracellular space, but they do not remain attached to the cell and do contribute to the structural stability at the cell level [16]. The secreted cellulose nanofibrils form an interesting layered three-dimensional (3D) hydrogel network [16], called a pellicle, at the interface between the growth medium and atmosphere. It is hypothesized that this hydrogel may confer benefits to bacteria by protecting against pathogens or foreign organisms, and ultraviolet light, thereby preventing dehydration, and trapping carbon di oxide, which in turn helps bacteria to float. Bacteria consume low molecular weight sugars and other carbon sources and the enzyme produced by cellulose synthase complex polymerizes glucose moieties from UDP-glucose into β-1, 4-glucan chains.

Bacteria also produce extracellular polymeric substances (EPS), which are organic polymers of microbial origin involved in the interaction of bacterial cells with the environment [17]. EPS comprises of polysaccharides, proteins, extracellular DNA and lipids. Within biofilms, EPS is distributed in a non-homogenous pattern [18]. EPS interacts and forms a matrix that encompasses living cells. The stability of the matrix depends upon the non-covalent interactions between EPS that involve physicochemical forces [19]. This EPS network interacts with the bacterial aggregates which confers cohesion and viscoelasticity to the structure. Fourier transform infrared (FTIR) spectroscopy is an implementation technique that helps in the detection and identification of organic molecules and the study of microbial attachment and development of microbial biofilm [20]. Attenuated total reflection Fourier transform infrared (ATR/FT-IR) spectroscopy can be used to analyse biopolymers, bacterial cells and biofilms. Although biopolymer synthesis consumes chemical energy and nutrients, it is maintained by the bacteria, as biopolymers enable bacteria to persist and grow in harsh environment under unfavou1rable conditions as well as helping in the immune response of the host during infection.

One such example is *Pseudomonas aeruginosa*, a gram-negative bacterium which is an opportunistic pathogen that becomes capable of carrying out extensive metabolism because of its ability to produce multiple secondary metabolites and polymers like EPS which help them in their quorum sensing. Production of a secondary messenger called the Bis-(3′-5′)-cyclic dimeric guanosine monophosphate (c-di-GMP) reduces the expression of flagellated proteins (and controls the expression of other proteins) and helps to make bacteria more non-motile and pathogenic [21]. C-di-GMP functions by allosterically binding to a variety of effectors, which not only include different types of proteins, but also RNA. It directly controls the central dogma processes including transcription elongation and translation of certain genes which are expressed at that stage of the bacterial life cycle [21]. These bacterial-derived polymers have diverse biological functions such as adhesion, energy storage and protection. Their synthesis is regulated not only by c-di-GMP but also by environmental stimuli. A wide variety of microorganisms including bacteria can accumulate polyhydroxyalkanoic acids (PHA) and polyhydroxybutyrate which are polyesters, in the intracellular water-insoluble inclusion bodies [21]. One such gram-negative bacteria of the *Pseudomonas sp* (*Pseudomonas putida*) has such ability to accumulate PHA, consisting of various 3-hydroxy fatty acids with carbon chain length, ranging from 6-15 carbon atoms [22]. The purified PHA_{MLC} synthase is the key enzyme for PHA synthesis.

P. aeruginosa is capable of producing various exoproducts, such as exoenzymes, pyocyanin, the exopolysaccharide (alginate), and some types of lipids. In 2006, about 350,000 metric tons of PHA bioplastic were produced which accounted for a market share of 0.2% of worldwide plastic production. The biodegradability of PHAs made them perfect candidates to substitute conventional plastics [23-25].

There are several factors affecting the production and purification of biopolymers. The control of common cultivation conditions, such as temperature, medium composition, aeration and agitation is necessary for almost every bioprocess. Some specific factors have to be considered when dealing with biopolymers. The rheology of the cultivation broth has a major impact on mass, oxygen and heat transfer characteristics and consequently on the outcome and success of a bioprocess. Due to the secretion of polymers, the viscosity of the culture fluid can increase dramatically, hence starts behaving like a non-Newtonian broth. In general, the relationship between shear stress and velocity gradient or shear rate (γ) is given by the Herschel Bulkley equation, which utilizes the consistency index K, the yield stress τ_0, and the flow index (n) [14].

The equation**: $\tau = \tau_0 + K * (\gamma)^n$**

Depending on the values for K, τ_0, and n, fluids can be classified as Newtonian and non-Newtonian (like Dilatant, Pseudoplastic, Bingham plastic or Casson fluids) [14]

The most frequent behaviour of biopolymer solutions is pseudoplastic also called "shear thinning". An example of a biopolymer with pseudoplastic behaviour is an aqueous solution of the biopolymer γ-PGA.

So, four major classes of polymers are produced by polysaccharides, polyamides, polyesters and polyanhydrides [26] [13] (Fig 1.2). These polymers serve various biological functions as reserve material or as part of structural composition, and can provide substantial advantages to bacteria under certain environmental conditions. A complex regulatory composition exists to control the biosynthesis and even the material properties of these polymers in response to the external queue. The tabular representation of all the four bacterial polymers with their producers, their precursors, their localization and their industrial applications are being listed in Table 1 [13, 26].

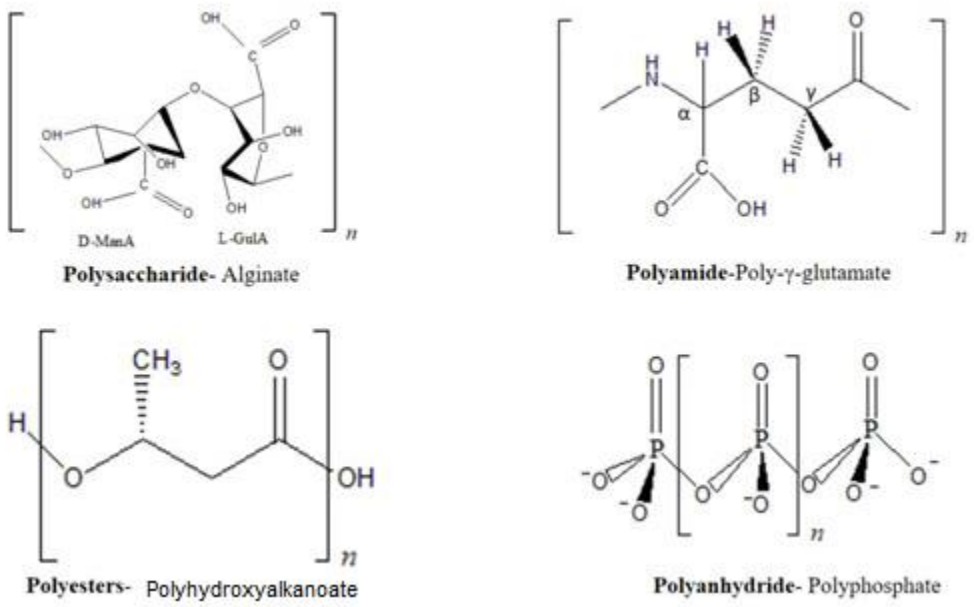

Fig 1.2: Chemical structure of different bacterial polymers (Modified from chemdraw)

Table 1: Bacterial polymers with their producers, their precursors, their localization and their industrial applications

Polymer class	Polymer localization	Main components	Precursors	Polymerizing enzyme	Producers	Industrial applications
Polysaccharide						
Glycogen	Intracellular	Glucose	ADP–glucose	Glycogen synthase (GlgA)	Bacteria and archaea	NA
Alginate	Extracellular	Mannuronic acid and guluronic acid	GDP–mannuronic acid	Glycosyl transferase (Alg8)	*Pseudomonas spp.* and *Azotobacter spp.*	Biomaterial (for example, as a tissue scaffold or for drug delivery)
Cellulose	Extracellular	D-glucose	UDP–D-glucose	Cellulose synthase (BcsA)	*Escherichia coli* (P), *Salmonella enterica* (P), *Sarcina spp.* (P	Food (nata De coco).

					and NP), *Agrobacterium* spp. (NP), *Rhizobium spp.* (NP), *Pseudomonas fluorescens* (NP)	
Polyamides						
Cyanophycin granule peptide	Intracellular	Aspartate and arginine	(β-aspartate arginine)-3-phosphate, ATP, l-arginine and l-aspartate	Cyanophycin synthetase (CphA)	*Cyanobacteria, Acinetobacter* spp. and *Desulfitobacterium* spp.	Dispersant and water softener (after removal of arginyl residues)
Poly-γ-glutamate	Extracellular or capsular	D-glutamate and/or l-glutamate	(Glutamate)n-phosphate, ATP and glutamate	Poly-γ-glutamate synthetase (PgsBC; also known as CapBC)	*Bacillus spp.* and a few Gram-positive bacteria, the Gram-negative bacterium *Fusobacterium nucleatum* and the archaea *Natronococcus occultus* and *Natrialba aegyptiaca*	Replacement of polyacrylate, thickener, humectant, drug delivery and cosmetics
Polyester						
Polyhydroxy alkanoates	Intracellular	(R)-3-hydroxy fatty acids	(R)-3-hydroxyacyl CoA	Polyhydroxy alkanoate synthase (PhaC)	Bacteria and archaea	Bioplastic, biomaterial and matrices for displaying or binding proteins
Polyanhydrides						
Polyphosphate	Intracellular	Phosphate	ATP	Polyphosphate kinase (PPK)	Most bacteria, *Janthinobacterium lividum, Citrobacter freundii* and other archaea	Replacement of ATP in enzymatic synthesis and flavour enhancer

BACTERIAL POLYSACCHARIDE

It is composed of sugar and sugar acids like glucuronic and iduronic acid, amino sugars like D-galactosamine, D-glucosamine, and their derivatives like N-acetylneuraminic acid and N-acetylmuramic acid. They are classified under homo polymers and hetero polymers. They can be charged or noncharged, non repeating or repeating, and branched or unbranched [13]. The homopolymer bacterial polysaccharides refer to bacterial cellulose (BC) and dextran, while alginate, hyaluronic acid or xanthan are heteropolymers. Polysaccharides are enormous groups of structurally diverse molecules with a linear or branched structure that includes a wide range of the same carbohydrate monomers or different types, linked by glycosidic bonds. Diverse bacteria produce polysaccharides and store them inside cells (for example, glycogen) or secrete them either as capsular polysaccharides that are linked to the cell surface or as free exopolysaccharides (for example, alginate and cellulose as well). The monosaccharide units are the building blocks of polysaccharides. D-glucose is the most predominant compound. Microbial polysaccharide exhibits a great diversity in their chemical composition and structure. Microbes can also produce a great diversity in terms of polysaccharides as different genera belonging to a specific class are capable of producing specific polysaccharides. The polysaccharide produced by the bacteria can be subdivided into exopolysaccharides like alginate, dextran, cellulose, hyaluronic acid, etc. which can be either secreted or synthesized in an extracellular pathway by cell wall anchoring enzymes [27-29]. Another class of polysaccharides are capsular like the K30 antigen and O antigen. The third type is the intracellular polysaccharide like the glycogens (Fig 2.2) [30, 31]. Exopolysaccharide and capsular-polysaccharide biosynthesis gene clusters are subject to extensive transcriptional regulation involving two-component signal transduction pathways, recruitment of different enzymes and proteins, alternative RNA polymerase σ-factor and quorum sensing as well as integrating host factor (IHF)-dependent and cyclic-di-GMP dependent processes [32]. Exopolysaccharides (EPSs) are usually built by monosaccharide units and non-carbohydrate components such as acetate, phosphate, pyruvate and succinate. These extracellular polysaccharides can be classified into four major categories, which include microcapsules and slime

polysaccharides, polyamides, inorganic polyanhydrides and polyesters which are collectively called EPS. Capsule polysaccharide represents a prevalent class of surface glycans, which are extensive hydrophilic layers called the capsules, surrounding the cells [32, 33]. Capsule polysaccharides protect against environmental factors like desiccation, promote biofilm formation and frequently serve as virulence factors by preventing clearance by the host immune responses. Capsule polysaccharides are anchored to the outer membrane of cells via lipid modification of 1, 2-diacylglycerols at the reducing end of the polymer. Polymerization and secretion of exopolysaccharides and capsular polysaccharides are often the rate-limiting steps and can substantially affect the flux of carbon towards the progressive formation of high-molecular-mass exopolymers.

The biosynthesis and assembly of capsules are extensively studied in *Escherichia coli* [34] and the protective capsules, found in *Klebsiella pneumoniae* and *Streptococcus pneumoniae*, can contribute to biofilm formation and pathogenesis [35]. *Streptococcus pneumoniae* causes severe lung infections and comprises more than 100 serotypes that produce different capsular polysaccharides to evade. However, when the bacteria switch to a sessile life style, they produce different types of exopolysaccharides as matrix components and this switching to sessile biofilms leads to different chronic infections as they are embedded. Alginates interact with divalent cations to form dense hydrogels with high water-holding capacity. Production of cellulose provides similar advantages to enterobacterial pathogens [36-38]. *Escherichia coli* produces phosphoethanolamine cellulose, which forms mortar-like structures to stabilize proteinaceous curly fibers. These fibers maintain a strong connection between cells in biofilm and provide resistance to higher shear force. *Streptococcus pyogenes* and *Bacillus cereus* (G9241) produce a capsule of hyaluronate, a linear negatively charged heteropolysaccharide that mimics the structure of hyaluronate found in human connective tissues. *Neisseria meningitidis* causes invasive meningococcal disease. It produces a capsular polysaccharide composed of homopolymers of sialic acid, also called N-acetylneuraminic acid, linked with α2→8 -sialic acid linkage. This structure resembles human antigenic polysialic acid moiety that imparts a poor immunogenic property like a low molecular hapten making the polysaccharide invisible in the host system [39]. Glycogen is secreted from the capsular polysaccharides that are associated with the surface of the cell and result in the development of the matrix of the biofilm. The synthesis of certain important materialistic polysaccharides like hydrogels, which participate in drug delivery system, have been used in many branches of medicine, including cardiology, oncology, immunology, wound healing, and pain relief [40]. Hydrogels are composed of a large amount of water and a cross-linked polymer network. This high water content (of about 70–99%) can offer the hydrogels excellent biocompatibility and the capability to easily encapsulate hydrophilic drugs. However, the production of such materialistic polysaccharides is tedious, expensive and restricted to low molecular weight molecules and could be done for a few simpler types of polysaccharides only. It becomes simple and cheaper when microbial cell factory comes in picture. Hydrophilic groups present on the polysaccharide confers high water binding capacity and allows intermolecular interaction and crosslinking like polymer-drug or polymer-host tissue, etc [41, 42]. Exopolysaccharides are mostly produced by bacteria and some archaea [43]. Depending upon subunit composition, structure and molecular mass, exopolysaccharide can be widely used in the production of commercially relevant materials that are of importance in industrial and medical application. A successful example of a bacterial polysaccharide used in biomedical applications is hyaluronate produced by non-pyogenic *Streptococcus zooepidemicus* [44]. Commercial formulations of gel-like fluids of hyaluronate were used for injection into the knee joint for arthritis pain. Dextran is a type of exopolysaccharide produced by *Leuconostoc* spp and *Streptococcus* spp and is water soluble. Its solution behaves as a Newtonian fluid and possesses viscosity that changes as a function of concentration, temperature and average molecular weight. Dextran sucrase is a glucan sucrase belonging to the glycoside hydrolase superfamily and is the key enzyme for dextran synthesis. Dextran sucrase is encoded by dsrS, the expression of which is induced in the presence of sucrose [45]; it is secreted and anchored to the cell wall, and has an average molecular weight. Production of hyaluronic acid (HA) which is also a polysaccharide requires a single protein HasA for polymerization and secretion. However, many exopolysaccharides are polymerized and secreted by the membrane spanning multiprotein complexes [16] (Fig. 2). Cellulose is a polysaccharide composed of β-(1→4)-linked D-glucose units. It is a major structural component of the cell wall of plants and many eukaryotic microorganisms [45]. Cellulose accounts for $\sim 1.5 \times 10^{12}$ tons of the annual biomass on Earth, which makes it the most abundant organic polymer on the planet [46]. In bacteria, cellulose is an exopolysaccharide, normally synthesized in the context of organized bacterial communities which inhibits bacterial motility by hindering flagellar rotation as in *Salmonella* spp [46]. The Wzy independent pathway is adopted for the production of exopolysaccharides such as cellulose and alginate [13]. The integral membrane protein Wzy has been proposed to be the polymerase that catalyses the transfer of the nascent polymer from its undecaprenyl phosphate carrier to the new lipid-linked repeat unit [47] about which there will be more explanation later in this chapter. Wzc belongs to the polysaccharide co-polymerase (PCP) family of proteins, which not only assists polymerization, but also control polymer length and guide the nascent polymer chain through the periplasm to the outer-membrane auxiliary protein Wza [48]. One component of the biosynthesis complex, Alg8, has substantial sequence similarities to the processive β-glycosyl transferases of the GT2 family. It was recently identified as a key membrane protein for

the production of alginate, with multiple copies of Alg8 contributing to substantial overproduction of alginate in *Pseudomonas aeruginosa* which is tailored genetically [49-51]. A storage polysaccharide glycogen produced by glycogen synthases belongs to the GT-B superfamily of retaining glycosyl transferases, which retain the anomeric stereochemistry of the donor sugar in the resulting polysaccharide. The cell surface of *E. coli* is a complex array of proteins and glycoconjugates. The capsular polysaccharides (CPSs) and the O-polysaccharides of the lipopolysaccharide (LPS) molecules are the major surface polysaccharides expressed at 37 °C. Resolution of the crystal structures of one archaeal and two bacterial glycogen synthases has improved the mechanistic understanding of their activity as retain glycosyl transferase [52-54].

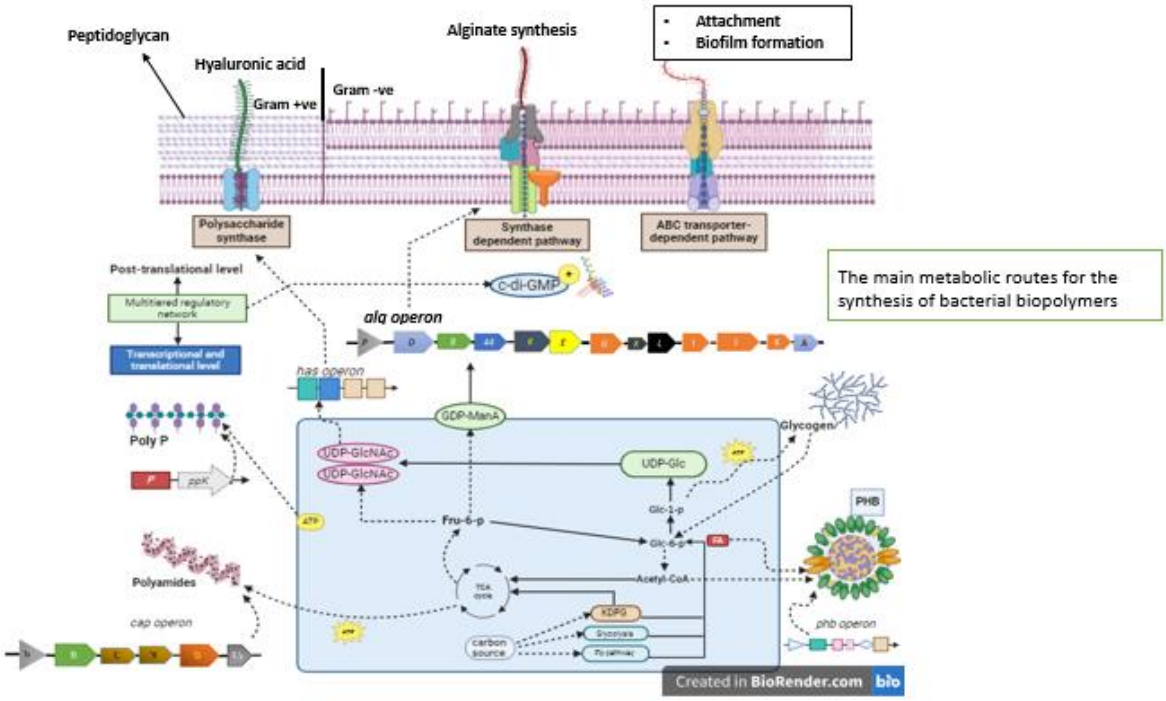

Fig. 2. Bacterial polymer synthesis and their regulation. Genome sequencing, functional genomics, advanced molecular tools and techniques, and new biochemical and biophysical approaches. Here are some examples of a wide variety of extracellular or secretory polymers and the polymers that are synthesized inside the bacterial cell cytoplasm. c-di-GMP not only plays an important role in the synthesis of the polymers, but its main role is in the bacterial pathogenesis for initiating attachment site for biofilm formation (Modified from Biorender).

The O antigen is a polysaccharide linked to the lipid A core component in the LPS molecule. In some bacteria (such as mucosal pathogens), the core oligosaccharide can be modified by a phase-variable (like an on-off mechanism) extension of one or a few sugars in a form known as lipooliogsaccharide. However, the more prevalent LPS format shows a tripartite structure where the core OS is further glycosylated by a long-chain repeat-unit polysaccharide. This is generally called the O-antigen (O-PS), with the term originating from the Kauffman–White serological classification system developed for *Salmonella* in the 1930s. LPS molecules carrying O-PS are called "smooth LPS" (S-LPS), whereas those lacking O-PS are termed as "rough LPS" [(R)-LPS]. All O-PSs are assembled as undecaprenyl diphosphate (Und-PP)-linked assembly intermediates in the cytoplasm and end with the ligation of Und-PP–linked O-PS glycans with defined chain-length distributions to lipid A core in the periplasm. Synthesis is initiated at the cytoplasmic face of the inner membrane by a phosphoglycosyltransferase (PGT) enzyme that transfers a hexose phosphate or acetamido sugar phosphate from the corresponding nucleotide diphosphoglycose donor to undecaprenyl phosphate [56-61]

BACTERIAL POLYAMIDES

These polymers are the products which are poly (amino acid) chains such as the secretory poly (γ-D-glutamic acid) and ε-poly-L-lysine (PL), along with the intracellular cyanophycin granule peptide (CGP) [62], having similar role like that of polysaccharide in the biofilm matrix. It is a polyamide capsule and is poorly immunogenic [63]. Polyglutamic acid (PGA) is a water-soluble, biodegradable biopolymer that is produced by microbial fermentation. PGA can be used in drug delivery for the controlled release of the anti-cancer drug [64]. The main property of PGA for drug delivery is its molecular weight. Its molecular weight is large to diffuse into tumor cells, while sparing normal cells. Poly-γ-glutamic acids (γ-PGAs) with different molecular weight are of

high application value in agriculture, cosmetics, medicine, and biosynthesis of γ-PGA with specific molecular weight [65]. Three poly-γ-glutamic (γ-PGA) hydrolase of *Bacillus subtilis* PgdS [66], *B. subtilis* YwtE and *B. licheniformis* SGH were heterologously expressed and their hydrolytic capacities were investigated. It was found that all three hydrolases can cleave high molecular weight γ-PGA into low molecular weight γ-PGA, particularly by *PgdS* gene. Poly-γ-glutamic acid (γ-PGA) is water soluble macromolecular peptide that consist of only D-glutamic acid or D- and L-glutamic acids and are polymerized by γ-glutamyl bonds [67]. Several strains of *B. subtilis* and *B. licheniformis* have been widely exploited for producing γ-PGA, as these organisms produce γ-PGA extracellularly, which simplifies recovery and purification of the polymers [68-71]. The PgdS enzyme (also known as YwtD) is a γ-PGA hydrolase from *B. subtilis* or *B. licheniformis*, which degrades γ-PGA and releases it extracellularly into the medium. PgdS is an endo-γ-glutamyl peptidase, belonging to the NlpC/P60 family [72], that cleaves only the γ-glutamyl bond between D-glutamic acid and L-glutamic acid of γ-PGA [73]. Released PGA might serve as a nitrogen and carbon source and is a water-binding component of the biofilm matrix. The chemical properties of PGA and of CGPs with chemically reduced arginine content resemble the properties of chemically synthesized and extensively applied polyacrylates. CGP synthesis has been proposed to resemble an amide ligase-dependent reaction [74]. Hence, bacterial polyamides could provide renewable, non-toxic and biodegradable alternatives to polyacrylates. In addition to being produced by Gram-positive bacteria and archaea, PGA is also produced by the Gram-negative bacterium *Fusobacterium nucleatum* [75, 76] (Fig 1.4). The membrane-anchored protein PGA synthase B (PgsB; also known as CapB), which has similarities to the amide ligases, is the catalytic subunit of the PGA synthetase [77]. Both PgsB and PgsC (also known as CapC) have ATPase activity that is increased by the addition of PgsA. The biosynthesis of the third natural polyamide, PL consists of at least 25-35 L-lysine residues, as found from studying PL synthetase. However, PL synthesis is distinguishable from PGA and CGP synthesis in that it does not require phosphorylation of the carboxyl terminus of the growing chain.

BACTERIAL POLYESTERS

Polyhydroxyalkanoates (PHAs) such as poly (R)-3-hydroxybutyrate are bacterially synthesized bioplastics. They are linear polyesters that are synthesized and assembled into hydrophobic spherical inclusions; they function in carbon and energy storage. A wide range of Gram-positive and Gram-negative bacteria produce PHAs; PHA^- mutants of *P. aeruginosa* were found to lack the means of attachment to glass surfaces and showed reduced stress tolerance in biofilms [78]. Polyhydroxyalkanoates (PHA) accumulate as carbon reserve when required during growth limitation owing to starvation of other nutrients like phosphorus [13] and nitrogen. PHA is deposited as spherical intracellular inclusions with an amorphous, hydrophobic PHA core that is mainly surrounded by proteins involved in PHA metabolism [79, 80]. Owing to the broad substrate specificity of PHA synthase (PhaC), any organic molecule, containing a carboxyl and a hydroxyl group, can be converted to the respective CoA thioester that can be incorporated into a high-molecular-mass PHA [81]. The biosynthesis pathways of the activated PHA precursor, (R)-3-hydroxyacyl-CoA, have been found and exploited through metabolic engineering, leading to the production of modified PHAs — that is, a range of heteropolymers and homopolymers containing (R)-3-hydroxy fatty acids and/or (R)-4-hydroxy fatty acids with different carbon chain lengths — that show more favourable physical properties (for example, melting temperature, glass transition temperature) for industrial and medical applications [81, 82]. Initiation of PHA synthesis requires activation of the thiol group of the cysteine residue in the PhaC active site by the conserved histidine in the same active site, enabling a nucleophilic attack on the thioester bond of the (R)-3-hydroxyacyl-CoA substrate, and also releasing CoA and forming a covalent enzyme–substrate intermediate [83, 84]. A conserved PhaC aspartic acid residue activates the hydroxyl group of the bound 3-hydroxy fatty acid, which then attacks the thioester bond between a second hydroxyl fatty acid unit and the active site cysteine of a second PhaC subunit, joining the two fatty acids together. The incoming substrate is then covalently bound to the free cysteine after the activation of the hydroxyl group, and then again another nucleophilic attack will extend the polyester by another unit and it continues. Experimental evidence was obtained for chain termination, occurring by transferring most of the polyester chain to a second, surface-exposed amino acid, which hydrolyses the chain [84]. The remaining already primed PhaC starts a new cycle of polyhydroxyalkanoates (PHA).

BACTERIAL POLYANHYDRIDES

Polyphosphate is a polymer of condensed phosphates (three to several hundred inorganic phosphates) that is highly negatively charged and rich in 'high-energy' anhydride bonds and used by bacteria as energy storage molecules [13]. We know ATP is the energy currency of the cell; phosphate groups form pyrophosphate linkage carrying a lot of energy. Polyphosphate synthesis is an evolutionarily ancient ability of bacteria. PolyPs, besides functioning in phosphate storage, also provide chemical energy for biosynthesis pathways, function as a buffer against alkali and as a metal-chelating agent, contributing to channel complexes for the uptake of DNA [85, 86]. In bacteria, polyphosphate can form intracellular storage particles but may also form a membrane-anchored complex with low-molecular-mass polyhydroxybutyrate, facilitating the uptake of DNA and various

ions [87], so that there exists a crosstalk between polyamides and polyanhydrides. As no polyP naturally exists on earth, living organisms, in particular, bacteria, are unique sources of polyP. Bacteria belonging to the genera *Mycobacterium* and *Corynebacterium* produce polyp granules with a high yield and therefore are potential production strains for the manufacture of polyps [88]. The highly conserved polyphosphate kinase (PPK) is the main enzyme for biosynthesis. In addition to synthesizing polyphosphate from ATP, PPK also catalyses the reverse reaction, viz., phosphorylating ADP to produce ATP [89]. The reaction mechanism of PPK has been elucidated by obtaining the crystal structure of PPK from *Escherichia coli* [90]. The chain elongation reaction, leading to the generation of polyphosphate, is still not elucidated but there might be some pyrophosphate linkages. Recently, a second PPK, PPK2, has been identified in *Pseudomonas aeruginosa*; PPK2 catalyses the synthesis of polyphosphate from GTP and prefers GDP over ADP as a phosphate group acceptor, for a greater energy carrier source [91]. PPK2 homologs have been found widely spread among a lot of bacteria [92].

A class of biopolymers called biopolysaccharides is typically associated with the structural (membrane and wall) and compositional (cytosolic polysaccharides) integrity of living organisms. Three different types of polysaccharides are produced by microorganisms: intracellular (for storage), extracellular (EPS) and structural forms. There are two types of EPS forms: capsular, which are integrated into the cell wall, and loose, which are created as large-scale accumulations outside the cell wall and disperse into the culture medium [93]. Over the past ten years, there has been a global increase in awareness regarding the intrinsic benefits that can be derived from biopolysaccharides obtained from various biogenetic resources. Exopolysaccharides with a variety of biotechnological, pharmacological, industrial and medicinal uses, as well as biosurfactants and bioemulsifiers that are cheap and variable in their bioactivity, are emerging as a result of this special inclination to biopolysaccharides [94] [95]. The paradigm shift towards the study of biopolysaccharides is due to the urgent need for safer, more ecologically and biologically compatible natural therapies than chemically synthesised polymers. Biopolysaccharides can be classified as either homo- or hetero-polysaccharides, possessing a branched or unbranched monomeric structure that enables them to exhibit unique bioactive, physicochemical and rheological properties [96, 97, 98]. Chitin [N-acetyl-D-glucosamine connected by β (1→4) glycosidic bonds] and cellulose [D-glucose residues joined by β (1→4) glycosidic bonds] are examples of homopolysaccharides. Conversely, hyaluronan, which is made up of 250–25,000 β (1→4)-linked D-glucuronic acid and β (1→3)-linked N-acetyl-D-glucosamine, comprise the heteropolysaccharide. Another heteropolysaccharide, chondroitin-4-sulfate, is made up of N-acetyl-D-galactosamine-4-sulfate and D-glucuronic acid. In contrast to biological proteins, which have a well-studied genetic code, the precise size of many biopolysaccharides is still unknown [99]. Microbial biopolymers are produced by a number of microorganisms that are now known to exist. These polymers are either recovered from the fermentation media or affixed to the cell surface. In reaction to specific environmental stressors, bacteria use these microbial biopolymers as storage resources [100].

Microbial polysaccharides can be broadly categorised, based on their biological roles into three groups: extracellular bacterial polysaccharides (like levan, xanthan, sphingan, alginate, pullulan, cellulose, etc.), intracellular storage polysaccharides (like glycogen), and capsular polysaccharides (K30 O-antigen). These groups are crucial for the formation of biofilms and pathogenicity [101].

Exopolysaccharides (EPSs) are microbial polysaccharides that are produced by microbes and secreted from the cell. They play a vital role in cell defence, bacterial attachment to solid surfaces and cell-to-cell contacts [102]. Due to their unique structural and functional characteristics, EPSs have attracted a lot of attention in recent years. For hydrocolloids utilised in the food, pharmaceutical, chemical and numerous other industries, EPSs are valuable resources [103]. Microbial enhanced oil recovery (MEOR), textiles, detergents, adhesives, wastewater treatment, dredging, brewing, downstream processing, cosmetology, pharmacology and food additives are just a few of the industrial sectors that make use of microbial EPSs, which are new biomaterials with a variety of intriguing physicochemical and rheological properties and novel functionality [104]. Because of their remarkable qualities, microbial polysaccharides such as xanthan, dextran and pullulan have a sizable market. Microbial EPSs are economically competitive with plant and algal origin polysaccharides, which are influenced by climatological and geological environmental variables, because they allow for quick and high yielding manufacturing procedures under controlled settings [105]. The utilisation of microbial EPSs in industrial and medicinal settings has become more popular. However, owing to their high manufacturing costs, only a limited number of bacterial EPSs, including xanthan, gellan and dextran have found commercial success [106][107]. Microbial EPSs were never able to find a suitable place in the polymer market due to the very high production costs; as a result, the industrial significance of high-level EPS-producing microbial systems is growing. In order to regulate and subsequently increase microbial productivity, the growing importance of EPSs in industrial and medical biotechnology necessitates the clarification of the relationships between metabolic pathways and biosynthetic mechanisms. As a result, in recent years, there has been a great deal of interest in understanding the mechanisms and pathways involved in the production of bacterial EPSs in order to increase productivity. To discover new EPS biosynthesis routes and comprehend the fundamentals of

EPS creation, omics technologies including genome sequencing, functional genomics, protein structure analysis and novel bioinformatics tools have been employed [101, 108].

MICROBIAL EXOPOLYSACCHARIDE

Four primary categories were identified for the biopolymers generated by microorganisms: polyesters, polyamides, inorganic polyanhydrides and polysaccharides [13, 109, 110]. Under some environmental circumstances, the microbes that create biopolymers have a great advantage because the microbial biopolymers act as a reserve resource or in defensive mechanism [13]. Midway through the 1800s, Pasteur found the first bacterial polymer, dextran, as a microbial product in wine [111]. Van Tieghem (1878) recognised the bacterium *Leuconostoc mesenteriodes* as a strain that produced dextran [112]. EPSs are the bacterial polysaccharides that are produced and released into the extracellular environment as soluble or insoluble polymer by different microorganisms. Different bacterial species have different compositions, roles and physical and chemical characteristics that determine their fundamental conformation. Acetate, pyruvate, succinate and phosphate are examples of non-carbohydrate substituents that make up EPSs; however, carbohydrates (a broad spectrum of sugar residues) make up the majority of them [113, 114, 115].

According to reports, the majority of bacteria that create EPSs can either manufacture homopolysaccharide or heteropolysaccharide. However, it has been shown that bacteria (*Aeromonas salominicida*, *Serratia marcescens*) are capable of producing two distinct polysaccharides [116]. Owing to the linking bonds and characteristics of monomeric units, homopolysaccharides are classified as fructans, polygalactans, α-d-glucans, and β-d-glucans. The repeating units of heteropolysaccharides are D-glucose, D-galactose, l-rhamnose, N-acetylglucosamine (GlcNAc), N-acetylgalactosamine (GalNAc) or glucuronic acid (GlcA), and rarely non-carbohydrate substituents like phosphate, acetate and glycerol. The synthetic enzymes and sites of synthesis of homopolysaccharides and heteropolysaccharides are also different. Specific substrates such as sucrose are needed for the biosynthesis of homopolysaccharides, whereas the intracellular production of heteropolysaccharide residues and the location of precursors for extracellular polymerization across the membrane are facilitated by isoprenoid glycosyl carrier lipids [117].

MECHANISM OF EXOPOLYSACCHARIDE SYNTHESIS BY BACTERIA

In recent years, significant progress has been made in understanding the genetic and metabolic pathways of bacterial polysaccharide production. The precursors needed and the biosynthesis pathway show how diverse the various kinds of EPSs are. Bacteria can produce extracellular or intracellular EPSs [118] [119] [106] [120]. Chain-length determination, repeat-unit assembly, polymerization and export are all controlled by genes necessary for the synthesis of EPS. Despite our understanding of the organisation of *EPS* genes, the mechanism controlling EPS biosynthesis remains a difficult subject to comprehend [121]. According to Delbarre-Ladrat et al. (2014), the availability of sugar precursors and the degree of enzyme expression are the two physiological and metabolic criteria that are linked to the regulation of EPS biomass. Genetic data on the synthesis of other EPS, such as pullulan, is still scarce, despite the abundance of information available on the genetics of some EPS, such as xanthan [122]. Most bacterial EPSs are produced intracellularly and exported to the extracellular environment, with the exception of homopolysaccharides like mutan, levan and dextran, which are produced extracellularly by secreted enzymes that polymerize the substrate. The four kinds of enzymes that are involved in EPS generation are located in distinct parts of the cell. Intracellular enzymes, including hexokinase, which phosphorylates glucose (Glc) to glucose-6-phosphate (Glc-6-P), comprise the first group. They participate in several cellular metabolic processes as well. The conversion of sugar nucleotides requires the second group.

The enzyme uridine-5′-diphosphate (UDP)-glucose pyrophosphorylase is responsible for converting Glc-1-P into UDP-Glc, a crucial molecule in the creation of EPS. Glycosyltransferases (GTFs) are another class of enzymes found in the periplasmic membrane of cells. GTFs transport the sugar nucleotides to a repeating unit that is connected to a glycosyl carrier lipid. A great deal of research has been done on the enzymatic activities, structures and gene identification of GTFs. As a result of commonalities in amino acid sequences, over 94 GTF families have been identified in the Carbohydrate-Active enzymes (CAZy) database (http://www.cazy.org) [123].

The Wzx/Wzy-dependent pathway, the ATP-binding cassette (ABC) transporter-dependent pathway, the synthase-dependent pathway, and the extracellular synthesis using a single sucrase protein are the general processes for the formation of bacterial polysaccharides. In the first three pathways, enzymes inside the cell convert the precursor molecules into activated sugars or sugar acids. On the other hand, the polymer strand is extended in the extracellular manufacturing route by the direct addition of monosaccharides produced by cleaving di- or tri-saccharides [124]. In the Wzx/Wzy-dependent process, GTFs bind the activated sugars to a lipid carrier in a predetermined order until a repeating unit comprising a Wzy protein is created. The cytoplasmic side of the inner membrane is where polymerization occurs in the Wzx/Wzy-independent (ABC transporter-dependent) pathway. Encoding an outer-membrane protein (wza), an acid phosphatase (wzb), and an

inner-membrane tyrosine autokinase (wzc), the corresponding genes are necessary for high-level polymerization and surface assembly. The majority of Gram-negative bacteria, including *Xanthomonas campestris*, *Rhizobium* spp., *Erwinia* spp., and *Methylobacillus* sp. strain 12S, have been shown to undergo EPS biosynthesis and export through the Wzx/Wzy-dependent and Wzx/Wzy-independent pathways [125] [126] [127].

In the synthase-dependent pathway, which secretes entire polymer strands across the membrane and cell wall, independent of a flippase for translocating repeat units, EPS secretion can take place in the presence or absence of a lipid acceptor molecule. In this system, a single synthase protein—a glycosyl transferase encased in a membrane—performs both the polymerization and the translocation processes concurrently. These routes are frequently employed in the building of homopolymers that need a single type of sugar precursor like bacterial cellulose [β-(1-4)-linked glucose units] or curdlan [β-(1-3)-linked glucose monomers] [128] [129] [101]. In Gram-negative synthase-dependent secretion systems like *Gluconacetobacter xylinus* cellulose and *P. aeruginosa* alginate, polymerization regulation is carried out by an inner-membrane receptor [129].

A monosaccharide is transferred from a disaccharide to a polymerizing polysaccharide chain in the extracellular environment during the polymerization step in extracellular synthesis. This kind of EPS formation is simple and does not depend on the central carbon metabolism, but there is some structural heterogeneity. For homopolysaccharides (dextran, levan and mutan), extracellular GTF can facilitate the synthesis of extracellular EPS [130], [131], [132]. The synthesis of irregular repeating units from sugar nucleotide precursors is a step in the intracellular biosynthesis of homo- and hetero-polysaccharides [133] [134]. These units are also involved in the manufacture of various cell wall components and are therefore thought to be necessary for growth. Intermediates of the core carbon metabolism are converted intracellularly to direct precursors for the production of EPS in bacteria. Sugar nucleotides, such as nucleoside diphosphate sugars (like ADP-glucose), nucleoside diphosphate sugar acids (like GDP-mannuronic acid), and nucleoside diphosphate sugar derivatives (like UDP-glucose, UDP-N-acetyl glucosamine, UDP-galactose, and deoxythymidine diphosphate (dTDP)-rhamnose), are the precursors and donor monomers for the biosynthesis of most repeating units [135] [136][137]. These sugars can be transported in essentially three different ways: (i) via the phosphoenolpyruvate (PEP) transport system (PTS), (ii) import connected to the transport of ions and other solutes, and (iii) by ATP hydrolysis linked to sugar translocation via a sugar transport ATPase [138, 139]. Heteropolysaccharide biosynthesis involves multiple intracellular processes that are catalysed by GTFs (EC 2.4.x.y). The polymerization of repeating units occurs in the last step, which is extracellular. The first stage in the uptake of sugars by the cell is accomplished by either an active or passive transport pathway, depending on the kind of substrate. Glycolysis is then used in the cytoplasm to catabolize the substrate, resulting in the formation of sugar nucleotides. From phosphorylated sugars, activated precursors—energy-dense monosaccharides, primarily nucleoside diphosphate sugars (NDP-sugars)—are biosynthesised. Lastly, because EPS are secreted into the extracellular environment, it can be difficult to secrete them from the cytoplasm via the cell membrane without sacrificing their essential barrier qualities [140] [141] [142].

The majority of EPSs generated by marine bacteria are heteropolysaccharides, which are repeating units made up of three or four distinct monosaccharides in groups of ten or fewer [143]. Monosaccharides like pentoses (as D-arabinose, D-Ribose, and D-Xylose), hexoses (as D-Glucose, D-Galactose, D-Mannose, D-Allose, L-Rhamnose, and L-Fucose), amino sugars (as D-Glucosamine and D-Galactosamine) or uronic acids (as D-Glucuronic acid, D-Galacturonic acid) are the most frequently found components in marine EPS. There may also be organic or inorganic substituents present, including phosphate, sulphate, acetic acid, succinic acid and pyruvic acid [144]. While uronic acids, ketal-linked pyruvate and inorganic residues like phosphate or sulphate can make most EPS polyanionic, some of them are neutral macromolecules [145] Furthermore, the most often observed connections among monosaccharides are 1,4-β- or 1,3-β-linkages in the highly rigid backbones and 1,2-α- or 1,6-α-linkages in the more flexible ones. The arrangement of the monosaccharides and the assembly of the single polymer chains have a significant impact on the physical properties of polysaccharides [146].

MARINE MICROBES PRODUCING EPS

One viable method to study microbial EPS production is to cultivate a single isolated strain in a controlled laboratory setting, as the analysis of EPSs in the natural marine environment is challenging because each polymer is rare [147][148]. Notwithstanding, no singular collection of culture settings may provide elevated yields of extracellular solid phase, as microorganisms vary in their utilisation of carbon and nitrogen sources, mineral needs, ideal pH and temperature. Furthermore, by employing a physiological control, the molecular mass, number of residues, and degree of branching of EPS can all be altered. It is true that the quality and output of microbial EPS can be affected by the environmental and nutritional factors (culture conditions) [132]. Sutherland [115] showed that when marine bacteria were cultivated in a laboratory setting with restricted nutrients (such as nitrogen, phosphorus, sulphur and potassium), the production of EPS increased. In general, physical constraints that limit growth, such as osmotic stress or suboptimal growth temperature, might increase the formation of EPS. Moreover, the initial stage in optimising the production of EPS is the selection of the

carbon source (sugar or non-sugar sources) in the growth medium. The microorganism *Hahella chejuensis*, which was isolated from the marine sediment collected from Marado on Cheju Island, Republic of Korea, was found to produce the highest EPS yield in a growth medium supplemented with sucrose [149, 150][151]. Meanwhile, *Halomonas alkaliantarctica*, strain CRSS, a haloalkalophilic bacteria, isolated from a saline lake in Antarctica, produced the most EPS [152] [153].

Specifically, strain CRSS demonstrated the ability to synthesise EPS with diverse chemical compositions on a range of substrates used as nutrients: two distinct complex media produced mannan or xylo-mannan, while minimal media containing acetate as the only carbon source produced fructo-glucan [152]. Since the yield and structure of polymers can be altered by growth conditions, this is a frequent event that has been reported for other strains [154]. Currently, the most often utilised nitrogen sources for EPS generation are ammonium sulphate, peptone, sodium nitrate, urea and yeast extract. The growth rate and EPS production are often enhanced by the presence of an organic nitrogen source [155, 156, 157]; however, some evidence indicated that EPS production was higher at lower nitrogen concentrations.

Marine EPS-Producing Microorganisms Isolated from Deep-Sea Hydrothermal Vents, Volcanic and Hydrothermal Marine Areas, Shallow Submarine Thermal Springs

Thermophilic microorganisms have not received as much attention as mesophilic heterotrophic bacteria, despite the ability of the latter to produce thermostable enzymes with potential biotechnological applications for large-scale industrial production. In actuality, only a small number of polymers have been thoroughly characterised to date, despite a great deal of information having been discovered regarding the chemical composition, rheological characteristics and metal binding capabilities.

A fresh source of EPS producing bacteria is provided by shallow submarine thermal springs, deep-sea hydrothermal vents and volcanic and hydrothermal marine regions. An EPS with an octasaccharide repeating unit with two side chains was generated by *Pseudoalteromonas* strain 721 [158, 159]. As the temperature increased, this EPS showed signs of gel formation and viscoelastic behaviour. Bacteria isolated from volcanic and hydrothermal marine areas are a valuable source of EPS. Specifically, some thermophiles such as *Methanosarcina*, *Haloferax* and *Bacillus* species, as well as more recently, *Thermotoga maritima* and *Thermococcus litoralis*, have been studied for their production of unique EPS [160][161]. It has been demonstrated that *Thermococcus* species can manufacture EPS in a specified growth medium free of sulphur at growth rates and cell yields that are comparable to those on complex media. This microbe, which has an ideal growth temperature of 88 °C, is a heterotrophic facultative hyperthermophilic Archaeon that is sulfur-dependent. It was isolated from a shallow underwater thermal spring. While extracellular polysaccharides have been found in a number of severe marine settings, very little research has been done on the ability of extremophiles to produce polysaccharides. Mannose is the only monosaccharidic component in EPS of *Thermococcus*, which is a highly unusual characteristic for a prokaryote because eukaryotes, such as plants or yeasts, are normally the ones that synthesise mannan-like elements [162][163][164]. Furthermore, *Thermococcus* species demonstrated the production of biofilms on hydrophilic surfaces in a range of settings. Notably, in culture medium supplemented with maltose or yeast extract, cell adherence was particularly strong. In fact, the amounts of polysaccharides in biofilms generated under various growth conditions were comparable. It is common for marine bacteria to adhere to surfaces, a process in which EPS probably have a major role. Even though more and more species are being isolated from hydrothermal vent locations, this phenomenon in hyperthermophilic archaea has not been thoroughly studied. Additional research on the EPS manufacturing may reveal fresh perspectives on the natural growth patterns or organisms and provide details on crucial ecological interactions in the microbiological world [165][166].

Furthermore, thermophilic bacteria from the *Geobacillus* species have been identified as makers of EPS after being isolated from shallow maritime hydrothermal vents in the flegrean area of Italy. Additionally, the surrounding marine shallow hydrothermal vents of the volcanic Eolian islands, which are on Sicily coast in Italy, offer fields that are easily accessible for the isolation of thermophilic bacteria. Previous research in Porto di Levante, Vulcano, revealed the presence of chemosynthetic, thermophilic, archaeal and bacterial strains while describing the variety and dispersion of bacterial communities within deep and shallow hydrothermal systems [160][167]. Moreover, a shallow hydrothermal vent on Vulcano Island (Eolian Islands, Italy) produced a thermophilic aerobic bacterium that was able to synthesise two exocellular polysaccharides (EPS1 and EPS2).

Marine EPS-Producing Microorganisms Isolated from Sea Environment That is Highly Salted: Marine Salterns and Salt Lakes

Numerous aquatic and terrestrial habitats contain hypersaline conditions. In addition to halophilic microorganisms (surviving in 0.5 M and 2.5 M NaCl) and extreme halophiles (surviving above 2.5 M NaCl), they are inhabited by halotolerant microorganisms. It has been possible to isolate moderate and extreme halophiles from both alkaline (alkaline lakes) and hypersaline (salt lakes, marine salterns and saline soils) areas. In order to survive in highly salinized environment, halophilic microbes have evolved a variety of metabolic

defence mechanisms, such as suitable solute production to preserve cell structure and function. Notably, their products are of industrial interest. These include ectoine, bacteriorhodopsins, exopolysaccharides, hydrolases and biosurfactants [168].

Since numerous species of *Halomonas* are able to produce sizable amounts of EPS with high surface activity and/or rheological features, the genus has in fact attracted more attention [169][170][171]. Interesting characteristics of the EPS generated from *Halomonas* species, like its capacity for emulsification, seem valuable for a wide variety of products and applications. Actually, hydrocarbons are used to synthesise large amounts of surface-active agents, which are essential components in food, cosmetic and pharmaceutical industries. This presents a problem because the non-renewable resource used to make these agents raises questions about their potential health and environmental effects. In contrast, because they are less harmful and renewable, biologically derived biosurfactants and bioemulsifiers have drawn more attention.

Marine EPS-Producing Microorganisms Isolated from Cold Marine Environment

Bacteria that are "cold loving," or psychrophilic, prefer a growing temperature below 15°C. Psychrotrophs or psychrotolerants are bacteria that prefer a high growth temperature but can tolerate such low temperature. It is now generally accepted that bacteria extracted from deep-sea vents will provide a significant supply of novel chemicals for biotechnological applications. A variety of deep-sea psychrotolerant bacterial species have been extensively studied because they may provide information on crucial ecological traits that allow bacteria to adapt morphologically, physiologically and metabolically to the constantly shifting deep-sea environment [172].

POLYSACCHARIDE PRODUCTION BY ARCHAEBACTERIA

Exopolysaccharides were found in several Archaea taxa, mainly in thermophiles and halophiles. Members of the halophilic genera, *Haloferax*, *Haloarcula*, *Halococcus* and *Natronococcus* expel large amounts of extracellular polymers [173-179]. It has been noted that a variety of thermoacidophilic archaea, such as those belonging to the genera *Sulfolobus* and *Thermococcus*, secrete sulphated heteropolysaccharide and mannan, respectively, and accumulate storage polysaccharides like glycogen [180, 181]. *Thermococcus litoralis* and *Archaeoglobus fulgidus* accumulate large volumes of EPS as biofilms [182], [183].

The well-characterized species of *Archaeoglobus fulgidus* is another example of the producer of an exopolysaccharide as a component of an archaea biofilm. Anaerobic marine hyperthermophile *A. fulgidus* uses lactate, pyruvate or H_2 as an electron donor to facilitate dissimilatory sulphate reduction, which releases energy.

It has been found that a soluble exopolysaccharide that *Thermococcus litoralis* generated was presumably involved in the production of a biofilm [184]. Mannose was the only monosaccharidic component identified by analysis of the exopolysaccharide degraded by acid. More recently, it was investigated how carbon and nitrogen sources affected the growth dynamics and exopolysaccharide production for the bacteria *Thermotoga maritima* and the archaeon *Thermococcus litoralis*. It was discovered that *T. litoralis* was not only unable to use NH_4Cl as a nitrogen source, but that at certain concentration, its growth was actually inhibited. Furthermore, both organisms produced a large amount of exopolysaccharides, which increased with dilution rate [185].

EXOPOLYSACCHARIDE SYNTHESIS BY FUNGI

Currently, it is known that a sizable range of fungi, including lower filamentous fungus, higher basidiomycetes, and yeasts from various ecological niches, can synthesise EPS in laboratory culture systems. EPSs are typically produced inside cells and released into the environment. Information about the manufacture of EPS from fungi is scarce [186],[187],[189],[190]. There are very few fungal EPS biosynthesis pathways that have been investigated, like EPS production from *Ganoderma lucidum* [190]. Most researchers have investigated how to best optimise the culture medium and environmental factors so that various fungal strains can produce EPS.

The type of carbohydrate utilised to produce EPS does not always affect its composition; nevertheless, the concentration and type of carbon source utilised greatly influence the intensity of EPS formation. Typically, the carbon sources in the culture media are glucose, sucrose, maltose, lactose, fructose, galactose, xylose, cellobiose, sorbitol, xylitol, mannitol and various agricultural byproducts. For the most part, it has been determined that the most important carbon sources for the ability of the fungus to produce EPS are glucose, sucrose and maltose [191]. These data suggest that different fungal strains have varied fascinations for sucrose absorption, that there may be some impacts of catabolic suppression of different sugars in different EPS production, or that these sugars may be readily metabolised by fungi. The true causes, however, remain unclear because fungal growth and EPS production are rarely directly correlated.

Another factor that has been shown to stimulate the generation of EPS is nitrogen supplementation. To determine which nitrogen source was best, multiple researchers evaluated both organic and inorganic sources. The majority of the organic sources that were evaluated were peptone, yeast extract polypeptone, Martone A-1,

soybean meal and corn steep powder. Yeast extract and maize steep powder have been found to be effective nitrogen supplements that stimulate the formation of EPS from a variety of fungal strains [192],[193],[194]. Among the different inorganic sources, researchers frequently examine ammonium chloride, ammonium sulphate, potassium, sodium and urea, as well as diammonium oxalate monohydrate. Numerous observations revealed that fungi create fewer extracellular polymers than supplements containing organic nitrogen when there are inorganic nitrogen sources present. Ammonium salts are often more effective than other inorganic salts as inorganic nitrogen suppliers. Other inorganic salts have been found in very few studies to be the most effective donors of nitrogen for EPS generation from fungus [195],[196]. The most efficient method for producing EPS from *Nigrospora oryzae* var. glucanicum was discovered to be urea [196]. Nitrogen limitation circumstances are typically present when EPS synthesis takes place [197]. Researchers investigated and found that varying concentrations of a particular nitrogen favoured maximum EPS synthesis for certain fungal strains [192] [193] [194].

PRODUCTION OF BIOPOLYMERS BY ENGINEERED MICROBES

It is possible to genetically modify bacteria so that they accumulate polymers inside their cells through the use of inexpensive, renewable substrates like sugars [198]. Through natural metabolism, the substrates can be converted into a wide variety of building blocks. Some of these building blocks can be polymerized into homo- or hetero-polymers by expressing new enzymes in the cells through genetic engineering, which will transform them into appropriate activated monomers. The assortment and kind of polymers that can be produced in this manner have significantly expanded over many decades of study and advancement [198], [199], [200].

In the same time frame, the same bacteria were also modified to produce small useful molecules from exogenously given substrates or their own natural metabolites. The aforementioned two processes are not mutually exclusive; they may compete with one another in the same cell for substrates, energy, and/or reducing equivalents. It has recently been shown that it is possible to create a single cell to make useful tiny molecules in addition to a polymer [201]. Furthermore, interactions between the polymer and the functional small molecules are possible [201]. This is a chance to create a unified fermentation process that can produce functionalized polymers from inexpensive substrates.

A number of approaches have been considered in order to produce biopolymers that have been altered [201]. Strains that have been created with higher yields and/or the ability to produce customised or modified polymers have been identified for the in vivo manufacture of polymers. This investigation pertains to metabolic engineering, which involves appropriately modifying the biosynthesis route by using knowledge of metabolic flux, primary enzymes and polymer biosynthesis pathways [201],[202].

The biopolymers that are now able to be made in large quantities by *E. coli* will need to be evaluated methodically in order to find a polymer that possesses the required qualities for controlled release applications as well as a sufficient affinity with L-DOPA. Many bacteria spontaneously create poly-(3-hydroxybutyrate) (P3HB) to store surplus carbon substrates [203]. When polyester is synthesised in *E. coli*, bioengineers have devised ways to include several monomers. Lactate, 2-hydroxybutyrate, glycolate and phenyl lactate are among the monomers [198],[199],[200],[201],[204]. These novel and intriguing developments offer ample opportunity to investigate in order to fulfil the two requirements: (1) they confer distinct hydrophobicity for interacting with L-DOPA; and (2) they alter the characteristics of polymer degradation in order to achieve the intended controlled release profile. Bioengineers adjust the composition, molecular weight, size and surface characteristics of the polymers. The mass ratio of the biopolymer to the mass of the dry cell is referred to as the content. Through metabolic engineering, it can be altered by adjusting the pace at which acetyl-CoA is supplied for PHB production [205]. These polyesters have proteins covering at least some of their surface [206]. These proteins can be genetically altered to add new residues that alter the hydrophobicity, charge and rate of polymer biodegradation.

First, by employing polymerizing or modified enzymes exposed to particular substrates, in vitro synthesis can produce novel biopolymers [13]. Second, the separated biopolymers may appear better after being subjected to chemical or enzymatic changes [207,208]. Genomic approaches can be used to reconstruct the structure and bioactivity relationships of EPS, which opens the door to the creation of customised polymers [209]. Protein function and the production of desired molecules can be enhanced by knowledge of the mechanisms, underlying the biosynthesis of carbohydrates as well as their molecular weight, acidic structure and functional substituents [210]. The xanthan production can also be changed by protein engineering [211].

Because functionalized polymers may include immunogenic or hazardous contaminants, their safety in tissue engineering applications is a difficulty [205]. By substituting a GRAS host species that can be genetically altered to deactivate contaminating compounds or disrupt interactions for *E. coli*, host selection and engineering can be enhanced. Human proteins can be produced by recombinant DNA technology to increase compatibility if the biopolymer is immunogenic. Appropriate separation methods are required to economically isolate polymer bodies [212].

The cytoplasm space of cells is what limits the creation of biopolymers, and fermentative industrial techniques are the main application for most microorganisms [213]. Yield per volume determines the cell density and biopolymer percentage. Poly(-D-glutamate), polysaccharides such as xanthan, dextran, curdlan, chitosan, pullulan and microbial cellulose are examples of biopolymers [214]. These biopolymers emerge from outside of cells [215].

Through overexpression experiments, GDP-mannose dehydrogenase has been widely established in *Pseudomonas aeruginosa* as a significant regulatory protein in alginate production [216]. However, genetic data also makes it possible to identify novel enzymatic tools that change glycopolymers. Enzymes would therefore be useful biotechnological tools in the event of in vitro synthesis or in vivo biosynthesis engineering. Additionally, marine biodiversity has demonstrated enormous promise as a biocatalyst [217].

With the advancement of biotechnology, scientists have developed novel techniques for studying and manipulating living systems. It is possible to alter the expression of genes in terms of time, place, intensity and form, using genetic engineering [177]. Recombinant DNA technology has the potential to create polymer chains that are similar in terms of length, content and spatial orientation.

CONCLUSION AND FUTURE PERSPECTIVES

The most promising approaches to boosting the synthesis of pure and high-quality EPS include either altering the encoded enzymes that catalyse the reactions in the processes or altering the signalling networks that affect gene expression and, consequently, enzyme performance. Further multidisciplinary research need to be conducted in the near future to investigate structural characterisation, establish bioactivity testing, comprehend biosynthesis, and address issues related to metabolic engineering and extensive screening. Therefore, synthetic biology techniques can be quite helpful in creating novel biopolymers more readily.

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