39. Marine-Derived Exopolysaccharides

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Marine biotechnology still remains a new and emergent science, which is closely linked to marine biodiversity and to the technological capacities of investigating more atypical ecosystems. Marine microorganisms show a unique biodiversity since they have to adapt to various marine environmental conditions such as low or high temperatures, alkaline or acidic water, high pressure, and limited nutrients. Marine natural products, especially marine polysaccharides, are attracting more and more attention. Microbial polysaccharides are of growing interest for many sectors of industry, resulting in isolation of new exopolysaccharide (EPS)-producing bacteria. The diversity of these polysaccharides arises from the structural variations (glycosidic bonds, side branching chains, monosaccharidic content) controlled through a genetic basis. A lower molecular weight and functionalized derivatives together with the native form of the polysaccharide have been shown to possess a variety of biotechnological activities. Therefore, the biophysical and biological properties have made them useful in many pharmaceutical, food, and industrial applications. This chapter gives information on EPS-producing bacteria from the marine environment, as well as on the carbohydrate molecule they produce, including the chemical composition or structure when available, the putative pathways of biosynthesis, and the potential applications in industry with a focus on healthcare and glycosaminoglycan-like compounds.

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39.1 In Search of New Polysaccharides

Polysaccharides constitute the most abundant and the most diverse materials found on earth and in the oceans. Polysaccharides (PSs) form a class of biotechnological molecules that find applications in many industrial fields. They consist of monosaccharides (sugar units) that are linked to each other, generally in a repeated sequence order. By composition, PSs may be divided into homopolysaccharides, composed of single monosaccharide type and heteropolysaccharides with several different monosaccharides. Interest in carbohydrates was first very low due to their extreme structural complexity and the lack of understanding of their key role in different biological systems or processes. The type of glycosidic bond, the chain length, the degree and

type of branching, as well as the presence of organic or chemical groups are important features of the molecular structure, which are also of great importance for the biophysical or biological properties. Except for the PS used traditionally in the food industry, the structure of most PS, as well as the structure–function relationships are mostly unknown.

For biotechnological purposes, marine PSs have been studied in animals (chitin from crustaceans, chondroitin sulfate from fish, sulfated PSs from sponge and glycosaminoglycans from scallops and abalone), plants (ulvans, agarans, carrageenans, alginates, fucans, laminarin from seaweeds), and microorganisms such as fungi, microalgae, and bacteria. In bacteria, several kinds of PS exist, depending on their cellular localization and structure. Exopolysaccharides (EPSs) represent an important component of the biofilm matrix and serve as functional elements for adhesion, protection, and recognition. They also have chelating properties especially of heavy metals. The main advantages of microbial EPSs over plants or macroalgal and animal PSs lie on a stable supply independently of climatic or physiologic variations. Moreover, new structures have been described in bacteria, in particular in marine bacteria, giving the opportunity for the development of new applications, especially in the pharmaceutical field.

Marine-derived PSs and their oligosaccharidic derivatives have been studied for a variety of biological activities such as antithrombotic, antitumor, antiviral, antioxidant, and immunomodulatory effects. A high specificity linked with highly beneficial properties compared with the reference molecules already existing on the market are the major conditions of the development of new molecules, especially if these molecules present a cheaper production cost.

There is in particular a big diversity in the abundant microbial life in the oceans. The marine environments include coastal ecosystems, the deep ocean with hydrothermal vents, sediments, and the cold water of the poles. The extremes of temperature, pressure, and acidity met in the ocean, and the availability of the sources of energy and nutrients results in different strategies of microorganisms to survive. The combination of the diversity, the quantity, and the rate of metabolism makes the oceanic world a vital for the earth. Indeed, marine microbes are responsible for the global cycle of nutriments and other elements and are involved in the chemistry of the ocean, the composition of the atmosphere, and climate. As microorganisms quickly reproduce when conditions are favorable, the huge number of generations allows, by selection and natural evolution, the appearance of new species or other groups of the classification. The microbial genetic diversity also results in a biochemical diversity offering opportunities of biotechnological and pharmaceutical applications.

This chapter will focus on PS from bacterial microorganisms, presenting an overview of microbial marine biodiversity, structures of the produced polymers, some molecular aspects of their biosynthesis, as well as some of their applications driven by their features and biological properties with a focus on the human health domain.

39.2 Marine Biodiversity

For a long time, oceans have been considered as unlimited to human beings, who have always wanted to exploit the attractive resources. However, the industrial exploitation of the oceans only began 100 years ago. Studies to understand oceans and their ecosystems with the aim to exploit molecules produced by marine organisms or to develop new drugs inspired by marine molecules have started to reveal secrets, new biodiversity, and innovative molecules of biotechnological interest.

39.2.1 Ecosystems in the Oceans

The oceans cover 71% of the earth's surface, with an average depth of 3800 m. Depths reach 6000 m for the abyssal plains; the deepest point is the Mariana Trench in the Pacific Ocean (estimated to be 11 000 m). Smaller seas of salt water also exist; they are partly or fully enclosed by land.

Except on the surface and in temperate or tropical areas, most of seawater is characterized by a temperature lower than 5 °C, low nutrients, and no sunlight to allow photosynthesis. The open ocean is a huge ecosystem but particular habitats sometimes qualified as extreme have also been discovered: underwater salt lakes, volcanos, mountains, hot smokers, cold seeps, ...

In the deep sea, pressure is very high, the temperature is between 2 and 5 °C, and there is total darkness. A remarkable variety of deep-sea habitats exist: cold seeping waters, sinking particles, sediments, animal

guts and surfaces, deep sediments, and hydrothermal vents [39.1]. These latter areas are unstable due to high volcanic activity; they are characterized by a large gradient of temperatures [39.2], high amounts of dissolved compounds, gas, as well as very low pH values. The first deep-sea hydrothermal vent was discovered in 1977, near the Galapagos islands, on the East Pacific Rise, and the first *black smoker* in 1979 on the East Pacific Rise, 21° North. Deep-sea hydrothermal vents are mainly located on the active Mid-Atlantic Ridge, the East Pacific Rise, and back-arc basins (Fiji, Okinawa, Lau Basin). They come from the seeping of seawater down into the crust through rock cracks in the seafloor. During course of the water, several chemical processes enrich it in minerals, such as calcium, sulfates, magnesium, sodium, hydrogen sulphide, and metals, and it also becomes hotter. Jetting upwards by chimneys called black or white smokers formed by compound deposits or diffusing out of cracks, the hydrothermal fluid gives rise to the development of a luxuriant life contrasting with the apparently deserted surrounding water. Various vent fauna, bivalves, crustaceans, and worms take their energy and resources from endosymbiotic and episymbiotic chemoautotrophic microorganisms capable of oxidation of H_2 and sulfur compounds [39.3–5]. To survive under extreme conditions, such organisms must be adapted by appropriate metabolic pathways and protective mechanisms.

Some other volcanic areas also exist near the coast [39.1]. Shallow hot springs have been studied [39.6, 7]. Cold seeps are also characterized by hydrocarbon-rich fluids emanating from the ocean floor and are also the center of ecosystems with a large biodiversity [39.8, 9].

Near-shore sediments and deep-sea sediments have also been explored (the Ocean Drilling Project) and have revealed microbial life down to 1600 m in the sediment under the sea floor [39.10].

In polar areas, the environment is perennially cold, and in some cases permanently covered with ice; therefore, microbes need to adapt to cold temperatures and fight against freezing. However, even in these extreme conditions, some bacteria have been collected at around 0 °C in the Antarctic [39.11–13].

High saline water in inland water (the Dead Sea) together with high pH, high saltwater lakes situated underwater of seas such as the Black Sea, the Red Sea, the Mediterranean sea, or Gulf of Mexico have also been explored [39.14].

French Polynesia microbial mats are characterized by variable parameters such as pH values between 6 and 10.5, salinity ranging from 5–42 $g\,L^{-1}$, and temperatures between 20 and 42 °C. Microbial mats are laminated abundant communities of phototrophic and chemotrophic microorganisms. *Pseudomonas*, *Alteromonas*, *Paracoccus*, and *Vibrio* bacteria from these environments have been shown to produce EPSs [39.15, 16].

Marine surfaces and especially marine eukaryotes are covered with microorganisms embedded in a matrix forming a biofilm. The microbial epibiotic consortia differ significantly from microorganisms living in the surrounding environment and highlight the close relationships between microbial epibionts and hosts [39.17].

From deep-sea vents to surface-sea ice and open ocean, nutrient sources and physic-chemical conditions may be highly fluctuant, making quick adaptive responses necessary; the adaptation is possible on the basis of genetic diversity and has led to huge biochemical diversity.

39.2.2 Microbial Diversity and the Limitation of Collections

A high diversity of ecosystems exists within the world's oceans; they are different from terrestrial and freshwater environments; some endemic species have, therefore, been identified resulting in specific diversity.

Most of the biodiversity is microbial. The genetic diversity of microorganisms is far larger than that of plants and animals all together. Due to their large population size and since they grow rapidly, prokaryotes have an enormous potential to accumulate mutations, allowing them to adapt, and thus acquire genetic diversity. By weight, more than 95% of all living organisms found in the oceans are microbial. Microorganisms are the most abundant life form in the ocean, they are the basis of the marine food web, drive energy and nutrient cycling, and are at the origin of life on the earth [39.18]. This genetic resource is mainly unknown and underexploited and will probably be the major source for biochemical diversity resulting in novel molecules in the future.

Isolating new species requires the ability to cultivate them. Cultivation efficiency only allows the isolation of 1–5% of microbial species present in marine ecosystems [39.19]. Therefore, microbial diversity is largely undiscovered. From oceanographic cruises or sampling campaigns, several academic or industrial teams all over the world have developed marine microbial collections. These they are a good source of screening for innovative biomolecules. These collections, except

private ones, offer marine microorganisms and other services such as various screening. However, these collections shelter only cultivable microorganisms and, therefore, are limited. Nevertheless cultivation is a prerequisite to isolate compounds of biotechnological value such as PSs [39.20].

Culture-independent molecular techniques such as metagenomics, also named environmental genomics, have been adopted to explore the actual diversity of natural assemblages of microbes. Their sensitivity allows the detection of taxa at very low abundance. They have also revealed the incredible richness of marine life.

For several years, J. Craig Venter's Sorcerer II (Venter's Global Ocean Sampling Expeditions) sailed all around the sea world to collect samples of near-surface sea water every 200 miles. After filtration and freezing to collect and store microorganisms in the samples, DNA was extracted in the J. Craig Venter Institute laboratory and sequenced. Results revealed the power of metagenomic approaches to obtain a good estimation of microbial diversity and biochemical diversity; they also revealed a high proportion of new bacterial and viral sequences, which suggested that until now marine microorganisms have not been well isolated, especially viruses [39.21]. Many of these sequences must have potential as sources of new drugs and they are also studied for protein evolution, discovery of new enzymes, and microbial ecology concerns.

Tara Oceans expeditions [39.22] started on 2004 to study the impact of climate change on marine ecosystems, especially marine plankton including microorganisms such as viruses and bacteria, and other organisms such as medusas. It sailed all over the seas to collect samples and data and provide them to the world's scientific community. In 9 years, 8 expeditions have been organized with the aim to establish a time zero of marine ecosystems, to discover new organisms in each size class, to obtain a good estimation of oceanic biodiversity and its distribution, and to develop new models to estimate this biodiversity.

Deep-sea microbial diversity has been explored by metagenomic [39.23] or cultivation approaches [39.24, 25]. Sea floor sediments as well as hydrothermal vents with black or white smokers have been sampled. Studies on the genomic diversity of water columns [39.26] or sea floor sediments [39.27] have also been carried out as well as on microbial communities as associations with marine invertebrates, sponges [39.28] or with corals [39.29, 30].

In all cases, sequences related to fungi, bacteria, archaea, viruses and phages have been detected in variable ratios in a site-specific manner or isolated on culture media. The orders Thermococcales, Archaeoglobales, and Methanococcales are widely encountered in extremophilic archaea. On the other hand, the vast majority of bacterial sequences are mainly assigned to Thermotogales: *Proteobacteria*, *Firmicutes*, *Cyanobacteria*, and *Actinobacteria*, and 80–95% of marine bacteria are Gram-negative rather than Gram-positive.

Global Ocean Sampling Expeditions revealed that the archaea are nearly absent from the list of dominant organisms in the near-surface samples. Based on 16S ribonucleic acid (RNA) data, the most abundant phyla or classes are *Proteobacteria*, *Bacteroidetes*, *Cyanobacteria*, *Firmicutes*, *Actinobacteria*, and *Planctomycetes* [39.21]. The sequences mainly cluster within *Alphaproteobacteria* and *Gammaproteobacteria*. However distribution of these ribotypes is not homogeneous all over the ocean and different microbial communities can be distinguished.

The diversity of seawater habitats results in diversity of the organisms, and subsequently of the metabolites they produce. Thus marine genomics provide sequence data for microbial ecology but also biodiscovery [39.31]. This renders marine ecosystems really interesting to discover innovative molecules for biotechnological purposes. Extremophiles will provide new molecules or pathways for novel biotechnological processes but are also models to investigate how biomolecules are stabilized under extreme conditions. Several studies on marine biodiversity for biotechnological applications have been carried out on particular habitats such as deep-sea hydrothermal vents for extremophiles and thermostable enzymes, polar areas for psychrophilic ones, for exopolysaccharides [39.1, 32–36], microbial mats from French Polynesia [39.16].

These bacteria may produce new enzymes and bioactive compounds, including exopolysaccharides (EPSs) with innovative structures (Table 39.1). Many known marine bacteria can produce molecules of biotechnological interest, some of which have unique properties, and the search for new microorganisms of biotechnological value is still promising.

Table 39.1 Some marine bacteria, cyanobacteria, and marine archaea, and the EPS they produce

Microorganism	Origin	EPS composition or repeating unit structure	References
Marine bacteria			
Alteromonas hispanica	Hypersaline lake, Southern Spain	Glc/Man/Rha/Xyl 18.1/62.7/6.9/12.3	[39.37]
Alteromonas infernus	Animal population, Guaymas Basin, Gulf of California	Sulfated PS [SO_3Na] ↓ 2 [→4)–β–Glc*p*–(1→4)–α–Gal*p*A–(1→4)–α–Gal*p*–(1→] 3 ↑ *1* β–Glc*p*–(1→6)–α–Gal*p*–(1→4)–β–Glc*p*A–(1→4)–β–Glc*p*A 2 3 ↑ ↑ *1* *1* α–Glc*p* α–Glc*p*	[39.38, 39]
Alteromonas macleodii subsp. *fijiensis*	Deep-sea hydrothermal vents, North Fidji Basin	→4)–β–D–Glc*p*–(1→4)–α–D–Gal*p*A–(1→4)–α–Gal*p*–(1→ 3 ↑ *1* α–D–Glc*p*A 3 ↑ *1* β–D–Glc*p*A 4 ↑ *1* 4,6–Pyr–β–D–Man*p*	[39.40, 41]
Alteromonas macleodii subsp. *fijiensis biovar deepsane*	Deep-sea hydrothermal vent, East Pacific Rise	16 to 18 Monosaccharides, including seven types of monosaccharide. Two fragments have been identified: β–D–Glc*p*A–(1→3)–α–D–Gal*p*–(1→4)–D–Glc*p*A *A* 3 *B* *C* ↑ *Lac* SO_3 ↓ *2* β–D–Glc*p*–(1→4)–α–D–Gal*p*A–(1→4)–α–D–Gal*p* *G* *D* 3 ? *E or E'F* ↓ *R*	[39.42, 43]
Alteromonas sp. Strain 1644	Polychaete tissue, East Pacific Rise	Glc/Gal/GlcA/GalA and 3-*O*-[(R)-1-carboxyethyl]-D-glucuronic acid	[39.44]
Bacillus licheniformis	Shallow hydrothermal vent, Vulcano Island, Italy	Tetrasaccharide repeating unit, Man/Glc 1/0.2	[39.6, 45, 46]
Bacillus thermoantarcticus	Sea sand in Ischia Island, Italy	2 Sulfated EPS: EPS1 contains α-Man and β-Glc (1.0/0.7) EPS2 contains α-Man and pyruvic acid	[39.47]
Bacillus thermodenitrificans	Shallow hydrothermal vent, Vulcano Island, Italy	2 EPS with uronic acid and sulfate	[39.46]
Geobacillus tepidamans V264	Bulgarian hot spring	Galacto-glucan, α linkage Glc/Gal/Fuc/Fru (1/0.07/0.04/0.02)	[39.36, 48]
Geobacillus thermodenitrificans		Man/Glc	[39.49]
Geobacillus sp.	Ischia Island	EPS 1 Man/Glc/Gal (0.5/1/0.3) EPS 2 Man/Glc/Gal (1 : 0.3 :trace) EPS 3 Gal/Man/GlcN/Ara (1 : 0.8/0.4/0.2) with pentasaccharide unit	[39.36]

Table 39.1 (continued)

Microorganism	Origin	EPS composition or repeating unit structure	References
Marine bacteria			
Geobacillus sp. 4001	Shallow marine hydrothermal vent, Italy	Mannan Man/Glc/Gal/NMan	[39.7]
Geobacillus sp. 4004	Shallow marine hot spring (Ischia, Italy)	Gal/Man/GlcN/Ara (1.0/0.8/0.4/0.2)	[39.46, 50]
Hahella chejuensis	Sediment of Marado, Cheju Island, Korea	EPS-R Glc/Gal/Rib/Xyl (0.68/1.0/trace/trace)	[39.46, 51]
Halomonas sp. AAD6		Levan Fru, β-2,6 linkage	[39.52]
Halomonas alkaliantarctica	Sediments in salt lake in Cape Russell, Antarctica	Glc/Fru/GlcN/GalN (1/0.7/0.3/0.2) xylan-mannan fructo-glucan	[39.46]
Halomonas almeriensis		Man/Glc and trace of Rha	[39.53]
Halomonas anticariensis	Saline wetland, Malaga, Spain	Glc/Man/AcGal	[39.46, 54]
Halomonas maura		Mauran Man/Gal/Glc (1/0.6/0.2)	[39.55]
Halomonas ventosae	Saline wetland, Malaga, Spain	Glc/Man/Gal	[39.46, 54]
Iodomarina fontislapidosi	Spanish hypersaline water	Glc/Man/Gal Anionic PS	[39.37]
Iodomarina ramblicola	Spanish hypersaline water	Glc/Man/Gal Anionic PS	[39.37]
Olleya marilimosa CAM030	Southern Ocean	Man/GlcA/GalNAc/Glc/GlcNAc/Ara/Gal/GalA/Xyl/Rha 48/10/10/9/8/6/4/2/2/1	[39.56, 57]
Paracoccus zeaxantifi-ciens subsp. *payriae*	Microbial mats, French Polynesia	Sulfated PS	[39.16, 58]
Pseudoalteromonas marinoglutinosa KMM232 Mucoid colonies		Sulfated PS →3)–β–D–Man*p*–(1→4)–α–L–Rha*p*–(1→ 2 | SO_3H	[39.59]
Pseudoalteromonas sp. HYD721	East Pacific Rise	→4)–β–D–Man*p*–(1→4)–β–D–Glc*p*–(1→4)α–D–Gal*p*–(1→4)–β–D–Glc*p*–(1→ 2 3 ↑ ↑ 1 1 α–L–Rha*p* β–D–Gal*p* 3 ↑ 1 β–D–Glc*p*A 4 ↑ 1 *[*SO_3H*]*→3–β–D–Man*p*	[39.60]
Pseudoalteromonas ruthenica SBT033	Sea water, coast of India	Man/Glc/Gal/Xyl, uronic acids	[39.61]
Pseudoalteromonas sp. strain CAM025	Sea water in Southern Ocean	Neutral sugar, uronic acid, acetyl, sulfate Glc/GalA/Rha/Gal (1/0.5/0.1/0.08)	[39.11, 56]
Pseudoalteromonas sp. strain CAM036	Sea water in Southern Ocean	Neutral sugar, uronic acid, acetyl, succinyl, sulfate GalA/Glc/Man/GalNAc/Ara (1/0.8/0.84/0.36/0.13)	[39.11, 56]
Pseudoalteromonas sp. strain SM9913	Deep-sea sediment, Yellow Sea, China	Glc with α-1,6 linkage and high degree of acetylation Glc/t-Ara/t-Glc/t-Gal/Xyl/Glc/Glc (0.62/0.11/0.11/0.03/0.04/0.05/0.05)	[39.46, 62]
Shewanella colwelliana	Eastern oyster		[39.63]
Thermotoga maritima		Glc/Rib/Man (1/0.05/0.02)	[39.64]

Table 39.1 (continued)

Microorganism	Origin	EPS composition or repeating unit structure	References
Marine bacteria			
Vibrio alginolyticus	Marine fouling material, Bengal	Glc/AraNRibN/Xyl	[39.65]
Vibrio diabolicus	Deep-sea hydrothermal vent, East Pacific Rise	→3)–β–D–Glc*p*NAc–(1→4)–β–D–Glc*p*A–(1→4)–β–D–Glc*p*A–(1→4)– –α–D–Gal*p*NAc–(1→	[39.66, 67]
Vibrio furnissii	Coastal regions, India	Neutral sugars (Glc/Gal), uronic acids	[39.68]
Vibrio harveyi		Neutral sugars, uronic acids, sulfate	[39.69]
Vibrio parahaemolyticus	Marine natural biofilm	Neutral sugars (Glc/Gal/Ara/Man), uronic acids	[39.70]
Marine cyanobacteria			
Aphanothece halophytica		Xanthan-like behavior	[39.71, 72]
Cyanothece sp. 113	Salt lakes in China	α-D-1,6-homoglucan	[39.73]
Cyanothece sp. Strain ATCC51142		Sulfated PS with uronic acids and methyl sugars	[39.74]
Arthrospira platensis	Blue-green cyanobacterium	Spirulan aldobiuronic acid, acofriose, sulfate	[39.75]
Marine archaea			
Haloarcula spp. T5		AcGlc/Man/Gal (1/0.6/0.3) pentasaccharide repeating unit	[39.76]
Haloferax denitrificans	San Francisco Bay	→ 4)-β-D-Glc*p*A2,3NAc-(1→4)-β-D-Glc*p*A2,3NAc-(1→4)-α-D-Glc*p*A2,3NAc-(1→3)-α-D-Gal*p*-(1→, where D-Glc*p*A2,3NAc is 2,3-diacetamido-2,3-dideoxy-D-glucopyranosiduronic acid	[39.77]
Haloferax gibbonsii		Man/Glc/Gal/Rha (0.6/0.3/1/0.3) heptasaccharide with side chains	[39.78]
Haloferax mediterranei	Mediterranean Sea	Man/Glc/Gal/amino sugars/ uronic acids, sulfate, 4-D-GlcNAcA-β-1,6-D-Man-α-1,4-D-GlcNAcA(3S)-1,	[39.79]
Sulfolobus solfataricus MT4 and MT3		MT4 Glc/Man/NGlc/Gal (1.2/1.0/0.18/0.13) and MT3 Glc/Man/NGlc/Gal (1.2/1.0/0.77/0.73) Sulfated PS	[39.80]
Thermococcus litoralis		Man	[39.64]

EPS were chosen when some structural data were available, CPS and O-antigens were excluded.
(3S): 3-*O*-sulfo, AcGal: acetylgalactose, AcGlc: acetylglucose, Ara: arabinose, Fru: Fructose, Fuc: L-Fucose, Gal: galactose, GalA: galacturonic acid, GalNac: *N*-acetylgalactosamine, GalNac: *N*-acetylgalactosamine, Glc: glucose, GlcA: glucuronic acid, GlcN:glucosamine, GlcNAc: *N*-acetylglucosamine, GlcNAcA: *N*-acetyl-glucuronic acid, Gro: Glycerophosphate, Man: mannose, ManN: mannosamine, p: pyranose, Rha: rhamnose, Rib: ribose, SO_3: sulfate, t- :terminal, Xyl: xylose)

39.3 Bacterial Polysaccharides

Different types of PSs exist within or around the bacterial cell depending on their location:

i) Polysaccharides found in the cytoplasm that serve as carbon and energy reserve,
ii) Cell wall PSs including teichoic acids and peptidoglycans,
iii) Extracellular PSs which are released outside the cell: exopolysaccharides (EPSs) or capsular polysaccharides (CPS).

CPSs are extracellular but remain attached to the cells through covalent bonds to other outer surface polymers thus forming the capsule.

Intracellular PSs are produced when bacteria are grown in excess of sugar and may serve as reserve, since the cell also produces enzymes to degrade them. They usually appear as granules of glycogen or amylopectin type PSs and may be related to sporulation of *Clostridium* sp., for example. However, bacteria synthesize only very few intracellu-

lar PSs but they produce many diverse extracellular PSs.

The cell wall is complex and rich in carbohydrate compounds. Among these, peptidoglycans are linear macromolecules consisting of PSs cross-linked by peptide moieties. The PS part is composed of alternating *N*-acetylmuramic acid (MurNAc) and *N*-acetylglucosamine (GlcNAc) linked by β-1,4-glycosidic linkages. Carboxylic groups from *N*-acetylmuramic acids are involved in the linkage to peptidic chains. Teichoic acids, which are only found in Gram-positive bacteria, may be glycerol phosphate units, or ribitol phosphate units linked by phosphodiester bonds. Glycerol and ribitol may also be associated with glucose, galactose, or *N*-acetylglucosamine. Teichoic acids are closely connected to the peptidoglycan network and make it more cohesive. Gram-negative bacteria also produce lipopolysaccharides (LPS) whose lipidic part (lipid A) is set inside the external membrane. They are composed of two carbohydrate parts, the O-specific chain, and the core [39.81]. The O-specific chain is highly variable, especially in pathogens such as *Vibrio cholerae* [39.81]. Lipid A is composed of aliphatic chains linked to glucosamine residue. LPS is also called endotoxin.

The following paragraph deals specifically with extracellular PSs and EPS.

39.3.1 Bacterial Exopolysaccharides

From different carbon sources, bacterial cells synthesize extracellular PSs; sugar units are synthesized within the cytoplasm before being excreted outside and polymerized. Extracellular PSs may remain attached to the cell membrane, forming a capsule or a slime, while the other part is released into the surrounding environment.

Depending on the enzymatic machinery, EPS are usually constituted of a regular repeating unit. They may be linear or branched. Homopolysaccharides are composed of a unique type of sugar residue type, while heteropolysaccharides contain different ones [39.82].

In heteropolysaccharides, glucose, galactose, and glucuronic or glacturonic acids are common. However, other neutral or acid sugars, hexosamines or *N*-acetylhexosamines, and some rarer sugars are also found. The presence or absence of a repeating unit containing up to 16 or 18 monomers [39.42], linear or branched, the presence of organic (lactate, pyruvate, *N* and *O*-acetates...) or chemical (sulfate, phosphate...) groups give a very large structural diversity of the molecules with consequently very diverse physicochemical or biological properties [39.83].

Depending on both sugar chain and osidic linkages, the behavior of the homopolysaccharides in solution may be different [39.84, 85]. Scleroglucan or pullulan are soluble and highly viscous, while curdlan lacking side-chains is poorly soluble and forms a gel; bacterial cellulose is insoluble; however, these three PSs are only composed of glucose. The presence of side-chains renders the polymer insoluble; acyl and non-sugar substituents may affect the physical features as well as uronic acids. β-1,4-linkages are rigid, while α-1,4-bonds found in dextrans confer flexibility of the structure [39.85].

EPS chain length may vary from one species to another, but also within a species; the molecular weight may range from 50 000 to over several million g mol^{-1}.

39.3.2 EPS from Marine Bacteria

A number of species of marine microorganisms can produce EPSs: cyanobacteria, bacteria, and archaea [39.86]. These strains are screened for mucoid phenotypes on a solidified medium supplemented with a carbohydrate source, for a high viscosity in liquid medium, by staining specifically EPSs.

Chitin, the main marine polymer composed of β-1,4-linked *N*-acetylglucosamine residues, is one of the most abundant PSs in nature after cellulose and is usually prepared from the shells of crabs and shrimps [39.87, 88]. Until now, unlike for cellulose [39.89, 90], no bacterium has been shown to produce chitin. Nevertheless, many marine bacteria species exhibit chitinolytic activity and also produce chitosan from chitin, the *N*-deacetylated product [39.91]; bacteria are, therefore, involved in the turnover of the PS in the marine environment [39.87]. Some non marine bacteria species still produce polyglucosamine exopolymers with a structural homology with chitosan [39.92].

Alginates, another kind of the most common marine PSs, are currently mainly obtained through brown seaweed harvesting; the non-marine bacteria *(Pseudomonas aeruginosa* and *Azotobacter vinelandii)* are also capable of producing acetylated alginates that bind some divalent ions but fail to form gels in the manner of their algal analogs.

Polysaccharide-producing bacteria have been isolated from the Gulf of Naples [39.7], from microbial mats [39.16, 58, 93], the Antarctic [39.11], and deep-sea hydrothermal vents [39.43, 94–98]. Psychrotolerant *Pseudoalteromonas*, *Shewanella*, *Polaribacter*, or

Flavobacterium sp. have been isolated from Antarctic water [39.56] and are able to produce high molecular weight EPS.

Some EPS-producing marine bacteria are listed in Table 39.1. The vast majority of marine bacteria producing exopolysaccharides are *Alteromonas* or the *Pseudoalteromonas*, *Pseudomonas*, *Shewanella*, and *Vibrio* species. In contrast only a few extremophilic bacteria or archaea have been studied for EPSs [39.36, 55, 64].

EPS production by microorganisms has the advantages of a rapid production (a few days compared to the 3–6 months in the case of plants), of a production in bioreactors on different substrates of possibly hydrocarbon residues and of easier extraction since they are exuded in the extracellular environment.

39.3.3 Benefits for the Bacterial Cell

Many marine bacteria produce EPS as a strategy for growth and survival, adhesion to surfaces, and resistance to adverse conditions. EPSs have also been involved in virulence and host contamination. The physiologic roles of EPS for bacteria have not been completely identified, but a protective or adhesive role has frequently been proposed. Most of the time, EPSs are not degraded by the bacteria producing them and thus do not represent a carbon source [39.99]. In a variable nutrient environment such as the ocean, bacteria need greater adaptability in the pathways involved in the detection of nutrients and in metabolism to utilize them. This could explain the diversity of already found polysaccharidic structures.

Therefore, EPSs have an effect on the interactions between the cells and the surrounding environment [39.86]. CPS or slime may help bacterial cells to overcome the various stresses encountered in the environment or to adhere to the surfaces, providing survival advantage. In aquatic environments, the majority of bacteria adhere in a selective manner or not to inert surfaces or living organisms. In biofilms, microbial cells aggregate within a matrix of extracellular polymeric substances composed of PSs, proteins, nucleic acids, peptidoglycan and lipids. Extracellular PSs are generally considered to be the main part of the biofilm matrix [39.100] and are involved in the flexibility of the biofilm shape under shear force as well as in some of the functional properties of the matrix [39.101].

Moreover, anionic EPSs slimes or from capsules allow the aggregation and storage of the soluble nutriments necessary for the growth of the cell or other particles and may chelate metals and ions [39.11]. Bacterial symbionts have been involved in the resistance of polychaetes to the high metal levels around deep-sea hydrothermal vents [39.3] and in environments anthropogenically polluted with metals [39.102]. In other respects, several anionic EPSs from marine bacteria have been shown to bind toxic compounds [39.13, 37, 62, 94]. The presence of bacteria on marine worms may protect them against toxic metals.

39.3.4 Putative Pathways of Biosynthesis

Oceans contain huge bioresources. To exploit them in a manner respectful to the environment, researchers are trying to decipher their production mechanisms. The first particular mechanism of biosynthesis was encountered for homopolysaccharides such as dextran, glucan, levan, inulin, and fructan; it involves an extracellular enzyme (glycan-sucrase) secreted at the cell surface of Gram-positive bacteria such as *Leuconostoc* and other lactic acid bacteria; it has not been yet described in Gram-negative bacteria [39.84, 103]. A specific glycosyltransferase (GT) transfers the sugar residue to the nascent polymer from a disaccharide, usually saccharose.

The other kinds of biosynthesis concern homo and heteropolysaccharides with repeating units. This biosynthesis usually involves three steps: sugar precursor production, repeating unit synthesis, export and polymerization (Fig. 39.1). The whole process is not fully understood, in particular for polymer exportation and synthesis regulations.

The synthesis of heteropolysaccharides has been widely studied in Gram-negative bacteria (*Escherichia coli* [39.104]) as well as lactic acid bacteria and appears to involve a common mechanism. Several enzymes are involved in the assemblage of the repeating unit; they are encoded by genes located within large EPS gene clusters ranging up to 15–20 kb. They are sometimes located on plasmids, especially when considering lactic acid bacteria [39.105]. The biosynthetic machinery involves activated sugar precursors such as nucleoside sugar diphosphate (UDP-sugar), which are produced in the central metabolic pathways. EPS biosynthesis from cytoplasmic activated UDP-sugar precursors is common. However, alternative activated sugar donors are encountered: nucleoside sugar monophosphates, lipid sugar phosphates or pyrophosphates, or unsubstituted sugar phosphate [39.106]. EPSs are usually synthesized inside the cytoplasm in the form of repeating units by glycosyltransferases before being ex-

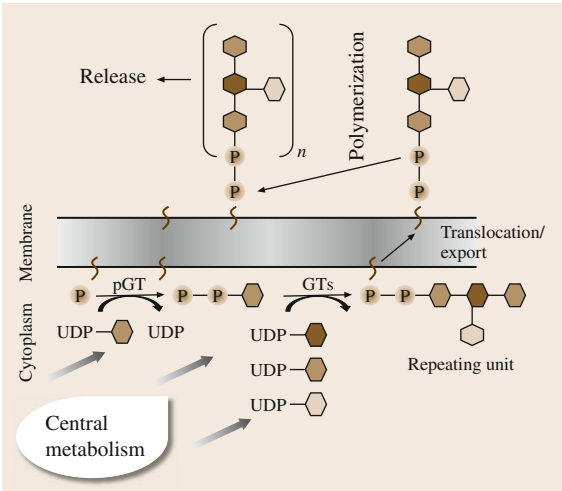

Fig. 39.1 Common biosynthetic mechanism of PSs with repeating units (the biosynthesis begins within the cytoplasm with the synthesis of activated precursors. The repeating unit is then assembled by GT on a carrier located on the membrane. The repeating unit is then translocated across the membrane, polymerized on the nascent polymer within the periplasm or outside the cell, and then released in the surrounding environment or slime)

creted [39.104]. The repeated unit is assembled on a carrier molecule, anchored in the cytoplasmic membrane. Different molecular acceptors on the membrane for the carbohydrate in formation have been proposed; in particular, a lipidic transporter such as undecaprenyl phosphate or isoprenoid pyrophosphate is used in EPS biosynthesis [39.95]. The first monomer is linked to the lipid-carrier by the priming-glycosyltransferase or phosphoglycosyltransferase. The following sugar monomers are linked by appropriate GTs to the growing repeating unit. After completion, the repeating unit is exported outside the cell and subsequently polymerized by addition to the reducing end of the growing EPS chain on the outer face of the cell membrane (Fig. 39.1). Regulations are also involved but not well understood. In particular, a chain length determination factor regulates the extension of the PS molecule; the efficiency of this regulation may depend on whether or not the chain length feature is important for the cell. Various exportation mechanisms have been described; some of them involve ATP (adenosine triphosphate)-binding-cassette (ABC)-transporters and others an externalization of the lipid carrier by a Wzx flippase [39.104, 107].

A specific biosynthesis mechanism involves a bifunctional glycosyltransferase called synthase. Cellulose [39.108] and hyaluronan (HA) [39.109, 110] are examples of the molecular pathway of that biosynthesis. The EPS chain is built by repetitive, non-processive addition of monosaccharides to the nascent polymer. Therefore, it seems that this mechanism can only exist for simple heteropolysaccharides (up to two sugar residues in the repeating unit). The EPS is subsequently exported out of the cell by an ABC transporter; synthase has also been described to be able to form a pore across the cell membrane, allowing the polymer to be exported while synthesized [39.107, 110].

Alginate is a linear, anionic heteropolysaccharide consisting of β-D-mannuronic acid (ManA) and α-D-guluronic acid (GulA). The primary structure of alginate relies on ManA-blocks containing homogeneous $(ManA)_n$, GulA-blocks or $(GulA)_m$, and alternating ManA–GulA-blocks. The relative amount of ManA and GulA as well as the length and distribution of the blocks are dependent on the producing species but are fundamental to the swelling and gelling features, as well as the solubility [39.111]. The bacterial alginate biosynthesis mechanism is particular since the polymer is not constituted of a repeating unit [39.112].

Except for xanthan production (up to 23 $g\,L^{-1}$ in the culture broth) or pullulan production, production yields upon fermentation seem low (up to 4 $g\,L^{-1}$ usually). However, an improved activity or a new one resulting in new applications may have a good chance of success for industrial applications.

Engineering of microbial production as well as of genetic material of bacteria, together with the exploration of biodiversity, are different promising approaches to find new PSs with innovative properties.

39.4 Applications of EPSs

Several PSs are well established in different industrial fields. The large range of these existing applications together with the potential ones reflects the many proposed functions in nature as well as their biochemical biodiversity.

Polysaccharides as texture agents of thickening or stabilizing additives are already widely used, such as xanthan, gellan, or some algal PSs (agars, alginates, carrageenans). Therefore, a large number of applications exploit the rheological properties of EPSs. Marine

EPSs having particular functional properties render them more resistant to extreme temperatures, pH, or salinity are of great value for industry [39.113].

Alginate PSs form hydrogels and have found applications in explosives as gelling agents, in the paper industry and textile printing as water-holding agents, in antifoams and lattices because of their emulsifying properties, and in cleaners and ceramics as stabilizers. Alginates are also used in the controlled release of active ingredients that are entrapped in calcium alginate beads, and as new biomaterials for cell immobilization and tissue engineering [39.111]. Research on chitin and chitosan have resulted in applications in various industrial and biomedical fields [39.88].

In plant and animal cells, PSs participate in many central biological processes through the interaction with key proteins such as chemokines, cytokines, growth factors, enzymes, and adhesion molecules involved in cell development, cell signaling, and cell integrity. Therefore, glycobiology, which studies these interactions, and impact on human health have resulted in an increased demand for PSs for therapeutic purposes in cancer, inflammatory diseases, pathogen infections, and thromboembolic disease [39.114, 115]. The potential in this domain of bacterial PSs is now well recognized [39.116, 117].

EPS from the bacteria *Xanthomonas campestris* (xanthan), *Sphingomonas paucimobilis*, or *Sphingomonas* (formerly *Pseudomonas*) *elodea* (gellan), *Acetobacter xylinum* now called *Gluconacetobacter xylinus* (cellulose), *Rhizobium* sp. (succinoglycan), *Agrobacterium* sp. (previously *Alcaligenes faecalis*) (curdlan), and from the fungi *Sclerotium rolfsii* (scleroglucan) and *Aureobasidium pullulans* (pullulan), as well as dextrans from various bacteria have been commercialized and are the most utilized PSs [39.84, 118]. Microbial hyaluronic acid (*Streptococcus* sp.) is also at commercial level and is mainly used in cosmetics, in ophthalmology, and in wound healing [39.119].

39.4.1 Food Products

Polysaccharides are used as ingredients in food products, as carbohydrate and dietary fibre source but also as hydrocolloid to influence rheology and texture. Therefore, in addition to the sensory benefits of EPS in food products, they also impact human health and nutrition [39.120]. EPSs are also produced in situ by bacteria involved in food processing, such as probiotic lactic acid bacteria, and act as functional or prebiotic ingredients. Alginates and various vegetal gums are thickeners, gelling agents, and emulsifiers in food products [39.121].

39.4.2 Environment

The biotechnologies applied to the domain of the environment are being developed and concern essentially the depollution and the rehabilitation of ground, water, and effluents by means of microbial techniques (bioremediation, biodetoxification). Thus, research projects relative to the applications of the bacterial biopolymers in the problems of environmental protection have been conducted for a few years. These concerns are mainly due to the biosorbent properties of the PSs to remove toxic metal pollutants [39.113].

39.4.3 Cosmetics

Cosmetics is a fast growing branch of industry that responds to a societal demand. The lifetime of new molecules ranges between 3–5 years, leading to a constant search for new molecules. Polysaccharides act in feeding, regenerating, and maintaining the skin. Among the most popular products are molecules with moisturizing, smoothing, and/or antiwrinkle activity, as well as those active in the cellular repair or the regeneration of dermic cells and UV protection. Polysaccharides represent more than 20% of the molecules used in the cosmetic field (hyaluronic acid, chitosan, and β-glucane).

The PS deepsane is produced by *Alteromonas macleodii* subsp. *fijiensis biovar deepsane* [39.43] and is used in cosmetics. This EPS shows a protective action of keratinocytes, which are the main cells of the skin, from a proinflammatory agent. Protective effects were also found for cells sensitive to ultraviolet attacks and involved in the cutaneous immune defense system. This EPS would, therefore, contribute to the repair of the skin [39.122].

39.4.4 Medical Applications

The most expected contribution from biotechnologies in the healthcare field concerns the development of new molecules with new biological activities or a better profit–risk ratio. This search inevitably passes by a phase of bioprospecting, and the marine environment constitutes a privileged field of investigations. Therefore, bacterial EPSs offer new and innovative approaches to handle a large number of diseases and to replace certain drugs. Moreover, bacterial EPSs are not

dependent on climatic or physiologic variation, since they are produced in a controlled environment in bioreactors.

The first kind of medical applications derives from the functional properties of the PS molecule (rheology, water-holding, or gelification) [39.123]. As an example, dextran, even widely used as chromatographic supports for purifications of molecules, is also developed in the therapeutic field as artificial plasma. Some bacterial EPSs may also serve as encapsulating agents for a controlled release of drugs near the target, in medical textiles for wound care, in artificial skin and tissue engineering scaffolds [39.121]. *Streptococcus zooepidermicus* produces a hyaluronate with a structure identical to the mammalian one. This EPS is used in ophthalmic surgery. Some other uses are under study, such as various biomaterials and prosthetic surgery to improve biocompatibility. Hydrogels based on natural polymers are close to living tissues and can help during the healing process. Most of these PSs are high molecular weight PSs.

Biological properties constitute the basis for the second type of applications. Some pathogenic bacteria exhibit PSs on their surface; this is the principal factor for immune response. Consequently, they are potential vaccines. Examples of PS vaccines are those against pneumonia (*Streptococcus pneumoniae*) and pneumococcal disease (*Neisseria meningitidis* serogroups A, C, Y, and W135).

Some EPSs have been studied for their antiviral effects [39.124–126], e.g., microbial β-1,3-D-glucans, including levan as well as sulfated dextran [39.127, 128]. Other bacterial PSs exhibit diverse bioactivities, which could also find interest in the biomedical field as therapeutics. Curdlan [39.127] shows antitumor activity. The marine *Geobacillus thermodenitrificans* produces an EPS having immunomodulatory and antiviral effects [39.49]. Some PSs may exhibit innovative structure similar to heparin, a mammalian PS with biological activities such as anticoagulant, antitumor or antiviral. Indeed within the structural diversity, some homologies of new bacterial EPSs have been identified with molecules from animal and bacterial molecules might advantageously replace animal ones. CPSs from *Escherichia coli* are usually antigenic but the *Escherichia coli* K5 strain produces an extracellular PS, called K5, whose structure, [4-D-GlcA β-1,4-D-GlcNAc α-1,]$_n$, is similar to a heparin precursor, *N*-acetylheparosan [39.129]. Because of this structural relationship, chemically and/or enzymatically modified K5 PS is of considerable interest for biomedical applications. Such a project is currently being undertaken by several research teams. This concern is developed in the following paragraph with a focus on marine EPSs.

39.5 Marine EPSs as Glycosaminoglycans (GAGs)

Glycosaminoglycans (GAGs) constitute a class of glycans found ubiquitously in mammalian tissues as components of proteoglycans that have various cellular and intercellular matrix functions. Vertebrates utilize GAGs in structural, recognition, adhesion, and signaling roles. GAG chains are negatively charged by the presence of uronic acids and sulfate groups. They are made up of hexuronic acid and *N*-acetylhexosamine in alternating linear sequence. These PSs exhibit polydisperse high molecular weights in the range of several thousands to

Table 39.2 Structural diversity of GAGs

Name	Hexuronic acid/hexose	Hexosamine	Major repeating unit
Chondroitin sulfate	GlcA or GlcA(2S)	GalNAc or GalNAc(4S) or GalNAc(6S) or GalNAc(4S,6S)	GlcA β-1,3 GalNAc(4S) β-1,4
Dermatan sulfate	GlcA or IdoA or IdoA(2S)	GalNAc or GalNAc(4S) or GalNAc(6S) or GalNAc(4S,6S)	IdoA α-1,3 GalNAc(4S) β-1,4
Keratan sulfate	Gal or Gal(6S)	GlcNAc or GlcNAc(6S)	Gal β-1,4 GlcNAc(6S) β-1,3
Heparin	GlcA or IdoA(2S)	GlcNAc or GlcNS or GlcNAc(6S) or GlcNS(6S)	IdoA(2S) α-1,4 GlcNS(6S) α-1,4
Hyaluronan	GlcA	GlcNAc	GlcA β-1,3 GlcNAc β-1,4

(2S): 2-*O*-sulfo, (4S): 4-*O*-sulfo, (6S): 6-*O*-sulfo, Gal:D-galactose, GalNac: D-*N*-acetylgalactosamine, GlcA: D-glucuronic acid, Glc: D-glucose, GlcNAc: D-*N*-acetylglucosamine, GlcNS: D-*N*-sulfoglucosamine, IdoA: L-iduronic acid

millions g mol^{-1}. GAG structural diversity comes from isomers differing in sulfate presence and position and uronic acid epimerization (Table 39.2). Among them, heparin and heparan sulfate (HS) represent the most heterogeneous structural group because of different sulfation degrees and position as well as various extents of uronic acid epimerization. Postpolymerization modifications of the PS backbone varies depending on the tissue and developmental stage [39.130]. HA is the only non-sulfated GAG.

Heparin is widely used in the clinical treatment of thrombosis as an intravenous anticoagulant with 100 000 kg produced annually through extraction from porcine intestinal mucous membranes.

39.5.1 Biological Activity and Structure–Activity Relationship

The high negative charge of GAG molecules allows binding to many cellular compounds (receptors, growth factors, ...), which results in various bioactivities [39.131]. Medical applications for GAGs are manifold. They are used as surgical aids, moisturizers, for drug delivery, in tissue engineering, as anticoagulants, and for their anticancer activities [39.123, 130, 132, 133]. These biological activities are dependent on the key structural features. Advances in the understanding of the relationships between biological activity and structure are important for the development of safer bioactive analogs.

Sulfation of carbohydrate compounds enhances their biological activity such as their antiviral [39.125, 126] or anticoagulant activity [39.115]. Although the mechanisms are not clear, it has been proposed that sulfates contribute to a molecular conformation that is essential for the activity which, in the case of spirulan, is dependent on calcium ion chelation by sulfates [39.125].

HA, heparin, and chondroitin sulfate (CS) are currently used in various medical applications. Linear HA is mainly used in cosmetics to reduce wrinkles, in drug delivery, in ophthalmology, and in wound healing for its hydratation capability and viscoelasticity [39.119]. Derivatized HA and cross-linked HA are also used in healthcare as non-surgical dermal tissue fillers and in the treatment of osteoarthritis and in tissue engineering [39.119].

The key pentasaccharide of heparin has been well studied and shown to be involved in the interaction with antithrombin, inhibiting blood coagulation [39.134]. In addition, some trisaccharides have been identified to be responsible for binding with fibroblast growth factors having an important role in cell proliferation, differentiation, and migration, as well as angiogenesis and, therefore, can inhibit cancer tumor development. The production of low molecular weight heparinoid would allow us to obtain more efficient preparations with lower side effects by targeting the structural features involved and consequently to obtain more specific preparations [39.131]. In addition, low molecular weight compounds are easier to inject as therapeutics drugs. The use of EPSs as active ingredients does, indeed, concern mainly low molecular weight polymers with bioactivity in cardiovascular and cancer domains.

39.5.2 Modifications to Create GAG-Like Molecules

When an active drug is extracted from animals (heparin from porcine intestinal mucosa, HA from rooster comb, CS from bovine trachea or shark cartilage) or marine invertebrates (scallop, whelk, crustacean, sea squirt), a risk of allergic response to contaminating compounds, or of contamination by non-conventional agents, e.g., prions or viruses [39.130], exists and some new molecules are searched for. In actual fact, GAGs also exhibit undesirable side effects such as hemorrhagic risk; therefore, new analog drugs are being developed [39.132].

For future applications, GAGs derived from bacteria seem to be safer materials with higher purity. Molecules similar to GAGs have been found in microbial sources [39.130]. As has already been presented, *Streptococci* as well as *Pasteurella* produce an EPS identical to HA acid [39.119]. Recently, the recombinant production of HA has been achieved in *Bacillus subtilis* [39.135] and *Lactococcus lactis* [39.136]. Both HA produced by extraction from rooster combs or by microbial fermentation are structurally identical and it can, therefore, be used for its physical properties such as viscosification in ophthalmology, orthopaedic surgery, rheumatology, dermatology, plastic surgery, and wound healing without any modifications of the structure [39.137].

The marine bacterium *Vibrio diabolicus* isolated on the Pompei worm from deep-sea hydrothermal vents has been shown to produce the HE800 polymer constituted of a tetrasaccharidic repeating unit [3-D-GlcNAc-β-(1,4)-D-GlcA-β-(1,4)-D-GlcA-β-(1,4)-D-GalNAc-α-(1] [39.66, 67]. This EPS shows structural similarities to HA. Bioactivity in bone re-

pair has also been described [39.138]. The HE800 EPS in its native form has found application in skin and cartilage therapy [39.133]. Microbial-derived HA has now been commercialized and even if research on marine bacteria may produce analogs, research is also directed at producing heparin via microbial fermentation [39.139].

Beside natural the PSs available, some PS derivatives exhibiting enhanced bioactivity or features can be generated by chemical, biochemical, and genetic methods, or a combination thereof to modify the PS structure through depolymerization and regioselective functionalization. The current challenge, in addition to improving the productivity of EPSs from bacteria, is to produce EPSs of a structure and size that allow the desired activity. In particular, the sulfation degree and pattern must be controlled since the biological activity of heparinoid relies greatly on the specific modification pattern (*N*-deacetylation, *N* and *O*-sulfation, and epimerization of glucuronic acid to iduronic acid). Tailoring the structure is currently performed in chemical ways, but enzymatic ways are also under study.

Pullulan was modified by chemical sulfation to produce new anticoagulant drugs as a potential heparin substituent [39.140]. Some analogs of GAG sugar backbones have also been found in bacteria such as *Escherichia coli* K4 and K5, as well as in marine bacteria. *E. coli* K4 and K5 have been shown to produce PSs with a basic structural similarity to chondroitin sulfate and heparin, respectively [39.139, 141]. The *E. coli* K5 polysaccharidic antigen is similar to a precursor of heparin (desulfoheparin). Much in vitro chemoenzymatic production of heparin, in which the EPS backbone of *E. coli* K5 is subjected to chemical and/or enzymatic modification, have been described [39.110, 139, 142–145].

Alteromonas infernus, a deep-sea marine bacterium has been isolated from seawater samples collected around a dense population of giant worms *Riftia pachyptila* [39.38]. It produces an anionic complex heteropolysaccharide with a high molecular weight and around 10% sulfate content [39.39]. Some other sulfated EPSs from marine bacteria have been described in *Alteromonas macleodii* strains and *Pseudoalteromonas* sp. [39.42, 60]. There are very few bacterial sulfated PSs whose structure has been determined (Table 39.1), but they provide the basis for future applications and basic research on GAG-like molecules from marine environments. On the other hand, L-iduronic acid resulting from 5-epimerization of glucuronic acid has been described in one bacterial PS [39.146]. Since sulfated PSs and L-iduronic acid exist in bacteria, one can expect the isolation of enzymes involved in their biosynthesis in the near future. These enzymes would then be very useful tools to produce engineered GAG-like PSs.

The *A. infernus* EPS repeating unit is a nonsaccharide composed of glucuronic acid, galacturonic acid, and neutral sugars (Gal and Glc), as well as one sulfate group on a galacturonic acid residue (Table 39.1). This PS prepared in a hydrogel exhibited interesting activity in cartilage tissue engineering applications [39.147]. Chemically oversulfated low molecular weight derivatives have been synthesized by two processes that differ in the order of the same steps (radical depolymerization and sulfation). Surprisingly, the two kinds of derivatives did not exhibit the same biological features: one was able to stimulate the proliferation of mature endothelial cells, whereas the other was not [39.148], which suggested that the modification pattern catalyzed by both processes is not the same; therefore, the sulfation pattern appears to be critical for bioactivity. However, *lteromonas infernus* EPS derivatives presented anticoagulant activity similar to that of heparin with a lower hemorrhagic risk [39.149]. These derivatives have also shown interesting properties for cell therapy and tissue engineering [39.133], and also in cartilage regeneration [39.150].

These studies have highlighted the crucial roles of molecular weight and the the sulfation pattern [39.151]. Chemical reactions may be selective to some extent but are not specific enough for such molecules. In particular, it is very difficult to obtain compounds with specific sulfated positions [39.151, 152]. Therefore, the use of enzymatic methods in the modification process leading to bioactive oligosaccharidic derivatives may have clear advantages, such as a better control of reaction selectivity and an easier reaction in a single step (without functional group protection and de-protection steps) under mild conditions. Most enzymes involved in GAG modification are very specific, especially sulfotransferases [39.114, 153, 154]. Moreover, the PAPS (3′-phosphoadenosine 5′-phosphosulfate) molecule required for the sulfation reaction as the sulfate donor is expensive. Therefore, approaches to regenerate this cofactor have been developed [39.153, 155]. However, finding active enzymes on each PS backbone is likely to be the main bottleneck of such a strategy; therefore, enzyme design and optimization by molecular techniques will surely need to be envisaged.

In the future, one can also expect that genetic engineering will be performed to tailor carbohydrate

biomolecules in vivo. This will depend on a deep understanding of biosynthetic mechanisms of EPSs to be able to engineer them and of how the structure of EPSs determines the final activity. The development of improved characterization methods is also required, especially to determine the distribution of functional groups as well as new methods to modify the PS backbone in a regiospecific manner.

39.6 Conclusion

Life emerged from the ocean; nowadays, oceans shelter large biomasses that are mainly microscopic. Among these, marine bacterial diversity, associated with the original ecosystem and biochemical metabolim, is a huge reservoir for the identification of new species and the extraction of new molecules of biotechnological interest, resulting in both fundamental and applied research. It is pointed out that the ocean provides renewable resources for different industrial fields.

The putative or demonstrated benefits of some new PSs and their derivatives are still being studied. Crucial knowledge has been gained on how the structural composition influences the functional properties and on how these features can be applied in biomedical research. In vitro and in vivo structural design of PSs to tailor targeted PS derivatives might produce important bioactive materials in the future. The growing demand will probably lead to the establishment of an adequate and efficient microbial production process.

Only a few marine products have reached commercial production. Indeed, the search for new molecules for healthcare rarely encounters success, and it is always a long time before they are released on the market. The number of marine molecules used as drugs is very low; anticancer, antiviral, and pain-fighting molecules are available on the market, but none are carbohydrates. However, oceans are not only a source of molecules but also a source of inspiration for the synthesis of new drugs [39.156].

In biotechnology, the development of new marine molecules is linked to the progress in the sustainable production process. Moreover, one will have to take into consideration the evolution of legislation regarding the use of natural products, intellectual property rights, the availability of the resource, the ease of implementation, and naturally the production costs.

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