Bacterial Extracellular Polymers: A Review

Hemlata Sharma¹, Jyoti Pal² and Deepesh Kumar Neelam¹*

¹Department of Microbiology, Faculty of Science, JECRC University, Ramchandrapura Industrial Area, Vidhani, Sitapura Extension, Jaipur - 303 905, Rajasthan, India.
²LNJN National Institute of Criminology and Forensic Sciences, Rohini, Delhi, India - 110 085, India.

Abstract

Prokaryotic microbial cells especially bacteria are highly emphases for their exopolysaccharides (EPS) production. EPS are the higher molecular weight natural extracellular compounds observe at the surface of the bacterial cells. Nowadays bacterial EPS represent rapidly emerging as new and industrially important biomaterials because it having tremendous physical and chemical properties with novel functionality. Due to its industrial demand as well as research studies the different extraction processes have been discovered to remove the EPS from the microbial biofilm. The novelities of EPS are also based on the microbial habitat conditions such as higher temperature, lower temperature, acidic, alkaliphilic, saline, etc. Based on its chemical structure they can be homopolysaccharide or heteropolysaccharide. EPSs have a wide range of applications in various industries such as food, textile, pharmaceutical, heavy metal recovery, agriculture, etc. So, this review focus on the understanding of the structure, different extraction processes, biosynthesis and genetic engineering of EPS as well as their desirable biotechnological applications.

Keywords: Exopolysaccharides, Biosynthesis, Genetic engineering, Industrial applications
INTRODUCTION

Exopolysaccharides are the key feature of most of the bacterial surfaces. The formation of biofilms takes place through the attachment of bacterial cells to the substratum or cells embedded in a protective extracellular matrix. It is a complex structure of a heterogeneous matrix consists of various molecules. These natural polymers have emerged as a new alternative to synthetic polymers with marvellous physical characteristics, so they have vast industrial applications. This term was first used by Sutherland in 1972 in his exclusive work on marine bacteria producing EPS. EPS finds in two forms viz. EPS (capsular EPS) and soluble EPS (slime). From the last decades, industries are more emphasises on natural polymers production and these natural polymers are used by various pharmaceuticals, food and other industries which are developing remarkable interest in polysaccharides produced by microorganisms. The total EPS yield depends upon microorganisms used and cultivation conditions provided to them. Bacterial EPS’s have been utilized as bio-absorbents, bio-flocculants and heavy metal removal agents. The main aim of writing this review is to give an overview of the structure, extraction process, biosynthesis, genetic engineering and applications of microbial EPS.

Structure of EPS

Based on the monomeric unit, EPS’s are classified as homopolysaccharides and heteropolysaccharides. Homopolysaccharides contain monosaccharides while heteropolysaccharides are composed of more than one type of monosaccharide. EPS is classified based on the number and nature of monomers, bonds between them and the type of linkage. EPS are generally polyanionic due to the presence of uronic acids or ketal-linked pyruvate or inorganic substances like phosphate or sulfate. Some are also neutral macromolecules. A few EPSs may even be polycationic, e.g. polymer obtained from Staphylococcus epidermidis strains. However, the physicochemical factors also affect the production of exopolysaccharides which includes pH, temperature, incubation time, and the constituents of culture media (with various organic and inorganic carbon (C) and nitrogen (N) sources).

Extraction of EPS

Various extraction processes have been developed to recover EPS from biofilm of microbes from different environments to identify the contents of EPS, to analyze various properties (chemical, physical and physicochemical) and to observe the functions of EPS. The various extraction methods include chemical or physical and physical and chemical, and analytical methods. It is estimated that extraction methods are dependent on the water solubility of the EPS separated. The extraction methods of EPS should be cost-effective, eco-friendly and do not damage the structure of EPS. The extraction efficiency is calculated as the overall amount of EPS separated from the entire microbial biomass for a particular sample.

Physical method

The physical process mainly involves three extraction techniques i.e. centrifugation, sonication and heating. Researchers explored the reason behind variations in extraction efficiency with different physical methods. These variations in results are because during the extraction procedure by heating, the components of EPS might be hydrolyzed, for this particular case, the proteins and polysaccharide content of EPS. Another study showed that the heating allowed extracting the capsular EPS to flocs. However, several studies suggested that the high EPS extraction yield by the heating methodology could also be thanks to meaningful cell lysis which can lead to high protein content in EPS.

Chemical method

In the chemical method, different chemicals are used which could break the linkage in the matrix so that EPS can be easily released to the external medium containing water. NaOH treatment can cause the ionization of a great number of charged groups, for instance, carboxylic groups in proteins and polysaccharides. Due to this, a strong repulsion occurs between EPS which enhances its solubility. A lot of polymers can suffer alkaline hydrolysis. In glycoproteins, disulfide bonds can be broken if exposed to basic environments (pH above 9), which will promote the extraction of these compounds. The repulsion and solubility between the compounds of the EPS matrix are resultant of the exchange of.
divalent cations with mono-valent cations. Resin, EDTA or EGTA are used for the removal of divalent cations. A high concentration of sodium chloride can also be used to carry out cation exchange. This process has been used in Pseudomonas sp. for the extraction of adhesive exopolymers. The extraction of EPS could be increased by destabilizing biofilm in an enzymatic digestion process. Furthermore, ethanol from activated sludge has been used to extract lipids.

**Combination of physical and chemical method**

Some studies proposed that the chemical extraction method could be of better performance once it is combined with the physical method i.e. defined shear. The shear is often provided by heat, sonication, or stirring under pre-established conditions. The alkaline and heat treatment has been combined to extract capsular EPS from varied microbial species. On the other hand, shear (stirring) and ion exchange by a Dowex extraction have been used in conjunction to extract EPS from activated sludge and biofilms. Formaldehyde (CH$_2$O) and NaCl was applied in combination with ultrasonication, to extract EPS from an anaerobic sludge. The formaldehyde is added to minimize cell disruption during the extraction process. However, formaldehyde (CH$_2$O) has the capacity to changes the properties of many EPS components.

**Analytical method**

**Colorimetric analyses**

The complex composition of EPS in biofilms is made up of carbohydrates, lipids, humic substances, proteins, nucleic acids, etc. Colorimetric analyses are may be used to quantify the components in EPS. The measurement of carbohydrate contents performed by two methodologies i.e. the anthrone method or the phenol–sulfuric acid method. The content of protein might be measured by the Lowry technique, the Press-Man technique, or the total N-content technique. The m-hydroxydiphenyl sulfuric acid method has been used to measure uronic acid content in EPS. To measure the nucleic acid content, three different methodologies could be used, which are the 4,6-diamidino-2-phenylindole (DAPI) fluorescence method, the UV absorbance method, or the diphenylamine method.

**Innovative methods**

EPS structure, functions and conformation examinations are very difficult to work due to their complex composition. However, recent studies in analytical chemistry develop new techniques such as transmission electron microscopy (TEM), scanning electron microscopy (SEM), atomic force microscopy (AFM), confocal laser scanning microscopy (CLSM), infrared spectroscopy (FTIR), X-ray photoelectron spectroscopy (XPS), nuclear magnetic resonance (NMR) and 3-dimensional excitation-emission matrix fluorescence spectroscopy (3D-EEM) which will help in examining the properties of EPS as well as their nature. The qualitative and quantitative analysis of EPS compositions was reported by using chromatography, mass spectrometry and their combination.

**Bioynthesis of EPS**

Bioynthesis of homopolysaccharides and heteropolysaccharides take place in different pathways.

**Synthase dependent pathway**

The synthesis of homopolysaccharides through synthase, a dependent pathway is quite complicated, but specific enzymes make this process easier, by making modifications in initially synthesized homopolysaccharides such as alginate (Table 1) via the polymerization of GDP-mannuronic acid monomers, the biosynthesis of alginate takes place too. The enzymes involved in alginate biosynthesis are epimerases, lyases and acetylases. Diverse alginate epimerases (AlgE1-7) outer of the cell are there that alter the final chemical polymer characteristics through the selective insertion of specific β-D- mannuronic acid (M) and α-L-guluronic acid (G) blocks. In recent years, the significance of P. aeruginosa was represented as depressingly induces alginate production. The DagL variant had maximum yields of alginate of equal MW. The higher O-acetylated alginate and lower molecular weight were observed after the overexpression of AlgL. Both are highly focused factors for the pathogenicity of P. aeruginosa. Therefore, the biosynthesis of alginate functions as a motley-enzyme complex. A pure EPS of biofilm is bacterial cellulose which is known by performs the polymerization
process through upend the mechanism. The structures of the principal subunit and another subunit, BcsA and BcsB both were identified from specific bacteria that was Rhodobacter sphaeroides, show the cell domain of the BcsAB comprised the GT activity and a PILZ domain for interaction with activator c-diGMP. The periplasmic domain is narrowly linked to a flavodoxin-like domain. The entire cell envelope is permeated by cellulose synthase complex and its productivity is very high. The interesting phenomenon of biosynthesis of cellulose was recently described.

**Dextrase/Sucrase dependent pathway**

The homopolysaccharide dextran and levan are formed and assembled from the cleavage of sucrose molecules by the action of the extracellular sucrase. After that, the monosaccharide unit is transported to a primer molecule, which may be ramified at distinct levels. Further, using different primer molecules leads to high oligosaccharide production.

**Wzx/Wzy pathway**

In this pathway activated sugar nucleotide monomers are transferred by an enzyme called glycosyltransferases (GTs). In this manner, the number of GTs available will determine the sequence of the final polymers. Both the side chain and substituents are incorporated adjacent to the backbone, before the completion of the polymer assembly, but the stage at which this incorporation occurs is not clear. The Wzx gene encodes the flippase protein which transfers the repeating unit by H+-dependent antiporter mechanism. Different numbers of trn membrane sequences are shown by the structures of the several Wzx proteins, besides lack in similarity. It points out that different types of Wzx protein exist. Evidence has been found to support the preference of the substrate Wzx on kindred O-units further than the first sugar. As soon are transferred the repeating units towards the periplasm another enzymes, the polymerase will recognize it and helps in the polymerization of repeating units. This procedure is carried out by the polymerase, sometimes accompanied by a co-polymerase, which may be associated with the process of determining the length of the polymer. The Wzx / Wzy pathway is defined by the involvement of the main protein in the transportation and polymerization of specific repeating units, on which the final structure of EPS depends. Various EPS such as xanthan and succinoglycan are synthesized through this pathway (Table 2).

**ABC transporter pathway**

Two synthesis strategies dependent on the ABC transporter have been identified. One of these strategies is combined with the synthesis and export of cytosolic glucans. The second is the synthesis and export of uncoupled glucans by modifying the non-reducing terminus of the polymer attached to Und-P that ends the chain extension. At this point, the terminator determines the glycan chain length and simultaneously serves as an export signal recognized by the transporter. A terminal residue linked by the WbdD protein and the end process depends on the chain size and the stereochemistry of the WbdD-WbdA complex. To assemble the glycan chain, the domains of GT activity are carried by the WbdA protein. Recently, a protein was described in Raoulettella terrigena, that was observed the significant role for polymerization, termination, and quality control within its protein structure. These types of findings show the complexity behind the biosynthesis mechanism. Additionally, specific domain scans could be within the protein complex. Hence, it was possible to understand an important phase of CPS biosynthesis, whose role is crucial in human pathogens. Recently, in Campylobacter jejuni, the ABC PglK transporter mechanism was described, which is highly dependent on ATP to achieve the transport of lipid-linked oligosaccharide units. The drop-interface-bilayer systems have been novel techniques that have helped to gather the most recent knowledge about the Wza homologs for the export of CPS, allowing to know the complexity of this transport mechanism and in turn, contributing to the construction of promising perspectives.

**Engineering strategies**

The specific operons present on the genome which are encrypted in genes for the biosynthesis of EPS. Thus, the number of open reading frames (ORFs) may differ from one to more than 30 ORFs. EPS is composed of all the genes that are essential for its biosynthesis of polysaccharides units, the turning of the repeating units, as well as the polymerization and the final
Table 1. List of homopolysaccharides and producing bacteria

| S.No. | EPS      | Localization | Polymerisation enzyme       | Precursors    | Micro-organism                  | Ref. |
|-------|----------|--------------|----------------------------|---------------|--------------------------------|------|
| 1.    | Dextran  | Extracellular | Dextransucrase             | Saccharose    | Leuconostoc sp.                | 72   |
| 2.    | Pullulan | Extracellular | UDPG-pyrophosphorylase      | UDP-d-glucose | Aureobasidium Pullulans        | 73,74|
| 3.    | Levan    | Extracellular | Levansucrase               | -             | Bacilluslicheniformis Acetobacter sp. Halomonas sp. | 75   |
| 4.    | Curdlan  | Extracellular | Curdlan synthase           | UDP-glucose   | Rhizobium spp.                 | 76   |
| 5.    | Cellulose | Extracellular | Cellulose synthase         | UDP-d-glucose | Acetobacter Xylinum            | 77   |

Besides these, there are some specific genes present on the operon which are involved in sugar precursors synthesis, whereas other genes that provide nucleotide sugar are spread all over the chromosomes. It was observed that most of the bacterial genes could encode more than one polysaccharide biosynthesis pathway. The production of EPS depends upon the regulatory effects and cultivation conditions. The various type of carbon (C) and nitrogen (N) sources can affect the polysaccharide’s expressions in bacteria. It was found that cdi-GMP has an impact on the biosynthesis of EPS, moreover, overexpression resultant as production of novel EPS. The new binding sites could be affecting the EPS biosynthesis.

Applications of EPS

Agriculture

EPS has a wide range of applications in agricultural fields and is known to have the ability to increase productivity. EPS’s secreted by microorganisms play a crucial role during soil development. It is also capable of entrapping nutrients and provides protection to microbes against unfavorable environmental conditions by forming niches. EPS also plays an important role in protecting a crop against desiccation and predation by other organisms. EPS also protects the seedlings from drought. The ability to freeze water, technically called ice nucleation activity (INA), is widely used in biotechnology, for example for the production of energy-saving artificial snow and ice. Additionally, in industries such as food processing, it has been used during ice-cream production and freezes concentration efficiently avoiding loss of flavor.

Heavy metal degradation

It has been reported in several studies that EPS has a high affinity for heavy metals present in wastewater. The binding affinity of EPS towards heavy metals depends upon the composition and binding sites present in EPS. EPS is associated with the surface so that it protects micro-organisms from heavy metal toxicity. It has been reported that the adsorption capacity of most of the heavy metals such as copper (Cu), lead (Pb), cadmium (Cd), and zinc (Zn), etc. depends on the components of EPS, which shows that the main reason for the adsorption performance of EPS is the protein.

The major issues of heavy metal contamination have been seen in agricultural soils because heavy metals can easily enter into the food chain and possess serious health hazards to the humans as well as ecosystem. Toxic heavy metals include cadmium, lead, copper, zinc and manganese.

Biomedical applications

EPS’s have a wide range of applications in the biomedical sector. It is used as a plasma volume expander for controlling wound shock, as an antiacid stomach protector in capsules and as a stabilizing agent in pharmaceutical suspensions and emulsions. It is also used during eye surgery, in wound healing, used in cosmetics, in the treatment of osteoarthritis, as a drug-controlled release carrier and also used in skin repair.

Food applications

The need of today’s hour is the healthier food without compromising with the safety of...
food. There are a lot of microbes producing EPS for eg. lactic acid bacteria mainly produce EPS which improves the quality, texture and safety of various food products and also inhibits the growth of disease-causing organisms in food. The EPS in the food industry has been used as an emulsifier, stabilizer and thickener. It is also used in the packaging of food products. Mostly xanthan, gellan and cellulose which are secreted by bacteria other than lactic acid bacteria, are predominantly used in the food industry.

CONCLUSION

As described in this review, it is now widely considered that bacterial EPS plays a very important role in various industrial applications. Moreover, EPSs biosynthesis is a complicated process through which various alterations occur and resultant many number of EPSs produces on bacterial cell surface, which have a valuable range of physicochemical properties and highly promising commercial applications. However, the EPS extraction methods from cell surface still required the some novel techniques or tricks that will be easy to handle, time consuming and more effective for understanding of mechanism involved in synthesis and excretion. This study showed the role of EPS in the food, pharmaceutical, heavy metal recovery and agriculture field, but there is still much to learn about their functions in the environment. To understand more about biopolymers synthesis, it will be necessary to explore insight into the some extremophiles from extreme condition these EPSs are highly stable at various physical as well as on chemical parameters more than mesophilic bacterial EPSs. On the other hand genetic engineering is the new tools for changes in the properties of molecules that will be possible by genome annotation and construction of EPS biosynthetic pathways in bacterial cell to understand about how they will incorporate and how they will be affected. The big research gaps still remain that no method exists to extract all microbial polysaccharides but in upcoming scientific studies it could be possibility to explain about EPSs with their specific structure and functions.

ACKNOWLEDGMENTS

All listed author(s) are thankful to JECRC.
University for providing the related support to compile this work.

CONFLICT OF INTEREST
The authors declare that there is no conflict of interest.

AUTHORS’ CONTRIBUTION
All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

FUNDING
None.

DATA AVAILABILITY
All datasets generated or analyzed during this study are included in the manuscript.

ETHICS STATEMENT
This article does not contain any studies with human participants or animals performed by any of the author.

REFERENCES
1. Whitfield C, Wear SS, Sande C. Assembly of bacterial capsular polysaccharides and exopolysaccharides. *Annu Rev Microbiol*. 2020;74:521-543. doi: 10.1146/annurev-micro-011420-075607
2. Pinto RM, Lopes-de-Campos D, Martins MCL, Van Dijck P, Nunes C, Reis S. Impact of nanosystems in *Staphylococcus aureus* biofilms treatment. *FEMS Microbiol Rev*. 2019;43(6):622-641. doi: 10.1093/femsre/fuz021
3. Mc Swain BS, Irvine RL, Hausner M, Wilderer PA. Composition and distribution of extracellular polymeric substances in aerobic flocs and granular sludge. *Appl Environ Microbiol*. 2005;71:1051-1057. doi: 10.1128/AEM.71.2.1051-1057.2005
4. Sutherland IW. Bacterial exopolysaccharides. *Adv Microb Phys*. 1972;8:143-213. doi: 10.1016/S0065-2911(08)60190-3
5. Nielsen PH, Jahn A. Extraction of EPS. *Microbial Extracellular Polymeric Substances*. 1999:49-72. doi: 10.1007/978-3-642-60147-7_3
6. Laspidou CS, Rittmann BE. A unified theory for extracellular polymeric substances,soluble microbial products, and active and inert biomass. *Water Res*. 2002;36(11):2711-2720. doi: 10.1016/S0043-1354(01)00413-4
7. Sutherland IW. Microbial polysaccharides from Gram-negative bacteria. *Int Dairy J*. 2001;11(9):663-674. doi: 10.1016/S0958-6946(01)00112-1
8. Angel SJ, Vidyadharani G, Santhosh S, Dhandapani R. Optimization and Characterisation of Thermo Stable Exopolysaccharide Produced from *Bacillus licheniformis* WSF-1 Strain. *J Polym Environ*. 2018;26(4):3824-3833. doi: 10.1007/s10924-018-1261-0
9. Hu X, Pang X, Wang PG, Chen M. Isolation and characterization of an antioxidant exopolysaccharide produced by *Bacillus sp.* S-1 from Sichuan Pickles. *Carbohydr Polym*. 2019;204:9-16. doi: 10.1016/j.carbpol.2018.09.069
10. Liang TW, Wang SL. Recent advances in exopolysaccharides from *Paenibacillus* spp.: production, isolation, structure, and bioactivities. *Mar Drugs*. 2015;13(4):1847-1863. doi: 10.3390/md13041847
11. Ruas-Madiedo P, Hugenholtz J, Zoon P. An overview of the functionality of exopolysaccharides produced by lactic acid bacteria. *Int Dairy J*. 2002;12(2-3):163-171. doi: 10.1016/S0958-6946(01)00160-1
12. Stewart-Tull DES. The immunological activities of bacterial peptidoglycans. *Annu Rev Microbiol*.1980;34:311-340. doi: 10.1146/annurev.mic.34.100180.001523
13. Sutherland M, Vuuren HJ, Martha M, Cloning, sequence and in vitro transcription/translation analysis of a 3.2-kb EcoRI HindIII fragment of *Leuconostoc oenos* bacteriophage L10 gene. *Gene*. 1994;148(1):125-129. doi: 10.1016/0378-1119(94)90245-3
14. Mack D, Haeder M, Siemssen N, Laufs R. Association of biofilm production of coagulase-negative staphylococci with expression of a specific polysaccharide intercellular adhesin. *J Infect Dis*. 1996;174(4):881-
43. Wang Y, Moradali MF, Gourdarzalejardi A, Sims IM, Rehm BHA. Biological function of a polysaccharide degrading enzyme in the periplasm. *Sci Rep*. 2015;6:31249. doi: 10.1038/srep31249

44. Maleki S, Almas E, Zotchv S, Valla S, Ertesvag H. Alginate biosynthesis factories in Pseudomonas fluorescens: Localization and correlation with alginate production level. *Appl Environ Microb*. 2016;82(4):1227-1236. doi: 10.1128/AEM.03114-15

45. Augiemi RV, Varley AJ, Strap JL. Establishing a role for bacterial cellulose in environmental interactions: lessons learned from diverse biofilm-producing proteobacteria. *Front Microbiol*. 2015;6:1282. doi: 10.3389/fmicb.2015.01282

46. McNamara JT, Morgan JLW, Zimmer J. A molecular description of cellulose biosynthesis. *Annu Rev Biochem*. 2015;84:895-921. doi: 10.1146/annurev-biochem-060614-033930

47. Morgan JL, Strumillo J, Zimmer J. Crystallographic snapshot of cellulose synthesis and membrane translocation. *Nature*. 2013;493(7431):181-186. doi: 10.1038/nature11744

48. Morgan JL, McNamara JT, Zimmer J. Mechanism of activation of bacterial cellulose synthase by cyclic di-GMP. *Nat Struct Mol Biol*. 2014;21:489-496. doi: 10.1038/nsmb.2803

49. Jang WD, Hwang JH, Kim HU, Ryu JY, Lee SY. Bacterial cellulose as an example product for sustainable production and consumption. *Microb Biotechnol*. 2017;10(5):1181-1185. doi: 10.1111/1751-7915.12744

50. Ua-Aarak T, Jakob F, Vogel RF. Fermentation pH modulates the size distributions and functional properties of *Glucconobacter albidus* TMW 2.1191 levan. *Front Microbiol*. 2017;8:807. doi: 10.3389/fmicb.2017.00807

51. Srikanth R, Reddy CH, Siddartha G, Ramaiyah MJ, Uppuluri KB. Review on production, characterization and applications of microbial levan. *Carbohydr Polym*. 2015;120:102-114. doi: 10.1016/j.carbpol.2014.12.003

52. Hu Y, Winter V, Chen XY, Ganze MG. Effect of acceptor carbohydrates on oligosaccharide and polysaccharide synthesis by dextrantransacssure DsrM from *Weissella cibaria*. *Food Res Int*. 2017;99(1):603-611. doi: 10.1016/j.foodres.2017.06.026

53. Baker P, Whitfield GB, Hill PJ, et al. Characterization of the *Pseudomonas aeruginosa* glycoside hydrolase PslG reveals that its levels are critical for Psl polysaccharide biosynthesis and biofilm formation. *J Biol Chem*. 2015;290(47):28374-28387. doi: 10.1074/jbc.M115.674929

54. Schmid J, Sieber V. Enzymatic transformations involved in the biosynthesis of microbial extrapoly saccharides based on the assembly of repeat units. *Chembiochem*. 2015;16(8):1141-1147. doi: 10.1002/cbic.201500035

55. Islsm ST, Lam JS. Synthesis of bacterial polysaccharides via the Wzx/Wzy-dependent pathway. *Can J Microbiol*. 2014;60(11):697-716. doi: 10.1139/cjm-2014-0595

56. Hong Y, Liu MA, Reeves PR. Progress in our understanding of Wzx flippase for translocation of bacterial membrane lipid-linked oligosaccharide. *J Bacteriol*. 2018;200(1):e00154-17. doi: 10.1128/JB.00154-17

57. Liu MA, Stent TL, Hong Y, Reeves PR. Inefficient translocation of a truncated O unit by a *Salmonella* Wzx affects both O-antigen production and cell growth. *FEMS Microbiol Lett*. 2015;362(9):fnv053. doi: 10.1093/femsle/fnv053

58. Whitfield C. Polymerases glycans chain-length control. *Nat Chem Biol*. 2010;6:403-404. doi: 10.1038/nchembio.376

59. Rehm BHA. Bacterial polymers: biosynthesis, modifications and applications. *Nat Rev Micro*. 2010;8(8):578-592. doi: 10.1038/nrmicro2354

60. Freitas F, Torres CA, Reis MAM. Engineering aspects of microbial exopolysaccharide production. *Bioreor Technol*. 2017;245(Part B):1674-1683. doi: 10.1016/j.biortech.2017.05.092

61. Schmid J, Sperl N, Sieber V. A comparison of genes involved in sphingan biosynthesis brought up to date. *Appl Microbiol Biotechnol*. 2014;98(18):7719-7733. doi: 10.1007/s00253-014-5940-z

62. Schmid J, Sieber V, Rehm B. Bacterial exopolysaccharides: biosynthesis pathways and engineering strategies. *Front Microbiol*. 2015;6:496. doi: 10.3389/fmicb.2015.00496

63. Kos V, Cuthbertson LT, Whitfield C. The Klebsiella pneumoniae O2a antigen defines a second mechanism for O antigen ATP binding cassette transporters. *J Biol Chem*. 2009;284(5):2947-2956. doi: 10.1074/jbc.M807213200

64. Mann E, Mallette E, Clarke BR, Kimber MS, Whitfield C. The *Klebsiella pneumoniae* O12 ATP-binding cassette (ABC) transporter recognizes the terminal residue of its O-antigen polysaccharide substrate. *J Biol Chem*. 2016;291(18):9748-9761. doi: 10.1074/jbc.M116.719344

65. Hagelueken G, Huang H, Clarke BR, Lebl T, Whitfield C, Naismith JH. Structure of WbdD: a bifunctional kinase and Methyl transferase that regulates the chain length of the O antigen in *Escherichia coli* O9a. *FEMS Microbiol*. 2012;86(3):730-742. doi: 10.1111/mmi.12014

66. King JD, Berry S, Clarke BR, Morris RJ, Whitfield C. Lipopolysaccharide O antigen size distribution is determined by a chain extension complex of variable stoichiometry in *Escherichia coli* O9a. *Proc Natl Acad Sci USA*. 2014;111:6407-6412. doi: 10.1073/pnas.1408014111

67. Williams DM, Ovchinnikova OG, Koizumi A, et al. Single polysaccharide assembly protein that integrates polymerization, termination, and chain-length quality control. *Proc Natl Acad Sci USA*. 2017;114:E1215-E1223. doi: 10.1073/pnas.1613609114

68. Perez C, Gerber S, Boilevin J, et al. Structure and mechanism of an active lipid-linked oligosaccharide flippase. *Nature*. 2015;524(7566):433-438. doi: 10.1038/nature14953

69. Kong L, Almond A, Bayley H, Davis BG. Chemical polyglycosylation and nanolitre detection enables single molecule recapitulation of bacterial sugar export. *Nat Chem*. 2016;8:461-469. doi: 10.1038/nchem.2487

70. Locher KP. Mechanistic diversity in ATP-binding cassette (ABC) transporters. *Nat Struct Mol Biol*. 2016;23(6):487-493. doi: 10.1038/nsmb.3216
71. Woodward L, Naismith JH. Bacterial polysaccharide synthesis and export. Curr Opin Struct Biol. 2016;40:81-88. doi: 10.1016/j.sbi.2016.07.016
72. Santos M, Teixeira J, Rodrigues A. Production of dextranulose, dextran and fructose from sucrose using Leuconostoc mesenteroides NRRL B512 (f). Biochem Eng J. 2000;4(3):177-188. doi: 10.1016/S1369-703X(99)00047-9
73. Jiang L. Optimization of fermentation conditions for pullulan production by Aureobasidium pullulans using response surface methodology. Carbohydr Polym. 2010;79(2):414-417. doi: 10.1016/j.carbpol.2009.08.027
74. Jiang L, Wu S, Kim JM. Effect of different nitrogen sources on activities of UDPG-pyrophosphorylase involved in pullulan synthesis and pullulan production by Aureobasidium pullulans. Carbohydr polymers. 2011;86(2):1085-1088. doi: 10.1016/j.carbpol.2011.05.016
75. Gojic-Cvijovic GD, Jakovljevic DM, Loncarevic BD, et al. Production of levan by Bacillus licheniformis N5032 in sugar beet molasses-based medium. Int J Biol Macromol. 2019;121:142-151. doi: 10.1016/j.ijbiomac.2018.10.019
76. Pavlova K, Panchev I, Hristozova T. Physico-chemical characterization of exo mannan from Rhodotorula acheniorum MC. World J Microbiol Biotechnol. 2005;21:279-283. doi: 10.1007/s11274-004-3632-z
77. Hwang JW, Yang YK, Hwang JK, Pyun YR, Kim YS. Effects of pH and dissolved oxygen on cellulose production by Acetobacter xylulon BRCS in agitated culture. J Biosci Bioeng. 1999;88:183-188. doi: 10.1016/S1389-1723(99)80199-6
78. Arnold MFF, Penterman J, Shabab M, Chen EJ, Walker GC. Important Late-Stage Symbiotic Role of fux017 in Xanthomonas campestris. Proc Natl Acad Sci USA. 2015;112(7):E757-E765. doi: 10.1073/pnas.1421748112
79. Perez-Mendoza D, Bertinetti D, Lorenz R, Gallegos M-T, Herberg FW, Sanjuan J. A novel c-di-GMP binding domain in glycosyltransferase BgsA is responsible for the synthesis of a mixed-linkage b-glucan. Sci Rep. 2017;7:8997. doi: 10.1038/s41598-017-09290-2
80. Schaper S, Steinchen W, Krol E, et al. AraC-like transcriptional activator CuxR binds c-di-GMP by a PILZ-like mechanism to regulate extracellular polysaccharide production. Proc Natl Acad Sci USA. 2011;108(24):E4822-E4831. doi: 10.1073/pnas.1012435114
81. Chou FL, Chou HC, Lin YS, et al. The Xanthomonas campestris gymnGenes Required for Synthesis of Xanthan Gum Is Involved in Normal Pigmentation and Virulence in Causing Black Rot. Biochem Biophys Res Commun. 1997;233(1):265-269. doi: 10.1016/bbcr.1996.12.074
82. Sugahara K, Schwartz NB, Dorfman A. Biosynthesis of hyaluronic acid by Streptococcus. J Biol Chem. 1979;254(14):6252-6261. doi: 10.1016/0021-9258(79)90356-2
83. Hashimoto W, Maesaka K, Sato N, et al. MicrobialSystem for Polysaccharide Depolymerization: Enzymatic Route for Gellan Depolymerization by Bacillus sp. GL1. Arch Biochem Biophys. 1997;339(1):17-23. doi: 10.1006/abbi.1996.0981
84. Martins LO, Sa-Correia I. Gellan gum biosynthetic enzymes in producing and nonproducing variants of Pseudomonas elodea. Biotechnol Appl Bioch. 1991;14(3):357-364.
85. Hay ID, Ur Rehman Z, Ghafoor A, Rehm BH. Bacterial biosynthesis of alginites. J Chem Technol Biotechnol. 2010;85(6):752-759. doi: 10.1002/jctb.2372
86. Pan YJ, Lin TL, Chen CT, et al. Genetic analysis of capsular polysaccharide synthesis gene clusters in 79 capsular types of Klebsiella spp. Sci Rep. 2015;5:15573. doi: 10.1038/srep15573
87. Zeidan AA, Poulsen VK, Janzen T, et al. Polysaccharide production by lactic acid bacteria: from genes to industrial applications. FEMS Microbiol Rev. 2017;41(1):S168-S200. doi: 10.1093/femsre/fux017
88. Cheng X, Zheng X, Zhou X, et al. Regulation of oxidative response and extracellular polysaccharide synthesis by a di adenylylate cyclase in Streptococcus mutans. Environ Microbiol. 2016;18(3):904-922. doi: 10.1111/1462-2920.13106
