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Bacterial extracellular matrix as a natural source of biotechnologically multivalent materials

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ABSTRACT

The extracellular matrix (ECM) is an intricate megastructure made by bacterial cells to form architecturally complex biostructures called biofilms. Protection of cells, modulation of cell-to-cell signalling, cell differentiation and environmental sensing are functions of the ECM that reflect its diverse chemical composition. Proteins, polysaccharides and eDNA have specific functionalities while cooperatively interacting to sustain the architecture and biological relevance of the ECM. The accumulated evidence on the chemical heterogeneity and specific functionalities of ECM components has attracted attention because of their potential biotechnological applications, from agriculture to the water and food industries. This review compiles information on the most relevant bacterial ECM components, the biophysical and chemical features responsible for their biological roles, and their potential to be further translated into biotechnological applications.

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Contents

1. Introduction

The extracellular matrix (ECM), both in eukaryotes and prokaryotes, is a mixture of high-molecular-weight polymers that are secreted to the external medium and are produced by nearly all types of cell (Dragos and Kovacs, 2017). By definition, the eukaryotic ECM can be understood as the non-cellular three-dimensional macromolecular network composed of a mixture of components such as collagens, proteoglycans/glycosaminoglycans (PGs), elastin, fibronectin, laminins, and several other glycoproteins. This structure can be found in tissues and organs providing support to the cellular components and providing biochemical and biomechanical cues for tissue morphogenesis, differentiation and homeostasis [1,2].

Fibril-forming collagen type I and II are the major constituents of the extracellular matrix of eukaryotic tissues, which can be found associated to other ECM proteins, collagens and PGs thus

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constructing large fibrilar structures [3]. These structures, in combination with other ECM molecules, define the 3D matrix network [2,4]. It is thus conceivable that the composition and structural organization of the ECM influences relevant biological processes such as adhesion, migration, proliferation and differentiation of eukaryotic cells [5]. Indeed, the ECM has been described as a reservoir for the localization and concentration of growth factors and signalling molecules, which form gradients critical for the establishment of developmental patterning during morphogenesis [6,7].

In contrast to eukaryotes, in which cells are intrinsically grouped forming tissues and organs, bacterial cells live as independent individuals or forming multicellular communities, known as biofilms, growing over surfaces and providing several benefits as the better adaptation to different environmental conditions, improved attachment to hosts and to the access to nutrients [8–11]. Analogous to eukaryotic tissues, bacterial cells within biofilms are embedded in a secreted and multifunctional ECM that provides i) structural support to the community, ii) improved cellular adhesion, iii) regulation of the flux of signals and nutrients to ensure cell differentiation [12,13], and iv) a formidable physico-chemical barrier against external assaults [14,15]. The microbial ECM is heterogeneously composed of proteins, exopolysaccharides, nucleic acids, lipids and secondary metabolites, each of which preserves similar functionality but is chemically variable across bacterial taxa. In this mini-review, we introduce the main components of the prokaryotic ECM, their functions in the maturation of the biofilm structure and bacterial interactions with the environment, and we highlight the biophysical peculiarities that allow their biotechnological exploitation.

2. Biotechnological applications of biofilms

Bacterial biofilms are widely distributed in nature and largely contribute to the modification of the environment in a variety of ways. However, the fact that bacterial biofilms have been extensively studied with human bacterial pathogens has led to biased negative perceptions associated with contamination and pathogenicity [16]. Water, food and agricultural industries, sustainable agriculture, and the production of recombinant proteins and chemicals are examples of biotechnology research fields that benefit from the unique properties of bacterial biofilms (Fig. 1) [17–19]. In addition, the possibility of combining diverse strains in multispecies biofilms expands their biotechnological applications, diversifying the variety of products that would be impossible to obtain with single strain cultures.

Bacterial biofilms can be immobilized in bioreactors using different strategies such as adsorption, entrapment, or covalent bond. Adsorption based on cell fixing is the most commonly used method in biofilm reactors (fluidized bed reactors, continuous stirred tank reactors, airlift reactors, and packed bed reactors), and it is probably the most natural method because it leverages the inherent ability of bacterial cells to adhere to any given support [18,20,21]. Immobilization of cells on alginate beads is also interesting for industrial bioprocesses and has been successfully used in the preservation of cell viability, the degradation and biotransformation of pollutants, and the production of enzymes, probiotics and other valuable products [22,23]. The water industry was the first to implement biofilm reactors, including the use of biofilters or moving bed biofilm reactors for wastewater treatment [24]. Recently, the development and use of biofilm-based systems has
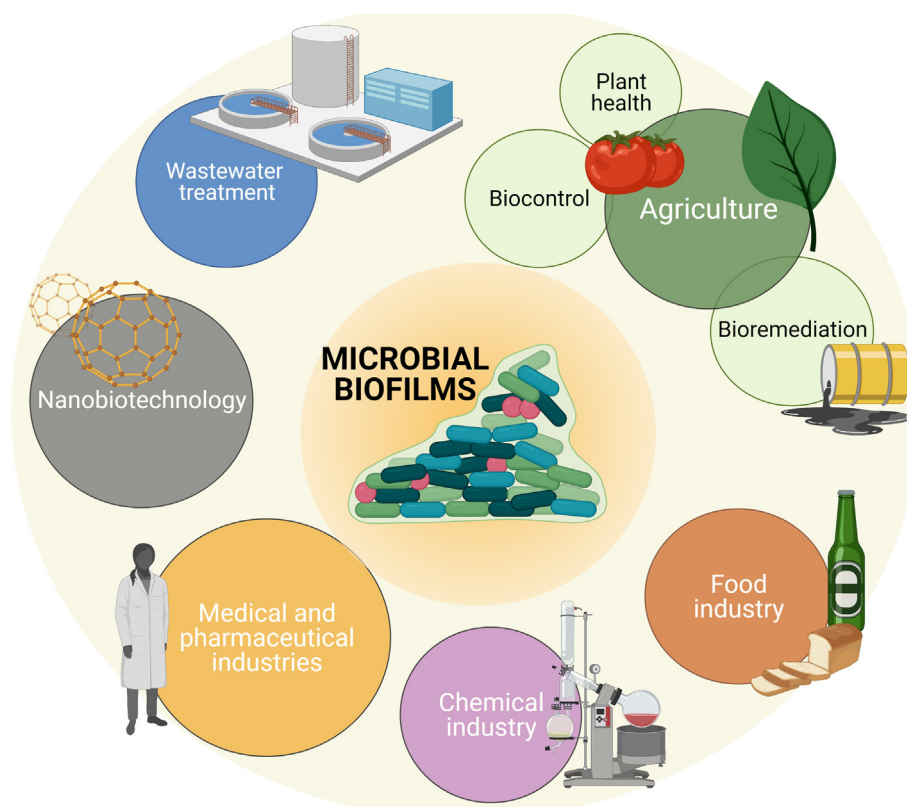

Fig. 1. Applicability of microbial biofilms to different branches of the biotechnological industry. Microbial biofilms are currently used in different agricultural techniques involved in biocontrol, bioremediation and plant health. In addition, they can be found in wastewater treatment plants, in the nanobiotechnology industry involved in the production of nanotubes and nanowires, in the food and chemical industries and also in areas of the pharmaceutical and medical industries. Created with BioRender.com.

increased to produce a variety of valuable chemicals, although many steps need to be completely understood to optimize the production and reach the highest yield [17,25].

Bacterial biofilms have also shown great potential in the food industry and the implementation of sustainable agricultural practices. In food industry, for instance, biofilms formed by probiotics in the gut epithelial mucosal surface have shown advantages and health benefits. Biofilms formed by probiotic bacteria such as *Lactotoccus reuteri* lead to the accumulation of bacteriocins which provides protection against foodborne pathogens [26,27]. In the agriculture field, biofilm-forming microbes have been shown to contribute to crop yield in multifaceted ways, including the promotion of plant growth and protection from abiotic disorders (dissection or high salinity) and microbial pathogens [28–30]. Root-colonizing bacteria, such as *Azotobacter* spp*., Azospirillum* spp*., Bacillus* spp*., Beijerinckia* spp*., Pseudomonas* spp*., Rhizobium, and Bradyrhizobium* spp. are known to enhance the growth of plants by improving the availability of phosphorous, potassium and zinc, fixing atmospheric di-nitrogen, or triggering the production of hormones such as auxins, gibberellins, and cytokinins [31]. Effective colonization and the establishment of biofilms on diverse plant organs create protective microbial barriers that reduce the growth of pathogens by limiting the availability of essential nutrients and micronutrients for growth and pathogenicity or by producing a variety of antimicrobials (i.e., 2, 4-diacetylphloroglucinol, cyclic lipopeptides, tropolone, pyrrolnitrin, pyoluteorin, phenazine, zwittermicin A, xanthobaccin, oligomycin A, or kanosamine) to effectively eradicate or reduce the population density of pathogenic competitors [32–38]. Novel strategies in biocontrol are using nanoparticle-entrapped biofilms to fight against bacterial and fungal pathogens [39]. This methodology has shown promising results as those observed by the combination of ZnO nanoparticles and *P. chlororaphis* O6 inhibiting *Fusarium* growth [40], and the combination of nano-silica and *Pseudomonas* sp. enhancing the biocontrol activity against maize pathogens [41].

A benefit of bacterial biofilms to agriculture is their use in soil bioremediation [42]. It has been demonstrated that cyanobacteria are able to accumulate high concentrations of toxic compounds such as insecticides or heavy metals that persist in crop soils after their application. This ability can be used for the immobilization of cyanobacteria forming biofilms on alginate and silica gel, thus increasing their resistance to toxic compounds and a series of procedural advantages such as a less water requirement for cultivation and easy harvesting [43]. *Pseudomonas putida* and other bacterial species are very interesting from the environmental and industrial points of view due to their remarkable ability to tolerate high concentration of toxic compounds and to degrade pollutants as xenobiotics, an effect that can be increased when bacterial cells are forming biofilms [44–47]. Accumulation of toxic compounds supposes a substantial threat to public health and the environment and the above-mentioned species are attracting attention as very promising microorganisms to be implemented in the bioremediation of contaminated soils and waters [48]. Finally, complex multispecies biofilms are of great interest for biotechnological applications as many possible combinations of diverse microbes can improve bioprocesses through cooperative methods, including metabolic cross-talk and sharing of resources. Examples are the degradation of crude oil [49] and the desulphurization of dibenzothiophene (DBT) to form sulphur-free 2-hydroxybiphenyl [50].

During biofilm lifestyle, bacterial cells also produce a wide array of secondary metabolites (lipopeptides, bacteriocins, antibiotics, amino acids, toxins, etc) which play critical roles in the ecology of bacterial communities, either as inhibitors of competitors growth or acting as signal molecules that modulate microbial interspecies or interkingdom communication and behaviour [51–53]. The diversity of secondary metabolites and their functionalities are reasons for their enormous biotechnological interest in a variety of fields of industry (medical, food industry or agricultural). Deeper review of the utilization of secondary metabolites as antimicrobials in medicine can be found in these works [54–56]. Different than antimicrobial therapies, secondary metabolites produced by microbial species (daunomycin, mitomycin C, adriamycin, etc.) are also applied in the treatment of different types of cancer [57], as anti-malarial compounds (gliotoxin) [58] or antiplasmodials (trichodermol) [59]. In addition, it should be specially mentioned how diverse bacterial secondary metabolites are contributing to plant health and crop yield by targeting microbial pathogens: i) Lipopeptides not only inhibit fungal and bacterial pathogen growth, but also trigger the plant immunity system or promote plant growth [55,60]; ii) small molecules as bacillaene, difficidin and macrolactin, produced by *B. amyloliquefaciens,* inhibit Gram-positive and Gram-negative pathogens such as *Erwinia amylovora* [61]. Food and flavour industries are also prominent areas where secondary metabolites are gaining interest. Examples of that are 3-octanone, 1-octen-3-ol and 3-octanol produced by *Trichoderma* spp. in mushroom flavour and aroma [62], and the use of probiotics for the production of antioxidant compounds [63,64].

3. Extracellular matrix components in bacterial biofilms

Biofilms are chemically complex and diverse, which may define their extensive impact in the environment. Thus, the knowledge on the individual structural components of this bacterial megastructures are essential to potentiate the aforementioned benefits of biofilms and to discover unprecedented biotechnological uses for each component.

3.1. Exopolysaccharides (EPSs).

The extracellular matrix of bacterial biofilms is commonly composed of proteins, exopolysaccharides, nucleic acids, lipids and other minor biomolecules such as secondary metabolites. EPSs are probably the most abundant component of the ECM and are considered important elements related to the virulence of bacterial pathogens or for bacterial protection [12,65]. The formation of biofilms is the result of a highly coordinated developmental programme; thus, different structural and regulatory elements are spatially and temporally expressed. Studies with diverse bacterial species have shown the importance of EPS in different stages of biofilm development, from the initial cellular adhesion to surfaces to the formation of complex structures and the final dispersion of the biofilm. Thus, it is not surprising that EPSs are important contributors to the architecture of biofilms composed of largely diverse bacterial species, as seen in *E. coli*, *S. mutans, Vibrio, B. subtilis* and *Pseudomonas*, among others [65]. In addition to this well-known structural function, the physicochemical properties of EPSs provide biofilms with a very effective impenetrable barrier that prevents or delays the entrance of antimicrobials into the biofilm, which gives ample time to initiate the expression of resistance genes by individual cells [66,67].

The staphylococcal polysaccharide intercellular adhesion (PIA) and PIA-related polymers in *Staphylococcus* and other Gram-negative species, cellulose in *Pseudomonas*, *Vibrio* and *Salmonella*, or alginate, Psl, Pel in *Pseudomonas* are common bacterial EPS that can be found in different bacteria [68,69]. The production of several EPSs with different compositions by the same strain, as exemplified by *P. aeruginosa*, allows for the adaptation to environmental changes, for the colonization of diverse niches, or for the fight against other microorganisms. *P. aeruginosa* isolated from cystic fibrosis patients overproduces alginate, leading to mucoid colonies; however, this EPS is not essential for biofilm formation

and mainly relies on the production of Pel and Psl in laboratory strains [70,71]. The EPS produced by *S. mutants* is one of the main contributors to the virulence of these strains and the formation of dental biofilms. The EPSs produced by glucosyltransferases encoded by *gtfB*, *gtfC* and *gtfD* and fructosyltransferases (Ftfs) leads to the synthesis of a mixture of different types of soluble and insoluble glucans and fructans thus promoting the local colonization of microorganisms on the teeth while forming a protective extracellular matrix [72–74]. The EPS produced by *B. subtilis* is encoded by the epsO-A operon which is mainly regulated by *sinI-sinR*, and is mainly composed of glucose, galactose, and N-acetylgalactosamine. At the genomic level, EpsA and EpsB constitute a sensor tyrosine kinase system, and they participate in the activation of other protein targets important for EPS synthesis, such as the glycosyltransferase EpsE [75]. EpsE, located in the cell membrane, seems to be involved in the inhibition of flagellar rotation through a clutch-like mechanism [76]. EpsC, EpsM and EpsN have been implicated in the synthesis of N,N'-diacetylbacillosamine, while EpsHIJK are involved in the synthesis of poly-N-acetylglucosamine [77]. Together, the EPSs of *B. subtilis* are directly involved in the formation of the wrinkles typical of *in vitro* biofilms formed by this strain [78] and are also involved in population motility [79]. In closely related *B. cereus,* different *eps* regions are found, and these regions appear to have complementary or specific roles. In the *B. cereus* type strain ATCC14567, EPS1 is orthologous to the EPS of *B. subtilis* and is more relevant in terms of social bacterial motility; however, EPS2 is involved in bacterial adhesion to surfaces, cell-to-cell interaction, cellular aggregation and biofilm formation [80]. The diversity of EPSs is interpreted as bacterial ecological adaptations to the different niches in which each strain is found during the different stages of the bacterial life cycle. Some of the most studied EPSs in *Bacillus* strains are levan type I and II, which consist of β-2,6-linked D-fructose units and a fructose polymer with a glucose residue linked to the terminal fructose by a α-glycoside bond, respectively, and they can be synthesized outside the cell following the extrusion of the extracellular enzyme levansucrase [81,82]. For example, these levan-based EPSs participate in the assembly of a levan capsule in *Pseudomonas* that protects cells against attack from bacteriophages or avoids desiccation of the cells and also as a polysaccharide that helps the aggregation of *Paenibacillus* on root-adhering soil on wheat plants [83].

In recent decades, the physicochemical characteristics and variability in the structure of EPSs have received special attention for their industrial and medical applications (Table 1). Industrial use of EPSs can be found in many different areas such as the food industry, agriculture, and cosmetics. Examples of the most relevant EPSs routinely considered in the food industry are chitosan, xanthan gum, kefiran and inulin. These polymers are used as viscosity-increasing agents, gelling agents, stabilizers in multiphase solutions, additionally to their use as lubricants, flocculants, or flavour enhancers contributing to increase food quality [84–86]. Gelrite and xanthan gum are used in agriculture as sprays and pesticides, in the biodegradation of gasoline and in the transportation of gel-encapsulated bacteria for bioaugmentation of contaminated aquifers [86,87]. In addition, EPSs are also interesting in the agricultural field given that they can potentiate beneficial effects such as the increase in root water retention, the improvement of salt tolerance or the avoidance of the toxicity provoked by flocculants derived from aluminium, polyacrylamide derivatives and polyethyleneimine used in water treatment plants [88]. In the cosmetics industry, EPSs such as xanthan gum, pullulan and kelcogels are also used as rheological stabilizers and fragrance carriers.

In addition to the above-mentioned fields, EPSs are also exploited for medical and pharmaceutical applications. The physiochemical properties of EPSs as xanthan, pullulan, dextran, hyaluronan, levan, alginate, cellulose and gellan (Table 1), in addition to chemical modifications such as acetylation or oxidation, have contributed to extend their utility [86]. For instance, alginate is used in the formation of nanoparticles for controlled drug release and it is also used as adjuvant for vaccines; pullulan, dextran, or bacterial cellulose are employed for the development of new micelle systems that can improve drug solubility and stability; and bacterial cellulose is also applied in the field of wound healing due to its permeability [86,89,90].

3.2. Proteins

In addition to the EPS, proteins are also important components of the ECM with very interesting roles and prospective biotechnological applications. Proteins in biofilms can be classified roughly into two main groups based on functionality: adhesins and functional amyloids. Adhesins are proteins that can be found in gram-negative and gram-positive species [91–93]. These proteins are cell-surface exposed proteins that promote cell-to-cell contacts within a biofilm or adhesion of bacterial cells to biotic or abiotic surfaces. Examples of adhesins are the biofilm-associated proteins Bap and SasG and fibronectin binding proteins EnBPA and EnBPB of *S. aureus* [94,95]; adhesin p1 of *Streptococcus mutans* [96], and members of the antigen-43 family of autotransporter adhesins in *E. coli*, such as TibA, and the autotransporter AIDA-I, are involved in the adherence of *E. coli* to human cells [97–99]. The adhesion proteins LapA and LapF and the recently discovered MapA in *Pseudomonas* strains have also been described as key elements in the colonization of surfaces, seed adhesion and biofilm development and maturation [91,100,101].

The other main group of proteinaceous components of biofilms is functional amyloids. These proteins, synthesized as monomers, progress timely into aggregates to finally render insoluble fibers with a common quaternary structure characterized by a cross-β pattern, in which hydrogen-bonded β-strands run perpendicularly to the axis of the fibril [102]. Amyloids were initially associated to diverse human disorders (Alzheimer, Parkinson and Huntington, among others) [103], however, amyloid fibres were later found in bacteria associated with the ECM of both gram-positive and gram-negative bacterial species. Functions of amyloids in microbes include the involvement in adhesion and biofilm formation, spore coating and protection, or in the dissemination of virulence factors and evasion of the host immune system, among others [104–106]. Examples of these proteins are the curli amyloid fibres of *E. coli* and *Salmonella* spp. [107], the Fap amyloid fibrils found in *Pseudomonas* spp. Biofilms [108], TasA of *B. subtilis* and *B. cereus* [109,110] and the harpins found in gram-negative pathogenic strains of *Erwinia amylovora* and *P. syringae,* among others [111–113]. Along with the curli fibres of *E. coli*, one of the best characterized functional amyloids is TasA produced by *B. subtilis* [114–117]. TasA is encoded by the *tapA-sipW-tasA* operon and needs the activity of TapA and SipW for correct fibre assembly in vivo. In fact, SipW is a bifunctional signal peptidase in charge of processing and translocating TasA and TapA to the exterior of the cell, and in addition, SipW seems to act as a regulatory element in the expression of the *tapA* and *eps* operons [118]. In addition, TapA is required for biofilm formation and polymerization of TasA fibres *in vivo* [115]. However, as exemplified in other amyloids, TasA has the intrinsic ability to form amyloid fibres *in vitro* in the absence of TapA, which also preserves the structural peculiarities of amyloid proteins but fails to form structurally defined fibres [110].

Although not included in the two main groups of proteins described above, BslA is another protein component of the ECM of *B. subtilis* with the outstanding ability to self-polymerize into a structural polymer. This protein is a cell surface-associated amphiphilic protein that forms a protective hydrophobic coat on the surface of the biofilm to prevent the penetration of hydrophobic

Table 1
Properties and applications of exopolysaccharides.

	Properties	Applications	Refs.
Exopolysaccharides			
Xanthan	Coating	Foods	[167,168]
	Emulsifying properties	Petroleum industry	
	Thickening agent	Pharmaceuticals	
	High viscosity at low shear rates	Cosmetics and personal care products	
	Freeze-thaw stability	Agriculture	
	Odorless		
Gellan	Hydrocolloid	Foods	[168,169]
	Stability over wide pH range	Pet food	
	Gelling capacity	Pharmaceuticals	
	Thermoreversible gels	Research: agar substitute and gel electrophoresis	
	Adhesive		
	Versatile texture		
	High clarity		
	Dispersibility		
	Biocompatibility		
Alginate	Hydrocolloid	Foods	[169,170]
	Gelling capacity	Medicine	
	Film-forming	- Surgical dressings	
	Stabilizer	- Wound management	
	Thickening agent	- Controlled drug release	
	Foam stabilizer		
Cellulose	High crystallinityInsolubility in most solvents	Foods	[171,172]
	High tensile strength	Biomedical	
	Moldability	- Wound healing	
		- Tissue engineered blood vessels	
Dextran	Non-ionic	Foods	[173]
	Good stability	Pharmaceutical industry	
	Newtonian fluid behavior	Chromatographic media	
	Improve moisture retention and viscosity		
	Low solubility in water		
	Antigenic properties		
Levan	Low viscosity	Food (prebiotic)	[174,175]
	High water solubility	Feed	
	Anti-tumor activity	Medicines	
	Anti-inflammatory	Cosmetics	
	Adhesive strength	Industry	
	Film-forming capacity		
	Biocompatibility		
Proteins			
Amyloids	Polymerization	Building blocks for nanostructures, nanosensors and nanotubes.	[131,132,135]
	Adhesive	Amyloid-based gels in cell adhesion and wound healing	
	Bioflocculants	Drug delivery systems	
BslA	Hydrophobicity	Food and cosmetic industries as stabilizers	[124]
Extracellular DNA			
eDNA	Easy digestion	Biomedicine	[157,159,166]
	Negative charge	Forensic (eukaryotic eDNA)	

fluids, and its deletion alters the microstructure of colonies [119,120]. BslA functions in cooperation with other ECM components as TasA/TapA and EPS to ease the maturation of the biofilm [99,120]. Hydrophobin proteins are typically found in fungi, where they assemble spontaneously into amphipathic monolayers at hydrophobic–hydrophilic interfaces. The surfactant and amphipathic nature of the hydrophobin layers helps in the formation of essential aerial structures of filamentous fungi, such as hyphae, fruiting bodies, and spores [121,122], in interactions with the environment and in protection against the host defence system [123]. Hydrophobins, such as the previously mentioned BslA, might also have applications in the food or cosmetic industries as stabilizers. The use of BslA in the production process of ice creams is a good example because the combination of the protein with air, fat and water yields a stable mixture permitting the ice cream to stay frozen for longer periods of time and retarding the growth of ice crystals [124].

In addition to the critical contribution of amyloid proteins to the progress towards the different stages of the biofilm life cycle, complementary roles in bacterial growth and survival, detoxifica-

tion of toxic compounds, resistance to antibiotics and even electron transport have been discovered [125,126]. Furthermore, the interesting biophysical properties of amyloid proteins, exemplified by their outstanding resistance to chemical and thermal denaturants or pH changes [127–129], are promoting studies on their use and implementation in a variety of biotechnological processes. In this sense, bacterial amyloids are attracting greater interest as potential natural building blocks for the design of new nanostructures and nanomaterials: nanowires and nanotubes for electronics, nanosensors, amyloid-based gels for cell adhesion and wound healing, or as drug delivery systems [130–132]. Specifically, studies performed with *E. coli* have permitted the development of a system to fabricate multiscale patterning fibres as versatile scaffolds able to synthetize fluorescent quantum dots, gold nanowires and nanoparticles [133]. A biofilm-integrated nanofiber display (BIND) system has served as the base for the exploitation of curli fibres to create a biocatalytic biofilm in which functional nanofibers immobilized the industrially relevant enzyme α-amylase [134]. Amyloids can also be used as bioflocculants in microalgae cultivation and are becoming popular as a preferred biomass for biofuel pro-

duction, promoting the use of cost- and energy-efficient technologies [135]. Because of their variability, functional amyloid proteins with their different biophysical properties are promising sources for the development of potential biotechnological tools and use in technological applications (Table 1).

3.3. Extracellular DNA.

The last main component of the bacterial extracellular matrix is extracellular genomic DNA (eDNA). Initially, it was assumed that eDNA was derived from lysed cells and that mere remnants of eDNA were present and had no relevance on biofilm structure [136,137]. However, this concept was rebutted by many studies showing species-specific amounts of eDNA in different single and multispecies biofilms and showing organized patterns forming grid-like structures of filamentous networks [138–142]. eDNA can be released from lysed cells by mechanisms analogous to holin-antiholin systems, as in *S. aureus* [143], but the possibility of secretion by different eDNA secretion mechanisms has also been described, such as secretion by outer membrane vesicles in *P. aeruginosa*, *S. aureus*, *B. subtilis*, *Klebsiella pneumoniae*, *S. epidermidis,* and *V. cholerae*. The addition of DNase to growing or mature biofilms resulted in the inhibition of biofilm formation or disruption of established biofilms, leading to the establishment of a direct relation between the age of the biofilm and its disruption (young biofilms were more sensitive to DNase than older biofilms) [144,145]. Currently, it is accepted that eDNA is a structural component of the ECM that provides structural stability to bacterial biofilms by interacting with other ECM components, such as exopolysaccharides and proteins, modulating cell surface properties and promoting cell-to-cell and cell-to-surface adhesion [146–148]. For example, eDNA in *P. aeruginosa* has been described to physically interact with the exopolysaccharide Psl, forming fibres facilitating bacterial adhesion and growth [149], while in *B. subtilis,* eDNA appears to interact with EPS in the early phases of biofilm development [150]. Several studies have also demonstrated the ability of eDNA to act as a chelator of cationic antimicrobials and explained their role in increasing resistance against antibiotics [139,151,152]. In addition to eDNA-polysaccharide interactions, eDNA has also been shown to attract and bind amyloid proteins, causing the polymerisation of the matrix and stimulating autoimmunity [153,154].

eDNA is rarely used as a biotechnological resource (Table 1); however, there are many applications where this component of the ECM has attracted attention. eDNA is mostly fragmented, which suggests an interesting way to fine tune the attachment of bacterial cells to surfaces, as experimentally reported for *Listeria monocytogenes* [155]. Therefore, eDNA seems to work as a unique element for the control of biofilm attachment and structural stability, and modification of the release of this molecule may be used to alter the mechanical properties of biofilms [140]. Another application is related to the increased resistance of biofilms to antibiotics. The negative charge of eDNA contributes to the creation of a shield that protects biofilm-associated cells from aminoglycosides and cationic antimicrobial peptides that are positively charged by its binding, thus avoiding the penetration of these peptides inside the bacterial cells [156].

Considering its relevance for biofilm formation, eDNA can also be used as a target in the search for antibiofilm agents [157–159]. Primarily, the use of DNase to digest eDNA leads to biofilm disruption, but the use of antibodies against DNA-binding proteins located at the intersection of crossed eDNA strands has also been investigated [160]. Continuing in this therapeutic direction, nanomaterial-cleaving eDNA in *S. aureus* biofilms has been proposed as a promising treatment against biofilm-related infections [161].

Very interestingly, although not bacterial, eukaryotic eDNA seems to be useful for medical diagnostics, given the correlation of eDNA concentration with different pathologies, including cancer and autoimmune disorders [162–164]. In fact, eukaryotic eDNA is used during pregnancy, as eDNA from foetal cells circulates in the maternal blood allowing the detection of foetal genetic disorders [165]. Finally, the application of eDNA in forensics is also interesting, as it can be located and quantified on human epithelial cells or other surfaces, providing a new tool in forensic analysis of touch samples [159,166].

4. Future perspectives and conclusions

The ECM is a complex structure that is chemically and functionally diverse. Mechanistic studies are providing increased knowledge on the chemical structure and biophysical peculiarities of the molecules that compose this structure. Additional studies on the features defining the stages of biofilm development are necessary to generate a solid body of knowledge that will enable further manipulation or even specific design of polymers for current or new biotechnological applications. The application of the bacterial biofilms or the different components of the extracellular matrix in industrial and agricultural processes is arising as a very promising strategy, both from economic and ecologic point of views, reducing production costs while increasing productivity, fighting against pollutants and serving as an ecological tool against agricultural plagues. Major challenges rely on the ability to scale the production of such biotechnological products at industrial level and thus, their commercial applications due to the high cost of the industrial processes and the low yield that is currently obtained from their production. Studies on the fine genetic regulation of the production of ECM components, the isolation of new producer strains and the utilization of the most adequate substrates should serve to select the more suitable natural microorganisms, especially when genetic manipulation is not permitted or affordable. The unspecific interaction of certain ECM with themselves or medium components impose limitations to further downstream processes. Thus, specific studies on the physicochemical singularities of each ECM component are a matter of interest to define the best ways to improve extraction and purification methods and how to proceed in the bioreactor, improving not only yield but also the quality and utility of the bioproduct.

CRediT authorship contribution statement

Carlos Molina-Santiago: Writing - original draft. **Antonio Vicente:** Writing - original draft. **Diego Romero:** Conceptualization, Writing - original draft, Writing - review & editing, Funding acquisition.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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