Prokaryotic Amyloids in Interspecies Interactions

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Amyloids are fibrillar protein aggregates with an ordered spatial structure called “cross-β”. While some amyloids are associated with development of approximately 50 incurable diseases of humans and animals, the others perform various crucial physiological functions. The greatest diversity of amyloids functions is identified within prokaryotic species where they, being the components of the biofilm matrix, function as adhesins, regulate the activity of toxins and virulence factors, and compose extracellular protein layers. Amyloid state is widely used by different pathogenic bacterial species in their interactions with eukaryotic organisms. These amyloids, being functional for bacteria that produce them, are associated with various bacterial infections in humans and animals. Thus, the repertoire of the disease-associated amyloids includes not only dozens of pathological amyloids of mammalian origin but also numerous microbial amyloids. Although the ability of symbiotic microorganisms to produce amyloids has recently been demonstrated, functional roles of prokaryotic amyloids in host–symbiont interactions as well as in the interspecies interactions within the prokaryotic communities remain poorly studied. Here, we summarize the current findings in the field of prokaryotic amyloids, classify different interspecies interactions where these amyloids are involved, and hypothesize about their real occurrence in nature as well as their roles in pathogenesis and symbiosis.

Keywords: amyloid ; bacteria ; interspecies interactions ; host–pathogen ; host–symbiont ; microbial community ; outer membrane protein ; biofilm ; Omp ; toxin

1. Introduction

The term “amyloid” dates back to the previous century. In 1838, Matthias Schleiden introduced “amyloid” (from Latin “amylum”—starch) to describe a starch material in plant cells [1]. In 1854, Rudolf Virchow first used “amyloid” to characterize cerebral inclusions that colored blue in a reaction with iodine [2]. Based on the iodine test reaction, Virchow hypothesized about polysaccharide nature of pathological inclusions in so-called “waxy” human organs that underwent irreversible changes called amyloidosis [1]. Five years later, in 1859, August Kekulé and Carl Friedreich showed that the inclusions in “waxy” spleen were enriched in nitrogen and were rather proteinaceous than starchy [3].

Currently, the term “amyloid” refers to the highly ordered protein aggregates formed by unbranched fibrils the protein monomers of which are stacked by intermolecular β-sheets [4] composed of β-strands running perpendicular to the fibril axis and connected via hydrogen bonds [5]. The spatial organization of amyloid fibrils determines “cross-β” diffraction pattern characterized by two scattering diffraction signals: ~4.7 Å, corresponding to the interstrand distance, and ~10 Å, corresponding to the distance between β-sheets [6][7]. This structure is typical of amyloids, yet there is no rule without exception: phenol-soluble modulin α3 (PSMα3), a secreted protein of *Staphylococcus aureus*, forms fibrils possessing physicochemical properties of amyloids but shows “cross-α” diffraction pattern that corresponds to perpendicularly stacked α-helices rather than to β-sheets in “cross-β” structure [8].

Unique physicochemical properties of amyloid fibrils allow discriminating amyloids from the non-amyloid protein aggregates. Two defining features of amyloids are: (i) their ability to form mainly unbranched fibrils that can be shown with electron or atomic force microscopy [9][10]; and (ii) their “cross-β” structure, which can be directly demonstrated by X-ray diffraction (XRD) [6] or solid state nuclear magnetic resonance (SS-NMR) [11]. Circular dichroism spectroscopy (CD), often used to evaluate the enrichment of protein aggregates in β-sheets, cannot prove their “cross-β” structure [12]. Amyloids are resistant to treatment with ionic detergents such as sodium dodecyl sulfate (SDS) [13] and proteases [14], although these properties vary significantly depending on the amyloids themselves and the proteins from which they are formed [15][16]. Amyloids also bind specific dyes [17], which was demonstrated for the first time in 1922 with Congo red (CR) dye [18]. Further, amyloids have been shown to exhibit “apple-green” birefringence in polarized light upon CR binding [19] and demonstrate specific fluorescence emission spectra [20]. Another widely used dye for the analysis of amyloids is Thioflavin T (ThT), which binding to amyloids leads to an increased fluorescence intensity [21].

For more than 150 years, amyloid studies were mainly focused on their roles in the development of incurable diseases in humans and animals, such as Alzheimer’s disease and various localized and systemic amyloidoses. Despite extensive study, the molecular mechanisms of amyloid toxicity remain unclear. To date, two dominant hypotheses have been proposed [22]. Amyloid hypothesis suggests that deposition of amyloid fibrils themselves causes toxicity leading to cell death [23]. The second hypothesis postulates that soluble oligomers rather than fibrillar polymers cause the main cytotoxic effect [24]. Both hypotheses are unable to fully explain observed data. Thus, there are new currently emerging hypotheses.

In particular, lipid-chaperone hypothesis is based on the observation that toxicity of amyloidogenic proteins is associated with their ability to damage cell membranes and suggests that phospholipids can act as molecular chaperones promoting interaction of amyloidogenic proteins with cell membranes [22][25].

The paradigm describing amyloid as harmful agents shifted with the discovery of functional amyloids that can perform various physiological roles [26][27]. To date, functional amyloids have been identified within all three domains of life, *Archaea*, *Bacteria*, and *Eukarya*, and their current number is approximately equal or even exceeds the number of pathogenic ones [27][28]. The greatest diversity of functional amyloids is identified within prokaryotic organisms. Since the discovery of amyloid properties of the curli fimbriae of *Escherichia coli* [29], more than 30 amyloidogenic proteins of *Bacteria* and *Archaea* have been identified. Part of them form amyloid fibrils under the native conditions and act as functional amyloids involved in the interspecies interactions with their hosts or within microbial communities. The variety of the prokaryotic amyloid-forming proteins, the methods that have been used to analyze their amyloid properties in vitro and in vivo, and their functions in the amyloid and soluble states are summarized in Table 1. In this paper, we review the published scientific data on the diversity of prokaryotic amyloids and discuss their biological functions with regard to their role in different types of interspecies interactions in both pathogenic and symbiotic aspects.

Table 1. Amyloidogenic proteins of prokaryotes, their properties, functions, and involvement in the interspecies interactions.

Species	**Protein**	**Function of Soluble Protein**	**Function of Amyloid**	**Amyloid Properties ***		**Type of Inter-Species Interactions Mediated by Amyloid ****	**Referer**
				In Vitro	**In Vivo**		
Domain: *Bacteria* Phylum: *Proteobacteria*							
Escherichia coli, Salmonella enterica	CsgA (curli), AgfA (tafi)	No data	Biofilm matrix protein; surface adhesion; intercellular adhesion	CR (Congo red) absorbance, ThT (Thioflavin T) fluorescence, CD (Circular dichroism), FTIR (Fourier-transform infrared spectroscopy), XDR (X-ray diffraction)	Extracellular fibrils formation	I	[29][30][31
Pseudomonas aeruginosa, P. fluorescens, P. putida	FapC	No data	Biofilm matrix protein; facilitates mechanical stiffness; enhances hydrophobic properties; binds quorum-sensing signal molecules	TEM (Transmission electron microscopy), FTIR, XDR	Extracellular fibrils formation; purified native fibrils: CD, FTIR, ThT fluorescence	I	[33][34][35

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Legionella pneumophila	Not identified	No data	Biofilm matrix protein	No data	ThT fluorescence, CR staining and WO1 antibodies binding of extracellular polymer matrix of biofilm	I	[36]
Escherichia coli	OmpA	Outer membrane porin	Virulence factor; amyloid function is unknown	ThT fluorescence, TEM, CD (for N-terminal domain)	No data	I ***	[37]
Escherichia coli	OmpC	Outer membrane porin	Virulence factor; amyloid function is unknown	Proteinase K resistance, TEM, ThT fluorescence, CR absorbance and birefringence	No data	I ***	[38]
Mannheimia haemolytica	OmpP2-like protein	Outer membrane porin	Biofilm matrix protein; adhesion to host's tissues	CR binding	Fibrils on the cell surface, binding anti-OmpP2-like protein antibodies	I	[39]
Rhizobium leguminosarum	RopA	Outer membrane porin	Component of extracellular capsule	CD, CR birefringence, ThT fluorescence, TEM, detergent-resistance, trypsin resistance	SDS (Sodium dodecyl sulfate)-resistant polymer formation, fibrils on the cell surface, binding anti-RopA antibodies	III ***	[40]

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Rhizobium leguminosarum	RopB	No data	Component of extracellular capsule	CD, CR birefringence, ThT fluorescence, TEM, detergent-resistance, trypsin and pepsin resistance	SDS-resistant polymer formation, fibrils on the cell surface, binding anti-RopB antibodies	III ***	[40]
Klebsiella pneumoniae	Microcin E492	Pore-forming toxin	Toxin inactivation	TEM, CD, ThT fluorescence, CR absorbance, proteinase K resistance, XDR	Fibril formation on the surface of Microcin E492 secreting strain (TEM)	II	[41][42]
Xanthomonas axonopodis	HpaG (harpin)	No data	Virulence factor; induces plant hypersensitive response	TEM, CD, CR absorbance and birefringence, proteinase K resistance	No data	I ***	[43]
Pseudomonas syringae	HrpZ (harpin)	No data	Virulence factor; induces plant hypersensitive response	TEM	No data	I ***	[43]
Erwinia amylovora	HrpN (harpin)	No data	Virulence factor; induces plant hypersensitive response	TEM	No data	I ***	[43]
Gallibacterium anatis	EF-Tu	Elongation factor	Biofilm matrix protein; surface adhesion	TEM	TEM, CR binding, antibodies against curli	N/a	[44]
Phylum: *Firmicutes*							

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Bacillus subtilis, Bacillus cereus	TasA	No data	Biofilm matrix protein; facilitates biofilm integrity; binds exopolysaccharides on the initial steps of multispecies biofilm formation	TEM, CD, NMR (Nuclear magnetic resonance), FTIR	Anti-TasA antibodies binding extracellular fibrils in biofilm matrix; native fibrils: TEM, CR absorbance, ThT fluorescence	I, II	[45][46][47
Staphylococcus aureus	PSMs	No data	Biofilm matrix protein	TEM, ThT fluorescence, NMR (cross-α structure)	Extracellular fibrils in biofilm matrix while *Δabpsm* mutant were unable to form fibrils	I	[8][48]
Staphylococcus aureus	SuhB	No data	Biofilm matrix protein; intercellular adhesion	CR absorbance, ThT fluorescence, FTIR, SEM, XDR	No data	I ***	[49]
Staphylococcus aureus	AgrD	Propeptide; autoinducing peptide pheromone (AIP) precursor	*N*-terminal peptide, cleaved during AIP maturation, forms amyloid; biofilm matrix component	*N*-terminal domain: ThT fluorescence, TEM, CR absorbance, CD	Fibrils formed by *N*-terminal domain of AgrD in biofilm matrix	I	[50]
Staphylococcus aureus	Bap	No data	Surface adhesion; intercellular adhesion; promotes biofilm formation in acidic conditions	Bap B-domain: ThT fluorescence, CR absorbance, TEM, FTIR, CD	Anti-Bap antibodies binding fibrils formation on the cell surface	I	[51]
Enterococcus faecalis	cOB1	Pheromone; part of the pheromone-based conjugation system	Prevention of conjugation; initiate the aggregation of biofilm matrix proteins (such as Esp)	ThT fluorescence, CR absorbance, CD, TEM	No data	II ***	[52]

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Enterococcus faecalis	Esp	No data	Biofilm matrix protein	C-DAG assay: CR binding, TEM; *N*-terminal domain: SEM (Scanning electron microscopy), FTIR, CD, CR absorbance, ThT fluorescence; Fibril formation on the surface of *Δbap S. aureus* expressing Esp_N	No data	I ***	[53]
Staphylococcus epidermidis	Aap	Intercellular adhesion	Biofilm matrix protein	ThT fluorescence, CR absorbance, TEM, CD	SDS-resistant aggregates, binding anti-Aap antibodies, were extracted from biofilm-forming bacteria	I	[54]
Staphylococcus epidermidis	Sbp	No data	Scaffolding protein in biofilms	TEM, AFM, FTIR, CR absorbance, ThT fluoerescence	ThS-binding inclusions, expressing Sbp	I	[55]
Streptococcus mutans	Adhesin P1	No data	Biofilm matrix protein; adhesion to tooth surface	CR birefringence, ThT fluorescence, TEM, XDR	No data	I ***	[56]
Streptococcus mutans	WapA	No data	Biofilm matrix protein	CR birefringence, ThT fluorescence, TEM, XDR	No data	I ***	[57][58]

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Streptococcus mutans	Smu_63c	No data	Biofilm matrix protein	CR birefringence, ThT fluorescence, TEM, XDR	No data	I ***	[57][58]
Bacillus subtilis	HelD	Helicase	Amyloid function is unknown	CD, ThT fluorescence, CR absorbance, XDR	ThS-binding inclusions in strain, overexpressing HelD	N/a	[59]
Clostridium botulinum	Rho****	Transcription terminator	Modulates transcription; causes genome-wide changes in transcriptome	Analysis of prion-like domain: ThT, ThS and CR fluorescence, FTIR, TEM; C-DAG (Curli-dependent amyloid generator) assay: CR birefringence, SDS-resistance	SDS-stable aggregate formation in *E. coli*	N/a	[60][61]
Solibacillus silvestris	Bioemulsifier BE-AM1	No data	Cell surface properties modulation; biofilm matrix protein	CR birefringence, FTIR, CD, TEM	No data	N/a	[62][63]
Listeria monocytogenes	Listeriolysin O	Toxin, that forms pores in phagolysosome's membrane	Toxin inactivation	CD, TEM, CR fluorescence and absorbance, ThT fluorescence, trypsin resistance	No data	I	[64]
Phylum: *Actinobacteria*							
Streptomyces coelicolor	ChpA-H (chaplin)	No data	Lowering of the surface tension; assists aerial hyphae formation	CD, TEM, XDR, FTIR	Native extracts: ThT fluorescence, TEM, CD	N/a	[65][66]

Species	Protein	Function of Soluble Protein	Function of Amyloid	Amyloid Properties *		Type of Inter-Species Interactions Mediated by Amyloid **	Referer
				In Vitro	In Vivo		
Streptomyces coelicolor	RdlB (rodlin)	No data	Rodlet layer formation; assists aerial hyphae formation	ThT fluorescence, TEM, CD, XDR	No data	N/a	[67]
Mycobacterium tuberculosis	CarD	Transcription factor	Amyloid function is unknown	ThT fluorescence, TEM, SDS-resistance, CD (increase in β-sheet content during heating)	ThS-binding (Thioflavin S) inclusions in strain, overexpressing CarD	N/a	[68]
Mycobacterium tuberculosis	MTP	No data	Adhesion to host's tissues	TEM, CR binding	TEM, SDS resistance of fibrils	I ***	[69]
Domain: *Archaea* Phylum: *Euryarchaeota*							
Haloferax volcanii	Not identified	No data	Biofilm matrix protein	No data	Fluorescence of biofilms stained with CR and ThT	N/a	[70]
Methanosaeta thermophila	MspA	No data	Tubular sheaths component; facilitates its stiffness	TEM, ThT, CD, FTIR, XDR	Intact sheaths: TEM, WO1 antibodies; purified sheaths: TEM, WO1 antibodies, ThT, FTIR, XDR	N/a	[71]
Methanospirillum hungatei	MspA	No data	Tubular sheaths component; facilitates its stiffness	No data	Intact sheaths: WO1 antibodies; purified sheaths: TEM, FTIR	N/a	[72]

* CR, Congo red; ThT, Thioflavin T; ThS, Thioflavin S; C-DAG, Curli-dependent amyloid generator; CD, Circular dichroism; FTIR, Fourier-transform infrared spectroscopy; XDR, X-ray diffraction; NMR, Nuclear magnetic resonance; SDS, Sodium dodecyl sulfate; SEM, Scanning electron microscopy; TEM, Transmission electron microscopy. ** Type of proven or hypothetical inter-species interactions: Type I, host–pathogen interactions; Type II, interactions between different microbial species in the communities; Type III, host–symbiont interactions; N/a, not applicable. *** Hypothetical interaction based on the structural protein function. **** This protein also possesses infectious prion properties [60].

2. Amyloids of Biofilms and Their Involvement in Host–Pathogen Interactions and Interspecies Interactions within Prokaryotic Communities

The highest number of the identified functional amyloids of prokaryotes is represented by the biofilm components. Biofilm is a community of microorganisms encapsulated in hydrated extracellular polymeric substances (EPS) [73]. EPS account for almost 90% of the dry weight of a biofilm and include polysaccharides, eDNA, lipids, and proteins [73]. The biofilm proteins include the extracellular enzymes, carrying out degradation and remodulation of EPS, and structural proteins, providing stability and integrity to biofilm [74]. The stability of amyloid fibrils, originating from their spatial structure, makes them perfect structural proteins of the biofilm EPS. Thus, amyloids, making the biofilms stable, serve as scaffolding proteins, as well as play a role in surface and intercellular adhesion [75]. At the same time, a biofilm formation is linked with the development of 65% of all bacterial infections and 80% of chronic bacterial infections [76] such as periodontitis, chronic rhinosinusitis, chronic otitis media, chronic urinary tract infections, and cystic fibrosis pneumonia [77]. Biofilm formation creates a local microenvironment (such as anaerobic conditions or zones with lowered pH), to protect the microbial community from the antibiotic treatment, host defense, and environmental stresses [78] and contributes to formation of so-called “persister” microbial sub-population formed by dormant, multi-drug resistant cells [79]. Thus, amyloids that have been identified within the pathogenic bacteria and being part and parcel of the biofilm matrix can act as virulence and pathogenesis factors [80].

The curli are the main structural proteins of EPS of *Escherichia coli* biofilms [29][31][81], adhering to both biotic and abiotic surfaces [82][83][84]. In 2002, amyloid properties were demonstrated for *E. coli* curlin CsgA [29] and in 2007 for *Salmonella enterica* curlin AgfA [31]. Curli amyloid formation involves secretion system Type VIII and is controlled by the expression of two operons—*csgABC* and *csgDEFG* (curli-specific genes)—in *E. coli* [29]. CsgA is the main structural protein while CsgB nucleates CsgA polymerization on the cell surface [85]. CsgC, the third gene from *csgABC* operon, is a periplasmic chaperone that prevents a premature CsgA polymerization [86]. Lipoprotein CsgG forms a pore in the outer membrane of bacterial cells and mediates the transport of the curli subunits to the cell surface [87]. CsgE and CsgF proteins facilitate CsgA and CsgB transport through CsgG pore [88]. CsgE interacts directly with the pore and secreted proteins and acts as a secretion adaptor [88]. The precise function of CsgF remains unclear, but it is required for the normal functioning of the CsgB nucleator [88][89].

Despite curli fimbriae were initially characterized within clinical isolates, the precise role of amyloid formation of those proteins in infection remained unclear [90][91]. Indeed, curli operons are not only present in the genomes of pathogenic strains of *Proteobacteria* but are also widespread within the non-pathogenic strains [92]; and curli homologs have also been found within *Firmicutes*, *Thermodesulfobacteria*, and *Bacteroidetes* phyla [92], including *Porphyromonas gingivalis* [93].

The curli fimbriae apparently take part in bacteria’s adhesion to host cells [94], interact with the host proteins [95][96], and trigger the host immune response [97] during an infection. The curli-producing *E. coli* and *Salmonella* spp. strains are highly adhesive to a variety of cell lines. Thus, curli-producing K-12 *E. coli* has demonstrated a higher level of adherence to human uroepithelial cells in comparison to curli deficient strains [94]. Similarly, higher levels of curli production in *S. typhimurium* SR-11 are linked to adherence to a murine small intestinal epithelial [98]. Nevertheless, Δ*csgA* strain of enteroaggregative *E. coli* (EAEC) has not shown any decrease in adherence to mammalian cells, suggesting that the *E. coli* system of adhesion to host cells includes not only the curli fimbriae but a broad repertoire of molecular factors [99]. Moreover, curli expression levels have been significantly lowered within the enterohemorrhagic *E. coli* [100][101] and invasive *Salmonella* spp. strains [102].

The curli interact with the host proteins including fibronectin, laminin, and plasminogen [90][103][104]. They also interact with Toll-like receptors, which leads to an innate immune system activation [105][106]. On the contrary, the curli can protect bacterial cells from the immune reactions via antimicrobial peptides sequestering [107] and inhibition of the classical pathway of the complement cascade activation [108].

The Gram-negative bacterium *Pseudomonas aeruginosa* is a cause of nosocomial and chronic infections associated with the biofilm formation, for example during cystic fibrosis pneumonia [109]. The biofilm matrix of *Pseudomonas* species includes amyloid fibrils formed by Fap proteins [33]. Amyloid fibril formation in *Pseudomonas* is controlled by a *fapABCDEF* operon, evolutionally distant from the curli system of *E. coli* [33]. Unlike the curli system, *fap* genes are unique for *Proteobacteria* species [110]. FapC is the main structural component of amyloid fibrils, whereas FapB, similar to CsgB from curlin system, acts as a nucleator of fibril polymerization [34]. Transport of FapB and FapC subunits to the cell surface is facilitated by FapF protein which forms trimer pores in the outer membrane of bacteria [111].

Fap amyloid fibrils increase the biofilm hydrophobicity, facilitate mechanical stiffness [112], and reversibly bind quorum sensing molecules, supporting their role as a reservoir for signal molecules that can modulate the reaction of the microbial community to turbulent environmental conditions [35]. Similar to curli, Fap proteins contribute to bacterial adhesion to a substrate. Thus, *Pseudomonas* strains overexpressing *fap* operon have a highly adhesive phenotype and an enhanced ability to form biofilms [33][34]. However, overexpression of *fap* operon notably changes the complete proteomic landscape, thus it is impossible to assume the direct connection between Fap amyloidogenesis and the altered phenotype [113]. The

role of Fap proteins in *Pseudomonas* virulence has been demonstrated using *P. aureginosa* mutant strain with *fapC* deletion. Strains with *fapC* deletion had lowered virulence to *Caenorhabditis elegans* [114]. In murine models of acute and chronic infections, *fap* operon transcription in *P. aureginosa* was also significantly elevated [115].

Gram-positive bacterium *Bacillus subtilis* forms biofilms on the surface of solid agar plates and floating biofilms, or pellicles, at the air–liquid interface [116]. TasA protein, the main component of *Bacillus* biofilm EPS [117], can form amyloids both in vitro and in vivo [45][46][47]. While *B. subtilis* is a soil-dwelling non-pathogenic bacterium, *Bacillus cereus* is a soil bacterium responsible for the development of food-borne disease. However, the role of biofilm formation and TasA amyloid formation in a particular disease development is unclear. At the same time, TasA amyloids of *Bacillus* apparently contribute to the interspecies interaction in complex biofilm communities as TasA amyloid fibrils adhere to *Streptococcus mutans* exopolysaccharides during the initial steps of multispecies biofilm formation [118].

Biofilms are the main form for *Streptococcus mutans*—a Gram-positive bacterium involved in the dental plaques and cavities formation [119][120]. Within the proteins of *S. mutans* amyloid formation in biofilm, EPS has been demonstrated for adhesin P1, WapA, and Smu_63c proteins [56][57]. Adhesin P1 and WapA protein represent substrates of sortase—an enzyme cleaving the C-terminal signal motif of proteins and attaching them to the cell wall through transpeptidase reaction [121]. As a result of adhesin P1 and WapA protein cleavage amyloid-forming fragments, C123 and AgA, respectively, are generated [57]. Smu_63c is a secreted protein that forms amyloids under acid conditions. These amyloids act as negative regulators of genetic competence and biofilm cell density [57]. The deletion of one of the genes encoding amyloid-forming proteins was shown not to affect the ability of *S. mutans* to form biofilms. At the same time, double (lacking in adhesin P1 and WapA) or triple deletions lead to decreased biofilm formation [57]. Mutants lacking in the adhesin P1 gene have a lowered virulence in the murine cavity models, but the precise role of adhesin P1 amyloidogenesis in virulence is still unclear [122].

Similar to *P. aeruginosa*, *Staphylococcus* species, *S. aureus* and *S. epidermidis*, are the leading causes of nosocomial infections [123]. At the same time, *S. aureus* as well as *S. epidermidis* can act not only as pathogens but as a part of the normal skin microbiome. *Staphylococcus* biofilm formation promotes adhesion and substrate colonization, including multicellular host tissues, as well as contributes to protection against antibiotic agents and immune system elements [124]. Thus, the biochemical content of *Staphylococcus* biofilms is a target of extensive research. The extracellular polymeric substances of staphylococcal biofilms include a variety of amyloid proteins, but their role in host–pathogen interactions have not yet been elucidated.

Sbp and Aap are amyloid-forming proteins of *Staphylococcus epidermidis* [54][55]. Sbp is a small (18 kDa) extracellular protein that forms the biofilm scaffolds [125]. The amyloid properties of Sbp have been demonstrated in vitro and in *E. coli* cells [55]. Aap is a multidomain protein associated with the bacterial cell wall. Aap includes the *N*-terminal region of tandem A-repeats, L-type lectin domain, the region of tandem B-repeats, the proline/glycine-enriched domain, and the C-terminal sortase recognition motif [126]. The ability to form amyloids was demonstrated in vitro for the B-repeats domain. Amyloid formation by B-repeats domain of Aap has a Zn^{2+}-dependent manner and requires metal ions for assembly. The peptides identified as B-repeats and lectin domains of Aap protein were also present in detergent-resistant aggregates from *S. epidermidis* biofilms [54]. These data are consistent with the research suggesting that Aap protein takes part in biofilm formation in a processed form, lacking the *N*-terminal domain [127][128]. Sbp and Aap colocalization in biofilms was demonstrated [125] unlike physical interaction in vitro [55].

There is a variety of the identified amyloid-forming proteins composing the extracellular biofilm matrix of *Staphylococcus aureus*. In 2012, phenol-soluble modulins (PSMs) were identified as a part of fibrils in the biofilm matrix of *S. aureus*. PSMs also form amyloid fibrils in vitro [48]. In the amyloid state, PSMs stabilize biofilms [48], whereas monomeric PSMs facilitate biofilm detachment [129]. Extracellular DNA (eDNA) is required for PSMs polymerization, so eDNA can act as a nucleator in the amyloid formation [130]. The amyloid properties have been demonstrated for the N-terminal leader peptide of ArgD propeptide (*N*-ArgD) as well. *N*-ArgD is a naturally occurring cleavage product of ArgD, appearing due to the AIP (autoinducing peptide) maturation [50] and identified as a part of fibrils, composing the biofilm matrix of *S. aureus*. The SuhB protein of *S. aureus* forms amyloids under overexpression in *E. coli* cells [49]. The precise function of SuhB remains unknown, but the *suhB* mutant strain is impaired (in terms) of biofilm formation [131]. Another *S. aureus* protein that can form amyloids extracellularly is called Bap (biofilm-associated protein) [132]. Bap is a multidomain protein anchored to the bacterial cell wall. The N-terminal domain of Bap is cleaved as a result of the Bap processing [133]. The cleaved fragment forms amyloid fibrils in the extracellular space at acidic conditions and low Ca^{2+} concentration. The Ca^{2+} concentration increase leads to acquiring a stable globular conformation of the *N*-terminal domain of Bap [51]. Thus, the *N*-terminal domain can act not only as a scaffold protein of biofilm but also as a sensor [75]. Local acidosis, the pH decrease, appears in vivo during staphylococcal infection due to glucose utilization by these microorganisms and are accompanied by the host's inflammatory response [132]. Within *S. aureus* strains, *bap* gene has been identified within bovine mastitis isolates [133] but not within human clinical isolates. Deletion in the *bap* gene leads to a lowered capacity to adhere to the bovine epithelial cells. *S. aureus* Δ*bap* strain cell titer is also significantly lower at 10 days post-infection [51]. Notably, Esp—the Bap ortholog of *Enterococcus faecalis*, a commensal bacterium capable of inducing nosocomial infection—forms amyloids, supporting the idea of the prevalence of amyloid formation by Bap-like proteins in biofilm matrix [53].

Pathogenic bacteria can also adhere to the host tissues in a biofilm-independent way. In particular, *Mycobacterium tuberculosis* possesses adhesive structures called pili. MTP (*Mycobacterium tuberculosis* pili) are structurally similar to *E. coli* curli and able to form amyloid fibrils [69]. The *mtp* gene has been identified only within the pathogenic strains of *M. tuberculosis*, supporting the key role of MTP in mycobacterial virulence [134]. MTPs bind laminin in vitro while Δ*mtp* strain is unable to bind it [69]. Moreover, mutants show a lowered ability to adhere and invade macrophages and alveolar epithelial cells [135].

Overall, amyloids are widespread structural components of prokaryotic biofilms. Interestingly, not only bacteria but also archaea can contain amyloids in their EPS. For instance, in 2014, the *Haloferax volcanii* biofilm extracellular matrix was demonstrated to bind ThT and CR dyes with the specific fluorescence [70]. In bacterial biofilms, the amyloids form a scaffold and facilitate their stiffness and integrity. Amyloids may also contribute to intercellular and surface adhesion, which makes them one of the key virulence factors of pathogenic bacteria. Thus, the crucial role of amyloids of biofilms in adhesion is apparently widespread across various prokaryotes, thus allowing us to suppose that there are numerous still unknown biofilm-associated amyloids underlying the pathogenesis and development of infectious diseases. Considering that the number of only human pathogenic bacteria species is about 1500 [136] and 65% of them form biofilms in disease-associated processes [55], the real number of such prokaryotic amyloids involved in pathogenesis in humans and animals could exceed hundreds and even thousands. The interactions between bacteria in microbial communities represent another type of interspecies interactions where the bacterial biofilm amyloids are involved by providing the cell adhesion to heterogeneous exopolysaccharides and where the number of yet unidentified prokaryotic amyloids could be remarkably high.

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