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Pseudomonas aeruginosa and Its 4 Arsenal of Proteases: Weapons 5 to Battle the Host

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Pseudomonas aeruginosa and Its Arsenal of Proteases: Weapons to Battle the Host

Anna Clara M. Galdino, Marta H. Branquinha, André L.S. Santos and Lívia Viganor

Abstract

Pseudomonas aeruginosa is a ubiquitous and opportunistic human pathogen that represents a critical problem to the clinician due to the increased number of resistant strains isolated from hospital settings. In addition, there is a great variety of pathologies associated with this versatile Gram-negative bacterium. *P. aeruginosa* cells are able to produce an incredible arsenal of virulence factors, especially secreted molecules that act singly or together to ensure the establishment, maintenance, and persistence of a successful infection in susceptible hosts. In this context, pseudomonal proteases roles are highlighted due to their ability to cleave key host proteinaceous substrates as well as to modulate several biological processes, for example, escaping and modulating the host immune responses in the bacterial own favor. Proteases secreted by *P. aeruginosa* include elastase A (LasA), elastase B (LasB), alkaline protease (AP), protease IV (PIV), *Pseudomonas* small protease (PASP), large protease A (LepA), MucD, and *P. aeruginosa* aminopeptidase (PAAP). In the present review, we discuss the role of each of these relevant proteases produced by

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P. aeruginosa taking into consideration their main biological functions in the bacterium–host interaction that favors the establishment of the infectious process.

Keywords
Pseudomonas aeruginosa · Proteases · Virulence factors

1 Introduction

Pseudomonads are bacteria well known for their metabolic versatility and widespread spatiotemporal distribution [1]. One of the most important species of pseudomonads is, with no doubt, *Pseudomonas aeruginosa*, which is a fascinating ubiquitous Gram-negative bacterium with rod shape measuring 0.5–0.8 μm × 1.5–3.0 μm (Fig. 1a) [1, 2]. *P. aeruginosa* presents the following metabolic features: non-fermentative, catalase positive, oxidase positive, ammonia producer, and usually aerobic, but it also can grow in an anaerobic environment if nitrate, citrate, and arginine are available [3]. The production of 2-aminoacetophenone by the bacterial cells generates the fruity grape-like odor that is characteristic of this pseudomonad species. On blood agar plates, colonies of *P. aeruginosa* often display beta-hemolysis and a greenish metallic sheen due to the production of pigments [2]. The characteristic that most distinguishes *P. aeruginosa* from the other pseudomonads, and from the other species of Gram-negative non-fermenting bacteria, is its ability to produce pyocyanin, a blue-green phenazine pigment that gives the green color to the bacterial colony (Fig. 1b) and also to the pus. This pigment and several others, such as pyochelin (purple-cyan), pyoverdin (yellow, green and

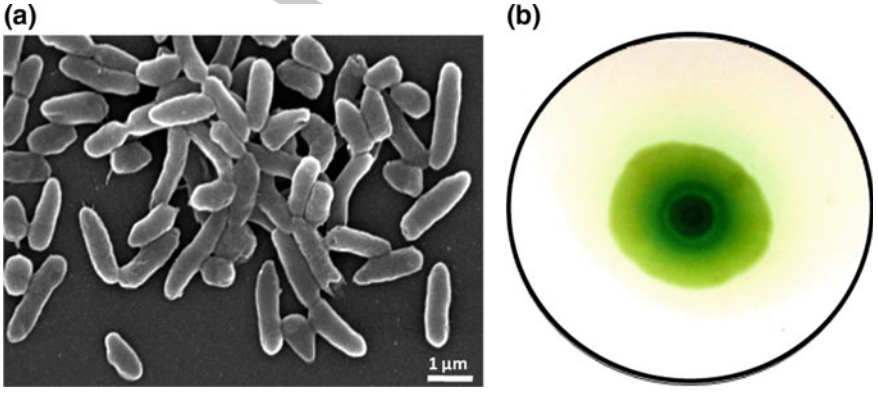

Fig. 1 Scanning electron microscopy (**a**), showing the characteristic bacterial rod shape, and colony morphology (**b**), evidencing the pyocyanin pigment, of *Pseudomonas aeruginosa*

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fluorescent), pyomelanin (light-brown), and pyorubin (red-brown), are secondary metabolites of *P. aeruginosa*, which play an important role in bacterial nutrition, such as iron acquisition and pathogenesis [2, 3]. Almost all *P. aeruginosa* strains are motile due to the presence of a single polar flagellum that facilitates the locomotion and colonization of a wide range of environmental niches [2]. This microorganism can grow within the temperature range from 4 to 42 °C in terrestrial (soil) and aquatic habitats (polluted, salt, and freshwater) as well as on the surface of animate hosts (insects, plants, animals, and humans) and inanimate surroundings, mainly in the hospital environment (distilled water, disinfectants, sinks, medical devices, and equipment), being an important causative agent of nosocomial infections, particularly in intensive care units (ICUs) [1–4]. One of the interesting characteristics of *P. aeruginosa* is its pan-genome, which presents a larger genetic repertoire than the human genome. This intriguing feature explains the broad metabolic capabilities of *P. aeruginosa* and its distribution and adaptability in diverse environments [5].

P. aeruginosa is one of the most important bacterial species for public health considerations due to its high resistance to different classes of antibiotics and its capability to cause serious health care-associated as well as nosocomial infections [6, 7]. Results reported from an International Nosocomial Infection Control Consortium (INICC) surveillance study, performed between 2007 and 2012, in Latin America, Asia, Africa, and Europe, in which prospective data were collected from 605,310 patients hospitalized in 503 ICUs, displayed frequencies of 42.8% of *Pseudomonas* isolates resistant to amikacin and 42.4% to imipenem [8]. In the USA, an estimated 51,000 health care-associated *P. aeruginosa* infections occur each year, in which more than 6,000 (13%) of these are multidrug-resistant and 400 deaths per year are attributed to these infections [9]. The analyses based on data extracted from the Public Health England (PHE) voluntary surveillance database in the period 2008–2012 showed that 92% of *Pseudomonas* spp. isolates identified from bacteremia in 3,457 reports were *P. aeruginosa* [10]. In Brazil, the National Health Surveillance Agency (ANVISA), through the National Monitoring Microbial Resistance Network Health Services (RM Network), published a report that shows the main etiologic agents and the resistance phenotypes responsible for causing primary bloodstream infections associated with the use of central venous catheter in adult patients interned at ICUs from Brazilian hospitals between January and December 2013. According to that study, 18,233 notifications were reported, of which 1,850 (10.1%) were caused by *P. aeruginosa*, being the fifth pathogen most often reported as the etiologic agent. The resistance rate to the carbapenems reached 37.4% (692 *P. aeruginosa* isolates) [11]. Additionally, the Infectious Diseases Society of America has highlighted *P. aeruginosa* as part of a faction of antibiotic-resistant bacteria, called 'the ESKAPE pathogens'—*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa,* and *Enterobacter* spp., capable of 'escaping' the bactericidal action of antibiotics and mutually representing new paradigms in pathogenesis, transmission, and resistance [12].

P. aeruginosa is extensively resistant to multiple drugs and is increasingly resistant to most available antibiotics, being a great emergency problem in the hospital settings worldwide [13]. Interestingly, *P. aeruginosa* has evolved over time in its ability to find new ways to be resistant to different classes of chemical compounds as well as to build strategies to exchange genetic materials, allowing that other bacteria also become drug-resistant [5]. Generally, resistance usually occurs due to a combination of factors acting synergistically: (i) *P. aeruginosa* is intrinsically resistant to antimicrobial agents due to its outer membrane/cell envelope composition that reduces the permeability of several drugs; and (ii) *P. aeruginosa* expresses a powerful repertoire of resistance mechanisms that can be developed through mutations in the genomic content that regulates resistance genes, and also acquired from other organisms via plasmids, transposons, or bacteriophages [14].

As a major opportunistic pathogen for humans, *P. aeruginosa* causes a plenty variety of acute and chronic infections and presents significant levels of morbidity and mortality [15, 16]. *P. aeruginosa* typically infects through airways, wounds, urinary tract, ear canal, via ocular and implanted medical devices (e.g., catheters or ventilators). Thereby, it is the main cause of eschars, conjunctivitis, keratitis, corneal ulcer, osteomyelitis, otitis, urinary infections, surgical site infections, bloodstream infections in ICUs and hospital-acquired pneumonia in immunocompromised individuals, mainly in patients with severe burn wounds, AIDS, lung cancer, chronic obstructive pulmonary disease, bronchiectasis, and cystic fibrosis [16–18].

It is known that Gram-negative bacteria are common causes of a huge diversity of infections including, intra-abdominal infections (IAIs), urinary tract infections (UTIs), ventilator-associated pneumonia (VAP), and bacteremia [19]. In particular, *P. aeruginosa* is one of the most important pathogens in the hospital setting, being responsible for 27% of all pathogens and 70% of all Gram-negative bacteria causing health care-associated infections in the USA, and it is the most common Gram-negative organism causing VAP and the second most common organism causing catheter-associated UTIs [7, 19]. The Centers for Disease Control and Prevention found that *P. aeruginosa* totalized 7.1% of health care-associated infection in the USA in 2011, being the second most common cause of pneumonia in hospital settings and the third most common Gram-negative bacterium to cause bloodstream infections [20]. *P. aeruginosa* is also a major cause of concern in the cystic fibrosis setting, being the most common pathogen isolated from cystic fibrosis sputum, and approximately 70% of adult cystic fibrosis patients are chronically colonized by this microorganism [21, 22].

The pathogenic potential of *P. aeruginosa* is not only due to its metabolic/genetic versatility and both intrinsic and acquired antibiotic resistance. Its ability to form biofilm and to produce an arsenal of virulence attributes, including cell-associated determinants (e.g., lipopolysaccharide, pili, and flagellum) and soluble secreted factors (e.g., extracellular polysaccharides, exotoxins, pigments, and proteases), is very important for the survival and adaptation of this pathogen in distinct environments [17, 22, 23].

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2 *Pseudomonas aeruginosa*: Establishing and Maintaining an Infection

In order to establish an infection, *P. aeruginosa* count on a suite of virulence factors (Fig. 2) [17, 24]. These factors act together not only causing injuries on the host epithelial cell lining but also induce dysfunctions in bacterial physiology, such as cell shape, membrane permeability, and protein synthesis, as well as manipulate/overcome host defenses, down-modulating the immune responses and preventing *P. aeruginosa* endocytosis and obstructing clearance mechanisms, thereby allowing this microbe to persist in cells/tissues and to establish an infection in the host [25, 26]. The virulence of *P. aeruginosa* is mediated by multiple mechanisms, but the major contributor is the production of extracellular proteases. In general, these enzymes regulate multiple cellular and physiological processes and are essential to

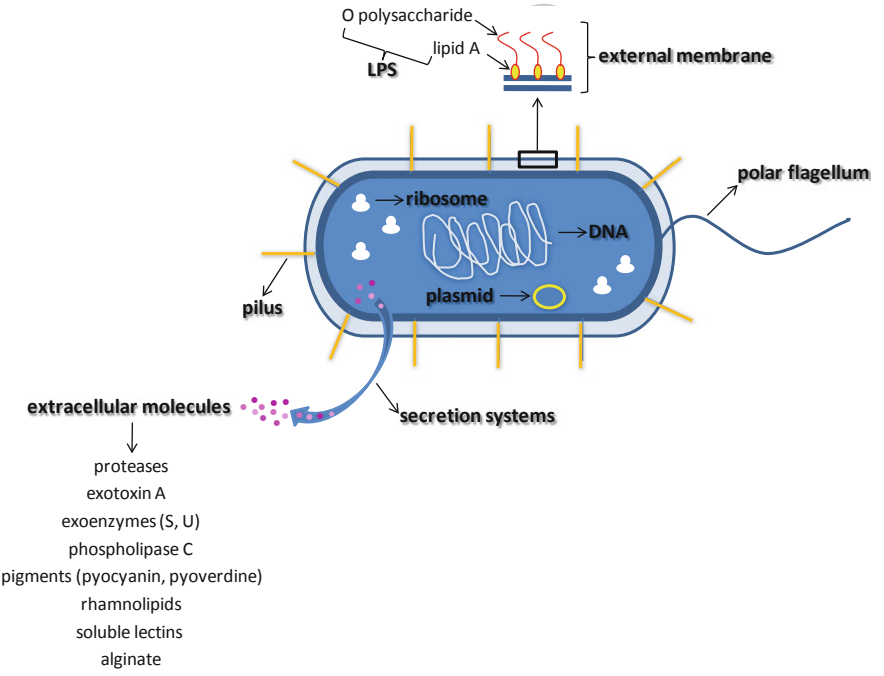

Fig. 2 Virulence factors expressed/produced by *P. aeruginosa* cells: (i) lipopolysaccharide (LPS) that induces cytokine production, (ii) pili that help bacterial adherence to the respiratory epithelial cells, (iii) flagellum that participates in mobility, adherence, and internalization events, (iv) extracellularly released molecules like proteases (responsible for the cleavage of key host proteins), exotoxin A (inhibition of host protein synthesis), exoenzyme S (induces cytotoxic effect), exoenzyme U (antiphagocytic effect), phospholipase C (cleavage of membrane phospholipids), pigments (many biological effects, like pyocyanin that induces free radicals in host cells), rhamnolipids (detergent action), soluble lectins (inhibition of beating of lung cells), and alginate (phagocytosis inhibition, antifungal action, and host immune responses)

the success of the infection. They degrade a wide array of host proteins, impairing host defenses and destroying physical barriers that normally prevent attachment and penetration of the bacteria [26–28].

3 Proteolytic Enzymes Produced by *Pseudomonas aeruginosa*

P. aeruginosa is able to extracellularly release different kinds of proteases (Fig. 3), which together are responsible for invasion and destruction of host tissues. Because of the relevant roles played by proteases on the physiopathology of *P. aeruginosa,* it has been shown that the majority of environmental and clinical strains of *P. aeruginosa* exhibited proteolytic activity, particularly elastase activity [29–31]. According to Stover and co-workers [32], approximately 3% of the whole *P. aeruginosa* genome is composed by open reading frames that encode proteases [32]. Thus, the high genomic variability allows the bacterium to adapt its virulence arsenal machinery to support the variations of environment conditions, and for that, protease production in *P. aeruginosa* can vary greatly (Fig. 4) [32].

The expression of extracellular proteolytic enzymes in *P. aeruginosa* is directly influenced by environmental factors and changes in the physicochemical properties of culture medium (e.g., nutrients, temperature, pH, and aeration), which significantly modulate the production of these crucial virulence factors [26, 33]. In addition, the amount of protease produced depends on the cell cycle moment (e.g., lag, exponential, or stationary growth phase) and on the growing lifestyle (e.g., planktonic or biofilm). For instance, the total protease production (Fig. 5a) as well as the specific elastase secretion increases along the first 48 h of in vitro cultivation

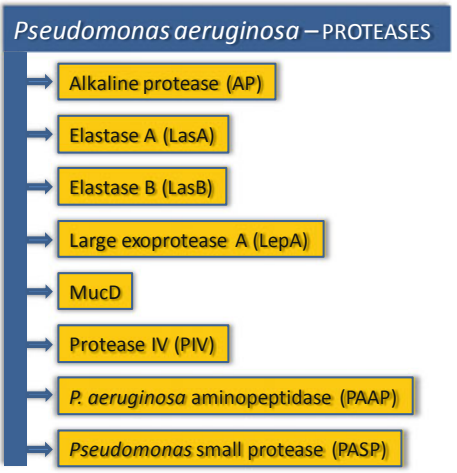

Fig. 3 Proteases secreted by *P. aeruginosa* cells

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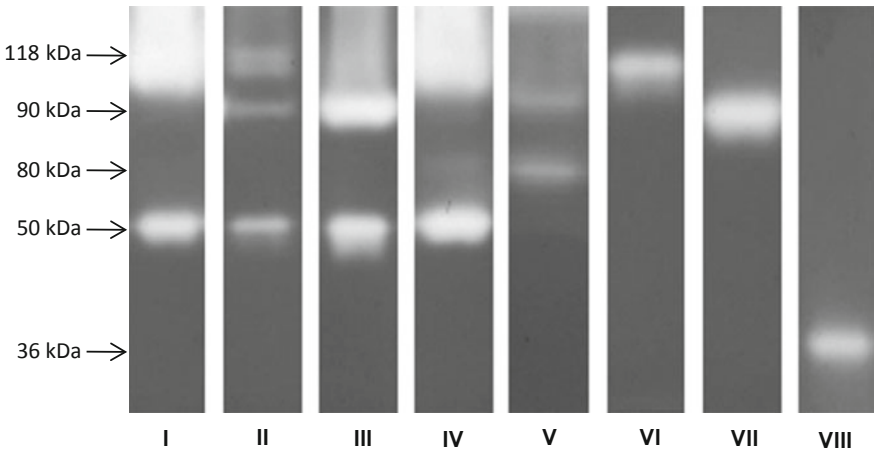

Fig. 4 Production of extracellular proteases in clinical isolates of *P. aeruginosa* recovered from different anatomical sites. The proteolytic profiles were characterized by sodium dodecyl sulfate-containing polyacrylamide gel electrophoresis (SDS-PAGE) containing 0.1% gelatin as the protein substrate. Profile I—118 + 50 kDa; Profile II—118 + 90 + 50 kDa; Profile III—90 + 50 kDa; Profile IV—118 + 80 + 50 kDa; Profile V—90 + 80 kDa; Profile VI—118 kDa; Profile VII—90 kDa, and Profile VIII—36 kDa

of *P. aeruginosa* planktonic cells (Fig. 5b). Further, according to Hastie and co-workers [34], after 85 h of bacterial growth, the elastase production dropped off.

3.1 Elastase B

One of the best proteases characterized in *Pseudomonas* is elastase B (LasB), also known as pseudolysin. This 33-kDa enzyme belongs to the M4 thermolysin-like family of neutral, Zn-dependent metallo-endopeptidases (Fig. 6). This enzyme is encoded by *lasB* gene as a pre-pro-protein, containing at the N-terminal region a signal peptide of 23 amino acids that transport the enzyme through the inner membrane to periplasmic place by bacterial secretory system [35].

The first and the most studied substrate of elastase B is bovine and human elastin [36–38]. Some reports correlate the elastinolytic activity of elastase B to *Pseudomonas* infections in cystic fibrosis patients [39–43]. Histological studies have detected altered elastin fibers in lung alveoli of cystic fibrosis patients on autopsy, indicating a probable elastase activity on cystic fibrosis lung [39]. In addition, the elastase activity is associated with vascular inflammation during *P. aeruginosa* infection, since the disorganization of elastin fiber in vascular tissue caused by protease degradation was observed [44]. Previously, our group analyzed the production of virulence attributes in 96 clinical strains of *P. aeruginosa* recovered from patients attended at hospitals located in three states of Brazil (Espírito Santo, Minas Gerais, and Rio de Janeiro), and it was shown that all bacterial strains exhibited a

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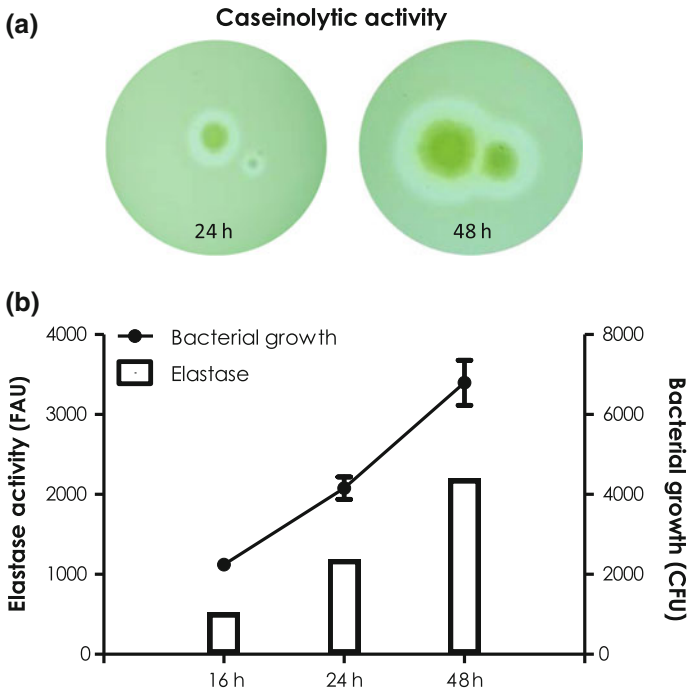

Fig. 5 Protease detection in *P. aeruginosa*. **a** Total extracellular protease production was analyzed by the degradation of casein (1%) incorporated into Luria Bertani agar medium up to 48 h at 37 °C. **b** The elastase activity was measured in the cell-free culture supernatant obtained from *P. aeruginosa* cells grown in tryptic soy broth up to 48 h at 37 °C, using the fluorogenic peptide substrate Abz-Ala-Gly-Leu-Ala-*p*-Nitro-Benzyl-Amide. Results were expressed as fluorescence arbitrary units (FAU). In parallel, the number of bacterial cells along each time point was evaluated by plating cells onto agar medium and expressed as colony-forming units (CFU)

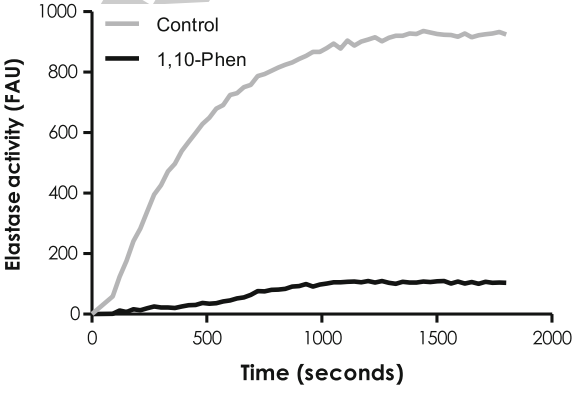

Fig. 6 Elastase of *P. aeruginosa* is a typical zinc-metalloprotease. The purified elastase B is able to cleave the fluorogenic peptide substrate Abz-Ala-Gly-Leu-Ala-*p*-Nitro-Benzyl-Amide along the time. Conversely, 1,10-phenanthroline (1,10-Phen), a metalloprotease inhibitor, at 10 μM was able to block the substrate cleavage. FAU, fluorescence arbitrary units

homogeneous elastase activity, with an average of 1069.28 ± 213.95 fluorogenic arbitrary units (FAU) with no correlation with the original anatomical site of isolation [16]. On the other hand, *P. aeruginosa* strains recovered from trachea, urinary tract, and wounds of patients attended at University Medical Center/Texas Tech Health Sciences Center were able to produce different amounts of elastase [45]. Woods and co-workers [46] showed that Canadian *P. aeruginosa* strains isolated from acute lung infections showed the highest production of elastase (0.053 ± 0.021 mg/ml) compared with elastase activity of strains isolated from burns, wounds, cystic fibrosis lung, and blood.

LasB is also able to cleave other host extracellular matrix proteins, such as collagen type III and IV. Interestingly, after subcutaneous injection of purified elastase B into mice, an intense degradation of basement membranes was observed, and elastase B was responsible for severe hemorrhage and tissue damage [47]. Several studies have demonstrated that LasB-associated epithelial disruption is mediated by the attack to intracellular tight junctions and cytoskeleton reorganization via inhibition of protein kinase C and activation of EGFR, ERK1/2 and NFκB, urokinase, and protease-activated receptor 2 (PAR-2) [48–53]. Elastase B can also interfere with the host bacterial clearance by degrading several components of innate and adaptive immune defense, including tumor necrosis factor-α (TNF-α), interferon-γ (IFN-γ) and interleukin-2 (IL-2), monocyte chemotactic protein-1 (MCP-1), and epithelial neutrophil activating protein-78 (ENA-78) [52–57]. In addition, it was shown that elastase B was efficient in the inactivation of key components of the complement system such as fluid-phase and cell-bound C1 and C3 and fluid-phase C5, C8, and C9 [44]. This multifunctional enzyme is also able to cleave surfactant protein A and D (SP-A and SP-D), also known as collectin. SP-A and SP-D are synthesized by alveolar type II epithelial cells and are responsible for the recognition and binding to oligosaccharides present on the cell surface of many bacteria to be phagocytized by host macrophages [58]. Previously, Meyer and co-workers [59] have reported that a decrease on the SP-A and SP-D levels in bronchoalveolar lavage (BAL) was observed in the lung of cystic fibrosis individuals. Also, SP-D knockout mice were more sensible to *P. aeruginosa* corneal infections when compared to wild-type animals, and only the wild-type mice recovered completely of the infection [60]. Based on this, elastase B was suggested to be responsible for the SP-D degradation in the eye [25, 26]. Furthermore, pseudomonal elastase can interact with host adaptive immune system by degrading immunoglobulins [61–63]. Bainbrigde and Flick [61] showed that elastase B was able to cleave IgG molecules recovered from cystic fibrosis patients and the degradation products bound to IgG-receptors of human neutrophils, thereby inhibiting the opsonization of bacterial invaders. Lomholt and Kilian [63] reported the IgA degradation in tears from patients infected with *P. aeruginosa.* They also observed that isogenic mutants of *P. aeruginosa* knockout to either elastase or alkaline protease were not able to completely inhibit the IgA degradation, indicating that several proteases were working in concert to cleave IgA.

Furthermore, elastase B plays a key role in the differentiation of pseudomonal biofilms. Tielen et al. [64] showed that strains that overexpress *lasB* gene were not

able to form robust biofilms, and they observed the formation of few microcolonies after 72 h of contact with glass surface. Those authors also assigned that *lasB*-overexpressed strain shifted the composition of its extracellular polymeric substances, reducing the alginate content as well as enhancing the rhamnolipids concentration [64]. However, Yu et al. [65] demonstrated that elastase B is crucial for biofilm formation. They observed that Δ*lasB* mutant decreased the biofilm formation through down-regulation of rhamnolipids synthesis.

3.2 Elastase A

Another extracellular protease produced by *P. aeruginosa* is elastase A (LasA), a metalloprotease that belongs to the subgroup A of M23 family of staphylolytic or β-lytic zinc metallo-endopeptidases. LasA is codified as an elastase A pre-pro-protein with molecular mass of 40 kDa [66, 67]. After its synthesis in intracellular bacterial environment, LasA is secreted via type II secretion machinery and when it is secreted to the extracellular space, LasA is immediately converted to its mature and active form of 27 kDa due to the cleavage by other pseudomonal-secreted endopeptidases, such as LasB, LysC, and protease IV [68, 69].

Elastase A is also called as staphylolysin, because it is able to cleave the pentaglycine bonds in the peptidoglycan of *Staphylococcus aureus* [70]. As well, LasA degrades several glycine-rich synthetic peptides [71]. However, LasA exhibited a limited elastinolytic activity [72]. Kessler and co-workers [71] showed that LasA prefers cleaving Gly–Ala peptide bonds within the Gly-Gly-Ala sequences surrounded by apolar sequences. Such sequences are uncommon in elastin, resulting in low elastinolytic activity [26, 73]. Besides its own intrinsic elastinolytic activity, LasA enhances significantly the elastinolytic activity of other proteases, including LasB in *P. aeruginosa*, but also human leukocyte elastase and human neutrophil elastase [74, 75]. Moreover, LasA is responsible for inducing shedding of the host cell surface proteoglycan syndecan-1 (co-receptor proteins), which has been shown to be important for *P. aeruginosa* survival [25, 26].

3.3 Alkaline Protease

Another pseudomonal protein shown to be important for phagocytic evasion is alkaline protease (AprA), which is also known as aeruginolysin. Alkaline protease is a 50-kDa zinc-metalloprotease, member of subfamily B of the M10 peptidase family and metzincin superfamily. AprA, encoded by *aprA* gene, has a C-terminal secretion signal located within the last 50 amino acids residues necessary to be translocated and secreted by AprD, APrE, and AprF membrane proteins, which form the bacterial type I secretory machinery [35].

It was reported that alkaline protease is able to degrade a large number of host proteins, including fibronectin and laminin, important components of basal lamina and endothelium. Therefore, alkaline protease develops an important function in

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invasion and hemorrhagic tissue necrosis in infections caused by *P. aeruginosa* [76]. Furthermore, this protease was found in many isolates of P. aeruginosa recovered from different human anatomical sites with especial elevated expression in clinical isolates from eyes, gastrointestinal tract, and mucoid wounds exacerbated in cystic fibrosis patients [25, 61]. AprA is important to bacterial escape from the host immunological defenses, degrading complement proteins (C1q, C2, and C3) and cytokines (IFN-γ, TNF-α and IL-6) [76]. Also, alkaline protease and elastase B are able to inhibit chemotaxis of neutrophils and block efficiently the phagocytosis, which gives the pathogen an advantage in escaping from phagocyte cells that are one of the first lines of host defense mechanisms [25, 31, 77, 78]. Moreover, alkaline protease is able to inhibit flagellin recognition by TLR5 due to the degradation of free flagellin monomers, helping *P. aeruginosa* cells to avoid the immune detection [79]. This enzyme has also been shown to aid *P. aeruginosa* survival in iron limitations conditions during human infections by cleaving transferrin that increase the siderophore-mediated iron uptake [80]. Gupta and co-workers [81] also reported that treatment of mouse corneal tissue with alkaline protease (50 ng) increases the binding of *P. aeruginosa* to the epithelial surface.

3.4 Protease IV

P. aeruginosa secretes a serine-type protease designated as protease IV (PIV) or lysyl endopeptidase (PrpL), a 26-kDa protease belonging to the chymotrypsin family S1 that has been demonstrated to be an important virulence factor in the rabbit cornea, but is found in clinical isolates recovered from all the anatomical sites analyzed [35, 82]. Its catalytic domain is formed by the triad His_{72}, Asp_{122}, and Ser_{198}. Moreover, it was demonstrated that the residue Ser_{197} adjacent to Ser_{198} is critical to the catalytic activity [83]. Protease IV is encoded by *piv* gene (PA4175), with a full length of 48 kDa, which is initially expressed in the cytoplasm in a pre-pro-enzyme form and then processed to the 26-kDa mature protease after its secretion into the extracellular milieu [83].

PIV participates in the tissue invasion/damage processes and hemorrhagic events due to the cleavage of fibrinogen. It is well known that fibrinogen is required after vascular damage, but the degradation of fibrinogen by PIV leads to hemorrhage during *P. aeruginosa* infection [84]. PIV is also important to evade host immune defenses because it is able to degrade plasminogen, immunoglobulin, C1q and C3, and host antimicrobial peptide LL-37 [25, 68]. Furthermore, Malloy and co-workers [82] observed that PIV degrades the surfactant proteins, SP-A, SP-D, and SP-B, by a time- and dose-depended way in cell-free bronchoalveolar lavage fluid. Those authors reported that degradation of SPs by protease IV reduced the association among bacteria and alveolar macrophage. Interestingly, the incubation of pulmonary surfactant with pseudomonal protease IV reduced the ability of the surfactant to diminish the superficial tension within the lung [82]. Protease IV has been shown to be an iron-regulated protein, suggesting that its expression is regulated irrespective of *quorum sensing* system, which is distinct from other pseudomonal

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proteases [69]. Protease IV has also been correlated to ring abscess lesions present in pseudomonal keratitis [68]. Corroborating this finding, Engel et al. [85] showed that protease IV-deficient mutants exhibited lower ocular virulence in rabbits when intrastromally infected.

3.5 Pseudomonas Small Protease

P. aeruginosa small protease (PASP) is described as a 18.5-kDa secreted zinc-dependent leucine aminopeptidase. *PASP* gene has been found in a large number of *P. aeruginosa* clinical strains, but its higher expression is found during the ocular infection [86]. Previous reports showed that PASP is found only in the bacterial supernatant culture. According to Tang and co-workers [86], the sequence of *PASP* gene appears to have a signal peptide consistent with that needed for type II secretion system.

Direct inoculation of purified PASP into the rabbit cornea causes severe ocular pathology, including epithelial erosion and ulcer in stroma, edema, and neutrophil infiltration into the corneal stroma [87]. PASP has also been demonstrated to cleavage host proteins required for maintaining structure of cornea, such as collagens, fibrinogen (but not fibrin), complement C3, and antimicrobial peptide LL-37. Studies of PASP, coupled with those of PIV, strongly support the hypothesis that *Pseudomonas* proteases play a major role in keratitis [87].

3.6 Large Exoprotease A

Large exoprotease A (LepA) is an exoprotease with molecular mass of ~100 kDa produced by *P. aeruginosa*. LepA, as well as thrombin and trypsin, cleaves human protease-activated receptors (PARs) 1, 2, and 4 in order to activate the critical transcription factor NF-κB, which is associated with host inflammatory and immune responses [49, 88].

3.7 MucD

MucD was reported to be a serine endoprotease that is localized within the periplasmic space. Data suggest that MucD induced a significant reduction on the levels of IL-1β, neutrophil-chemoattractant chemokines KC, and macrophage-inflammatory protein-2 (MIP-2) in the early stages of bacterial infection as well as it inhibited the recruitment of polymorphonuclear (PMN) cells into the cornea. Furthermore, a decrease in PMN cells recruited to infection site favored the establishment of infection by *P. aeruginosa*. MucD may be secreted to the extracellular space, interfering with the biological functions of cytokines and chemokines, but further investigation is needed to understand the mechanisms underlying the role of MucD in keratitis [89, 90].

3.8 Aminopeptidase

The *P. aeruginosa* aminopeptidase (PAAP) or leucine aminopeptidase has been speculated as complementary enzyme to the activity of other endopeptidases. PAAP has an important function in bacterial physiology; it acts releasing free amino acids/small peptides from protein fragments produced by the others *P. aeruginosa* endopeptidases, thereby providing low molecular mass nutrients that can be taken up by the bacterium, which in turn may promote bacterial growth and proliferation [26].

4 Conclusions

P. aeruginosa is a metabolically versatile bacterium that can cause a wide range of severe opportunistic infections in hospitalized patients. To cause this huge variety of infections, *P. aeruginosa* has an arsenal of proteases that are involved in critical events of bacterial pathogenicity and virulence, which are important for survival in the host, tissue invasion, and evasion of host immune defenses. Therefore, this review has highlighted the importance of each pseudomonal protease in bacterial physiology and/or in infectious events. In this context, inhibitors able to block the proteases produced by *P. aeruginosa* cells would represent a new drug class quite promising to combat this widespread bacterial pathogen.

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