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

A.C.M. Galdino

Universidade Federal do Rio de Janeiro (UFRJ)

M.H.B Branquinha

Instituto de Microbiologia Paulo de Góes (IMPG)

A.L.S. Santos

Instituto de Química (IQ)

See next page for additional authors

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Authors

A.C.M. Galdino, M.H.B Branquinha, A.L.S. Santos, and L. Viganor



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

A.C.M. Galdino · A.L.S. Santos
Programa de Pós-Graduação em Bioquímica (PPGBq), Instituto de Química (IQ),
Universidade Federal do Rio de Janeiro (UFRJ), Rio de Janeiro, Brazil

A.C.M. Galdino · M.H. Branquinha · A.L.S. Santos (✉) · L. Viganor
Departamento de Microbiologia Geral, Instituto de Microbiologia Paulo de Góes (IMPG),
Centro de Ciências Da Saúde (CCS), Universidade Federal do Rio de Janeiro, Rio de Janeiro,
Brazil
e-mail: andre@micro.ufrj.br

L. Viganor (✉)
The Centre for Biomimetic & Therapeutic Research, Focas Research Institute,
Dublin Institute of Technology, Camden Row, Dublin 8, Dublin, Ireland
e-mail: liviaviganor@gmail.com

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

30 **Keywords**

32 *Pseudomonas aeruginosa* • Proteases • Virulence factors

33 **1 Introduction**

35 Pseudomonads are bacteria well known for their metabolic versatility and wide-
 36 spread spatiotemporal distribution [1]. One of the most important species of
 37 pseudomonads is, with no doubt, *Pseudomonas aeruginosa*, which is a fascinating
 38 ubiquitous Gram-negative bacterium with rod shape measuring $0.5\text{--}0.8\ \mu\text{m} \times 1.5\text{--}$
 39 $3.0\ \mu\text{m}$ (Fig. 1a) [1, 2]. *P. aeruginosa* presents the following metabolic features:
 40 non-fermentative, catalase positive, oxidase positive, ammonia producer, and
 41 usually aerobic, but it also can grow in an anaerobic environment if nitrate, citrate,
 42 and arginine are available [3]. The production of 2-aminoacetophenone by the
 43 bacterial cells generates the fruity grape-like odor that is characteristic of this
 44 pseudomonad species. On blood agar plates, colonies of *P. aeruginosa* often dis-
 45 play beta-hemolysis and a greenish metallic sheen due to the production of pig-
 46 ments [2]. The characteristic that most distinguishes *P. aeruginosa* from the other
 47 pseudomonads, and from the other species of Gram-negative non-fermenting bac-
 48 teria, is its ability to produce pyocyanin, a blue-green phenazine pigment that gives
 49 the green color to the bacterial colony (Fig. 1b) and also to the pus. This pigment
 50 and several others, such as pyochelin (purple-cyan), pyoverdine (yellow, green and

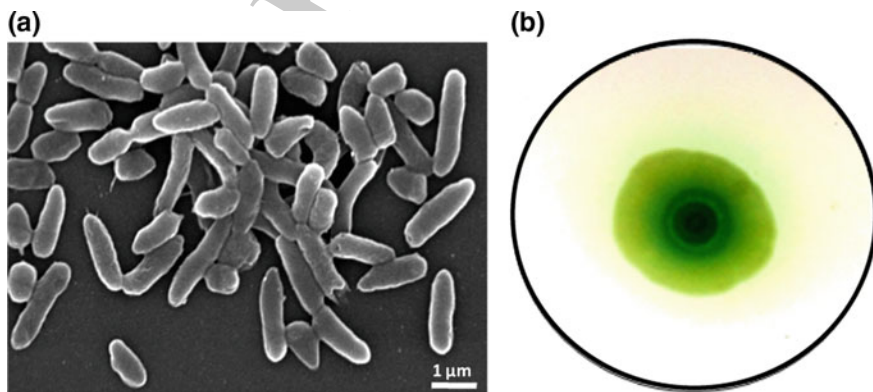


Fig. 1 Scanning electron microscopy (a), showing the characteristic bacterial rod shape, and colony morphology (b), evidencing the pyocyanin pigment, of *Pseudomonas aeruginosa*

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

66 *P. aeruginosa* is one of the most important bacterial species for public health
67 considerations due to its high resistance to different classes of antibiotics and its
68 capability to cause serious health care-associated as well as nosocomial infections
69 [6, 7]. Results reported from an International Nosocomial Infection Control Con-
70 sortium (INICC) surveillance study, performed between 2007 and 2012, in Latin
71 America, Asia, Africa, and Europe, in which prospective data were collected from
72 605,310 patients hospitalized in 503 ICUs, displayed frequencies of 42.8% of
73 *Pseudomonas* isolates resistant to amikacin and 42.4% to imipenem [8]. In the
74 USA, an estimated 51,000 health care-associated *P. aeruginosa* infections occur
75 each year, in which more than 6,000 (13%) of these are multidrug-resistant and 400
76 deaths per year are attributed to these infections [9]. The analyses based on data
77 extracted from the Public Health England (PHE) voluntary surveillance database in
78 the period 2008–2012 showed that 92% of *Pseudomonas* spp. isolates identified
79 from bacteremia in 3,457 reports were *P. aeruginosa* [10]. In Brazil, the National
80 Health Surveillance Agency (ANVISA), through the National Monitoring Micro-
81 bial Resistance Network Health Services (RM Network), published a report that
82 shows the main etiologic agents and the resistance phenotypes responsible for
83 causing primary bloodstream infections associated with the use of central venous
84 catheter in adult patients interned at ICUs from Brazilian hospitals between January
85 and December 2013. According to that study, 18,233 notifications were reported,
86 of which 1,850 (10.1%) were caused by *P. aeruginosa*, being the fifth pathogen most
87 often reported as the etiologic agent. The resistance rate to the carbapenems reached
88 37.4% (692 *P. aeruginosa* isolates) [11]. Additionally, the Infectious Diseases
89 Society of America has highlighted *P. aeruginosa* as part of a faction of
90 antibiotic-resistant bacteria, called ‘the ESKAPE pathogens’—*Enterococcus fae-*
91 *cium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*,
92 *Pseudomonas aeruginosa*, and *Enterobacter* spp., capable of ‘escaping’ the bac-
93 tericidal action of antibiotics and mutually representing new paradigms in patho-
94 genesis, transmission, and resistance [12].

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

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

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

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

2 *Pseudomonas aeruginosa*: Establishing and Maintaining an Infection

In order to establish an infection, *P. aeruginosa* [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 shape, membrane permeability, and protein synthesis, as well as 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

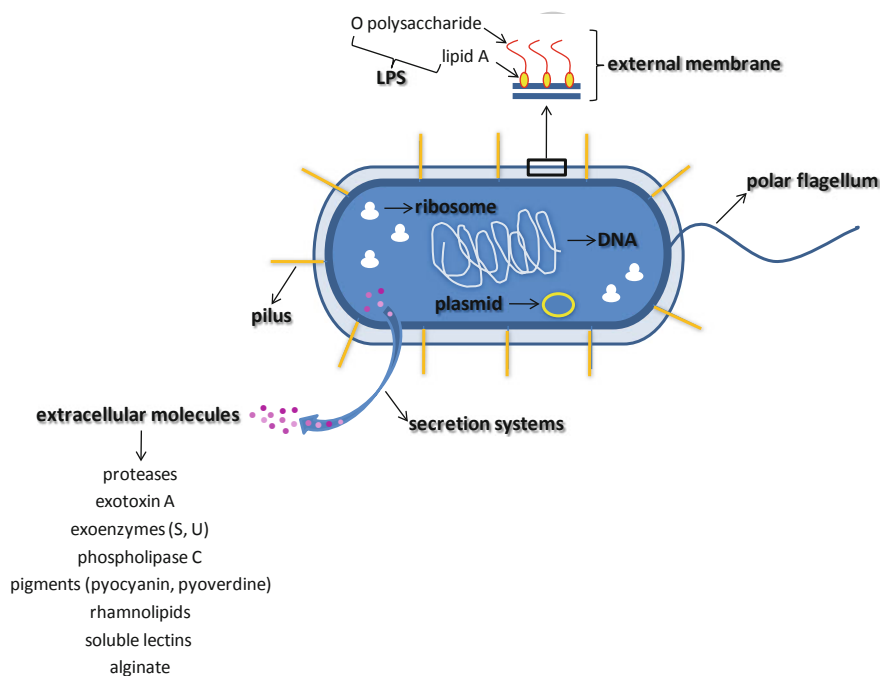


Fig. 2 Virulence factors expressed/produced by *P. aeruginosa* cells: (i) lipopolysaccharide (LPS) that induces cytokine production, (ii) pili that help bacterial adherence to the respiratory epithelial cells, (iii) flagellum that participates in mobility, adherence, and internalization events, (iv) 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)

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

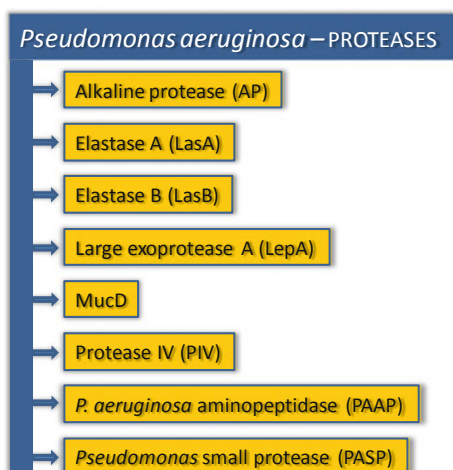
154 3 Proteolytic Enzymes Produced by *Pseudomonas* 155 *aeruginosa* 156

157 *P. aeruginosa* is able to extracellularly release different kinds of proteases (Fig. 3),
158 which together are responsible for invasion and destruction of host tissues. Because
159 of the relevant roles played by proteases on the physiopathology of *P. aeruginosa*,
160 it has been shown that the majority of environmental and clinical strains of
161 *P. aeruginosa* exhibited proteolytic activity, particularly elastase activity [29–31].
162 According to Stover and co-workers [32], approximately 3% of the whole
163 *P. aeruginosa* genome is composed by open reading frames that encode proteases
164 [32]. Thus, the high genomic variability allows the bacterium to adapt its virulence
165 arsenal machinery to support the variations of environment conditions, and for that,
166 protease production in *P. aeruginosa* can vary greatly (Fig. 4) [32].

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

AQ3

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



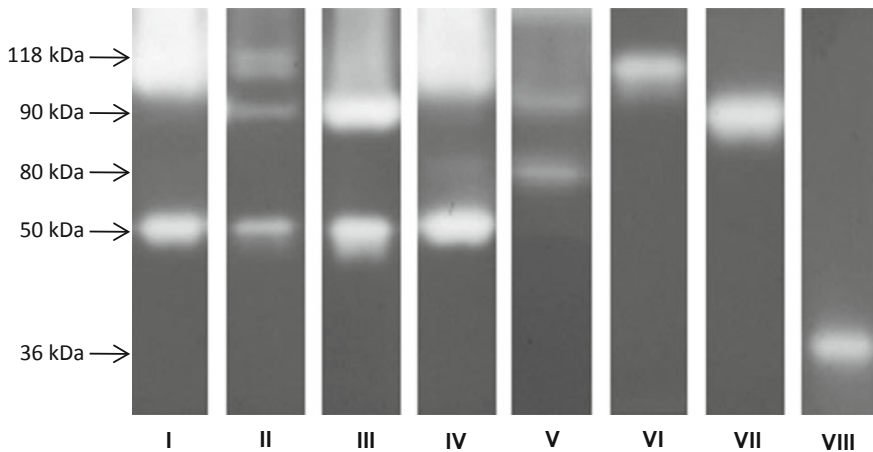


Fig. 4 Production of extracellular proteases in clinical isolates of *P. aeruginosa* recovered from different anatomical sites. The proteolytic profiles were characterized by sodium dodecyl sulfate-containing polyacrylamide gel electrophoresis (SDS-PAGE) containing 0.1% gelatin as the protein substrate. 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

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

177 3.1 Elastase B

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

184 The first and the most studied substrate of elastase B is bovine and human elastin
185 [36–38]. Some reports correlate the elastinolytic activity of elastase B to *Pseu-*
186 *domonas* infections in cystic fibrosis patients [39–43]. Histological studies have
187 detected altered elastin fibers in lung alveoli of cystic fibrosis patients on autopsy,
188 indicating a probable elastase activity on cystic fibrosis lung [39]. In addition, the
189 elastase activity is associated with vascular inflammation during *P. aeruginosa*
190 infection, since the disorganization of elastin fiber in vascular tissue caused by
191 protease degradation was observed [44]. Previously, our group analyzed the pro-
192 duction of virulence attributes in 96 clinical strains of *P. aeruginosa* recovered from
193 patients attended at hospitals located in three states of Brazil (Espírito Santo, Minas
194 Gerais, and Rio de Janeiro), and it was shown that all bacterial strains exhibited a

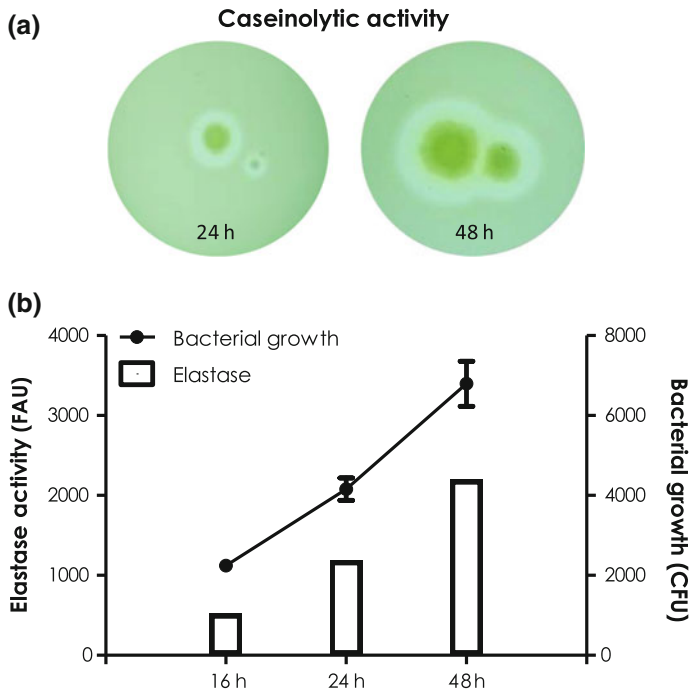


Fig. 5 Protease detection in *P. aeruginosa*. **a** Total extracellular protease production was analyzed by the degradation of casein (1%) incorporated into Luria Bertani agar medium up to 48 h at 37 °C. **b** The elastase activity was measured in the cell-free culture supernatant obtained from *P. aeruginosa* 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)

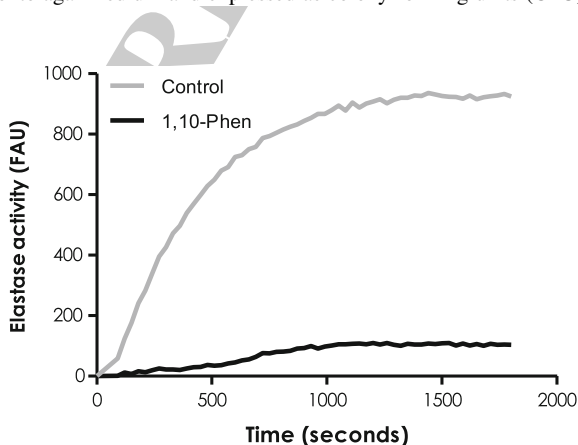


Fig. 6 Elastase of *P. aeruginosa* is a typical zinc-metalloprotease. The purified elastase B is able to cleave the fluorogenic peptide substrate Abz-Ala-Gly-Leu-Ala-*p*-Nitro-Benzyl-Amide along the time. Conversely, 1,10-phenanthroline (1,10-Phen), a metalloprotease inhibitor, at 10 μ M was able to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

195 homogeneous elastase activity, with an average of 1069.28 ± 213.95 fluorogenic
196 arbitrary units (FAU) with no correlation with the original anatomical site of iso-
197 lation [16]. On the other hand, *P. aeruginosa* strains recovered from trachea, uri-
198 nary tract, and wounds of patients attended at University Medical Center/Texas
199 Tech Health Sciences Center were able to produce different amounts of elastase
200 [45]. Woods and co-workers [46] showed that Canadian *P. aeruginosa* strains
201 isolated from acute lung infections showed the highest production of elastase
202 (0.053 ± 0.021 mg/ml) compared with elastase activity of strains isolated from
203 burns, wounds, cystic fibrosis lung, and blood.

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

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

240 able to form robust biofilms, and they observed the formation of few microcolonies
241 after 72 h of contact with glass surface. Those authors also assigned that *lasB*-
242 overexpressed strain shifted the composition of its extracellular polymeric sub-
243 stances, reducing the alginate content as well as enhancing the rhamnolipids con-
244 centration [64]. However, Yu et al. [65] demonstrated that elastase B is crucial for
245 biofilm formation. They observed that $\Delta lasB$ mutant decreased the biofilm for-
246 mation through down-regulation of rhamnolipids synthesis.

247 3.2 Elastase A

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

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

268 3.3 Alkaline Protease

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

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

279 invasion and hemorrhagic tissue necrosis in infections caused by *P. aeruginosa*
280 [76]. Furthermore, this protease was found in many isolates of *P. aeruginosa*
281 recovered from different human anatomical sites with especial elevated expression
282 in clinical isolates from eyes, gastrointestinal tract, and mucoid wounds exacerbated
283 in cystic fibrosis patients [25, 61]. AprA is important to bacterial escape from the
284 host immunological defenses, degrading complement proteins (C1q, C2, and C3)
285 and cytokines (IFN- γ , TNF- α and IL-6) [76]. Also, alkaline protease and elastase B
286 are able to inhibit chemotaxis of neutrophils and block efficiently the phagocytosis,
287 which gives the pathogen an advantage in escaping from phagocyte cells that are
288 one of the first lines of host defense mechanisms [25, 31, 77, 78]. Moreover,
289 alkaline protease is able to inhibit flagellin recognition by TLR5 due to the
290 degradation of free flagellin monomers, helping *P. aeruginosa* cells to avoid the
291 immune detection [79]. This enzyme has also been shown to aid *P. aeruginosa*
292 survival in iron limitations conditions during human infections by cleaving trans-
293 ferrin that increase the siderophore-mediated iron uptake [80]. Gupta and
294 co-workers [81] also reported that treatment of mouse corneal tissue with alkaline
295 protease (50 ng) increases the binding of *P. aeruginosa* to the epithelial surface.

296 3.4 Protease IV

297 *P. aeruginosa* secretes a serine-type protease designated as protease IV (PIV) or
298 lysyl endopeptidase (PrpL), a 26-kDa protease belonging to the chymotrypsin
299 family S1 that has been demonstrated to be an important virulence factor in the
300 rabbit cornea, but is found in clinical isolates recovered from all the anatomical sites
301 analyzed [35, 82]. Its catalytic domain is formed by the triad His₇₂, Asp₁₂₂, and
302 Ser₁₉₈. Moreover, it was demonstrated that the residue Ser₁₉₇ adjacent to Ser₁₉₈ is
303 critical to the catalytic activity [83]. Protease IV is encoded by *piv* gene (PA4175),
304 with a full length of 48 kDa, which is initially expressed in the cytoplasm in a
305 pre-pro-enzyme form and then processed to the 26-kDa mature protease after its
306 secretion into the extracellular milieu [83].

307 PIV participates in the tissue invasion/damage processes and hemorrhagic events
308 due to the cleavage of fibrinogen. It is well known that fibrinogen is required after
309 vascular damage, but the degradation of fibrinogen by PIV leads to hemorrhage
310 during *P. aeruginosa* infection [84]. PIV is also important to evade host immune
311 defenses because it is able to degrade plasminogen, immunoglobulin, C1q and C3,
312 and host antimicrobial peptide LL-37 [25, 68]. Furthermore, Malloy and co-workers
313 [82] observed that PIV degrades the surfactant proteins, SP-A, SP-D, and SP-B, by
314 a time- and dose-dependent way in cell-free bronchoalveolar lavage fluid. Those
315 authors reported that degradation of SPs by protease IV reduced the association
316 among bacteria and alveolar macrophage. Interestingly, the incubation of pul-
317 monary surfactant with pseudomonal protease IV reduced the ability of the sur-
318 factant to diminish the superficial tension within the lung [82]. Protease IV has been
319 shown to be an iron-regulated protein, suggesting that its expression is regulated
320 irrespective of *quorum sensing* system, which is distinct from other pseudomonal

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

325 **3.5 Pseudomonas Small Protease**

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

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

340 **3.6 Large Exoprotease A**

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

346 **3.7 MucD**

347 *MucD* was reported to be a serine endoprotease that is localized within the
348 periplasmic space. Data suggest that *MucD* induced a significant reduction on the
349 levels of IL-1 β , neutrophil-chemoattractant chemokines KC, and macrophage-
350 inflammatory protein-2 (MIP-2) in the early stages of bacterial infection as well as it
351 inhibited the recruitment of polymorphonuclear (PMN) cells into the cornea. Fur-
352 thermore, a decrease in PMN cells recruited to infection site favored the estab-
353 lishment of infection by *P. aeruginosa*. *MucD* may be secreted to the extracellular
354 space, interfering with the biological functions of cytokines and chemokines, but
355 further investigation is needed to understand the mechanisms underlying the role of
356 *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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References

1. Tümmler B, Wiehlmann L, Klockgether J et al (2014) Advances in understanding *Pseudomonas*. F1000 Prime 6:9
2. Jayaseelan S, Ramaswamy D, Dharmaraj S (2014) Pyocyanin: production, applications, challenges and new insights. World J Microbiol Biotechnol 30:1159–1168
3. Vasil ML (1986) *Pseudomonas aeruginosa*: biology, mechanisms of virulence, epidemiology. J Pediatr 108:800–805
4. Wiehlmann L, Wagner G, Cramer N et al (2007) Population structure of *Pseudomonas aeruginosa*. PNAS 104:8101–8106
5. Ghodhbane H, Elaidi S, Sabatier JM et al (2015) Bacteriocins active against multi-resistant gram negative bacteria implicated in nosocomial infections. Infect Disord Drug Targets 15:2–12
6. Bartram J, Cotruvo J, Exner M, Fricker C, Glasmacher A (2003) WHO—World Health Organization. Heterotrophic plate counts and drinking-water safety. IWA Publishing, London. ISBN: 1 84339 025 6
7. Nielsen SL (2015) The incidence and prognosis of patients with bacteremia. Dan Med J 62: B5128
8. Rosenthal VD, Maki DG, Mehta Y, Leblebicioglu H et al (2014) International nosocomial Infection Control Consortium. International Nosocomial Infection Control Consortium

- 399 (INICC) report, data summary of 43 countries for 2007–2012. Device-associated module.
400 Am J Infect Control 42:942–956
- 401 9. Centers for Disease Control and Prevention. Healthcare-associated Infections (HAIs).
402 *Pseudomonas aeruginosa* in Healthcare Settings. Available in: [http://www.cdc.gov/hai/](http://www.cdc.gov/hai/organisms/pseudomonas.html)
403 [organisms/pseudomonas.html](http://www.cdc.gov/hai/organisms/pseudomonas.html). Accessed on 17 June 2016
- 404 10. Public Health England (2012) *Pseudomonas aeruginosa*: guidance, data and analysis—
405 voluntary surveillance of *Pseudomonas* spp. and *Stenotrophomonas* spp. causing bacteraemia in
406 England, Wales and Northern Ireland. Available in: [https://www.gov.uk/government/](https://www.gov.uk/government/publications/pseudomonas-spp-and-stenotrophomonas-spp-voluntary-surveillance-2012)
407 [publications/pseudomonas-spp-and-stenotrophomonas-spp-voluntary-surveillance-2012](https://www.gov.uk/government/publications/pseudomonas-spp-and-stenotrophomonas-spp-voluntary-surveillance-2012).
408 Accessed on 17 June 2016
- 409 11. ANVISA—Agência Nacional de Vigilância Sanitária. Boletim Informativo Segurança do
410 Paciente e Qualidade em Serviços de Saúde—Ano V nº 09|Dezembro de 2014. Available in:
411 <http://portal.anvisa.gov.br>. Accessed on 17 June 2016
- 412 12. Pendleton JN, Gorman SP, Gilmore BF (2013) Clinical relevance of the ESKAPE pathogens.
413 Expert Rev Anti Infect Ther 11:297–308
- 414 13. Buhl M, Peter S, Willmann M (2013) Prevalence and risk factors associated with colonization
415 and infection of extensively drug-resistant *Pseudomonas aeruginosa*: a systemic review.
416 Expert Rev Anti-infect Ther 13:1159–1170
- 417 14. El Zowalaty ME, Al Thani AA, Webster TJ et al (2015) *Pseudomonas aeruginosa*: arsenal of
418 resistance mechanisms, decades of changing resistance profiles, and future antimicrobial
419 therapies. Future Microbiol 10:1683–1706
- 420 15. Bentzmann S, Plésiat P (2011) The *Pseudomonas aeruginosa* opportunistic pathogen and
421 human infections. Environ Microbiol 13:1655–1665
- 422 16. Silva LV, Galdino ACM, Nunes APF et al (2014) Virulence attributes in Brazilian clinical
423 isolates of *Pseudomonas aeruginosa*. Int J Med Microbiol 304:990–1000
- 424 17. Balasubramanian D, Schnepfer L, Kumari H, Mathee K (2013) A dynamic and intricate
425 regulatory network determines *Pseudomonas aeruginosa* virulence. Nucleic Acids Res 41:1–20
- 426 18. Savoia D (2014) New perspectives in the management of *Pseudomonas aeruginosa*
427 infections. Future Microbiol 9:917–928
- 428 19. Kaye KS, Pogue JM (2015) Infections caused by resistant Gram-negative bacteria:
429 epidemiology and management. Pharmacotherapy 35:949–962
- 430 20. McCarthy K (2015) *Pseudomonas aeruginosa*: evolution of antimicrobial resistance and
431 implications for therapy. Semin Respir Crit Care Med 36:44–55
- 432 21. Sousa AM, Pereira MO (2014) *Pseudomonas aeruginosa* diversification during infection
433 development in cystic fibrosis lungs—a review. Pathogens 3:680–703
- 434 22. Oliver A, Mulet X, López-Causapé C, Juan C (2015) The increasing threat of *Pseudomonas*
435 *aeruginosa* high-risk clones. Drug Resist Update 22:41–59
- 436 23. Kung VL, Ozer EA, Hauser AR (2010) The accessory genome of *Pseudomonas aeruginosa*.
437 Microbiol Mol Biol 74:621–664
- 438 24. Crousilles A, Maunders E, Bartlett S, Fan C et al (2015) Which microbial factors really are
439 important in *Pseudomonas aeruginosa* infections? Future Microbiol 10:1825–1836
- 440 25. Ballok AE, O’Toole GA (2013) Pouring salt on a wound: *Pseudomonas aeruginosa* virulence
441 factors alter Na⁺ and Cl⁻ flux in the lung. J Bacteriol 195:4013–4019
- 442 26. Kessler E, Safrin M (2014) Elastinolytic and proteolytic enzymes. In *Pseudomonas* methods
443 and protocols. Methods Mol Biol 1149:135–169
- 444 27. McCarty SM, Cochrane CA, Clegg PD, Percival SL (2012) The role of endogenous and
445 exogenous enzymes in chronic wounds: a focus on the implications of aberrant levels of both
446 host and bacterial proteases in wound healing. Wound Repair Regen 20:125–136
- 447 28. Gellatly SL, Hancock REW (2013) *Pseudomonas aeruginosa*: new insights into pathogenesis
448 and host defenses. Pathog Dis 67:159–173
- 449 29. Schmidtchen A, Wolff H, Hansson C (2001) Differential proteinase expression by
450 *Pseudomonas aeruginosa* derived from chronic leg ulcers. Acta Derm Venereol 81:406–409

- 451 30. Tingpej P, Smith L, Rose B et al (2007) Phenotypic characterization of clonal and nonclonal
452 *Pseudomonas aeruginosa* strains isolated from lungs of adults with cystic fibrosis. *J Clin*
453 *Microbiol* 45:1697–1704
- 454 31. Thibodeau PH, Butterworth MB (2013) Proteases, cystic fibrosis and the epithelial sodium
455 channel (ENaC). *Cell Tissue Res* 351:309–323
- 456 32. Stover CK, Pham XQ, Erwin AL et al (2000) Complete genome sequence of *Pseudomonas*
457 *aeruginosa* PAO1, an opportunistic pathogen. *Nature* 406:959–964
- 458 33. Marquart ME, Dajcs JJ, Caballero AR et al (2005) Calcium and magnesium enhance the
459 production of *Pseudomonas aeruginosa* protease IV, a corneal virulence factor. *Med*
460 *Microbiol Immunol* 194:39–45
- 461 34. Hastie AT, Hingley ST, Kueppers F (1983) Protease production by *Pseudomonas aeruginosa*
462 isolates from patients with cystic fibrosis. *Infect Immun* 40:506–513
- 463 35. Hoge R, Pelzer A, Rosenau F, Wilhelm S (2010) Weapons of a pathogen: proteases and their
464 role in virulence of *Pseudomonas aeruginosa*. In: Méndez-Vilas A (ed) Current research,
465 technology and education topics in applied microbiology and microbial biotechnology.
466 Formatex Research Center, Badajoz, pp. 383–395
- 467 36. Hamdaoui A, Wund-Bisseret F, Bieth JG (1987) Fast solubilization of human lung elastin by
468 *Pseudomonas aeruginosa* elastase. *Am Rev Respir Dis* 135:860–863
- 469 37. Saulnier JM, Curtil FM, Duclos MC, Wallach JM (1989) Elastolytic activity of *Pseudomonas*
470 *aeruginosa* elastase. *Biochim Biophys Acta* 995:285–290
- 471 38. Yang J, Zhao HL, Ran YL et al (2015) Mechanistic insights into elastin degradation by
472 pseudolysin, the major virulence factor of the opportunistic pathogen *Pseudomonas*
473 *aeruginosa*. *Sci Rep* 9936
- 474 39. Bruce MC, Poncz L, Klinger JD et al (1985) Biochemical and pathologic evidence for proteolytic
475 destruction of lung connective tissue in cystic fibrosis. *Am Rev Respir Dis* 132:529–535
- 476 40. Erickson DL, Endersby R, Kirkham A et al (2002) *Pseudomonas aeruginosa* quorum-sensing
477 systems may control virulence factor expression in the lungs of patients with cystic fibrosis.
478 *Infect Immun* 70:1783–1790
- 479 41. Kosorok MR, Zeng L, West SE et al (2001) Acceleration of lung disease in children with
480 cystic fibrosis after *Pseudomonas aeruginosa* acquisition. *Pediatr Pulmonol* 32:277–287
- 481 42. Voynow JA, Fischer BM, Zheng S (2008) Proteases and cystic fibrosis. *Int J Biochem Cell*
482 *Biol* 40:1238–1245
- 483 43. Van't Wout EF, van Schadewijk A, van Boxtel R et al (2015) Virulence factors of
484 *Pseudomonas aeruginosa* induce both the unfolded protein and integrated stress responses in
485 airway epithelial cells. *PLoS Pathog* 11:e1004946
- 486 44. Schultz DR, Miller KD (1974) Elastase of *Pseudomonas aeruginosa*: inactivation of
487 complement components and complement-derived chemotactic and phagocytic factors. *Infect*
488 *Immun* 10:128–135
- 489 45. Hamood A, Griswold G, Colmer J (1996) Characterization of elastase-deficient clinical
490 isolates of *Pseudomonas aeruginosa*. *Infect Immun* 64:3154–3160
- 491 46. Woods DE, Schaffer MS, Rabin HR et al (1988) Phenotypic comparison of *Pseudomonas*
492 *aeruginosa* strains isolated from a variety of clinical sites. *J Bacteriol* 170:4309–4314
- 493 47. Komori Y, Nonogaki T, Nikai T (2001) Hemorrhagic activity and muscle damaging effect of
494 *Pseudomonas aeruginosa* metalloproteinase (elastase). *Toxicon* 39:1327–1332
- 495 48. Bentzmann S, Polette M, Zahm JM et al (2000) *Pseudomonas aeruginosa* virulence factors
496 delay airway epithelial wound repair by altering the actin cytoskeleton and inducing
497 overactivation of epithelial matrix metalloproteinase-2. *Lab Invest* 80:209–219
- 498 49. Kida Y, Higashimoto Y, Inoue H et al (2008) A novel secreted protease from *Pseudomonas*
499 *aeruginosa* activates NF-kappaB through protease-activated receptors. *Cell Microbiol*
500 *10*:491–504
- 501 50. Clark CA, Thomas LK, Azghani AO (2011) Inhibition of protein kinase C attenuates
502 *Pseudomonas aeruginosa* elastase-induced epithelial barrier disruption. *Am J Respir Cell Mol*
503 *Biol* 45:1263–1271

- 504 51. Cosgrove S, Chotirmall SH, Greene CM et al (2011) Pulmonary proteases in the cystic
505 fibrosis lung induce interleukin 8 expression from bronchial epithelial cells via a
506 heme/mepprin/epidermal growth factor receptor/Toll-like receptor pathway. *J Biol Chem*
507 286:692–704
- 508 52. Kuang Z, Hao Y, Walling BE et al (2011) *Pseudomonas aeruginosa* elastase provides an
509 escape from phagocytosis by degrading the pulmonary surfactant protein-A. *PLoS ONE* 6:
510 e27091
- 511 53. Nomura K, Obata K, Keira T et al (2014) *Pseudomonas aeruginosa* elastase causes transient
512 disruption of tight junctions and downregulation of PAR-2 in human nasal epithelial cells.
513 *Respir Res* 18:15–21
- 514 54. Parmely M, Gale A, Clabaugh M et al (1990) Proteolytic inactivation of cytokines by
515 *Pseudomonas aeruginosa*. *Infect Immun* 58:3009–3014
- 516 55. Horvat RT, Clabaugh M, Duval-Jobe C, Parmely MJ (1989) Inactivation of human gamma
517 interferon by *Pseudomonas aeruginosa* proteases: elastase augments the effects of alkaline
518 protease despite the presence of alpha 2-macroglobulin. *Infect Immun* 57:1668–1674
- 519 56. Theander TG, Kharazmi A, Pedersen BK et al (1988) Inhibition of human lymphocyte
520 proliferation and cleavage of interleukin-2 by *Pseudomonas aeruginosa* proteases. *Infect*
521 *Immun* 56:1673–1677
- 522 57. Leidal KG, Munson KL, Johnson MC et al (2003) Metalloproteases from *Pseudomonas*
523 *aeruginosa* degrade human RANTES, MCP-1, and ENA-78. *J Interferon Cytokine Res*
524 23:307–318
- 525 58. Mariencheck WI, Alcorn JF, Palmer SM (2003) *Pseudomonas aeruginosa* elastase degrades
526 surfactant proteins A and D. *Am J Respir Cell Mol Biol* 28:528–537
- 527 59. Meyer KC, Sharma R, Brown M et al (2000) Function and composition of pulmonary
528 surfactant and surfactant-derived fatty acid profiles are altered in young adults with cystic
529 fibrosis. *Chest* 118:164–174
- 530 60. McCormick CC, Hobden JA, Balzli CL et al (2007) Surfactant protein D in *Pseudomonas*
531 *aeruginosa* keratitis. *Ocular Immun Inflam* 15:371–379
- 532 61. Bainbridge T, Fick RB (1989) Functional importance of cystic fibrosis immunoglobulin G
533 fragments generated by *Pseudomonas aeruginosa* elastase. *J Lab Clin Med* 114:728–733
- 534 62. Heck LW, Alarcon PG, Kulhavy RM et al (1990) Degradation of IgA proteins by
535 *Pseudomonas aeruginosa* elastase. *J Immunol* 144:2253–2257
- 536 63. Lomholt JA, Kilian M (2008) Degradation of uniquely glycosylated secretory immunoglob-
537 ulin A in tears from patients with *Pseudomonas aeruginosa* keratitis. *Invest Ophthalmol Vis*
538 *Sci* 49:1944–4939
- 539 64. Tielen P, Rosenau F, Wilhelm S et al (2010) Extracellular enzymes affect biofilm formation of
540 mucoid *Pseudomonas aeruginosa*. *Microbiology* 156:2239–2252
- 541 65. Yu H, He X, Xie W et al (2014) Elastase LasB of *Pseudomonas aeruginosa* promotes biofilm
542 formation partly through rhamnolipid-mediated regulation. *Can J Microbiol* 60:227–235
- 543 66. Schad PA, Iglewski BH (1988) Nucleotide sequence and expression in *Escherichia coli* of the
544 *Pseudomonas aeruginosa* lasA gene. *J Bacteriol* 170:2784–2789
- 545 67. Kessler E, Safrin M, Gustin JK et al (1998) Elastase and the LasA protease of *Pseudomonas*
546 *aeruginosa* are secreted with their propeptides. *J Biol Chem* 273:30225–30231
- 547 68. Engel LS, Hill JM, Caballero AR (1998) Protease IV, a unique extracellular protease and
548 virulence factor from *Pseudomonas aeruginosa*. *J Biol Chem* 273:16792–16797
- 549 69. Wilderman PJ, Vasil AI, Johnson Z (2001) Characterization of an endoprotease (prp1)
550 encoded by a pvds-regulated gene in *Pseudomonas aeruginosa*. *Infect Immun* 69:5385–5394
- 551 70. Barequet IS, Bourla N, Pessach YN et al (2012) Staphylolysin is an effective therapeutic agent
552 for *Staphylococcus aureus* experimental keratitis. *Graefes Arch Clin Exp Ophthalmol*
553 250:223–229
- 554 71. Kessler E, Safrin M, Abrams WR, Rosenbloom J, Ohman DE (1997) Inhibitors and specificity
555 of *Pseudomonas aeruginosa* LasA. *J Biol Chem* 272:9884–9889

- 556 72. Kessler E, Safrin M, Blumberg S, Ohman DE (2004) A continuous spectrophotometric assay
 557 for *Pseudomonas aeruginosa* LasA protease (staphylolysin) using a two-stage enzymatic
 558 reaction. *Anal Biochem* 328:225–232
- 559 73. Vessillier S, Delolme F, Bernillon J, Saulnier J, Wallach J (2001) Hydrolysis of
 560 glycine-containing elastin pentapeptides by LasA, a metalloelastase from *Pseudomonas*
 561 *aeruginosa*. *Eur J Biochem* 268:1049–1057
- 562 74. Peters JE, Galloway DR (1990) Purification and characterization of an active fragment of the
 563 LasA protein from *Pseudomonas aeruginosa*: enhancement of elastase activity. *J Bacteriol*
 564 172:2236–2240
- 565 75. Peters JE, Park SJ, Darzins A et al (1992) Further studies on *Pseudomonas aeruginosa* LasA:
 566 analysis of specificity. *Mol Microbiol* 6:1155–1162
- 567 76. Laarman AJ, Bardoel BW, Ruyken M et al (2012) *Pseudomonas aeruginosa* alkaline protease
 568 blocks complement activation via the classical and lectin pathways. *J Immunol* 188:386–393
- 569 77. Kharazmi A, Hoiby N, Doring G, Valerius NH (1984) *Pseudomonas aeruginosa* exoproteases
 570 inhibit human neutrophil chemiluminescence. *Infect Immun* 44:587–591
- 571 78. Hong YQ, Ghebrehiwet B (1992) Effect of *Pseudomonas aeruginosa* elastase and alkaline
 572 protease on serum complement and isolated components C1q and C3. *Clin Immunol*
 573 *Immunopathol* 62:133–138
- 574 79. Bardoel BW, van Kessel KP, van Strijp JA, Milder FJ (2012) Inhibition of *Pseudomonas*
 575 *aeruginosa* virulence: characterization of the AprA-AprI interface and species selectivity.
 576 *J Mol Biol* 415:573–583
- 577 80. Kim SJ, Park RY, Kang SM (2006) *Pseudomonas aeruginosa* alkaline protease can facilitate
 578 siderophore-mediated iron-uptake via the proteolytic cleavage of transferrins. *Biol Pharm Bull*
 579 29:2295–22300
- 581 81. Gupta SK, Masinick SA, Hobden JA et al (1996) Bacterial proteases and adherence of
 582 *Pseudomonas aeruginosa* to mouse cornea. *Exp Eye Res* 62:641–650
- 583 82. Malloy JL1, Veldhuizen RA, Thibodeaux BA et al (2005) *Pseudomonas aeruginosa* protease
 584 IV degrades surfactant proteins and inhibits surfactant host defense and biophysical functions.
 585 *Am J Physiol Lung Cell Mol Physiol* 288:409–418
- 586 83. Traidej M, Caballero AR, Marquart ME et al (2003) Molecular analysis of *Pseudomonas*
 587 *aeruginosa* protease IV expressed in *Pseudomonas putida*. *Invest Ophthalmol Vis Sci*
 588 44:190–196
- 589 84. Matsumoto K (2004) Role of bacterial proteases in pseudomonal and serratial keratitis. *Biol*
 590 *Chem* 385:1007–1016
- 591 85. Engel LS, Hobden JA, Moreau JM et al (1997) *Pseudomonas* deficient in protease IV has
 592 significantly reduced corneal virulence. *Invest Ophthalmol Vis Sci* 38:1535–1542
- 593 86. Tang A, Marquart ME, Fratkin JD et al (2009) Properties of PASP: a *Pseudomonas* protease
 594 capable of mediating corneal erosions. *Invest Ophthalmol Vis Sci* 50:3794–3801
- 595 87. Tang A, Caballero AR, Marquart ME, O'callaghan RJ (2013) *Pseudomonas aeruginosa* small
 596 protease (PASP), a keratitis virulence factor. *Invest Ophthalmol Vis Sci* 54:2821–2828
- 597 88. Kida Y, Shimizu T, Kuwano K (2011) Cooperation between LepA and PlcH contributes to
 598 the in vivo virulence and growth of *Pseudomonas aeruginosa* in mice. *Infect Immun*
 599 79:211–219
- 600 89. Mochizuki Y, Suzuki T, Oka N, Zhang Y et al (2014) *Pseudomonas aeruginosa* MucD
 601 protease mediates keratitis by inhibiting neutrophil recruitment and promoting bacterial
 602 survival. *Invest Ophthalmol Vis Sci* 55:240–246
- 603 90. Okuda J, Hayashi N, Tanabe S et al (2011) Degradation of interleukin 8 by the serine protease
 MucD of *Pseudomonas aeruginosa*. *Infect Chemother* 17:782–792

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