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Review Paper

From Simple to its Extreme: Proteases Serving as Innovative Bacterial Weapon

Sikander Ali; Muhammad Zain Ul Abbdin; Muhammad Faiq Ali and Waqas Ali Awan

Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan.

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Corresponding Author
Sikander Ali
Institute of Industrial Biotechnology, Government College University, Lahore, Pakistan.

Abstract
Proteases are group of enzymes that catalyse protein digestion. Large numbers of bacterial proteases perform different roles in bacteria. Most of bacteria especially saprotrophic bacteria uses proteases to digest organic matter. Somehow bacteria proved to be smart enough to cash protein digesting ability of proteases to execute different cellular functions. These normal cellular functions include protein digestion (pepsin), quality control (FtsH), control of cell secretions (Lon), regulation of flagella synthesis (clpXP) and signal transduction (YluC). However, some bacteria proved to be even smarter as they managed to use proteases as an effective weapon. As a bacterial weapon these have potential to disfigure host tissues (glutamylen- o-peptidase SspA) confront host defences (ScpC) and to escalate infection (V8 serine protease). Collectively these functional shifts are symbol of bacterial innovation. All these mechanism used by bacteria can be exploited and can pave way in antibiotics production.

Keywords-*Proteases, Protein digestion, Quality control, Signal transduction, Disfigure host tissues, Confronting defenses, Escalating infection.*

Introduction

Proteases are group of enzymes that function as protein degraders. The most commonly known function of protease is digestion of protein components of organic matter. Most of the bacteria especially saprotrophic bacteria use proteases to digest or decay organic matter. However, proteases are also a very useful tool to bring about normal cellular functions. But it could be regarded as innovation after that some of bacteria had been reported to use protease a destructive weapon and a very important virulent factor. So, for bacteria the protease is something more than just degrading protein content. Bacteria have moulded this protein digester to perform number of different cellular functions. For instance it regulates signal transmission through cell membrane and play role in quality control of cellular proteins. Protease also regulates protein secretions and maintains cell homeostasis. Furthermore, Proteases are also reported to help proper bacterial motility by ensuring normal biosynthesis of flagella. Proteases play direct role in signal transductions of a bacterial cell. Site-2 proteases (S2Ps) are common in bacteria and known to participate in different pathways which share the requirement for proteolysis of a transmembrane protein [3]. In this context S2Ps can be defined as multipass transmembrane proteins bearing a conserved zinc metalloprotease active site within a transmembrane domain while a motif within another transmembrane domain [1], [2]. The S2Ps of *B. subtilis* (YluC and SpoIVFB) and *E. coli* (RseP) have been intensely studied and are the best understood in terms of signal transduction mechanism, upstream activating signals along with downstream regulons. Further investigation of S2Ps in bacterial pathogens has revealed roles for S2Ps in sensing host signals and regulating virulence gene expression during infection period [3]. Generally, the signalling cascades in which S2Ps participate follow the same general pathway. For example, site-1 protease usually cleaves extra cytoplasmic segment of the transmembrane substrate in response to inducer signal. This site-1 cleavage is instantly followed by cleavage of substrate present with in membrane by S2P, So it results liberating the cytosolic fragment of the substrate. Normally, the fragment released into the cytosol by the S2P cleavage is a transcriptional regulator [3].

In quality control of cellular proteins FtsH is a membrane bounded protease with N-terminal part embedded in transmembrane along with a cytosolic region comprising of an AAAþ ATPase and a Zn2þ metalloprotease. Enzyme FtsH is known to degrade number different short lived proteins and to degrade proteins that are misassembled in membrane [4]. Proteases also take part in control of cell secretions. A Gram negative pathogen *Salmonella typhimurium*, causative agent gastroenteritis in humans colonizes the small intestine. *S. typhimurium* mutants that lack Lon protease was unable to colonize. Salmonella pathogenicity island 1, SPI1 has been found to have ability to induce apoptosis in macrophages and enhanced ability to invade epithelial cells [5], [6]. Type III secretion system that is encoded by SPI1 is critical **[7]**. The SPI1 system composed of many proteins that arrange to form a specific needle shaped structure to inject effector proteins in cytosol of host. In host actin cytoskeleton is rearranged by these injected proteins helping

bacterial uptake. Expression of this secretion system is regulated by activators of AraC/XylS family like HilC and HilD [8]. Studies had shown that both these activators are target of Lon protease and absence of Lon stimulates of SPI1 gene expression [9]. Flagella for many bacteria play their role in pathogenesis either serving as factors for bacterial adhesion [10]. In *S. typhimurium*, inactivation of clpP resulted in a hyper flagellated phenotype (abnormal flagella biosynthesis) due to overproduction of flagella proteins and increased expression of fliC encoding flagellin, the flagellum filament protein [11], [12]. The increased fliC expression is the result of FlhD/FlhC serving as flagella biosynthesis regulator. FlhD/FlhC regulators are degraded by ClpXP to regulate their concentrations. However Protease is also a multi role bacterial weapon. As all the Bacterial pathogens carry some factors that directly or indirectly contribute in virulence. Many of the proteases are toxic in nature for a host cell for instance metalloproteases. A metalloprotease is a type of protease that contain zinc (II) ion in catalytic site [13]. *Vibrio vulnificus*, an opportunistic human pathogen causative agent of septicemia along with edematous lesions, secretes metalloprotease that enhance the vascular permeability by increased production of mediators involved in inflammation. Moreover, metalloprotease of *V. cholerae* play role in the activation of cholera toxin. Another metalloprotease from *V. cholerae* serovar O1, a type of human enteropathogenic vibrios, are involved in attachment of bacteria to epithelial cells of intestine by digestion of intestinal mucosa which serves as barrier on epithelial surface. These digested fragments can serve as nutritional source for microorganisms hence supporting their reproduction [13].

Metalloprotease of *P. aeruginosa* [14] or *S. marcescens* [15] causes necrosis along with liquid release from cornea that is result of digestion of proteoglycan, a chief structural constituent of the cornea. *P. aeruginosa* elastase (An enzyme from thermolysin family) or *V. vulnificus* metalloprotease, are known to induce haemorrhagic damage to tissue upon inoculation into lung or dorsal skin [16]. Some proteases also show toxic effects, For instance exfoliative toxin, also known as epidermolytic toxins (ETs), is produced by *S. aureus* and is responsible to cause blisters during diseases [17]. Epidermolytic toxins are known to recognize and cleave desmoglein 1, a cadherin that connects the junctions in epithelial cell sheets, so lead to loss of superficial skin layer in infants [17]. Proteases can be further explained as performing different tasks utilising an existing product by bringing slight changes in it. All the evidences discussed above sounds enough to portray proteases as a deadly bacterial weapon that is the symbol of a bacterial innovation.

Weapon deployments

Proteases are the bacterial weapons having multi roles which can attack host cells or tissues directly or indirectly which makes it an important factor in regard to virulence. Proteases are also involved in communication among bacterial cells through a process called quorum sensing which serve as an efficient virulence factor in some bacteria that aids in spread of infection by dispersion of cells from biofilm and some are also involved in altering permeability of tissues. Proteases are also helpful in enhancement of uptake some of essential elements required for growth. Bacterial proteases can directly hydrolyze tissues of host cells and defense components by the release of hydrolytic proteases, and indirectly can exploit host own proteases for mass destruction of its own tissues by targeting the components involved in regulation of expression of host protease. Furthermore, proteases are an important bacterial weapon when it comes to tackle host immune responses and complement systems. Some Bacterial proteases and their action are listed in Table 1.

Table 1: Some bacterial proteases and their role in pathogenesis.

Pathogen	Protease	Function	Reference
S. aureus	SpLA-F ScpA	Biofilm dispersal	(Shaw *et al*., 2004)
	Ssp(V8)	Bacterial adhesion	(Koziel & Potempa, 2013)
	ClpP	Help to withstand oxidative stress	(Ingmer & Brondsted, 2009)
	Aureolysin	Complement inactivation	(Koziel & Potempa, 2013)
Staphylococcus Epidermitis	SepA Ecp	Colonisation Connective tissue destruction	(Koziel & Potempa, 2013)
Streptococcus pyogenes	Streptolysin S	Skin penetration	(Koziel & Potempa, 2013)
	SpeB	Intracellular survival	(Koziel & Potempa, 2013)
Pseudomonas aeruginosa	Elastase	Host immune dysregulation, inhibiting neutrophil function	(Matsumoto, 2004)
Proteus mirabilis	ZapA	Colonisation	(Koziel & Potempa, 2013)

A. Altering tissue or organ permeability for colonization

Contact system plays an important role in innate immunity of skin. If it is activated it provides peptides having bactericidal action and these are derived from kininogens which play their role in entrapping pathogen and carry out its phagocytes [19]. Some peptides called kinins i.e. bradykinin (BK) and its associated metabolite have ability to act as co-mitogens stimulating proliferation of cells and can also serve as proinflammatory factors which have important role in vascular permeability and pain spread [22]. At start antimicrobial factors like antibodies and complement factors are supplied by vascular leakage and it results in leukocyte penetration that now eliminates pathogen. Bacterial proteases have ability to induce a high inflammatory reaction like that results in serious tissue damage. Some bacteria use this process as their pathological weapon. However, most virulent action of *V. vulnificus* metalloprotease

lies in ability to enhance vascular permeability [13]. The metalloprotease act on mast cell and stimulate release of histamine from these cells, involved in enhancement of permeability whose expression was abolished by synchronised release of an anti-histaminic agent. In an experiment it was demonstrated that the metalloprotease of *V. vulnificus* in direct interaction with mast cells isolated from rat cells induced the release of exocytotic histamine within five seconds in rat [13]. However, when this experiment was performed in guinea pig skin a factor other than histamine was involved in permeability enhancement, most likely because of activation of a Hageman factor-plasma kallikrein-kinin cascade shown in figure 1 [13].

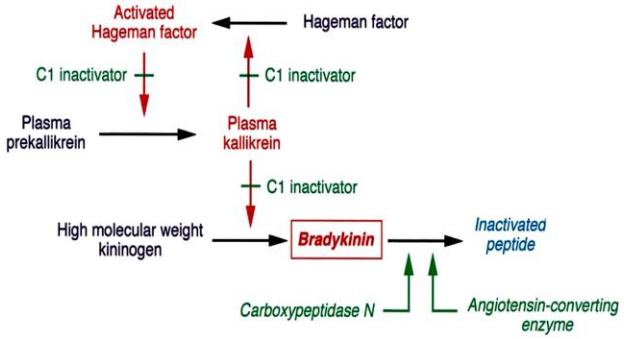

Fig 1: Kallikrein-kinin system is activated by release of Hageman factor in activated form and is involved in production of bradykinin stimulating permeability.

Kallikrein-kinin system is activated by release of Hageman factor in activated form, involved in production of kallikrein (serine protease subgroup) from plasma pre-kallikrein [19]. Plasma kallikrein act on high molecular weight kininogen that act as its substrate and which releases bradykinin. First of all kallidin is produced as a result of kallikrein action that is then further converted in to bradykinin and in turn bradykinin increases vascular permeability [23], [24]. Bacterial proteases have ability to activate pre-kallikrein [19]. Proteases of some bacteria activate Hageman Factor and Pre-kallikrein while some have ability to release kinins directly by attacking kininogen and cleaving its peptide bonds [19]. Exploiting these mechanism bacterial proteases enhances permeability of a tissue or organ and this helps them to colonize into deeper layers of tissue or organs.

B. Host tissue damage

Bacterial proteases are known to damage host tissue by two contrasting mechanisms that include direct digestion of protein components of a tissue i.e. by cleavage of structural proteins in tissue by proteases released by bacteria and indirect damage by targeting components which regulates expression of host protease and by induction of an over expressed host inflammatory response. An example of direct tissue damage includes release of exfoliative toxin, also known as epidermolytic toxins (ETs), by *S. aureus*. Epidermolytic toxins (ETs) are responsible to cause blisters during diseases like bullous impetigo and staphylococcal scaled-skin syndrome (SSSS). These diseases especially staphylococcal scalded skin syndrome predominantly affects infants and is characterized by the loss of superficial skin layers, dehydration and secondary infections. The toxin is actually a serine protease of size ~30 kDa with narrow substrate specificity. ET is known to recognize and cleaves desmoglein 1 only. The desmoglein 1 is a type of desmosomal cadherin that connects the junctions in epithelial cell sheets [17]. However Damage of desmoglein 1 by ETs in the deep layers of skin can be compensated by another type of desmoglein, desmoglein 3. Therefore considerable damage only occurs in upper layers like Stratum granulosum (SG), the second layer of epidermis from surface, where desmoglein 3 would not be available as alternative [17]. Three types of exfoliative toxins (ET), namely A, B and D, are yet identified which are encoded by eta, etb and etd genes respectively [25]. However, expression of 2 genes eta and etb is known to be regulated by Accessory gene regulator (agr) system [26]. Another example of direct tissue damage by proteases includes the *S. aureus* extracellular serine protease, glutamylen- o-peptidase SspA, commonly called as V8 protease. It is also regulated by the agr system. SspA cleaves peptide bonds with glutamate to a greater degree or sometimes with aspartate at the carboxyl-terminal side [27]. Recently SspA has been reported to impair the epidermal permeability barrier in nude mice by disturbing the structure of the Stratun corneum (SC), an epidermal layer adjacent to dermis. Furthermore, electron microscopy of Stratun corneum has shown significant drop in number of adhesive corneocytes on the skin of mice on which protease is applied. On the bases of high degree of similarity between the primary and tertiary SspA structures and exfoliative toxins for Glu-Xaa peptide bonds it can be speculated that the epidermal permeability results after damage of desmoglein 1 present in corneo desmosomes. Due to disruption of the epithelial barrier by *S. aureus* extracellular protease protective functions of the skin are adversely affected due to entry of allergens and microorganisms [28] - [30]. This can additionally aggravate allergic reactions attributable to mast cell activation by infection [31]. Once keratinocyte barrier is disrupt by proteolytic activity of enzymes released by skin pathogens, and then it became comparatively easier for them to penetrate further to underlying tissue layers on behalf of their by-products. For instance recent studies have shown that the translocation of *S. pyogenes* through damaged skin has been facilitated by an oxygen stable hemolytic exotoxin called as Streptolysin S which cleavages of transmembrane junctional proteins including E-cadherin. Interestingly, Streptolysin works in collaboration with a host cysteine protease calpain [32]. Elastin and collagen are important connective tissue components of the skin. Two cysteine proteases staphopain A (ScpA) and staphopain B (SspB) produced

by *S. aureus* are known to target connective tissues present in skin. ScpA bears elastinolytic properties, which can be a possible reason behind destruction of connective tissue during staphylococcal infections [33]. Furthermore, staphopains are also reported to degrade collagen at concentrations as low as 10 nM [34]. In addition with ScpA cell wall associated cysteine protease Ecp produced by a predominant inhabitant of human skin, *S. epi dermidis* also possesses elastinolytic activity and possibly contributes towards the invasiveness and pathogenic potential of *S. epidermidis* [35]. Direct tissue damage caused by bacterial proteases is not restricted to skin only. *P. aeruginosa* elastase directly affects endothelial cells and destroys the basement membrane of blood vessels to cause hemorrhage [36]. Furthermore, it extensively degrades intact basement membranes from bovine anterior lens capsules, bovine glomeruli, and bovine lung [37]. These conclusions are evidence based as hydroxyproline was released when collagen IV was degraded by P. aeruginosa elastase in a test study [37]. Furthermore, purified *P. aeruginosa* elastase and alkaline protease are reported to rapidly cleave soluble laminin (a protein that helps cementing cells together). However, each enzyme yields different cleavage products i.e. Pseudomonas elastase produced rapid and extensive degradation of both A and B chains of laminin protein along with disulfide-rich regions. Alkaline protease on the other hand rapidly degraded the A chain while slowly degrading the B chain [38]. Another evidence include release of immune reactive laminin from authentic basement membranes after incubation with either enzyme indicates a direct role for elastase and alkaline protease in both tissue invasion and hemorrhagic tissue necrosis in infections caused by *P. aeruginosa* [38]. There are also evidences present for a role of elastase in localized infections such as experimental pseudomonas keratitis, pneumonia, and burn infection [39].

The metalloprotease from *P. aeruginosa* [14] or S. marcescens [15] are also known to target eyes by causing liquefactive necrosis of the cornea through digestion of the proteoglycan ground substance, a major structural component of the cornea. Furthermore, according to studies *P. aeruginosa* elastase or *V. vulnificus* metalloprotease induces hemorrhagic tissue damage when inoculated into the lung or dorsal skin [16]. Mass destruction of host tissues can also be done by host's own protease so bacterial proteases have to play just an indirect role here. Human as host deals with its own proteases by strict regulation and restricting their activities. Host appears to activate proteolytic enzymes only when and where they are needed and to inactivate them as soon as they are no longer needed [40], [41]. Host proteases are usually expressed in inactive or precursor forms called zymogens. These zymogens are often further restricted by compartmentalization in the cells or in the body as in the case of lysosomal enzymes [42]. For instance Matrix metalloproteinases are produced as zymogens by a variety of host cell types and specific inhibitors of these enzymes are instantly present in plasma like a2-macroglobulin and in tissue like TIMPs that are ready to inactivate these enzymes when they are no longer needed [41].

The protease cascade systems of plasma are based on highly regulated zymogen systems. These systems include the complement system, the kinin-generating system (Bradykinin is a type of plasma hormone that are responsible for dilation of blood vessels and drop in blood pressure, the contraction of muscles in the lungs, intestines, and uterus, and pain). Each system generates a group of serine proteases and for each of them a group of specific inhibitors called serpins (serine protease inhibitors) are also synthesized by the host [43]. Such complex, multilevel control mechanisms operating protease cascade systems of plasma are frequent in the fibrinolytic system. A Functional end product of the fibrinolytic system is a serine protease called as Plasmin, which further mediates process of thrombolysis. This Plasmin plays an important role in many normal cell processes, including ovulation, wound healing and spermatogenesis, development of embryo, mammary gland involution and prohormone processing [44]. Presence of free active plasmin in the body is a rare event, However the zymogen form of plasmin, plasminogen, is abundantly present in plasma and in most body fluids. A single step activation of plasminogen to plasminis is accomplished by the serine proteases [45]. So a bacterium can cause indirect tissue damage by simply by using host proteases against its own tissues. In host the concentration or amount of host proteases are regulated in appropriate fashion, the protein based regulatory enzymes can be an easy target for bacterial proteases. If any of the proteins part of regulatory cascade is cut down the whole system collapse resulting in uncontrolled release of host proteases which can cause serious damage to host tissues. For instance Bacterial pathogens with plasminogen receptors may mimic host cells and carry out pericellular proteolysis using a pirated host enzyme (by virtue of being cell-bound) that cannot be inactivated. These organisms have obtained a cell surface protease that will activate host protease cascade systems and lyse most proteins in basement membranes and the extracellular matrix [45].

C. Role of proteases in biofilm detachment & quorum sensing

Role of proteases in dispersal of biofilm was first of all described during the examination of *S. aureus* strains that were deficient in the global regulators like sarA and sigB [46]. These strains were unable to form biofilm. Study of these mutants exposed, that the observed biofilm ability was resulted from increased protease activity [47]. Important matrix proteins were degraded by high protease activity and this resulted in destabilization of the biofilm helping biofilm dispersal [48]. Degradation ability of different proteases like the staphopains (SspB and ScpA), V8 serine protease (SspA) and Aureolysin (Aur) with the relative importance to degrade biofilms have been established, varying between different strains and under different conditions [49]. Staphopains even have ability disrupt the matrix of biofilm but its target proteins have not been characterized despite some of the specific matrix proteins have been identified as targets for proteases degradation. Quorum sensing is important in regulation of process of biofilm degradation like increased production of proteases is controlled through the quorum sensing system. *S. aureus* Quorum sensing system contains *agr* system [50]. An auto inducing peptide (AIP) plays role in activation of *agr* system. Agr system detects this AIP and gets activated. *Agr* operon encodes and produces this AIP that is detected by a two-component system which than produces regulatory RNA, RNAIII involved in

virulence [49]. This virulence state is regulated by the activation of production of secreted enzymes and toxins and down regulating the surface factors. Now the enzymes secreted by *agr* system play their role in biofilms dispersal [51]. Thus the activation *agr* system can be effective in shifting of a biofilm state to a planktonic phase of growth.

D. Enhancement of uptake of essential elements

All bacteria require some essential factor for growth. Iron is one of these factors that are essential for growth of bacteria. To persist infection bacteria must have ability to uptake these elements because most of these elements are not freely available. For instance iron in human body is stored in the form of heme proteins i.e. hemoglobin and myoglobin while small quantity of iron is linked with some carrier proteins of host like lactoferrin. In an experiment metalloprotease containing *V. vulnificus* L-180, was found to have ability to extract iron from these proteins instead of mutant strains deficient in these enzymes was not able to as use the iron associated to proteins [13]. Upon addition of protease bacteria was able to assimilate iron. These results show important role of the protease regarding uptake of iron bound to proteins. *V. vulnificus* uptake iron using metalloprotease is shown in figure 2 [13]. The metalloprotease degrade heme proteins and in result releases heme residue which is then easily up taken by bacteria. While iron attached to carrier protein lactoferrin is transferred to an iron chelator named vulnibactin that is secreted by *V. vulnificus*, following breakdown of carrier protein. The iron attached to vulnibactin is conveyed to the bacterial cells [13].

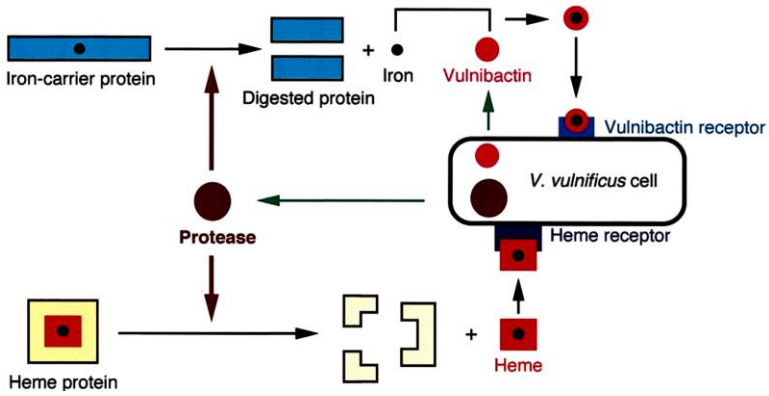

Fig 2: The metalloprotease degrade heme proteins and in result releases heme residue which is then directly up taken by bacteria. In case of carrier protein an iron chelator is involved in transport of iron to bacterial cell.

E. Tackling Host defence systems

Defence mechanism involves macrophages and neutrophils that are transported to subepithelial tissues following an infection. Most important step is recognition of pathogens, when recognized engulfed by macrophages and neutrophils. Antibody opsonisation is an approach in which a pathogen is labelled for ingestion and elimination by phagocytes. Some bacteria like *S. pyogenes* have ability to evade such antibody-mediated process of phagocytosis by producing protease IdeS that cleaves IgG [19]. Following cleavage of these IgG some bacterial proteins capture these broken antibodies components on their surface, this helps bacteria to build coat of host molecules which protect against proteolysis [52]. ScpC protease of *S. pyogenes* affects a chemokine interleukin-8 that is involved in migration of neutrophils. In an experiment, bacteria producing ScpC was able to evade phagocytosis because of inhibition of transport of neutrophils to infected tissue in mice [53]. Complement system contributes in recognition of antigens that enter body. To survive pathogen must have ability to tackle complement system. Some bacteria have ability to stop whole pathway activation or destabilizing it by degradation of components of complement system using proteases [13]. In an experiment metalloprotease of *V. vulnificus* was added to human serum and its activity was checked and in result this metalloprotease was able to inactivate both antibody dependent and antibody independent pathways of recognition in dose dependant manner. C5 component of complement system produces C5a peptide which recruits neutrophils for action on the site of infection, while C5a was inhibited on treatment with a bacterial protease serralysin [13].

A component of complement system C3b is first released and deposited on bacterial surface and in turn C5a is released. Bacterial protease serralysin has ability to inhibit deposition of C3b disarming antibacterial functions that are complement-dependent [19]. C3b deposition is inhibited by breakdown of C3 which forms C3b [54]. *S. pyogenes*, protease SpeB also have ability to degrade C3 [55]. SpeB protease also has ability to cleave properdin a protein that positively regulates complement system by stabilizing the formation of C5 complex [56]. C5a peptidase is a strong contributor of pathogenicity in *S. pyogenes* and might play indispensable role in protecting streptococci from phagocytosis [57]. The humoral immune response is also controlled by a number of cytokines which can also be effected metalloproteases of bacteria, in a study cytokines lost their proper functioning on incubation with these proteases of bacteria [13]. It was proposed that these liberate certain proteins serving as receptors for these cytokines [13]. Some proteases show a wide range of activity like a bacterial protease called protease IV can degrade lysine containing peptides and thus can degrade some important proteins like immunoglobulins and components of complement system, plasminogen and fibrinogen [58].

Conclusion

These normal cellular functions of proteases include protein digestion (pepsin), quality control (FtsH), control of cell secretions (Lon), regulation of flagella synthesis (clpXP) and signal transduction (YluC). However, some bacteria proved to be even smarter as they managed to use proteases as an effective weapon. As a bacterial weapon these have potential to disfigure host tissues (glutamylen- o-peptidase SspA) confront host defenses (ScpC) and to escalate infection (V8 serine protease). Studies presented above portray proteases as useful bacterial weapon in pathogenesis besides performing normal functions in body.

Future recommendations

There is need to go in depth of these mechanisms used by bacteria during pathogenesis and find out ways to target these, paving the way in development of diverse antibacterial drugs as world is facing antibiotic crisis.

References

1. D. Z. Rudner, P. Fawcett and R. Losick, "A family of membrane-embedded metalloproteases involved in regulated proteolysis of membrane-associated transcription factors," *Proceedings of the National Academy of Sciences*, vol. 96, no. 26, pp. 14765-14770, 1999.
2. L. N. Kinch, K. Ginalski and N. V. Grishin, "Site-2 protease regulated intramembrane proteolysis: Sequence homologs suggest an ancient signaling cascade," *Protein science*, vol. 15, no. 1, pp. 84-93, 2006.
3. J. S. Schneider and M. S. Glickman, "Function of site-2 proteases in bacteria and bacterial pathogens," *Biochimica et Biophysica Acta (BBA)-Biomembranes*, vol. 1828, no. 12, pp. 2808-2814, 2013.
4. K. Ito and Y. Akiyama, "Cellular functions, mechanism of action, and regulation of FtsH protease," *Annu. Rev. Microbiol.*, vol. 59, pp. 211-231, 2005.
5. A. Takaya, T. Tomoyasu, A. Tokumitsu, M. Morioka and T. Yamamoto, "The ATP-dependent lon protease of *Salmonella enterica* serovar typhimurium regulates invasion and expression of genes carried on Salmonella Pathogenicity Island 1," *J. Bacteriol.*, vol. 184, pp. 224-232, 2002.
6. J. D. Boddicker and B. D. Jones, "Lon protease activity causes downregulation of Salmonella pathogenicity island 1 invasion gene expression after infection of epithelial cells," *Infect. Immun.*, vol. 72, pp. 2002- 2013, 2004.
7. D. Zhou and J. Galán, "Salmonella entry into host cells: the work in concert of type III secreted effector proteins," *Microbes and Infection*, vol. 3, no. 14, pp. 1293-1298, 2001.
8. L. M. Schechter and C. A. Lee, 2001, "AraC/XylS family members, HilC and HilD, directly bind and derepress the *Salmonella typhimurium* hilA promoter," *Molecular microbiology*, vol. 40, no. 6, pp. 1289-1299, 2001.
9. A. Takaya, Y. Kubota, E. Isogai and T. Yamamoto, "Degradation of the HilC and HilD regulator proteins by ATP-dependent Lon protease leads to downregulation of Salmonella pathogenicity island 1 gene expression," *Mol. Microbiol*, vol. 55, pp. 839-852, 2005.
10. H. C. Ramos, M. Rumbo and J. C. Sirard, "Bacterial flagellins: mediators of pathogenicity and host immune responses in mucosa," *Trends Microbiol*., vol. 12, pp. 509-517, 2004.
11. T. Tomoyasu, T. Ohkishi, Y. Ukyo, A. Tokumitsu, A. Takaya, M. Suzuki, K. Sekiya, H. Matsui, K. Kutsukake and T. Yamamoto, "The ClpXP ATP-dependent protease regulates flagellum synthesis in Salmonella enterica serovar Typhimurium," *Journal of bacteriology*, vol. 184, no. 3, pp. 645-653, 2002.
12. T. Tomoyasu, A. Takaya, E. Isogai and T. Yamamoto, "Turnover of FlhD and FlhC, master regulator proteins for Salmonella flagellum biogenesis by the ATP-dependent ClpXP protease," *Mol. Microbiol*., vol. 48, pp. 443-452, 2003.
13. S. I. Miyoshi and S. Shinoda, "Microbial metalloproteases and pathogenesis," *Microbes and infection*, vol. 2, no. 1, pp. 91-98, 2000.
14. A. S. Kreger and L. D. Gray, "Purification of *Pseudomonas aeruginosa* proteases and microscopic characterization of pseudomonal protease-induced rabbit corneal damage," *Infection and immunity*, vol. 19, no. 2, pp. 630-648, 1978.
15. D. Lyerly, L. Gray and A. Kreger, "Characterization of rabbit corneal damage produced by *Serratia keratitis* and by a serratia protease," *Infection and immunity*, vol. 33, no. 3, pp. 927-932, 1981.
16. S. I. Miyoshi, H. Nakazawa, K. Kawata, K. I. Tomochika, K. Tobe and S. Shinoda, "Characterization of the hemorrhagic reaction caused by *Vibrio vulnificus* metalloprotease, a member of the thermolysin family," *Infection and immunity*, vol. 66, no. 10, pp. 4851-4855, 1998.
17. M. Amagai, K. Nishifuji, T. Yamaguchi, Y. Hanakawa, M. Sugai and J. R. Stanley, "Staphylococcal exfoliative toxin B specifically cleaves desmoglein 1," *Journal of investigative dermatology*, vol. 8, no. 5, pp. 845-850, 2002.
18. L. Shaw, E. Golenka, J. Potempa and S. J. Foster, "The role and regulation of the extracellular proteases of *Staphylococcus aureus*," *Microbiology*, vol. 150, pp. 217–228, 2004.
19. J. Koziel and J. Potempa, "Protease-armed bacteria in the skin," *Cell and tissue research*, vol. 351, no. 2, pp. 325-337, 2013.
20. H. Ingmer and L. Brøndsted, "Proteases in bacterial pathogenesis," *Research in microbiology*, vol. 160, no. 9, pp. 704-710, 2009.
21. K. Matsumoto, "Role of bacterial proteases in pseudomonal and serratial keratitis," *Biological chemistry*, vol. 385, no. 11, pp. 1007-1016, 2004.

22. E. Schremmer-Danninger, A. Hermann, E. Fink, H. Fritz and A. A. Roscher, "Identification and occurrence of mRNAs for components of the kallikrein–kinin system in human skin and in skin diseases," *Immunopharmacology*, vol. 43, no. 2 pp. 287-291, 1999.
23. C. G. Cochrane and J. H. Griffin, "The biochemistry and pathophysiology of the contact system of plasma," *Adv. Immunol.*, vol. 33, pp. 241–306, 1982.
24. J. Chao, B. Shen, L. Gao, C. F. Xia, G. Bledsoe and L. Chao, "Tissue kallikrein in cardiovascular, cerebrovascular and renal diseases and skin wound healing," *Biol Chem.*, vol. 391, pp. 345–355, 2010.
25. C. Y. Lee, J. J. Schmidt, A. D. Johnson-Winegar, L. Spero and J. J. Iandolo, "Sequence determination and comparison of the exfoliative toxin A and toxin B genes from *Staphylococcus aureus*," *Journal of bacteriology*, vol. 169 no.9, pp. 3904-3909, 1987.
26. B. J. Sheehan, T. J. Foster, C. J. Dorman, S. Park and G. S. Stewart, "Osmotic and growth-phase dependent regulation of the eta gene of *Staphylococcus aureus*: a role for DNA supercoiling," *Molecular and General Genetics MGG*, vol. 232, no. 1, pp. 49-57, 1992.
27. T. Ono, Y. Ohara-Nemoto, Y. Shimoyama, H. Okawara, T. Kobayakawa, T. T. Baba, S. Kimura and T.K. Nemoto, "Amino acid residues modulating the activities of staphylococcal glutamyl endopeptidases," *Biological chemistry*, vol. 39, no.10, pp. 1221-1232, 2010.
28. T. Kato, T. Takai, K. Mitsuishi, K. Okumura and H. Ogawa, "Cystatin A inhibits IL-8 production by keratinocytes stimulated with Der p 1 and Der f 1: biochemical skin barrier against mite cysteine proteases," *Journal of allergy and clinical immunology*, vol. 116 no. 1, pp. 169-176, 2005.
29. Y. Kikuchi, T. Takai, T. Kuhara, M. Ota, T. Kato, H. Hatanaka, S. Ichikawa, T. Tokura, H. Akiba, K. Mitsuishi and S. Ikeda, "Crucial commitment of proteolytic activity of a purified recombinant major house dust mite allergen Der p1 to sensitization toward IgE and IgG responses," *The journal of immunology*, vol. 177, no. 3, pp. 1609-1617, 2006.
30. T. Nakamura, Y. Hirasawa, T. Takai, K. Mitsuishi, M. Okuda, T. Kato, K. Okumura, S. Ikeda and H. Ogawa, "Reduction of skin barrier functions by proteolytic activity of a recombinant house dust mite major allergen Der f 1," *J. Inves.t Dermatol.*, vol. 126, pp. 2719–2723, 2006.
31. S. M. McAlpine, M. Enoksson, C. Lunderius-Andersson and G. Nilsson, "The effect of bacterial, viral and fungal infection on mast cell reactivity in the allergic setting," *Journal of innate immunity*, vol. 3, no. 2, pp. 120-130, 2011.
32. T. Sumitomo, M. Nakata, M. Higashino, Y. Jin, Y. Terao, Y. Fujinaga and S. Kawabata, "Streptolysin S contributes to group A streptococcal translocation across an epithelial barrier," *Journal of Biological Chemistry*, vol. 286, no. 4, pp. 2750-2761, 2011.
33. J. Potempa, A. Dubin, G. Korzus and J. Travis, "Degradation of elastin by a cysteine proteinase from *Staphylococcus aureus*," *J. BiolChem*., vol. 263, pp. 2664–2667, 1988.
34. T. Ohbayashi, A. Irie, Y. Murakami, M. Nowak, J. Potempa, Y. Nishimura, M. Shinohara and T. Imamura, "Degradation of fibrinogen and collagen by staphopains, cysteine proteases released from *Staphylococcus aureus*," *Microbiology*, vol. 157, pp. 786–792, 2010.
35. A. Oleksy, E. Golonka, A. Bańbuła, G. Szmyd, J. Moon, M. Kubica, D. Greenbaum, M. Bogyo, T. J. Foster, J. Travis and J. Potempa, "Growth phase-dependent production of a cell wall-associated elastinolytic cysteine proteinase by Staphylococcus epidermidis," *BiolChem.*, vol. 385, pp. 525–535, 2004.
36. Y. Komori, T. Nonogaki and T. Nikai, "Hemorrhagic activity and muscle damaging effect of *Pseudomonas aeruginosa* metalloproteinase (elastase)," *Toxicon*, vol. 39, no. 9, pp. 1327-1332, 2001.
37. P. A. Bejarano, J. P. Langeveld, B.G. Hudson and M. E. Noelken, "Degradation of basement membranes by Pseudomonas aeruginosa elastase," *Infect. Immun*., vol. 57, pp. 3783-3787, 1989.
38. L. W. Heck, K. Morihara and D. R. Abrahamson, "Degradation of soluble laminin and depletion of tissue-associated basement membrane laminin by *Pseudomonas aeruginosa* elastase and alkaline protease," *Infect. Immun.*, vol. 54, pp. 149–153, 1986.
39. B. Wretlind and O. R. Pavlovskis, "*Pseudomonas aeruginosa* elastase and its role in pseudomonas infections," *Review of Infectious Diseases*, vol. 5, no. 5, pp. 998-1004, 1983.
40. O. Saksela and D. B. Rifkin, "Cell associated plasminogen activation. Regulation and physiological functions," *Ann. Rev. Cell Biol*., vol. 4, pp. 93-126, 1988.
41. L. M. Matrisian and B. L. Hogan, "8 Growth Factor-Regulated Proteases and Extracellular Matrix Remodeling during Mammalian Development," *Current topics in developmental biology*, vol. 24, pp. 219-259, 1990.
42. A. A. Salyers and D. D. Whitt, "Host defenses against bacterial pathogens: defenses of tissue and blood," *Bacterial Pathogenesis a Molecular Approach*, pp. 16-29, 1994.
43. L. T. Hunt and M. O. Dayhoff, "A surprising new protein superfamily containing ovalbumin, antithrombin-III, and alpha1-proteinase inhibitor," *Biochemical and biophysical research communications*, vol. 95, no. 2, pp. 864-871, 1980.
44. B. M. Schäfer, K. Maier, U. Eickhoff, R. F. Todd, and M. D. Kramer, "Plasminogen activation in healing human wounds," *The American journal of pathology*, vol. 144, no. 6, pp. 1269, 1994.
45. M.S. Lantz, "Are bacterial proteases important virulence factors?," *Journal of periodontal research*, vol. 32, no. 1, pp. 126-132, 1997.
46. S. Bronner, H. Monteil and G. Prévost, "Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications," *FEMS microbiology reviews*, vol. 28, no. 2, pp. 183-200, 2004.

47. L. H. Tsang, J. E. Cassat, L .N. Shaw, K. E. Beenken, and M. S. Smeltzer, "Factors contributing to the biofilm-deficient phenotype of *Staphylococcus aureus sarA*mutants," *PLoSONE,* vol. 3, no. 10, pp. 3361, 2008.
48. A. K. Zielinska, K. E. Beenken, L. N. Mrak, H. J. Spencer, G. R. Post, R. A. Skinner, A. J. Tackett, A. R. Horswill and M. S. Smeltzer, "SarA-mediated repression of protease production plays a key role in the pathogenesis of *Staphylococcus aureus* USA300 isolates," *Molecular microbiology,* vol. 86, no. 5, pp. 1183-1196, 2012.
49. J. L. Lister and A. R. Horswill, "Staphylococcus aureus biofilms: recent developments in biofilm dispersal. Biofilm formation by staphylococci and streptococci: Structural, functional and regulatory aspects and implications for pathogenesis," *Front. Cell. Infect. Microbiol.,* vol. 4, Dec. 2014.
50. M. Thoendel, J. S. Kavanaugh, C. E. Flack, and A. R. Horswill, "Peptide Signalling in the staphylococci," *Chem. Rev.,* Vol. 111, pp. 117–151, 2011.
51. A. Peschel and M. Otto, "Phenol-soluble modulins and staphylococcal infection," *Nat. Rev. Microbiol*., vol. 11, pp. 667–673, 2013.
52. Y. F. Su, W. J. Chuang, S. M. Wang, W. Y. Chen, C. Chiang-Ni, Y. S. Lin, J. J. Wu and C. C. Liu, "The deficient cleavage of M protein-bound IgG by IdeS: insight into the escape of *Streptococcus pyogenes* from antibody-mediated immunity," *Molecular immunology,* vol. 49, no. 1, pp. 134-142, 2011.
53. H. Sjölinder, L. Lövkvist, L. Plant, J. Eriksson, H. Aro, A. Jones and A. B. Jonsson, "The ScpC protease of *Streptococcus pyogenes* affects the outcome of sepsis in a murine model," *Infection and immunity*, vol. 76, no. 9, pp. 3959-3966, 2008.
54. A. J. Laarman, M. Ruyken, C. L. Malone, J. A. van Strijp, A. R. Horswill and S. H. Rooijakkers, "*Staphylococcus aureus* metalloprotease aureolysin cleaves complement C3 to mediate immune evasion," *The Journal of Immunology*, vol. 186, no. 11, pp. 6445-6453, 2011.
55. C.F. Kuo, Y. S. Lin, W. J. Chuang, J. J. Wu and N. Tsao, "Degradation of complement 3 by streptococcal pyrogenic exotoxin B inhibits complement activation and neutrophil opsonophagocytosis," *Infection and immunity*, vol. 76, no. 3, pp. 1163-1169, 2008.
56. N. Tsao, W. H. Tsai, Y. S. Tsai, W. J. Chuang, C. H. Wang and C. F. Kuo, "Streptococcal pyrogenic exotoxin B cleaves properdin and inhibits complement-mediated opsono phagocytosis," *Biochemical and biophysical research communications*, vol. 339, no. 3, pp. 779-784, 2006.
57. Y. Ji, L. McLandsborough, A. Kondagunta and P. P. Cleary, "C5a peptidase alters clearance and trafficking of group A streptococci by infected mice," *Infection and immunity*, vol. 64, no. 2, pp. 503-510, 1996.
58. L. S. Engel, J. M. Hill, A. R. Caballero, L. C. Green and R. J. O'Callaghan, "Protease IV, a unique extracellular protease and virulence factor from *Pseudomonas aeruginosa*," *J. Biol. Chem.,* vol. 273, pp. 16792–16797, 1998.