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Bacterial Extracellular Zinc-Containing Metalloproteases

CLAUDIA C. HÄSE AND RICHARD A. FINKELSTEIN*

Department of Molecular Microbiology and Immunology, School of Medicine, University of Missouri, Columbia, Missouri 65212

PROLOGUE

In 1961, when we began studies of hemagglutinins (HAs) produced by *Vibrio cholerae* (71) and their possible role as adhesins/colonization factors, we were unaware that there were zinc-containing bacterial metalloproteases or that we would find ourselves involved with them 30 years later. In 1976, we became interested in a specific HA which is found in supernatants of cultures of *V. cholerae* (67). It didn't seem a particularly smart thing to do for the vibrios to give off a factor which theoretically might occupy receptors and thereby prevent colonization. Thus this soluble HA was all the more interesting to us. Attempts at purification of the protein met with little success until we entertained the notion that perhaps we were copurifying a protease which was destroying the HA during purification. To our amazement, the HA turned out to be a protease (70), and we subsequently found that other proteases were HAs (23). The HA/protease contained zinc and calcium (23) and was active on a number of putatively relevant physiological substrates (69), including fibronectin, lactoferrin, and mucin (we had actually rediscovered the enzyme studied by Sir Macfarlane Burnet in the 1940s). The enzyme can also nick, and thus activate, the A-subunit proteins of cholera toxin (24) and other cholera toxin-related enterotoxins. A major breakthrough occurred when Bever and Iglewski cloned and sequenced the elastase gene of *Pseudomonas aeruginosa* (15). Elastase is a zinc-containing metalloprotease which is believed to participate in pathogenesis and the tissue changes associated with pseudomonas infections, especially in the lung. The N-terminal amino acid sequence of elastase was practically the same as that of our enzyme, and that observation helped us to clone the *V. cholerae* HA/protease (92). Subsequently, we were unable to escape the recognition that there is, in fact, a large world of zinc-containing bacterial endopeptidases distributed among pathogens and nonpathogens and among industrially important microorganisms. It is the purpose of this review to gather them under one roof, so to speak, to enable their further examination by subsequent generations of investigators. The field is made all the more exciting by the observations, published during construction of this review, that both botulinum B and tetanus neurotoxins—whose mode of action had long eluded us—are zinc metalloproteases which act specifically on synaptobrevin, an integral membrane protein of small synaptic vesicles, thus blocking neurotransmitter release (206). It may safely be predicted that additional bacterial metalloproteases will be found to be involved in pathogenesis and to serve useful functions as well. The recent announcements (94, 185) of "designer proteins" illustrate the point.

Following submission of this manuscript, Klimpel et al. (120a) reported that the lethal factor (LF) of the anthrax toxin complex has a short region of homology with zinc-binding sites of other metalloproteases containing the HEXXH motif, which is essential for activity. Protease activity and toxicity were inhibited by known inhibitors of metalloproteases.

* Corresponding author.

INTRODUCTION

Proteases are enzymes that catalyze the hydrolysis of peptide bonds in proteins or peptides. They are either exopeptidases, whose actions are restricted to the amino or carboxyl termini of proteins, or endopeptidases, which cleave internal peptide bonds. Proteases are present in all living organisms, in which they display a variety of physiological functions. Microbial proteases are predominantly extracellular and can be classified into four groups based on the essential catalytic residue at their active site. They include serine proteases (EC 3.4.21), cysteine proteases (also called thiol proteases) (EC 3.4.22), aspartate proteases (EC 3.4.23), and the metalloproteases (EC 3.4.24). Most metalloproteases are zinc-containing proteins. Zinc is an integral component of many proteins which are involved in virtually all aspects of metabolism of the different species of all phyla. X-ray crystallographic analyses of several zinc-containing proteins have defined the features of the catalytic and structural zinc-binding sites (reviewed in reference 243). In all zinc enzymes whose crystal structures are known, a catalytic zinc atom is coordinated to three amino acid residues of the protein and an active water molecule, whereas structural zinc atoms are coordinated to four Cys residues (243). A combination of His, Glu, Asp, or Cys residues creates a tridentate active zinc site, and an activated water molecule fills and completes the coordination sphere (242, 243). A potential benefit of our increased understanding of structure-activity relationships of metal-dependent enzymes is the possibility of designing engineered metalloproteins for various purposes (94, 185). The introduction of metal-binding sites into proteins could induce specific and predictable conformational changes as well as allow the regulation of enzymatic activity. Computer analysis may be useful to identify suitable sites in the three-dimensional structure of an enzyme that, when substituted with His amino acid residues, would form a metal-binding site (94). Additionally, a further understanding of the contribution(s) of the remaining framework residues will offer the opportunity of artificially simulating the remarkable specificity of this family of enzymes to create new and useful proteases.

Well-studied metalloproteins have served as standards of reference for the structures of other proteins, and similarities in the primary structure alone have frequently been used to group these proteins. The number of families of eucaryotic zinc proteins is extensive and continues to increase. A recent review classified all metalloendopeptidases into five distinct families based on sequence alignments (107); however, the present review will be restricted to secreted zinc metalloproteases from bacterial species. Among the bacterial metalloproteases that have been examined by X-ray crystallography are the zinc-containing, calcium-stabilized, neutral metalloendopeptidases; thermolysin from *Bacillus thermoproteolyticus* (40, 98), neutral protease from *Bacillus cereus* (184, 218), and elastase from *Pseudomonas aeruginosa* (231). The structures of thermolysin, *B. cereus* neutral protease, and elastase have served for comparison with sequences of other metalloproteases for which no X-ray crystallography standards exist as yet.

The availability of sequence information for the family of zinc-dependent metalloproteases has grown rapidly over the last few years. The first "consensus sequence" for members of the metalloprotease family was based on homology found within the human fibroblast collagenase and the 11 amino acids flanking the zinc-binding site of the *Serratia* protease, a bacterial metalloprotease that also shares strong homology with thermolysin at this site (153). Later, the primary sequence motif HEXXH was found in many zinc-containing proteases, including several eucaryotic zinc metalloproteases, and was suggested to be indicative of membership in the family (108).

Many extracellular bacterial proteases from pathogenic organisms that have been studied in detail have either been demonstrated or suggested to play important roles in virulence. Secreted bacterial metalloproteases have been identified in both gram-positive and gram-negative pathogens, but they are certainly not unique to pathogenic species. There has been greater interest in the proteases of organisms which are pathogenic or of industrial importance than in those of organisms which lack either property.

METALLOPROTEASES FROM GRAM-POSITIVE BACTERIA

Bacillus spp.

Members of the *Bacillus* neutral protease family for which amino acid sequences have been determined, such as neutral proteases from *B. thermoproteolyticus* (thermolysin) (232), *B. stearothermophilus* (124, 175, 226), *B. subtilis* (238, 260), *B. cereus* (213, 253), *B. brevis* (1), *B. polymyxa* (227), *B. caldolyticus* (244), *B. megaterium* (126), *B. amyloliquefaciens* (212, 245), and *B. mesentericus* (221), typically have a high degree of amino acid sequence homology with each other. The zinc-binding sites and catalytic residues among these metalloproteases are conserved (Fig. 1), and all these proteases have large propeptides between their signal sequence and the mature proteins which are removed during secretion. Among known neutral proteases produced by bacilli, thermolysin is the best characterized structurally and enzymatically; its primary and tertiary structures, its active site, and its substrate-binding site have been characterized (29, 97, 149–151). The enzyme consists of a single polypeptide chain lacking thiol or disulfide groups with a zinc ion located at the active site and four calcium ions involved in protein stability (40, 98). The crystal structure of the closely related *B. cereus* neutral protease was shown to be very similar to that of thermolysin, and the proteases contain the same zinc ligands (184, 218). The structure and mechanism of activity of a neutral metalloprotease from *B. subtilis* were very similar to those of thermolysin (139, 214, 235). Site-directed mutagenesis of amino acids predicted to be involved in catalytic activity dramatically affected both catalysis and secretion, indicating that the *B. subtilis* neutral protease is autocatalytically processed (234). At sublethal levels of expression, the gene product is correctly expressed and secreted in *Escherichia coli*, again suggesting that its processing is autogenous (251). The activity of the *B. subtilis* neutral protease has been used as an indicator system for cloning in *B. subtilis* by using milk-clearing halos as a direct screening marker for recombinant clones (258). A minor extracellular metalloprotease from *B. subtilis* was characterized, and its gene was cloned and sequenced; however, this novel cysteine-containing protease had a very short prosequence and showed little similarity to other known bacterial proteases (216). The protease genes of *B. subtilis* have been recently reviewed elsewhere (93). The amino acid sequence of a neutral protease from *B. subtilis* subsp. *amylosaccharitics* was found to be identical to that of *B. subtilis* (122). Studies involving chemical modification and computer-aided modeling indicated that the three-dimensional structure and reaction mechanism of this protease are quite similar to

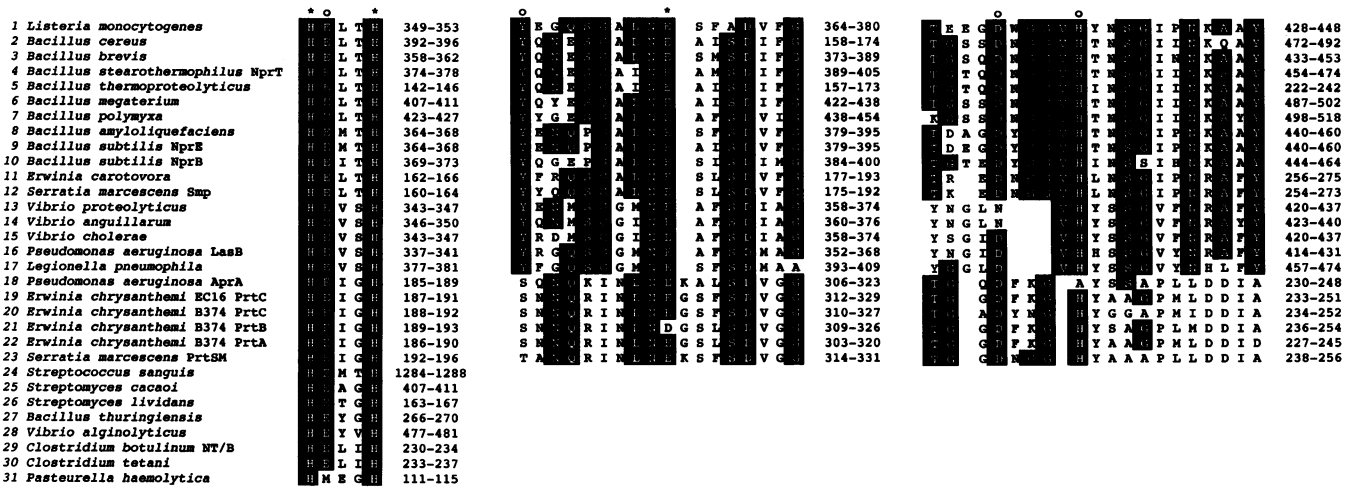

FIG. 1. Amino acid comparison of some conserved domains among bacterial metalloproteases. Identical amino acids are indicated by black boxes. Putative zinc-binding and active-site residues are indicated by asterisks and open circles, respectively. References, in the sequence cited above, are as follows: 1 (54, 155); 2 (213, 253); 3 (1); 4 (226); 5 (232); 6 (126); 7 (227); 8 (212, 245); 9 (260); 10 (238); 11 (129); 12 (128); 13 (49); 14 (157); 15 (92); 16 (15, 76); 17 (16); 18 (60, 180); 19 (45); 20 (52); 21 (51); 22 (80); 23 (27, 169); 24 (82); 25 (34); 26 (30, 140); 27 (142); 28 (228); 29 (127); 30 (64); 31 (2). The amino acid sequences of the *B. mesentericus* (221) and *B. subtilis* subsp. *amylosacchariticus* (122) neutral proteases were identical to that of the *B. subtilis* NprE protease. The sequences of the mature *B. stearothermophilus* NprM (124) and NprS (175) were identical to each other and are the same as the sequence of thermolysin (*B. thermoproteolyticus*) in the regions shown in this figure. Similarly, the *B. caldolyticus* neutral protease (244) is identical to the *B. stearothermophilus* NprT in the regions shown. The *Streptomyces* sp. strain C5 (132) and *S. coelicolor* (47) metalloproteases are identical to the *S. lividans* protease in the amino acid region shown. The amino acid sequence of the *C. botulinum* NT/E neurotoxin (190) is the same as the NT/B sequence shown.

those of thermolysin (121, 240). The amino acid sequence of the neutral zinc metalloprotease from *B. mesentericus* has been derived by using peptide cleavage and was found to be identical to that of the *B. subtilis* protease (221): inhibition experiments confirmed the participation of a predicted histidine residue in catalytic activity (220). Differences in their thermostability are interesting and well-studied aspects of neutral proteases from various *Bacillus* species (72, 104). The thermostabilities of the *B. stearothermophilus* and *B. subtilis* neutral proteases have been studied extensively by site-directed mutagenesis (62, 63, 225, 235, 246). A *B. stearothermophilus* strain which produces a more thermostable neutral protease has been identified, and the gene encoding this protease has been cloned and sequenced (124). The metalloprotease gene from a different *B. stearothermophilus* strain encoded an identical mature enzyme (175). Site-directed mutagenesis of the *B. caldolyticus* protease revealed that different amino acid residues contribute to differences in thermostability (244). The effects of metal ions on activity and stability of *Bacillus* metalloproteases have also been studied (41, 99). It has become evident that the enhanced stability of thermophilic enzymes is the result of a variety of stabilizing effects and cannot be attributed to a common determinant.

As mentioned below with regard to zinc metalloproteases from other genera, some of the *Bacillus* proteases have, or may have, useful applications. A neutral metalloprotease from *B. polymyxa* may be useful therapeutically, especially in dermatology, since it selectively cleaves fibronectin and type IV collagen (219). The *B. polymyxa* enzyme also cleaves the amylase precursor into β- and α-amylases (227) for starch conversion into fermentable sugars, for example. An extracellular collagenase produced by a human oral *B. cereus* strain was characterized as a zinc- and calcium-containing metalloprotease that resembled the *Clostridium histolyticum* collagenases (146). A phosphate-repressed metalloprotease activity was detected in culture supernatants of a *B. cereus* strain isolated from soil; however, it is not clear whether this enzyme activity participates in phosphate scavenging (85). *B. thuringiensis* is an insect pathogen and secretes a protease called immune inhibitor A because it specifically degrades antibacterial proteins produced by the insect host (46). The sequence for this metalloprotease gene has been determined, and, although the amino acid sequence showed no extensive homology to other bacterial proteases, a putative zinc-binding domain can be found (142) (Fig. 1). Protease production of *B. megaterium* was suppressed by temperature at the level of mRNA transcription and was associated with decreased sporangial development (125). The anticancer drug netropsin increased the formation of mRNA coding for the neutral metalloprotease of *B. megaterium* (33) but did not affect its repression by increased temperature.

Clostridium spp.

Protease production by clostridia has been associated with pathogenicity and with food spoilage. *C. histolyticum*, a causative agent of clostridial myonecrosis or gas gangrene, produces a mixture of collagenases, also known as clostridiopeptidase A or collagenase A, which has been studied extensively (209). Six individual collagenases present in the culture filtrate of *C. histolyticum* have been purified and were shown to be calcium-dependent zinc metalloproteases that can be divided into two classes according to structural and biochemical differences (21, 22). Comparison of the six purified collagenases indicated that their secondary structures are very similar and the enzymes are immunologically cross-reactive (22). Class I and class II collagenases had extensive protein sequence homology within each class,

and, although it was suggested that one class evolved from the other by gene duplication, the enzymes in the two classes showed substantially different sequences (22). Studies on the effects of various divalent metal ions on enzyme activity of the clostridial collagenases have suggested differences in the mechanisms of catalysis (10, 11, 113). Six proteases, each differing in activity against various collagen substrates and sensitivity to inhibitors, produced by *C. sporogenes* had characteristics of metalloproteases (9). Proteases secreted by the potential pathogen *C. bifermentans* were predominantly of the metalloprotease type, and although a wide variety of proteins were hydrolyzed, none of the enzymes degraded collagen (145). Although it is known to produce a substantial number of extracellular toxins and other virulence factors, including collagenase, and calcium-dependent serine proteases have been reported (182), the extracellular proteases of *C. perfringens* have not been well characterized (8). Acidolysin is an acidic calcium- and zinc-containing metalloprotease produced by *C. acetobutylicum*, and its N-terminal amino acid sequence showed a high degree of similarity with that of *B. subtilis* neutral metalloprotease (43). Clostridial neurotoxins, including tetanus toxin and seven serotypes of botulinum toxin, are produced by toxigenic strains of *C. tetani* and *C. botulinum*, respectively. Recently, the DNA-deduced amino acid sequences of five botulinum neurotoxin serotypes have been determined and aligned with that of tetanus toxin (172), showing an overall low degree of homology with a few segments of strong similarity including a segment with the HEXXH zinc-binding motif of metalloproteases (Fig. 1). Zinc was then shown to bind to tetanus toxin (207, 257) and to be essential for tetanus and botulinum B toxin inhibition of neurotransmitter release in *Aplysia* neurons (207). Light chains from both toxin types were demonstrated to have proteolytic activities; botulinum toxin type E has been reported to cleave actin (48), whereas botulinum toxin serotype B, but not serotype A or E, and tetanus toxin showed high specificity for synaptobrevin (206, 207). Most recently, it has been shown (103a) that additional *C. botulinum* neurotoxins are proteases which cleave other target proteins. This family of toxins should be useful tools in the elaboration of the mechanism(s) of neurotransmitter release.

Staphylococcus spp.

Staphylococcus aureus is a frequent cause of human disease, and the majority of strains produce several extracellular proteases. A neutral calcium-dependent zinc-containing metalloprotease from *S. aureus* V8 was isolated and characterized (12, 58). This metalloprotease plays a role in the activation of the precursor of a well-studied serine protease, called staphylococcal or V8 protease, secreted by the same organism (58); however, the metalloprotease itself is degraded by the serine protease when both calcium and zinc (but not zinc selectively) are chelated (189). Irreversible loss of activity and conformational changes in the tertiary structure of the protease upon the removal of calcium ions have been observed (13, 252). Although the *S. aureus* metalloprotease exerted no cytotoxic effects on mononuclear leukocytes and did not stimulate proliferation and differentiation of lymphocytes, it was able to affect the stimulation of lymphocytes by polyclonal activators in vitro and therefore may influence the host immune response to infection (193). Some staphylococci also produce a staphylolytic glycylglycine endopeptidase, lysostaphin (207a), which is also called a peptidoglycan hydrolase (171a) and which was stated to be a zinc metalloenzyme (238a). The DNA sequence of the lysostaphin gene does not reveal the presence of the HEXXH motif (93a, 199a).

After our manuscript was submitted, *S. epidermidis* was shown to produce an elastase with pronounced sequence homology to thermolysin and *P. aeruginosa* elastase (230a).

Streptococcus spp.

Streptococcus sanguis, clinically important as a cause of bacterial endocarditis and as a constituent of dental plaque, produces an extracellular immunoglobulin A (IgA) protease that was characterized as a metalloprotease (130, 187). The DNA-deduced amino acid sequence of the *S. sanguis* IgA protease lacked significant homology with that of IgA proteases from other bacterial species and did not show a recognizable signal sequence, and no precursor form could be detected (82). However, a pentapeptide analogous to the zinc-binding signature in other metalloproteases (Fig. 1) was directly shown to be involved in catalytic activity (82). By using enzyme-neutralizing antisera, four distinct IgA proteases were detected in various *Streptococcus* species, and a cooperative activity of protease and neuraminidase was suggested (201). The *S. sanguis* gene probe showed no detectable hybridization with chromosomal DNA from *S. pneumoniae*, which also produces an extracellular metal-dependent IgA protease (81, 186, 192). These IgA proteases have been suggested to play a role in pathogenesis by promoting adherence; additionally, the bacteria become coated with incompetent Fab_{α} fragments as a consequence of the protease activity (4, 200). *S. faecalis*, frequently identified as the etiological agent of various opportunistic infections, produces an extracellular zinc-containing metalloprotease (19, 31). This enzyme was recently further characterized biochemically and had a similar substrate specificity to that of thermolysin (147). The enzyme was active on gelatin, Azocoll, and collagens, and its amino acid composition showed some similarity with that of *Staphylococcus aureus* (147). With regard to *S. pyogenes*, an exposed protease (considered to be a virulence factor) was recently shown to resemble a serine protease (36); to our knowledge, zinc metalloproteases have not been reported.

Streptomyces spp.

Pronase P, a commercial protease mixture from *Streptomyces griseus*, contains several kinds of proteases, including several neutral metalloproteases (170). Recently, two zinc endopeptidases from pronase P were purified and characterized (109, 241). A protein inhibitor of metalloproteases, which is produced extracellularly by *S. nigrescens* and specifically inhibits metalloproteases such as thermolysin (166, 178), also inhibited these proteases (109). Unexpectedly, these proteases were strongly inhibited by serine-protease inhibitors secreted by *Streptomyces* species (109, 241). The DNA sequence for an extracellular metalloprotease from *S. cacaoi* indicated that the mature enzyme is processed from a prepropeptide (34). Although the amino acid sequence similarity to other proteases was not strong, a putative zinc-binding region was recognized (34) (Fig. 1). Site-specific mutagenesis of amino acid residues putatively involved in the zinc-binding and active sites resulted in production of enzymatically inactive protein and extracellular accumulation of the larger proprotein (35). The mutant protein could be converted to the mature form by using active enzyme, indicating an extracellular autoprocessing

event in the maturation of this protease (35). The gene encoding a milk protein-degrading metalloprotease from *Streptomyces* sp. strain C5 also indicated a conserved zinc-binding site (132) (Fig. 1). However, the substrate and inhibition characteristics of this protease were markedly different from those of other known neutral proteases: calcium ions, which are frequently found associated with other metalloproteases, were not found associated with this purified protease (132). Homologous metalloprotease genes from *S. coelicolor* (47) and *S. lividans* (30, 140) were sequenced, and the predicted amino acid sequences showed regions that correspond to the zinc-binding motif found in other zinc-dependent metalloproteases (Fig. 1). Whereas the *S. coelicolor* metalloprotease does not appear to be produced as a preproprotein, the *S. lividans* and C5 proteases undergo proteolytic processing. Although its amino acid sequence is not known, X-ray crystallography of a neutral, zinc-containing protease from *S. caespitosus* showed no structural homology to other neutral proteases whose three-dimensional structures have been determined (90).

Listeria spp.

Listeria monocytogenes is an opportunistic intracellular pathogen that causes listeriosis, a serious disease of humans, particularly those who are immunocompromised. Although no extracellular protease has previously been described for *L. monocytogenes*, an open reading frame located downstream of the gene encoding the secreted hemolysin, listeriolysin, was found to encode a protein highly homologous to the secreted neutral metalloproteases produced by various *Bacillus* species and to a lesser extent to those produced by gram-negative bacteria (54, 155) (Fig. 1). The gene sequence indicated a putative signal sequence followed by a large propeptide and the mature protein and was present only in *L. monocytogenes* strains (54). The metalloprotease gene was the first gene of an operon that is potentially involved in virulence of *L. monocytogenes* (155, 197). By using specific antiserum against thermolysin, only the larger unprocessed proform of the protease could be detected in culture supernatants, which might explain the lack of proteolytic activity (54). A lecithinase-negative mutant of *L. monocytogenes*, obtained by a transposon insertion in the metalloprotease gene, was strongly impaired in virulence in a mouse model (155, 197). This mutant strain produced a larger unprocessed form of the phosphatidylcholine phospholipase C, an exoenzyme probably involved in cell-to-cell spreading, and the metalloprotease gene restored the lecithinase phenotype and the production of active phosphatidylcholine phospholipase C and partially restored the level of virulence (191).

METALLOPROTEASES FROM GRAM-NEGATIVE BACTERIA

Pseudomonas spp.

Pseudomonas aeruginosa is an opportunistic pathogen that can cause fatal infections especially in compromised hosts. Among the numerous extracellular products of *P. aeruginosa* are two well-characterized metalloproteases, elastase and alkaline protease (165), as well as a more recently recognized elastolytic enzyme, LasA, that appears to act in concert with the other proteases in elastolysis (reviewed in reference 77). These metalloproteases and their possible roles in pathogenicity of *P. aeruginosa* infections have been reviewed elsewhere (77, 96, 183, 256). Elastase is a zinc-containing metalloprotease that degrades a variety of biologically important substances, including elastin, laminin, fibrin, human collagens, several complement components, and immunoglobulins. The structural gene encoding elastase was cloned from two *P. aeruginosa* strains (205, 259). The mature elastases from both strains were preceded by a signal sequence and a large propeptide and differed only in one residue (15, 76). Additionally, elastase genes from three elastase-deficient *P. aeruginosa* strains had almost identical sequences to the genes from elastase-producing strains (229). One of the three strains had a single-base deletion in the coding region, which resulted in early termination. These observations suggest that elastase production is repressed by gene regulation in the other strains (229). Elastase shares amino acid homology with the neutral proteases from the *Bacillus* species, especially in regions that are considered to contain the active and zinc-binding sites of thermolysin (Fig. 1). Recently, X-ray crystallographic analysis of elastase showed that the overall tertiary structure of elastase is similar to that of thermolysin and that the zinc ligands and nearly all the active-site residues are identical (231). Site-directed mutagenesis of presumed active-site amino acid residues in elastase confirmed involvement in catalysis and suggested autoproteolytic processing of proelastase (116, 152). By using monoclonal antibodies, immunologic variations as well as common epitopes were found among elastases from different *P. aeruginosa* strains (131). Other monoclonal antibodies could neutralize protease activity against high-molecular-weight substrates, whereas only one also inhibited peptidase activity (261). Purified elastase used as a vaccine provided some protection against severe lung lesions and reduced the incidence of inflammation in a rat model (83). A genetically engineered mutant elastase with diminished proteolytic activity elicited protective activity against *Pseudomonas* infection in mice, as did an inactivated elastase preparation (116).

The alkaline protease from *P. aeruginosa* is also a metalloprotease; however, its properties are very different from those of elastase (165). The *P. aeruginosa* alkaline protease gene was cloned (14, 87) and sequenced (60, 180), and the deduced amino acid sequence shows strong homology with extracellular metalloproteases from *Serratia marcescens* and *Erwinia chrysanthemi* (60, 180). Limited homology is also found to thermolysin and elastase, particularly in the regions that include structurally and functionally important residues, although it is interesting that the order of these conserved regions within the alkaline protease is different (Fig. 1). An extracellular metalloprotease produced by *P. cepacia*, an important etiological agent of clinical infections, was shown to have antigenic similarity with the *P. aeruginosa* elastase (154). Recently, the cloning of the gene encoding this protease was reported (42). A zinc/calcium-dependent metalloprotease from *P. fragi* was purified and had properties similar to those of other extracellular neutral proteases (188). Mutant *P. fragi* strains have been found to produce similar metalloproteases with altered substrate specificities, including protease V, which is now commercially available (59, 105, 176).

Legionella spp.

Legionella pneumophila is a facultative intracellular pathogen capable of causing an acute pneumonitis referred to as Legionnaires' disease. The major secreted protein is a neutral zinc metalloprotease that has been suggested to be an important virulence determinant and was thoroughly re-

viewed recently (57). This enzyme has many properties that suggest its involvement in pathogenesis, including cytotoxic and tissue-destructive activities, inhibitory effects on phagocytes, and proteolytic activity against a broad spectrum of physiologically important substrates (57). The gene encoding the extracellular protease from *L. pneumophila* has been cloned (196, 224). The nucleotide sequence of the protease gene revealed that the open reading frame was significantly larger than expected from the previously reported molecular weight of the mature protease (16). Significant amino acid identity with *P. aeruginosa* elastase (and the HA/protease of *V. cholerae* [see below]) was detected; similarities are most pronounced in the regions forming the zinc-binding and enzymatic sites (Fig. 1). Inhibition studies suggested that the *L. pneumophila* metalloprotease shares similar molecular mechanisms of proteolysis with *P. aeruginosa* elastase and *B. thermoproteolyticus* thermolysin (16). Vaccination with purified protease has been shown to induce cell-mediated immunity in a guinea pig model (17). However, recent studies indicated that the metalloprotease is not required for the ability of *L. pneumophila* to grow in or kill macrophages (224) or for lethality in vivo (18). Despite its potential, the enzyme may not be a major or primary virulence factor in Legionnaires' disease, although it could contribute to pathogenesis by modifying the host inflammatory response (57, 198, 199). Other *Legionella* species, including *L. dumoffii*, *L. micdadei*, and *L. jordanis*, produced proteolytic activities that biochemically resembled *L. pneumophila* protease, but none produced secreted proteins that reacted with the specific antisera and none reacted with the cloned protease DNA probe (195).

Vibrio spp.

The HA/protease of *V. cholerae* O1, the causative agent of epidemic cholera, was discovered as a secreted HA (70, 89), which was subsequently shown to be a zinc- and calcium-dependent neutral protease (23) with the ability to cleave several physiologically important substrates, including mucin, fibronectin, and lactoferrin (69). The HA/protease, which can also nick and thus activate the A subunit of cholera toxin (24), was considered to potentially play a role in the pathogenesis of cholera (25, 44, 208). Almost all *V. cholerae* O1 and most non-O1 strains produce an extracellular protease that is inhibited by specific antiserum (26). Although similarities and some dissimilarities between O1 and non-O1 HA/proteases were reported (100), more recent biochemical and immunological analyses suggested that the proteins are identical, or nearly so (101, 102, 254). Monoclonal antibodies against the *V. cholerae* non-O1 HA/protease neutralized its proteolytic but not its hemagglutinating activity (101), whereas other monoclonal antibodies against the *V. cholerae* O1 HA/protease inhibited both activities but were reported to have no effect on the mucinase activity (254). The *V. cholerae* O1 HA/protease was found to be immunologically and functionally related to the *P. aeruginosa* elastase (91). The cloned HA/protease structural gene suggested that a large propeptide preceded the mature protein (92). The deduced amino acid sequence of the mature HA/protease showed strong homology with that of *P. aeruginosa* elastase and *L. pneumophila* protease and also shared the conserved domains shown in Fig. 1. An HA/protease-negative *V. cholerae* O1 mutant strain was constructed (92) and was found to be fully virulent in an animal model (68). However, further experiments indicated that the HA/protease may play an important role in facilitating detachment of the vibrios from the intestinal cells (68), thus enabling them to find another human host. Another protease, "Detach," has been reported to protect rabbits against experimental diarrhea disease due to *E. coli*, presumably by destroying receptors and preventing attachment (167). The mucolytic ability of such proteases as the *V. cholerae* HA/protease and the *P. aeruginosa* elastase might prove useful therapeutically in clearing airways obstructions as in cystic fibrosis. This would have to be evaluated carefully, because the ability of *P. aeruginosa* proteases to release mucin from airways goblet cells has been suggested to have potential deleterious effects (20).

The halophilic bacterium *V. vulnificus*, which causes wound infections and septicemia in humans, elaborates a neutral metalloprotease with elastolytic activity that was toxic for mice and elicited dermonecrosis (123, 217). The enzyme contributes to edema formation during *V. vulnificus* infections by enhancing vascular permeability (158, 159). This protease is inhibited by plasma α_2-macroglobulin (160, 161). The recent development of a protease derivative which is resistant to this inhibition could clarify the involvement of this enzyme in *V. vulnificus* infections (171). The N-terminal amino acid sequence of this protease is highly homologous to that of metalloproteases from other *Vibrio* species, the *P. aeruginosa* elastase, and *L. pneumophila* protease (123). Protease-deficient mutants cannot utilize heme as an iron source, indicating that the protease may be involved in iron scavenging (174). *V. proteolyticus* (formerly *Aeromonas proteolytica*) is a halotolerant bacterium that secretes a thermostable neutral protease that has industrial applications for enzyme-mediated synthesis of dipeptides. The enzyme is a zinc metalloprotease which appeared to be homologous to thermolysin and elastase in many properties (61, 84). The nucleotide sequence of the *V. proteolyticus* neutral protease gene indicated a large prosequence following the signal sequence, and the deduced amino acid sequence shared extensive homology with that of the *V. cholerae* HA/protease and other bacterial metalloproteases (Fig. 1). The purified zinc metalloprotease secreted by the fish pathogenic bacterium *V. anguillarum* shares some properties with proteases from other *Vibrio* species such as *V. vulnificus* and *V. cholerae* (65). The protease had elastolytic activity and was strongly suggested to be associated with host invasion (177). Recently, this protease gene sequence indicated a signal peptide and prosequence and strong amino acid homology to metalloproteases from several gram-negative organisms and also showed the putative zinc-binding and active-site regions (157) (Fig. 1). A chromosomal marker exchange metalloprotease mutant strain had only slightly reduced virulence; however, the mutant strain expressed two additional proteases not detected in the wild-type strain that might also contribute to pathogenesis (157). *V. mimicus* has been found to be responsible for various types of human illness, including enteric infections. A purified extracellular metalloprotease from this organism was reported to enhance vascular permeability in skin (37) and fluid accumulation in rabbit ileal loops (39). The enzyme had both protease and hemagglutination activity and was immunologically cross-reactive with the HA/protease produced by *V. cholerae* (38, 55). *V. alginolyticus* chemovar *iophagus* (formerly *Achromobacter iophagus*) produces an extracellular collagenase which is a zinc-containing metalloprotease (117). Three active forms of the collagenase have been isolated that are structurally related and are autodegradation products of a larger single polypeptide chain (117, 237). The collagenase gene was cloned (75), and although no strong overall se-

quence homology to other proteases could be detected (228), a putative zinc-binding motif is present (Fig. 1).

Aeromonas spp.

Aeromonas hydrophila, an opportunistic pathogen of humans and fish, produces a variety of extracellular products, including proteases which may contribute to virulence (103). There are disparities among the reports of the number and types of metalloproteases secreted by *A. hydrophila* (137, 173, 203). Most strains of *A. hydrophila* produce a single heat-stable metalloprotease, and some strains also secrete an unrelated serine protease (137). A similar thermostable metalloprotease was found in some strains of *A. sobria* and *A. caviae* but not in the *A. salmonicida* strains that were tested (137). The *A. hydrophila* metalloprotease was shown to have esterase but no cytotoxic activity and was lethal for fish (203). Although the purified protease was inhibited by EDTA and *o*-phenanthroline (112), an association of the protein with zinc has not yet been shown. Tn*5*-induced protease-deficient mutants of an *A. hydrophila* strain that produces a single metalloprotease had reduced virulence for fish (138). The metalloprotease appeared to contribute to the establishment of the infection in fish by overcoming host defenses and by providing nutrients for growth (138). The gene for a heat-stable extracellular metalloprotease from *A. hydrophila* has been cloned; however, this protease acted differently from the previously characterized protease (202). Interestingly, a secreted metalloprotease from an *A. hydrophila* strain isolated from milk was partially purified and shown to be a heat-labile, calcium-dependent enzyme that was actually inhibited by zinc ions (6).

Serratia spp.

Serratia sp. strain E-15 produces an extracellular zinc metalloprotease that has been used as an anti-inflammatory agent. The nucleotide sequence of the protease gene has been determined. The mature protein was preceded by a short peptide that is different from typical signal peptides of secreted proteins (169). Three zinc ligands and an active site of the *Serratia* protease were predicted from comparison with thermolysin (169) (Fig. 1). Recently, examination of metal-induced conformational changes of this protease revealed that the position and coordination of the zinc ion appear to be essential both for enzymatic activity and for the overall tertiary structure (115). *S. marcescens* is now well recognized as an opportunistic pathogen, and its major extracellular metalloprotease has been suggested to be an important virulence factor in both keratitis and pulmonary infections (110, 143, 144). This protease degrades several physiologically important substrates, including fibronectin, collagen, and several serum proteins; it enhances vascular permeability; and it suppresses the complement-derived chemotactic activity of C5a (111, 162, 163, 179). Moreover, the pathogenesis of influenza virus infection in mice is enhanced by the *S. marcescens* protease (5). Transposon-induced mutagenesis of *S. marcescens* affecting extracellular metalloprotease activity suggested that several genes may be required for production and secretion of this protease (95). The metalloprotease gene from *S. marcescens* was cloned; it had a sequence almost identical to that of the *Serratia* sp. strain E-15 protease (27). Metalloproteases from *S. liquefaciens* strains showed serological cross-reactivity with the *S. marcescens* metalloprotease as well as very similar proteolytic activity against human serum proteins (255). Recently, the gene encoding a minor extracellular metalloprotease from *S. marcescens* revealed high overall homology to a metalloprotease from *Erwinia caratovora* and only low homology to other proteases, including the *S. marcescens* major metalloprotease (128) (Fig. 1).

Erwinia spp.

Erwinia chrysanthemi, a phytopathogenic bacterium, secretes several proteins including three distinct but antigenically related metalloproteases, A, B, and C (249), and produces a protease inhibitor which specifically binds to these proteases and also strongly inhibits the *S. marcescens* metalloprotease (133). The genes encoding these metalloproteases have been sequenced, and the deduced proteins share strong amino acid homology with each other, the *S. marcescens* metalloprotease, and the *P. aeruginosa* alkaline protease (45, 51, 52, 80). The predicted zinc-binding and active-site residues of these proteases are also found in the *E. chrysanthemi* proteases, and they also appear in a different order from others in Fig. 1. The *E. chrysanthemi* proteases, like the *Serratia* and *Pseudomonas* enzymes, lack typical signal sequences at their N-termini and are preceded by short propeptides. Marker exchange mutants of *E. chrysanthemi*, defective in production of one or all of the extracellular proteases, are not impaired in virulence on plant tissue (45). *E. carotovora* subsp. *carotovora* causes soft rot in many plant species, and a possible role for an extracellular protease in pathogenesis has been suggested (239). The gene encoding an extracellular protease was cloned and complemented a transposon-induced protease-deficient mutant (7). The nucleotide sequence of the protease gene revealed strong amino acid homology of the deduced protein with the neutral proteases from *Bacillus* species and other metalloproteases in certain regions (Fig. 1) and very little overall similarity with the *E. chrysanthemi* proteases (129).

Others

Xanthomonas campestris pv. *campestris*, the causal agent of the black rot disease of cruciferous plants, produces two proteases, of which one is a zinc-requiring metalloprotease with properties similar to those of the *E. chrysanthemi* proteases (56). A protease-deficient mutant that lacked both proteases showed considerable loss of virulence for nicked leaves (56, 230). An extracellular metalloprotease which has been purified from a psychrophilic strain of *X. maltophilia* resembles proteases from mesophilic organisms (148). *Pasteurella haemolytica*, associated with bovine pneumonic pasteurellosis, secretes a glycoprotease that is highly specific for O-glycosylated glycoproteins (3, 223). The gene for this neutral metalloprotease has recently been cloned and sequenced (2). Although the gene product showed no significant homology with other proteases, a putative zinc-binding site was proposed (2) (Fig. 1). The hydrothermal vent bacterium *Hyphomonas jannaschiana* produces a thermostable alkaline zinc/calcium-dependent metalloprotease (211). Several IgA proteases from a variety of bacterial species, including *Proteus*, *Capnocytophaga*, and *Bacteroides* species, can be inhibited by chelating agents, indicating that these enzymes are metalloproteases; however, the presence of zinc has not been demonstrated (73, 186, 210). An extracellular collagenase from *Porphyromonas gingivalis* apparently is a calcium-containing metalloprotease that does not contain zinc (114).

PROCESSING OF METALLOPROTEASES

Many extracellular bacterial proteases are synthesized as inactive precursors with an additional polypeptide segment (propeptide) that is removed from the mature secreted protein (247). Several roles have been proposed for the propeptides of bacterial proteases. The propeptide may function to keep the protease inactive inside the cell, thus protecting the host cell against an "untethered" protease; it may play a role in the folding of the proenzyme into the proper conformation necessary for activity or the secretion process; and/or it might temporarily anchor the protease to the membrane. Neither the exact function of the propeptide nor the mechanism of cleavage to the mature form of the enzyme is known, although, in many cases, an autoproteolytic processing has been suggested. Some amino acid homology was found between the prosequences of several neutral proteases from *Bacillus* species and the *P. aeruginosa* elastase (253). The prosequences of *Bacillus* neutral proteases have been reviewed recently (215). *P. aeruginosa* elastase is produced as a larger inactive precursor, indicating that proteolytic processing is required for activation of the enzyme (118). Three cell-associated elastase precursors were identified in *P. aeruginosa* cells, and it was suggested that the propeptide forms a noncovalent complex with the elastase portion after proteolytic cleavage in the periplasmic space, thus inhibiting its proteolytic activity (119). The cleavage sites involved in proteolytic processing of preproelastase were defined (120). Site-directed mutagenesis of catalytic amino acid residues resulted in accumulation of enzymatically inactive proelastase, indicating autocatalytic processing (116, 152), and a model of the possible events of elastase processing in *E. coli* was proposed (116). Homologous metalloproteases from several *Vibrio* species were suggested to undergo an additional processing by autocatalytic removal of a small polypeptide from the C terminus (49, 92, 123, 157, 168). However, the small cleavage product has so far been demonstrated only for *V. vulnificus* (123).

SECRETION OF METALLOPROTEASES

Protein secretion by gram-negative and gram-positive bacteria has been extensively reviewed recently (141, 194, 204, 215, 236, 248). Most bacterial proteins, including many metalloproteases, are secreted by a *sec*-dependent general secretory pathway; however, a small group of metalloproteases do not have conventional N-terminal signal sequences, including the *S. marcescens* metalloprotease; proteases A, B, and C from *E. chrysanthemi*; and the *P. aeruginosa* alkaline protease. Specific secretion proteins have been identified for the *Erwinia* proteases and the alkaline protease, which appear to be homologous to those required for secretion of several bacterial toxins such as the *E. coli* alpha-hemolysin (52, 60, 86, 88, 134, 204, 236). As with *E. coli* alpha-hemolysin, the C termini of these proteases have been shown to be essential for their secretion. They contain a repeated consensus sequence also found in alpha-hemolysin and related cytotoxins (45, 53, 135, 136, 222). The secretion proteins for the *E. chrysanthemi* proteases can mediate the secretion of the *S. marcescens* metalloprotease (135) and the *P. aeruginosa* alkaline protease (86) from *E. coli*. Similarly, the *E. coli* alpha-hemolysin secretion proteins can complement secretion of the *P. aeruginosa* alkaline protease (86), the *Serratia* metalloprotease (135, 222), and *Erwinia* proteases (52). However, alpha-hemolysin is not exported through the *Erwinia* protease system (66). A recent study suggested that lipopolysaccharide may be involved in the secretion of alpha-hemolysin and the *E. chrysanthemi* proteases (250).

REGULATION OF METALLOPROTEASES

In many instances, only the physiological and nutritional factors affecting extracellular protease production have been studied (9, 145). However, in several organisms regulatory proteins involved in transcription of metalloprotease genes were identified. Production of the neutral proteases in *B. subtilis* is controlled by a number of regulatory genes that also control several other extracellular proteins (93). A transcriptional activator gene specific for the neutral metalloprotease gene of *B. stearothermophilus* was located upstream of the protease gene, and possible target regions were identified in the 5′ regions of both genes (175). Mutations in regulatory proteins showing pleiotropic effects on extracellular proteins, including metalloprotease production, have been described in *S. aureus* (106, 164). A gene has been identified from *Streptomyces griseus* that resulted in an increase in the production of several extracellular activities in various *Streptomyces* species (50). Recently, adjacent divergent open reading frames upstream of the metalloprotease genes from *S. lividans*, *S. coelicolor*, and *Streptomyces* sp. strain C5 showed strong sequence homology to transcriptional activators of the LysR family (30, 47, 132, 140). In *S. lividans*, the protein encoded by this gene was shown to bind to the intergenic region between the regulator and protease genes and to activate transcription of the metalloprotease (47). The metalloprotease gene of *L. monocytogenes* is the first gene of an operon that is transcriptionally activated by a regulatory protein that recognized a palindromic sequence in the upstream region of the protease gene and several other putative virulence genes (32, 74, 156). The *lasR* gene of *P. aeruginosa* is required for the transcription of the genes for elastase, alkaline protease, and LasA and thus appears to be a global regulator of proteases in *P. aeruginosa* (78, 79, 233). LasR shows sequence homology to LuxR, a regulatory protein of *Vibrio fischeri*, and a putative dyad recognition sequence upstream of the protease genes was proposed (79). Among several environmental factors, zinc, iron, and calcium are involved in efficient elastase production (28, 181). Both zinc and iron seem to regulate elastase expression at the translational level, although they probably use separate mechanisms (28).

CONCLUSIONS

In gram-negative bacteria there are at least two closely related families of bacterial zinc metalloproteases that can be differentiated by amino acid homology, genetic organization, and mechanism of secretion. The "elastase-like" proteases (numbers 13 to 17 in Fig. 1) initially contain N-terminal signal sequences followed by propeptides and the mature proteases that are then proteolytically processed and are secreted via the general secretion pathway. The "*Serratia* protease-like" metalloproteases (numbers 18 to 23 in Fig. 1) do not contain conventional signal sequences or propeptides and are secreted by specific secretion functions. The "thermolysin-like" proteases (numbers 1 to 12 in Fig. 1) are more similar to the *P. aeruginosa* elastase, suggesting a common ancestral gene for these proteases. Additionally, several bacterial zinc metalloproteases (numbers 24 to 31 in Fig. 1) have been described that do not share extensive homology with any of these groups. This implies an independent

evolution of these enzymes and emphasizes the potential importance of these proteolytic activities. The ubiquity and conservation of extracellular zinc-containing metalloproteases in the microbial world, in both pathogenic and nonpathogenic species, suggest that they must provide survival advantages which are not necessarily associated with virulence. From their remarkable diversity and specificity, as stated in the Prologue, it may safely be predicted that additional bacterial metalloproteases will be found to be involved in pathogenesis (like the neurotoxins) and to serve useful functions as well (like "designer proteins").

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REFERENCES

1. **Abakov, A. S., A. P. Bolotin, and A. V. Sorokin.** 1990. The structure of *Bacillus brevis* metalloprotease gene. Mol. Biol. (Moscow) **24**:1363–1372.
2. **Abdullah, K. M., R. Y. C. Lo, and A. Mellors.** 1991. Cloning, nucleotide sequence, and expression of the *Pasteurella haemolytica* A1 glycoprotease gene. J. Bacteriol. **173**:5597–5603.
3. **Abdullah, K. M., E. A. Udoh, P. E. Shewen, and A. Mellors.** 1992. A neutral glycoprotease of *Pasteurella haemolytica* A1 specifically cleaves O-sialoglycoproteins. Infect. Immun. **60**: 56–62.
4. **Ahl, T., and J. Reinholdt.** 1991. Detection of immunoglobulin A1 protease-induced Fab_{α} fragments on dental plaque bacteria. Infect. Immun. **59**:563–569.
5. **Akaike, T., A. Molla, M. Ando, S. Araki, and H. Maeda.** 1989. Molecular mechanism of complex infection by bacteria and virus analyzed by a model using serratial protease and influenza virus in mice. J. Virol. **63**:2252–2259.
6. **Alichanidis, E.** 1988. Partial purification and characterization of an extracellular proteinase from *Aeromonas hydrophila* strain A4. J. Dairy Res. **55**:97–107.
7. **Allen, C., V. K. Stromberg, F. D. Smith, G. H. Lacy, and M. S. Mount.** 1986. Complementation of an *Erwinia carotovora* subsp. *carotovora* protease mutant with a protease-encoding cosmid. Mol. Gen. Genet. **202**:276–279.
8. **Allison, C., and G. T. Macfarlane.** 1989. Protease production by *Clostridium perfringens* in batch and continuous culture. Lett. Appl. Microbiol. **9**:45–48.
9. **Allison, C., and G. T. Macfarlane.** 1992. Physiological and nutritional determinants of protease secretion by *Clostridium sporogenes*: characterization of six extracellular proteases. Appl. Microbiol. Biotechnol. **37**:152–156.
10. **Angleton, E. L., and H. E. Van Wart.** 1988. Preparation and reconstruction with divalent metal ions of class I and class II *Clostridium histolyticum* apocollagenases. Biochemistry **27**: 7406–7412.
11. **Angleton, E. L., and H. E. Van Wart.** 1988. Preparation by direct metal exchange and kinetic study of active site metal substituted class I and class II *Clostridium histolyticum* collagenases. Biochemistry **27**:7413–7418.
12. **Arvidson, S.** 1973. Studies on extracellular proteolytic enzymes from *Staphylococcus aureus*. II. Isolation and characterization of an EDTA-sensitive protease. Biochim. Biophys. Acta **302**:149–157.
13. **Arvidson, S.** 1973. The role of calcium for stability and activity of an extracellular proteolytic enzyme from *Staphylococcus aureus*. Acta Pathol. Microbiol. Scand. Sect. B **81**:545–551.
14. **Atsumi, Y., S. Yamamoto, K. Morihara, J. Fukushima, H. Takeuchi, N. Mizuki, S. Kawamoto, and K. Okuda.** 1989. Cloning and expression of the alkaline proteinase gene from *Pseudomonas aeruginosa* IFO 3455. J. Bacteriol. **171**:5173–5175.
15. **Bever, R. A., and B. H. Iglewski.** 1988. Molecular characterization and nucleotide sequence of the *Pseudomonas aeruginosa* elastase structural gene. J. Bacteriol. **170**:4309–4314.
16. **Black, W. J., F. D. Quinn, and L. S. Tompkins.** 1990. *Legionella pneumophila* zinc metalloprotease is structurally and functionally homologous to *Pseudomonas aeruginosa* elastase. J. Bacteriol. **172**:2608–2613.
17. **Blander, S. J., and M. A. Horwitz.** 1989. Vaccination with the major secretory protein of *Legionella pneumophila* induces cell-mediated and protective immunity in a guinea pig model of Legionnaires' disease. J. Exp. Med. **169**:691–705.
18. **Blander, S. J., L. Szeto, H. A. Shuman, and M. A. Horwitz.** 1990. An immunoprotective molecule, the major secretory protein of *Legionella pneumophila*, is not a virulence factor in a guinea pig model of Legionnaires' disease. J. Clin. Invest. **86**:817–824.
19. **Bleiweis, A. S., and L. N. Zimmerman.** 1964. Properties of proteinase from *Streptococcus faecalis* var. *liquefaciens*. J. Bacteriol. **88**:653–659.
20. **Boat, T. B., P. W. Cheng, J. D. Klinger, C. M. Liedtke, and B. Tandler.** 1984. Proteinases release mucin from airways goblet cells. CIBA Found. Symp. **109**:72–87.
21. **Bond, M. D., and H. E. Van Wart.** 1984. Characterization of the individual collagenases from *Clostridium histolyticum*. Biochemistry **23**:3085–3091.
22. **Bond, M. D., and H. E. Van Wart.** 1984. Relationship between the individual collagenases of *Clostridium histolyticum*: evidence for evolution by gene duplication. Biochemistry **23**: 3092–3099.
23. **Booth, B. A., M. Boesman-Finkelstein, and R. A. Finkelstein.** 1983. *Vibrio cholerae* soluble hemagglutinin/protease is a metalloenzyme. Infect. Immun. **42**:639–644.
24. **Booth, B. A., M. Boesman-Finkelstein, and R. A. Finkelstein.** 1984. *Vibrio cholerae* hemagglutinin/protease nicks cholera enterotoxin. Infect. Immun. **45**:558–560.
25. **Booth, B. A., T. J. Dyer, and R. A. Finkelstein.** 1990. Adhesion of *Vibrio cholerae* to cultured human cells, p. 19–35. *In* R. B. Sack and Y. Zinnaka (ed.), Advances in research on cholera and related diarrheas. KTK Scientific Publishers, Tokyo.
26. **Booth, B. A., and R. A. Finkelstein.** 1986. Presence of hemagglutinin/protease and other potential virulence factors in O1 and non-O1 *Vibrio cholerae*. J. Infect. Dis. **154**:183–186.
27. **Braunagel, S. C., and M. J. Benedik.** 1990. The metalloprotease gene of *Serratia marcescens* strain SM6. Mol. Gen. Genet. **222**:446–451.
28. **Brumlik, M. J., and D. G. Storey.** 1992. Zinc and iron regulate translation of the gene encoding *Pseudomonas aeruginosa* elastase. Mol. Microbiol. **6**:337–344.
29. **Burstein, Y., K. A. Walsh, and H. Neurath.** 1974. Evidence of an essential histidine residue in thermolysin. Biochemistry **13**:205–210.
30. **Butler, M. J., C. C. Davey, P. Krygsman, E. Walczyk, and L. T. Malek.** 1992. Cloning of genetic loci involved in endoprotease activity in *Streptomyces lividans* 66: a novel neutral protease gene with an adjacent divergent putative regulatory gene. Can. J. Microbiol. **38**:912–920.
31. **Casas, I. A., and L. N. Zimmerman.** 1969. Dependence of protease secretion by *Streptococcus faecalis* var. *liquefaciens* on arginine and its possible relation to site of synthesis. J. Bacteriol. **97**:307–312.
32. **Chakraborty, T., M. Leimeister-Wächter, E. Domann, M. Hartl, W. Goebel, T. Nichterlein, and S. Notermans.** 1992. Coordinate regulation of virulence genes in *Listeria monocytogenes* requires the product of the *prfA* gene. J. Bacteriol. **174**:568–574.
33. **Chaloupka, J., and H. Kucerova.** 1988. Netropsin increases formation of mRNA coding for neutral metalloproteinase in *Bacillus megaterium*. J. Basic Microbiol. **28**:11–16.
34. **Chang, P. C., T.-C. Kuo, A. Tsugita, and Y.-H. W. Lee.** 1990. Extracellular metalloprotease gene of *Streptomyces cacaoi*: structure, nucleotide sequence and characterization of the cloned gene product. Gene **88**:87–95.
35. **Chang, P.-C., and Y.-H. W. Lee.** 1992. Extracellular autopro-

cessing of a metalloprotease from *Streptomyces cacaoi*. J. Biol. Chem. **267**:3952–3958.
36. **Chen, C. C., and P. P. Cleary.** 1990. Complete nucleotide sequence of the streptococcal C5a peptidase gene of *Streptococcus pyogenes*. J. Biol. Chem. **265**:3161–3167.
37. **Chowdhury, M. A. R., S. Miyoshi, and S. Shinoda.** 1991. Vascular permeability enhancement by *Vibrio mimicus* protease and the mechanisms of action. Microbiol. Immunol. **35**:1049–1058.
38. **Chowdhury, M. A. R., S.-I. Miyoshi, and S. Shinoda.** 1990. Purification and characterization of a protease produced by *Vibrio mimicus*. Infect. Immun. **58**:4159–4162.
39. **Chowdhury, M. A. R., S.-I. Miyoshi, and S. Shinoda.** 1991. Role of *Vibrio mimicus* protease in enterotoxigenicity. J. Diarrhoeal Dis. Res. **9**:332–334.
40. **Colman, P. M., J. N. Jansonius, and B. W. Matthews.** 1972. The structure of thermolysin: an electron density map at 2.3 Å resolution. J. Mol. Biol. **70**:701–724.
41. **Coolbear, T., J. M. Whittaker, and R. M. Daniel.** 1992. The effect of metal ions on the activity and thermostability of the extracellular proteinase from a thermophilic *Bacillus*, strain EA.1. Biochem. J. **287**:367–374.
42. **Cox, A. D., and P. A. Sokol.** 1992. Cloning of a protease gene from *Pseudomonas cepacia*, abstr. D-53, p. 104. Abstr. 92nd Gen. Meet. Am. Soc. Microbiol. 1992. American Society for Microbiology, Washington, D.C.
43. **Croux, C., V. Paquet, G. Goma, and P. Soucaille.** 1990. Purification and characterization of acidolysin, an acidic metalloprotease produced by *Clostridium acetobutylicum* ATCC 824. Appl. Environ. Microbiol. **56**:3634–3642.
44. **Crowther, R. S., N. W. Roomi, R. E. F. Fahim, and J. F. Forstner.** 1987. *Vibrio cholerae* metalloproteinase degrades intestinal mucin and facilitates enterotoxin-induced secretion from rat intestine. Biochim. Biophys. Acta **924**:393–402.
45. **Dahler, G. S., F. Barras, and N. T. Keen.** 1990. Cloning of genes encoding extracellular metalloproteases from *Erwinia chrysanthemi* EC16. J. Bacteriol. **172**:5803–5815.
46. **Dalhammar, G., and H. Steiner.** 1984. Characterization of inhibitor A, a protease from *Bacillus thuringiensis* which degrades attacins and cecropins, two classes of antibacterial proteins in insects. Eur. J. Biochem. **139**:247–252.
47. **Dammann, T., and W. Wohlleben.** 1992. A metalloprotease gene from *Streptomyces coelicolor* 'Müller' and its transcriptional activator, a member of the LysR family. Mol. Microbiol. **6**:2267–2278.
48. **DasGupta, B. R., and W. Tepp.** 1993. Protease activity of botulinum neurotoxin type E and its light chain: cleavage of actin. Biochem. Biophys. Res. Commun. **190**:470–474.
49. **David, V. A., A. H. Deutch, A. Sloma, D. Pawlyk, A. Ally, and D. R. Durham.** 1992. Cloning, sequencing and expression of the gene encoding the extracellular neutral protease, vibriolysin, of *Vibrio proteolyticus*. Gene **112**:107–112.
50. **Daza, A., J. A. Gil, T. Vigal, and J. F. Martin.** 1990. Cloning and characterization of a gene of *Streptomyces griseus* that increases production of extracellular enzymes in several species of *Streptomyces*. Mol. Gen. Genet. **222**:384–392.
51. **Delepelaire, P., and C. Wandersman.** 1989. Protease secretion by *Erwinia chrysanthemi*. Proteases B and C are synthesized and secreted as zymogens without a signal peptide. J. Biol. Chem. **264**:9083–9089.
52. **Delepelaire, P., and C. Wandersman.** 1990. Protein secretion in Gram-negative bacteria. The extracellular metalloprotease B from *Erwinia chrysanthemi* contains a C-terminal secretion signal analogous to that of *Escherichia coli* α-hemolysin. J. Biol. Chem. **265**:17118–17125.
53. **Delepelaire, P., and C. Wandersman.** 1991. Characterization, localization and transmembrane organization of the three proteins PrtD, PrtE and PrtF necessary for protease secretion by the Gram-negative bacterium *Erwinia chrysanthemi*. Mol. Microbiol. **5**:2427–2434.
54. **Domann, E., M. Leimeister-Wächter, W. Goebel, and T. Chakraborty.** 1991. Molecular cloning, sequencing, and identification of a metalloprotease gene from *Listeria monocytogenes* that is species specific and physically linked to the listeriolysin gene. Infect. Immun. **59**:65–72.
55. **Dotevall, H., G. Jonson-Strömberg, S. Sanyal, and J. Holmgren.** 1985. Characterization of enterotoxin and soluble hemagglutinin from *Vibrio mimicus*: identity with *V. cholerae* O1 toxin and hemagglutinin. FEMS Microbiol. Lett. **27**:17–22.
56. **Dow, J. M., B. R. Clarke, D. E. Milligan, J.-L. Tang, and M. J. Daniels.** 1990. Extracellular proteases from *Xanthomonas campestris* pv. campestris, the black rot pathogen. Appl. Environ. Microbiol. **56**:2994–2998.
57. **Dowling, J. N., A. K. Saha, and R. H. Glew.** 1992. Virulence factors of the family *Legionellaceae*. Microbiol. Rev. **56**:32–60.
58. **Drapeau, G. R.** 1978. Role of a metalloprotease in activation of the precursor of staphylococcal protease. J. Bacteriol. **136**:607–613.
59. **Drapeau, G. R.** 1980. Substrate specificity of a proteolytic enzyme isolated from a mutant of *Pseudomonas fragi*. J. Biol. Chem. **255**:839–840.
60. **Duong, F., A. Lazdunski, B. Cami, and M. Murgier.** 1992. Sequence of a cluster of genes controlling synthesis and secretion of alkaline protease in *Pseudomonas aeruginosa*: relationships to other secretory pathways. Gene **121**:47–54.
61. **Durham, D. R.** 1990. The unique stability of *Vibrio proteolyticus* neutral protease under alkaline conditions affords a selective step for purification and use in amino acid-coupling reactions. Appl. Environ. Microbiol. **56**:2277–2281.
62. **Eijsink, V. G. H., J. R. van der Zee, B. van den Burg, G. Vriend, and G. Venema.** 1991. Improving the thermostability of the neutral protease of *Bacillus stearothermophilus* by replacing a buried asparagine by leucine. FEBS Lett. **282**:13–16.
63. **Eijsink, V. G. H., G. Vriend, B. van den Burg, and B. K. Stulp.** 1990. Contribution of the C-terminal amino acid to the stability of *Bacillus subtilis* neutral protease. Protein Eng. **4**:99–104.
64. **Eisel, U., W. Jarausch, K. Goretzki, A. Henschen, J. Engels, U. Weller, M. Hudel, E. Habermann, and H. Niemann.** 1986. Tetanus toxin: primary structure, expression in *E. coli*, and homology with botulinum toxins. EMBO J. **5**:2495–2502.
65. **Farrell, D. H., and J. H. Crosa.** 1991. Purification and characterization of a secreted protease from the pathogenic marine bacterium *Vibrio anguillarum*. Biochemistry **30**:3432–3436.
66. **Fath, M. J., R. C. Skvirsky, and R. Kolter.** 1991. Functional complementation between bacterial MDR-like export systems: colicin V, alpha-hemolysin, and *Erwinia* protease. J. Bacteriol. **173**:7549–7556.
67. **Finkelstein, R. A., M. Arita, J. D. Clements, and E. T. Nelson.** 1978. Isolation and purification of an adhesive factor ("cholera lectin") from *Vibrio cholerae*, p. 137–151. *In* Proceedings of the 13th Joint Conference on Cholera, U.S.-Japan Cooperative Medical Science Program. Department of Health, Education, and Welfare publication no. 78-1590. National Institutes of Health, Bethesda, Md.
68. **Finkelstein, R. A., M. Boesman-Finkelstein, Y. Chang, and C. C. Häse.** 1992. *Vibrio cholerae* hemagglutinin/protease, colonial variation, virulence, and detachment. Infect. Immun. **60**:472–478.
69. **Finkelstein, R. A., M. Boesman-Finkelstein, and P. Holt.** 1983. *Vibrio cholerae* hemagglutinin/lectin/protease hydrolyzes fibronectin and ovomucin: F. M. Burnet revisited. Proc. Natl. Acad. Sci. USA **80**:1092–1095.
70. **Finkelstein, R. A., and L. F. Hanne.** 1982. Purification and characterization of the soluble hemagglutinin (cholera lectin) produced by *Vibrio cholerae*. Infect. Immun. **36**:1199–1208.
71. **Finkelstein, R. A., and S. Mukerjee.** 1963. Hemagglutination: a rapid method for differentiating *Vibrio cholerae* and El Tor vibrios. Proc. Soc. Exp. Biol. Med. **112**:355–359.
72. **Fontana, A.** 1988. Structure and stability of thermophilic enzymes. Studies on thermolysin. Biophys. Chem. **29**:181–193.
73. **Frandsen, E. V. G., J. Reinholdt, and M. Kilian.** 1987. Enzymatic and antigenic characterization of immunoglobulin A1 proteases from *Bacteroides* and *Capnocytophaga* spp. Infect. Immun. **55**:631–638.
74. **Freitag, N. E., P. Youngman, and D. A. Portnoy.** 1992. Tran-

scriptional activation of the *Listeria monocytogenes* hemolysin gene in *Bacillus subtilis*. J. Bacteriol. **174:**1293–1298.
75. **Fukushima, J., H. Takeuchi, E. Tanaka, K. Hamajima, Y. Sato, S. Kawamoto, K. Morihara, B. Keil, and K. Okuda.** 1990. Molecular cloning and partial DNA sequencing of the collagenase gene of *Vibrio alginolyticus*. Microbiol. Immunol. **34:**977–984.
76. **Fukushima, J., S. Yamamoto, K. Morihara, Y. Atsumi, H. Takeuchi, S. Kawamoto, and K. Okuda.** 1989. Structural gene and complete amino acid sequence of *Pseudomonas aeruginosa* IFO 3455 elastase. J. Bacteriol. **171:**1698–1704.
77. **Galloway, D. R.** 1991. *Pseudomonas aeruginosa* elastase and elastolysis revisited: recent developments. Mol. Microbiol. **5:**2315–2321.
78. **Gambello, M. J., and B. H. Iglewski.** 1991. Cloning and characterization of the *Pseudomonas aeruginosa lasR* gene, a transcriptional activator of elastase expression. J. Bacteriol. **173:**3000–3009.
79. **Gambello, M. J., S. Kaye, and B. H. Iglewski.** 1993. LasR of *Pseudomonas aeruginosa* is a transcriptional activator of the alkaline protease gene (*apr*) and an enhancer of exotoxin production. Infect. Immun. **61:**1180–1184.
80. **Ghigo, J.-M., and C. Wandersman.** 1992. Cloning, nucleotide sequence and characterization of the gene encoding the *Erwinia chrysanthemi* B374 PrtA metalloprotease: a third metalloprotease secreted via a C-terminal secretion signal. Mol. Gen. Genet. **236:**135–144.
81. **Gilbert, J. V., A. G. Plaut, Y. Fishman, and A. Wright.** 1988. Cloning of the gene encoding streptococcal immunoglobulin A protease and its expression in *Escherichia coli*. Infect. Immun. **56:**1961–1966.
82. **Gilbert, J. V., A. G. Plaut, and A. Wright.** 1991. Analysis of the immunoglobulin A protease gene of *Streptococcus sanguis*. Infect. Immun. **59:**7–17.
83. **Gilleland, H. E., L. B. Gilleland, and M. R. Fowler.** 1993. Vaccine efficacies of elastase, exotoxin A, and outer-membrane protein F in preventing chronic pulmonary infection by *Pseudomonas aeruginosa* in a rat model. J. Med. Microbiol. **38:**79–86.
84. **Griffin, T. B., and J. M. Prescott.** 1970. Some physical characteristics of a proteinase from *Aeromonas proteolytica*. J. Biol. Chem. **245:**1348–1356.
85. **Guddal, P. H., T. Johansen, K. Schulstad, and C. Little.** 1989. Apparent phosphate retrieval system in *Bacillus cereus*. J. Bacteriol. **171:**5702–5706.
86. **Guzzo, J., F. Duong, C. Wandersman, M. Murgier, and A. Lazdunski.** 1991. The secretion genes of *Pseudomonas aeruginosa* alkaline protease are functionally related to those of *Erwinia chrysanthemi* proteases and *Escherichia coli* α-haemolysin. Mol. Microbiol. **5:**447–453.
87. **Guzzo, J., M. Murgier, A. Filloux, and A. Lazdunski.** 1990. Cloning of the *Pseudomonas aeruginosa* alkaline protease gene and secretion of the protease into the medium by *Escherichia coli*. J. Bacteriol. **172:**942–948.
88. **Guzzo, J., J.-M. Pages, F. Duong, A. Lazdunski, and M. Murgier.** 1991. *Pseudomonas aeruginosa* alkaline protease: evidence for secretion genes and study of secretion mechanism. J. Bacteriol. **173:**5290–5297.
89. **Hanne, L. F., and R. A. Finkelstein.** 1982. Characterization and distribution of the hemagglutinins produced by *Vibrio cholerae*. Infect. Immun. **36:**209–214.
90. **Harada, S., K. Kitadokoro, T. Kinoshita, Y. Kai, and N. Kasai.** 1991. Crystallization and main-chain structure of neutral protease from *Streptomyces caespitosus*. J. Biochem. **110:**46–49.
91. **Häse, C. C., and R. A. Finkelstein.** 1990. Comparison of the *Vibrio cholerae* hemagglutinin/protease and the *Pseudomonas aeruginosa* elastase. Infect. Immun. **58:**4011–4015.
92. **Häse, C. C., and R. A. Finkelstein.** 1991. Cloning and nucleotide sequence of the *Vibrio cholerae* hemagglutinin/protease (HA/protease) gene and construction of an HA/protease-negative strain. J. Bacteriol. **173:**3311–3317.
93. **He, X.-S., R. Brückner, and R. H. Doi.** 1991. The protease genes of *Bacillus subtilis*. Res. Microbiol. **142:**797–803.
93a. **Heinrich, P., R. Rosenstein, M. Böhmer, P. Sonner, and F. Götz.** 1987. The molecular organization of the lysostaphin gene and its sequences repeated in tandem. Mol. Gen. Genet. **209:**563–569.
94. **Higaki, J. N., R. J. Fletterick, and C. S. Craik.** 1992. Engineered metalloregulation in enzymes. Trends Biochem. Sci. **17:**100–104.
95. **Hines, D. A., P. N. Saurugger, G. M. Ihler, and M. J. Benedik.** 1988. Genetic analysis of extracellular proteins of *Serratia marcescens*. J. Bacteriol. **170:**4141–4146.
96. **Holder, I. A.** 1983. Experimental studies of the pathogenesis of infections due to *Pseudomonas aeruginosa*: effect of treatment with protease inhibitors. Rev. Infect. Dis. **5**(Suppl.)**:**S914–S921.
97. **Holland, D. R., D. E. Tronrud, H. W. Pley, K. M. Flaherty, W. Stark, J. N. Jansonius, D. B. McKay, and B. W. Matthews.** 1992. Structural comparison suggests that thermolysin and related neutral proteases undergo hinge-bending motion during catalysis. Biochemistry **31:**11310–11316.
98. **Holmes, M. A., and B. W. Matthews.** 1982. Structure of thermolysin refined at 1.6 Å resolution. J. Mol. Biol. **160:**623–639.
99. **Holmquist, B., and B. L. Vallee.** 1974. Metal substitutions and inhibition of thermolysin: spectra of the cobalt enzyme. J. Biol. Chem. **249:**4601–4607.
100. **Honda, T., B. A. Booth, M. Boesman-Finkelstein, and R. A. Finkelstein.** 1987. Comparative study of *Vibrio cholerae* non-O1 protease and soluble hemagglutinin with those of *Vibrio cholerae* O1. Infect. Immun. **55:**451–454.
101. **Honda, T., A. Hata-Naka, K. Lertpocasombat, and T. Miwatani.** 1991. Production of monoclonal antibodies against a hemagglutinin/protease of *Vibrio cholerae* non-O1. FEMS Microbiol. Lett. **78:**227–230.
102. **Honda, T., K. Lertpocasombat, A. Hata, T. Miwatani, and R. A. Finkelstein.** 1989. Purification and characterization of a protease produced by *Vibrio cholerae* non-O1 and comparison with a protease of *V. cholerae* O1. Infect. Immun. **57:**2799–2803.
103. **Hsu, T. C., W. D. Waltman, and E. B. Shotts.** 1981. Correlation of extracellular enzymatic activity and biochemical characteristics with regard to virulence of *Aeromonas hydrophila*. Dev. Biol. Stand. **49:**101–111.
103a. **Huttner, W. B.** 1993. Snappy exocytotoxins. Nature (London) **365:**104–105.
104. **Imanaka, T., M. Shibazaki, and M. Takagi.** 1986. A new way of enhancing the thermostability of proteases. Nature (London) **324:**695–697.
105. **Ingrosso, D., A. V. Fowler, J. Bleibaum, and S. Clarke.** 1989. Specificity of endoproteinase Asp-N (*Pseudomonas fragi*): cleavage at glutamyl residues in two proteins. Biochem. Biophys. Res. Commun. **162:**1528–1534.
106. **Janzon, L., and S. Arvidson.** 1990. The role of the δ-lysin gene (*hld*) in the regulation of virulence genes by the accessory gene regulator (*agr*) in *Staphylococcus aureus*. EMBO J. **9:**1391–1399.
107. **Jiang, W., and J. S. Bond.** 1992. Families of metalloendopeptidases and their relationships. FEBS Lett. **312:**110–114.
108. **Jongeneel, C. V., J. Bouvier, and A. Bairoch.** 1989. A unique signature identifies a family of zinc-dependent metallopeptidases. FEBS Lett. **242:**211–214.
109. **Kajiwara, K., A. Fujita, H. Tsuyuki, T. Kumazaki, and S. Ishii.** 1991. Interactions of *Streptomyces* serine-protease inhibitors with *Streptomyces griseus* metalloendopeptidase II. J. Biochem. **110:**350–354.
110. **Kamata, R., K. Matsumoto, R. Okamura, T. Yamamoto, and H. Maeda.** 1985. The serratial 56K protease as a major pathogenic factor in serratial keratitis: clinical and experimental study. Ophthamology **92:**1452–1459.
111. **Kamata, R., T. Yamamoto, K. Matsumoto, and H. Maeda.** 1985. A serratial protease causes vascular permeability reaction by activation of the Hageman factor-dependent pathway in guinea pigs. Infect. Immun. **48:**747–753.
112. **Kanai, K., and H. Wakabayashi.** 1984. Purification and some

properties of protease from *Aeromonas hydrophila*. Bull. Jpn. Soc. Sci. Fish. **50**:1367–1374.
113. **Karakiulakis, G., E. Papadimitriu, E. Missirlis, and M. E. Maragoudakis.** 1991. Effect of divalent metal ions on collagenase from *Clostridium histolyticum*. Biochem. Int. **24**:397–404.
114. **Kato, T., N. Takahashi, and H. K. Kuramitsu.** 1992. Sequence analysis and characterization of the *Porphyromonas gingivalis prtC* gene, which expresses a novel collagenase activity. J. Bacteriol. **174**:3889–3895.
115. **Katsuya, Y., M. Sato, Y. Katsube, Y. Matsuura, and K. Tomoda.** 1992. Small-angle X-ray scattering study of metal ion-induced conformational changes in *Serratia* protease. J. Biol. Chem. **267**:12668–12672.
116. **Kawamoto, S., Y. Shibano, J. Fukushima, N. Ishii, K. Morihara, and K. Okuda.** 1993. Site-directed mutagenesis of Glu-141 and His-223 in *Pseudomonas aeruginosa* elastase: catalytic activity, processing, and protective activity of the elastase against *Pseudomonas* infection. Infect. Immun. **61**:1400–1405.
117. **Keil-Dlouha, V.** 1976. Chemical characterization and study of the autodigestion of pure collagenase from *Achromobacter iophagus*. Biochim. Biophys. Acta **429**:239–251.
118. **Kessler, E., and M. Safrin.** 1988. Partial purification and characterization of an inactive precursor of *Pseudomonas aeruginosa* elastase. J. Bacteriol. **170**:1215–1219.
119. **Kessler, E., and M. Safrin.** 1988. Synthesis, processing, and transport of *Pseudomonas aeruginosa* elastase. J. Bacteriol. **170**:5241–5247.
120. **Kessler, E., M. Safrin, M. Peretz, and Y. Burstein.** 1992. Identification of cleavage sites involved in proteolytic processing of *Pseudomonas aeruginosa* preproelastase. FEBS Lett. **299**:291–293.
120a. **Klimpel, K. R., N. Arora, and S. H. Leppla.** 1993. Anthrax toxin lethal factor has homology to the thermolysin-like proteases and displays proteolytic activity, abstr. B-111, p. 45. Abstr. 93rd Gen. Meet. Am. Soc. Microbiol. 1993. American Society for Microbiology, Washington, D.C.
121. **Kobayashi, R., A. Kanatani, T. Yoshimoto, and D. Tsuru.** 1989. Chemical modification of neutral protease from *Bacillus subtilis* var. *amylosacchariticus* with tetranitromethane: assignment of tyrosyl residues nitrated. J. Biochem. **106**:1110–1113.
122. **Kobayashi, R., T. Yoshimoto, and D. Tsuru.** 1989. Complete amino acid sequence of neutral protease from *Bacillus subtilis* var. *amylosacchariticus*. Agric. Biol. Chem. **53**:2737–2749.
123. **Kothary, M. H., and A. S. Kreger.** 1987. Purification and characterization of an elastolytic protease of *Vibrio vulnificus*. J. Gen. Microbiol. **133**:1783–1791.
124. **Kubo, M., and T. Imanaka.** 1988. Cloning and nucleotide sequence of the highly thermostable neutral protease gene from *Bacillus stearothermophilus*. J. Gen. Microbiol. **134**: 1883–1892.
125. **Kucerova, H., and J. Chaloupka.** 1985. Suppression by temperature of sporulation and of exocellular metalloproteinase synthesis in *Bacillus megaterium*. FEMS Microbiol. Lett. **28**:293–296.
126. **Kühn, S., and P. Fortnagel.** 1993. Molecular cloning and nucleotide sequence of the gene encoding a calcium-dependent exoproteinase from *Bacillus megaterium* ATCC 14581. J. Gen. Microbiol. **139**:39–47.
127. **Kurazono, H., S. Mochida, T. Binz, U. Eisel, M. Quanz, O. Grebenstein, K. Wernars, B. Poulain, L. Tauc, and H. Niemann.** 1992. Minimal essential domains specifying toxicity of the light chains of tetanus toxin and botulinum neurotoxin type A. J. Biol. Chem. **267**:14721–14729.
128. **Kwon, Y. T., H. H. Lee, and H. M. Rho.** 1993. Cloning, sequencing, and expression of a minor protease-encoding gene from *Serratia marcescens* ATCC21074. Gene **125**:75–80.
129. **Kyöstiö, S. R. M., C. L. Cramer, and G. H. Lacy.** 1991. *Erwinia carotovora* subsp. *carotovora* extracellular protease: characterization and nucleotide sequence of the gene. J. Bacteriol. **173**:6537–6546.
130. **Labib, R. S., N. J. Calvanico, and T. B. Tomasi.** 1978. Studies on extracellular proteases of *Streptococcus sanguis*. Purification and characterization of a human IgA1 specific protease. Biochim. Biophys. Acta **526**:547–559.
131. **Lagace, J., and M. Frechette.** 1991. Four epitopes of *Pseudomonas aeruginosa* elastase defined by monoclonal antibodies. Infect. Immun. **59**:712–715.
132. **Lampel, J. S., J. S. Aphale, K. A. Lampel, and W. R. Strohl.** 1992. Cloning and sequencing of a gene encoding a novel extracellular neutral proteinase from *Streptomyces* sp. strain C5 and expression of the gene in *Streptomyces lividans* 1326. J. Bacteriol. **174**:2797–2808.
133. **Letoffe, S., P. Delepelaire, and C. Wandersman.** 1989. Characterization of a protein inhibitor of extracellular proteases produced by *Erwinia chrysanthemi*. Mol. Microbiol. **3**:79–86.
134. **Letoffe, S., P. Delepelaire, and C. Wandersman.** 1990. Protease secretion by *Erwinia chrysanthemi*: the specific secretion functions are analogous to those of *Escherichia coli* α-hemolysin. EMBO J. **9**:1375–1382.
135. **Letoffe, S., P. Delepelaire, and C. Wandersman.** 1991. Cloning and expression in *Escherichia coli* of the *Serratia marcescens* metalloprotease gene: secretion of the protease from *E. coli* in the presence of the *Erwinia chrysanthemi* protease secretion functions. J. Bacteriol. **173**:2160–2166.
136. **Letoffe, S., and C. Wandersman.** 1992. Secretion of CyaA-PrtB and HlyA-PrtB fusion proteins in *Escherichia coli*: involvement of the glycine-rich repeat domain of *Erwinia chrysanthemi* protease B. J. Bacteriol. **174**:4920–4927.
137. **Leung, K.-Y., and R. M. W. Stevenson.** 1988. Characteristics and distribution of extracellular proteases from *Aeromonas hydrophila*. J. Gen. Microbiol. **134**:151–160.
138. **Leung, K. Y., and R. M. W. Stevenson.** 1988. Tn*5*-induced protease-deficient strains of *Aeromonas hydrophila* with reduced virulence for fish. Infect. Immun. **56**:2639–2644.
139. **Levy, P. L., M. K. Pangburn, Y. Burstein, L. H. Ericsson, H. Neurath, and K. A. Walsh.** 1975. Evidence of homologous relationship between thermolysin and neutral protease A of *Bacillus subtilis*. Proc. Natl. Acad. Sci. USA **72**:4341–4345.
140. **Lichenstein, H. S., L. A. Busse, G. A. Smith, L. O. Narhi, M. O. McGinley, M. F. Rhode, J. L. Katzowitz, and M. M. Zukowski.** 1992. Cloning and characterization of a gene encoding extracellular metalloprotease from *Streptomyces lividans*. Gene **111**:125–130.
141. **Lory, S.** 1992. Determinants of extracellular protein secretion in gram-negative bacteria. J. Bacteriol. **174**:3423–3428.
142. **Lövgren, A., M. Zhang, A. Engström, G. Dalhammar, and R. Landen.** 1990. Molecular characterization of immune inhibitor A, a secreted virulence protease from *Bacillus thuringiensis*. Mol. Microbiol. **4**:2137–2146.
143. **Lyerly, D., L. Gray, and A. Kreger.** 1981. Characterization of rabbit corneal damage produced by serratia keratitis and by a serratia protease. Infect. Immun. **33**:927–932.
144. **Lyerly, D. M., and A. S. Kreger.** 1983. Importance of serratia protease in the pathogenesis of experimental *Serratia marcescens* pneumonia. Infect. Immun. **40**:113–119.
145. **Macfarlane, G. T., and S. Macfarlane.** 1992. Physiological and nutritional factors affecting synthesis of extracellular metalloproteases by *Clostridium bifermentans* NCTC 2914. Appl. Environ. Microbiol. **58**:1195–1200.
146. **Makinen, K. K., and P.-L. Makinen.** 1987. Purification and properties of an extracellular collagenolytic protease produced by the human oral bacterium *Bacillus cereus* (strain Soc 67). J. Biol. Chem. **262**:12488–12495.
147. **Mäkinen, P.-L., D. B. Clewell, F. An, and K. K. Mäkinen.** 1989. Purification and substrate specificity of a strongly hydrophobic extracellular metalloendopeptidase ("gelatinase") from *Streptococcus faecalis* (strain 0G1-10). J. Biol. Chem. **264**:3325–3334.
148. **Margesin, R., and F. Schinner.** 1991. Characterization of a metalloprotease from psychrophilic *Xanthomonas maltophilia*. FEMS Microbiol. Lett. **79**:257–262.
149. **Matthews, B. W.** 1988. Structural basis of the action of thermolysin and related zinc peptidases. Acc. Chem. Res. **21**:333–340.
150. **Matthews, B. W., J. N. Jansonius, P. M. Colman, B. P. Schoenborn, and D. Dupourque.** 1972. Three-dimensional

structure of thermolysin. Nature (London) New Biol. **238**:37–41.
151. **Matthews, B. W., L. H. Weaver, and W. R. Kester.** 1974. The conformation of thermolysin. J. Biol. Chem. **249**:8030–8044.
152. **McIver, K., E. Kessler, and D. E. Ohman.** 1991. Substitution of active-site His-223 in *Pseudomonas aeruginosa* elastase and expression of the mutated *lasB* alleles in *Escherichia coli* show evidence for autoproteolytic processing of proelastase. J. Bacteriol. **173**:7781–7789.
153. **McKerrow, J. H.** 1987. Human fibroblast collagenase contains an amino acid sequence homologous to the zinc-binding site of *Serratia* protease. J. Biol. Chem. **262**:5943.
154. **McKevitt, A. I., S. Bajaksouzian, J. D. Klinger, and D. E. Woods.** 1989. Purification and characterization of an extracellular protease from *Pseudomonas cepacia*. Infect. Immun. **57**:771–778.
155. **Mengaud, J., C. Geoffroy, and P. Cossart.** 1991. Identification of a new operon involved in *Listeria monocytogenes* virulence: its first gene encodes a protein homologous to bacterial metalloproteases. Infect. Immun. **59**:1043–1049.
156. **Mengaud, J., M. F. Vicente, and P. Cossart.** 1989. Transcriptional mapping and nucleotide sequence of the *Listeria monocytogenes hlyA* region reveal structural features that may be involved in regulation. Infect. Immun. **57**:3695–3701.
157. **Milton, D. L., A. Norqvist, and H. Wolf-Watz.** 1992. Cloning of a metalloprotease gene involved in the virulence mechanism of *Vibrio anguillarum*. J. Bacteriol. **174**:7235–7244.
158. **Miyoshi, N., S.-I. Miyoshi, K. Sugiyama, Y. Suzuki, H. Furuta, and S. Shinoda.** 1987. Activation of the plasma kallikrein-kinin system by *Vibrio vulnificus* protease. Infect. Immun. **55**:1936–1939.
159. **Miyoshi, S., and S. Shinoda.** 1988. Role of the protease in the permeability enhancement by *Vibrio vulnificus*. Microbiol. Immunol. **32**:1025–1032.
160. **Miyoshi, S., and S. Shinoda.** 1989. Inhibitory effect of α_2-macroglobulin on *Vibrio vulnificus* protease. J. Biochem. **106**: 299–303.
161. **Miyoshi, S., and S. Shinoda.** 1991. α-Macroglobulin-like plasma inactivator for *Vibrio vulnificus* metalloprotease. J. Biochem. **110**:548–552.
162. **Molla, A., T. Akaike, and H. Maeda.** 1989. Inactivation of various proteinase inhibitors and the complement system in human plasma by the 56-kilodalton proteinase from *Serratia marcescens*. Infect. Immun. **57**:1868–1871.
163. **Molla, A., K. Matsumoto, I. Oyamada, T. Katsuki, and H. Maeda.** 1986. Degradation of protease inhibitors, immunoglobulins, and other serum proteins by *Serratia* protease and its toxicity to fibroblasts in culture. Infect. Immun. **53**:522–529.
164. **Morfeldt, E., L. Janzon, S. Arvidson, and S. Löfdahl.** 1988. Cloning of a chromosomal locus (*exp*) which regulates the expression of several exoprotein genes in *Staphylococcus aureus*. Mol. Gen. Genet. **211**:435–440.
165. **Morihara, K., and J. Y. Homma.** 1985. *Pseudomonas* proteases, p. 41–79. *In* I. A. Holder (ed.), Bacterial enzymes and virulence. CRC Press, Inc., Boca Raton, Fla.
166. **Murai, H., S. Hara, T. Ikenaka, K. Oda, and S. Murao.** 1985. Amino acid sequence of *Streptomyces* metallo-proteinase inhibitor from *Streptomyces nigrescens* TK-23. J. Biochem. **97**:173–180.
167. **Mynott, T. L., D. S. Chandler, and R. K. J. Luke.** 1991. Efficacy of enteric-coated protease in preventing attachment of enterotoxigenic *Escherichia coli* and diarrheal disease in the RITARD model. Infect. Immun. **59**:3708–3714.
168. **Naka, A., K. Yamamoto, T. Miwatani, and T. Honda.** 1992. Characterization of two forms of hemagglutinin/protease produced by *Vibrio cholerae* non-O1. FEMS Microbiol. Lett. **98**:197–200.
169. **Nakahama, K., K. Yoshimura, R. Marumoto, M. Kikuchi, I. S. Lee, T. Hase, and H. Matsubara.** 1986. Cloning and sequencing of *Serratia* protease gene. Nucleic Acids Res. **14**:5843–5855.
170. **Narahashi, Y., K. Shibuya, and M. Yanagita.** 1968. Studies on proteolytic enzymes (pronase) of *Streptomyces griseus* K-1. J. Biochem. **64**:427–437.
171. **Narukawa, H., S. Miyoshi, and S. Shinoda.** 1993. Chemical modification of *Vibrio vulnificus* metalloprotease with activated polyethylene glycol. FEMS Microbiol. Lett. **108**:43–46.
171a. **Neumann, V. C., H. E. Heath, P. A. LeBlanc, and G. L. Sloan.** 1993. Extracellular proteolytic activation of bacteriolytic peptidoglycan hydrolases of *Staphylococcus simulans* biovar *staphylolyticus*. FEMS Microbiol. Lett. **110**:205–212.
172. **Niemann, H.** 1991. Molecular biology of clostridial neurotoxins, p. 303–348. *In* J. E. Alouf and J. H. Freer (ed.), A sourcebook of bacterial protein toxins. Academic Press Ltd., London.
173. **Nieto, T. P., and A. E. Ellis.** 1986. Characterization of extracellular metallo- and serine-proteases of *Aeromonas hydrophila* strain B_{51}. J. Gen. Microbiol. **132**:1975–1979.
174. **Nishina, Y., S.-I. Miyoshi, A. Nagase, and S. Shinoda.** 1992. Significant role of an exocellular protease in utilization of heme by *Vibrio vulnificus*. Infect. Immun. **60**:2128–2132.
175. **Nishiya, Y., and T. Imanaka.** 1990. Cloning and nucleotide sequence of the *Bacillus stearothermophilus* neutral protease gene and its transcriptional activator gene. J. Bacteriol. **172**: 4861–4869.
176. **Noreau, J., and G. R. Drapeau.** 1979. Isolation and properties of the protease from the wild-type and mutant strains of *Pseudomonas fragi*. J. Bacteriol. **140**:911–916.
177. **Norqvist, A., B. Norrman, and H. Wolf-Watz.** 1990. Identification and characterization of a zinc metalloprotease associated with invasion by the fish pathogen *Vibrio anguillarum*. Infect. Immun. **58**:3731–3736.
178. **Oda, K., T. Koyama, and S. Murao.** 1979. Purification and properties of a proteinaceous metallo-proteinase inhibitor from *Streptomyces nigrescens* TK-23. Biochim. Biophys. Acta **571**: 147–156.
179. **Oda, T., Y. Kojima, T. Akaike, S. Ijiri, A. Molla, and H. Maeda.** 1990. Inactivation of chemotactic activity of C5a by the serratial 56-kilodalton protease. Infect. Immun. **58**:1269–1272.
180. **Okuda, K., K. Morihara, Y. Atsumi, H. Takeuchi, S. Kawamoto, H. Kawasaki, K. Suzuki, and J. Fukushima.** 1990. Complete nucleotide sequence of the structural gene for alkaline proteinase from *Pseudomonas aeruginosa* IFO 3455. Infect. Immun. **58**:4083–4088.
181. **Olson, J. C., and D. E. Ohman.** 1992. Efficient production and processing of elastase and LasA by *Pseudomonas aeruginosa* require zinc and calcium ions. J. Bacteriol. **174**:4140–4147.
182. **Park, K. B., and R. G. Labbe.** 1990. Proteolysis of *Clostridium perfringens* type A enterotoxin during purification. Infect. Immun. **58**:1999–2001.
183. **Parmely, M. J.** 1993. *Pseudomonas* metalloproteases and the host-microbe relationship, p. 79–94. *In* R. B. Fick (ed.), *Pseudomonas aeruginosa* the opportunist: pathogenesis and disease. CRC Press, Inc., Boca Raton, Fla.
184. **Pauptit, R. A., R. Karlsson, D. Picot, J. A. Jenkins, A.-S. Niklaus-Reimer, and J. N. Jansonius.** 1988. Crystal structure of neutral protease from *Bacillus cereus* refined at 3.0 Å resolution and comparison with the homologous but more thermostable enzyme thermolysin. J. Mol. Biol. **199**:525–537.
185. **Pessi, A., E. Bianchi, A. Crameri, S. Venturini, A. Tramontano, and M. Sollazzo.** 1993. A designed metal-binding protein with a novel fold. Nature (London) **362**:367–369.
186. **Plaut, A. G.** 1983. The IgA1 proteases of pathogenic bacteria. Annu. Rev. Microbiol. **37**:603–622.
187. **Plaut, A. G., R. J. Genco, and T. B. Tomasi.** 1974. Isolation of an enzyme from *Streptococcus sanguis* which specifically cleaves IgA. J. Immunol. **113**:289–291.
188. **Porzio, M. A., and A. M. Pearson.** 1975. Isolation of an extracellular neutral proteinase from *Pseudomonas fragi*. Biochim. Biophys. Acta **384**:235–241.
189. **Potempa, J., Z. Porwit-Bobr, and J. Travis.** 1989. Stabilization vs. degradation of *Staphylococcus aureus* metalloproteinase. Biochim. Biophys. Acta **993**:301–304.
190. **Poulet, S., D. Hauser, M. Quanz, H. Niemann, and M. R. Popoff.** 1992. Sequences of the botulinal neurotoxin E derived from *Clostridium botulinum* type E (strain Beluga) and *Clostridium butyricum* (strains ATCC 43181 and ATCC 43755).

Biochem. Biophys. Res. Commun. **183:**107–113.
191. **Poyart, C., E. Abachin, I. Razafimanantsoa, and P. Berche.** 1993. The zinc metalloprotease of *Listeria monocytogenes* is required for maturation of phosphatidylcholine phospholipase C: direct evidence obtained by gene complementation. Infect. Immun. **61:**1576–1580.
192. **Proctor, M., and P. J. Manning.** 1990. Production of immunoglobulin A protease by *Streptococcus pneumoniae* from animals. Infect. Immun. **58:**2733–2737.
193. **Prokesova, L., Z. Porwit-Bobr, K. Baran, J. Potempa, M. Pospisil, and C. John.** 1991. Effect of metalloproteinase from *Staphylococcus aureus* on in vitro stimulation of human lymphocytes. Immunol. Lett. **27:**225–230.
194. **Pugsley, A. P.** 1993. The complete general secretory pathway in gram-negative bacteria. Microbiol. Rev. **57:**50–108.
195. **Quinn, F. D., M. G. Keen, and L. S. Tompkins.** 1989. Genetic, immunological, and cytotoxic comparisons of *Legionella* proteolytic activities. Infect. Immun. **57:**2719–2725.
196. **Quinn, F. D., and L. S. Tompkins.** 1989. Analysis of a cloned sequence of *Legionella pneumophila* encoding a 38 kD metalloprotease possessing haemolytic and cytotoxic activities. Mol. Microbiol. **3:**797–805.
197. **Raveneau, J., C. Geoffroy, J.-L. Beretti, J.-L. Gaillard, J. E. Alouf, and P. Berche.** 1992. Reduced virulence of a *Listeria monocytogenes* phospholipase-deficient mutant obtained by transposon insertion into the zinc metalloprotease gene. Infect. Immun. **60:**916–921.
198. **Rechnitzer, C., and A. Kharazmi.** 1992. Effect of *Legionella pneumophila* cytotoxic protease on human neutrophil and monocyte function. Microb. Pathog. **12:**115–125.
199. **Rechnitzer, C., A. Williams, J. B. Wright, A. B. Dowsett, N. Milman, and R. B. Fitzgeorge.** 1992. Demonstration of the intracellular production of tissue-destructive protease by *Legionella pneumophila* multiplying within guinea-pig and human alveolar macrophages. J. Gen. Microbiol. **138:**1671–1677.
199a. **Recsei, P. A., A. D. Gruss, and R. P. Novick.** 1987. Cloning, sequence, and expression of the lysostaphin gene from *Staphylococcus simulans*. Proc. Natl. Acad. Sci. USA **84:**1127–1131.
200. **Reinholdt, J., and M. Kilian.** 1987. Interference of IgA protease with the effect of secretory IgA on adherence of oral streptococci to saliva-coated hydroxyapatite. J. Dent. Res. **66:**492–497.
201. **Reinholdt, J., M. Tomana, S. B. Mortensen, and M. Kilian.** 1990. Molecular aspects of immunoglobulin A1 degradation by oral streptococci. Infect. Immun. **58:**1186–1194.
202. **Rivero, O., J. Anguita, C. Paniagua, and G. Naharro.** 1990. Molecular cloning and characterization of an extracellular protease gene from *Aeromonas hydrophila*. J. Bacteriol. **172:**3905–3908.
203. **Rodriguez, L. A., A. E. Ellis, and T. P. Nieto.** 1992. Purification and characterization of an extracellular metalloprotease, serine protease and haemolysin of *Aeromonas hydrophila* strain B_{32}: all are lethal for fish. Microb. Pathog. **13:**17–24.
204. **Salmond, G. P. C., and P. J. Reeves.** 1993. Membrane traffic wardens and protein secretion in Gram-negative bacteria. Trends Biochem. Sci. **18:**7–12.
205. **Schad, P. A., R. A. Bever, T. I. Nicas, F. Leduc, L. F. Hanne, and B. H. Iglewski.** 1987. Cloning and characterization of elastase genes from *Pseudomonas aeruginosa*. J. Bacteriol. **169:**2691–2696.
206. **Schiavo, G., F. Benfenati, B. Poulain, O. Rossetto, P. P. de Laureto, B. R. DasGupta, and C. Montecucco.** 1992. Tetanus and botulinum-B neurotoxins block neurotransmitter release by proteolytic cleavage of synaptobrevin. Nature (London) **359:**832–835.
207. **Schiavo, G., B. Poulain, O. Rossetto, F. Benfenati, L. Tauc, and C. Montecucco.** 1992. Tetanus toxin is a zinc protein and its inhibition of neurotransmitter release and protease activity depend on zinc. EMBO J. **11:**3577–3583.
207a. **Schindler, C. A., and V. T. Schuhardt.** 1965. Purification and properties of lysostaphin—a lytic agent for *Staphylococcus aureus*. Biochim. Biophys. Acta **97:**242–250.
208. **Schneider, D. R., and C. D. Parker.** 1978. Isolation and characterization of protease-deficient mutants of *Vibrio cholerae*. J. Infect. Dis. **138:**143–151.
209. **Seifert, S., and E. Harper.** 1971. The collagenases, p. 649–697. *In* P. D. Boyer (ed.), The enzymes. Academic Press, Inc., New York.
210. **Senior, B. W., L. M. Loomes, and M. A. Kerr.** 1991. Microbial IgA proteases and virulence. Rev. Med. Microbiol. **2:**200–207.
211. **Shi, J., V. E. Coyne, and R. M. Weiner.** Unpublished data.
212. **Shimada, H., M. Honjo, I. Mita, A. Nakayama, A. Akaoka, K. Manabe, and Y. Furutani.** 1985. The nucleotide sequence and some properties of the neutral protease gene of *Bacillus amyloliquefaciens*. J. Biotechnol. **2:**75–85.
213. **Sidler, W., E. Niederer, F. Suter, and H. Zuber.** 1986. The primary structure of *Bacillus cereus* neutral proteinase and comparison with thermolysin and *Bacillus subtilis* neutral proteinase. Biol. Chem. Hoppe-Seyler **367:**643–657.
214. **Signor, G., C. Vita, A. Fontana, F. Frigerio, M. Bolognesi, S. Toma, R. Gianna, E. De Gregoriis, and G. Grandi.** 1990. Structural features of neutral protease from *Bacillus subtilis* deduced from model-building and limited proteolysis experiments. Eur. J. Biochem. **189:**221–227.
215. **Simonen, M., and I. Palva.** 1993. Protein secretion in *Bacillus* species. Microbiol. Rev. **57:**109–137.
216. **Sloma, A., C. F. Rudolph, G. A. Rufo, B. J. Sullivan, K. A. Theriault, D. Ally, and J. Pero.** 1990. Gene encoding a novel extracellular metalloprotease in *Bacillus subtilis*. J. Bacteriol. **172:**1024–1029.
217. **Smith, G. C., and J. R. Merkel.** 1982. Collagenolytic activity of *Vibrio vulnificus*: potential contribution to its invasiveness. Infect. Immun. **35:**1155–1156.
218. **Stark, W., R. A. Pauptit, K. S. Wilson, and J. N. Jansonius.** 1992. The structure of neutral protease from *Bacillus cereus* at 0.2-nm resolution. Eur. J. Biochem. **207:**781–791.
219. **Stenn, K. S., R. Link, G. Moellmann, J. Madri, and E. Kuklinska.** 1989. Dispase, a neutral protease from *Bacillus polymyxa*, is a powerful fibronectinase and type IV collagenase. J. Invest. Dermatol. **93:**287–290.
220. **Stoeva, S.** 1991. Modification of a zinc proteinase from *Bacillus mesentericus* strain 76 by diethylpyrocarbonate. Int. J. Peptide Protein Res. **37:**325–330.
221. **Stoeva, S., T. Kleinschmidt, B. Mesrob, and G. Braunitzer.** 1990. Primary structure of a zinc protease from *Bacillus mesentericus* strain 76. Biochemistry **29:**527–534.
222. **Suh, Y., and M. J. Benedik.** 1992. Production of active *Serratia marcescens* metalloprotease from *Escherichia coli* by α-hemolysin HlyB and HlyD. J. Bacteriol. **174:**2361–2366.
223. **Sutherland, D. R., K. M. Abdullah, P. Cyopick, and A. Mellors.** 1992. Cleavage of the cell-surface O-sialoglycoproteins CD34, CD43, CD44, and CD45 by a novel glycoprotease from *Pasteurella haemolytica*. J. Immunol. **148:**1458–1464.
224. **Szeto, L., and H. A. Shuman.** 1990. The *Legionella pneumophila* major secretory protein, a protease, is not required for intracellular growth or cell killing. Infect. Immun. **58:**2585–2592.
225. **Takagi, M., and T. Imanaka.** 1989. Addition of a methyl group changes both the catalytic velocity and thermostability of the neutral protease from *Bacillus stearothermophilus*. FEBS Lett. **254:**43–46.
226. **Takagi, M., T. Imanaka, and S. Aiba.** 1985. Nucleotide sequence and promoter region for the neutral protease gene from *Bacillus stearothermophilus*. J. Bacteriol. **163:**824–831.
227. **Takekawa, S., N. Uozumi, N. Tsukagoshi, and S. Udaka.** 1991. Proteases involved in generation of β- and α-amylases from a large amylase precursor in *Bacillus polymyxa*. J. Bacteriol. **173:**6820–6825.
228. **Takeuchi, H., Y. Shibano, K. Morihara, J. Fukushima, S. Inami, B. Keil, A.-M. Gilles, S. Kawamoto, and K. Okuda.** 1992. Structural gene and complete amino acid sequence of *Vibrio alginolyticus* collagenase. Biochem. J. **281:**703–708.
229. **Tanaka, E., S. Kawamoto, J. Fukushima, K. Hamajima, H. Onishi, Y. Miyagi, S. Inami, K. Morihara, and K. Okuda.** 1991.

Detection of elastase production in *Escherichia coli* with the elastase structural gene from several non-elastase-producing strains of *Pseudomonas aeruginosa*. J. Bacteriol. **173**:6153–6158.

230. **Tang, J. L., C. L. Gough, C. E. Barber, J. M. Dow, and M. J. Daniels.** 1987. Molecular cloning of protease gene(s) from *Xanthomonas campestris* pv. *campestris*: expression in *Escherichia coli* and role in pathogenicity. Mol. Gen. Genet. **210**:443–448.

230a. **Teufel, P., and F. Götz.** 1993. Characterization of an extracellular metalloprotease with elastase activity from *Staphylococcus epidermidis*. J. Bacteriol. **175**:4218–4224.

231. **Thayer, M. M., K. M. Flaherty, and D. B. McKay.** 1991. Three-dimensional structure of the elastase of *Pseudomonas aeruginosa* at 1.5-Å resolution. J. Biol. Chem. **266**:2864–2871.

232. **Titani, K., M. A. Hermodson, L. H. Ericsson, K. A. Walsh, and H. Neurath.** 1972. Amino-acid sequence of thermolysin. Nature (London) New Biol. **238**:35–37.

233. **Toder, D. S., M. J. Gambello, and B. H. Iglewski.** 1991. *Pseudomonas aeruginosa* LasA: a second elastase under the transcriptional control of *lasR*. Mol. Microbiol. **5**:2003–2010.

234. **Toma, S., S. Campagnoli, E. De Gregoriis, R. Gianna, I. Margarit, M. Zamai, and G. Grandi.** 1989. Effect of Glu-143 and His-231 substitutions on the catalytic activity and secretion of *Bacillus subtilis* neutral protease. Protein Eng. **2**:359–364.

235. **Toma, S., S. Campagnoli, I. Margarit, R. Gianna, G. Grandi, M. Bolognesi, V. De Filippis, and A. Fontana.** 1991. Grafting of a calcium-binding loop of thermolysin to *Bacillus subtilis* neutral protease. Biochemistry **30**:97–106.

236. **Tommassen, J., A. Filloux, M. Bally, M. Murgier, and A. Lazdunski.** 1992. Protein secretion in *Pseudomonas aeruginosa*. FEMS Microbiol. Rev. **103**:73–90.

237. **Tong, N. T., A. Tsugita, and V. Keil-Dlouha.** 1986. Purification and characterization of two high-molecular-mass forms of *Achromobacter* collagenase. Biochim. Biophys. Acta **874**:296–304.

238. **Tran, L., X.-C. Wu, and S.-L. Wong.** 1991. Cloning and expression of a novel protease gene encoding an extracellular neutral protease from *Bacillus subtilis*. J. Bacteriol. **173**:6364–6372.

238a. **Trayer, H. R., and C. E. Buckley.** 1970. Molecular properties of lysostaphin, a bacteriolytic agent specific for *Staphylococcus aureus*. J. Biol. Chem. **245**:4842–4846.

239. **Tseng, T. C., and M. S. Mount.** 1974. Toxicity of endopolygalacturonate trans-eliminase, phosphatidase and protease to potato and cucumber tissue. Phytopathology **64**:229–236.

240. **Tsuru, D., S. Imajo, S. Morikawa, T. Yoshimoto, and M. Ishiguro.** 1993. Zinc protease of *Bacillus subtilis* var. *amylosacchariticus*: construction of a three-dimensional model and comparison with thermolysin. J. Biochem. **113**:101–105.

241. **Tsuyuki, H., K. Kajiwara, A. Fujita, T. Kumazaki, and S. Ishii.** 1991. Purification and characterization of *Streptomyces griseus* metalloendopeptiodases I and II. J. Biochem. **110**:339–344.

242. **Vallee, B. L., and D. S. Auld.** 1990. Active-site zinc ligands and activated H_2O of zinc enzymes. Proc. Natl. Acad. Sci. USA **87**:220–224.

243. **Vallee, B. L., and D. S. Auld.** 1990. Zinc coordination, function, and structure of zinc enzymes and other proteins. Biochemistry **29**:5647–5659.

244. **van den Burg, B., H. G. Enequist, M. E. van der Haar, V. G. H. Eijsink, B. K. Stulp, and G. Venema.** 1991. A highly thermostable neutral protease from *Bacillus caldolyticus*: cloning and expression of the gene in *Bacillus subtilis* and characterization of the gene product. J. Bacteriol. **173**:4107–4115.

245. **Vasantha, N., L. D. Thompson, C. Rhodes, C. Banner, J. Nagle, and D. Filpula.** 1984. Genes for alkaline protease and neutral protease from *Bacillus amyloliquefaciens* contain a large open reading frame between the regions coding for signal sequence and mature protein. J. Bacteriol. **159**:811–819.

246. **Vriend, G., H. J. C. Berendsen, J. R. van der Zee, B. van den Burg, G. Venema, and V. G. H. Eijsink.** 1991. Stabilization of the neutral protease of *Bacillus stearothermophilus* by removal of a buried water molecule. Protein Eng. **4**:941–945.

247. **Wandersman, C.** 1989. Secretion, processing and activation of bacterial extracellular proteases. Mol. Microbiol. **3**:1825–1831.

248. **Wandersman, C.** 1992. Secretion across the bacterial outer membrane. Trends Genet. **8**:317–322.

249. **Wandersman, C., P. Delepelaire, S. Letoffe, and M. Schwartz.** 1987. Characterization of *Erwinia chrysanthemi* extracellular proteases: cloning and expression of the protease genes in *Escherichia coli*. J. Bacteriol. **169**:5046–5053.

250. **Wandersman, C., and S. Letoffe.** 1993. Involvement of lipopolysaccharide in the secretion of *Escherichia coli* α-hemolysin and *Erwinia chrysanthemi* proteases. Mol. Microbiol. **7**:141–150.

251. **Wang, L.-F., S. M. Ekkel, and R. J. Devenish.** 1990. Expression in *Escherichia coli* of the *Bacillus subtilis* neutral protease gene (*nprE*) lacking its ribosome binding site. Biochem. Int. **22**:1085–1093.

252. **Wasylewski, Z., W. Stryjewski, A. Wasniowska, J. Potempa, and K. Baran.** 1986. Effect of calcium binding on conformational changes of staphylococcal metalloproteinase measured by means of intrinsic protein fluorescence. Biochim. Biophys. Acta **871**:177–181.

253. **Wetmore, D. R., S.-L. Wong, and R. S. Roche.** 1992. The role of the pro-sequence in the processing and secretion of the thermolysin-like neutral protease from *Bacillus cereus*. Mol. Microbiol. **6**:1593–1604.

254. **Wikström, M., G. Jonsson, and A.-M. Svennerholm.** 1991. Production and characterization of monoclonal antibodies to *Vibrio cholerae* soluble haemagglutinin. APMIS **99**:249–256.

255. **Wolf, U., D. Bauer, and W. H. Traub.** 1991. Metalloproteases of *Serratia liquefaciens*: degradation of purified human serum proteins. Zentralbl. Bakteriol. Mikrobiol. Hyg. Ser. A **276**:16–26.

256. **Wretlind, B., and O. R. Pavlovskis.** 1983. *Pseudomonas aeruginosa* elastase and its role in pseudomonas infections. Rev. Infect. Dis. **5**(Suppl.):S998–S1004.

257. **Wright, J. F., M. Pernollet, A. Reboul, C. Aude, and M. G. Colomb.** 1992. Identification and partial characterization of a low affinity metal-binding site in the light chain of tetanus toxin. J. Biol. Chem. **267**:9053–9058.

258. **Wu, Z.-R., B.-J. Qi, R.-Q. Jiao, F.-D. Chen, and L.-F. Wang.** 1991. Development of a novel *Bacillus subtilis* cloning system employing its neutral protease as screening marker. Gene **106**:103–107.

259. **Yamamoto, S., J. Fukushima, Y. Atsumi, H. Takeuchi, S. Kawamoto, K. Okuda, and K. Morihara.** 1988. Cloning and characterization of elastase structural gene from *Pseudomonas aeruginosa* IFO 3455. Biophys. Biophys. Res. Commun. **152**:1117–1122.

260. **Yang, M. Y., E. Ferrari, and D. J. Henner.** 1984. Cloning of the neutral protease gene of *Bacillus subtilis* and the use of the cloned gene to create an in vitro-derived deletion mutation. J. Bacteriol. **160**:15–21.

261. **Yokota, S., H. Ohtsuka, and H. Noguchi.** 1992. Monoclonal antibodies against *Pseudomonas aeruginosa* elastase: a neutralizing antibody which recognizes a conformational epitope related to an active site of elastase. Eur. J. Biochem. **206**:587–593.