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(54) **Titre : LYSINES D'ACINETOBACTER**
(54) **Title: ACINETOBACTER LYSINS**

(57) **Abrégé/Abstract:**
Acinetobacter lysin polypeptides and variants peptides with killing activity against gram negative bacteria. Methods for treating bacterial infections or bacterial colonization using Acintobacter lysin polypeptides.

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(54) **Title**: ACINETOBACTER LYSINS

(57) **Abstract**: Acinetobacter lysin polypeptides and variants peptides with killing activity against gram negative bacteria. Methods for treating bacterial infections or bacterial colonization using Acintobacter lysin polypeptides.

ACINETOBACTER LYSINS

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This PCT application claims priority to U.S. Provisional Application Serial No. 62/017,618, filed June 26, 2014, the disclosure of which is hereby incorporated by reference in its entirety.

SEQUENCE LISTING

[0002] This application incorporates by reference in its entirety the sequence listing entitled "235932-373399_Sequence_Listing_ST25", (57KB) which was created on June 24, 2015, and filed electronically herewith.

FIELD

[0003] Compositions comprising a bacteriophage lytic enzyme specific for Acinetobacter and method for treating Acinetobacter infections.

BACKGROUND

[0004] Acinetobacter baumannii-calcoaceticus complex and other members of this species frequently colonize the human skin without harm. However, injuries to the skin from scrapes, wounds or surgery, can result in Acinetobacter infection of the wound, blood, soft tissues, and central nervous system. Given that >80% of Acinetobacter sp. are also multiply drug resistant (MDR) (at least three classes of antibiotics), these infections may result in adverse clinical outcomes, including high rates of morbidity and mortality, prolonged hospital stay, and substantial health care expenses. Military personnel and athletes have an increased the risk of injuries (from skin abrasions to severe wounds) that would be susceptible to infection by Acinetobacter spp., thus methods to remove them quickly and effectively would reduce or eliminate downstream complications. Outbreaks caused by MDR Acinetobacter have been reported in hospitals all over the world; more recently, they have become a serious problem in military medical facilities. Because of its MDR, Acinetobacter infections are difficult to treat so infections by these organisms usually result in a poor outcome. Thus, new and better ways of controlling this pathogen are needed.

[0005] Acinetobacter baumannii strains resistant to all known antibiotics have now been reported. Acting in synergy with this emerging resistance profile is the uncanny ability of A. baumannii to survive for prolonged periods throughout a hospital environment, thus potentiating its ability for nosocomial spread. The organism commonly targets hospitalized subjects, who are critically ill with breaches in skin integrity and airway protection. As such, hospital-acquired pneumonia is still the most common infection caused by A. baumannii. However, recently, infections involving the central nervous system, skin and soft tissue, and bone have emerged as

highly problematic for certain institutions. Because of this resistance problem, new methods to control these pathogens must be developed.

[0006] Antimicrobial agents known as bacteriophage-encoded lysins have been identified. Bacteriophages are viruses that infect bacterial and it is estimated that there are 10^6 distinct bacteriophage species. Bacteriophage lysins are generally genus- or species-specific, i.e., a *Staphylococcus aureus* phage lysin may have activity only against *Staphylococcus aureus* providing a targeted therapeutic approach. In some cases, lysins may have activity against several genera or species.

[0007] Bacteriophage infect their host bacteria to produce more virus particles. At the end of the reproductive cycle they are faced with a problem, how to release the progeny phage trapped within the bacterium. They solve this problem by producing an enzyme called "lysin" that degrades the cell wall of the infected bacteria to release the progeny phage. The lytic system consists of a holin and at least one peptidoglycan hydrolase, or lysin, capable of degrading the bacterial cell wall. Typically, the holin is expressed in the late stages of phage infection forming a pore in the cell membrane, allowing the lysin(s) to gain access to the cell wall peptidoglycan resulting in release of progeny phage. Significantly, exogenously added lysin, in the absence of a holin, can lyse the cell wall of healthy, uninfected cells, producing a phenomenon known as "lysis from without".

SUMMARY

[0008] We have recently identified, purified and characterized several phage lysins that specifically attack Acinetobacter bacteria. This is a breakthrough since most lysins have antibacterial activity only against gram-positive bacteria. The purified phage lysins of the present invention are well suited for a variety of applications such as treatment of bacterial infections, and disinfection.

BRIEF DESCRIPTION OF THE DRAWINGS

[0009] **Figure 1A**. Negative staining electron micrograph showing phage induced from A. baumannii strain 1790.

[0010] **Figure 1B**. Negative staining electron micrograph showing phage induced from A. baumannii strain 1794

[0011] **Figure 1C**. Negative staining electron micrograph showing phage induced from *A. baumannii* strain 1796

[0012] **Figure 2**. A representative image of lysin clone activity in clearing live *A. baumannii* imbedded in the agar.

[0013] **Figure 3**. Schematic of amino acid sequences of cloned lysins showing four classes of lytic activity: i) glycosyl hydrolase family, ii) phage baseplate lysozymes, iii) lysozyme

autolysins, and iv) lysins.

[0014] **Figure 4**. Alignment of nucleotide sequences for cloned lysins.

[0015] **Figure 5**. Alignment of amino acid sequences of cloned lysins.

[0016] **Figure 6.** Is a graph showing the lytic activity of 21 cloned constructs against thirteen. different A. baumannii clinical isolates.

[0017] **Figure 7A, and 7B**. Blebbing of the cytoplasmic membrane containing cytosolic contents from A. baumannii cells are observed after treatment with F307 (arrows).

[0018] **Figure 8**. Scanning electron micrograph of 3-day biofilms of strain 1791 A. baumannii before and after treatment with F307 polypeptide.

[0019] **Figure 9**. Is a graph showing reduction in bacterial counts on whole catheter pieces with Acinetobacter biofilm after treatment with F307 polypeptide.

[0020] **Figure 10**. Is a graph showing the survival of mice infected with *A. baumannii* treated with F307 polypeptide versus control.

[0021] **Figure 11**. Sequence of F307, P307 polypeptide without and with short extension (P307Ex).

[0022] **Figure 12.** Figure 12A is a graph a comparison of *in vitro* bactericidal activities of P307, P307SQ-8C and $P307_{AE-8}$ against *A. baumannii* strains #1791, S5 and ATCC17978. Figure 12B shows the comparative *in* vivo bactericidal activity of P307, P307SQ-8C, and P307CS-8 against *A. baumannii* strains #1791 and S5. Figure 12C shows a comparison of the comparative *in* vivo bactericidal activity of P307SQ-8C and P307CS-8 against *A. baumannii* strains #1791, S5 and ATCC17978.

[0023] **Figure 13.** The i*n vitro* bactericidal activities of P307 and P307SQ-8C against *A. baumannii* strain #1791 to investigate the pH optimum (13A), and NaCl optimum (13B). The same conditions, except for the variables, were used with 50 mM Tris-HCl, pH 7.5 to determine the concentration optimum (13C), and killing kinetics (13D). The error bars show standard deviation and the black horizontal line marks the limit of detection.

[0024] **Figure 14.** Is a graph showing the sensitivity of different bacterial species to P307 and P307SQ-8C. The error bars show standard deviation and the black horizontal line marks the limit of detection.

[0025] **Figure 15.** Figures 15A and 15B are graphs that show the bactericidal activities of P307 and P307SQ-8C against the log phase and stationary phase of *A. baumannii* strain No. 1791 (15A) and the biofilm phase (15B).

[0026] **Figure 16.** Figure 16 shows the cytotoxic effects of P307 and P307SQ-8C as measured by B cell survival (16A) and hemolysis (16B).

[0027] **Figure 17.** Figure 17A shows the effect of DTT at 0, 0.1 and 1 mM on the activity

of P307 and P307SQ-8C. Figure 17 B shows the effect of substitution of the terminal cysteine residue of P307SQ-8C with alanine (P307SQ-8A).

[0028] **Figure 18.** Is a DNA shift gel showing the shift for control peptide and P307.

[0029] **Figure 19.** Figures 7 A-C show representative transmission electron microscopy images of *A. baumanii* strain no.1791: untreated control (19A), treated with 300 μg/mL P307SQ-8C for 5 minutes (19B) and for 2 hours (19C). Magnification, ×2600 (left, scale bar = 2 μm) and ×5000 (right top and bottom, scale bar = 0.5 μm) . Figure 7D shows the bactericidal activity of P307SQ-8C on gram negative bacteria *K. pneumoniae* and *E. coli* at pH 7.5 and 8.8.

[0030] **Figure 20.** Shows the membrane permeability of *A. baumannii* strains #1791 and S5 treated with P307 and P307SQ-8C.

[0031] **Figure 21.** Shows the inhibition of bactericidal activity of P307 or P307SQ-8C by hydroxyl radical scavenger, thiourea and anaerobic condition.

[0032] **Figure 22.** Shows the effect of treatment of a skin infection with polymyxin B and P307SQ-8C.

DETAILED DESCRIPTION

[0033] The present invention provides polypeptides having antibacterial activity and for methods for using the disclosed polypeptides. As used herein, the singular forms “a”, “an” and “the” include plural references unless the context clearly dictates otherwise.

[0034] Terms such as “comprises”, “comprised”, “comprising”, “contains”, “containing” and the like have the meaning attributed in United States Patent law; they are inclusive or open-ended and do not exclude additional, un-recited elements or method steps. Terms such as “consisting essentially of” and “consists essentially of” have the meaning attributed in United States Patent law; they allow for the inclusion of additional ingredients or steps that do not materially affect the basic and novel characteristics of the claimed invention. The terms “consists of” and “consisting of” have the meaning ascribed to them in United States Patent law; namely that these terms are close ended

[0035] In a first aspect, the invention provides polypeptides that comprise an amino acid sequence that has at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0036] In another embodiment of the first aspect, the polypeptides comprise an amino acid

sequence that has at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0037] In yet another embodiment of the first aspect, the polypeptides comprise an amino acid sequence that has 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0038] In a second aspect, the invention provides polypeptides that consist of an amino acid sequence that has at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0039] In another embodiment of the second aspect, the polypeptides consist of an amino acid sequence that has at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0040] In yet another embodiment of the second aspect, the polypeptides consists of an amino acid sequence that has 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0041] In a third aspect, the invention provides polypeptides that comprise an amino acid

sequence that has at least at least 80%, or at least 85%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

[0042] In one embodiment of the third aspect the polypeptide comprises an amino acid sequence that has at least 90%, or at least 92%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

[0043] In a fourth aspect, the invention provides polypeptides that consists of an amino acid sequence that has at least at least 80%, or at least 85%, or at least 90%, or at least 91%, or at least 92%, or at least 93%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

[0044] In one embodiment of the fourth aspect the polypeptide consists of an amino acid sequence that has at least 90%, or at least 92%, or at least 94%, or at least 95%, or at least 96%, or at least 97%, or at least 98%, or at least 99%, or 100%, identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or

fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

[0045] In some embodiments of the third or fourth aspect, the antimicrobial peptide comprises the amino acid sequence SQSRESQC (SEQ ID NO:44) wherein at least one amino is cysteine and 0, 1, 2, 3, 4, 5, 6, or 7 amino acids of the antimicrobial peptide are conservatively substituted. 0, 1, 2, 3, 4, 5, 6, or 7 amino acids of the antimicrobial peptide are conservatively substituted. In other embodiments of the third or fourth aspects, the antimicrobial peptide comprises the amino acid sequence SQSRESQC (SEQ ID NO:44). In still other embodiments of the third or fourth aspect, the antimicrobial peptide comprises the amino acid sequence SQSRESQC (SEQ ID NO:44) wherein 0, 1, 2, 3, 4, 5, 6, or 7 amino acids of the antimicrobial peptide are conservatively substituted and the antimicrobial peptide consists of 8 amino acids. In yet other embodiments of the third or fourth aspect, the antimicrobial peptide consists of the amino acid sequence SQSRESQC (SEQ ID NO:44).

[0046] In some embodiments of the third or fourth aspect, the antimicrobial peptide comprises the amino acid sequence CSQRQSES (SEQ ID NO:50) wherein at least one amino is cysteine and 0, 1, 2, 3, 4, 5, 6, or 7 amino acids of the antimicrobial peptide are conservatively substituted. In other embodiments of the third or fourth aspects, the antimicrobial peptide comprises the amino acid sequence CSQRQSES (SEQ ID NO:50). In still other embodiments of the third or fourth aspect, the antimicrobial peptide comprises the amino acid sequence CSQRQSES (SEQ ID NO:50) wherein 0, 1, 2, 3, 4, 5, 6, or 7, amino acids of the antimicrobial peptide are conservatively substituted and the antimicrobial peptide consists of 8 amino acids. In yet other embodiments of the third or fourth aspect, the antimicrobial peptide consists of the amino acid sequence CSQRQSES (SEQ ID NO:50).

[0047] In some embodiments of the third or fourth aspect, the C-terminus of the polypeptide or the fragment is conjugated to the antimicrobial peptide. In other embodiments of the third or fourth aspect, the C-terminus of the polypeptide or the fragment is conjugated to the N-terminus of the antimicrobial peptide. In still other embodiments of the third or fourth aspect, the N-terminus of the polypeptide or fragment is conjugated to the antimicrobial peptide. In yet other embodiments of the third or fourth aspect, the N-terminus of the polypeptide or fragment is conjugated to the C-terminus of the antimicrobial peptide. For any of the embodiments of the third or fourth aspect the antimicrobial peptide can be conjugated to the polypeptide or fragment via a peptide bond.

[0048] Another embodiment of the peptides of the present disclosure is a peptide having the amino acid sequence NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRKSQSRESQA (SEQ ID NO: 45). Another embodiment is a peptide having the amino acid sequence

NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRKCSQRQSES (SEQ ID NO:51).

[0049] In some embodiments the polypeptides, polypeptide fragments or conjugated polypeptides have antibacterial activity against a gram-negative bacterium. In some embodiments, the gram-negative bacterium is of the genus Acinetobacter.

[0050] In some embodiments the polypeptides, polypeptide fragments or conjugated polypeptides have antibacterial activity against E. coli, P. aeruginosa or A. baumannii.

[0051] In some embodiments the polypeptides, polypeptide fragments or conjugated polypeptides have antibacterial activity against a gram-positive bacterium. In some embodiments, the gram-positive bacterium is *S. aureus* or *B. anthracis*.

[0052] In some embodiments, the polypeptide is lyophilized.

[0053] Specific embodiments of the polypeptides of the invention are provided in Table 1.

Table 1

SEQ ID NO:1	F307	SEQ ID NO:11	F330	SEQ ID NO:21	F311
SEQ ID NO:2	F376	SEQ ID NO:12	F328	SEQ ID NO:43	P307
SEQ ID NO:3	F351	SEQ ID NO:13	F324	SEQ ID NO:44	SQSRESQC
SEQ ID NO:4	F347	SEQ ID NO:14	F321	SEQ ID NO:45	P307SQ-8C (P307Ex)
SEQ ID NO:5	F344	SEQ ID NO:15	F320	SEQ ID NO:48	AEMLFLK
SEQ ID NO:6	F340	SEQ ID NO:16	F315	SEQ ID NO:49	P307AE-8
SEQ ID NO:7	F338	SEQ ID NO:17	F306	SEQ ID NO:50	CSQRQSES
SEQ ID NO:8	F336	SEQ ID NO:18	F303	SEQ ID NO:51	P307CS-8
SEQ ID NO:9	F334	SEQ ID NO:19	F301	SEQ ID NO:52	SQSRESQA
SEQ ID NO:10	F332	SEQ ID NO:20	F309	SEQ ID NO:53	P307SQ-8A

[0054] P307SQ-8C and P307Ex are used interchangeably herein.

[0055] The invention also provides for pharmaceutical compositions comprising the polypeptides, polypeptide fragments or conjugated polypeptides of the invention. In some embodiments, the compositions are pharmaceutical compositions, which comprise a pharmaceutically acceptable carrier, buffering agent, or preservative.

[0056] In some embodiments, the pharmaceutical composition is formulated for topical administration. In other embodiments, the pharmaceutical composition is formulated for subcutaneous delivery. In still other embodiments, the pharmaceutical composition is formulated for intravenous delivery. In yet other embodiments, the pharmaceutical composition is formulated for oral delivery.

[0057] In some embodiments, the composition further comprises an antibiotic. Examples of suitable antibiotics include, but are not limited to, amoxicillin, augmentin, amoxicillin, ampicillin, azlocillin, flucloxacillin, mezlocillin, methicillin, , penicillin G, penicillin V, cephalexin, cefazedone, cefuroxime, loracarbef, cemetazole, cefotetan, cefoxitin, ciprofloxacin, levaquin, and floxacin, tetracycline, doxycycline, or minocycline, gentamycin, amikacin, and tobramycin, clarithromycin, azithromycin, erythromycin, daptomycin, neomycin, kanamycin, or streptomycin.

[0058] In some embodiments, the pharmaceutical composition further comprises a clotting agent.

[0059] In some embodiments, the pharmaceutical composition is lyophilized.

[0060] The present invention also provides for methods for treating a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a polypeptide, polypeptide fragment or conjugated polypeptide of the invention, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

[0061] In one embodiment the method is a method for treating a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a polypeptide of the invention, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

[0062] In another embodiment the method is a method for treating a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a polypeptide fragment of the invention, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

[0063] In one embodiment the method is a method for treating a subject in need thereof, comprising administering to the subject a pharmaceutical composition comprising a conjugated polypeptide of the invention, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

[0064] In one embodiment the method is a method for treating having a bacterial infection and the treatment is therapeutic treatment comprising administering to the subject a pharmaceutical composition comprising a conjugated polypeptide of the invention, and a pharmaceutically acceptable carrier, buffering agent, or preservative. In some embodiments, the subject has a bacterial infection that is non-responsive to other treatment modalities. For example, the bacterial infection may be resistant to one or more antibiotic. In one embodiment, the bacterial infection is a wound infection.

[0065] In one embodiment the method is a method for prophylactically treating a subject in need thereof comprising administering to the subject a pharmaceutical composition comprising a conjugated polypeptide of the invention, and a pharmaceutically acceptable carrier, buffering

agent, or preservative. In some embodiments the subject has undergone, or is undergoing surgery and the surgical wound is contacted with a pharmaceutical composition of the invention. In certain embodiments, the surgical wound is irrigated with the pharmaceutical composition prior to closure of the wound. In other embodiments the pharmaceutical composition is applied to the wound after closure, for example the pharmaceutical composition is applied to the sutured or stapled area of the wound.

[0066] In some embodiments, the method comprises administering a pharmaceutical composition of the invention is administered in combination with an antibiotic. In some embodiments, the method comprises topically administering a pharmaceutical composition of the invention. In other embodiments, the method comprises administering a pharmaceutical composition of the invention subcutaneously. In still other embodiments, the method comprises administering a pharmaceutical composition of the invention by intravenous injection. In yet other embodiments, the method comprises administering a pharmaceutical composition of the invention orally.

[0067] In some embodiments, the pharmaceutical composition is in a unit dosage form. In other embodiments, the pharmaceutical composition is in the form of a cream, ointment, salve, gel, lozenge, spray, or aerosol.

[0068] Also provided, are methods for treating a bacterial infection comprising inhibiting the formation of or disrupting a bacterial biofilm comprising administering to a subject in need thereof, a composition comprising a polypeptide, polypeptide fragment or conjugated polypeptide of the invention in an amount effective to kill bacteria in the biofilm.

[0069] Additionally provided, are methods of disinfecting an article comprising contacting the article with a composition comprising a polypeptide, polypeptide fragment or conjugated polypeptide of the invention to the article for a time sufficient to disinfect the article. In some embodiments, the article is a hard surface. In some embodiments, the article is a countertop, keyboard, surgical instrument, or medical device.

[0070] Additionally provided, are methods for inhibiting the formation of or disrupting a bacterial biofilm on an article comprising contacting the article with a polypeptide, polypeptide fragment or conjugated polypeptide of the invention, in an amount effective to kill bacteria in the biofilm.

[0071] Also provided, are articles of manufacture that contain a composition comprising a polypeptide, polypeptide fragment or conjugated polypeptide of the invention. In some embodiments, the article of manufacture is a spray bottle that contains a polypeptide, polypeptide fragment or conjugated polypeptide of the invention.

[0072] In some embodiments, the article of manufacture contains a pharmaceutical

composition comprising a polypeptide, polypeptide fragment or conjugated polypeptide of the invention and a carrier, buffering agent or preservative. In some embodiments, the article of manufacture is a vial. In some embodiments, the article of manufacture is a delivery device. In some embodiments, the composition contained by the article of manufacture is lyophilized.

[0073] Modifications and changes can be made in the structure of the polypeptides of the disclosure and still obtain a molecule having similar characteristics as the polypeptide (*e.g.*, a conservative amino acid substitution). For example, certain amino acids can be substituted for other amino acids in a sequence without appreciable loss of activity. Because it is the interactive capacity and nature of a polypeptide that defines that polypeptide's biological functional activity, certain amino acid sequence substitutions can be made in a polypeptide sequence and nevertheless obtain a polypeptide with like properties.

[0074] Such amino acid substitutions are generally based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions that take various of the foregoing characteristics into consideration are well known to those of skill in the art and include (original residue: exemplary substitution): (Ala: Gly, Ser), (Arg: Lys), (Asn: Gln, His), (Asp: Glu, Cys, Ser), (Gln: Asn), (Glu: Asp), (Gly: Ala), (His: Asn, Gln), (Ile: Leu, Val), (Leu: Ile, Val), (Lys: Arg), (Met: Leu, Tyr), (Ser: Thr), (Thr: Ser), (Tip: Tyr), (Tyr: Trp, Phe), and (Val: Ile, Leu). Embodiments of this disclosure thus contemplate functional or biological equivalents of a polypeptide as set forth above. In particular, embodiments of the polypeptides can include variants having about 50%, 60%, 70%, 80%, 90%, and 95% sequence identity to the polypeptide of interest.

[0075] "Identity"" as known in the art, is a relationship between two or more polypeptide sequences, as determined by comparing the sequences. "Identity" can be readily calculated by known algorithms well known in the art. Preferred methods to determine identity are designed to give the largest match between the sequences tested. Methods to determine identity are codified in publicly available computer programs. The percent identity between two sequences can be determined using analysis software (*i.e.*, Sequence Analysis Software Package of the Genetics Computer Group, Madison Wis.) that incorporates the *Needelman and Wunsch*, (J. Mol. Biol., 48: 443-453, 1970) algorithm (*e.g.*, NBLAST, and XBLAST).

[0076] Identity can be measured as "local identity" or "global identity". Local identity refers the degree of sequence relatedness between polypeptides as determined by the match between strings of such sequences. Global identity refers to the degree of sequence relatedness of a polypeptide compared to the full-length of a reference polypeptide. Unless specified otherwise, as used herein identity means global identity. The percentages for global identity herein are calculated using the ClustalW algorithm used through the software MacVector, using

the default settings; both for local and global identity.

Production of polypeptides

[0077] Polypeptides of the present invention can be produced by any known method. For example, polypeptides can be produced in bacteria including, without limitation, *E. coli*, or in other existing system for polypeptide (e.g., *Bacillus subtilis,* baculovirus expression systems using Drosophila Sf9 cells, yeast or filamentous fungal expression systems, mammalian cell expression systems), or they can be chemically synthesized.

[0078] If the a polypeptide is to be produced in bacteria, e.g., *E. coli*, the nucleic acid molecule encoding the peptide may also encode a leader sequence that permits the secretion of the mature peptide from the cell. Thus, the sequence encoding the peptide can include the pre sequence and the pro sequence of, for example, a naturally occurring bacterial ST peptide. The secreted, mature peptide can be purified from the culture medium.

[0079] The sequence encoding a peptide described herein is can be inserted into a vector capable of delivering and maintaining the nucleic acid molecule in a bacterial cell. The DNA molecule may be inserted into an autonomously replicating vector (suitable vectors include, for example, pGEM3Z and pcDNA3, and derivatives thereof). The vector may be a bacterial or bacteriophage DNA vector such as bacteriophage lambda or M13 and derivatives thereof. Construction of a vector containing a nucleic acid described herein can be followed by transformation of a host cell such as a bacterium. Suitable bacterial hosts include but are not limited to, *E. coli,* B *subtilis*, *Pseudomonas*, *Salmonella*. The genetic construct also includes, in addition to the encoding nucleic acid molecule, elements that allow expression, such as a promoter and regulatory sequences. The expression vectors may contain transcriptional control sequences that control transcriptional initiation, such as promoter, enhancer, operator, and repressor sequences. A variety of transcriptional control sequences are well known to those in the art. The expression vector can also include a translation regulatory sequence (e.g., an untranslated 5' sequence, an untranslated 3' sequence, or an internal ribosome entry site). The vector can be capable of autonomous replication or it can integrate into host DNA to ensure stability during peptide production.

[0080] One embodiment of a nucleic acid according to the present invention is a nucleic acid that encodes a polypeptide comprising an amino acid sequence that has at least 90% sequence identity to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ

ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0081] In another embodiment, the nucleic acid encodes a polypeptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0082] In yet another embodiment, the nucleic acid encodes a polypeptide consisting of an amino acid sequence nucleic acid of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0083] In still another embodiment, the nucleic acid comprises the nucleotide sequence of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, or SEQ ID NO:42.

[0084] In still another embodiment, the nucleic acid consists of the nucleotide sequence of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, or SEQ ID NO:42.

[0085] Another embodiment is an expression vector that comprises a nucleic acid that encodes a polypeptide comprising an amino acid sequence that has at least 90% sequence identity to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0086] In another embodiment, the expression vector comprises a nucleic acid that encodes a polypeptide comprising an amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID

NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0087] In yet another embodiment, the expression vector comprises a nucleic acid that encodes a polypeptide consisting of an amino acid sequence nucleic acid of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, or SEQ ID NO:45, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

[0088] In still another embodiment, the expression vector comprises a nucleic acid that comprises the nucleotide sequence of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, or SEQ ID NO:42.

[0089] In still another embodiment, the expression vector comprises a nucleic acid that consists of the nucleotide sequence of SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33, SEQ ID NO:34, SEQ ID NO:35, SEQ ID NO:36, SEQ ID NO:37, SEQ ID NO:38, SEQ ID NO:39, SEQ ID NO:40, SEQ ID NO:41, or SEQ ID NO:42.

[0090] Table 2 provides specific embodiments of the nucleic acids of the invention showing the nucleotide SEQ ID NO: that corresponds to the polypeptides they encode.

Table 2

Nucleotide SEQ ID NO:	Corresponding Amino Acid SEQ ID NO:	Nucleotide SEQ ID NO:	Corresponding Amino Acid SEQ ID NO:
22	1	33	12
23	2	34	13
24	3	35	14
25	4	36	15

Nucleotide SEQ ID NO:	Corresponding Amino Acid SEQ ID NO:
26	5
27	6
28	7
29	8
30	9
31	10
32	11

Nucleotide SEQ ID NO:	Corresponding Amino Acid SEQ ID NO:
37	16
38	17
39	18
40	19
41	20
42	21

[0091] The nucleic acid that encodes a polypeptide described herein can also be fused to a nucleic acid encoding a peptide affinity tag, e.g., glutathione S-transferase (GST), maltose E binding protein, protein A, FLAG tag, hexa-histidine, myc tag or the influenza HA tag, in order to facilitate purification. The affinity tag or reporter fusion joins the reading frame of the peptide of interest to the reading frame of the gene encoding the affinity tag such that a translational fusion is generated. Expression of the fusion gene results in translation of a single peptide that includes both the peptide of interest and the affinity tag. In some instances where affinity tags are utilized, DNA sequence encoding a protease recognition site will be fused between the reading frames for the affinity tag and the peptide of interest.

[0092] Genetic constructs and methods suitable for production of immature and mature forms of the polypeptides and variants described herein in protein expression systems other than bacteria, and well known to those skilled in the art, can also be used to produce polypeptides in a biological system.

[0093] Polypeptides and variants thereof can be synthesized by the solid-phase method using an automated peptide synthesizer. For example, the peptide can be synthesized on Cyc(4-CH_2 Bxl)-OCH_2-4-(oxymethyl)-phenylacetamidomethyl resin using a double coupling program. Peptides can also be synthesized by many other methods including solid phase synthesis using traditional FMOC protection (i.e., coupling with DCC-HOBt and deprotection with piperdine in DMF).

Therapeutic and Prophylactic Compositions and Their Use

[0094] This invention provides methods of treatment comprising administering to a subject in need thereof an effective amount of a polypeptide of the invention. The subject is human or another animal, including but not limited to primates such as monkeys and chimpanzees;

livestock animals such as cows, pigs, horse or chickens; and companion animals such as dogs cats, and rodents. In a specific embodiment the subject is a human. In another specific embodiment the subject is a non-human mammal. In one embodiment the polypeptides are administered as the sole antibacterial agent. In another embodiment the polypeptides are administered in combination with one or more other antibacterial agents.

[0095] Methods of administration of the disclosed pharmaceutical compositions can be oral or parenteral and include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, intra-articular, intra-synovial, subcutaneous, intranasal, epidural, topical and oral routes. The compounds may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings *(e.g.,* oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Administration can be systemic or local. In addition, it may be desirable to introduce the pharmaceutical compositions of the invention into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.,* by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. In a specific embodiment, it may be desirable to administer the pharmaceutical compositions of the invention locally to the area in need of treatment, such as topical use on the skin; any suitable method known to the art may be used.

[0096] In one aspect of the invention provides for pharmaceutical compositions comprising the polypeptides of the present disclosure for therapeutic or prophylactic treatment of bacterial infections. An embodiment of the invention is a pharmaceutical composition formulated for topical treatment. Another embodiment of the invention is a pharmaceutical composition formulated for systemic infections.

[0097] Such compositions comprise a therapeutically effective amount of a polypeptide of the invention and a pharmaceutically acceptable carrier, buffering agent, or preservative. The term "pharmaceutically acceptable carrier" as used herein, includes, but is not limited to, solvents, diluents, or other liquid vehicle, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, solid binders, lubricants and the like, as suited to the particular dosage form desired. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose,

gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition can also contain of wetting or emulsifying agents, preservatives, or pH buffering agents. These compositions can take the form of a solution, suspension, emulsion, tablet, pill, lozenge, capsule, powder, patches for topical administration and the like. For topical applications, the pharmaceutically acceptable compositions may be formulated in a suitable ointment, lotion or cream containing the active component suspended or dissolved in one or more carriers. Carriers for topical administration include, but are not limited to, mineral oil, liquid petrolatum, white petrolatum, propylene glycol, polyoxyethylene-polyoxypropylene compounds, emulsifying wax, polysorbate 60, cetyl esters wax, ceteary alcohol, 2-octyldodecanol, benzyl alcohol and water. The composition can be formulated as a suppository with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. One of skill in the art is well versed in formulation of therapeutic agents. *See* e.g. *Remington* The Science and Practice of Pharmacy, 20th Edition, Lippincott Williams & White, Baltimore, Md. (2000); *Remington's Pharmaceutical Sciences,* 19th Edition (Mack Publishing Company, 1995).

[0098] The invention also provide a pharmaceutical pack or kit comprising one or more containers filled with one or more of the ingredients of the pharmaceutical compositions of the invention. Optionally associated with such container(s) is a notice in the form prescribed by a governmental agency regulating the manufacture, use, or sale of pharmaceuticals or biologic products, which notice reflects (a) approval by the agency of manufacture, use or sale for human administration, (b) directions for use, or both.

EXAMPLES

[0099] The following examples are put forth so as to provide additional information to one of skill in the art of how to make and use the polypeptides described herein, and are not intended to limit the scope of what the inventors regard as their invention. Efforts have been made to ensure accuracy with respect to numbers used (e.g., amounts, temperature, etc.) however, some experimental errors and deviations should be accounted for. Unless indicated otherwise, molecular weight is average molecular weight, and the temperature is in degrees Centigrade.

Example 1

[00100] Identification of polypeptides having antibacterial activity.

[00101] Fifteen clinical isolates of *A. baumannii* were obtained from a New York hospital. Strains of A. baumannii were isolated and treated with mitomycin C to induce prophage induction. The supernatants were collected and phage were precipitated with polyetheylene

glycol (PEG). Supernatants from three of the *A. baumannii* isolates were examined by negative staining EM and images taken of the phage (Figures 1A, 1B, and 1C).

[00102] Phage DNA was separated from co-precipitated compounds by agarose gel electrophoresis and extraction of high-molecular-weight DNA. From this DNA, an expressible linker shotgun library (E-LASL) was constructed as previously described. (Schmitz JE. et al., 2008, Appl. Environ. Microbiol. 74:1649-1652.) Briefly, for all samples, 100 ng of DNA was fragmented with the restriction enzyme TSP509I (consensus sequence AATT) Following phenol-chloroform extraction and ethanol precipitation, the DNA was ligated to 40 ng of linker sequence, with a complementary 5' overhang (AATTCGGCTCGAG, where the overhang is underlined (SEQ ID NO:46). The ligation mixture was used as the template for Taq-based PCR using the linker-targeted primer CCATGACTCGAGCCGAATT (SEQ ID NO:47).

[00103] The amplified inserts were ligated into the arabinose-inducible pBAD plasmid using the pBAD TOPO® TA expression kit; Invitrogen, per the manufacturer's directions. The recombinant vectors were transformed into competent *E. Coli* TOP10 (Invitrogen). To determine which clones had lytic activity, the *E. coli* were plated on LB agar supplemented with 100 µg/ml ampicillin and 5% defibrinated sheep's blood. Following overnight growth at 37°C, the plates were placed in a sealed container that was attached to the outlet of a commercial nebulizer. Nebulized arabinose was continuously pumped into the container for 1 hour. The plates were returned to 37°C and colonies were identified that developed a zone of hemolysis in the surrounding blood agar. Chosen clones were streaked onto separate LB-ampicillin plates (lacking arabinose) and allowed to propagate without induced expression. (Schmitz JE, et al., 2010 Appl. Environ. Microbiol.76:7181-7187).

[00104] To determine killing activity for A. baumannii, a secondary screen was done essentially as described in Schmitz JE, et al., 2010 Appl. Environ. Microbiol.76:7181-7187. Hits were streaked as approximately 1-cm by 2-cm patches onto LB-ampicillin plates supplemented with 0.2% arabinose. Following overnight incubation at 37°C, the plates were exposed to chloroform vapor to kill and permeabilize any still-viable *E. coli*. The patches were then overlaid with molten soft agar containing *A. baumannii* and observed for clearing zones. Twenty-one positive clones were identified that exhibited a clear zone around the clone. Figure 2 shows a representative screen of lysin clone activity in clearing live *A. baumannii* imbedded in the agar.

[00105] The inserts of the positive closes were sequenced and compared to the sequences in the NCBI protein database. The alignments showed that among the 21 clones there are four classes of lytic activity: i) nine were in the glycosyl hydrolase family, ii) seven were phage baseplate lysozymes, iii) two were lysozyme autolysins, and iv) three were lysins. (Fig. 3). For

ease of reference here in, regardless of class, the polypeptides encoded by these sequences are referred to as "lysins". Figure 4 shows a sequence alignment based on similarity of the nucleotide sequences encoding the 21 clones. Figure 5 shows a sequence alignment based on similarity of the polypeptide sequences of the 21 clones.

Example 2

[00106] Activity of positive clones.

[00107] Twenty-one different constructs were screened for activity against thirteen different *A. baumannii* clinical isolates. The constructs were recombinantly expressed in E. coli. Cells were grown at 30°C 200rpm, and when reaching mid-log phase they were induced by adding 0.2% arabinose. Induction continued overnight. In the morning, cells were spun down, washed 3x with 50 mM sodium phosphate buffer pH 7.0, before being homogenized in an Emulsiflex homogenizer. Cell debris was removed by centrifugation (16000 g, 45 min) and the lysate passed through a 0.22 um sterile filter to generate the crude lysate.

[00108] A. baumannii grown overnight in TSB, were mixed with 50°C soft agar TSB and poured onto a TSB agar plate as a top agar layer. The plate was allowed to solidify in room temperature. Crude lysates (10 ul) were added to a soft agar plate with *A. baumannii*, and incubated for 2 hours at room temperature each day, while being kept at 4°C for the remainder of the time. Plates were incubated until clearing zones were visible (4-5 days). A clearing zone larger than the original spot of the crude lysate was scored. The number above each lysin indicates how many stains for which that lysin was the most efficient.

[00109] Results are shown in in Figure 6. The lysin construct is shown on the x-axis and the percent of Acinetobacter strains lysed is shown on the y-axis. The numbers above each bar indicates the number of strains for which that lysin was the most efficient, no number indicates one strain. As can be seen, Lysin F307 lysed about 90% of the strains tested and was the most active against seven strains.

Example 3

[00110] Lysis of *A. baumannii* by F307.

[00111] Figure 7 shows representative Transmission Electron Micrographs of *A. baumannii* strain 1791 cells after treatment with F307 polypeptide. The micrographs show that F307 caused lysis through extrusion of the cytoplasmic membrane to the outside of the cell. (See Figure 7, arrows). Two 100 mL culture of *A. baumannii* strain 1791 were started in BHI media and grown in a 500 mL flask for 1.5h at 37˚C, 200 rpm. The cells were then centrifuged and washed one time with 1X PBS buffer. They were then re-suspended in 1.2 mL of 1X PBS. EDTA at a final concentration of 250 μM was added to each sample. To the experimental sample 300 μL of lysin (~1.2 mg final concentration) and incubated the control (EDTA alone) and experimental

(EDTA+F307 lysin) at 25°C. Time points were taken at 0.5, 1, 5, 10, 15, and 30 minutes. The reactions were quenched and cells were fixed using 2.5% Gluteraldhyde in CAC buffer (10 mm sodium cacodylate, 0.1 m CaCl2, pH 6.5).

Example 4

[00112] Effect of F307 polypeptide on *A. baumannii* biofilms on catheters *in vitro* and *in vivo*.

[00113] In vitro treatment of catheter adherent *A. baumannii* 1791 with F307 lysin

[00114] Catheter tubing (CareFusion Ref#72023E) was cut using a sterile scalpel into 3-inch long sections. An overnight culture of *A. baumannii* 1791 was used to inoculate 1:1000 a 50 mL of TSB 0.2% Glucose (~1X105 CFU/mL). Each 3-inch catheter tube was seeded with 300-350 µL of the 1:1000 diluted culture. The catheters were then clamped and placed in plastic containers in a 37°C incubator for 3 days to allow for biofilm formation to occur. After the 3 days catheters were washed twice with either PBS or Sodium Phosphate buffer pH 7.5 and then had 300-350 µL of F307 added to the tube (~1 mg final concentration). The catheters were then clamped. Catheters were taken at time points 0, 15 minutes, 30 minutes, and 1 hour. The catheters were washed twice with 50 mM Sodium Phosphate pH 7.5 and were cut into small pieces. These were placed into a 1.5 mL ependorff tube and 500 µL of 50 mM NaP buffer pH 7.5 was added. The tubes were sonicated for 20 minutes, and vortexed for 1 minute. The samples were then serial diluted and 20 µL was plated onto a quadrant of a BHI agar plate and incubated at 37°C overnight. CFU's were calculated the following morning.

[00115] An approximate 4-log drop in the number of colony forming units (CFU's of *A. baumannii* was observed after 30 minutes of treatment. Table 3 shows CFU counts. Figure 8 shows Scanning Electron micrographs of 3-day biofilms of *A. baumannii* strain 1791 before and after treatment with 250 µg F307 polypeptide.

Table 3. Treatment of *A. baumannii* biofilms on catheters.

Sample	**CFU**
no treatment	1.4×10^7
no treatment replicate	3.0×10^6
15 minutes F307 treatment	9.0×10^4
30 minutes F307 treatment	6.0×10^3

Example 5

[00116] Mouse Catheter Model: Several 3 inch section of catheter tubing were seeded (1:1000) with *A. baumannii* strain 1791. *A. baumannii* biofilms were formed as described above. The back of twenty BALB/C mice were shaved, their backs were sterilized, and then an incision

was made to place a 1 inch section of the catheter with a biofilm already formed under the dermis of the back. The incisions were then stapled shut. After 24 hours, 250 µl of F307 (1 mg) (n=10) or 250 µl control vehicle (n=10) was injected directly into the catheter that was under the dermis of the mouse. The treatment was repeated after 4 hours. After 3 hours the catheters were removed from the mice, and assayed as described in Experiment 4. Figure 9 shows the reduction of bacterial counts by approximately 2-logs in mice treatment with F307 polypeptide compared with control.

Example 6

[00117] F307 polypeptide rescues mice from death after a lethal injection of *A. baumannii*.

[00118] Twenty-two C57BL/6 mice were given 10^8 CFU of *A. baumannii* strain intraperitoneally (IP). Two hours later, two mice were euthanized and organs examine as described below, ten mice injected IP with 1 mg of F307 and ten mice were injected IP with control vehicle. Treated animals showed 50% survival with this dose of lysin, whereas control mice showed only 10% survival 14 days after infection (Figure 10).

[00119] The organs from the two mice that were euthanized after infection were examined to confirm that the organs were infected with *A. baumannii* at the time of treatment with F307 polypeptide. Liver, spleen, kidney, and heart were dissected from the mice. The organs were then homogenized in 500 µl of 1X PBS. Dilutions were made and were plated onto Brain Heart infusion (BHI) plates. The plates were incubated at 37°C overnight. The number of colony forming units was counted. Control mice were sacrificed at the two hour time point and showed Acinetobacter in all organs examined indicating that the organs were infected with *A. baumannii* at the time of treatment.

Example 7

[00120] P307 polypeptide (SEQ ID NO:43) was tested in duplicate against 18 clinical isolates of *A. baumannii* strains. *A. baumannii* strains were cultured ON to reach stationary phase. Cells were washed 3x in 20 mM Tris pH 7.5 and resuspended in the same buffer to an OD (595nm) of around 0.7. To these cells, P307(250 ug/ml) or a corresponding volume of buffer, was added, and the mixture was allowed to incubate for 60 minutes at room temperature. Dilutions of the mixtures were made and plated on TSB Agar plates for subsequent counting of colony forming units.

[00121] P307 polypeptide treatment resulted in a 1 to 8-log drop in bacterial viability, versus control, after incubation for 60 minutes with 250 µg of P307. Results are show in Table 4. When P307 was compared with the full length F307 polypeptide (SEQ ID NO:1) the P307 polypeptide had higher activity.

Table 4. P307 activity against 18 *A. baumannii* strains.

Strain	Control 1	Control 2	Control Average	P307 1	P307 2	P307 Average	Difference	Log drop
1775	1.00E+08	4.50E+08	2.75E+08	1.00E+07	1.50E+07	1.25E+07	2.20E+01	1.34
1776	5.50E+08	3.50E+08	4.50E+08	8.80E+05	7.50E+05	8.15E+05	5.52E+02	2.74
1777	7.00E+08	4.00E+08	5.50E+08	6.50E+06	9.00E+06	7.75E+06	7.10E+01	1.85
1788	2.00E+08	3.00E+08	2.50E+08	1.50E+07	1.20E+07	1.35E+07	1.85E+01	1.27
1789	4.50E+08	3.50E+08	4.00E+08	1.10E+07	1.30E+07	1.20E+07	3.33E+01	1.52
1790	1.50E+08	2.00E+08	1.75E+08	5.50E+05	1.80E+05	3.65E+05	4.79E+02	2.68
1791	9.0E+08	4.5E+08	6.8E+08	2.2E+05	2.2E+05	2.2E+05	3.1E+03	3.49
1792	1.2E+09	8.5E+08	1.0E+09	7.1E+05	7.5E+05	7.3E+05	1.4E+03	3.15
1793	3.5E+08	5.0E+08	4.3E+08	6.5E+05	5.6E+05	6.1E+05	7.0E+02	2.85
1794	7.5E+08	4.0E+08	5.8E+08	7.0E+05	6.0E+05	6.5E+05	8.8E+02	2.95
1795	9.5E+08	1.3E+09	1.1E+09	9.0E+06	2.5E+07	1.7E+07	6.6E+01	1.82
1796	1.0E+09	7.0E+08	8.5E+08	8.2E+05	8.2E+05	8.2E+05	1.0E+03	3.02
1797	1.2E+09	9.0E+08	1.1E+09	6.7E+05	6.5E+05	6.6E+05	1.6E+03	3.20
1798	4.0E+08	4.0E+08	4.0E+08	2.7E+05	6.5E+05	4.6E+05	8.7E+02	2.94
1799	5.5E+08	3.5E+08	4.5E+08	2.9E+07	7.0E+06	1.8E+07	2.5E+01	1.40
S1	1.4E+09	1.1E+09	1.3E+09	4.2E+07	3.0E+07	3.6E+07	3.5E+01	1.54
S3	2.5E+08	2.0E+08	2.3E+08	6.8E+05	6.5E+05	6.7E+05	3.4E+02	2.53
S5	1.1E+09	8.5E+08	9.8E+08	1.0E+00	1.0E+00	1.0E+00	9.8E+08	8.99

Example 8

[00122] Addition of a short extension peptide resulted in increased antibacterial activity of P307.

[00123] The peptide SQSRESQC (SEQ ID NO:44) is derived from hepatitis C virus and has been shown to have antimicrobial activity against gram-positive and gram-negative bacteria. We conjugated this sequence to P307 (P307Ex) to determine its effect on the activity. The sequence of F307, p307 and the P307Ex (SEQ ID Nos: 1, 43 and 45 respectively) are provided in figure 11 where a portion of the sequence of F307 is underlined to show the location of P307 and a portion of the sequence of P307 is double underline to show the location of the antimicrobial sequence.

[00124] P307 and P307Ex were assayed in duplicate against six bacterial strains. Antibacterial acidity was measured as described in Example 5. Treatment with P307Ex resulted

in a 3.2 log drop in ***A. baumannii* 1791** whereas treatment with P307 resulted in a 2.9 log drop demonstrating that the addition of the antimicrobial peptide increased the activity of P307. The results are shown in Table 5.

Table 5

Strain	Control 1	P307 EX1	P307 EX 2	P307Ex Average	Difference	Log drop
1775	5.00E+08	1.60E+05	1.10E+05	1.35E+05	3.70E+03	3.5
1776	5.00E+08	5.50E+05	6.50E+05	6.00E+05	8.33E+02	2.9
1777	6.50E+08	6.50E+04	2.80E+05	1.73E+05	3.77E+03	3.5
1788	3.50E+08	8.80E+05	5.80E+05	7.30E+05	4.79E+02	2.6
1789	4.00E+08	1.10E+07	1.30E+07	1.20E+07	3.33E+01	1.5
1790	2.00E+08	1.50E+04	2.00E+04	1.75E+04	1.14E+04	4.0
1791	3.50E+08	4.00E+04	4.50E+04	4.25E+04	8.24E+03	3.9
1792	1.00E+08	4.00E+04	5.00E+03	2.25E+04	4.44E+03	3.6
1793	1.50E+08	3.50E+04	2.00E+04	2.75E+04	5.45E+03	3.7
1794	5.00E+07	1.40E+05	1.00E+05	1.20E+05	4.17E+02	2.6
1795	4.00E+08	5.50E+04	1.30E+05	9.25E+04	4.32E+03	3.6
1796	2.50E+08	3.80E+05	2.50E+05	3.15E+05	7.94E+02	**2.8**
1797	2.50E+08	5.50E+06	8.50E+06	7.00E+06	3.57E+01	**1.5**
1798	3.50E+08	3.40E+05	3.70E+05	3.55E+05	9.86E+02	**3.0**
1799	3.50E+08	5.00E+03	3.00E+04	1.75E+04	2.00E+04	**4.3**
S1	8.50E+08	5.90E+05	7.00E+05	6.45E+05	1.32E+03	**3.1**
S3	3.00E+08	1.60E+07	1.40E+07	1.50E+07	2.00E+01	**1.3**
S5	1.50E+09	5.00E+05	2.90E+05	3.95E+05	3.80E+03	**3.57**

[00125] P307 and P307Ex were tested for activity against *A. baumannii* strain 1791, *E. coli*, *P. aeruginosa* strain PAO1, *S. aureus* strain RN4220, *S. aureus* strain 8325 and *B. anthracis*. As shown in Table 6, P307 and P307 were most active against *A. baumannii* and *B. anthracis*.

Table 6. P307 and P307Ex against other bacterial species.

Sample	*A. baumannii* 1791	*E. coli*	*B. anthracis* Δsterne	*Pseudomonas aeruginosa* PAO1	*S. aureus* RN4220	*S. aureus* 8325
control 1	5.50E+08	5.50E+08	2.40E+07	1.30E+09	1.50E+09	6.50E+08
control 2	2.60E+08	4.50E+08	2.80E+07	4.50E+08	7.50E+08	9.50E+08
P307EX 1	1.10E+05	3.50E+08	2.60E+03	4.70E+07	5.20E+07	3.20E+07

Sample	*A. baumannii* 1791	*E. coli*	*B. anthracis* Δsterne	*Pseudomonas aeruginosa* PAO1	*S. aureus* RN4220	*S. aureus* 8325
P307EX 2	3.90E+05	3.00E+08	2.90E+03	3.70E+07	5.80E+07	3.60E+07
P307 1	5.80E+05	3.50E+08	3.10E+03	5.60E+07	8.00E+08	4.80E+07
P307 2	3.70E+05	3.50E+08	4.00E+03	4.00E+07	4.50E+08	4.40E+07
average control	4.05E+08	5.00E+08	2.60E+07	8.75E+08	1.13E+09	8.00E+08
average P307EX	2.50E+05	3.25E+08	2.75E+03	4.20E+07	5.50E+07	3.40E+07
average P307	4.75E+05	3.50E+08	3.55E+03	4.80E+07	6.25E+08	4.60E+07
difference P307EX	1.62E+03	1.54E+00	9.45E+03	2.08E+01	2.05E+01	2.35E+01
difference P307	8.53E+02	1.43E+00	7.32E+03	1.82E+01	1.80E+00	1.74E+01
log drop P307EX	**3.2**	**0.2**	**4.0**	**1.3**	**1.3**	**1.4**
log drop P307	**2.9**	**0.2**	**3.9**	**1.3**	**0.3**	**1.2**

Example 9

[00126] P307 is not toxic to B cells or red blood cells.

[00127] P307 was mixed with red blood cells to determine if it would cause lysis. No lysis was observed at 200 µg of P307. When P307 was tested for lysis of a B cell line it was found to have only a slight effect on cell number after 24 hours. The results are shown in Table 7.

Table 7 (% viable)

Sample	0 min	5 min	30 min	1 hour	2 hour	3 hour	24 hours
Initial cell only	97%			83.60%		85%	88.20%
Tris-HCl pH=6.8		97.20%	89.70%	94.70%	81.50%	90.90%	89.90%
200 µg P307		94.60%	1100%	71.40%	71.90%	58.60%	76.30%
20 µg P307		97.20%	88.60%	89.70%	90.30%	91.00%	94.20%
2 µg P307		95.20%	76.90%	89.30%	92.50%	93.80%	96.30%

[00128] The peptides used in Examples 10-20 were chemically synthesized.

[00129] Peptides were created using a Protein Technologies Symphony™ peptide synthesizer (PTI Tucson, Arizona, USA) on pre-coupled Wang (*p*-alkoxy-benzyl alcohol) resin (Bachem, Torrance, CA, USA). Reaction vessels were loaded at 25 µM and peptides were elongated using Fmoc protected amino acids (Anaspec, San Jose, CA, USA) (1997. Standard Fmoc protocols.

289:44–67). Deprotection of the amine was accomplished with 20% piperidine (Sigma-Aldrich) in NMP (N-methylpyrrolidinone). Repetitive coupling reactions were conducted using 0.6 M HATU/Cl-HOBT (azabenzotriazol tetramethyluronium hexafluorophosphate/6-chloro-1-hydroxybenzotriazole)(P3 Biosystems, Shelbyville, KY, USA) and 0.4 M NMM (N-methylmorpholine) using NMP (EMD) as the primary solvent (1989. New Coupling Reagents in Peptide Chemistry 30:1927–1930.). Resin cleavage and side-chain deprotection were achieved by transferring to a 100 ml round bottom flask and reacted with 4.0 ml concentrated, sequencing grade, trifluoracetic acid (Fisher) with triisopropylsilane (Fluka), degassed water, and 3,6-dioxa-1,8-octanedithiol (DODT, Sigma-Aldrich) in a ratio of 95:2:2:1 over a 6 hour time frame. This was followed by column filtration to a 50 ml round bottom flask and TFA volume reduced to 2 ml using a rotary evaporator. A standard ether precipitation was performed on the individual peptides by transferring to a 50 ml falcon tube containing 40 ml cold tert-butyl methyl ether (TBME, Sigma-Aldrich). Samples were placed in an ice bath for 2 hours to aid precipitation followed by pellet formation using centrifugation (3300 rpm, 5 min). Excess ether was removed by vacuum aspiration and the peptide pellets were allowed to dry overnight in a fume hood. Dried peptide pellets were resolved in 20% acetonitrile and 10 ml HPLC grade water, subsampled for LC/MS and lyophilized. All crude products were subsequently analyzed by reverse-phase Aquity™ UPLC (Waters Chromatography, Milford, MA, USA) using a Waters BEH C18 column. Individual peptide integrity was verified by tandem electrospray mass spectrometry using a ThermoFinnigan LTQ™ (Thermo Fisher, Waltham, MA, USA) spectrometer system. Preparative chromatography was accomplished on a Vydac C18 RP preparative column on a Waters 600 Prep HPLC. Individual fractions were collected in 30 seconds intervals, characterized using LC/MS and fractions containing desired product were lyophilized. These were stored at -20°C until being resuspended in autoclaved Milli-Q water for various assays. The stock solutions were then stored at 4°C. The peptides are summarized with their amino acid sequences, isoelectric points (pI) and molecular weights (MW) in table 8.

Table 8

Names	Amino acid sequences	pI	MW	SEQ ID NO:
F307		10.12	16 kDa	1
P307	NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK	10.71	3.4 kDa	43
P307AE-8	NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK**AEMELFLK**	10.21	4.4 kDa	49
P307SQ-8C	NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK**SQSRESQC**	10.38	4.3 kDa	45
P307CS-8	NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK**CSQRQSES**	10.38	4.3 kDa	51
P307SQ-8A	NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK**SQSRESQA**	10.69	4.3 kDa	53

Example 10

[00130] Comparison of *in vitro* bactericidal activities of peptides of the present disclosure.

[00131] To determine the *in vitro* bactericidal activities of the peptides, P307, P307AE-8, P307SQ-8C, and P307CS-8, bacteria were treated with the peptides for 2 hours at room temperature. The survived cells were serially diluted and plated on TSB agar plates to determine the activity.

[00132] The bactericidal activities of 50 μg/mL the peptides, P307, P307AE-8 and P307SQ-8C were compared by treating *A. baumannii* strains #1791, S5 and ATCC17978. P307SQ-8C was the most active, reducing about 10^6 cfu/mL of bacteria to below the limit of detection (<10 cfu/mL). P307 was slightly more active than P307AE-8, but both peptides induced about a 3.8-log-unit decrease in viable bacteria (Fig. 2A). To investigate how the eight amino acids, SQSRESQC, contributed to the higher activity of P307SQ-8C, the same molar concentration of peptide SQSRESQC as 50 μg/mL P307 was added by itself or in combination with P307 to *A. baumannii* strains #1791 and S5. The activities were compared with 50 μg/mL of P307 and P307SQ-8C. The combination was only as active as P307 while SQSRESQC peptide alone has no activity (Fig. 2B). Hence the linkage is essential for the high bactericidal activity of P307SQ-8C. Next, we investigated the importance of sequence and composition. By scrambling the last eight amino acids in P307SQ-8C, we synthesized P307CS-8 with a C-terminal addition of CSQRQSES to P307. The activities of P307SQ-8C and P307CS-8 were comparable (Fig. 2C). The error bars show standard deviation and the black horizontal line marks the limit of detection. Thus, we concluded that the superior activity of P307SQ-8C derives from the composition of the last eight amino acids, regardless of the order of the last eight amino acids. For further investigation, we used P307SQ-8C because it is the most active, and compared its activity with P307.

Example 11

[00133] Bactericidal activities of P307 and P307SQ-8C

[00134] The effects of pH and NaCl on the *in vitro* activities of P307 and P307SQ-8C were investigated. *A. baumannii* strain #1791 were treated with 50 μg/mL of peptides to test each condition. Two buffer systems (sodium phosphate and Tris-HCl) were used to test pH 6.8, 7.5, 8.0 and 8.8. The peptides were more active in Tris-HCl and higher pH elicited better killing (Fig. 13A). Thus, we elected to continue our *in vitro* experiments with 50 mM Tris-HCl, pH 7.5, which approximates physiological pH. The activities of both peptides were inversely proportional to the concentration of NaCl (Fig. 13B). Next, titration of P307 and killing kinetics of P307 and P307SQ-8C were investigated by treating *A. baumannii* strain #1791. The activity of P307 was concentration-dependent, beginning from 4 μg/mL (Fig. 13C). P307SQ-8C acted

faster than P307, resulting in about 3.2-log-unit decrease already at the 5 minute time point (Fig. 13D). There was no difference in activities of either peptide at room temperature or 37°C (data not shown). From these *in vitro* characterization experiments, we decided our optimal experimental conditions to be 50 mM Tris-HCl, pH 7.5, 50 μg/mL peptides and 2 hours at room temperature (22-25°C), unless otherwise indicated.

Example 12

[00135] Next, we investigated the in vitro bactericidal spectra of P307 and P307SQ-8C against different bacterial species, A. baumannii (strain Nos. 1775, 1776, 1777, 1788, 1789, 1790, 1791, 1792, 1793, 1794, 1796, 1797, 1798, 1799, ATCC 17978 and S1, S3, D5), Bacillus anthracis (ΔSterne), Escherichia coli (DH5α), Pseudomonas aeruginosa (PA01), Staphylococcus aureus (RN4220) and two strains of Klebsiella pneumonia (ATCC 700603 and ATCC10031). These bacterial species were treated with 50 μg/mL of P307 or P307SQ-8C in 50 mM Tris-HCl, pH 7.5 for 2 hours at room temperature to investigate the specificity of the peptides. Among the bacteria tested, A. baumannii strains were consistently most sensitive to the peptides, showing an average of 2.7- and 6.2-log-unit decrease with P307 and P307SQ-8C, respectively. Bacillus anthracis, Pseudomonas aeruginosa and Staphylococcus aureus are moderately sensitive. P307 and P307SQ-8C produced an average of about 1.3- and 2.9-log unit decrease, respectively, for these bacteria. However, Escherichia coli and Klebsiella pneumoniae are resistant to both peptides (Fig. 14).

Example 13.

[00136] In addition, to investigate the activities of the peptides against A. baumannii at different growth phases, we compared the sensitivities of strain #1791 at log phase, stationary phase and biofilm state. The bacteria in log phase (3 hours post inoculation of 1:100 overnight culture in fresh media) and stationary phase (overnight culture) were treated with 50 μg/mL of P307 or P307SQ-8C for 2 hours at room temperature. The survived cells were serially diluted and plated on TSB agar plates to determine cfu/mL. (figure 15A). *A. baumannii* biofilms were established by incubating about 10^5 cfu/mL of strain #1791 in TSB with 0.2% glucose inside about 2.5 cm long catheters for 72 hours at 37°C. The catheters were then washed to remove planktonic cells and treated with 250 μg/mL of P307 or P307SQ-8C. After 2 hours and 24 hours at room temperature, the biofilm was thoroughly disrupted and survived cells re-suspended to be plated and counted to determine the killing efficiency of the peptides against *in vitro* biofilm. (figure 15B) The log phase organisms were slightly more sensitive to P307 than stationary phase (about 3.7- versus 2.4-log-unit decrease). There seems to be no such difference with P307SQ-8C (Figure 15A). The biofilms were the most resistant of all growth phases. Biofilms were treated with 250 μg/mL P307 or P307SQ-8C for 2 or 24 hr. After 2 hours, about 3- and 4-log-unit

decrease in cfu/mL was observed with P307 and P307SQ-8C, respectively. After 24 hours, P307 produced an additional about 1.3-log-unit decrease while P307SQ-8C did not (figure 15B).

Example 14

[00137] In order to compare the efficiency of the peptides P307 and P307SQ-8C with some clinically used antibiotics, we performed a minimal inhibitory concentration assay for two *A. baumannii* strains, #1791 and ATCC17978. Microtiter dilution method was used to determine the MICs of levofloxacin, ceftazidime, polymyxin B, P307 and P307SQ-8C for *A. baumannii* strains #1791, #1798, S5 and ATCC17978. For the antibiotics, 1.5–2 fold serial dilutions (three lower and three higher) of the MICs determined by Etest Lood R, et al., 2015 Antimicrob. Agents Chemother. 59:1983–1991.) were included. For the peptides, two-fold serial dilutions (500–31.25 μg/mL) were tested. The overnight cultures were re-suspended to OD_{600} of 0.001 (about10^5 cfu/mL) in Mueller-Hinton broth (pH 7.9). The antibiotics or peptides were added to final 100 μL for each dilution. The bacteria were allowed to grow at 37°C for 24 hour at 220 rpm. The absorbance at 595 nm was then read in a SpectraMax Plus Reader (Molecular Devices). The MICs were determined as the lowest concentrations of antimicrobial agents that completely inhibit bacterial growth. Alamar®Blue was used to confirm the data obtained from OD_{595}. The experiments were conducted at least twice in duplicate.

[00138] The strains displayed varying degree of sensitivity to all antimicrobial agents (Table 9).

Table 9

A. baumannii strains	Levofloxacin		Ceftazidime		Polymyxin B		P307		P307SQ-8C	
	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
#1791	6	16.6	250	457	0.25	0.19	375	110	125	29
ATCC17978	≤0.1	0.3	12	21.9	0.25	0.19	750	220	≤500	≤116

[00139] P307SQ-8C has a lower MIC than P307, which is in accordance with the *in vitro* killing activity (Fig. 2 and 3).

Example 15

[00140] Cytotoxic effects of P307 and P307SQ-8C as measured by B cell survival and hemolysis.

[00141] Human B-cells obtained from a rheumatic fever patient at The Rockefeller University Hospital were grown in RPMI media supplemented with 10% bovine serum, penicillin and streptomycin. Cells were harvested by low speed centrifugation, washed once in media, and resuspended in pre-warmed media to a concentration of 10^7 cells/ml, as determined by trypan blue exclusion tests. The peptides (P307, P307SQ-8C and melittin) were serially diluted (80-

0.3125 μM) in culture media, and added to $5x10^4$ live cells. Cells were incubated for 1 hour at 37°C in a humidified 5% CO_2 atmosphere, after which they were stained (CellTiter 96 Non-radioactive cell proliferation assay; Promega) according to manufacturer's instructions. The samples were incubated for additionally 4 hours, before a Solubilization/Stop solution was added, and incubated overnight. The absorbance at 570 nm was measured in SpectraMax Plus Reader (Molecular Devices). The reactions were carried out twice in triplicate and representative data are shown as mean ± standard deviation.

[00142] Human blood from a healthy individual was gathered in an EDTA-tube, and red blood cells (RBC) collected through low speed centrifugation. Cells were washed in PBS, and resuspended to a 10% RBC solution. P307 and P307SQ-8C were serially diluted in PBS (80-0.3125 μM). PBS and 1% Triton X-100 were used as negative and positive controls, respectively. Samples were mixed, and incubated for 1 hour at 37°C. The supernatant was collected, and absorbance at 405 nm recorded through SpectraMax Plus Reader (Molecular Devices). The reactions were carried out twice in triplicate and representative data are shown as mean ± standard deviation.

[00143] Serial dilutions of peptides were incubated with about $5x10^4$ live B cells for 1 hr at 37°C in a humidified 5% CO_2 atmosphere, and melittin was used as a positive control. CellTiter 96® Non-Radioactive Cell Proliferation Assay (Promega) was conducted according to manufacturer's protocol to quantify the survival of B cells. Red blood cells (RBCs) were incubated with serial dilutions of the peptides and the release of hemoglobin into the supernatant was measured by OD_{405} to determine hemolysis. Triton X-100 was used as a positive control. The error bars show standard deviation.

[00144] The peptides were tested for their cytotoxicity using human B cells and red blood cells (RBCs). In contrast to the melittin positive control, the membranes of B cells are not affected by either P307 or P307SQ-8C. Even at the highest concentration tested (80 μM), the viability of the cells remains the same as the buffer control (figure 16A). Similarly, the integrity of RBCs are also not affected by either peptide in comparison to the Triton X-100 positive control (figure 16B).

Example 16

[00145] A portion of P307SQ-8C (about 25%) runs at twice the theoretical molecular weight in comparison to P307SQ-8A, which runs at 4.3 kD (data not shown). To determine the importance of disulfide bond formation for the high activity of P307SQ-8C the bactericidal activities of P307 and P307SQ-8C were compared in the presence of 0, 0.1 and 1 mM dithiothreitol (DTT). *A. baumannii* strain #1791 was treated with 50 μg/mL P307 or 10 μg/mL

P307SQ-8C in 50 mM Tris-HCl, pH 7.5 for 2 hours at room temperature. The survived cells were serially diluted and plated on TSB agar. P307SQ-8C becomes less active with higher DTT concentration whereas the activity of P307 slightly increases (figure 17A). To further confirm the importance of disulfide formation for P307SQ-8C activity, we synthesized P307SQ-8A with the last cysteine changed to alanine. *A. baumannii* strains no. 1791 and ATCC17978 were treated with 10 μg/mL of each peptide. The bactericidal assays of P307SQ-8C and P307SQ-8A showed that the former is slightly more active than the latter (figure 17B). These results altogether pointed out that part of the superior activity of P307SQ-8C derives from disulfide bond formation between two molecules.

Example 17

[00146] Next, we investigated whether P307 binds to DNA, given the positive charges on the peptides (net charge of +7). The peptide P307 was mixed with DNA at different peptide:DNA ratios (0:1-15:1) and incubated for 1 hour before being analyzed on an agarose gel. In comparison to positive control peptide, no shift in molecular weight was observed for P307 at any of the ratios of peptide to DNA tested (Fig. 18).

Example 18

[00147] Because the peptides did not appear to kill the bacteria by interacting with DNA, we investigated whether they affect the bacterial membrane using transmission electron microscopy (TEM). *A. baumannii* strain #1791 was treated with buffer (control) or 300 μg/mL P307SQ-8C for 5 minutes or 2 hours. Comparing the TEM images of the samples reveals disruption of inner membrane and changes in intracellular density (Figures 19A, B and C). In addition, we found that the resistant bacteria at pH 7.5 (figure 3) were sensitive to P307 at pH 8.8, including *E. coli* and *K. pneumoniae* (figure 19D). Because the charges on the peptide do not vary as pH changes from 7.5 to 8.8, we reasoned that the changes occur on the bacterial membrane. At higher pH, the bacterial membrane becomes more negatively charged, allowing the positively charged peptides to establish stronger ionic interactions.

Example 19

[00148] Without wishes to be bound by theory, we hypothesize the following mechanism of action: P307SQ-8C interacts with the bacterial membrane to gain entry into the cell, and in the process, disrupts the cytoplasmic membrane. Membrane permeabilization is more effective when the peptide is dimerized. The disruption induces the production of reactive oxygen species such as hydroxyl radicals, which disturbs the intracellular content. To investigate this hypothesis, we determined membrane disruption using SYTOX® Green uptake assay.

[00149] Overnight cultures of bacteria were washed in 50 mM Tris-HCl pH 7.5, and resuspended to an OD_{600} of 0.3 (about 10^7 cfu/ml). Benzonase® nuclease (25 U/ml)(Novagen)

and SYTOX® Green (1 μM)(Invitrogen) was added to the bacterial cells, and incubated for 15 minutes at room temperature in the dark. Peptides were added (50 μg/ml; 14.7 μM P307 and 11.6 μM P307SQ-8C, and melittin (14.7 μM)(Sigma) was used as a control. Relative fluorescence units (RFU) were measured in a SpectraMax Plus reader (Molecular Devices) at room temperature (ex: 485 nm, em: 520 nm) for 2 hours. The reactions were carried out twice in duplicate and representative data are shown as mean ± standard deviation.

[00150] Both peptides permeabilize the membranes of sensitive bacteria, giving rise to an increase in fluorescent signals of SYTOX® Green dye as it binds to intracellular DNA (figure 20). Hydroxyl radical formation was investigated by treating the bacteria with P307 and P307SQ-8C in the presence of hydroxyl radical scavenger, thiourea. Polymyxin B was included as a control since it has been reported that its bactericidal activity partially relies on hydroxyl radical death pathway. Thiourea (300 mM) inhibits the activity of P307 and P307SQ-8C completely (Fig. 21A). However, it cannot be disregarded that thiourea affects the activities by other pathways. Therefore, bactericidal activities were also compared under aerobic and anaerobic conditions. Since *A. baumannii* is a strictly aerobic bacteria, *E. coli* was used for the bactericidal assay with 50 mM Tris-HCl, pH 8.8. Both peptide activities were completely inhibited by anaerobic condition (Fig. 21B). Although we cannot rule out other possibilities such as effect on oxygen-dependent transport mechanism, the current results support our hypothesis of hydroxyl radical formation.

Example 20

[00151] We investigated the *in vivo* activity of P307SQ-8C using mouse skin model because skin infection is a common route of disease by *A. baumannii*. The backs of 40 female CD-1 mice (6 to 8 weeks of age; Charles River Laboratories) were shaved with an electric razor. Nair™ (Hair remover lotion for body and legs, aloe and lanolin) was applied to the shaved areas to remove any remaining hair. The areas were then disinfected with alcohol wipes, and skin abrasion was induced by tape-stripping. An area of ~ 1 cm^2 of the tape striped skin was then marked and infected with 10 μL of about 10^8 cfu/mL *A. baumannii* strain no. 1791. The bacteria were allowed to colonize for 16-18 hours, after which the infected area was either left untreated or treated with 200 μg of P307SQ-8C or 2 μg of polymyxin B for 2 hours. To harvest the remaining bacteria on the skin, the mice were sacrificed and the infected skin was processed in 500 μL PBS for 1 minute in a Stomacher® 80 Biomaster using a microbag (Seward Ltd., UK). The solution was serially diluted and plated on LB agar containing 4 μg/mL levofloxacin and 12 μg/mL ampicillin for selection. The resulting cfu/mL from each animal is shown as a point and the horizontal bars represent the means. Both treatments reduce the bacterial load significantly

(p-value = 0.0023, ordinary one-way ANOVA) (figure 22).

CLAIMS

What is claimed is:

1. A polypeptide comprising an amino acid sequence that has at least 90% sequence identity to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

2. The polypeptide according to claim 1, wherein the polypeptide consists of an amino acid sequence that has at least 90% sequence identity to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment has antibacterial activity.

3. A polypeptide comprising an amino acid sequence that has at least 80% sequence identity to the amino acid sequence of SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

4. The polypeptide according to claim 3, wherein the polypeptide consists of an amino acid that has at least 80% sequence identity to SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

5. The polypeptide according to claim 3, wherein the polypeptide consists of an amino acid having the SEQ ID NO:1, SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ

ID NO:18, SEQ ID NO:19, SEQ ID NO:20, or SEQ ID NO:21, or a fragment of the polypeptide, wherein the polypeptide or fragment is conjugated to an antimicrobial peptide to yield a conjugated polypeptide and the conjugated polypeptide has antibacterial activity.

6. The polypeptide according to any one of claims 3, 4 or 5, wherein the antimicrobial peptide comprises the eight amino acids C, S, Q, R, S, E and S in any order.

7. The polypeptide according to claim 6, wherein the antimicrobial peptide has the amino acid sequence SQSRESQC (SEQ ID NO:44) or CSQRQSES (SEQ ID NO:50) .

8. The polypeptide according to any one of claims 3, 4 or 5, wherein the polypeptide comprises the amino acid sequence of SEQ ID NO:45 or SEQ ID NO:51.

9. The polypeptide or the fragment according to anyone of claims 3-8, wherein the C-terminus of the polypeptide or fragment is conjugated to the antimicrobial peptide.

10. The polypeptide or fragment according to claim 9, wherein the C-terminus of the polypeptide or fragment is conjugated to the N-terminus of the antimicrobial peptide via a peptide bond.

11. The polypeptide or the fragment according to anyone of claims 3, 4, 6, or 8, wherein the N-terminus of the polypeptide or fragment is conjugated to the antimicrobial peptide.

12. The polypeptide or fragment according to claim 11, wherein the N-terminus of the polypeptide or fragment is conjugated to the C-terminus of the antimicrobial peptide via a peptide bond.

13. The polypeptide or fragment according to any one of claims 1-12, wherein the polypeptide or fragment has antibacterial activity against a gram negative bacterium.

14. The polypeptide according to claim 13, wherein the bacterium is of the genus *Acinetobacter*.

15. The polypeptide according to claim 13, wherein the bacterium is *E.coli*, *P. aeruginosa* or *A. baumannii.*

16. The polypeptide or fragment according to any one of claims 1-12, wherein the polypeptide or fragment has antibacterial activity against a gram positive bacterium.

17. The polypeptide according to claim 16, wherein the bacterium is *S. aureus* or *B. anthracis*.

18. The polypeptide according to any one of claims 1-17, wherein the polypeptide is lyophilized.

19. A composition comprising one or more polypeptide according to any one of claims 1-17.

20. The composition according to claim 19, wherein the composition comprises a pharmaceutically acceptable carrier, buffering agent, or preservative.

21. The composition according to claim 20, wherein the composition is formulated for topical administration, subcutaneous deliver, intravenous delivery or oral delivery.

23. The composition according to claim 20, wherein the composition further comprises an antibiotic or a clotting agent.

24. The composition according to any one of claims 19-23, wherein the composition is lyophilized.

25. A method for treating a subject in need thereof, comprising administering to the subject a composition comprising one or more polypeptide according to any one of claims 1-17, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

26. The method according to claim 25, wherein the subject has a bacterial infection.

27. The method according to claim 26, wherein the bacterial infection is non-responsive to other treatment modalities.

28. The method according to claim 25 or claim 26, wherein the composition is administered in combination with an antibiotic.

29. The method according to claim 28, wherein the antibiotic is amoxicillin, augmentin, amoxicillin, ampicillin, azlocillin, flucloxacillin, mezlocillin, methicillin, , penicillin G, penicillin V, cephalexin, cefazedone, cefuroxime, loracarbef, cemetazole, cefotetan, cefoxitin, ciprofloxacin, levaquin, and floxacin, tetracycline, doxycycline, or minocycline, gentamycin, amikacin, and tobramycin, clarithromycin, azithromycin, erythromycin, daptomycin, neomycin, kanamycin, or streptomycin.

30. The method according to anyone of claims 25-29, wherein the bacterial infection is a wound infection.

31. The method according to anyone of claims 25-30, wherein the composition is administered topically, subcutaneously, by intravenous injection or orally.

32. The method according to anyone of claims 25-31, wherein the composition is in a unit dosage form.

33. The method according to claim 25, wherein the composition is in the form of a cream, ointment, salve, gel, lozenge, spray, or aerosol.

34. A method for treating a bacterial infection comprising inhibiting the formation of or disrupting a bacterial biofilm comprising administering to a subject in need thereof, a composition comprising a polypeptide according to any one of claims 1-17, in an amount effective to kill bacteria in the biofilm.

35. A method of disinfecting an article comprising contacting the article with a composition comprising a polypeptide according to any one of claims 1-17.

36. The method according to claim 35, wherein the article is a hard surface.

37. A method for inhibiting the formation of or disrupting a bacterial biofilm on an article comprising contacting the article with a peptide according to any one of claims 1-17, in an amount effective to kill bacteria in the biofilm.

38. The method according to any one of claims 35-37, wherein the article is a countertop, keyboard, surgical instrument, or medical device.

39. An article of manufacture containing a composition comprising a polypeptide according to any one of claims 1-17.

40. The article of manufacture of claim 39, wherein the composition is a composition wherein the composition further comprises a carrier, buffering agent or preservative.

41. The article of manufacture according to claim 40, wherein the article of manufacture is a vial or a delivery device.

42. The article of manufacture according to claim 40 or 41, wherein the composition is lyophilized.

43. A method for treating a subject having a surgical wound comprising irrigating the wound with a composition comprising a polypeptide according to any one of claims 1-17, and a pharmaceutically acceptable carrier, buffering agent, or preservative.

44. The method according to claim 43, wherein the surgical wound is irrigated prior to surgical closure of the wound.

FIG. 1A

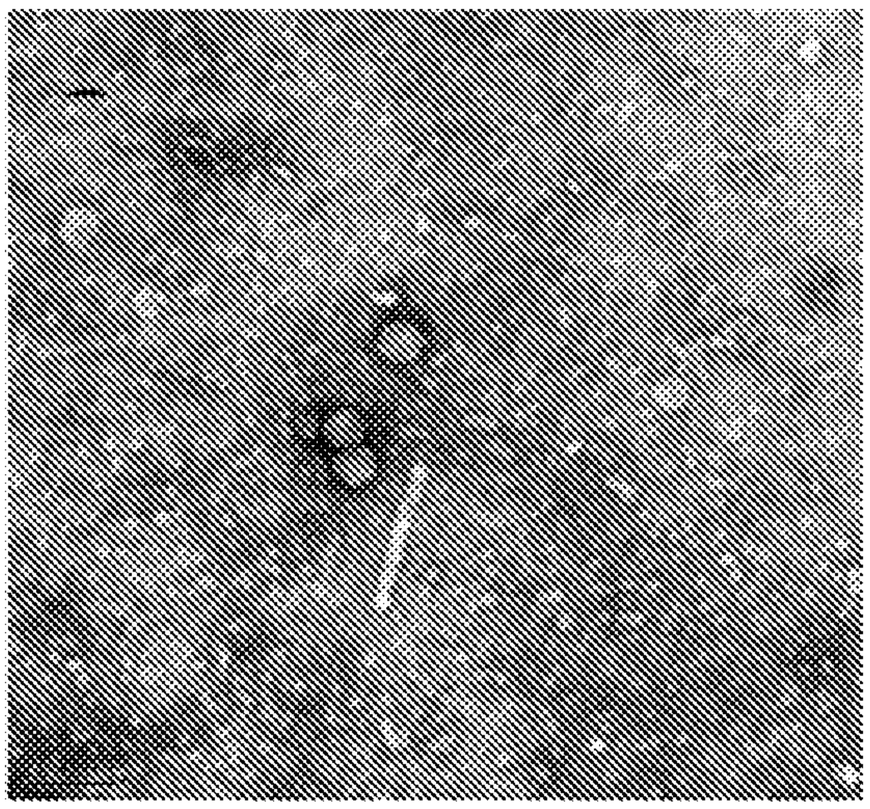


FIG. 1B

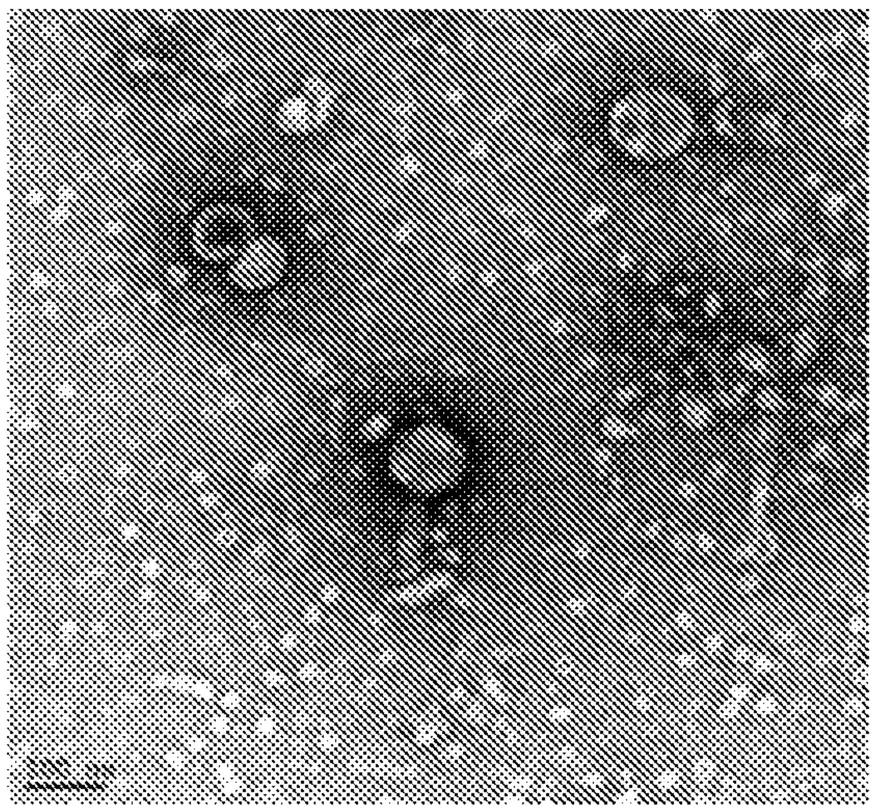


FIG. 1C

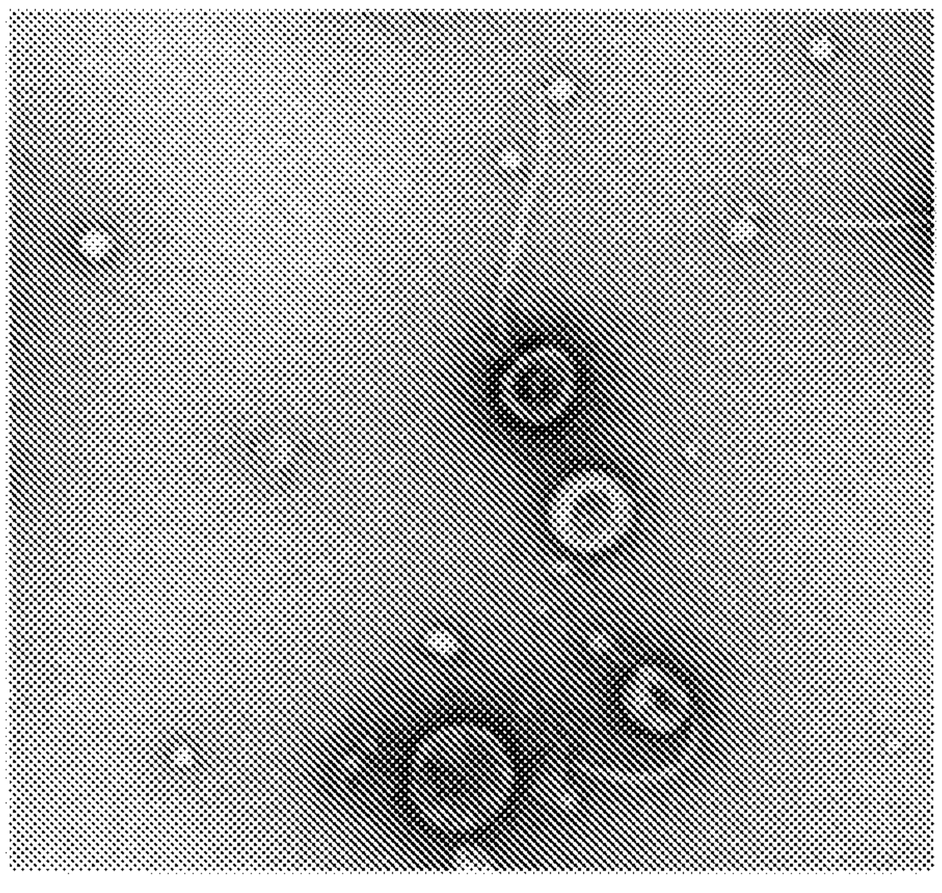


FIG. 2

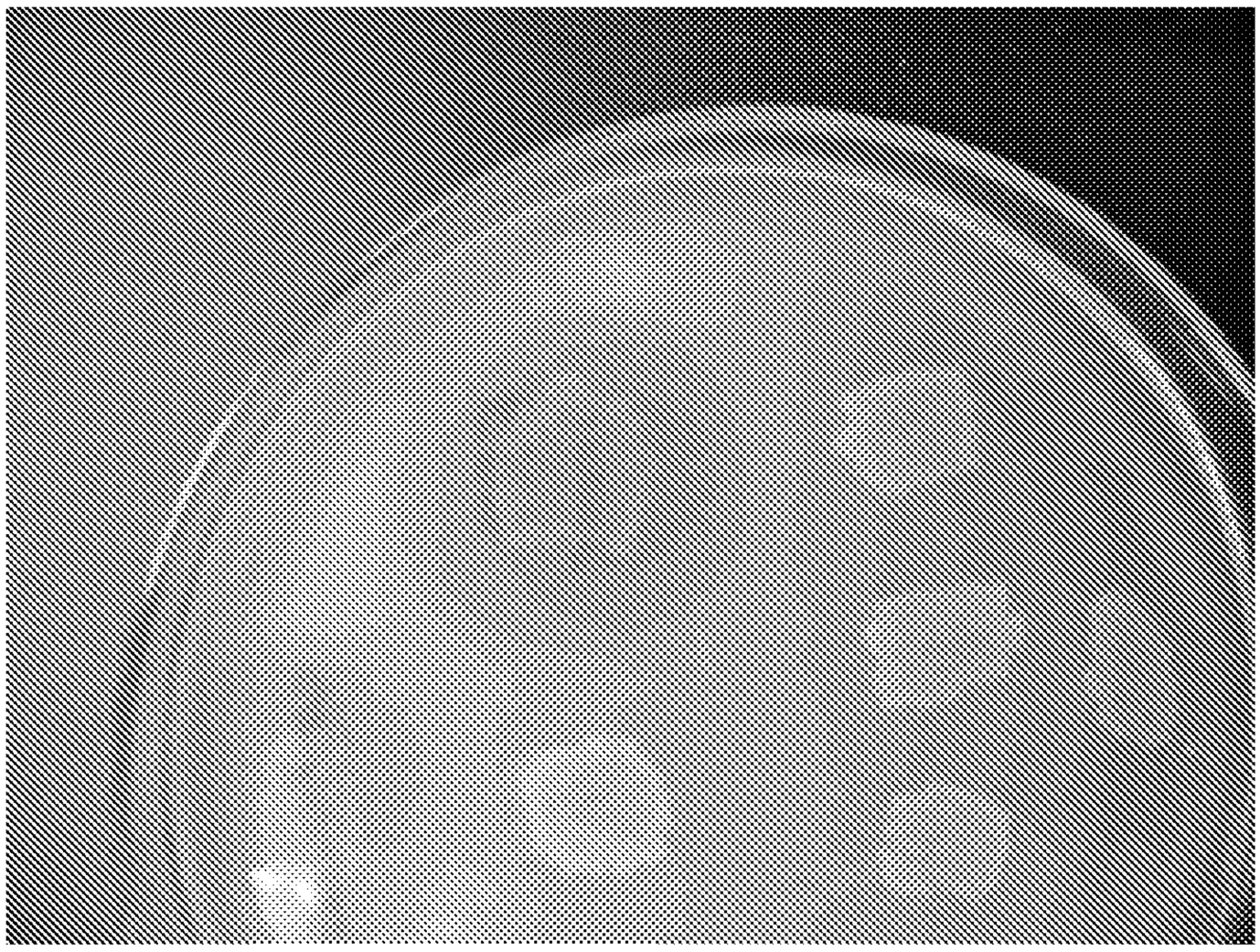
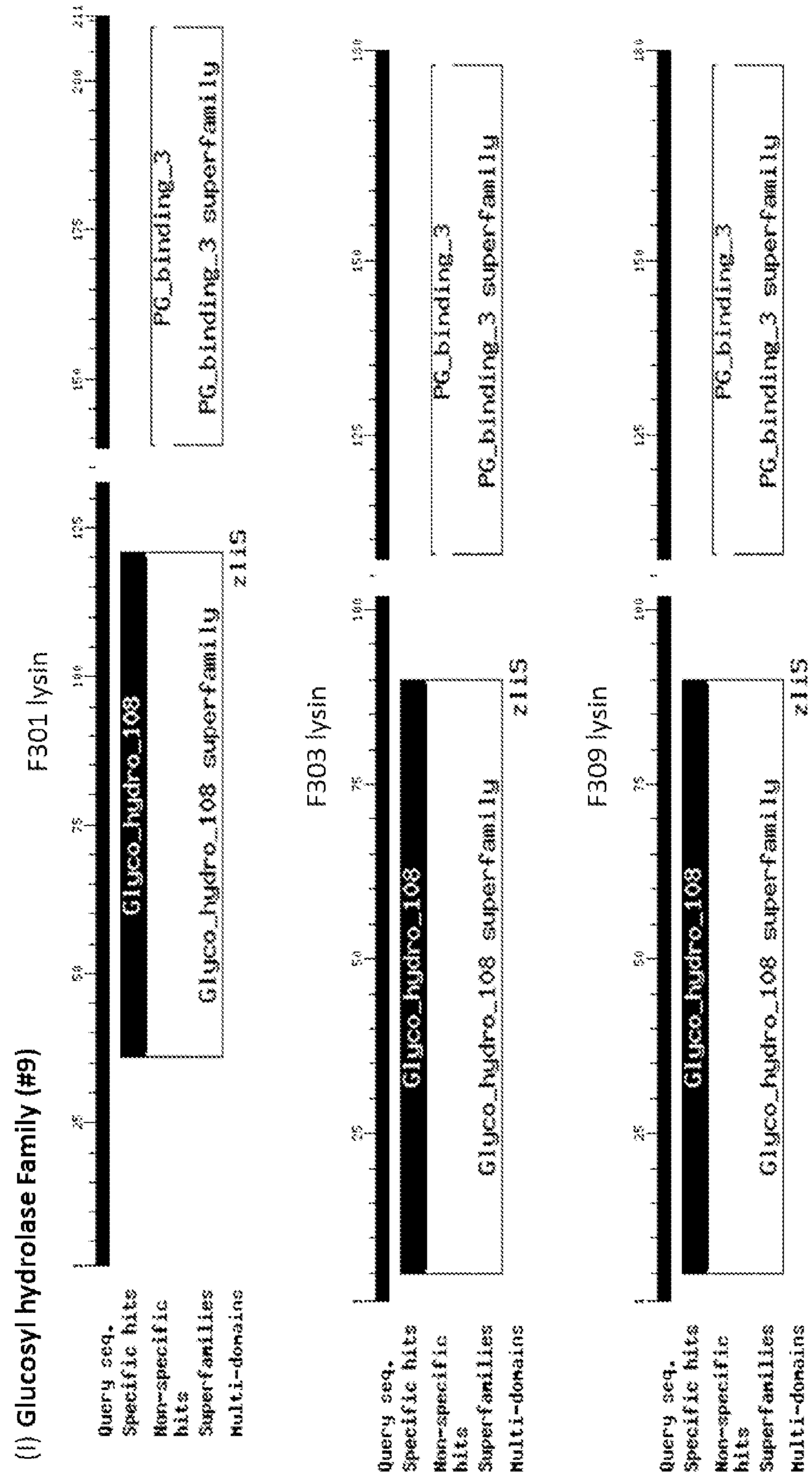


FIG. 3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F303 lysin
F309 lysin
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Specific hits
Non-specific hits
Superfamilies
Multi-domains
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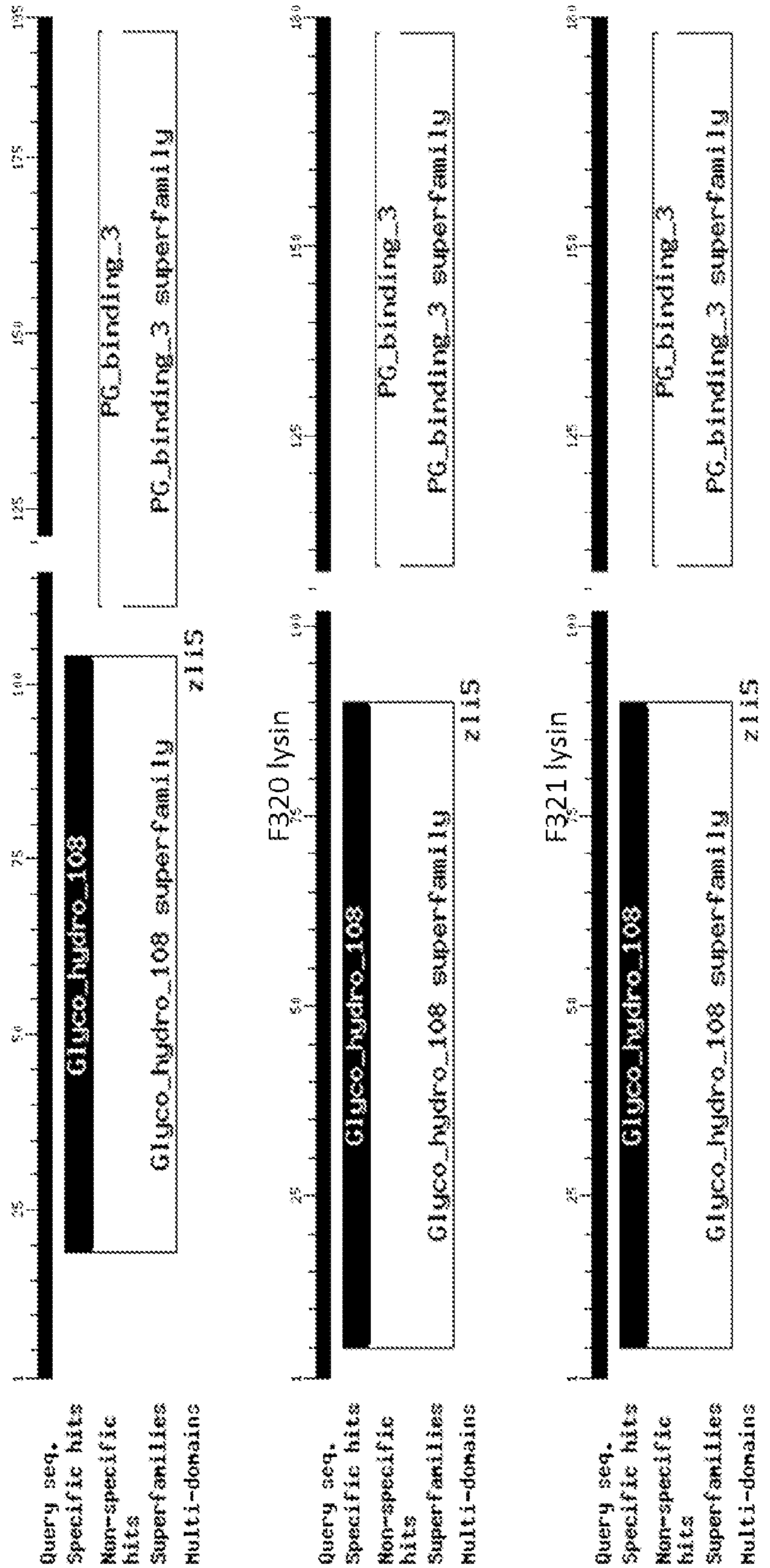
Glyco_hydro_108 superfamily
PG_binding_3
PG_binding_3 superfamily
211

FIG. 3 Cont.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F321 lysin
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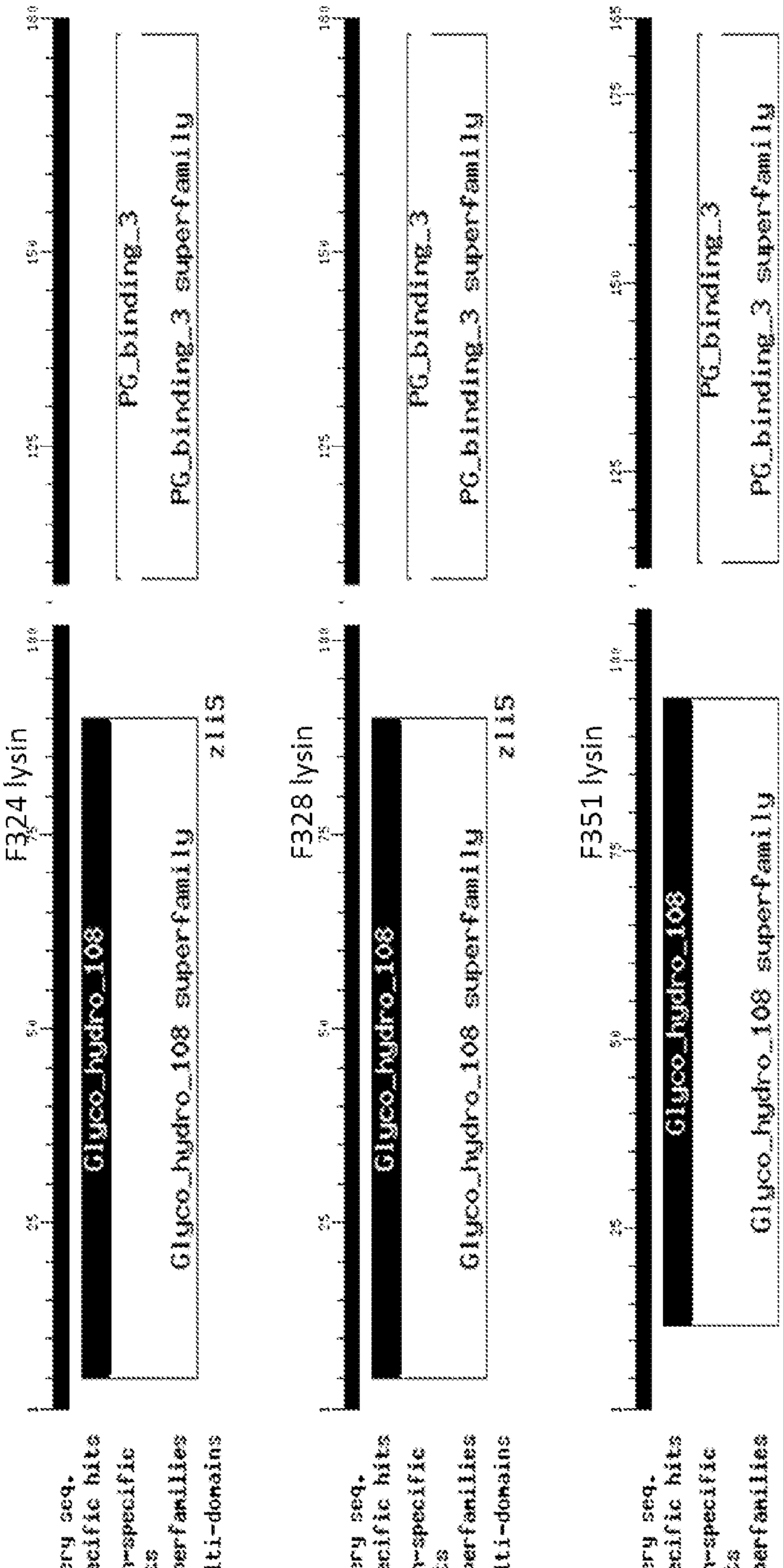
Specific hits
Non-specific hits
Superfamilies
Multi-domains

FIG. 3 Cont.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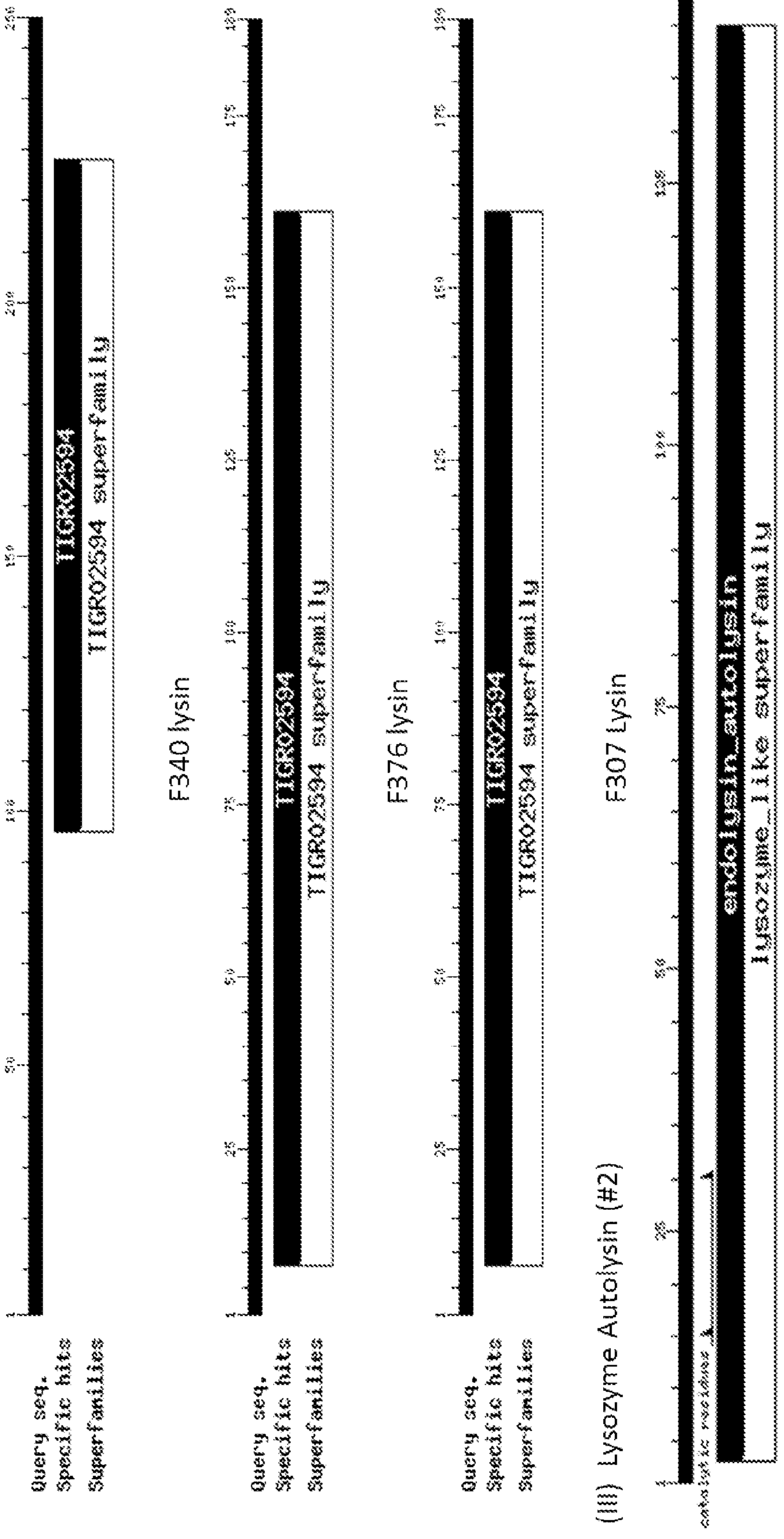
F328 lysin
F351 lysin
Glyco_hydro_108
Glyco_hydro_108 superfamily
PG_binding_3
PG_binding_3 superfamily
2IIS
Query seq.
Specific hits
Non-specific hits
Superfamilies
Multi-domains

FIG. 3 Cont.

(iii) Baseplate Lysozyme (#7)

FIG. 3 Cont.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TIGR02594
TIGR02594 superfamily
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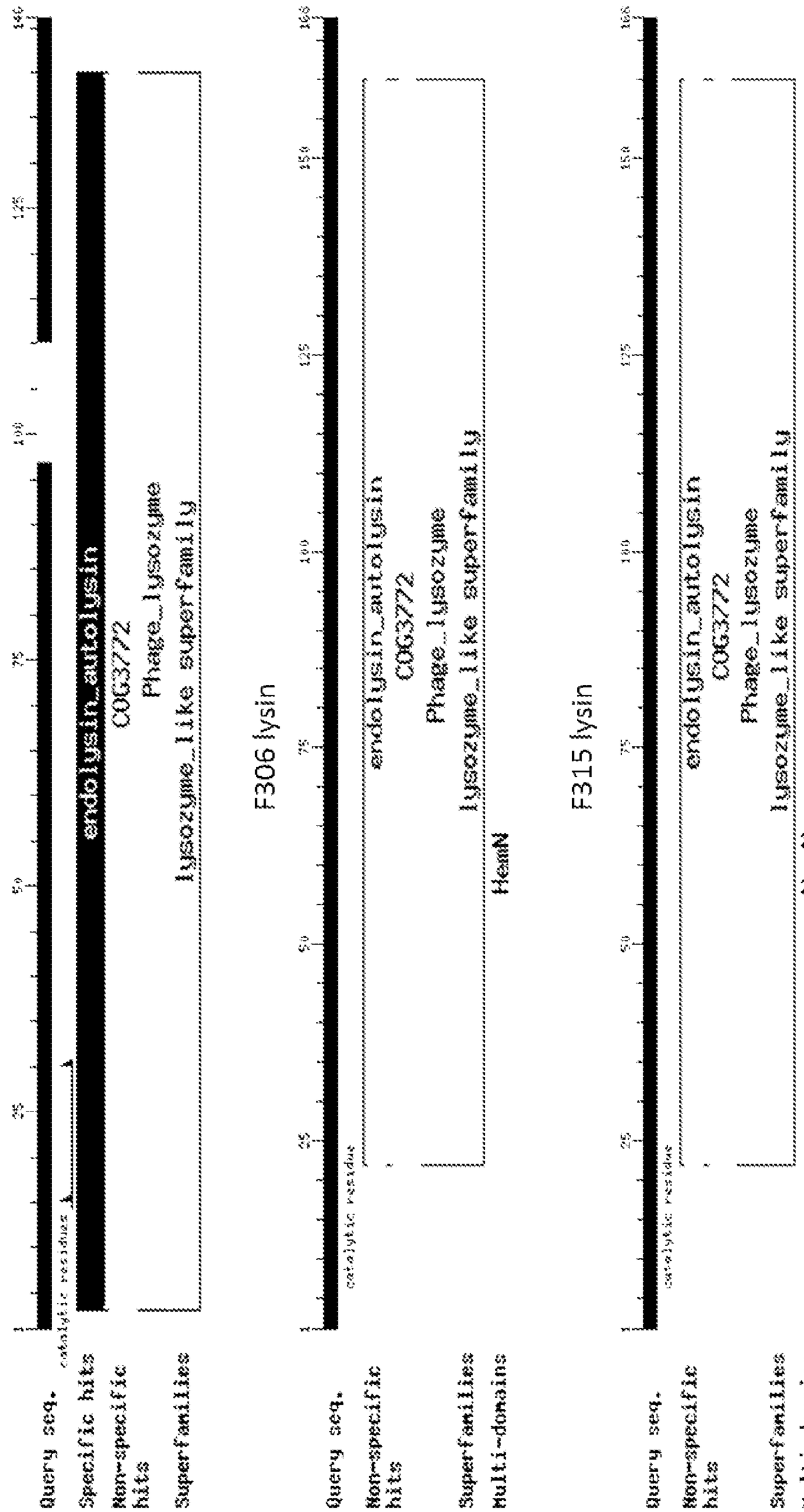
TIGR02594
TIGR02594 superfamily
F376 lysin
TIGR02594
TIGR02594 superfamily
F307 Lysin
endolysin_autolysin
lysozyme_like superfamily
Autolysin (#2)
(IIII) Lysozyme
Query seq.
Specific hits
Superfamilies

FIG. 3 Cont.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COG3772
Phage_lysozyme
lysozyme_like superfamily
F306 lysin
endolysin_autolysin
COG3772
Phage_lysozyme
lysozyme_like superfamily
F315 lysin
endolysin_autolysin
COG3772
Phage_lysozyme
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catalytic residues
HemN
Query seq.
Non-specific hits
Superfamilies
Multi-domains

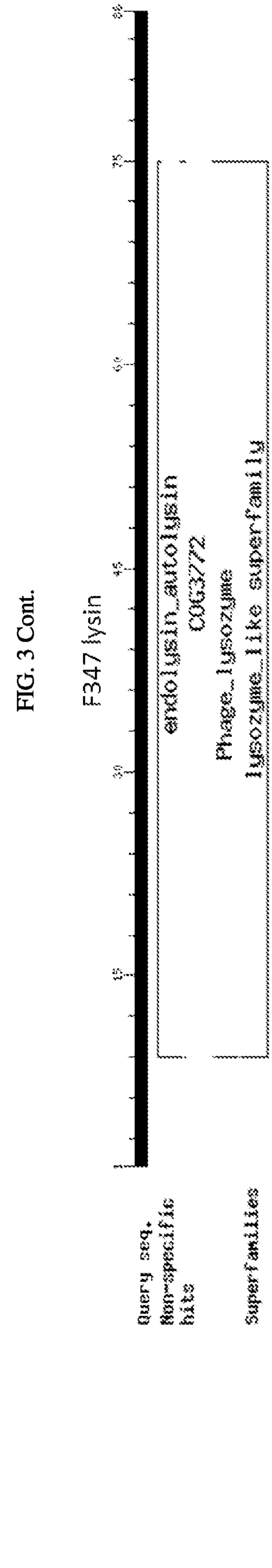
Query seq.
Non-specific hits
Superfamilies
Phage_lysozyme
lysozyme_like superfamily
endolysin_autolysin
COG3772
F347 lysin
1
15
30
45
60
75

FIG. 3 Cont.

FIG. 4

```
ClustalW (v1.83) multiple sequence alignment


21 Sequences Aligned              Processing time: 1.8 seconds
Gaps Inserted = 540               Conserved Identities = 1
Score = 0

Pairwise Alignment Mode: Slow
Pairwise Alignment Parameters:
    Open Gap Penalty = 10.0   Extend Gap Penalty = 5.0

Multiple Alignment Parameters:
    Open Gap Penalty = 10.0   Extend Gap Penalty = 5.0
    Delay Divergent = 40%     Transitions: Weighted

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F311     1 ------------------------------------------------------------   0
F328     1 ------------------------------------------------------------   0
F324     1 ------------------------------------------------------------   0
F347     1 ------------------------------------------------------------   0
F351     1 ------------------------------------------------------------   0
F344     1 ------------------------------------------------------------   0
F340     1 ------------------------------------------------------------   0
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F336     1 ------------------------------------------------------------   0
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F330     1 ----------------CTGGATCCGGTGATGACGAT--GACAAG---CTCGCCCT---T   35
F307     1 ------------------------------------------------------------   0
F309     1 ------------------------------------------------------------   0
F303     1 ------------------------------------------------------------   0
F301     1 ------------------------------------------------------------   0
F320     1 ------------------------------------------------------------   0
F306     1 ------------------------------------------------------------   0
F321     1 ------------------------------------------------------------   0
F315     1 ------------------------------------------------------------   0
F376     1 ------------------------------------------------------------   0
F311     1 ------------------------------------------------------------   0
```

FIG. 4 Cont.

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F328     1 ----------------------------------------------------------    0
F324     1 ----------------------------------------------------------    0
F347     1 ----------------------------------------------------------    0
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F344     1 ----------------------------------------------------------    0
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F336     1 ----------------------------------------------------------    0
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F332    61 GCATTCATCATGCT-AATTTTATTGCCAGCACTACAATGGGGTGTTGATTATGAAGTTAT  119
F330    36 CCACAACTCGAGCCGAATTTTATTGCCAGCACTACAATGGGGTGTTGATTATGAAGTTAT   95
F307     1 ----------------------------------------------------------    0
F309     1 ----------------------------------------------------------    0
F303     1 ----------------------------------------------------------    0
F301     1 ----------------------------------------------------------    0
F320     1 ----------------------------------------------------------    0
F306     1 ----------------------------------------------------------    0
F321     1 ----------------------------------------------------------    0
F315     1 ----------------------------------------------------------    0
F376     1 ----------------------------------TTAAGCCTCTGAAACACTCGTAACAGA   27
F311     1 ----------------------------------------------------------    0
F328     1 ----------------------------------------------------CTAGCCGAT    9
F324     1 --------------ATGAAAAT-TGAACAATATCTTGATGATT------TGATTAAACGC   39
F347     1 ----------------------------------------------------TCACTGC    7
F351     2 TGAAGGAAACCGAAATGAATAT-TGAAAAATATCTTGATGAAT------TAATTAAGCGT   54
F344     1 ----------------------------------------------TCATATAAC-AAC   12
F340     1 ----------------------------------TTAAGCCTCTGAAACACTCGTAACAGA   27
F338   120 TCCTGAAAAATATCATGCATTTGTTACTGGTACTTTGATGCTTGTTCTGTCATGGATTGG  179
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F332   120 TCCTGAAAAATATCATGCATTTGTTACTGGTACTTTGATGCTTGTTCTGTCATGGATTGG  179
F330    96 TCCTGAAAAATATCATGCATTTGTTACTGGTACTTTGATGCTTGTTCTGTCATGGATTGG  155
F307     1 ----------------------------------------------------------GT    2
F309     1 --------------ATGAAAAT-TGAACAATATCTTGATGATT------TGATTAAACGC   39
F303     1 ----------------------------------------------------CTAGCCGAT    9
F301     1 ----------------------------------------------------CTAGCCGAT    9
F320     1 ----------------------------------------------------CTAGCCGAT    9
```

FIG. 4 Cont.

```
F306     1 ----------------------------------ATGTCAAACAAGACTAAAATCATAGT  26
F321     1 --------------ATGAAAAT-TGAACAATATCTTGATGATT------TGATTAAACGC  39
F315     1 ------------------------------------------------TCACTGC   7
F376    28 AACGATTTTTAGGACTGGTAAATCGTATCGGTGAGCAG---CTGGTTTACTTGTTTTGCC  84
F311     1 ------------------------------------------TCAT-TTTAA-AAA  12
F328    10 CCGGTTAGCGATCCAGCCATAAAAAAACTGCTCTTGGC---TTTTATTACGTTCAC-AGA  65
F324    40 GAAGGCGGTTATGTAA--ATAATCCAGTGGATCGAGG---AGGTGCTACCAAATAC-GGT  93
F347     8 GCTCCTATACATTT-------TGCGTGTCGTT--CTAC---TTGTCTGGTCCAGAC-GCC  54
F351    55 GAAGGCGGGTATGTAA--ATAACCCAGCTGATCGGGG---CGGTGCAACTAAATAT-GGC 108
F344    13 TCGATTGGCGATCCAACCATAGAAAAACTGTTCCTGGC---TAGGATTGCGTTCAC-AGA  68
F340    28 AACGATTTTTAGGACTGGTAAATCGTATCGGTGAGCAG---CTGGTTTACTTGTTTTGCC  84
F338   180 AAAGAAAATTTCTCAACCACGACTTAATGGCCCGCAATTAACAGGCCAGTTAGTA-GGG  237
F336    28 TACGCCTTTTAATTGTGGCAATGTATAACGCTTACTTG---CTGGTTGAGTTGTACGACC  84
F334    28 TACGCCTTTTAATTGTGGCAATGTATAACGCTTACCTG---CTGGTTGAGTTGTACGACC  84
F332   180 AAAGAAAATTTCTCAACCACGACTTAATGGCCCGCAATTAACAGGCCAGTTAGTA-GGG  237
F330   156 AAAGAAAATTTCTCAACCACGACTTAATGGCCCGCAATTAACAGGCCAGTTAGTA-GGG  213
F307     3 GAAAACAAGTAACCCA--GGAGTGGATTTAATCAAA----------GGCTTTGAA-GGT  48
F309    40 GAAGGCGGTTATGTAA--ATAATCCAGTAGATCGAGG---AGGTGCTACCAAATAC-GGT  93
F303    10 CCGGTTAGCAATCCAGCCATAGAAGAATTGCTCTTGCT---TAGGATTACGCTCAC-AAA  65
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F320    10 CCGGTTAGCAATCCAGCCATAGAAGAATTGCTCTTGCT---TGGGATTACGCTCAC-AAA  65
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F321    40 GAAGGTGGTTATGTAA--ATAATCCAGTAGATCGAGG---AGGTGCTACCAAATAC-GGT  93
F315     8 ACCGCCATACACTT-------GCTATAACAAT--CTTG---TTGGCGTGTCCAGAC-ACC  54
F376    85 ATACCACATAAAGGCTTCAAAAGCCGAAACGTCATATACTGCAAAACAAACTT-TATTTG 143
F311    13 T----AACTCCATTTCAGCTTTGCGACGT-TTCACCAGTCCTGC-CAATACACGACCGCC  66
F328    66 TTT-CAATGTATCGTTGGCCTTGCATAATATTTAACACGCGCAC-TAAGACCTTTTCGCC 123
F324    94 AT--TACTGAAGCTGTAGCACGTGAAAACGGCTATAAGGGCAAT-ATGAA-AGATTTGC  148
F347    55 ATAACACCCGTTTTTACGAA---CAGAGCAATCGCGCTTTGCA-ACGTACTTATATTTA  109
F351   109 AT--CACACAAGCTGTTGCGCGTGAAAATGGCTGGAATGGCAAT-ATGAA-AGATTTGC  163
F344    69 TTT-CAATGTAACGTTGCCCTTGCATGATATTAAGAACACGCAC-CAGGACTTTTTCGCC 126
F340    85 ATACCACATAAAGGCTTCAAAAGCCGAAACGTCATATACTGCAAAACAAACTT-TATTTG 143
F338   238 AT--CAATTCT-TTATTGAATATCCCAACACCAACAAAGCCTG-ATGAATTAGCTTGGA  292
F336    85 ATACCATCTGAATTCTTGAAAGTCAGAGTCATTATAAAGTGCATAACAAACTT-TATTTG 143
F334    85 ATACCATCTGAATTCTTGAAAGTCAGAGTCATTATAAAGTGCATAACAAACTT-TATTTG 143
F332   238 AT--CAATTCT-TTATTGAATATCCCAACACCAACAAAGCCTG-ATGAATTAGCTTGGA  292
F330   214 AT--CAATTCT-TTATTGAATATCCCAACACCAACAAAGCCTG-ATGAATTAGCTTGGA  268
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FIG. 4 Cont.

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F309    94 AT--TACTGAAGCTGTAGCACGTGAAAACGGCTATAAGGGCAAT-ATGAA-AGATTTGC  148
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F320    66 TTT-CGATATATCGCTGGCCTTGCATGATATTAAGAACTCGCAC-TAGGACTTTCTCACC 123
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F315    55 ATAACAACCATTGGACCGAA---TCGAGCAATCACGCTTTGCA-ACATATTTGTATTTC  109
F376   144 ACTGATTACCACCTAAGCAAATTA-ATTTACCAGTAGGTGTTTTAC---CAACAACGAAA 199
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F324   149 CTCTTGATGTGGCCAAA-GCAATTTA--TCGGAAACAGTACTGGA-TAGA-GCCAC-GTT 202
F347   110 AGTAAAGAGTCGCAAGCTGCTTTATATTGACCAGC-----CTTTA-----AATGCTTAAG 159
F351   164 CGCTTGATGTGGCCAAA-GCTATTTA--CAAGAAGCAATACTGGA-CAGC-TCCGC-GAT 217
F344   127 GTCTTTTCCACGCTTGGCCAGATAAGTTTTGAGTGCATTAAGAGTTGCTGGACCATAAAT 186
F340   144 ACTGATTACCACCTAAGCAAATTA-ATTTACCAGTGGGTGTTTTAC---CAACAACGAAA 199
F338   293 TTGCGGAAGCAAA-AAA-GCATCTTGGCCTTCAAGAAATACCTGG-TAAACAGCAT-AAC 348
F336   144 ACTGATTGCCTCCAAGGCATACTA-ACTTTCCAGACTTTTTGTCACGGCCAACTACAAAA 202
F334   144 ACTGATTGCCTCCAAGGCATACTA-ACTTTCCAGACTTTTTGTCACGGCCAACTACAAAA 202
F332   293 TTGCAGAAGCAAA-AAA-GCATCTTGGCCTTCAAGAAATACCTGG-TAAACAGCAT-AAC 348
F330   269 TTGCAGAAGCAAA-AAA-GCATCTTGGCCTTCAAGAAATACCTGG-TAAACAGCAT-AAC 324
F307   103 ACCATCAAATACCCGAACGGTGTGCGAGTCAAAAAAGGCGATA---CATGCACTGA-ATC 158
F309   149 CTCTTGATGTGGCCAAA-GCAATTTA--TCGGAAACAGTACTGGA-TAGA-GCCAC-GTT 202
F303   124 TTCTTTCCCACGTTTGGCCAAGTAAGTTTTGAGAGCTCCTAATGTGCTAGAACCATAAAC 183
F301   124 TTCTTTGCCACGTTTGGCCAAGTAAGTTTTTAGAGCTCCTAAAGTGTTAGAACCATAAAC 183
F320   124 TTCTTTCCCACGTTTGGCCAAGTAAGTTTTGAGAGCTCCTAATGTGCTAGAACCATAAAC 183
F306   136 TCCACTTCCTATGAAAACGGTACCAAGGTCAAAATGACTGACAAGCCGATTACAAA-AGA 194
F321   149 CTCTTGATGTGGCCAAA-GCAATTTA--TCGGAAACAGTACTGGA-TAGA-GCCAC-GTT 202
F315   110 AGTAATGAAGCACAAGCTTGCTTATATTGCCCTGC-----TTTGA-----GATTACGAAG 159
F376   200 GTTACATGGCCACCGCCCTCTCTTGATTTTACCGCAACACACCCATAACAAGGCTTGTYT 259
F311   125 TTTTTTAGCAAAGTAGATTTGCTAAGATTGCCTTC-GCCTAAGT-TAT-AAGTGAATG-A 180
F328   184 GCCATCAACCTT-CAAGTCTGCATAACCAGCTTT--ACCTTGAT-TGTTAAGCAAGTTCA 239
F324   203 TTGATCAGGTTAATA---CTCTTAGCTCTGCAGT-------AGC-TG--AAGA------- 242
F347   160 CATTGATG------ATTT-TGCG-AATGTTGGCACACCGTATTGATACGTGAA---ATCA 208
F351   218 TTGACCAAGTAAATG---CTGTTTCTTCTGCAGT-------AGC-TG--AAGA------- 257
F344   187 TCCGTCAACTGT-TAAATCTGGCCAACCTGCTTT--ACCTTGGT-TATTCAGCAAATTTA 242
F340   200 GTTACATGGCCACCGCCCTCTCTTGATTTTACCGCAACACACCCATAACAAGGCTTGTCT 259
```

FIG. 4 Cont.

```
F338  349 CCAACTATTTTAAAATGGCTCTCGGAGCTAAAGGCTTGGTGGGC-TG-ACGATGAAACG  405
F336  203 CAAACATGCCCACCACCCTTTCGAGTTTTAATAGCTACACAACCGTAAGCGGGTTTAGCT 262
F334  203 CAAACATGCCCACCACCCTTTCGAGTTTTAATAGCTACACAACCGTAAGCGGGTTTAGCT 262
F332  349 CCAACTATTTTAAAATGGCTCTCGGAGCTAAAGGCTTGGTGGGC-TG--ACGATGAAACG 405
F330  325 CCAACTATTTTAAAATGGCTCTCGGAGCTAAAGGCTTGGTGGGC-TG--ACGATGAAACG 381
F307  159 TCAAGCGGAAGAATA---CCTTCGCAA-TGACTT-------AGT-TG--TATTTGAAA-202
F309  203 TTGATCAGGTTAATA---CTCTTAGCTCTGCAGT-------AGC-TG--AAGA------- 242
F303  184 GCCATCAACCTT-CAAGTCTGCATAACCAGCTTT--ACCTTGAT-TGTTAAGCAAGTTCA 239
F301  184 GCCATCAACCTT-CAAGTCTGCATAACCAGCTTT--ACCTTGAT-TGTTAAGCAAGTTCA 239
F320  184 GCCATCAACCTT-CAAGTCTGCATAACCAGCTTT--ACCTTGAT-TGTTAAGCAAGTTCA 239
F306  195 ACGTGCTGTTCAA-A---TTGCCAAAGCTCACAT-------TGC-TA--AAGATGAGGTG 240
F321  203 TTGATCAGGTTAATA---CTCTTAGCTCTGCAGT-------AGC-TG--AAGA------- 242
F315  160 CATTGATG------AGCCGTTCC-AAT-TTGCTTGGCCATAGTTGTAAACAAA---GTCT 208
F376  260 AATTTGACTCCGCCTTCTTTGATGTATTC--AAGTGCGGCAT------ACCAGTTGAAAG 311
F311  181 GGCCAAAGCATCGAATTGGTTTTGATTAA-GTGGTACT----------TTCAC-CAAGC 227
F328  240 AAGCACGTTGTAAAAGTGGTTTTGCAAAG-TTGATACCACAG-------TTCACACCAGT 291
F324  243 ACTT----TTAGACACTGGT-GTGAACTG--TGGTATCAACT----TTGCAAAACCA--- 288
F347  209 AGGTA---TAGGTCATATTCAGTCTGTGA--TAATT---CGG------CT-----CGAGT 249
F351  258 GCTT----CTAGACACTGGT-GTGAATTG--CGGTACCGGAT----TTGCAAAACCT--- 303
F344  243 AAGCACGCTGTAAGAGTGGTTTTGCAAAT-CCGGTACCGCAA-------TTTACCCCAGT 294
F340  260 AATTTGACTCCGCCTTCTTTGATGTATTC--AAGTGCGGCAT------ACCAGTTGAAAG 311
F338  406 GCTTGGTGTGGGACCTTCGTTGCACATTGCTTGAAATCAGCTGGAATTGCTTATCCTAAG 465
F336  263 AATTT---TGTACCATAATTCACATAATC--CAATGCACGGT------ACCAATGCTTAG 311
F334  263 AATTT---TGTACCATAATTCACATAATC--CAATGCACGGT------ACCAATGCTTAG 311
F332  406 GCTTGGTGTGGGACCTTCGTTGCACATTGCTTGAAATCAGCTGGAATTGCTTATCCTAAG 465
F330  382 GCTTGGTGTGGGACCTTCGTTGCACATTGCTTGAAATCAGCTGGAATTGCTTATTCTAAG 441
F307  203 GCGC----TAT-CAATCGTTTGGTGAAAG--TTCCGCTTAAT-------CAAAACCA--- 245
F309  243 ACTT----TTAGACACTGGT-GTGAACTG--TGGTATCAACT----TTGCAAAACCA--- 288
F303  240 AAGCACGTTGTAAAAGTGGTTTTGCAAAG-TTGATACCACAG-------TTCACACCAGT 291
F301  240 AAGCACGTTGTAAAAGTGGTTTTGCAAAG-TTGATACCACAG-------TTCACACCAGT 291
F320  240 AAGCACGTTGTAAAAGTGGTTTTGCAAAG-TTGATACCACAG-------TTCACACCAGT 291
F306  241 GCAT----TTCGCAAGTCGTTGCAGGGCG--TGAGGCTAACT-------CAGACTGA--- 284
F321  243 ACTT----TTAGACACTGGT-GTGAACTG--TGGTATCAACT----TTGCAAAACCA--- 288
F315  209 AAGTA---CACATCATATTCAGTCTGAGT--TAGCCTCACGC------CCTGCAACGACT 257
F376  312 GATAA--AAGCCTGGTGGGGCCTTTGTTCC-TGATTT---ATAAGTTTTGGAACGCGAGT 365
F311  228 GATTG--ATAGCATTTTCAAATGCGACCAAGTCATTGCGAAGATATCCTTCTGCTTGAGA 285
F328  292 GTCTA--AAAGT-TCTTCAGCTACTGCAGAGCTAAGAGTATTAACCTGATCAAAACGTGG 348
```

FIG. 4 Cont.

```
F324  289 CTTTT--ACAACGTGCTTTGAACTTGCTTAACAATCAAGGTAAAGCTGGTTATGCAG--A 344
F347  250 TGTGG--AAGGCAG---------------------------------------------- 261
F351  304 CTTTT--ACAACGAGCTTTGAACTTGCTTAATAACCAAGGTAAAGCTGGATATGCAG--A 359
F344  295 ATCTA--AAAGC-TCTTCAGCAACTAACGAGCTAATTACATTCACTTGATCAAATCGCGG 351
F340  312 GATAA--AAGCCTGGTGGGGCCTTTGTTCC-TGATTT---ATAAGTTTTGGAACGCGAGT 365
F338  466 CATTG--GTACCGTGCATTGGATTATGTGAATTAT-GGTACAAAATTAGCTAAACCC--G 520
F336  312 GATAAGCAATTCCAGCTGATTTCAAGCAATGTGCAAC---GAAGGTCCCACACCAAGCCG 368
F334  312 GATAAGCAATTCCAGCTGATTTCAAGCAATGTGCAAC---GAAGGTCCCACACCAAGCCG 368
F332  466 CATTG--GTACCGTGCATTGGATTATGTGAATTAT-GGTACAAAATTAGCTAAACCC--G 520
F330  442 CATTG--GTACCGTGCATTGGATTATGTGAATTAT-GGTACAAAATTAGCTAAACCC--G 496
F307  246 -ATTC--GATGCTTTGGCCTCATTCACTTA-CAACCTTGGTGAGGGCAATCTTAGTA--T 299
F309  289 CTTTT--ACAACGTGCTTTGAACTTGCTTAACAATCAAGGTAAAGCTGGTTATACAG--A 344
F303  292 GTCTA--AAAGT-TCTTCAGCTACTGCAGAGCTAAGAGTATTAACCTGATCAAAACGTGG 348
F301  292 GTCTA--AAAGT-TCTTCAGCTACTGCAGAGCTAAGAGTATTAACCTGATCAAAACGTGG 348
F320  292 GTCTA--AAAGT-TCTTCAGCTACTGCAGAGCTAAGAGTATTAACCTGATCAAAACGTGG 348
F306  285 -ATAT--GATGTGTACTTAGACTTTGTTTA-CAACTATGGCCAAGCAAATTGGAACG--G 338
F321  289 CTTTT--ACAACGTGCTTTGAACTTGCTTAACAATCAAGGTAAAGCTGGTTATGCAG--A 344
F315  258 TGCGA--AATGCCACCTCATCTTTAGCAATGTGAGCTTTGGCAATTTGAACAGCACGTTC 315
F376  366 TTACACT-TCCTCTTTGAAATCCCGCCGTTTGG-AGA--CAGTGCGCAACGAATGTTCCG 421
F311  286 CTCAGTGCATATATCGCCTTTTTTGACACGC----ACTCCATTAGG------ATATTTAA 335
F328  349 CTCTATCCA-GTACTGTTTCCGATAAATTGCTTTGGCCACATCAAGAGGCAAATCTTTCA 407
F324  345 CTTGAAGGTTG-ATGGCGTTT-ATGGTTCTA---ACACTT--TAGGA-----GCTCTAAA 392
F347  262 ---------------------------------------------------------- 261
F351  360 TTTAGAGGTTG-ATGGTGTTT-ATGGCTCAG---CAACGC--TAGGT-----GCCCTTAA 407
F344  352 ATCTGTCCA-ATACTGCTTTTTATAAATGGCTTTGGCCACATCAAGCGGTAAATCTTTCA 410
F340  366 TTACACT-TCCTCTTTGAAATCCCGCCGTTTGG-AGA--CAGTGCGCAACGAATGTTCCG 421
F338  521 CTT-ACGGTTGTGTAGCTATT-AAAACTCGA---AAGGGTGGTGGGC-----ATGTTTGT 570
F336  369 TTTCATCGTCAGCCCACCAAGCCTTTAGCTCCG-AGAGCCATTTTAAAATAGTTGGGTTA 427
F334  369 TTTCATCGTCAGCCCACCAAGCCTTTAGCTCCG-AGAGCCATTTTAAAATAGTTGGGTTA 427
F332  521 CTT-ACGGTTGTGTAGCTATT-AAAACTCGA---AAGGGTGGTGGGC-----ATGTTTGT 570
F330  497 CTT-ACGGTTGTGTAGCTATT-AAAACTCGA---AAGGGTGGTGGGC-----GTGTTTGT 546
F307  300 ATC-AACTTTGCTAAAAAAGC-TTAATGCCA---AAGACTA-TAAAG-----GTGCTGCA 348
F309  345 CTTGAAGGTTG-ATGGCGTTT-ATGGTTCTA---GCACAT--TAGGA-----GCTCTCAA 392
F303  349 CTCTATCCA-GTACTGTTTCCGATAAATTGCTTTGGCCACATCAAGAGGCAAATCTTTCA 407
F301  349 CTCTATCCA-GTACTGTTTCCGATAAATTGCTTTGGCCACATCAAGAGGCAAATCTTTCA 407
F320  349 CTCTATCCA-GTACTGTTTCCGATAAATTGCTTTGGCCACATCAAGAGGCAAATCTTTCA 407
F306  339 CTC-ATCAATGCTTCGTAATC-TCAAAGCAG---GGCAATA-TAAGC-----AAGCTTGT 387
```

FIG. 4 Cont.

```
F321  345 CTTGAAGGTTG-ATGGCGTTT-ATGGTTCTA---ACACTT--TAGGA-----GCTCTAAA 392
F315  316 TTTTGTAATCGGCTTGTCAGTCATTTTGACCTT-GGTACCGTTTTCATAGGAAGTGGATC 374
F376  422 CACCATG---CAGTTTCATCGTCAAG---CCAAGAGG---ACTTTAAGTCCTTCAGCCAT 472
F311  336 T--TGTTC-C-AAATCCAATG----GTCCAAACGCCC-----ACACCAT----CGTCATA 378
F328  408 TATTGCCC-TTATAGCCGTTTTCACGTGCTACAGCTTCAGTAATACCGTATTTGGTAGCA 466
F324  393 A-ACTTAC-TTGGCCA-AACGTGGCAA--AGAAGGCG-----AAAAGGTATTAGTGCGCG 442
F347  262 ------------------------------------------------------------ 261
F351  408 A-ACATAC-TTGTCAA-AACGTGGGAA--AGAAGGTG-----AGAAGGTTCTGGTGCGAG 457
F344  411 TGTTGCCC-TTAAAGCCGTTAGTACGTGCTACTGCTTCAGTAATACCGTACTTTGTTTCA 469
F340  422 CACCATG---CAGTTTCATCGTCAAG---CCAAGAGG---ACTTTAAGTCCTTCAGCCAT 472
F338  571 T-TTGTAG-TTGGCCGTGACAAAAAGTCTGGAAAGTT-----AGTATGCCTTGGAGGCAA   6
F336  428 TGCTGTTTACCAGGTATTTCTTGAAGG--CCAAGATG---CTTTT--TTGCTTCTGCAAT 480
F334  428 TGCTGTTTACCAGGTATTTCTTGAAGG--CCAAGATG---CTTTT--TTGCTTCTGCAAT 480
F332  571 T-TTGTAG-TTGGCCGTGACAAAAAGTCTGGAAAGTT-----AGTATGCCTTGGAGGCAA 623
F330  547 T-TTGTAG-TTGGCCGTGACAAAAAGTCTGGAAAGTT-----AGTATGCCTTGGAGGCAA 599
F307  349 G-CTGAAT-TT--CCTAAATG-GAATA--AG--G--C-----GG---GTGGTCGT--GTC 387
F309  393 A-ACTTAC-TTGGCCA-AACGTGGGAA--AGAAGGTG-----AGAAAGTCCTAGTGCGAG 442
F303  408 TATTGCCC-TTATAGCCGTTTTCACGTGCTACAGCTTCAGTAATACCGTATTTGGTAGCA 466
F301  408 TATTGCCC-TTATAGCCGTTTTCACGTGCTACAGCTTCAGTAATACCGTATTTGGTAGCA 466
F320  408 TATTGCCC-TTATAGCCGTTTTCACGTGCTACAGCTTCAGTAATACCGTATTTGGTAGCA 466
F306  388 G-CTTCAT-TA--CTGAAATACAAATA--TGTTG--C-----AAAGCGTGATTGCTCGAT 434
F321  393 A-ACTTAC-TTGGCCA-AACGTGGCAA--AGAAGGCG-----AAAAGGTCTTAGTGCGCG 442
F315  375 CGATACCAATCGTTGCCACTTTAGCGCTATCTAAATA---TGGCTTTGATTTGTACCCCT 431
F376  473 TTGA--TGATT-GTCTGGTTGTGTTTGGGGCCGGCGATTTCAGCCAA--------CCCTA 521
F311  379 GGCTTTCAAAC-----GTTTACCTTC---AAA---GCCTTTG-ATT-----AGATTGATT 421
F328  467 CCTCCTCGATCTACTGGATTATTTAC---ATAACCGCCTACGCGTT-----TAATCAAAT 518
F324  443 T--GTTAAATA-------TTATGCA----AGGCCAACGAT-ACATT----GAAATC---- 480
F347  262 ------------------------------------------------------------ 261
F351  458 T--GCTCAATA-------TTATGCA----AGGGCAACGCT-ACATT----GAAATC---- 495
F344  470 CCGCCTCGATCTGCTGGGTTGTTTAC---GTACCCGCCCTCACGCT-----TAATTAACT 521
F340  473 TTGA--TGATT-GTCTGGTTGTGTTTGGGGCCGGCGATTTCAGCCAA--------CCCTA 521
F338  624 TCAGTCAAATAAAGTTTGTTATGCACTTTATAATGACTCTGACTTTCAA-GAATTCAGAT 682
F336  481 CCAAGCTAATTCATCAGGCTTTGTT-GGTGTTGGGATATTCAATAAAGAATTGATCCCTA 539
F334  481 CCAAGCTAATTCATCAGGCTTTGTT-GGTGTTGGGATATTCAATAAAGAATTGATCCCTA 539
F332  624 TCAGTCAAATAAAGTTTGTTATGCACTTTATAATGACTCTGACTTTCAA-GAATTCAGAT 682
F330  600 TCAGTCAAATAAAGTTTGTTATGCACTTTATAATGACTCTGACTTTCAA-GAATTCAGAT 658
F307  388 TTGGCTGGAT-----TAGTTA-AAC----GTCGCAAAGCTGAAAT-----GGAGTTAT-T 431
```

FIG. 4 Cont.

```
F309   443 T--TCTTAATA-------TCATGCA----AGGCCAGCGAT-ATATC----GAAATT---- 480
F303   467 CCTCCTCGATCTACTGGATTATTTAC---ATAACCGCCTTCGCGTT-----TAATCAAAT 518
F301   467 CCTCCTCGATCTACTGGATTATTTAC---ATAACCGCCTTCGCGTT-----TAATCAAAT 518
F320   467 CCTCCTCGATCTACTGGATTATTTAC---ATAACCGCCTTCGCGTT-----TAATCAAAT 518
F306   435 TCGGTCCAATGG---TTGTTATGGT----GTCTGGACACGCCAAC-----AAGATCGT-T 481
F321   443 T--GTTAAATA-------TTATGCA----AGGCCAACGAT-ACATT----GAAATC---- 480
F315   432 CATAGCCAATTAAAGATGCAAAAAAAAGCGCTGATGCGCTTAATGTTGTTACTATGATTT 491
F376   522 TGTGACGGCGAGCCT-CTGCGATCCACG-----GTAATTCTGGA-TTTGCACTC--AT-- 570
F311   422 CCT---GA-GTT--ACTTG-TTTTCAT------------------------------- 441
F328   519 CATCAAGATATT--GTTCAATTTTCAT------------------------------- 543
F324   481 --TGTGAACGTA--ATAAAA--GCCAAGAGCAGTTTTTTTATGGCTG-GATCGCTAACCG 533
F347   262 ------------------------------------------------------- 261
F351   496 --TGTGAGCGTA--ATCCAA--AGCAGGAACAGTTTTTCTATGGCTG-GATTGCTAACCG 548
F344   522 CGTCCAGATATT--GTTCAATGTTCATTTCGGTTTCCTTCAGATGTAAAAAACCGCCCGA 579
F340   522 TGTGACGGCGAGCCT-CTGCGATCCACG-----GTAATTCTGGA-TTTGCACTC--AT-- 570
F338   683 GGTATGGTCGTACAACTCAACCAGCAAGTAAGCGTTATACATTGCCACAATTAAAAGGCG 742
F336   540 CTAACTGGCCTGTTAATTGCGGGCCATTAAGTCGTGGTTGAGAAATTTTCTTTCCAATCC 599
F334   540 CTAACTGGCCTGTTAATTGCGGGCCATTAAGTCGTGGTTGAGAAATTTTCTTTCCAATCC 599
F332   683 GGTATGGTCGTACAACTCAACCAGCAAGTAAGCGTTATACATTGCCACAATTAAAAGGCG 742
F330   659 GGTATGGTCGTACAACTCAACCAGCAAGTAAGCGTTATACATTGCCACAATTAAAAGGCG 718
F307   432 TTTA-AAATGA------------------------------------------------ 441
F309   481 --TGTGAGCGTA--ATCCTA--AGCAAGAGCAATTCTTCTATGGCTG-GATTGCTAACCG 533
F303   519 CATCAAGATATT--GTTCAATTTTCAT------------------------------- 543
F301   519 CATCAAGATATT--GTTCAATTTTCATTTCACTTTCCTTTAGACGTAAAAAAGCCACCAG 576
F320   519 CATCAAGATATT--GTTCAATTTTCAT------------------------------- 543
F306   482 ATAGCAAGTGTATGGCGGTGCAATGA---------------------------------- 507
F321   481 --TGTGAACGTA--ATAAAA--GCCAAGAGCAGTTTTTTTATGGCTG-GATCGCTAACCG 533
F315   492 TAGTCTTGTTTGACAT------------------------------------------- 507
F376   571 ------------------------------------------------------- 570
F311   442 ------------------------------------------------------- 441
F328   544 ------------------------------------------------------- 543
F324   534 GATCGGCTAG------------------------------------------------ 543
F347   262 ------------------------------------------------------- 261
F351   549 GATCGGCTAG------------------------------------------------ 558
F344   580 AGGCGGCAT------------------------------------------------- 588
F340   571 ------------------------------------------------------- 570
F338   743 TAACAGCTACTAGGGTTTTGGAAGCCTAA------------------------------ 771
```

FIG. 4 Cont.

```
F336  600 ATGACAGAACAAGCATCAAAGTACCAGTAACAAATGCATGATATTTTTCAGGAATAACTT 659
F334  600 ATGACAGAACAAGCATCAAAGTACCAGTAACAAATGCATGATGTTTTTCAGGAATAACTT 659
F332  743 TAACAGCTACTAGGGTTTTGGAAGCCTAA---------------------------- 771
F330  719 TAACAGCTACTAGGGTTTTGGAAGCCTAA---------------------------- 747
F307  442 ---------------------------------------------------------- 441
F309  534 GATCGGCTAG------------------------------------------------ 543
F303  544 ---------------------------------------------------------- 543
F301  577 TTGATCCGTGAACTTGATATTTATATTGATCCAGAAGTGCAGGCTGAGGTTTTTGGTCAG 636
F320  544 ---------------------------------------------------------- 543
F306  508 ---------------------------------------------------------- 507
F321  534 GATCGGCTAG------------------------------------------------ 543
F315  508 ---------------------------------------------------------- 507
F376  571 ---------------------------------------------------------- 570
F311  442 ---------------------------------------------------------- 441
F328  544 ---------------------------------------------------------- 543
F324  544 ---------------------------------------------------------- 543
F347  262 ---------------------------------------------------------- 261
F351  559 ---------------------------------------------------------- 558
F344  589 ---------------------------------------------------------- 588
F340  571 ---------------------------------------------------------- 570
F338  772 ---------------------------------------------------------- 771
F336  660 CATAATCAACACCCCATTGTAGTGCTGGCAATAAAATTAGCATGATGAATGCACCTACGG 719
F334  660 CATAATCAACACCCCATTGTAGTGCTGGCAATAAAATTAGCATGATGAATGCACCTACGG 719
F332  772 ---------------------------------------------------------- 771
F330  748 ---------------------------------------------------------- 747
F307  442 ---------------------------------------------------------- 441
F309  544 ---------------------------------------------------------- 543
F303  544 ---------------------------------------------------------- 543
F301  637 ---------------------------------------------------------- 636
F320  544 ---------------------------------------------------------- 543
F306  508 ---------------------------------------------------------- 507
F321  544 ---------------------------------------------------------- 543
F315  508 ---------------------------------------------------------- 507
F376  571 ---------------------------------------------------------- 570
F311  442 ---------------------------------------------------------- 441
F328  544 ---------------------------------------------------------- 543
F324  544 ---------------------------------------------------------- 543
```

FIG. 4 Cont.

```
F347  262 ---------------------------------------------------------- 261
F351  559 ---------------------------------------------------------- 558
F344  589 ---------------------------------------------------------- 588
F340  571 ---------------------------------------------------------- 570
F338  772 ---------------------------------------------------------- 771
F336  720 CGGGTAACTTAACAGATAGATACTGCCAAGCATTGTTTTCAATTAACTTCAT-------- 771
F334  720 CGGGTAACTTAACAGATAGATACTGCCAAGCATTGTTTTCAATTAACTTCAT-------- 771
F332  772 ---------------------------------------------------------- 771
F330  748 ---------------------------------------------------------- 747
F307  442 ---------------------------------------------------------- 441
F309  544 ---------------------------------------------------------- 543
F303  544 ---------------------------------------------------------- 543
F301  637 ---------------------------------------------------------- 636
F320  544 ---------------------------------------------------------- 543
F306  508 ---------------------------------------------------------- 507
F321  544 ---------------------------------------------------------- 543
F315  508 ---------------------------------------------------------- 507
```

FIG. 5

```
                    ClustalW (v1.83) multiple sequence alignment

          21 Sequences Aligned              Processing time: 0.3 seconds
            Gaps Inserted = 50                Conserved Identities = 1
                                    Score = 83346

                                Pairwise Alignment Mode: Slow
                                Pairwise Alignment Parameters:
                            Open Gap Penalty = 10.0   Extend Gap Penalty = 0.1
                                    Similarity Matrix: gonnet

                                Multiple Alignment Parameters:
                            Open Gap Penalty = 10.0   Extend Gap Penalty = 0.1
                                Delay Divergent = 40%     Gap Distance = 8
                                    Similarity Matrix: gonnet

F307     1 ------------------------------------------------------------   0
F376     1 ------------------------------------------------------------   0
F351     1 ------------------------------------------------------------   0
F347     1 ------------------------------------------------------------   0
F344     1 ------------------------------------------------------------   0
F340     1 ------------------------------------------------------------   0
F338     1 MKLIENNAWQYLSVKLPAVGAFIMLILLPALQWGVDYEVIPEKYHAFVTGTLMLVLSWIG  60
F336     1 MKLIENNAWQYLSVKLPAVGAFIMLILLPALQWGVDYEVIPEKYHAFVTGTLMLVLSWIG  60
F334     1 MKLIENNAWQYLSVKLPAVGAFIMLILLPALQWGVDYEVIPEKHHAFVTGTLMLVLSWIG  60
F332     1 MKLIENNAWQYLSVKLPAVGAFIMLILLPALQWGVDYEVIPEKYHAFVTGTLMLVLSWIG  60
F330     1 ----LDPVMTMTSSPFHNS----SRILLPALQWGVDYEVIPEKYHAFVTGTLMLVLSWIG  52
F328     1 ------------------------------------------------------------   0
F324     1 ------------------------------------------------------------   0
F321     1 ------------------------------------------------------------   0
F320     1 ------------------------------------------------------------   0
F315     1 ------------------------------------------------------------   0
F306     1 ------------------------------------------------------------   0
F303     1 ------------------------------------------------------------   0
F301     1 ----------------------------------------------------------LTKN   4
F309     1 ------------------------------------------------------------   0
F311     1 ------------------------------------------------------------   0
```

FIG. 5 Cont.

```
F307     1 ----------------------------VKTSNPGVDLIKGFEGLRLKAYDDGVGVWTIGF  33
F376     1 ----------------------------MSANP-ELPWIAEARRHIGLAEIAGPKHNQTII  32
F351     1 ------------------------LKETEMNIEKYLDELIKREGGYVNNPADRGGATKYGIT  38
F347     1 ------------------------------------------------LPSTTR-----   6
F344     1 -------------MPPSGGFLHLKETEMNIEQYLDELIKREGGYVNNPADRGGETKYGIT  47
 F340    1 ----------------------------MSANP-ELPWIAEARRHIGLAEIAGPKHNQTII  32
 F338   61 KKISQPRLNGPQLTGQLVGINSLLNIPTPTKPDELAWIAEAKKHLGLQEIPGKQHNPTIL 120
 F336   61 KKISQPRLNGPQLTGQLVGINSLLNIPTPTKPDELAWIAEAKKHLGLQEIPGKQHNPTIL 120
 F334   61 KKISQPRLNGPQLTGQLVGINSLLNIPTPTKPDELAWIAEAKKHLGLQEIPGKQHNPTIL 120
 F332   61 KKISQPRLNGPQLTGQLVGINSLLNIPTPTKPDELAWIAEAKKHLGLQEIPGKQHNPTIL 120
 F330   53 KKISQPRLNGPQLTGQLVGINSLLNIPTPTKPDELAWIAEAKKHLGLQEIPGKQHNPTIL 112
 F328    1 ----------------------------MKIEQYLDDLIKRVGGYVNNPVDRGGATKYGIT  33
F324     1 ----------------------------MKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  33
F321     1 ----------------------------MKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  33
F320     1 ----------------------------MKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  33
F315     1 ----------------MSNKTKIIVTTLSASALFFASLIGYEGYKSKPYLDSAKVATIGI  44
F306     1 ----------------MSNKTKIIVTTLSASALFFASLIGYEGYKSKPYLDSAKVATIGI  44
F303     1 ----------------------------MKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  33
F301     5 LSLHFWININIKFTDQLVAFLRLKESEMKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  64
F309     1 ----------------------------MKIEQYLDDLIKREGGYVNNPVDRGGATKYGIT  33
F311     1 ----------------------------MKTSNSGINLIKGFEGKRLKAYDDGVGVWTIGF  33
F307    34 GTIKYPNGVRVKKGDTCTESQAEEYLRNDLVV--------------------------FES  68
F376    33 KWLKDLKSSWLDDETAWCGTFVAHCLQTAGFQRGSVNSRSKTYKSGTKAPPGFYPFNWYA  92
F351    39 QAVARENGWNGNMKDLPLDVAKAIYKKQYWTAPRFDQVNA-----------VSSAVAEE  86
F347     7 ------------------------------------------------------------   6
F344    48 EAVARTNGFKGNMKDLPLDVAKAIYKKQYWTDPRFDQVNV-----------ISSLVAEE  95
F340    33 KWLKDLKSSWLDDETAWCGTFVAHCLQTAGFQRGSVNSRSKTYKSGTKAPPGFYPFNWYA  92
F338   121 KWLSELKAWWADDETAWCGTFVAHCLKSAGIA--------------------YPKHWYR 159
 F336  121 KWLSELKAWWADDETAWCGTFVAHCLKSAGIA--------------------YPKHWYR 159
 F334  121 KWLSELKAWWADDETAWCGTFVAHCLKSAGIA--------------------YPKHWYR 159
 F332  121 KWLSELKAWWADDETAWCGTFVAHCLKSAGIA--------------------YPKHWYR 159
 F330  113 KWLSELKAWWADDETAWCGTFVAHCLKSAGIA--------------------YSKHWYR 151
 F328   34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
 F324   34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
 F321   34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
 F320   34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
 F315   45 GSTSYENGTKVKMTDKPITKERAVQIAKAHIA-------------------------KDE  79
```

FIG. 5 Cont.

```
F306    45 GSTSYENGTKVKMTDKPITKERAVQIAKAHIA-----------------------KDE  79
F303    34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
F301    65 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE 112
F309    34 EAVARENGYKGNMKDLPLDVAKAIYRKQYWIEPRFDQVNT-----------LSSAVAEE  81
F311    34 GTIKYPNGVRVKKGDICTESQAEGYLRNDLVA-----------------------FEN  68
F307    69 AINR--LVKVPLNQNQFDALASFTYNLG------EGNLSISTLLKKLNAKDYKGAAAEFP 120
F376    93 ALEYIKEGGVKLXKPCYGCVAVKSREGGGHVTFVVGKTP-TGKLICLGGNQSNKVCFAVY 151
F351    87 LLDTGVNCGTGFAKPLLQRALNLLNNQG------KAGYADLEVDGVYGSATLGALKTYLS 140
F347     7 ---------AELSQTEYDLYLDFTYQYG------VPTFAKSSMLKHLKAGQYKAACDSLL  51
F344    96 LLDTGVNCGTGFAKPLLQRALNLLNNQG------KAGWPDLTVDGIYGPATLNALKTYLA 149
F340    93 ALEYIKEGGVKLDKPCYGCVAVKSREGGGHVTFVVGKTP-TGKLICLGGNQSNKVCFAVY 151
F338   160 ALDYVNYG-TKLAKPAYGCVAIKTRKGGGHVCFVVGRDKKSGKLVCLGGNQSNKVCYALY 218
F336   160 ALDYVNYG-TKLAKPAYGCVAIKTRKGGGHVCFVVGRDKKSGKLVCLGGNQSNKVCYALY 218
F334   160 ALDYVNYG-TKLAKPAYGCVAIKTRKGGGHVCFVVGRDKKSGKLVCLGGNQSNKVCYALY 218
F332   160 ALDYVNYG-TKLAKPAYGCVAIKTRKGGGHVCFVVGRDKKSGKLVCLGGNQSNKVCYALY 218
F330   152 ALDYVNYG-TKLAKPAYGCVAIKTRKGGGRVCFVVGRDKKSGKLVCLGGNQSNKVCYALY 210
F328    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSNTLGALKTYLA 135
F324    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSNTLGALKTYLA 135
F321    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSNTLGALKTYLA 135
F320    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSSTLGALKTYLA 135
F315    80 VAFRKSLQGVRLTQTEYDVYLDFVYNYG------QANWNGSSMLRNLKAGQYKQACASLL 133
F306    80 VAFRKSLQGVRLTQTEYDVYLDFVYNYG------QANWNGSSMLRNLKAGQYKQACASLL 133
F303    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSSTLGALKTYLA 135
F301   113 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYADLKVDGVYGSNTLGALKTYLA 166
F309    82 LLDTGVNCGINFAKPLLQRALNLLNNQG------KAGYTDLKVDGVYGSSTLGALKTYLA 135
F311    69 AINR--LVKVPLNQNQFDALASFTYNLG------EGNLSKSTLLKKLNAKDYKGAAAEFP 120
F307   121 KWNKAGGR-----------VLAGLVKRRKAEMELFLK----------------------146
F376   152 DVSAFEAFMWYGKTSKPAAHRYDLPVLKIVSVTSVSEA---------------------189
F351   141 KRGKEGEKVLVRVLNIMQGQRYIEICERNPKQEQFFYGWIANRIG---------------185
F347    52 KYKYVAKRDCS-----VRKNGCYGVWTRQVERHAKCIGAQ---------------------86
F344   150 KRGKDGEKVLVRVLNIMQGQRYIEICERNPSQEQFFYGWIANRVVI--------------195
F340   152 DVSAFEAFMWYGKTSKPAAHRYDLPVLKIVSVTSVSEA---------------------189
F338   219 NDSDFQEFRWYGRTTQPASKRYTLPQLKGVTATRVLEA----------------------256
F336   219 NDSDFQEFRWYGRTTQPASKRYTLPQLKGVTATRVLEA----------------------256
F334   219 NDSDFQEFRWYGRTTQPAGKRYTLPQLKGVTATRVLEA----------------------256
F332   219 NDSDFQEFRWYGRTTQPASKRYTLPQLKGVTATRVLEA----------------------256
F330   211 NDSDFQEFRWYGRTTQPASKRYTLPQLKGVTATRVLEA----------------------248
```

FIG. 5 Cont.

```
F328   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNKSQEQFFYGWIANRIG----------------180
F324   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNKSQEQFFYGWIANRIG----------------180
F321   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNKSQEQFFYGWIANRIG----------------180
F320   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNPKQEQFFYGWIANRIG----------------180
F315   134 KYKYVAKRDCS-----IRSNGCYGVWTRQQDCYSKCMAVQ----------------------168
F306   134 KYKYVAKRDCS-----IRSNGCYGVWTRQQDRYSKCMAVQ----------------------168
F303   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNPKQEQFFYGWIANRIG----------------180
F301   167 KRGKEGEKVLVRVLNIMQGQRYIEICERNKSQEQFFYGWIANRIG----------------211
F309   136 KRGKEGEKVLVRVLNIMQGQRYIEICERNPKQEQFFYGWIANRIG----------------180
F311   121 KWNKAGGR-----------VLAGLVKRRKAEMELFLK-----------------------146
```

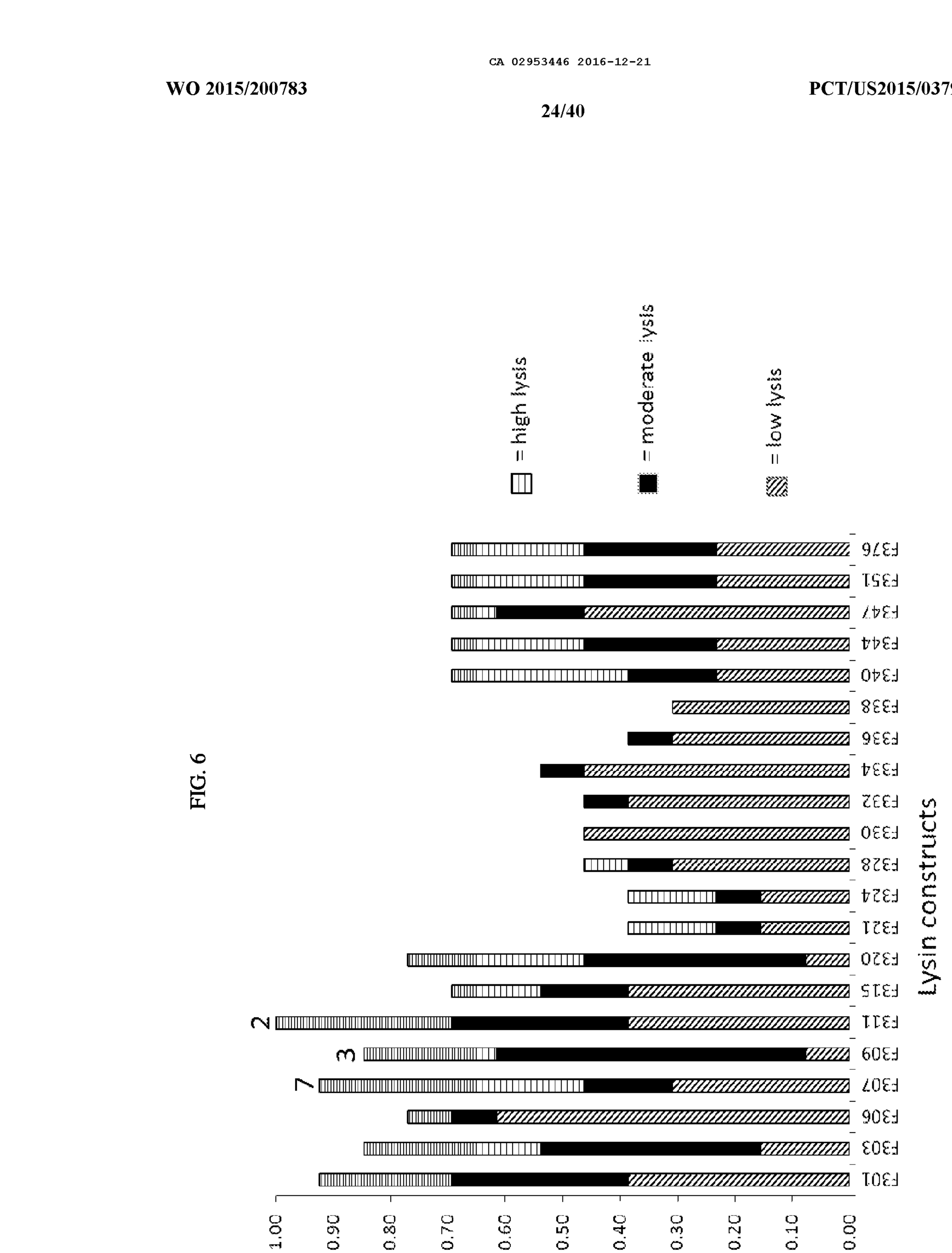

high lysis
moderate lysis
low lysis
F376
F351
F347
F344
F340
F338
F336
F334
F332
F330
F328
F324
F321
F320
F315
F311
F309
F307
F306
F303
F301
Lysin constructs
2
3
7
1.00
0.90
0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
% Acinetobacter strains lysed

FIG. 6

FIG. 7A

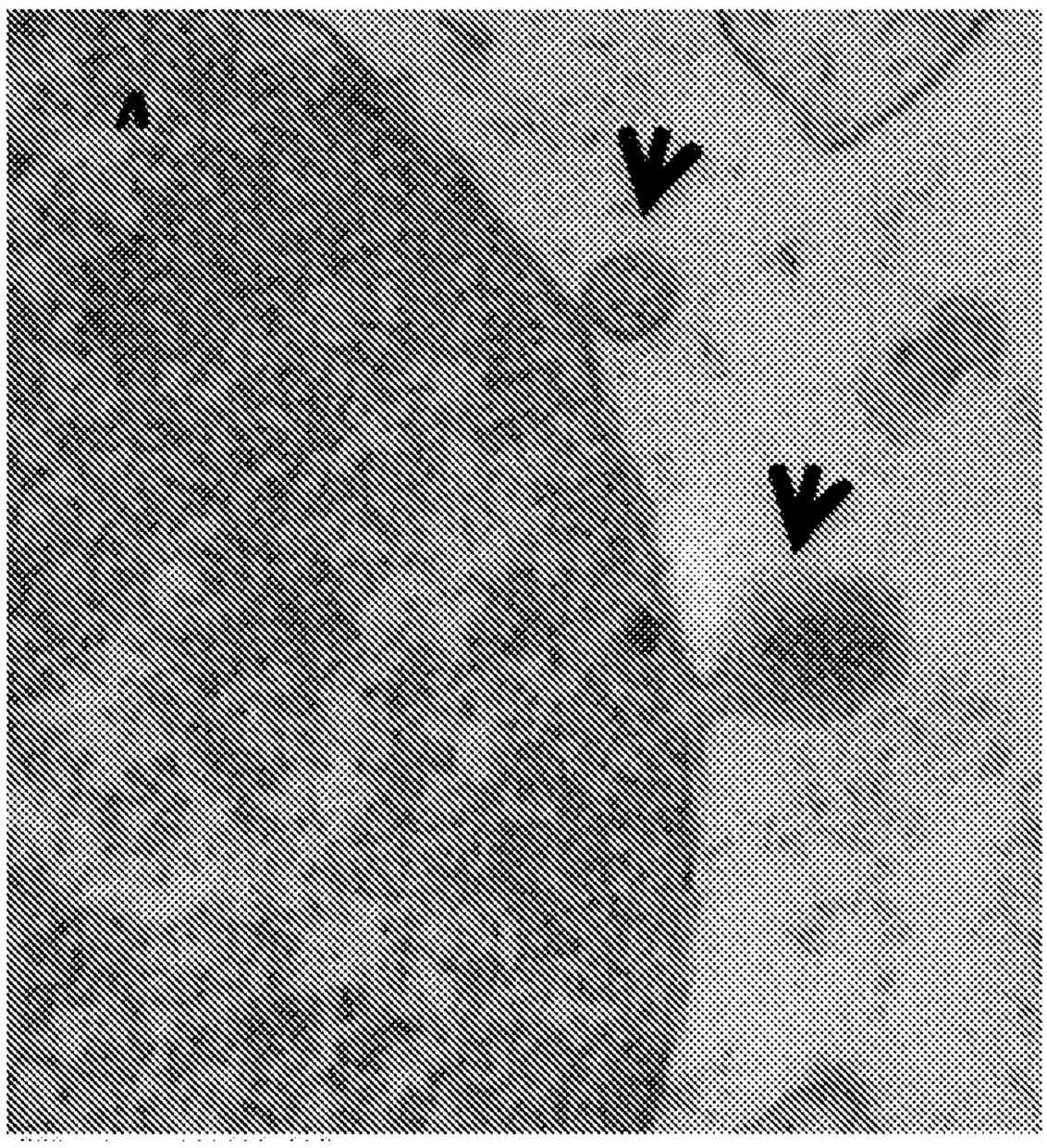


FIG. 7B

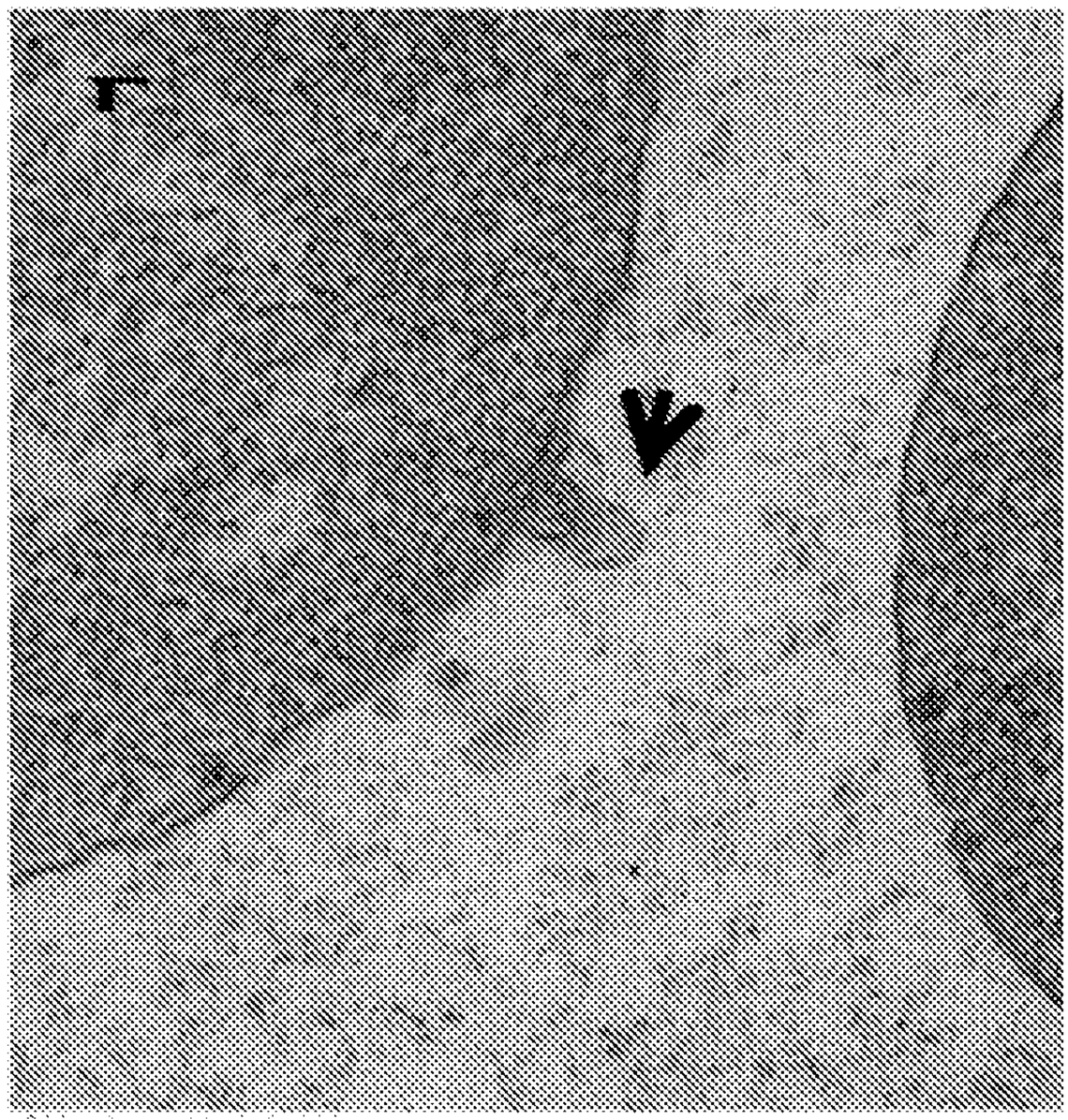
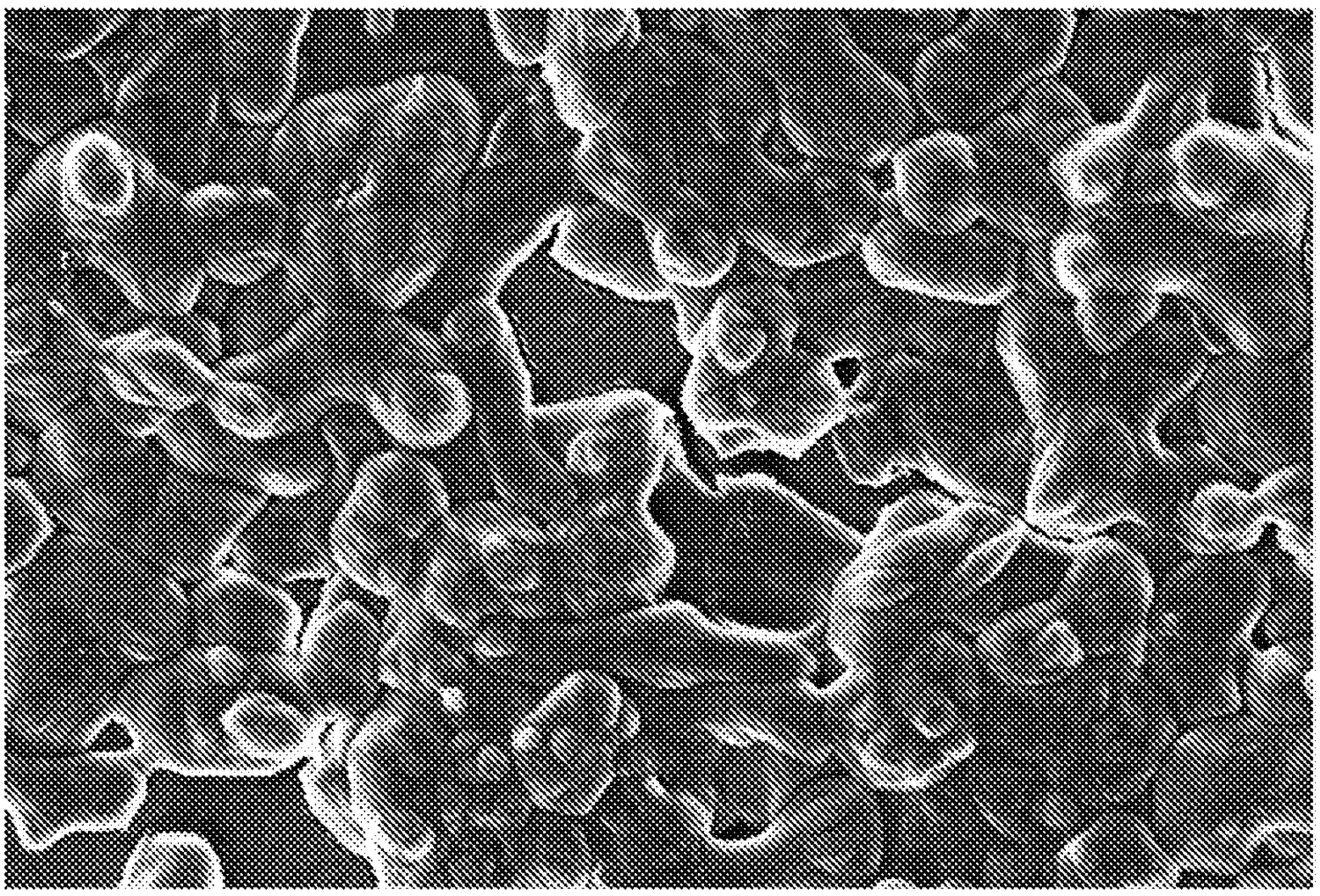


FIG. 8

0 Time



30 Min

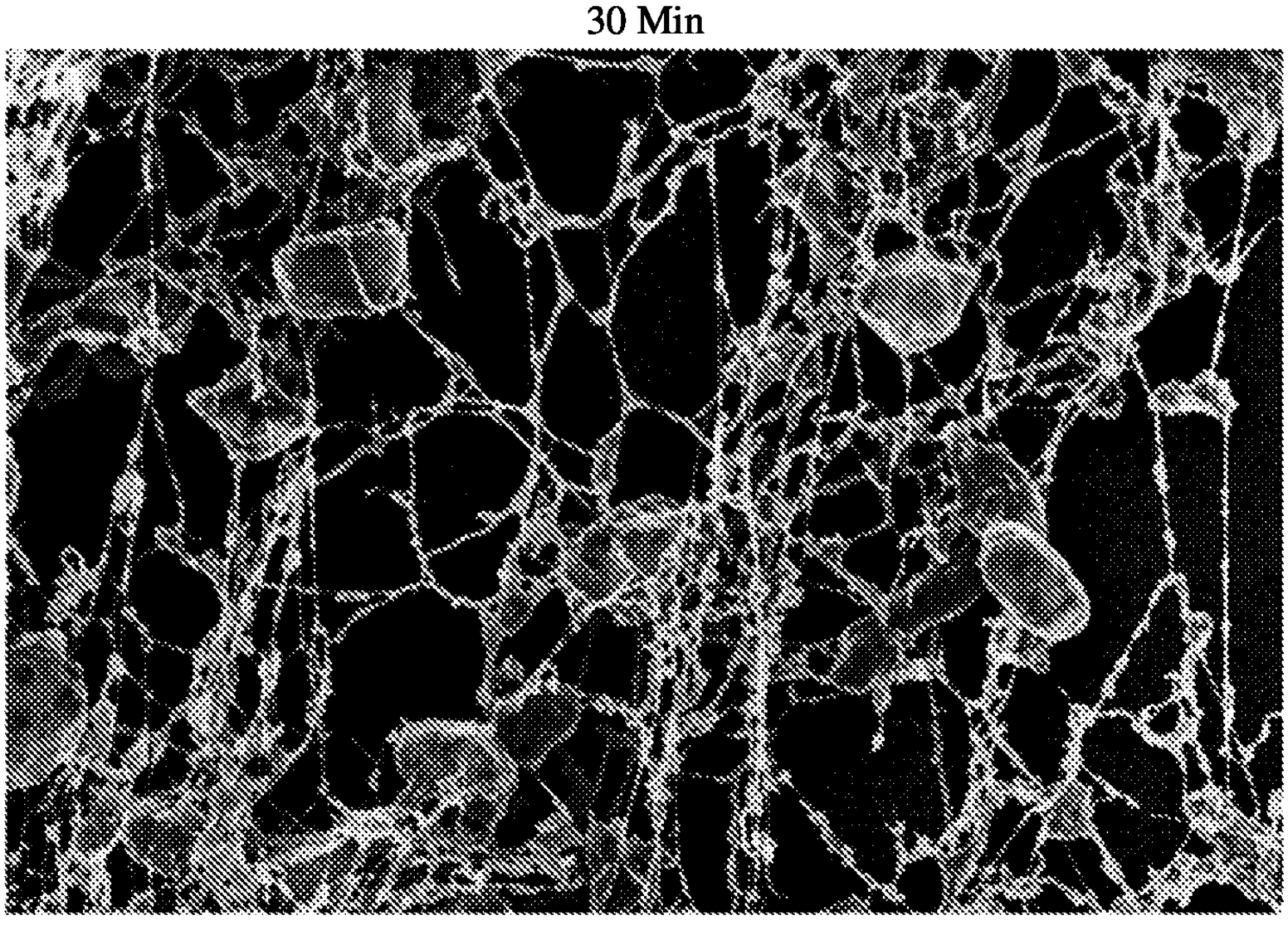


FIG. 9

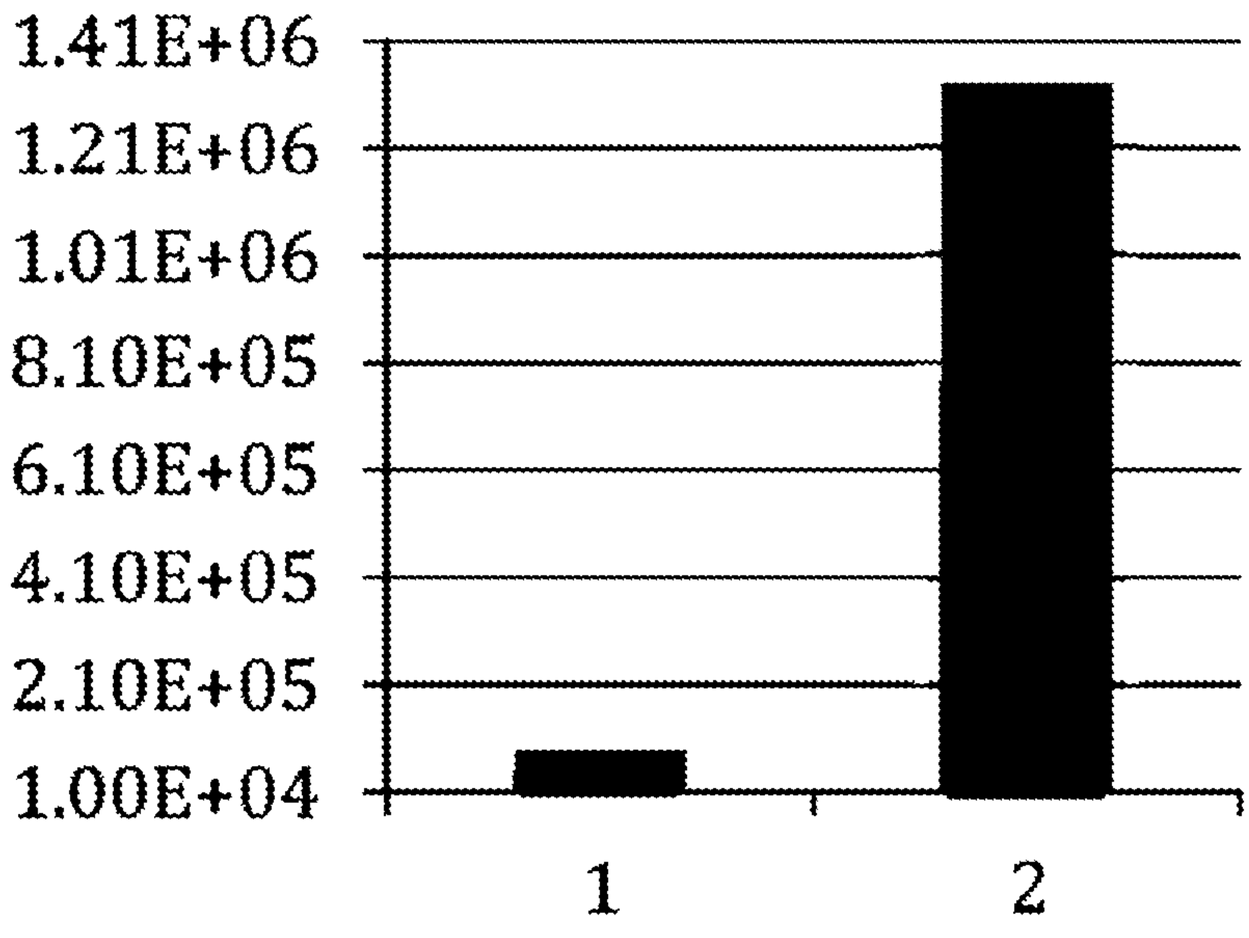

1.41E+06
1.21E+06
1.01E+06
8.10E+05
6.10E+05
4.10E+05
2.10E+05
1.00E+04
1
2

FIG. 10

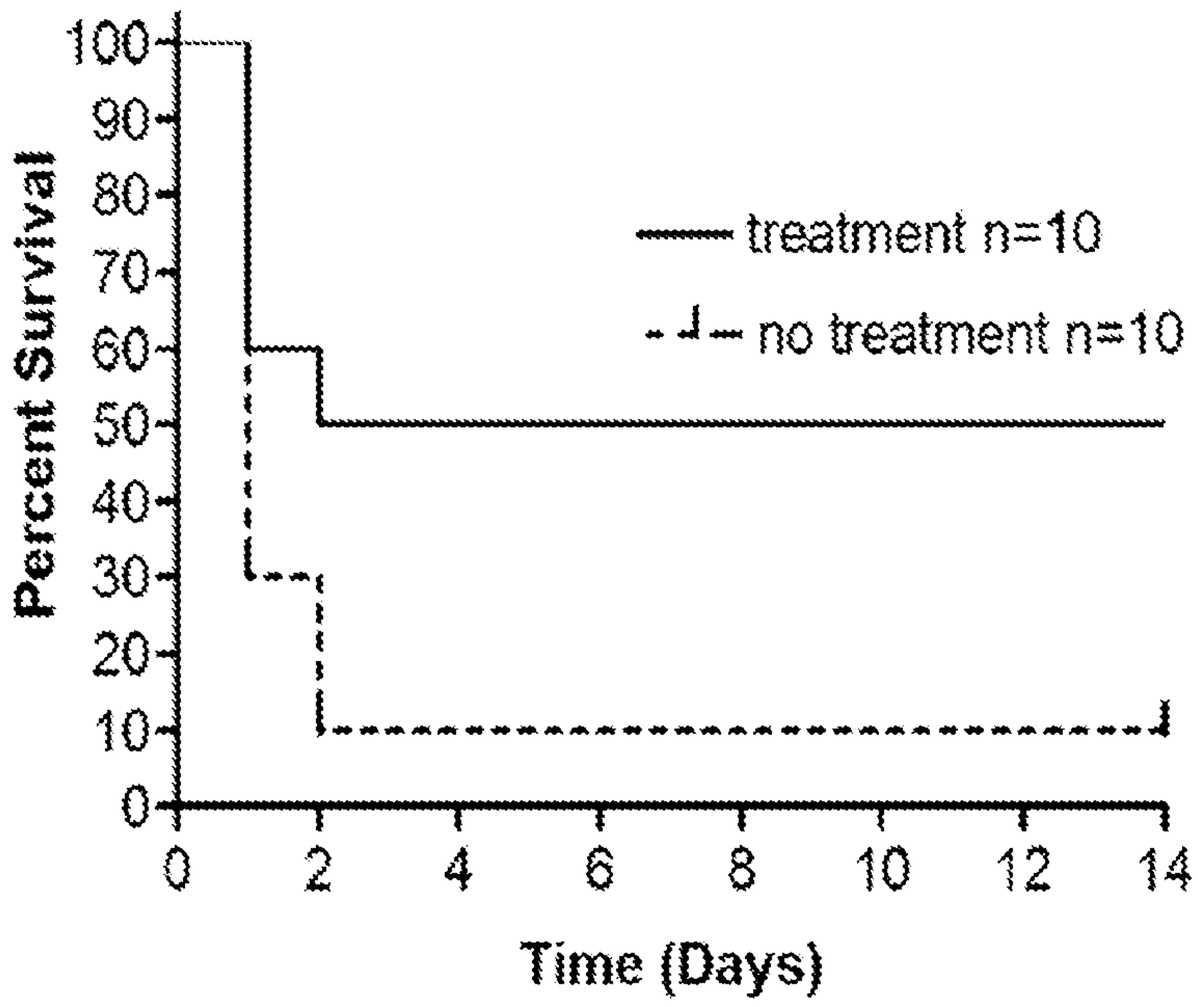

Percent Survival
100
90
80
70
60
50
40
30
20
10
0
0
2
4
6
8
10
12
14
Time (Days)
treatment n=10
no treatment n=10

FIG. 11

F307

VKTSNPGVDL IKGFEGLRLK AYDDGVGVWT IGFGTIKYPN GVRVKKGDTC TESQAEEYLR NDLVVFESAI NRLVKVPLNQ NQFDALASFT YNLGEGNLSI STLLKKL**NAK DYKGAAAEFP KWNKAGGRVL AGLVKRRK**AE MELFLK (SEQ ID NO:1)

P307- NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRK **-- 3.4 kDa** (SEQ ID NO:43)

P307Ex - NAKDYKGAAAEFPKWNKAGGRVLAGLVKRRKSQSRESQC **-- 4.3 kDa** (SEQ ID NO:45)

FIG. 12

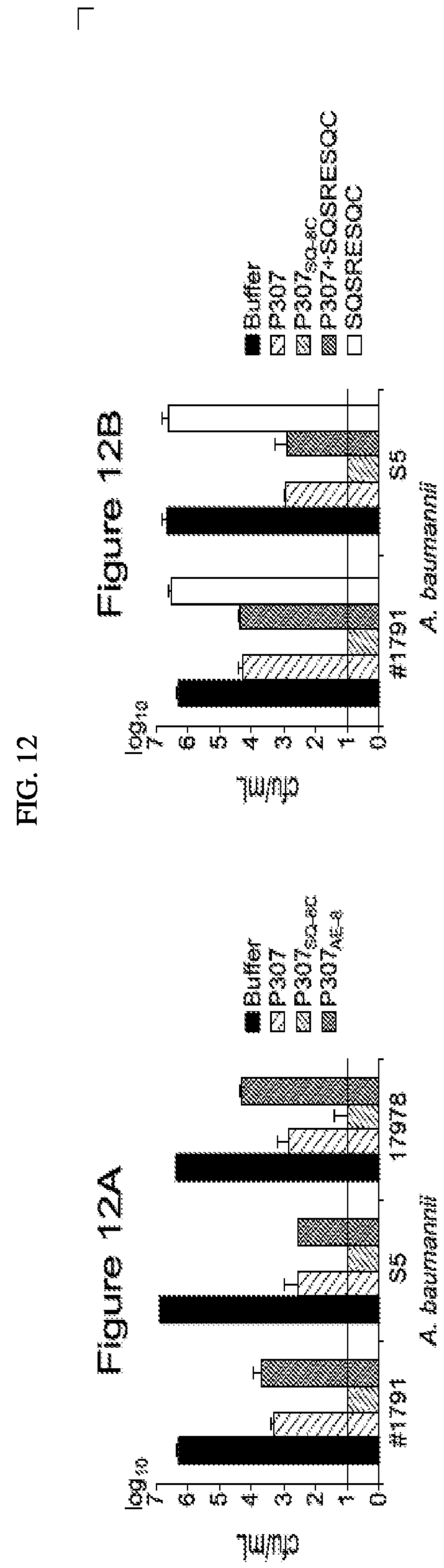


Figure 12A
Buffer
P307
P307SQ-8C
P307CS-8
log10 cfu/mL
7
6
5
4
3
2
1
0
#1791
S5
17978
A. baumannii

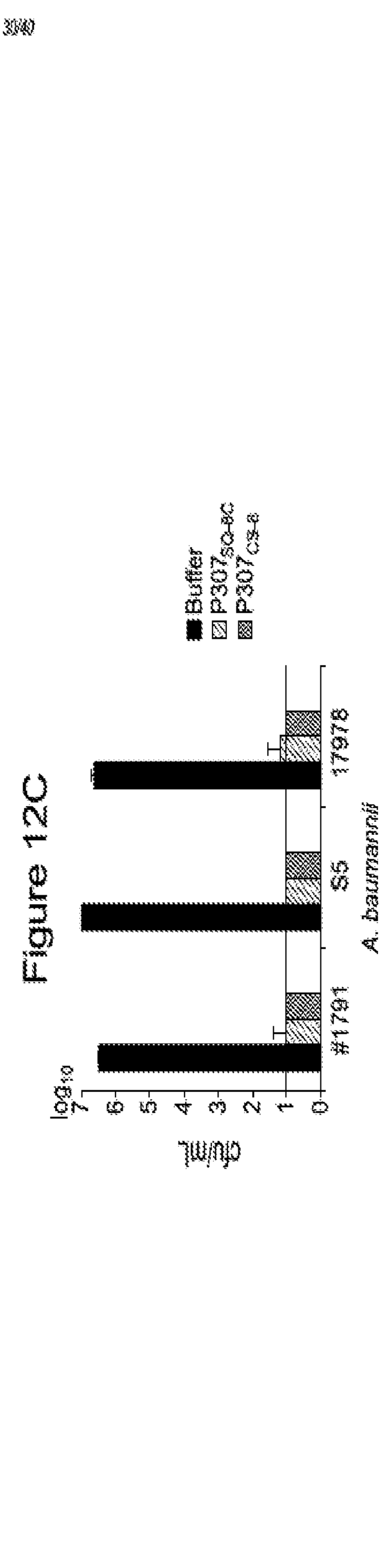


Figure 12B
Buffer
P307
P307SQ-8C
P307CS-8
log10 cfu/mL
7
6
5
4
3
2
1
0
#1791
S5
17978
A. baumannii

Figure 12C
Buffer
P307SQ-8C
P307CS-8
log10 cfu/mL
7
6
5
4
3
2
1
0
#1791
S5
17978
A. baumannii

FIG. 13

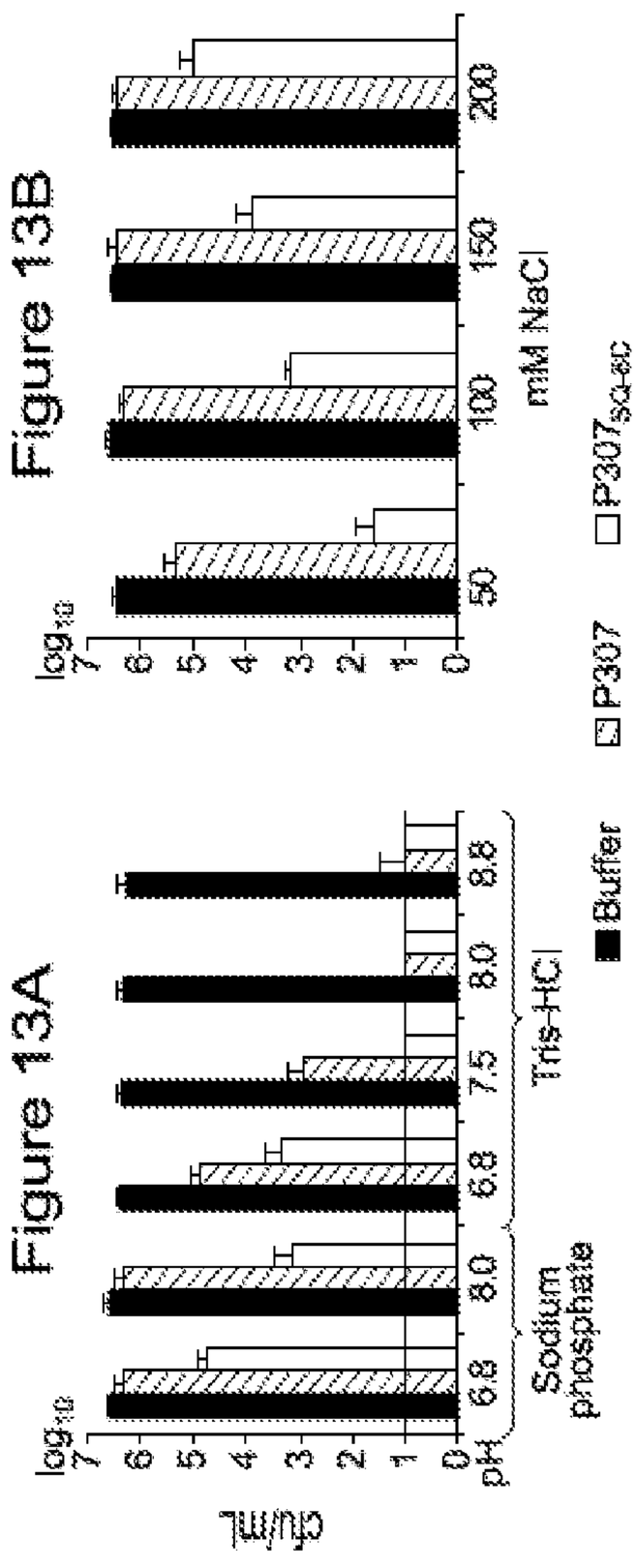


Figure 13A
log10
cfu/mL
pH
6.8
8.0
6.8
7.5
8.0
8.8
Sodium phosphate
Tris-HCl
Buffer
Figure 13B
log10
mM NaCl
50
100
150
200
P307
P307SQ-sec
Figure 13C
log10
cfu/mL
μg/mL
0
1
2
4
8
16
32
63
125
Figure 13D
log10
Minutes
1
5
10
15
30
90
120

FIG 14

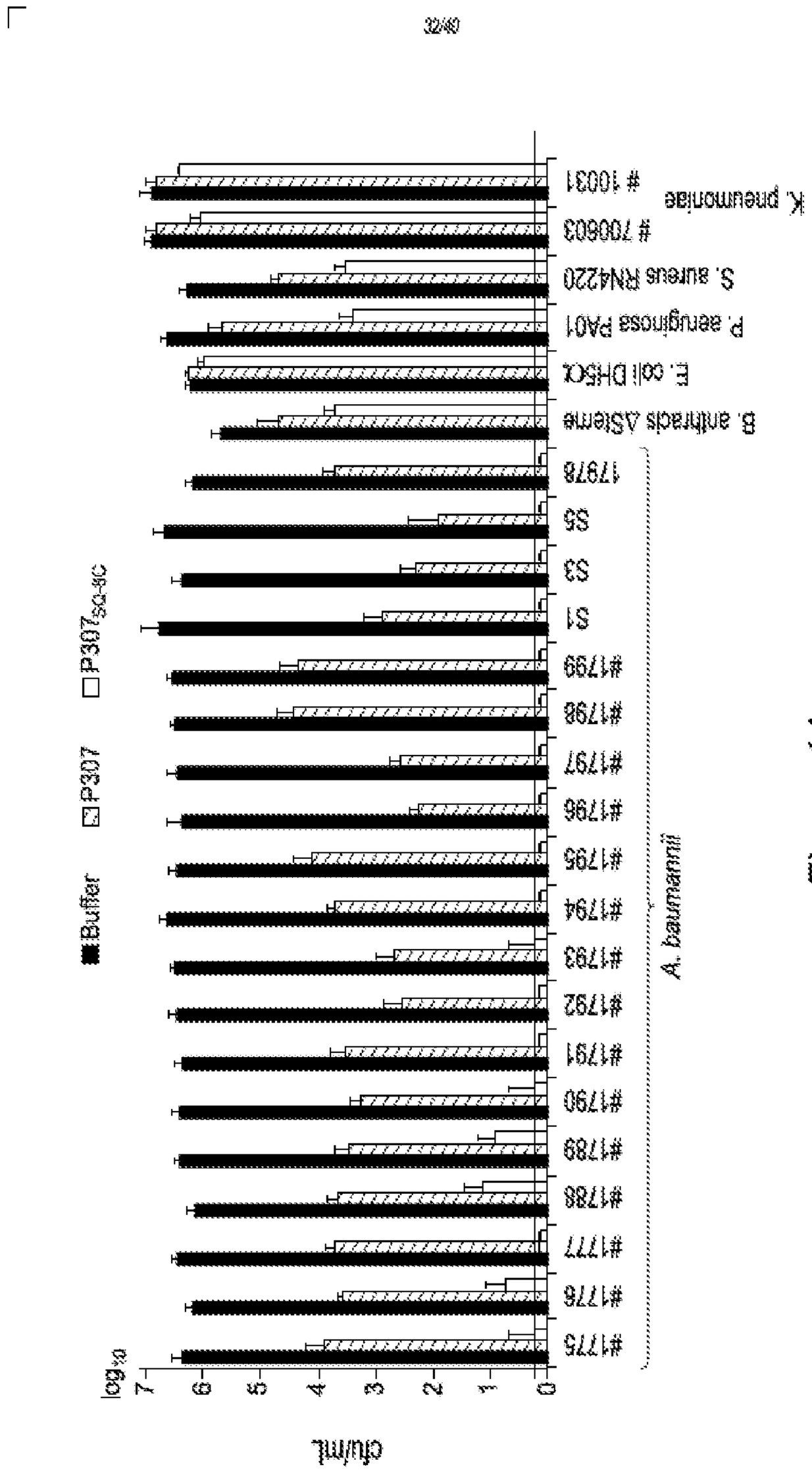


Figure 14

FIG 15

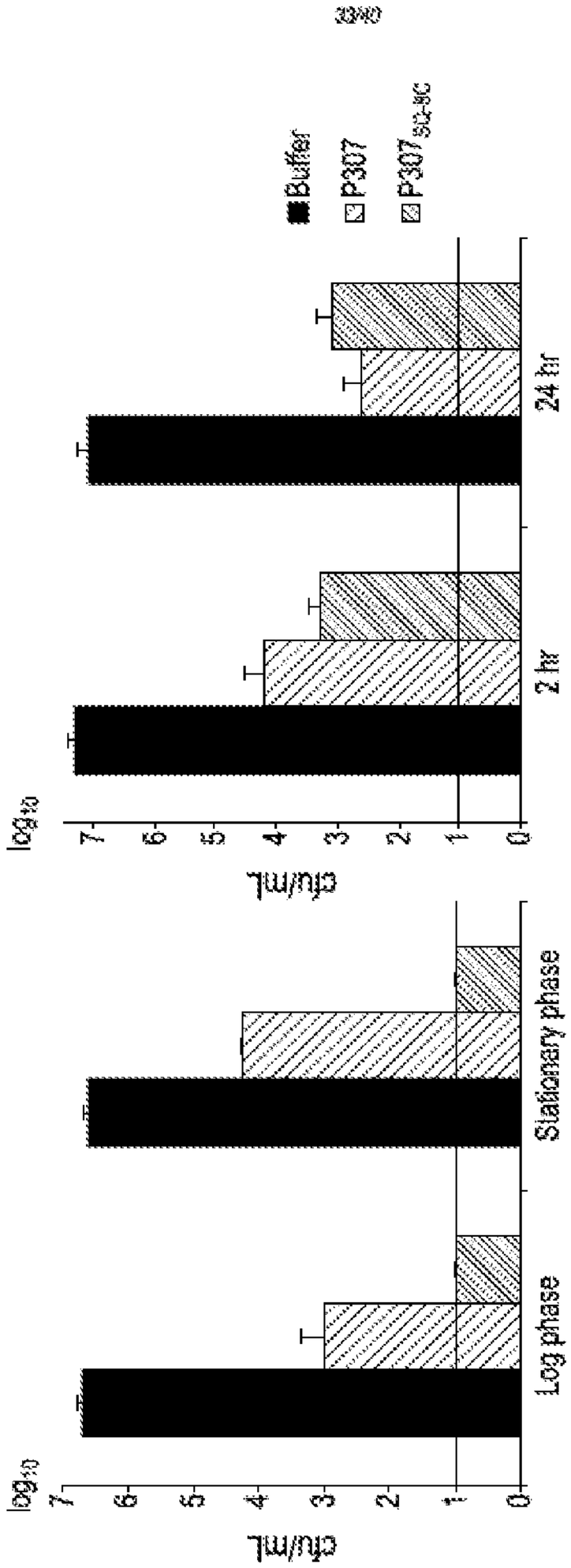


Figure 15B

Figure 15A

FIG. 16

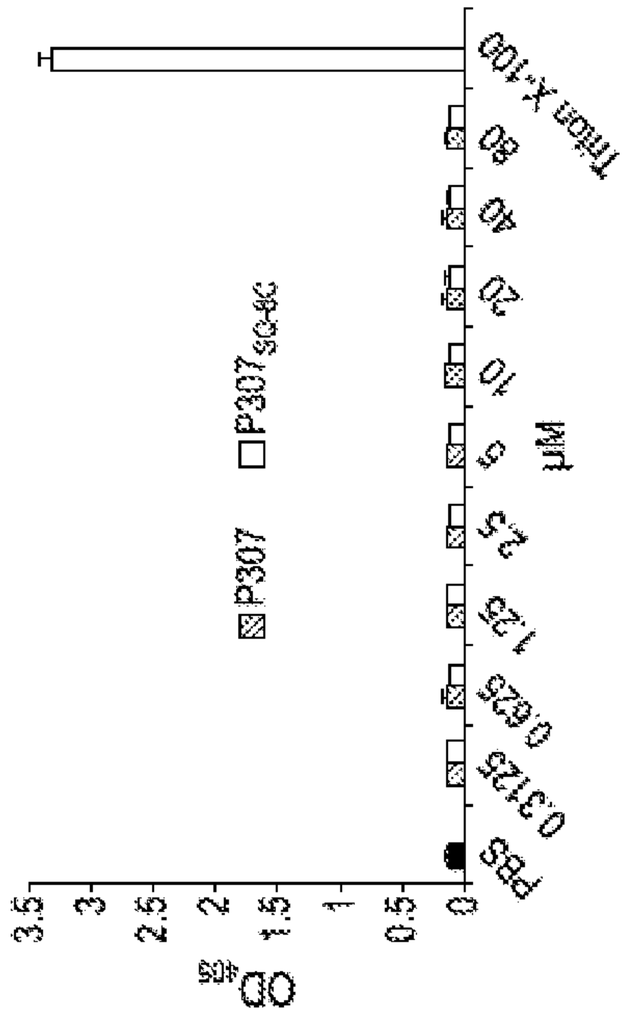


Figure 16B

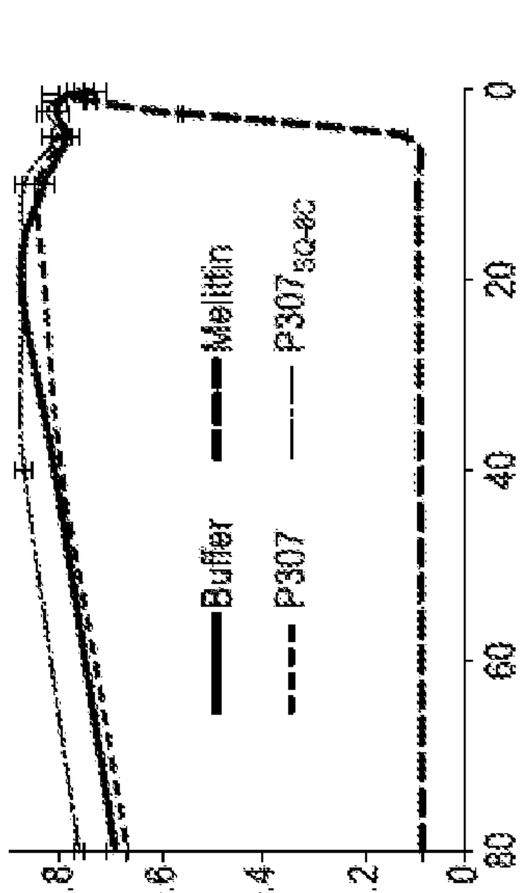


Figure 16A

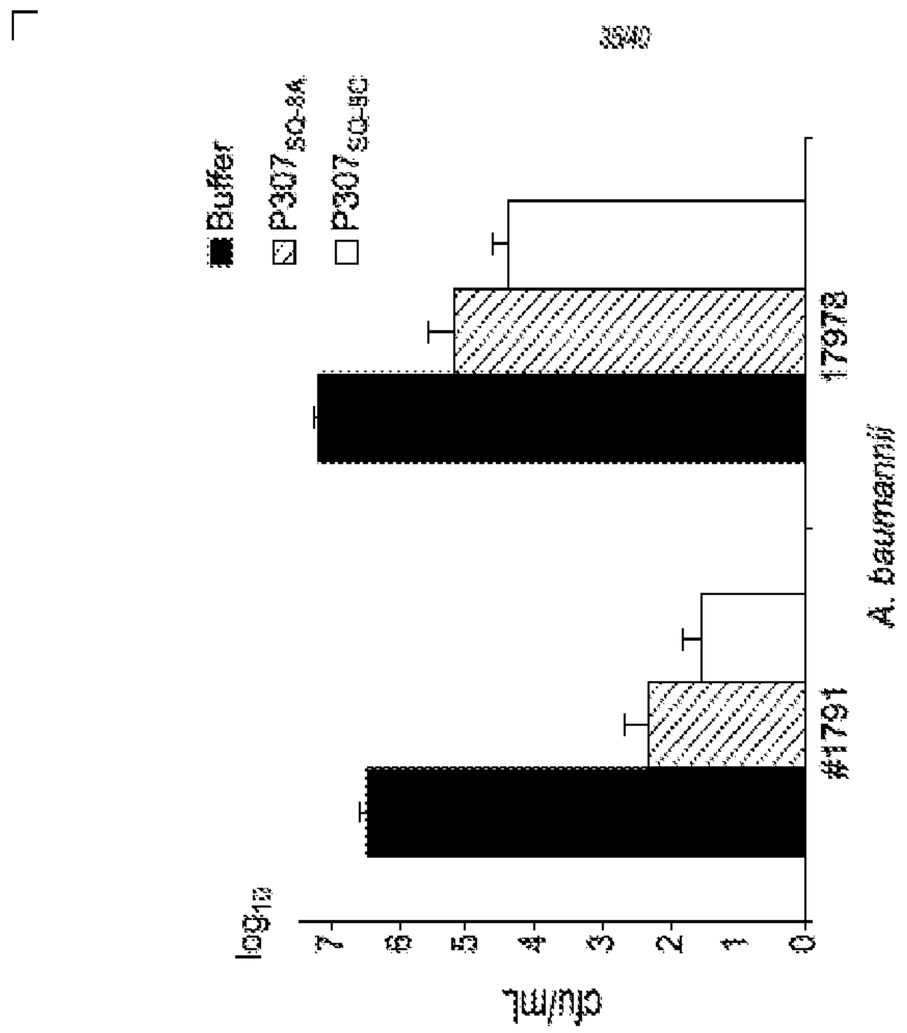


Figure 17A

Figure 17B

FIG. 17

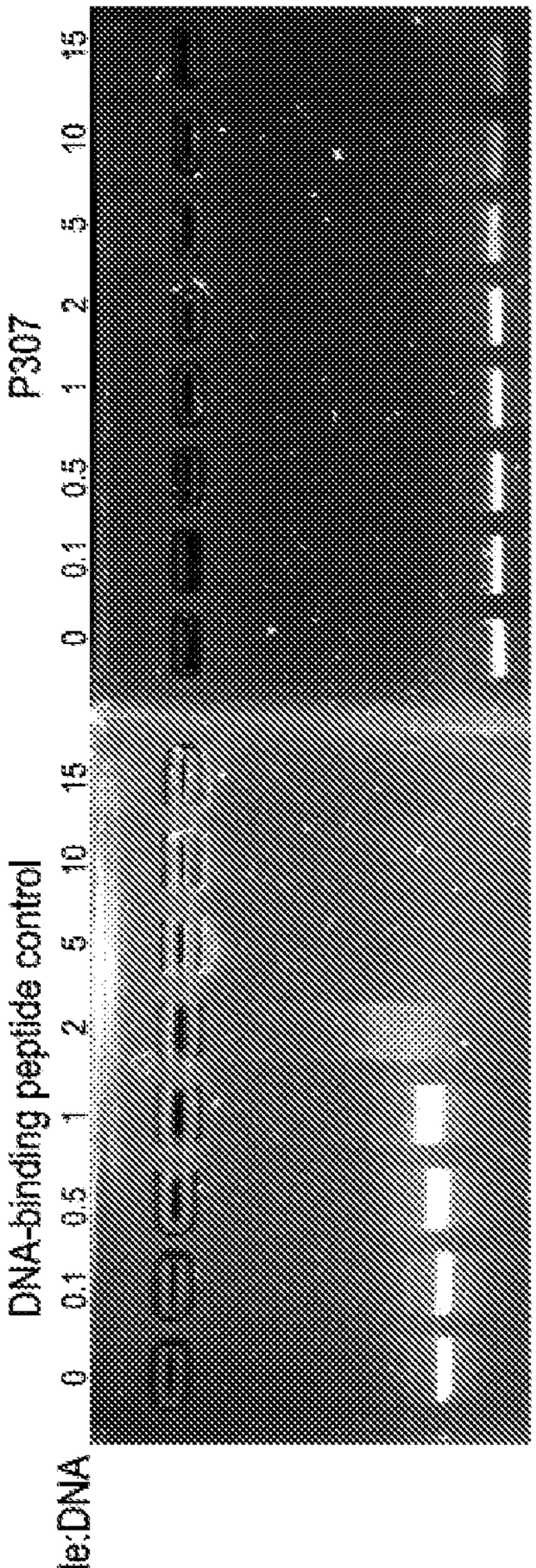


FIG. 18

Figure 18

FIG. 19

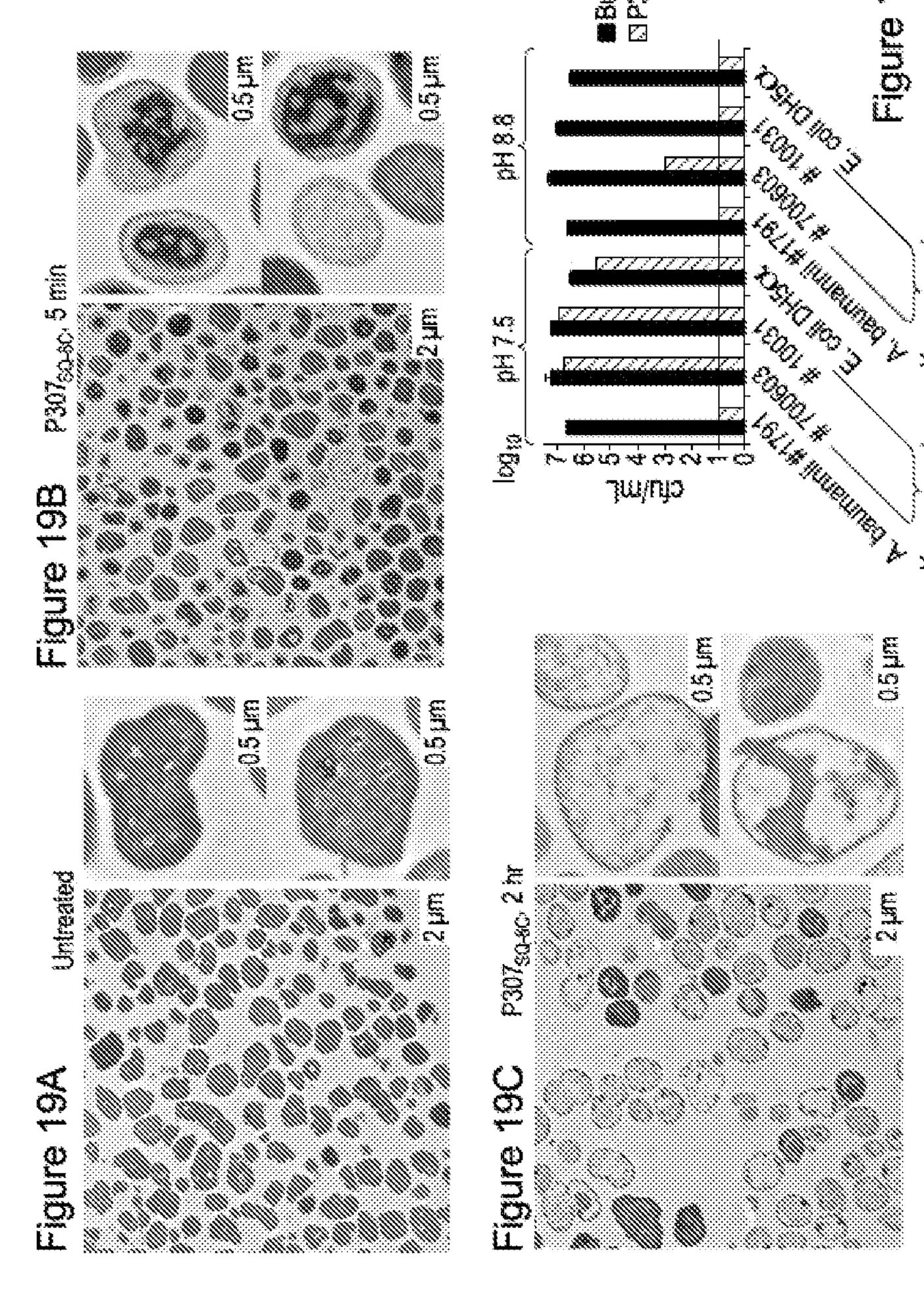


Figure 19B P307SQ-SC, 5 min
0.5 µm
0.5 µm
2 µm
Figure 19A Untreated
0.5 µm
0.5 µm
2 µm
Figure 19C P307SQ-SC, 2 hr
0.5 µm
0.5 µm
2 µm
Figure 19D
Buffer
P307
log10 cfu/mL
pH 7.5
pH 8.8
A. baumannii #1791
700603
E. coli 1031
E. coli DH5α
K. pneumoniae

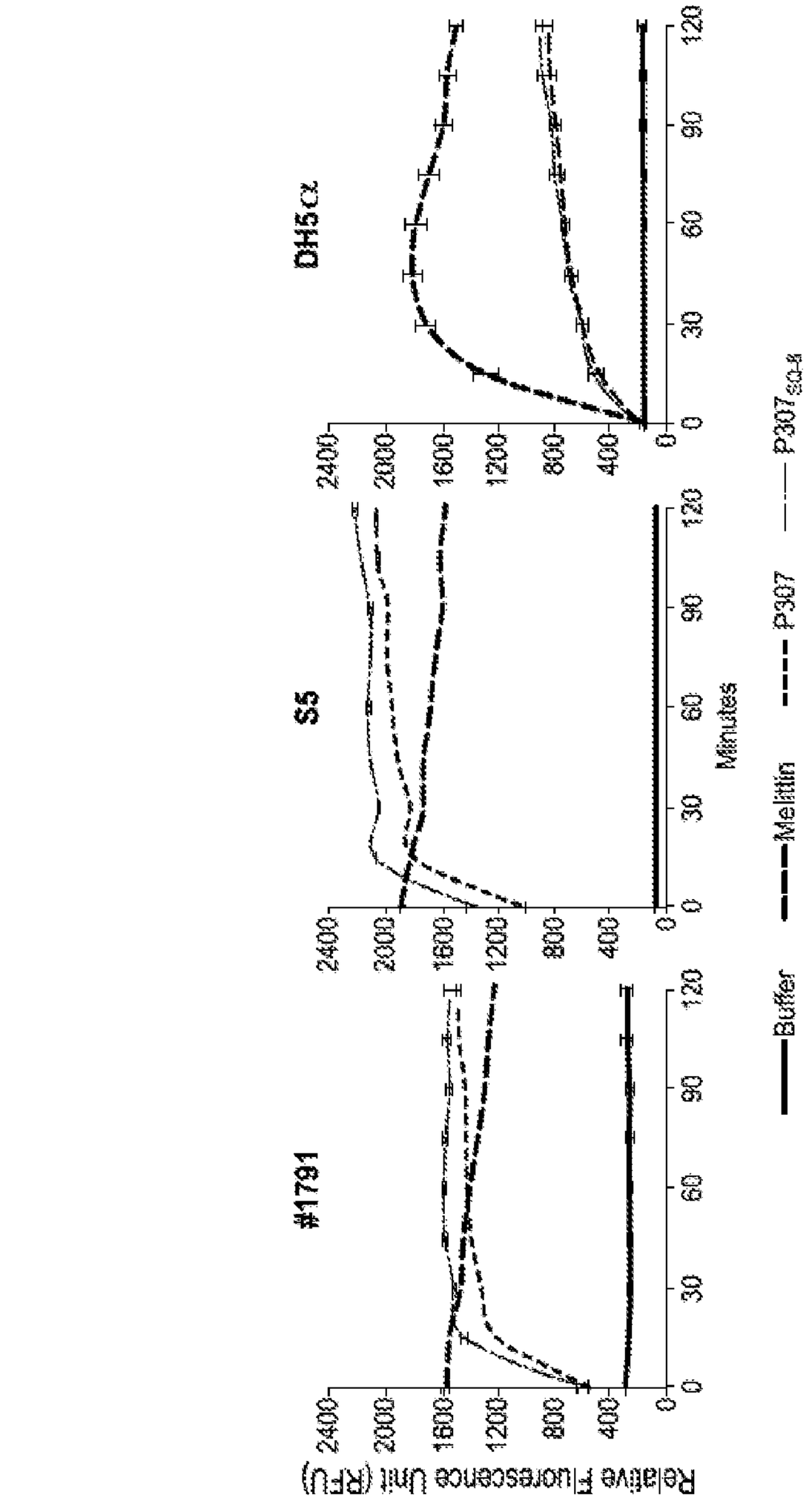

S5
DH5α
#1791
Relative Fluorescence Unit (RFU)
Minutes
Buffer
Melittin
P307
P307eco-s

FIG. 20

FIG. 21

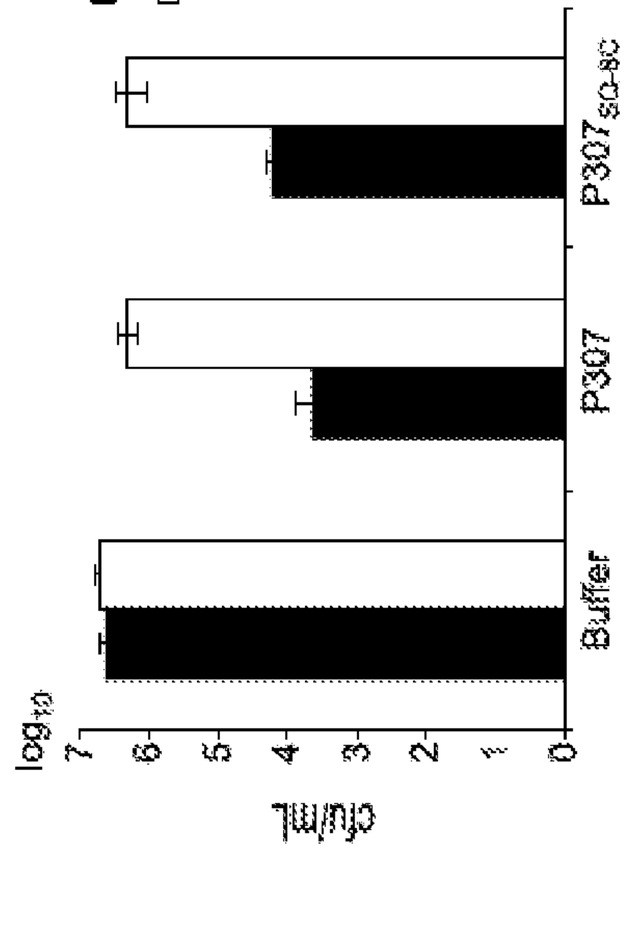


Figure 21A

Figure 21B

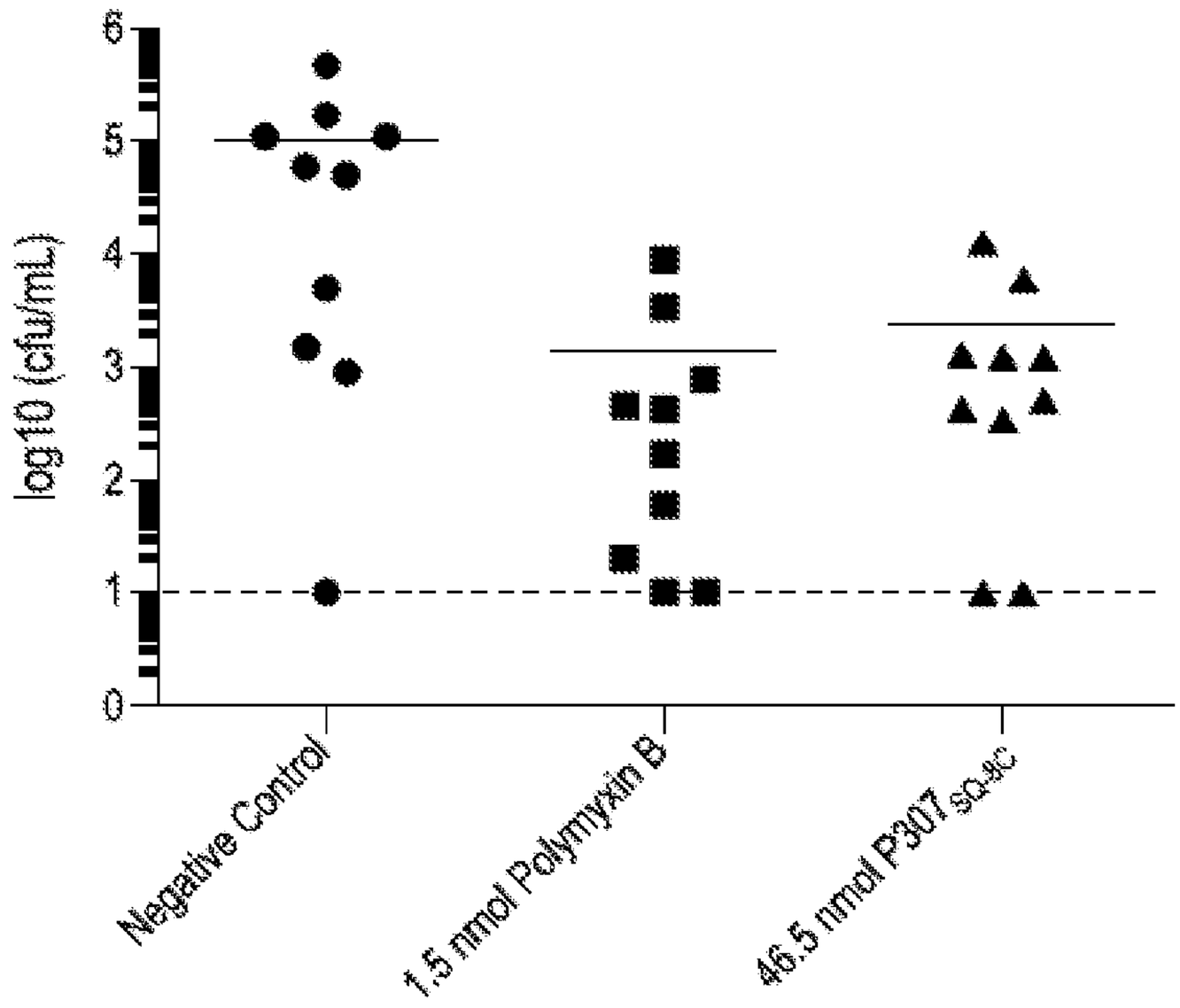

log10 (cfu/mL)
0
1
2
3
4
5
6
Negative Control
1.5 nmol Polymyxin B
46.5 nmol P307 SQ-9C

FIG. 22