(19) Europäisches Patentamt
European Patent Office
Office européen des brevets

(11) **EP 4 289 949 A1**

(12) **EUROPEAN PATENT APPLICATION**
published in accordance with Art. 153(4) EPC

(43) Date of publication:
13.12.2023 Bulletin 2023/50

(21) Application number: **22749015.8**

(22) Date of filing: **27.01.2022**

(51) International Patent Classification (IPC):
C12N 9/88 *(2006.01)* ***A61K 38/51*** *(2006.01)*
A61P 31/04 *(2006.01)* ***C12N 15/60*** *(2006.01)*
A61K 35/76 *(2015.01)* ***A61P 17/10*** *(2006.01)*

(52) Cooperative Patent Classification (CPC):
A61K 8/02; A61K 8/66; A61K 9/00; A61K 35/76; A61K 38/51; A61L 27/22; A61L 27/54; A61L 29/04; A61L 29/16; A61P 17/10; A61P 31/04; A61Q 17/00; A61Q 19/00; C12N 9/88; C12N 15/70;

(Cont.)

(86) International application number:
PCT/CN2022/074221

(87) International publication number:
WO 2022/166736 (11.08.2022 Gazette 2022/32)

(84) Designated Contracting States:
AL AT BE BG CH CY CZ DE DK EE ES FI FR GB GR HR HU IE IS IT LI LT LU LV MC MK MT NL NO PL PT RO RS SE SI SK SM TR
Designated Extension States:
BA ME
Designated Validation States:
KH MA MD TN

(30) Priority: **08.02.2021 CN 202110187030**

(71) Applicant: **Wuhan Lysigen Bio-Tech Company Limited**
Wuhan, Hubei 430075 (CN)

(72) Inventors:
- **ASSAF, Raz**
  Wuhan, Hubei 430075 (CN)
- **MYA, Thandar**
  Wuhan, Hubei 430075 (CN)

(74) Representative: **Michalski Hüttermann & Partner Patentanwälte mbB**
Kaistraße 16A
40221 Düsseldorf (DE)

Remarks:
The complete document including Reference Table(s) and the Sequence Listing(s) can be downloaded from the EPO website

(54) BACTERIOPHAGE LYSINE, CHIMERA THEREOF AND APPLICATION THEREOF

(57) The invention relates to the field of medical biology, and specifically provides a phage lysin or a chimera thereof used in the preparation of medicines, cosmetics or drugs for preventing, treating or improving acne or infection caused by Propionibacterium acnes or diseases related to Propionibacterium acnes. For application in medical equipment, the phage lytic enzymes include phage lytic enzymes derived from Nocardioidaceae and Propionibacteriaceae bacteria. The phage lytic enzyme or its chimera provided by the present invention can specifically and effectively kill Propionibacterium acnes while keeping other commensal bacteria intact, and can provide a safe and effective acne solution suitable for long-term use without a high risk of dysbiosis and acquired resistance.

EP 4 289 949 A1

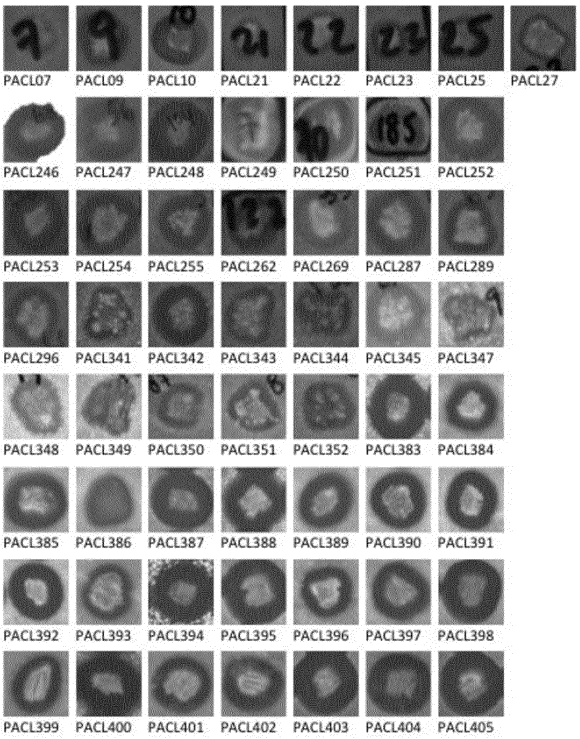

Figure 1

(52) Cooperative Patent Classification (CPC): (Cont.)
C12R 2001/19

Description

[0001] This application claims the following priority: CN202110187030.8, filing date 2021.02.08.

Technical field

[0002] The invention relates to the field of medical biology, and specifically provides a bacteriophage lysin with anti-Cutibacterium acnes activity, a chimera and application thereof.

Background technique

[0003] Acne is an inflammatory disease of the skin, especially on the face, neck, shoulders, chest, back and upper arms. The pilosebaceous unit is inflamed due to the accumulation of dead skin cells due to clogging with excess oil secretion and proliferation of the pathogenic Propionibacterium acnes (C. acnes, the same below). According to the National Institutes of Health, 80 percent of people ages 11 to 30 have acne to some extent. Acne usually begins in adolescence and may last into the forties and fifties. Different factors can trigger acne breakouts such as stress, changes in hormone levels, allergens, etc. The 2010 Global Burden of Disease study estimated that acne affects 9.4% of the global population and ranks it as the eighth most prevalent disease in the world. The American Academy of Dermatology estimated that in 2013 the cost of treating acne patients and lost productivity was more than $1.2 billion. While rarely life-threaten in itself, it can cause long-lasting scarring on the skin and considerable emotional distress for patients.

[0004] Current options for treating mild acne are the use of over-the-counter topical medications containing chemicals such as benzoyl peroxide, resorcinol, salicylic acid, and sulfur, as well as prescription topical medications such as antibiotics, benzoyl peroxide, nonyl Diacids, dapsone, corticosteroids, and vitamin A derivatives called retinoids. Moderate to severe disease can be treated with prescription topical or oral medications. The goal of the intervention is to remove dead skin cells and excess oil to unclog the pilosebaceous unit and reduce the bacterial load. Since acne is a chronic disease, it requires prolonged treatment and sometimes prevention of recurrence. However, none of the existing treatment options are suitable for long-term treatment. When these medications are used for an extended period of time, even the mildest chemicals can cause allergic reactions and irritation of the skin, leading to dryness, flaking, cracking, and more. Antibiotic treatment options are increasingly limited as resistant P. acnes become more prevalent in the world. In addition, chronic antibiotic use has been shown to disturb the homeostasis of the microbiota by exerting selective pressure on resistant bacterial species. This in turn leads to myriad complications such as dysbiosis and immune abnormalities. It has been shown in the literature that the skin Staphylococcus flora of acne patients changes from mostly antibiotic-sensitive to mostly resistant during antibiotic treatment. Not only the patient himself, but also the close contacts of the patient are also affected by multidrug resistant bacteria. Notably, acne patients who received long-term antibiotic treatment were two times more likely to develop a respiratory infection. Therefore, there is an urgent need for a safer, more effective, and more suitable for long-term use of treatment options. One such approach is pathogen-specific antimicrobials, which specifically kill P. acnes without affecting other commensal bacteria, regardless of antibiotic resistance.

[0005] In addition, P. acnes has been associated with a range of invasive infections, including postoperative shoulder infections and intervertebral disc infections. Among such infections, P. acnes exhibits a slowly progressive biofilm-like infection. Bacteria found in biofilms are inherently resistant to antibiotics. A P. acnes anti-microbial with strong anti-biofilm properties is ideal for the treatment of such infections.

[0006] An alternative for pathogen-specific antimicrobials is the bacteriophage endolysin. Bacteriophages use these enzymes to destabilize the cell wall for lysis, releasing phage progeny from the interior of the cell. They are classified as peptidoglycan hydrolases that cleave various bonds in bacterial peptidoglycan. With few exceptions, each lytic enzyme targets bacteria belonging to a single genus or species. Recombinant lysin against Gram-positive bacteria have been shown to be effective bactericides capable of causing hypotonic lysis (eg PlyC against several Streptococci, PlyG against Bacillus anthracis, etc.). Given that lysin have been under selective evolutionary pressure over billions of years to continue to successfully infect cells, the peptidoglycan bonds of interest are highly conserved and unlikely to be easily altered by bacteria. This property has little chance of developing resistance to the lyase in the target bacteria, making it suitable for long-term therapeutic use. Bacterial resistance to the respective phage lytic enzymes has not been detected so far. Generally, lysin against Gram-positive bacteria are composed of one or two N-terminal catalytic domains (CD) and a C-terminal cell wall-binding domain (BD), with linkers of different lengths between the domains). Lysin are modular proteins, and chimeric lysins can be generated by pairing catalytic and binding domains from different lysins. Linkers are also variable in length and sequence. However, the major bottleneck in the development of lysin as antibacterial agents is the inability to soluble express highly active lysin. In fact, no lysin with high level soluble expression and effective killing of P. acnes has been reported so far.

[0007] Patent CN102482655A discloses an antimicrobial agent, which is composed of an endolysin with the activity

of degrading the cell wall of Gram-positive bacteria and an amphiphilic peptide segment fused to the endolysin at the N-terminal or C-terminal or both ends, can be used for the treatment or prevention of Gram-positive bacterial infections, as a diagnostic means or as a cosmetic substance. However, although the patent describes that PA6 has anti-P. acnes DSMZ 1897 strain and DSMZ 16379 strain activity, it does not provide the initial cfu/mL; in addition, PA6 cannot solve the problem of increasing the soluble expression level.

[0008] In summary, there is clearly a great need for new therapeutic modalities against P. acnes, and so far no phage lysin with high P. acnes killing activity suitable for industrial scale production has been reported. The ability of phage lytic enzymes to specifically and efficiently kill P. acnes while leaving other commensal bacteria intact may provide a safe and effective acne solution for long-term use without high dysbiosis and gain risk of drug resistance.

Contents of the invention

[0009] Aiming at the above technical status, the present invention provides phage lysin with anti-Propionibacterium acnes activity, its chimera and application. The details of the invention are as follows:

The invention provides the use of a phage lysin or its chimera in the preparation of medicines, cosmetics, or medical devices for preventing, treating or improving acne or infection caused by Propionibacterium acnes or diseases related to Propionibacterium acnes, the bacteriophage lysin includes bacteriophage lysin derived from Nocardioidaceae and Propionibacteriaceae bacteria.

[0010] In the use of the present invention, as one of the embodiments, the Nocardioidaceae bacteria include Micropruina, Propionicimonas, and Propionicimonas ,Propionicicella, Friedmanniella; the bacteria of the family Propionibacteriaceae include the Propioniferax, Mariniluteicoccus, Granulicoccus, Naumannella, Propioniciclava, Auraticoccus, Microlunatus, Aestuariimicrobium, Luteococcus, Tessaracoccus, Brooklawnia, Propionimicrobium, Propionibacterium, Cutibacterium, Acidipropionibacterium, or Pseudopropionibacterium; preferably Propionibacterium, Cutibacterium, Acidipropionibacterium, or Pseudopropionibacterium bacteria.

[0011] In the application of the present invention, as one of the embodiments, the bacteriophage lysin includes lysins derived from Cutibacterium acnes, Propionibacterium humerusii, Cutibacterium avidum, Cutibacterium granulosum, Acidipropionibacterium thoenii, Acidipropionibacterium jensenii, Acidipropionibacterium acidipropionici, Aestuariimicrobium kwangyangense, Granulicoccus phenolivorans, Microlunatus phosphovorus, Pseudopropionibacterium propionicum, Tessaracoccus sp., Propionicicella superfundia, Propionibacterium freudenreichii, Propionibacterium freudenreichii subsp. Freudenreichii, Propionibacterium freudenreichii subsp. Shermanii, Propionibacterium acidifaciens, Propionibacterium lymphophilum, Propionibacteriaceae bacterium, Propionibacterium sp. oral taxon 192, Propioniferax innocua, Naumannella halotolerans, Propioniciclava tarda, Micropruina glycogenica, Propionicimonas paludicola, Auraticoccus monumenti, Luteococcus japonicus, Tessaracoccus oleiagri, Tessaracoccus bendigoensis, Tessaracoccus lapidicaptus, Acidipropionibacterium microaerophilum, Acidipropionibacterium olivae, Acidipropionibacterium damnosum.

[0012] In the application of the present invention, as one of the embodiments, the bacteriophage lysin comprises lysin derived from Acidipropionibacterium jensenii, Acidipropionibacterium thoenii, Acidipropionibacterium acidipropionici, Acidipropionibacterium microaerophilum, Acidipropionibacterium olivae, Acidipropionibacterium damnosum, Cutibacterium acnes, Cutibacterium avidum, Cutibacterium granulosum and Pseudopropionibacterium propionicum.

In the use of the present invention, as one of the embodiments, the bacteriophage lysin has the amino acid sequence shown in any one of SEQ ID NO:1~SEQ ID NO:28;

In the use of the present invention, as one of the embodiments, the bacteriophage lysin has the nucleotide sequence shown in any one of SEQ ID NO:29~ SEQ ID NO:56;

In the use of the present invention, as one of the embodiments, the bacteriophage lysin preferably has PACL10 having the amino acid sequence shown in SEQ ID NO:10;

In the use of the present invention, as one of the embodiments, the bacteriophage lysin is preferably PACL10 having the nucleotide sequence shown in SEQ ID NO:38.

[0013] In the use of the present invention, as one of the embodiments, the chimera comprises a catalytic domain derived from bacteriophage lysin, or a combination of a catalytic domain and a binding domain derived from bacteriophage lysin.

[0014] In the use of the present invention, as one of the embodiments, the catalytic domain has a full C-terminal linker, a half C-terminal linker, no C-terminal linker, or any part of the linker; the binding domain has full N-terminal linker, half N-terminal linker, no N-terminal linker, or any part of the linkers.

[0015] In the application of the present invention, as one of the embodiments, a synthetic linker may be used, which also includes a synthetic linker. Non-limiting examples of possible linkers derived from phage lysin are given in Table 3 below. See Table 4 below for non-limiting examples of synthetic linkers.

[0016] In the use of the present invention, as one of the embodiments, the chimera is formed by pairing catalytic

domains and binding domains derived from different bacteriophage lysins.

[0017] In the application of the present invention, as one of the embodiments, the chimera includes one or two catalytic domains without a binding domain;

As one of the embodiments, the chimera includes one or more catalytic domains and one or more binding domains;
As one of the embodiments, the chimera can be one or more catalytic domains connected to a single binding domain;
As one of the embodiments, the chimera can be a single catalytic domain connected to a single binding domain;
As one of the embodiments, the chimera can be a single catalytic domain in the N-terminal of the lysin linked to a single binding domain in the C-terminal of the lysin.

[0018] Furthermore, one or more catalytic domains may be used in the absence of a binding domain. Further, for specialized applications such as detection, one or more binding domains can be used in the absence of the catalytic domain and fused to a different molecule to facilitate detection. Such molecules include but are not limited to biotin, flag tag, myc tag, avidin, streptavidin, ovalbumin, firefly luciferase, biotin, fluorescent molecules such as FITC, TRITC, Alexafluor, etc.

[0019] In the use of the present invention, as one of the embodiments, the chimera further comprises a linker between the catalytic domain and the binding domain, and the linker includes:

1) the linker region or any part thereof derived from the parent lysin molecule, the catalytic domain is derived from the parent lysin molecule;
In the application of the present invention, as one of the embodiments, the linker region derived from the parent lyase molecule has the amino acid sequence shown in any one of SEQ ID NO:343~SEQ ID NO:367.
2) the linker region or any part thereof derived from the parent lysin molecule, the binding domain is derived from the parent lysin molecule;
In the use of the present invention, as one of the embodiments, the linker region derived from the parent lysin molecule has any nucleotide sequence shown in SEQ ID NO: 368~SEQ ID NO: 392; or
3) The amino acid sequence shown in any one of SEQ ID NO:393~SEQ ID NO:406, or the synthetic linker domains be (GGGS)n, (GGGGS)n, (GGGGGS)n, (Gly)3-8, (EAAAK)n, (Ala-Pro)n, A(EAAAK)nALEA(EAAAK) nA amino acid sequences (wherein $1 \leq n \leq 15$, n is an integer), as an exemplary illustration, n can be 1, 2, 3, 4 , 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15.

[0020] In the use of the present invention, as one of the embodiments, the chimera includes that the catalytic domain has the amino acid sequence shown in SEQ ID NO:57, SEQ ID NO:58, SEQ ID NO:59, SEQ ID NO:61, SEQ ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO:71, SEQ ID NO:73, SEQID NO:74, SEQID NO:77, SEQ ID NO:80, SEQID NO:81, SEQID NO:85, SEQID NO:86, SEQID NO:87, SEQID NO:91, SEQID NO:93, SEQID NO:95, SEQ ID NO:97, SEQ ID NO:98, SEQ ID NO:99, SEQ ID NO:100, SEQ ID NO:102, SEQ ID NO:104, SEQ ID NO:105, SEQ ID NO:106. SEQ ID NO:107, SEQ ID NO:108. SEQ ID NO:110, SEQ ID NO:111, SEQ ID NO:113. SEQ ID NO:114, SEQ ID NO:117, SEQ ID NO:118, SEQ ID NO:121, SEQ ID NO:122, SEQ ID NO:125, SEQ ID NO:127, SEQ ID NO:128, SEQ ID NO:131, SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:135 或 SEQ ID NO:136.

[0021] The binding domain has the amino acid sequence shown as SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:68, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO:94, SEQ ID NO:96, SEQ ID NO:101, SEQ ID NO:103, SEQ ID NO:109. SEQ ID NO:112, SEQ ID NO:115. SEQ ID NO:116. SEQ ID NO:119, SEQ ID NO:120. SEQ ID NO:123, SEQ ID NO:124. SEQ ID NO:126, SEQ ID NO:129, SEQ ID NO:130, SEQ ID NO:134, SEQ ID NO: 137 , SEQ ID NO:138, or SEQ ID NO:139.

[0022] In the application of the present invention, as one of the embodiments, the catalytic domain has the nucleotide sequence shown in SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO: 157, SEQ ID NO: 160, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 176, SEQ ID NO: 178, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO:193, SEQ ID NO:194, SEQ ID NO:196, SEQ ID NO:197, SEQ ID NO:200, SEQ ID NO: 201, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216, SEQ ID NO:218, SEQ ID NO:219;

[0023] The binding domain has the nucleotide sequence shown in SEQ ID NO:143, SEQ ID NO:145, SEQ ID NO:151, SEQ ID NO:155, SEQ ID NO:158, SEQ ID NO:159, SEQ ID NO:161, SEQ ID NO:162, SEQ ID NO:165, SEQ ID NO:166,

SEQ ID NO:167, SEQ ID NO:171, SEQ ID NO:172, SEQ ID NO:173, SEQ ID NO:175, SEQ ID NO:177, SEQ ID NO:179, SEQ ID NO:184, SEQ ID NO:186, SEQ ID NO:192, SEQ ID NO:195, SEQ ID NO:198, SEQ ID NO:199, SEQ ID NO:202, SEQ ID NO:203, SEQ ID NO:206, SEQ ID NO:207. SEQ ID NO:209, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:217, SEQ ID NO:220, SEQ ID NO:221, or SEQ ID NO:222.

[0024] In the use of the present invention, as one of the embodiments, the chimera has the amino acid sequence shown in any one of SEQ ID NO:223~SEQ ID NO:282; preferably the amino acid sequence shown in SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:271 or SEQ ID NO:272, more preferably the amino acid sequence shown in SEQ ID NO:260 or SEQ ID NO:271;

[0025] In the use of the present invention, as one of the embodiments, the chimera has a nucleotide sequence shown in any one of SEQ ID NO:283~SEQ ID NO:342; preferably the nucleotide sequence shown in SEQ ID NO:316, SEQ ID NO 317, SEQ ID NO: 318, SEQ ID NO: 329 or SEQ ID NO: 330; More preferably the nucleotide sequence shown in SEQ ID NO: 318 or SEQ ID NO: 329.

[0026] In the use of the present invention, as one of the embodiments, the infection caused by Propionibacterium acnes includes invasive infection, postoperative infection and/or instrument-related infection.

[0027] In the use of the present invention, as one of the embodiments, the device-related infection includes joint prosthesis, shunt tube and artificial heart valve-related infection.

[0028] In the use of the present invention, as one of the embodiments, the infection includes bone and/or joint infection, especially postoperative shoulder infection, as well as oral cavity, eye, intervertebral disc and brain infection.

[0029] In the use of the present invention, as one of the embodiments, the diseases related to Propionibacterium acnes include prostatitis leading to cancer, SAPHO (synovitis, acne, impetigo, hypertrophy, osteitis) syndrome, knot arthritis, or sciatica.

[0030] In the use of the present invention, as one of the embodiments, the medical device includes any device for releasing the lysin or its chimera to the affected area, preferably a clamp, patch or spray applied to the skin surface, devices that use microneedles to enhance the skin penetration of lysin or their chimeras, fine needles used by cosmetic professionals to apply lysin or their chimeras specifically to acne-affected hair follicles, or other similar devices.

[0031] In the use of the present invention, as one of the embodiments, the medical device includes that fixes the lysin or its chimera in a position prone to infection of P. acnes, preferably for prosthetic implants in shoulder surgery which infected by Propionibacterium particularly.

[0032] The present invention also provides the phage lysin chimera described in the application. As one of the embodiments, the chimera has the amino acid sequence shown in any one of SEQ ID NO:223~SEQ ID NO:282; preferably the amino acid sequence shown in SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:271 or SEQ ID NO:272; more preferably the amino acid sequence shown in SEQ ID NO:260 or SEQ ID NO:271;

[0033] In the application of the present invention, as one of the embodiments, the chimera has a nucleotide sequence shown in any one of SEQ ID NO:283~SEQ ID NO:342; preferably the nucleotide sequence shown in SEQ ID NO:316, SEQ ID NO: 317, SEQ ID NO: 318, SEQ ID NO: 329 or SEQ ID NO: 330; More preferably the nucleotide sequence shown in SEQ ID NO: 318 or SEQ ID NO: 329.

[0034] The present invention also provides a method for preparing the aforementioned chimera, comprising:

(1) Synthetic domain sequences and primers for amplifying domain sequences;
(2) Using Taq DNA polymerase PCR to amplify the domain sequence;
(3) The PCR product was gel-purified and ligated with the expression plasmid pET28;
(4) Transfer the recombinant plasmid to Escherichia coli BL21 (DE3);
(5) Cultivate Escherichia coli BL21 (DE3) containing the recombinant plasmid, induce expression, collect the cells by centrifugation, lyse, and purify to obtain the chimera.

[0035] In the preparing method of the present invention, as one of the embodiments, the method further includes: Escherichia coli BL21 (DE3) containing recombinant chimeric lysin expression plasmid was cultured in self-inducing medium at 37°C and 300 rpm until the OD_{600} reached 0.6-0.8, and then at 18°C and 300 rpm for continuous incubate for 16-18 hours. Cells were collected by centrifugation, resuspended in 50mM sodium phosphate pH 7.4, and lysed by homogenization under high pressure. The lysin was centrifuged again to collect the soluble crude lysate. The soluble fraction was mixed with an equal volume of 5 M NaCl, and the mixture was loaded onto a hydrophobic column. After sample loading, the column was washed with 5 column volumes of 20 mM sodium phosphate (pH 7.4), 2.5 M NaCl. The recombinant chimeric lyase was then eluted with 10 mM sodium phosphate (pH 7.4). Alternatively, expression and purification can be optimized by those skilled in the art, and the lysin can be expressed in LB using different concentrations of IPTG at different temperatures, aeration and induction times. Cells can be resuspended in different buffers to increase solubility and lysed by mechanical or chemical means. Different protein chromatography methods can be used to purify the lysin. These variables can be optimized for better lysin protein yield.

[0036] The present invention also provides preparations containing the chimera, which further comprises antibiotics,

other lysin, or inactive excipients.

[0037] The present invention also provides the amino acid sequence encoding the chimera, which is 80% or more, 85% or more, 90% or more, 95% or more or 99% or more of the amino acid sequence similarity to the above sequence, or an alternative amino acid sequence having similar functional group. As an exemplary illustration, the present invention also provides amino acid sequences with 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91 %, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% similarity to the aforesaid sequences.

[0038] As one of the embodiments, the replacement amino acid sequence is a conservative substitution using amino acids in the same group of amino acids, and the amino acid group includes:

Aliphatic: glycine, alanine, valine, leucine, isoleucine;
Hydroxyl or sulfur/selenium: serine, cysteine, threonine, methionine;
Cyclic: proline;
Aromatic: phenylalanine, tyrosine, tryptophan;
Basic: histidine, lysine, arginine;
Acidic and their amides: aspartic acid, glutamic acid, asparagine, glutamine.

[0039] The present invention also provides the nucleotide sequence encoding the chimera, or its synonymous codon sequence.

[0040] The present invention also provides the use of the chimera as a reagent for bacterial lysis for DNA extraction and typing using a PCR-based kit.

[0041] The present invention also provides the use of the chimera as a disinfectant or sterilizing agent on an abiotic surface to prevent infection by removing P. acnes in planktonic form or in biofilms, preferably, surgical equipment or prosthetic implant devices are sterilized during the surgical procedure.

[0042] The present invention also provides an application of the chimera, a single binding domain in the chimera, or a series combination of similar or different binding domains in the chimera in the preparation of a diagnostic tool for P. acnes, wherein the lysin, chimera or binding domain is used in combination with a detection marker.

[0043] As one of the embodiments, the lysin, the chimera or the binding domain and the signal molecule form a fusion through gene fusion or chemical coupling.

[0044] As one of the embodiments, the fusion is used to directly detect Propionibacterium acnes on a microscope slide by fluorescence or other means, to label Propionibacterium acnes by immunohistochemistry, and to be used as a detection reagent in an ELISA assay, Use as a detection reagent on Western blot, for attachment to magnetic beads in MACS or other pull-down assays, or as a detection reagent in assays in which antibodies are used as detection reagents. As one of the embodiments, the signal molecules include proteins or chemical fluorescent dyes, protein labels, enzymes, avidin, streptavidin, ovalbumin, biotin, labels sensitive to click chemical labels, inclusion Peptides, or other molecules that can cause the recruitment of secondary proteins or molecules that produce signals,

The fluorescent dyes include GFP, RFP, mCherry, FITC, TRITC, Alexafluor 488, Cy3 or Cy5;
The protein tag includes Flag-tag, myc-tag, halo-tag, his-tag, or any other tag that can be combined with antibodies or other high-affinity molecules to generate signals;
The enzyme includes firefly luciferase, β-lactamase, alkaline phosphatase, horseradish peroxidase, or any other enzyme that causes reactions such as light, color change, substrate deposition or other reactions that can be detected in the assay.

[0045] The present invention also provides the use of the catalytic domain in the chimera in the preparation of a drug for treating P. acnes infection, where the catalytic domain is combined with a targeting module.

[0046] The present invention provides compositions and methods for preventing and treating P. acnes infection and skin colonization and acne lesions associated with such infection or colonization. In a broad aspect, the present invention provides the use and application of a lysin having broad killing activity against skin-related organisms involved in the development or exacerbation of acne lesions and secondary infection of acne lesions, including but not limited to Propionibacterium, Pseudopropionibacterium, Cutibacterium acnes. In particular, the present invention describes methods for the decolonization, dispersion and removal of established bacterial flora in the skin, with particular emphasis on Cutibacterium acnes, (formerly known as Propionibacterium acnes). In addition, the present invention provides methods for producing chimeric lysin comprising catalytic domains and binding domains from different lysin resulting in various advantageous properties including, but not limited to, improved activity, host range, expression, solubility, stability or more suitable for commercialization.

[0047] According to the present invention, in the method and application of the present invention, the phage lysin used is derived from Propionibacterium, Cutibacterium, Acidipropionibacterium, Pseudopropionibacterium. Table 2 provides lysin and polypeptides used in the present invention.

[0048] In one aspect of the present invention, the lysin shown in SEQ ID NO:1~SEQ ID NO:28 is encoded by the nucleotide sequence shown in SEQ ID NO:29~SEQ ID NO:56.

[0049] The present invention also provides a method for generating chimeras between the binding and catalytic domains of different phage lysin. SEQ ID NO:57~SEQ ID NO:139 shows a non-limiting sequence listing providing examples of polypeptide sequences encoding the catalytic domain and the binding domain derived from phage lysin of the present invention.

[0050] In one aspect of the invention, the catalytic domain and binding domain shown in SEQ ID NO:57~SEQ ID NO:139 are encoded by the nucleotide sequence shown in SEQ ID NO:140~SEQ ID NO:222.

[0051] The present invention also provides non-limiting examples of chimeric lysin produced by pairing the catalytic and binding domains of different lysin as described herein. SEQ ID NO:223-SEQ ID NO:282 provides a non-limiting sequence listing of chimeric lysin active against P. acnes produced by the methods of the present invention.

[0052] In one aspect of the present invention, the nucleotide sequence described in SEQ ID NO:283~SEQ ID NO:342 is used to generate the chimeric lysin described in the present invention.

[0053] In one aspect of the present invention, the polypeptide sequence is encoded by the nucleotide sequence inserted into the expression vector pET28.

[0054] In one aspect of the present invention, the expression vector encoding the polypeptide of the present invention is expressed in Escherichia coli (E. coli) BL21 (DE3) strain.

[0055] In addition, the present invention provides a method for screening and evaluating the activity of phage lysin against Propionibacterium acnes.

[0056] Furthermore, the present invention demonstrates the activity of defined phage lysin against P. acnes.

[0057] In one aspect of the invention, the lysin for treating Propionibacterium is PACL10. The invention provides a method for expressing and purifying PACL10. In addition, the present invention proves that PACL10 has higher antibacterial activity against Propionibacterium acnes.

[0058] The activity of PACL10 against a series of P. acnes strains of different clades was evaluated by minimum inhibitory concentration (MIC) test and time killing assay, and the results consistently showed that PACL10 has high effectiveness.

[0059] The phage lysin and its derivatives produced in the present invention are suitable for industrial scale production, can specifically and effectively kill Propionibacterium acnes, while keeping other commensal bacteria intact, and can provide a safe and effective acne solution, suitable for long-term use without high risk of dysbiosis and acquired resistance. The phage lysin and derivatives thereof produced in the present invention have the following advantages:

(1) It can kill Propionibacterium acnes (C. acnes) without damaging the natural flora.
(2) Rapid activity (within minutes) compared to antibiotics that take a long time to act.
(3) No drug resistance. Bacteria quickly develop resistance to antibiotics, so lysin are a solution. C. acnes is a strongly secreting biofilm organism. Even for organisms that are sensitive to antibiotics, when they are found in the form of biofilms, they can become resistant to antibiotics. Lysin is highly effective against biofilms.
(4) The invention is more efficient compared to other lyases - low MIC and high activity in other assays.
(5) It can be expressed in a soluble manner, and its yield is suitable for large-scale and industrial production.
(6) Active against all clades of C. acnes..

Definition

[0060] Due to the reclassification of Propionibacterium by Scholz et al., a non-limiting example of the comparison of old and new names is shown in Table 1 below:

Table 1

Chinese Name	New Name	Old Name
Cuochuangbingsuanganjun	*Cutibacterium acnes*	*Propionibacterium acnes*
Tanlanbingsuanganjun	*Cutibacterium avidum*	*Propionibacterium avidum*
Kelibingsuanganjun	*Cutibacterium granulosum*	*Propionibacterium granulosum*
Hongbingsuanganjun	*Cutibacterium humerusii*	*Propionibacterium humerusii*
Teshibingsuanganjun	*Acidipropionibacterium thoenii*	*Propionibacterium thoenii*
Zhanshibingsuanganjun	*Acidipropionibacterium jensenii*	*Propionibacterium jensenii*
Chanbingsuanganjun	*Acidipropionibacterium acidipropionici*	*Propionibacterium acidipropionici*

(continued)

Chinese Name	New Name	Old Name
Weishiqibingsuanganjun	*Acidipropionibacterium microaerophilum*	*Propionibacterium microaerophilum*
Dannuoshibingsuanganjun	*Acidipropionibacterium damnosum*	*Propionibacterium damnosum*
Ganlantibingsuanganjun	*Acidipropionibacterium olivae*	*Propionibacterium olivae*
bingsuanbingsuan ganjun	*Pseudopropionibacterium propionicum*	*Propionibacterium propionicum*

[0061] Persons in the field should understand that the reclassification, new and old names, and Chinese names of bacteria should not affect the identification of the bacteria. No matter whether the new name or the old name is adopted in the present invention, it represents the same bacterial species.

[0062] Non-limiting examples of sequences related to the present invention are as follows in Table 2:

Table 2

Name	Amino acid sequence	Name	Nucleotide sequence
PACL01	SEQ ID NO:1	PACL01	SEQ ID NO:29
PACL02	SEQ ID NOT	PACL02	SEQ ID NO:30
PACL03	SEQ ID NOT	PACL03	SEQ ID NO:31
PACL04	SEQ ID NOT	PACL04	SEQ ID NO:32
PACL05	SEQ ID NO:5	PACL05	SEQ ID NO:33
PACL06	SEQ ID NOT	PACL06	SEQ ID NO:34
PACL07	SEQ ID NOT	PACL07	SEQ ID NO:35
PACL08	SEQ ID NOT	PACL08	SEQ ID NO:36
PACL09	SEQ ID NO:9	PACL09	SEQ ID NO:37
PACL10	SEQ ID NO:10	PACL10	SEQ ID NO:38
PACL 11	SEQ ID NO:11	PACL 11	SEQ ID NO:39
PACL12	SEQ ID NO:12	PACL12	SEQ ID NO:40
PACL13	SEQ ID NO:13	PACL13	SEQ ID NO:41
PACL14	SEQ ID NO:14	PACL14	SEQ ID NO:42
PACL15	SEQ ID NO:15	PACL15	SEQ ID NO:43
PACL16	SEQ ID NO:16	PACL16	SEQ ID NO:44
PACL17	SEQ ID NO:17	PACL17	SEQ ID NO:45
PACL18	SEQ ID NO:18	PACL18	SEQ ID NO:46
PACL19	SEQ ID NO:19	PACL19	SEQ ID NO:47
PACL20	SEQ ID NO:20	PACL20	SEQ ID NO:48
PACL21	SEQ ID NO:21	PACL21	SEQ ID NO:49
PACL22	SEQ ID NO:22	PACL22	SEQ ID NO:50
PACL23	SEQ ID NO:23	PACL23	SEQ ID NO:51
PACL24	SEQ ID NO:24	PACL24	SEQ ID NO:52
PACL25	SEQ ID NO:25	PACL25	SEQ ID NO:53
PACL26	SEQ ID NO:26	PACL26	SEQ ID NO:54
PACL27	SEQ ID NO:27	PACL27	SEQ ID NO:55
PACL28	SEQ ID NO:28	PACL28	SEQ ID NO:56

(continued)

Name	Amino acid sequence	Name	Nucleotide sequence
PACL01 CD1	SEQ ID NO:57	PACL01 CD1	SEQ ID NO:140
PACL01 CD2	SEQ ID NO:58	PACL01 CD2	SEQ ID NO:141
PACL01 CD1_2	SEQ ID NO:59	PACL01 CD1_2	SEQ ID NO:142
PACL01 BD	SEQ ID NO:60	PACL01 BD	SEQ ID NO:143
PACL02 CD	SEQ ID NO:61	PACL02 CD	SEQ ID NO:144
PACL02 BD	SEQ ID NO:62	PACL02 BD	SEQ ID NO:145
PACL03 CD	SEQ ID NO:63	PACL03 CD	SEQ ID NO:146
PACL04 CD	SEQ ID NO:64	PACL04 CD	SEQ ID NO:147
PACL05 CD1	SEQ ID NO:65	PACL05 CD1	SEQ ID NO:148
PACL05 CD2	SEQ ID NO:66	PACL05 CD2	SEQ ID NO:149
PACL05 CD1_2	SEQ ID NO:67	PACL05 CD1_2	SEQ ID NO:150
PACL05 BD	SEQ ID NO:68	PACL05 BD	SEQ ID NO:151
PACL06 CD1	SEQ ID NO:69	PACL06 CD1	SEQ ID NO:152
PACL06 CD2	SEQ ID NO:70	PACL06 CD2	SEQ ID NO:153
PACL06 CD1 _ 2	SEQ ID NO:71	PACL06 CD1_2	SEQ ID NO:154
PACL06 BD	SEQ ID NO:72	PACL06 BD	SEQ ID NO:155
PACL07 CDf	SEQ ID NO:73	PACL07 CDf	SEQ ID NO:156
PACL07CD0	SEQ ID NO:74	PACL07CD0	SEQ ID NO:157
PACL07 BDf	SEQ ID NO:75	PACL07 BDf	SEQ ID NO:158
PACL07BD0	SEQ ID NO:76	PACL07BD0	SEQ ID NO:159
PACL08 CD	SEQ ID NO:77	PACL08 CD	SEQ ID NO:160
PACL08 BDf	SEQ ID NO:78	PACL08 BDf	SEQ ID NO:161
PACL08BD0.5	SEQ ID NO:79	PACL08BD0.5	SEQ ID NO:162
PACL09 CDf	SEQ ID NO:80	PACL09 CDf	SEQ ID NO:163
PACL09CD0	SEQ ID NO:81	PACL09CD0	SEQ ID NO:164
PACL09 BDf	SEQ ID NO:82	PACL09 BDf	SEQ ID NO:165
PACL09BD0	SEQ ID NO:83	PACL09BD0	SEQ ID NO:166
PACL09BD0.5	SEQ ID NO:84	PACL09BD0.5	SEQ ID NO:167

(continued)

Name	Amino acid sequence	Name	Nucleotide sequence
PACL10 CDf	SEQ ID NO:85	PACL10 CDf	SEQ ID NO:168
PACL10CD0	SEQ ID NO:86	PACL10CD0	SEQ ID NO:169
PACL10CD0.5	SEQ ID NO:87	PACL10CD0.5	SEQ ID NO:170
PACL10 BDf	SEQ ID NO:88	PACL10 BDf	SEQ ID NO:171
PACL10BD0	SEQ ID NO:89	PACL10BD0	SEQ ID NO:172
PACL10BD0.5	SEQ ID NO:90	PACL10BD0.5	SEQ ID NO:173
PACL11 CD	SEQ ID NO:91	PACL11 CD	SEQ ID NO:174
PACL11 BD	SEQ ID NO:92	PACL11 BD	SEQ ID NO:175
PACL12 CD	SEQ ID NO:93	PACL12 CD	SEQ ID NO:176
PACL12 BD	SEQ ID NO:94	PACL12 BD	SEQ ID NO:177
PACL13 CD	SEQ ID NO:95	PACL13 CD	SEQ ID NO:178
PACL13 BD	SEQ ID NO:96	PACL13 BD	SEQ ID NO:179
PACL14 CD 1	SEQ ID NO:97	PACL14 CD1	SEQ ID NO:180
PACL14 CD2	SEQ ID NO:98	PACL14 CD2	SEQ ID NO:181
PACL14 CD	SEQ ID NO:99	PACL14 CD	SEQ ID NO:182
PACL15 CD	SEQ ID NO:100	PACL15 CD	SEQ ID NO:183
PACL15 BD	SEQ ID NO:101	PACL15 BD	SEQ ID NO:184
PACL16 CD	SEQ ID NO:102	PACL16 CD	SEQ ID NO:185
PACL16 BD	SEQ ID NO:103	PACL16 BD	SEQ ID NO:186
PACL17 CD 1	SEQ ID NO:104	PACL17 CD1	SEQ ID NO:187
PACL17 CD	SEQ ID NO:105	PACL17 CD	SEQ ID NO:188
PACL18 CD1	SEQ ID NO:106	PACL18 CD1	SEQ ID NO:189
PACL18 CD	SEQ ID NO:107	PACL18 CD	SEQ ID NO:190
PACL19 CD	SEQ ID NO:108	PACL19 CD	SEQ ID NO:191
PACL19 BD	SEQ ID NO:109	PACL19 BD	SEQ ID NO:192
PACL20 CDf	SEQ ID NO:110	PACL20 CDf	SEQ ID NO:193
PACL20CD0	SEQ ID NO:111	PACL20CD0	SEQ ID NO:194
PACL20 BD	SEQ ID NO:112	PACL20 BD	SEQ ID NO:195

(continued)

Name	Amino acid sequence	Name	Nucleotide sequence
PACL21 CDf	SEQ ID NO:113	PACL21 CDf	SEQ ID NO:196
PACL21CD0	SEQ ID NO:114	PACL21CD0	SEQ ID NO:197
PACL21 BDf	SEQ ID NO:115	PACL21 BDf	SEQ ID NO:198
PACL21BD0	SEQ ID NO:116	PACL21BD0	SEQ ID NO:199
PACL22 CDf	SEQ ID NO:117	PACL22 CDf	SEQ ID NO:200
PACL22CD0	SEQ ID NO:118	PACL22CD0	SEQ ID NO:201
PACL22 BDf	SEQ ID NO:119	PACL22 BDf	SEQ ID NO:202
PACL22BD0	SEQ ID NO:120	PACL22BD0	SEQ ID NO:203
PACL23 CDf	SEQ ID NO:121	PACL23 CDf	SEQ ID NO:204
PACL23CD0	SEQ ID NO:122	PACL23CD0	SEQ ID NO:205
PACL23 BDf	SEQ ID NO:123	PACL23 BDf	SEQ ID NO:206
PACL23BD0	SEQ ID NO:124	PACL23BD0	SEQ ID NO:207
PACL24 CD	SEQ ID NO:125	PACL24 CD	SEQ ID NO:208
PACL24 BD	SEQ ID NO:126	PACL24 BD	SEQ ID NO:209
PACL25 CDf	SEQ ID NO:127	PACL25 CDf	SEQ ID NO:210
PACL25CD0	SEQ ID NO:128	PACL25CD0	SEQ ID NO:211
PACL25 BDf	SEQ ID NO:129	PACL25 BDf	SEQ ID NO:212
PACL25BD0.5	SEQ ID NO:130	PACL25BD0.5	SEQ ID NO:213
PACL26 CDf	SEQ ID NO:131	PACL26 CDf	SEQ ID NO:214
PACL26CD0	SEQ ID NO:132	PACL26CD0	SEQ ID NO:215
PACL26CD0.5	SEQ ID NO:133	PACL26CD0.5	SEQ ID NO:216
PACL26 BD	SEQ ID NO:134	PACL26 BD	SEQ ID NO:217
PACL27 CDf	SEQ ID NO:135	PACL27 CDf	SEQ ID NO:218
PACL27CD0	SEQ ID NO:136	PACL27CD0	SEQ ID NO:219
PACL27 BDf	SEQ ID NO:137	PACL27 BDf	SEQ ID NO:220
PACL27BD0	SEQ ID NO:138	PACL27BD0	SEQ ID NO:221
PACL27BD0.5	SEQ ID NO:139	PACL27BD0.5	SEQ ID NO:222
PACL246	SEQ ID NO:223	PACL246	SEQ ID NO:283
PACL247	SEQ ID NO:224	PACL247	SEQ ID NO:284
PACL248	SEQ ID NO:225	PACL248	SEQ ID NO:285

(continued)

Name	Amino acid sequence	Name	Nucleotide sequence
PACL249	SEQ ID NO:226	PACL249	SEQ ID NO:286
PACL250	SEQ ID NO:227	PACL250	SEQ ID NO:287
PACL251	SEQ ID NO:228	PACL251	SEQ ID NO:288
PACL252	SEQ ID NO:229	PACL252	SEQ ID NO:289
PACL253	SEQ ID NO:230	PACL253	SEQ ID NO:290
PACL254	SEQ ID NO:231	PACL254	SEQ ID NO:291
PACL255	SEQ ID NO:232	PACL255	SEQ ID NO:292
PACL256	SEQ ID NO:233	PACL256	SEQ ID NO:341
PACL262	SEQ ID NO:234	PACL262	SEQ ID NO:293
PACL269	SEQ ID NO:235	PACL269	SEQ ID NO:294
PACL287	SEQ ID NO:236	PACL287	SEQ ID NO:295
PACL289	SEQ ID NO:237	PACL289	SEQ ID NO:296
PACL296	SEQ ID NO:238	PACL296	SEQ ID NO:297
PACL306	SEQ ID NO:239	PACL306	SEQ ID NO:342
PACL341	SEQ ID NO:240	PACL341	SEQ ID NO:298
PACL342	SEQ ID NO:241	PACL342	SEQ ID NO:299
PACL343	SEQ ID NO:242	PACL343	SEQ ID NO:300
PACL344	SEQ ID NO:243	PACL344	SEQ ID NO:301
PACL345	SEQ ID NO:244	PACL345	SEQ ID NO:302
PACL347	SEQ ID NO:245	PACL347	SEQ ID NO:303
PACL348	SEQ ID NO:246	PACL348	SEQ ID NO:304
PACL349	SEQ ID NO:247	PACL349	SEQ ID NO:305
PACL350	SEQ ID NO:248	PACL350	SEQ ID NO:306
PACL351	SEQ ID NO:249	PACL351	SEQ ID NO:307
PACL352	SEQ ID NO:250	PACL352	SEQ ID NO:308
PACL383	SEQ ID NO:251	PACL383	SEQ ID NO:309
PACL384	SEQ ID NO:252	PACL384	SEQ ID NO:310
PACL385	SEQ ID NO:253	PACL385	SEQ ID NO:311
PACL386	SEQ ID NO:254	PACL386	SEQ ID NO:312
PACL387	SEQ ID NO:255	PACL387	SEQ ID NO:313
PACL388	SEQ ID NO:256	PACL388	SEQ ID NO:314
PACL389	SEQ ID NO:257	PACL389	SEQ ID NO:315
PACL390	SEQ ID NO:258	PACL390	SEQ ID NO:316
PACL391	SEQ ID NO:259	PACL391	SEQ ID NO:317
PACL392	SEQ ID NO:260	PACL392	SEQ ID NO:318
PACL393	SEQ ID NO:261	PACL393	SEQ ID NO:319
PACL394	SEQ ID NO:262	PACL394	SEQ ID NO:320
PACL395	SEQ ID NO:263	PACL395	SEQ ID NO:321

(continued)

Name	Amino acid sequence	Name	Nucleotide sequence
PACL396	SEQ ID NO:264	PACL396	SEQ ID NO:322
PACL397	SEQ ID NO:265	PACL397	SEQ ID NO:323
PACL398	SEQ ID NO:266	PACL398	SEQ ID NO:324
PACL399	SEQ ID NO:267	PACL399	SEQ ID NO:325
PACL400	SEQ ID NO:268	PACL400	SEQ ID NO:326
PACL401	SEQ ID NO:269	PACL401	SEQ ID NO:327
PACL402	SEQ ID NO:270	PACL402	SEQ ID NO:328
PACL403	SEQ ID NO:271	PACL403	SEQ ID NO:329
PACL404	SEQ ID NO:272	PACL404	SEQ ID NO:330
PACL405	SEQ ID NO:273	PACL405	SEQ ID NO:331
PACL406	SEQ ID NO:274	PACL406	SEQ ID NO:332
PACL407	SEQ ID NO:275	PACL407	SEQ ID NO:333
PACL408	SEQ ID NO:276	PACL408	SEQ ID NO:334
PACL409	SEQ ID NO:277	PACL409	SEQ ID NO:335
PACL410	SEQ ID NO:278	PACL410	SEQ ID NO:336
PACL411	SEQ ID NO:279	PACL411	SEQ ID NO:337
PACL412	SEQ ID NO:280	PACL412	SEQ ID NO:338
PACL413	SEQ ID NO:281	PACL413	SEQ ID NO:339
PACL414	SEQ ID NO:282	PACL414	SEQ ID NO:340

[0063] Non-limiting examples of natural linkers described in the present invention are shown in Table 3 below:

Table 3

Original Lysin	Linker Name	Amino Acid Sequence	Nucleic Acid Sequence
PACL01	01linker1	SEQ ID NO:343	SEQ ID NO:368
PACL01	01linker2	SEQ ID NO:344	SEQ ID NO:369
PACL02	02linker	SEQ ID NO:345	SEQ ID NO:370
PACL05	05linker1	SEQ ID NO:346	SEQ ID NO:371
PACL05	05linker2	SEQ ID NO:347	SEQ ID NO:372
PACL06	06linker1	SEQ ID NO:348	SEQ ID NO:373
PACL06	06linker2	SEQ ID NO:349	SEQ ID NO:374
PACL07	07 linker	SEQ ID NO:350	SEQ ID NO:375
PACL08	08linker	SEQ ID NO:351	SEQ ID NO:376
PACL09	09linker	SEQ ID NO:352	SEQ ID NO:377
PACL10	10linker	SEQ ID NO:353	SEQ ID NO:378
PACL11	11linker	SEQ ID NO:354	SEQ ID NO:379
PACL12	12linker	SEQ ID NO:355	SEQ ID NO:380
PACL13	13linker	SEQ ID NO:356	SEQ ID NO:381
PACL14	14linker	SEQ ID NO:357	SEQ ID NO:382

(continued)

Original Lysin	Linker Name	Amino Acid Sequence	Nucleic Acid Sequence
PACL15	15linker	SEQ ID NO:358	SEQ ID NO:383
PACL16	16linker	SEQ ID NO:359	SEQ ID NO:384
PACL19	19linker	SEQ ID NO:360	SEQ ID NO:385
PACL20	20linker	SEQ ID NO:361	SEQ ID NO:386
PACL21	21linker	SEQ ID NO:362	SEQ ID NO:387
PACL22	22linker	SEQ ID NO:363	SEQ ID NO:388
PACL23	23linker	SEQ ID NO:364	SEQ ID NO:389
PACL25	25linker	SEQ ID NO:365	SEQ ID NO:390
PACL26	26linker	SEQ ID NO:366	SEQ ID NO:391
PACL27	27linker	SEQ ID NO:367	SEQ ID NO:392

[0064] Non-limiting examples of synthetic linkers described in the present invention are shown in Table 4 below:

Table 4

S/N	Linker Name	Amino Acid Sequence
1	(GGGS)n, 1≤n≤8, n is an integer	-
2	(GGGGS)n, 1≤n≤8, n is an integer	-
3	(GGGGGS)n, 1≤n≤8, n is an integer	-
4	$(Gly)_{3-8}$	-
5	(EAAAK)n, 1≤n≤8, n is an integer	-
6	PAPAP	SEQ ID NO:393
7	AEAAAKEAAAKA	SEQ ID NO:394
8	$(Ala\text{-}Pro)_n$, 1≤n≤15, n is an integer	-
9	$A(EAAAK)_nALEA(EAAAK)_nA$, 1≤n≤8, n is an integer	-
10	VSQTSKLTRAETVFPDV	SEQ ID NO:395
11	PLG LWA	SEQ ID NO:396
12	RVLAEA	SEQ ID NO:397
13	EDVVCCSMSY	SEQ ID NO:398
14	GGIEGRGS	SEQ ID NO:399
15	TRHRQPRGWE	SEQ ID NO:400
16	AGNRVRRSVG	SEQ ID NO:401
17	RRRRRRRRR	SEQ ID NO:402
18	GFLG	SEQ ID NO:403
19	KESGSVSSEQLAQFRSLD	SEQ ID NO:404
20	EGKSSGSGSESKST	SEQ ID NO:405
21	GSAGSAAGSGEF	SEQ ID NO:406

Description of drawings

[0065]

Figure 1: Example 3 shows the effective killing effect of selected lysin on P. acnes. Lysin was expressed in E. coli BL21(DE3) on LB agar containing IPTG and released by E. coli osmotic lysis. A clearing zone or halo around the E. coli indicates that the expressed lysin inhibits growth or kills P. acnes embedded in the agar overlay.

Figure 2: Example 3 shows the clearing zone formed on the P. acnes overlay by crude E. coli lysates containing induced selected lysin.

Figure 3: Example 4 demonstrates that PACL10 can be soluble expressed and purified. All samples were run at 150 V for 50 mins in a 5-12% tris-glycine gel and stained with Coomassie brilliant blue. PACL10 was expressed in E. coli BL21 (DE3) using the pET expression system and purified by hydrophobic interaction. The eluted fractions show purified PACL10 at 29.4 kDa.

Figure 4: Example 7 shows the kinetics of PACL10 in reducing cfu/mL of P. acnes 34A strain. When the MIC was 6.4 μg/mL and the 2x MIC was 12.8 μg/mL, the cfu/mL could drop below the detection limit within 3 hours and 2 hours, respectively.

Figure 5: shows a single-step purified lysin for MIC determination. Lysin concentrations loaded on 5-12% Tris-HCl SDS gels were: 0.62 mg/mL PACL10, 1.41 mg/mL PACL390, 2.12 mg/mL PACL391, 2.10 mg/mL PACL392, 1.49 mg/mL PACL403 and 1.60 mg/mL PACL404.

Figure 6: shows PACL392 purified by mixed mode and ion exchange chromatography.

Figure 7: shows the bactericidal activity of PACL392 relative to strain 10.

Figure 8: shows PACL403 purified by mixed mode chromatography.

Figure 9: shows the bactericidal activity of PACL403 relative to strain 10 - OD reduction.

Figure 10: shows the bactericidal activity of PACL403 relative to strain 10 - CFU reduction.

Figure 11: shows the bactericidal activity of PACL403 relative to biofilm-associated strain 10.

Specific embodiment

[0066] The following examples and/or experimental examples are only used to further illustrate the present invention, but do not limit the effective scope of the present invention in any way. Experiments were carried out using standard experimental methods to obtain the results shown in the examples.

Example 1

[0067] By analyzing the sequences of prophages in the genomes of Propionibacteriaceae bacteria, we identified P. acnes phage lysin that could be soluble expressed and purified. The gene sequence expressing phage lysin was synthesized by Sangon and ligated with the expression plasmid pET28. Each recombinant plasmid was then transformed into E. coli BL21 (DE3) strain.

Example 2

[0068] The chimera was constructed as an N-terminal catalytic domain (CD) and a C-terminal binding domain (BD), with a variable-length linker in between. Domains were identified by sequence analysis using the NCBI constant region database and the RaptorX web server for in silico protein structure prediction. The catalytic domain has a full C-terminal, half C-terminal or no C-terminal linker. Likewise, the binding domain has a full N-terminal, half N-terminal or no N-terminal linkage. Primers for amplifying domain sequences were synthesized by GeneCreate. Domain sequences were amplified using Taq DNA polymerase PCR at an annealing temperature of 55°C. The PCR product was gel purified and ligated with the expression plasmid pET28. The recombinant plasmid was then transferred to E. coli BL21 (DE3).

Example 3

[0069] Propionibacterium acnes coverage test confirmed the antibacterial activity of lysin. Briefly, E. coli BL21 (DE3) clones containing the recombinant lysin plasmid were plated on LB agar containing IPTG for induction. Once the lysin is overexpressed, the E. coli clone osmotically releases the lysin. Soft agar with P. acnes embedded was overlaid and cultured to allow P. acnes to grow. The presence of active lysin forms a zone of clearing or halo around the E. coli. The experimental results are shown in Figure 1.

[0070] In a similar experiment, E. coli BL21 (DE3) clones containing the recombinant lysin plasmid were induced in liquid medium. Cells were harvested by centrifugation and sonicated in 50 mM sodium phosphate pH 7.4 buffer. The lysin is centrifuged to separate soluble and insoluble fractions. Soluble crude lysin were spotted on soft agar embedded with P. acnes. Active lysin in the soluble crude lysate formed a clearing zone after culturing to grow P. acnes. The experimental results are shown in Figure 2.

Example 4

Expression and purification of PACL10

[0071] The expression and purification of PACL10 were as follows. Briefly, Escherichia coli BL21 (DE3) containing the recombinant PACL10 expression plasmid was cultured in an autoinduction medium at 37°C and 300 rpm until the OD_{600} reached 0.6-0.8, and then continued to culture at 18°C and 300 rpm for 16-18 hours. Cells were collected by centrifugation, resuspended in 50mM sodium phosphate pH 7.4, and homogeneously lysed under high pressure. The lysin was centrifuged again to collect the soluble crude lysin. The soluble fraction was mixed with an equal volume of 5 M NaCl, and the mixture was loaded onto a hydrophobic column. After sample loading, the column was washed with 5 column volumes of 20 mM sodium phosphate (pH 7.4), 2.5 M NaCl. PACL10 was then eluted with 10 mM sodium phosphate (pH 7.4). The experimental results are shown in Figure 3.

Example 5

[0072] The chimeric lysin was expressed and purified in a similar manner to PACL10. Escherichia coli BL21 (DE3) containing recombinant chimeric lysin expression plasmid was cultured in self-inducing medium at 37°C and 300 rpm until the OD_{600} reached 0.6-0.8, and then at 18°C and 300 rpm for continuous incubation for 16-18 hours. Cells are collected by centrifugation, resuspended in 50mM sodium phosphate pH 7.4, and lysed by homogenization under high pressure. The lysate was centrifuged again to collect the soluble crude lysate. The soluble fraction was mixed with an equal volume of 5 M NaCl, and the mixture was loaded onto a hydrophobic column. After sample loading, the column was washed with 5 column volumes of 20 mM sodium phosphate (pH 7.4), 2.5 M NaCl. The recombinant chimeric lysin was then eluted with 10 mM sodium phosphate (pH 7.4).

Example 6

Minimum Inhibitory Concentration Determination

[0073] The minimal inhibitory concentration (MIC) determination method is as follows. First prepare a colony suspension in 0.9% saline to an OD_{600} of 0.05 to prepare an inoculum, which corresponds to 10^7 colony-forming units per mL (cfu/mL). The suspension was diluted 20 times in agar-free enhanced Clostridium medium (RCM) to an inoculum of $5*10^5$cfu/mL. Prepare 2-fold serial dilutions of PACL10 or vancomycin with 0.9% saline and add no more than one-tenth of the total culture. Cultures were grown anaerobically at 37°C for 3 days. The MIC is the lowest concentration of PACL10 or vancomycin at which no growth of P. acnes can be observed with the naked eye. The minimum bactericidal concentration (MBC) was determined by subculturing the above cultures on enhanced Clostridium agar (RCA) plates containing 0.1% Tween-80. MBC is the lowest concentration of PACL10 or vancomycin at which no P. acnes colonies are seen on the enhanced Clostridium agar (RCA) plate.

[0074] Experimental results: Table 6-1 shows the minimal inhibitory concentration (MIC) and minimal bactericidal concentration (MBC) of PACL10 to 12 strains of Propionibacterium acnes bacterial strains from IA1, IA2, and II clades compared with vancomycin.

Table 6-1

Clade	**Strai n**	**#10 (29380 g/mol)**				**Vancomycin (1485.7 g/mol)**			
		MIC		MBC		MIC		MBC	
		μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
IA_1	1	6.4	0.218	25.6	0.871	0.5	0.337	0.5	0.337
	5	12.8	0.436	12.8	0.436	1.0	0.673	1.0	0.673
	7	6.4	0.218	12.8	0.436	0.5	0.337	1.0	0.673
	15	6.4	0.218	25.6	0.871	1.0	0.673	1.0	0.673
	27	12.8	0.436	>25.6	>0.871	1.0	0.673	1.0	0.673

(continued)

Clade	Strai n	#10 (29380 g/mol)				Vancomycin (1485.7 g/mol)			
		MIC		MBC		MIC		MBC	
		μg/mL	μM	μg/mL	μM	μg/mL	μM	μg/mL	μM
IA_2	10	6.4	0.218	12.8	0.436	0.5	0.337	1.0	0.673
	14	6.4	0.218	>25.6	>0.871	0.5	0.337	1.0	0.673
	17	6.4	0.218	25.6	0.871	0.5	0.337	0.5	0.337
	31	12.8	0.436	>25.6	>0.871	0.5	0.337	0.5	0.337
	33A	12.8	0.436	>25.6	>0.871	0.5	0.337	1.0	0.673
IB	13	12.8	0.436	25.6	0.871	1.0	0.673	1.0	0.673
II	34A	6.4	0.218	6.4	0.218	0.5	0.337	0.5	0.337

Example 7

Time sterilization test

[0075] The time sterilization test method is as follows. Resuspend the P. acnes 34A strain colony in 0.9% saline to an OD_{600} of 0.05, which corresponds to 10^7 cfu/mL. The suspension was diluted 10-fold in 50 mM sodium phosphate (pH 6.0) to ~10^6 cfu/mL. Add 0, 0.5x, 1x, 2x MIC of two-fold serial dilutions of PACL10 to the 34A strain, respectively. Ten-fold serial dilutions (0, 1, 2, 3 log) from the assay were plated on enhanced Clostridium agar (RCA) at each time point and incubated anaerobically at 37°C for 3 days. The experimental results are shown in Figure 4.

Example 8

Minimal inhibitory concentrations of selected lysin after single-step purification

[0076] Express PACL 10, 390, 391, 392, 403 and 404 according to the method of Examples 1-4, and perform the following single-step purification: put Escherichia coli BL21 (DE3) containing each recombinant expression plasmid in the self-induction medium incubate at 37°C, 300 rpm for 3 hours, then at 18°C, 300 rpm for 16-18 hours. Cells were collected by centrifugation and lysed by homogenizatoin under high pressure. Clarified lysate from 1 L of E. coli culture medium expressing the protein of interest was loaded onto a 26mm/200mm column containing 70 mL of purification resin. The lysin was purified according to the conditions in Table 8-1, and the purity determination of the lyase after single-step purification is shown in Figure 5. These lysin are used to determine the minimal inhibitory concentration (MIC).

[0077] The minimal inhibitory concentration (MIC) determination method is as follows. Propionibacterium acnes is streaked on Fortified Clostridium Agar (Fortified Clostridium Medium RCM with 1.5% agar, RCM was prepared according to the revised recipe, per liter - 10g acid hydrolyzed casein, 10g beef extract, 3g yeast extract, 5g D - glucose, 5 g sodium chloride, 3 g sodium acetate, 0.5 g L-cysteine hydrochloride). Plates were incubated anaerobically at 37°C for 72 hours. The inoculum for MIC determination was first prepared by resuspending single clones from RCA in 0.9% saline to an OD_{600} of 0.05, corresponding to 10^7 colony-forming units per milliliter (cfu/mL). The suspension was diluted 20-fold in RCM to an inoculum of $5*10^5$ cfu/mL. Two-fold serial dilutions of each lyase were prepared in 0.9% saline and added not to exceed one-tenth of the total culture volume. Cultures were grown anaerobically at 37°C for 48-72 hours. The MIC is the lowest concentration of lyase at which no growth of P. acnes can be observed with the naked eye. The MIC results are shown in Table 8-2.

Table 8-1 Purification conditions of selected lysin

PACL	homogenization buffer	binding buffer	Chromatography	Elution buffer
10	sodium phosphate, pH 7.4	25 mM sodium phosphate, 2.5 M NaCl, pH 7.4	Hydrophobic Interaction, Phenyl Sepharose (Low Substitution)	Gradient to 10 mM sodium phosphate

(continued)

PACL	homogenization buffer	binding buffer	Chromatography	Elution buffer
390	50 mM sodium phosphate, pH 7.4	25 mM sodium phosphate, 2.5 M NaCl, pH 7.4	Hydrophobic Interaction, Phenyl Sepharose (Low Substitution)	Gradient to 10 mM sodium phosphate
391	50 mM sodium phosphate, pH 7.4	25 mM sodium phosphate, 2.5 M NaCl, pH 7.4	Hydrophobic Interaction, Phenyl Sepharose (Low Substitution)	Gradient to 10 mM sodium phosphate
392	50 mM sodium phosphate, pH 7.4	25 mM sodium phosphate, 2.5 M NaCl, pH 7.4	Hydrophobic Interaction, Phenyl Sepharose (Low Substitution)	Gradient to 10 mM sodium phosphate
403	20 mM sodium phosphate, pH 7.4	20 mM sodium phosphate, pH 7.4	Ion exchange, SP Sepharose	Gradient to 20 mM NaP, 0.5 M NaCl, pH 7.4
404	20 mM sodium phosphate, pH 7.4	20 mM sodium phosphate, pH 7.4	Ion exchange, SP Sepharose	Gradient to 20 mM NaP, 0.5 M NaCl, pH 7.4

Table 8-2 MIC of selected lyases relative to Propionibacterium acnes (μg/mL)

Clade	Strain	PACL10	PACL390	PACL391	PACL392	PACL403	PACL404	Tetracycline
IA_1	1	8	8	8	8	16	8	8
	5	8	8	16	8	16	16	8
	7	8	8	8	8	16	16	8
IA_2	10	8	8	32	16	8	16	8
	17	8	8	32	16	8	8	8
	33	8	8	16	8	8	8	8
II	34	8	8	8	8	16	8	8

Example 9

Purification and bactericidal activity of PACL392

[0078] PACL392 was expressed in Escherichia coli BL21(DE3) containing the recombinant expression plasmid by placing it in autoinduction medium and culturing at 37°C, 300 rpm for 3h, followed by 18°C, 300 rpm for 16-18h. Cells were collected by centrifugation and homogeneously lysed in 20 mM sodium phosphate, pH 7.4, under high pressure. The lysate was centrifuged at 12,000 rpm, 4°C for 1 h. The supernatant was mixed with an equal volume of 20 mM sodium phosphate, pH 7.4, 1 M ammonium sulfate, and centrifuged again at 12,000 rpm, 4°C for 1 h. The supernatant was filtered with a 0.22 μm filter membrane and loaded onto a mixed-mode chromatographic column equilibrated with 20mM sodium phosphate, pH 7.4, and 0.5M ammonium sulfate (70 mL purified resin 26mm/200mm column). After sample loading, the column was washed with a series of buffers: 20 mM sodium phosphate, pH 7.4, 0.5 M ammonium sulfate; 20 mM sodium phosphate, pH 7.4, 2.5 M NaCl; 20 mM sodium phosphate, pH 7.4, and 50 mM, piperazine, pH 10.02, 50 mM NaCl. PACL392 was eluted with 50 mM piperazine, pH 10.02, and 750 mM NaCl. The eluate was dialyzed against 50 mM sodium phosphate, pH 7.4 to neutralize the pH and remove cations. Dialyzed samples were filtered and loaded onto an ion exchange column (70 mL of 26mm/200mm column of purification resin). The final result of PACL392 purification is shown in Figure 6.

[0079] The bactericidal activity of PACL392 was tested by 50 mM, pH 7.0 in the presence of a series of additives such as NaCl, $CaCl_2$, $MgCl_2$, EDTA, DTT, Tween-20, Tween-80 and hyaluronic acid (concentrations shown in Figure 7). CFU reduction in HEPES was determined.

Example 10

Purification and bactericidal activity of PACL403

[0080] PACL403 was expressed in Escherichia coli BL21(DE3) containing the recombinant expression plasmid by placing it in autoinduction medium and culturing at 37°C, 300 rpm for 3 h, followed by 18°C, 300 rpm for 16-18 h. Cells were collected by centrifugation and lysed by homogenization in 20 mM sodium phosphate, pH 7.4, under high pressure. The lysate solution was adjusted to 20 mM sodium phosphate, pH 7.4, 1 M NaCl, and centrifuged at 12,000 rpm, 4°C for 1 h. The supernatant was filtered with a 0.22 μm filter membrane and loaded onto a mixed-mode chromatographic column (26mm/200mm column of 70 mL purified resin) equilibrated with 20mM sodium phosphate, pH 7.4, and 1 M NaCl. After loading the column was washed with a series of buffers: 20 mM sodium phosphate, pH 7.4, 1 M NaCl; 20 mM sodium phosphate, pH 7.4, 2.5 M NaCl; 20 mM sodium phosphate, pH 7.4; 20 mM, Sodium phosphate pH 7.4, 0.1% Triton X-114 and again 20 mM sodium phosphate pH 7.4. PACL403 was eluted with 50 mM piperazine, pH 9.5, 1M NaCl. Neutralize the high pH by adding a fifth volume of 500 mM sodium phosphate, pH 7.4. The eluate was then dialyzed against 50 mM sodium phosphate, pH 7.4, to remove cations. Dialyze sample filtration. The final result of PACL403 purification is shown in Figure 8.

[0081] The bactericidal activity of PACL403 was determined by the reduction in OD in 50 mM sodium phosphate, pH 7.0, in the presence of Tween-20 and Tween-80 at the concentrations shown in FIG. 9.

[0082] The bactericidal activity of PACL403 was determined by the reduction of CFU in 50 mM HEPES, pH 7.0, in the presence of Tween-20 and Tween-80 at the concentrations shown in FIG. 10.

[0083] PACL403 also killed biofilm-associated P. acnes (Fig. 11). Strain 10 with an OD_{600} of 1.0 was added to a 96-well polystyrene plate to inoculate the biofilm overnight in an anaerobic gas mixture. The supernatant was removed and the wells were gently rinsed with 0.9% NaCl. Biofilm formation medium (RCM + 5% glucose) was added to allow biofilm growth overnight in the anaerobic gas mixture. The medium was removed and the wells were gently rinsed with 0.9% NaCl. Biofilms were then treated with buffer or PACL403 overnight at 25°C under anaerobic conditions. The buffer or PACL403 was removed and the wells were rinsed with 0.9% NaCl. Resuspend in 0.9% NaCl, plate on RCM agar and count biofilm-associated CFU.

Claims

1. Use of a bacteriophage lysin or its chimera in the preparation of drugs, cosmetics, or medical devices for the prevention, treatment, or improvement of acne or infections caused by Propionibacterium acnes , or diseases related to Propionibacterium acnes, wherein the bacteriophage lysin comprises a bacteriophage lysin derived from the Nocardioidaceae or Propionibacteriaceae family.

2. The use according to claim 1, wherein the Nocardioidaceae bacteria include Micropruina, Propionicimonas, Propionicicella, Friedmanniella; the Propionibacteriaceae bacteria include Propioniferax, Mariniluteicoccus, Granulicoccus, Naumannella, Propioniciclava, Auraticoccus, Microlunatus, Aestuariimicrobium, Luteococcus, Tessaracoccus, Brooklawnia, Propionimicrobium, Propionibacterium, Cutibacterium, Acidipropionibacterium, or Pseudopropionibacterium;
Preferably Propionibacterium, Cutibacterium, Acidipropionibacterium, or Pseudopropionibacterium.

3. The use according to claim 1, wherein the bacteriophage lysin comprises lysins derived from Cutibacterium acnes, Propionibacterium humerusii, Cutibacterium avidum, Cutibacterium granulosum, Acidipropionibacterium thoenii, Acidipropionibacterium jensenii, Acidipropionibacterium acidipropionici, Aestuariimicrobium kwangyangense, Granulicoccus phenolivorans, Microlunatus phosphovorus, Pseudopropionibacterium propionicum, Tessaracoccus sp., Propionicicella superfundia, Propionibacterium freudenreichii, Propionibacterium freudenreichii subsp. Freudenreichii, Propionibacterium freudenreichii subsp. Shermanii, Propionibacterium acidifaciens, Propionibacterium lymphophilum, Propionibacteriaceae bacterium, Propionibacterium sp. oral taxon 192, Propioniferax innocua, Naumannella halotolerans, Propioniciclava tarda, Micropruina glycogenica, Propionicimonas paludicola, Auraticoccus monumenti, Luteococcus japonicus, Tessaracoccus oleiagri, Tessaracoccus bendigoensis, Tessaracoccus lapidicaptus, Acidipropionibacterium microaerophilum, Acidipropionibacterium olivae, or Acidipropionibacterium damnosum.

4. The use according to claim 1, wherein the bacteriophage lysin comprises lysin derived from Acidipropionibacterium jensenii, Acidipropionibacterium thoenii, Acidipropionibacterium acidipropionici, Acidipropionibacterium microaerophilum, Acidipropionibacterium olivae, Acidipropionibacterium damnosum, Cutibacterium acnes, Cutibacterium avidum, Cutibacterium granulosum, or Pseudopropionibacterium propionicum..

5. The use according to claim 1, wherein the bacteriophage lysin has the amino acid sequence shown in any one of SEQ ID NO: 1 to SEQ ID NO: 28.

6. The use according to claim 1, wherein the bacteriophage lysin has the amino acid sequence shown in SEQ ID NO:10.

7. The use according to claim 1 wherein the bacteriophage lysin has the nucleotide sequence shown in any one of SEQ ID NO:29 to SEQ ID NO:56.

8. The use according to claim 1, wherein the bacteriophage lysin has the nucleotide sequence shown in SEQ ID NO:38.

9. The use according to claim 1, wherein the chimera comprises the catalytic domain of bacteriophage lysin, or a combination of the catalytic domain and the binding domain of bacteriophage lysin.

10. The use according to claim 9, wherein the catalytic domain has a full C-terminal linker, a half C-terminal linker, no C-terminal linker, or any part of the linker; the binding domain has a full N-terminal linker, a half N-terminal linker, no N-terminal linker, or any portion of the linker.

11. The use according to claim 9, wherein the catalytic domain comprises one, two or more than two catalytic domains.

12. The use according to claim 9, wherein the binding domain comprises one, two or more than two binding domains.

13. The use according to claim 9, wherein the chimera further comprises a linker between the catalytic domain and the binding domain, the linker comprising:

1) The amino acid sequence shown in any one of SEQ ID NO:343~SEQ ID NO: 367;
2) The amino acid sequence shown in any one of SEQ ID NO:368~SEQ ID NO: 392;
3) the amino acid sequence shown in any one of SEQ ID NO:393~SEQ ID NO: 406; or
4) (GGGS)n, (GGGGS)n, (GGGGGS)n, (Gly)3-8, (EAAAK)n, (Ala-Pro)n, or A(EAAAK)nALEA(EAAAK)nA, where $1 \leq n \leq 15$, n is an integer.

14. The use according to claim 9, wherein said chimera comprises said catalytic domain having the amino acid sequence shown in SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO: 61, SEQ ID NO: ID NO:62, SEQ ID NO:63, SEQ ID NO:64, SEQ ID NO:65, SEQ ID NO:66, SEQ ID NO:67, SEQ ID NO:69, SEQ ID NO:70, SEQ ID NO :71, SEQ ID NO:73, SEQ ID NO:74, SEQ ID NO:77, SEQ ID NO:80, SEQ ID NO:81, SEQ ID NO:85, SEQ ID NO:86, SEQ ID NO:87 , SEQ ID NO: 91, SEQ ID NO: 93, SEQ ID NO: 95, SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, SEQ ID NO: 100, SEQ ID NO: 102, SEQ ID NO: 102, SEQ ID NO: ID NO: 104, SEQ ID NO: 105, SEQ ID NO: 106, SEQ ID NO: 107, SEQ ID NO: 108, SEQ ID NO: 110, SEQ ID NO: 111, SEQ ID NO: 113, SEQ ID NO : 114, SEQ ID NO: 117, SEQ ID NO: 118, SEQ ID NO: 121, SEQ ID NO: 122, SEQ ID NO: 125, SEQ ID NO: 127, SEQ ID NO: 128, SEQ ID NO: 131 , SEQ ID NO:132, SEQ ID NO:133, SEQ ID NO:135, or SEQ ID NO:136; The binding domain has the amino acid sequence shown in SEQ ID NO:60, SEQ ID NO:62, SEQ ID NO:68, SEQ ID NO:72, SEQ ID NO:75, SEQ ID NO:76, SEQ ID NO:78, SEQ ID NO:79, SEQ ID NO:82, SEQ ID NO:83, SEQ ID NO:84, SEQ ID NO:88, SEQ ID NO:89, SEQ ID NO:90, SEQ ID NO:92, SEQ ID NO: 94. SEQ ID NO: 96, SEQ ID NO: 101, SEQ ID NO: 103, SEQ ID NO: 109, SEQ ID NO: 112, SEQ ID NO: 115, SEQ ID NO: 116, SEQ ID NO: 119, SEQ ID NO: 120, SEQ ID NO: 123, SEQ ID NO: 124, SEQ ID NO: 126, SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 134, SEQ ID NO: 137, SEQ ID NO:138 or SEQ ID NO:139.

15. The use according to claim 9, wherein the catalytic domain has the nucleotide sequence shown in SEQ ID NO: 140, SEQ ID NO: 141, SEQ ID NO: 142, SEQ ID NO: 144, SEQ ID NO: 146, SEQ ID NO: 146, SEQ ID NO: ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, SEQ ID NO: 156, SEQ ID NO : 157, SEQ ID NO: 160, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 168, SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 174, SEQ ID NO: 176 , SEQ ID NO: 178, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 185, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 188, SEQ ID NO: ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 196, SEQ ID NO: 197, SEQ ID NO: 200, SEQ ID NO :201, SEQ ID NO:204, SEQ ID NO:205, SEQ ID NO:208, SEQ ID NO:210, SEQ ID NO:211, SEQ ID NO:214, SEQ ID NO:215, SEQ ID NO:216 , SEQ ID NO:218, or SEQ ID NO:219;
The binding domain has the nucleotide sequence shown in SEQ ID NO: 143, SEQ ID NO: 145, SEQ ID NO: 151, SEQ ID NO: 155, SEQ ID NO: 158, SEQ ID NO: 159, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 175, SEQ ID NO:

177. SEQ ID NO: 179, SEQ ID NO: 184, SEQ ID NO: 186, SEQ ID NO: 192, SEQ ID NO: 195, SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 202, SEQ ID NO:203, SEQ ID NO:206, SEQ ID NO:207, SEQ ID NO:209, SEQ ID NO:212, SEQ ID NO:213, SEQ ID NO:217, SEQ ID NO:220, SEQ ID NO:221, or SEQ ID NO:222.

16. The use according to claim 9, wherein the described chimera has the amino acid sequence shown in any one of SEQ ID NO:223 ~ SEQ ID NO:282; Preferably the amino acid sequence shown in SEQ ID NO:258, SEQ ID in NO:259, SEQ ID NO:260, SEQ ID NO:271 or SEQ ID NO:272; more preferably the amino acid sequence shown in SEQ ID NO:260 or SEQ ID NO:271.

17. The use according to claim 9, wherein the chimera has a nucleotide sequence shown in any one of SEQ ID NO:283 ~ SEQ ID NO:342; preferably the nucleotide sequence shown in SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:329 or SEQ ID NO:330; more preferably the nucleotide sequence shown in SEQ ID NO:318 or SEQ ID NO:329.

18. The use according to claim 1, wherein the infection caused by Propionibacterium acnes includes invasive infection, postoperative infection and/or instrument-related infection.

19. The use according to claim 18, wherein the device-related infection includes joint prosthesis, shunt tube and artificial heart valve-related infection.

20. The use according to claim 18, wherein the infection includes bone and/or joint infection, especially postoperative shoulder infection, as well as oral cavity, eye, intervertebral disc and brain infection.

21. The use according to claim 1, wherein the diseases related to Propionibacterium acnes include prostatitis leading to cancer, SAPHO (synovitis, acne, impetigo, hypertrophy, osteitis) syndrome syndrome, sarcoidosis, or sciatica.

22. The use according to claim 1, wherein the medical device comprises any device for releasing the lysin or chimera thereof to the affected area, preferably a clamp or patch applied to the skin surface or sprays, devices that use microneedles to enhance skin penetration of lysin or their chimeras, fine needles used by cosmetic professionals to apply lysin or their chimeras specifically to acne-affected hair follicles, or other similar device.

23. The use according to claim 1, wherein the medical device comprises a prosthetic device that will immobilize the lysin or its chimera in a location susceptible to P. acnes infection, preferably for a particularly susceptible infection Prosthetic implants in shoulder surgery for Propionibacterium acnes.

24. A bacteriophage lysin chimera in the application of any one of claims 1 to 23, said chimera has an amino acid sequence shown in any one of SEQ ID NO:223~SEQ ID NO:282; preferably the amino acid sequence shown in SEQ ID NO:258, SEQ ID NO:259, SEQ ID NO:260, SEQ ID NO:271 or SEQ ID NO:272; more preferably the amino acid sequence shown in SEQ ID NO:260 or SEQ ID NO:271.

25. The chimera according to claim 24, wherein the chimera has a nucleotide sequence shown in any one of SEQ ID NO:283 ~ SEQ ID NO:342; preferably the nucleotide sequence shown in SEQ ID NO:316, SEQ ID NO:317, SEQ ID NO:318, SEQ ID NO:329 or SEQ ID NO:330; more preferably the nucleotide sequence shown in SEQ ID NO:318 or SEQ ID NO:329.

26. A method for preparing the chimera described in any one of claims 1 to 23 or any one of claims 24 to 25, wherein the method comprises:

(1) Synthetic domain sequences and primers for amplifying domain sequences;
(2) Using Taq DNA polymerase PCR to amplify the domain sequence;
(3) The PCR product was gel purified and ligated with the expression plasmid pET28
(4) Transfer the recombinant plasmid to Escherichia coli BL21 (DE3);
(5) Cultivate Escherichia coli BL21 (DE3) containing the recombinant plasmid, induce expression, collect the cells by centrifugation, lyse, and purify to obtain the chimera.

27. The method according to claim 26, further comprises:
Escherichia coli BL21 (DE3) containing recombinant chimeric lysin expression plasmid was cultured in self-inducing medium at 37°C and 300 rpm until the OD_{600} reached 0.6-0.8, and then at 18°C and 300 rpm for continuous cultivate

for 16-18 hours; collect the cells by centrifugation, resuspend in 50mM sodium phosphate pH 7.4, and homogeneously lyse under high pressure; centrifuge the lysate again to collect the soluble crude lysate. The soluble fraction was mixed with an equal volume of 5 M NaCl, and the mixture was loaded onto a hydrophobic column; after loading, the column was washed with 5 times the column volume of 20 mM sodium phosphate (pH 7.4), 2.5 M NaCl; then washed with 10 mM Sodium phosphate (pH 7.4) eluted the recombinant chimeric lysin.

28. preparations containing the bacteriosphage lysin or its chimera in application according to any one of claims 1 to 23, or a chimera according to any one of claims 24 to 25, wherein the preparations further comprise antibiotics, other lysin, or inactive excipients.

29. The amino acid sequence encoding the phage lysin or its chimera used in any one of claims 1 to 23, or the chimera described in any one of claims 24 to 25, or the chimera prepared in claim 26, or an amino acid sequence with a similarity of 80% and above, 85% and above, 90% and above, 95% and above, or 99% and above, or an alternative amino acid sequence with the same functional group.

30. The amino acid sequence according to claim 29, wherein the replacement amino acid sequence is a conservative substitution using amino acids in the same group of amino acids, the amino acid group comprising:

Aliphatic: glycine, alanine, valine, leucine or isoleucine;
Hydroxyl or sulfur/selenium containing: serine, cysteine, threonine or methionine;
Cyclic: proline;
Aromatic: phenylalanine, tyrosine or tryptophan;
Basic: histidine, lysine, or arginine; or
Acidic and their amides: aspartic acid, glutamic acid, asparagine, glutamine.

31. Nucleotide sequence encoding the phage lysin or its chimera in any one of claims 1 to 23, or the chimera of any one of claims 24 to 25, or the chimera prepared in claim 26, or its synonymous codon sequence.

32. The bacteriophage lysin or its chimera in the application of any one of claims 1 to 23, or the chimera described in any one of claims 24 to 25, or the chimera prepared by claim 26 is used for bacterial lysis application of reagents, wherein said bacterial lysates are used for DNA extracting and typing using a PCR-based kit.

33. The bacteriophage lysin or its chimera in the application of any one of claims 1 to 23, or the chimera of any one of claims 24 to 25, or the chimera prepared by claim 26 as a application of a disinfectant or sterilant on abiotic surfaces, wherein said disinfectant or sterilant prevents infection by removing P. acnes in planktonic form or in biofilms, preferably on surgical equipment during surgery or prosthetic implants.

34. Use of The phage lysin or its chimera in any one of claims 1 to 23, or the chimera of any one of claims 24 to 25, or the chimera prepared in claim 26, or a single binding domain in the above-mentioned molecule, or a series combination of similar or different binding domains in the above-mentioned molecules, in the preparation of a diagnostic tool for Propionibacterium acnes, wherein said the lysin, Chimera or binding domains are used in combination with signaling molecules.

35. The use according to claim 34, wherein the fusion of the lysin, chimera or binding domain and the signal molecule is formed by gene fusion or chemical coupling.

36. The use according to claim 35, wherein the fusion is used to directly detect Propionibacterium acnes on a microscope slide by fluorescence or other means, for marking Propionibacterium acnes by immunohistochemistry, and use as a detection reagent in ELISA assaysand on Western blot, for attachment to magnetic beads in MACS or other pull-down assays, or as a detection reagent in assays where antibodies are used as detection reagents.

37. The use according to claim 34, wherein the signal molecules include proteins or chemical fluorescent dyes, protein tags, enzymes, avidin, streptavidin, ovalbumin, biotin, para Click chemical label-sensitive tags, inteins, or other molecules that can cause the recruitment of secondary proteins or molecules that produce signals,

The fluorescent dyes include GFP, RFP, mCherry, FITC, TRITC, Alexafluor 488, Cy3 or Cy5;
The protein tag includes Flag-tag, myc-tag, halo-tag, his-tag, or any other tag that can be combined with antibodies or other high-affinity molecules to generate signals;

The enzymes include firefly luciferase, beta-lactamase, alkaline phosphatase, horseradish peroxidase, or any reaction that causes a reaction such as light, color change, substrate deposition, or other enzymes that can be detected in the assay.

38. The Use of bacteriophage lysin or its chimera of any one of claims 1 to 23, or the chimera of any one of claims 24 to 25, or the catalitic domain in the chimera prepared by method of claim 26in the preparation of medicines for the treatment of P. acnes infection, wherein the catalytic domain is combined with a targeting module.

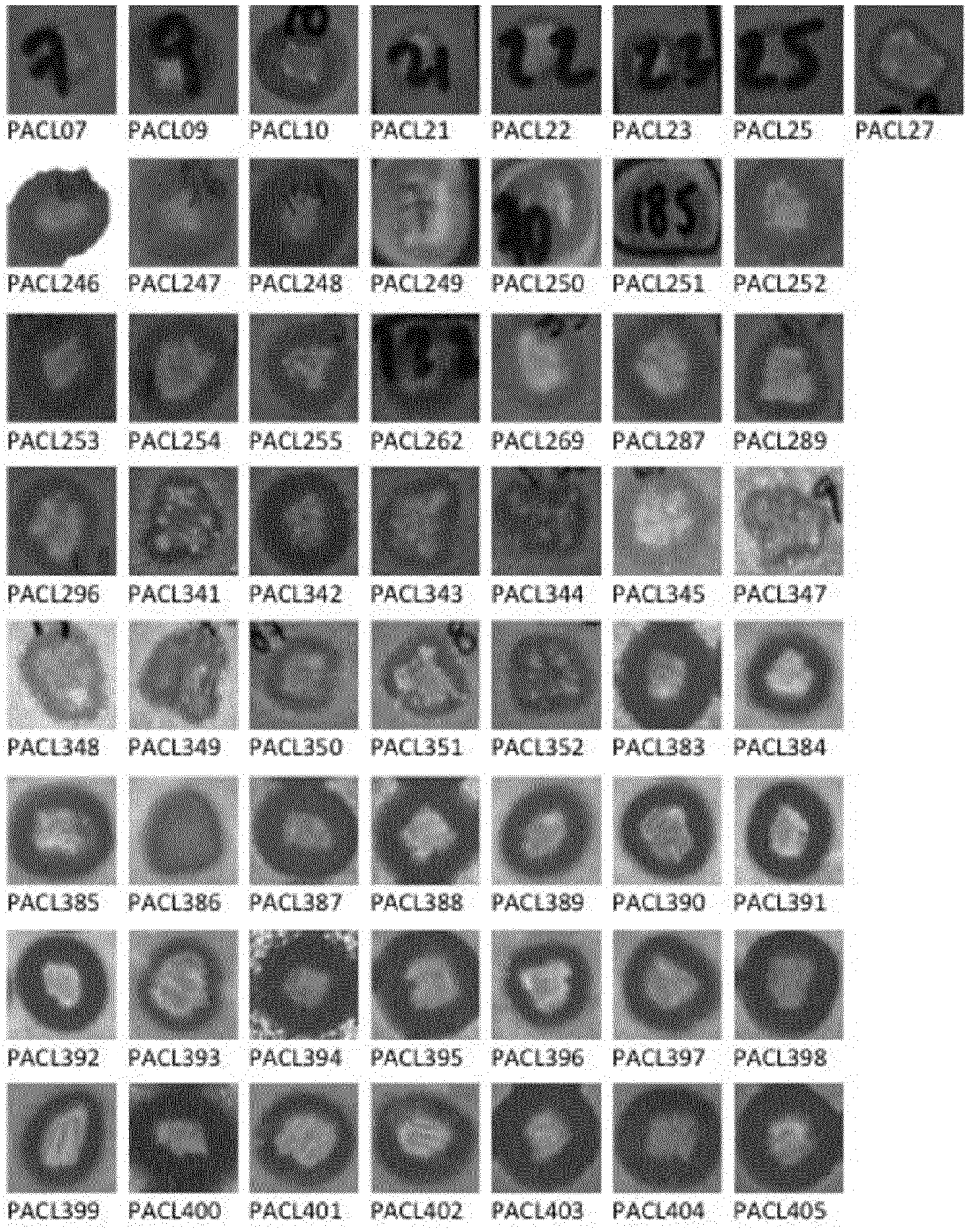
PACL07
PACL09
PACL10
PACL21
PACL22
PACL23
PACL25
PACL27
PACL246
PACL247
PACL248
PACL249
PACL250
PACL251
PACL252
PACL253
PACL254
PACL255
PACL262
PACL269
PACL287
PACL289
PACL296
PACL341
PACL342
PACL343
PACL344
PACL345
PACL347
PACL348
PACL349
PACL350
PACL351
PACL352
PACL383
PACL384
PACL385
PACL386
PACL387
PACL388
PACL389
PACL390
PACL391
PACL392
PACL393
PACL394
PACL395
PACL396
PACL397
PACL398
PACL399
PACL400
PACL401
PACL402
PACL403
PACL404
PACL405

Figure 1

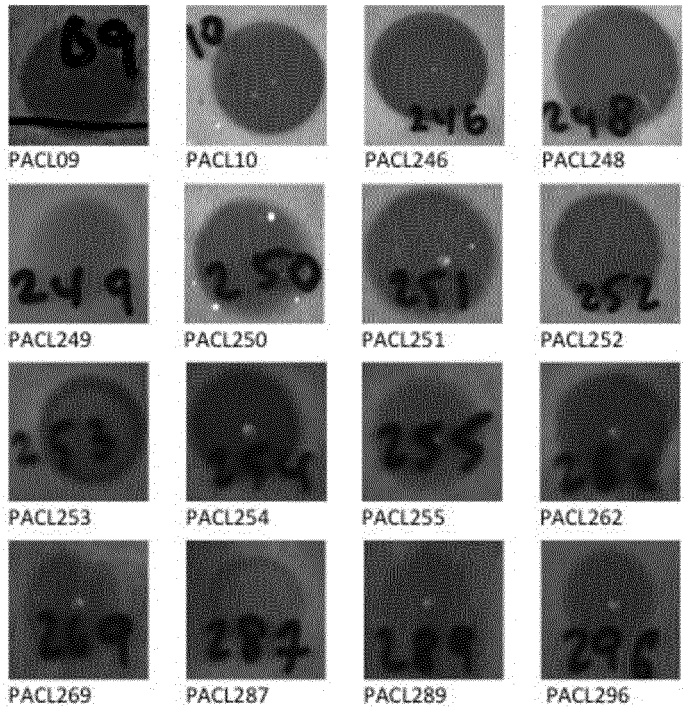

Figure 2

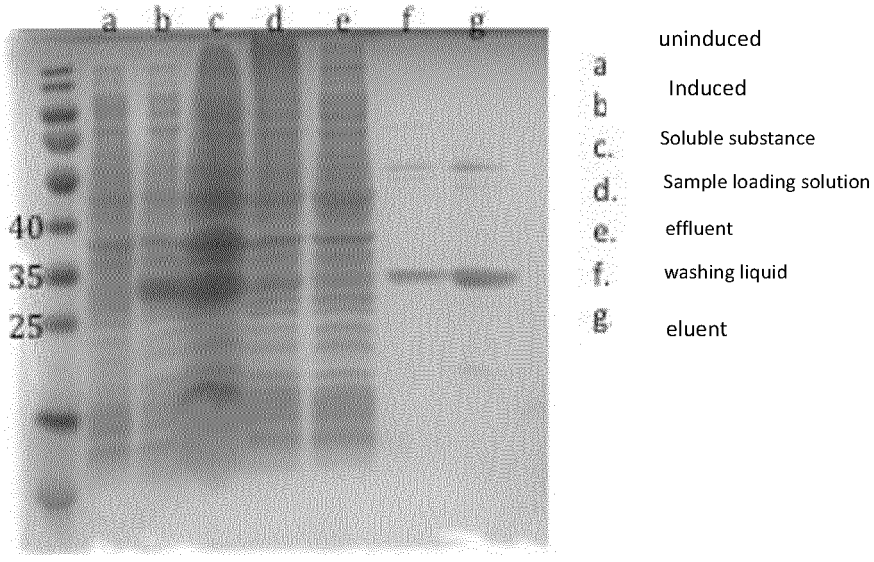

Figure 3

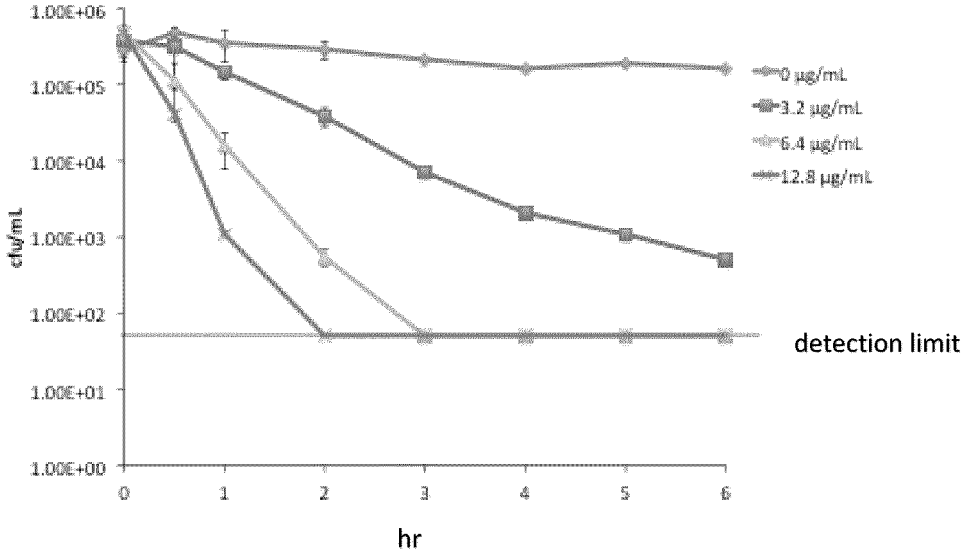

Figure 4

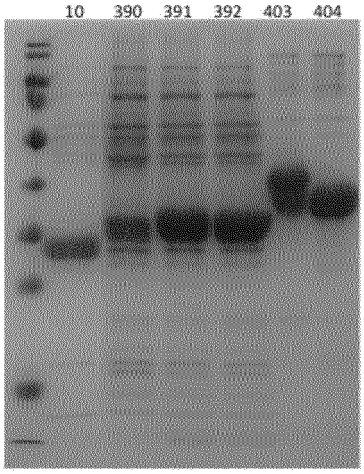

Figure 5

Figure 6

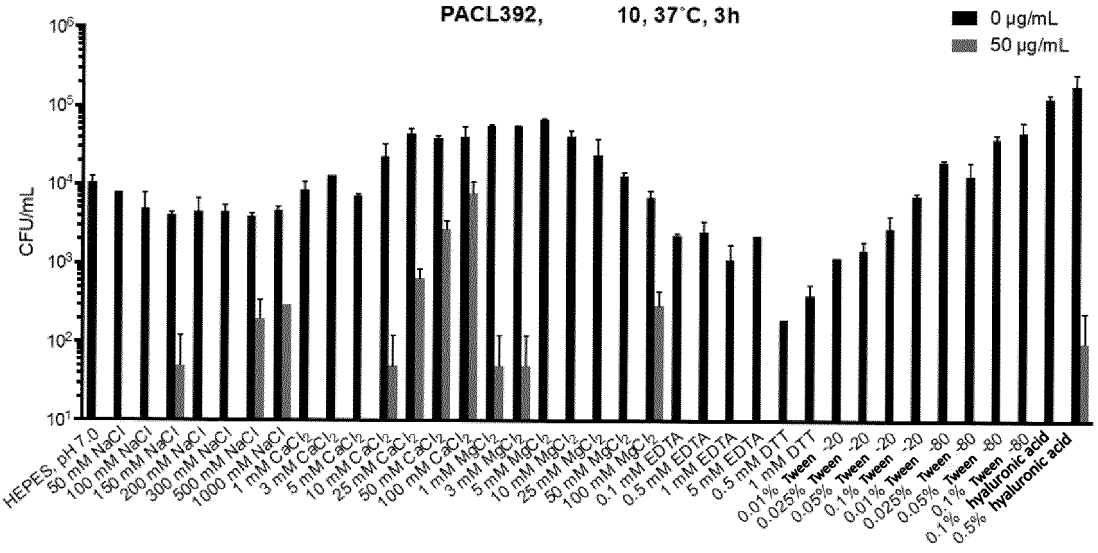

Figure 7

Figure 8

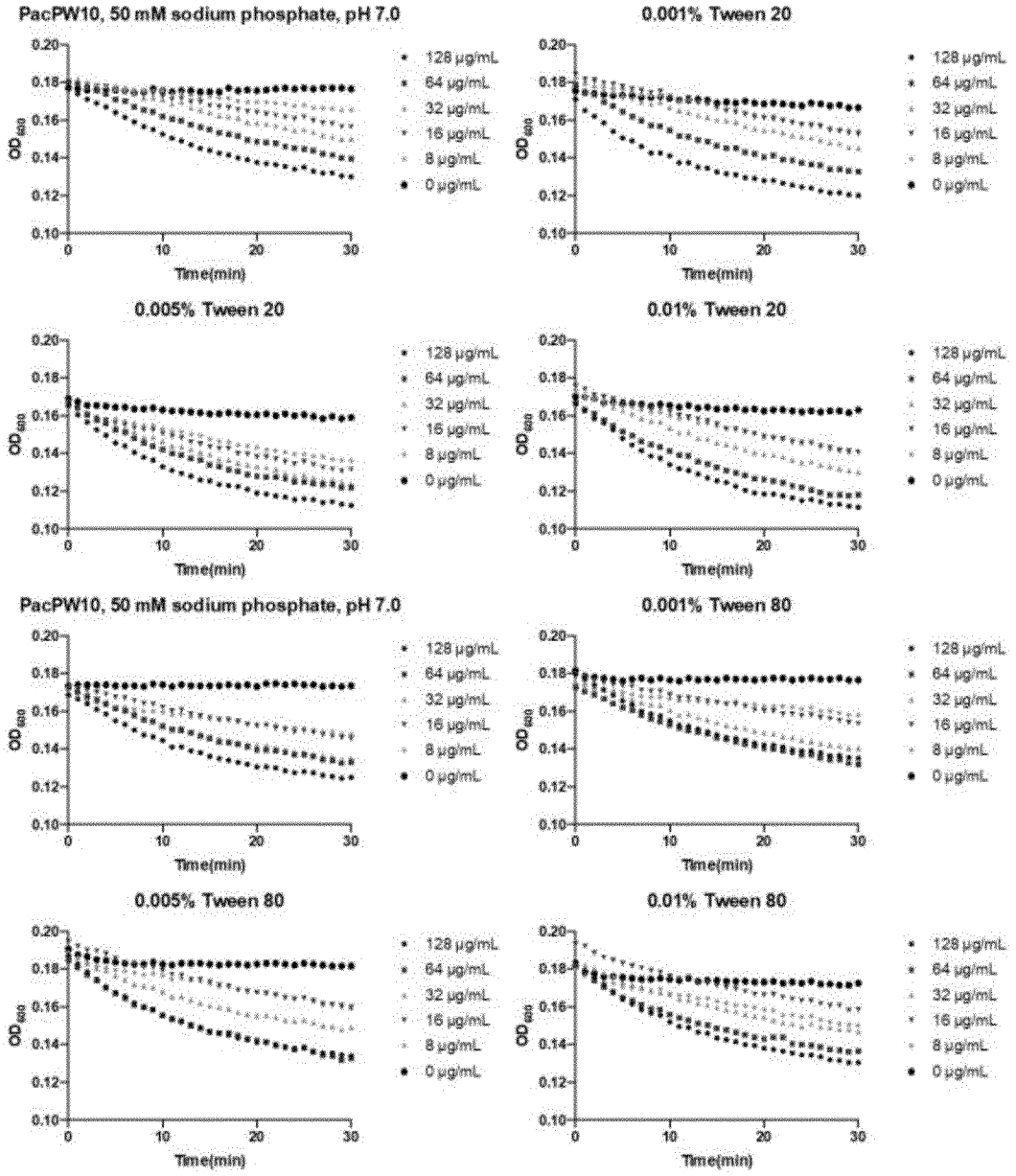
PacPW10, 50 mM sodium phosphate, pH 7.0
0.001% Tween 20
0.005% Tween 20
0.01% Tween 20
PacPW10, 50 mM sodium phosphate, pH 7.0
0.001% Tween 80
0.005% Tween 80
0.01% Tween 80
OD620
Time(min)
128 μg/mL
64 μg/mL
32 μg/mL
16 μg/mL
8 μg/mL
0 μg/mL

Figure 9

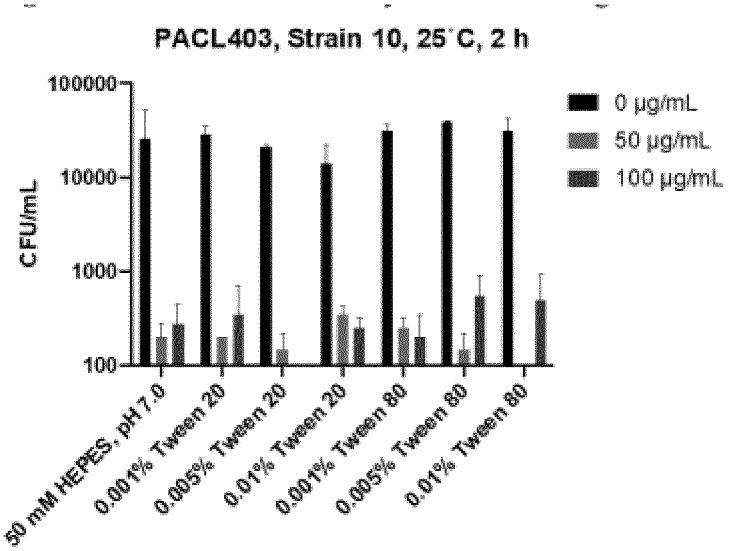

Figure 10

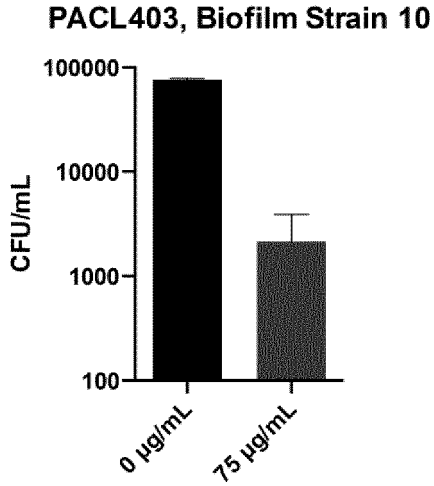

Figure 11

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/074221

A. CLASSIFICATION OF SUBJECT MATTER

C12N 9/88(2006.01)i; A61K 38/51(2006.01)i; A61P 31/04(2006.01)i; C12N 15/60(2006.01)i; A61K 35/76(2015.01)i; A61P 17/10(2006.01)i

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

C12N; A61K; A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CNABS, DWPI, SIPOABS, CNTXT, USTXT, EPTXT, WOTXT, CNKI, 万方数据库, WANFANG DATABASE, WEB OF SCIENCE, PUBMED: 噬菌体, 裂解酶, 内溶素, 嵌合, 痤疮, 痤疮丙酸杆菌, 类诺卡氏菌, 丙酸杆菌, phage, lytic enzymes, lysin, Endolysin, Chimeri+, acne, Cutibacterium acnes, Propionobacterium acnes, C.acne, Nocardioidaceae, Propionibacteriaceae; GenBank+EMBL+中国专利生物序列检索系统, GenBank+EMBL+Chinese Patent Biological Sequence Retrieval System: 对SEQ ID NO:260和SEQ ID NO:318的序列检索, search for SEQ ID NO: 260 and SEQ ID NO: 318

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	CN 1390134 A (NEW HORIZONS DIAGNOSTICS CORPORATION) 08 January 2003 (2003-01-08) see entire document	1-38 (all in part)
A	CN 103667230 A (INSTITUTE OF MICROBIOLOGY AND EPIDEMIOLOGY, THE ACADEMY OF MILITARY MEDICAL SCIENCES) 26 March 2014 (2014-03-26) see entire document	1-38 (all in part)
A	CN 101275146 A (ZHEJIANG SHUREN UNIVERSITY) 01 October 2008 (2008-10-01) see entire document	1-38 (all in part)
A	US 2013052182 A1 (MILLER, S. et al.) 28 February 2013 (2013-02-28) see entire document	1-38 (all in part)
A	NCBI. "GENBANK Accession number: WP_154563654.1" *GENBANK*, 06 October 2020 (2020-10-06), see sequence and feature parts	1-38 (all in part)

☑ Further documents are listed in the continuation of Box C. ☑ See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" earlier application or patent but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
18 April 2022	**28 April 2022**
Name and mailing address of the ISA/CN **China National Intellectual Property Administration (ISA/CN)** **No. 6, Xitucheng Road, Jimenqiao, Haidian District, Beijing 100088, China** Facsimile No. **(86-10)62019451**	Authorized officer Telephone No.

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/074221

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	NCBI. "GENBANK Accession number: WP_138574014.1" *GENBANK,* 17 October 2019 (2019-10-17), see sequence and feature parts	1-38 (all in part)
A	黄振华等 (HUANG, Zhenhua et al.). "噬菌体裂解酶结构特征、重组策略及其在控制食源性致病菌中的应用 (Structural Characteristics and Recombination Strategies of Phage Lysins and Their Application in Controlling Foodborne Pathogens)" *食品科学 (Food Science),* Vol. 42, No. 23, 20 November 2020 (2020-11-20), ISSN: 1002-6630, pp. 315-324, see entire document	1-38 (all in part)
A	CASTILLO, D. E. et al. "Propionibacterium (Cutibacterium) acnes Bacteriophage Therapy in Acne: Current Evidence and Future Perspectives" *DERMATOLOGY AND THERAPY,* Vol. 9, No. 1, 11 December 2018 (2018-12-11), ISSN: 2193-8210, pp. 19-31, see entire document	1-38 (all in part)

Form PCT/ISA/210 (second sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/074221

Box No. I Nucleotide and/or amino acid sequence(s) (Continuation of item 1.c of the first sheet)

1. With regard to any nucleotide and/or amino acid sequence disclosed in the international application, the international search was carried out on the basis of a sequence listing:
   a. ☑ forming part of the international application as filed:
      ☑ in the form of an Annex C/ST.25 text file.
      ☐ on paper or in the form of an image file.
   b. ☐ furnished together with the international application under PCT Rule 13*ter*.1(a) for the purposes of international search only in the form of an Annex C/ST.25 text file.
   c. ☐ furnished subsequent to the international filing date for the purposes of international search only:
      ☐ in the form of an Annex C/ST.25 text file (Rule 13*ter*.1(a)).
      ☐ on paper or in the form of an image file (Rule 13*ter*.1(b) and Administrative Instructions, Section 713).

2. ☐ In addition, in the case that more than one version or copy of a sequence listing has been filed or furnished, the required statements that the information in the subsequent or additional copies is identical to that forming part of the application as filed or does not go beyond the application as filed, as appropriate, were furnished.

3. Additional comments:

Form PCT/ISA/210 (continuation of first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/074221

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

[1] Invention 1: claims 1-5, 7, 9-23, and 28-38 (all in part), relating to a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 1 (a corresponding nucleotide sequence is SEQ ID NO: 29), a nucleotide sequence encoding the bacteriophage lytic enzyme, a preparation containing the bacteriophage lytic enzyme, and an application of the bacteriophage lytic enzyme;

[2] Invention 2: claims 1-5, 7, 9-23, and 28-38 (all in part), relating to a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 2 (a corresponding nucleotide sequence is SEQ ID NO: 30), a nucleotide sequence encoding the bacteriophage lytic enzyme, a preparation containing the bacteriophage lytic enzyme, and an application of the bacteriophage lytic enzyme;

[3] ...

[4] Invention 10: claims 1-5, 7, 9-23, 28-38 (all in part), 6, and 8, relating to a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 10 (a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 10 (a corresponding nucleotide sequence is SEQ ID NO: 38), a nucleotide sequence encoding the bacteriophage lytic enzyme, a preparation containing the bacteriophage lytic enzyme, and an application of the bacteriophage lytic enzyme;

[5] Invention 11: claims 1-5, 7, 9-23, and 28-38 (all in part), relating to a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 11 (a corresponding nucleotide sequence is SEQ ID NO: 39), a nucleotide sequence encoding the bacteriophage lytic enzyme, a preparation containing the bacteriophage lytic enzyme, and an application of the bacteriophage lytic enzyme;

[6] ...

[7] Invention 28: claims 1-5, 7, 9-23, and 28-38 (all in part), relating to a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 28 (a corresponding nucleotide sequence is SEQ ID NO: 56), a nucleotide sequence encoding the bacteriophage lytic enzyme, a preparation containing the bacteriophage lytic enzyme, and an application of the bacteriophage lytic enzyme;

[8] Invention 29: claims 1-38 (all in part), relating to a chimera of a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 223 (a corresponding nucleotide sequence is SEQ ID NO: 283), a method for preparing the chimera, a nucleotide sequence encoding the chimera, a preparation containing the chimera, and an application of the chimera;

[9] ...

[10] Invention 88: claims 1-38 (all in part), relating to a chimera of a bacteriophage lytic enzyme having an amino acid sequence shown as SEQ ID NO: 282 (a corresponding nucleotide sequence is SEQ ID NO: 342), a method for preparing the chimera, a nucleotide sequence encoding the chimera, a preparation containing the chimera, and an application of the chimera;

[11] Inventions 1-88 do not share a same or corresponding technical feature to form a special technical feature that makes a contribution over the prior art. Therefore, inventions 1-88 do not share a same or corresponding special technical feature, do not belong to a single general inventive concept, and thus lack unity, and do not comply with PCT Rule 13.1.

Form PCT/ISA/210 (continuation of first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/CN2022/074221

Box No. III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☑ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: **the portions of claims 1-38 (all in part) relating to a chimera of a bacteriophage lytic enzyme of SEQ ID NO: 260 (a corresponding nucleotide sequence is SEQ ID NO: 318), a method for preparing the chimera, a nucleotide sequence encoding the chimera, a preparation containing the chimera, and an application of an application of the chimera.**

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☐ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet) (January 2015)

INTERNATIONAL SEARCH REPORT
Information on patent family members

International application No.
PCT/CN2022/074221

Patent document cited in search report			Publication date (day/month/year)	Patent family member(s)			Publication date (day/month/year)
CN	1390134	A	08 January 2003	KR	20020043585	A	10 June 2002
				US	6056954	A	02 May 2000
				MX	PA02002920	A	19 April 2005
				IL	148651	D0	12 September 2002
				US	2002127212	A1	12 September 2002
				NZ	518058	A	30 July 2004
				TR	200201484	T2	21 February 2003
				US	6238661	B1	29 May 2001
				HK	1047888	A1	14 March 2003
				EP	1212080	A2	12 June 2002
				AU	7702100	A	17 April 2001
				ZA	200202305	B	24 December 2002
				BR	0013998	A	14 May 2002
				RU	2002109596	A	20 November 2003
				JP	2003509375	A	11 March 2003
				HU	0203523	A2	28 February 2003
				CA	2384881	A1	22 March 2001
				WO	0119385	A2	22 March 2001
				PL	354259	A1	29 December 2003
CN	103667230	A	26 March 2014	None			
CN	101275146	A	01 October 2008	None			
US	2013052182	A1	28 February 2013	BR	112012026880	A2	22 September 2015
				SG	184836	A1	29 November 2012
				JP	2013532955	A	22 August 2013
				US	2017173121	A1	22 June 2017
				IN	2493MUMNP2012	A	17 January 2014
				AU	2011247584	A1	11 October 2012
				CN	103119158	A	22 May 2013
				CA	2794603	A1	03 November 2011
				IL	222713	D0	31 December 2012
				TW	201204262	A	01 February 2012
				DK	2563916	T3	05 October 2015
				KR	20130100062	A	09 September 2013
				EA	201270774	A1	30 April 2013
				WO	2011134998	A1	03 November 2011
				EP	2563916	A1	06 March 2013
				MX	2012012345	A	20 May 2013
				JP	2016208985	A	15 December 2016

Form PCT/ISA/210 (patent family annex) (January 2015)

REFERENCES CITED IN THE DESCRIPTION

This list of references cited by the applicant is for the reader's convenience only. It does not form part of the European patent document. Even though great care has been taken in compiling the references, errors or omissions cannot be excluded and the EPO disclaims all liability in this regard.

Patent documents cited in the description

- CN 202110187030 **[0001]**
- CN 102482655 A **[0007]**