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- (73) Patenthaver: **Crown Laboratories, Inc., 349 Lafe Cox Drive, Johnson City, TN 37604, USA**
- (72) Opfinder: **HITCHCOCK, Thomas, 1873 Robinson Avenue, San Diego, California 92103, USA**
RHEE, Mun Su, Apt 118, 8775 Costa Verde Blvd., San Diego, California 92122, USA
- (74) Fuldmægtig i Danmark: **Budde Schou A/S, Dronningens Tværgade 30, 1302 København K, Danmark**
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DESCRIPTION

Description

Introduction

[0001] The present disclosure relates to a compositions comprising genetically modified microbes, methods of makings genetically modified microbes, and uses thereof. In some aspects subject matter described herein relates to the field of dermatology and compositions for topical dermatological purposes. The present invention is defined by the claims.

Summary

[0002] The invention provides genetically modified microbes. In a first aspect, the invention refers to a composition formulated for topical or mucosal administration to a mammal, the composition comprising a genetically modified *Propionibacterium acnes* (*P. acnes*) type II, ribotype 6 strain comprising a nucleic acid encoding a mammalian growth factor or a mammalian cytokine, wherein the nucleic acid includes a regulatory element that drives the expression of the mammalian growth factor or the mammalian cytokine in the *P. acnes* strain, the *P. acnes* strain comprises an endogenous CRISPR array, and the nucleic acid and regulatory element are integrated into the genome of the *P. acnes* strain.

[0003] In a preferred embodiment of the first aspect, the present invention, the mammalian growth factor is (a) a hormone; (b) a human or bovine hormone; (c) a somatotrophin, wherein preferably the somatotrophin comprises SEQ ID NO:45, SEQ ID NO:46, SEQ ID NO:47, SEQ ID NO:48 or SEQ ID NO:49; (d) secreted by the *P. acnes* strain; (e) a human growth factor; (f) selected from the group consisting of transforming growth factor beta (TGF- β), vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), fibroblast growth factor (FGF), insulin-like growth factor-1 (IGF1), platelet derived growth factor (PDGF), granulocyte monocyte colony stimulating factor (GMCSF) and epidermal growth factor (EGF); (g) a transforming growth factor comprising any one of SEQ ID NO:63 to SEQ ID NO:69, or a hepatocyte growth factor comprising SEQ ID NO:70; (h) a vascular endothelial growth factor (VEGF) comprising any one of SEQ ID NO:71 to SEQ ID NO:91; (i) a platelet derived growth factor (PDGF) comprising any one of SEQ ID NO:93 to SEQ ID NO: 102; (j) an epidermal growth factor (EGF) comprising any one of SEQ ID NO:103 to SEQ ID NO: 106; or (k) a fibroblast growth factor (FGF) comprising any one of SEQ ID NO: 107 to SEQ ID NO:144.

[0004] In another preferred embodiment of the first aspect, (a) the *P. acnes* strain secretes the

mammalian cytokine; (b) the mammalian cytokine is an immunosuppressive cytokine; (c) the mammalian cytokine is a human cytokine; (d) the cytokine is selected from the group consisting of interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-7 (IL-7) and interleukin-8 (IL-8), wherein preferably the cytokine is selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27 and SEQ ID NO:28; or (e) the mammalian cytokine comprises IL-10, wherein preferably the IL-10 comprises SEQ ID NO:25.

[0005] In another preferred embodiment of the first aspect, the nucleic acid comprises an inducible promoter configured to regulate the expression of the mammalian growth factor or the mammalian cytokine.

[0006] In another preferred embodiment of the first aspect, the nucleic acid (a) is under control of an endogenous promoter that directs expression of the mammalian growth factor or the mammalian cytokine; or (b) comprises an endogenous promoter configured to direct expression of the mammalian growth factor or the mammalian cytokine; wherein preferably the inducible promoter or endogenous promoter comprises a lacZ promoter or lacZ operon, or an ara operon promoter.

[0007] In another preferred embodiment of the first aspect, the nucleic acid encoding the mammalian growth factor or the mammalian cytokine is inserted into all or a part of an endogenous gene encoding a pathogenic protein.

[0008] In another preferred embodiment of the first aspect, expression of an endogenous protein is substantially reduced or eliminated.

[0009] In another preferred embodiment of the first aspect, expression of a pathogenic protein is substantially reduced or eliminated, wherein preferably the pathogenic protein comprises an endotoxin and/or an exotoxin, or the endogenous pathogenic protein comprises a glyceraldehyde 3-phosphate dehydrogenase (GADPH) protein or a CAMP protein.

[0010] In another preferred embodiment of the first aspect, the present invention refers to a composition comprising an inducible promoter regulating expression of an essential protein, wherein preferably (a) the essential protein is selected from the group consisting of a chromosome replication initiator protein DnaA, FtsA, FtsI, FtsL, FtsK, FtsN, FtsQ, FtsW, FtsZ, ZipA, aroE, atpD, gmk, guaA, lepA, recA, and sodA; (b) the inducible promoter is inducible by a sugar, preferably lactose or arabinose; (c) the inducible promoter is inducible by an amino acid; or (d) the inducible promoter is inducible by a synthetic amino acid.

[0011] In another preferred embodiment of the first aspect, the *P. acnes* strain has multiple genetic modifications, wherein the *P. acnes* strain has a modification in which 1) expression of an endogenous protein is substantially reduced or eliminated; and 2) an inducible promoter regulates expression of an essential protein.

[0012] In a second aspect, the present invention refers to a composition comprising a plurality

of genetically modified *P. acnes* strains of claim 1, wherein each of the *P. acnes* strains have multiple genetic modifications, and wherein the *P. acnes* strain has a modification in which 1) expression of an endogenous protein is substantially reduced or eliminated; and 2) an inducible promoter regulates expression of an essential protein.

[0013] In a third aspect, the present invention refers to a genetically modified *Propionibacterium acnes* (*P. acnes*) type II, ribotype 6 strain comprising: a) a mammalian growth factor or a human IL-10, wherein the mammalian growth factor or the IL-10 is secreted, respectively; and b) an inducible promoter directing the expression of an essential protein selected from the group consisting of a chromosome replication initiator protein DnaA, FtsA, FtsI, FtsL, FtsK, FtsN, FtsQ, FtsW, FtsZ, ZipA, aroE, atpD, gmk, guaA, lepA, recA, and sodA; wherein the expression of an endogenous CAMP2 and/or endogenous GADPH protein is substantially reduced or eliminated in the genetically modified *P. acnes* strain.

[0014] In a fourth aspect, the present invention refers to a composition comprising the genetically modified *P. acnes* type II, ribotype 6 strain of claims 12, wherein preferably the composition is (a) configured for topical or mucosal administration to a mammal; or (b) comprises a sugar or amino acid, wherein the inducible promoter is induced or stimulated by the presence of the sugar or amino acid.

[0015] In a fifth aspect, the present invention refers to a genetically modified *P. acnes* strain or composition of any one of claims 1-13 for use in a method of treating a skin or nail disorder.

Brief Description of Drawings

[0016] The drawings illustrate certain embodiments of the technology and are not limiting. For clarity and ease of illustration, the drawings are not made to scale and, in some instances, various aspects may be shown exaggerated or enlarged to facilitate an understanding of particular embodiments.

Fig. 1 shows a plasmid map of *dnaA-Bs-Cm* : A self-ligated DNA for construction of the growth arrest strain of *Bacillus subtilis*

Fig. 2 shows a plasmid map of *18-araRE-ftsOp-erm* : A plasmid for construction of the growth arrest strains of *P. acnes* I, Arabinose inducible *fts* operon.

Fig. 3 shows a plasmid map of *18-bgalpro-dnaA-erm-cm* : A plasmid for construction of the growth arrest strains of *P. acnes* II, Lactose inducible *dnaA*.

Fig. 4A shows a plasmid map of protein expression vector pET15-IL10 : An expression vector for IL-10.

Fig. 4B shows a plasmid map of protein expression vector pET15-EGF : An expression vector for EGF.

Fig. 4C shows a plasmid map of protein expression vector pET15-GH : An expression vector for GH.

Fig. 5 shows a plasmid map of *18-CAMP1I-erm-cm* : A plasmid for construction of CAMP1I mutant *P. acnes* strain.

Fig. 6 shows a plasmid map of *beta-gal pro-IL10* : A plasmid for construction of lactose inducible IL10 secretion *P. acnes* strain.

Fig. 7 shows a plasmid map of *19-CAMP1I-sig-IL10* : A plasmid for construction of CAMP1I mutant and IL-10 expression strain of *P. acnes*.

Fig. 8 shows a plasmid map of *19-sig-IL10-gapdh* : A plasmid for construction of GAPDH mutant and IL-10 expression strain of *P. acnes*.

Fig. 9 shows a scheme for construction of a markerless mutant construction.

Fig. 10 shows an alignment of the RecA gene from a type II strain of *P. acnes* (RecA ATCC 11828, SEQ ID NO: 274) and the RecA gene from a type I strains of *P. acnes* (RecA NCTC737, SEQ ID NO: 275). Shaded areas indicated nucleotide base identity at the same base position. Numbering shown is relative to the position of the sequence within the full length gene.

Fig. 11 shows an alignment of RecA genes sequenced from putative RT6 clones (RecA NCTC737, SEQ ID NO: 277; NC_017_41_RecA, SEQ ID NO: 278; NC_001_4RecA, SEQ ID NO: 279; NC_003_18_RecA, SEQ ID NO: 280; NC_005_31_RecA, SEQ ID NO: 281; NC_007_33_RecA, SEQ ID NO: 282; NC_009_34_RecA, SEQ ID NO: 283; NC_011_35_RecA, SEQ ID NO: 284; NC_015_39_RecA, SEQ ID NO: 285; NC_019_43_RecA, SEQ ID NO: 286; NC_021_44_RecA, SEQ ID NO: 287; NC_023_45_RecA, SEQ ID NO: 288; NC_025_46_RecA, SEQ ID NO: 289) with the region 720-1191 of the RecA gene from ATCC11828 strain (RecA 11828, SEQ ID NO: 276). Shaded areas indicated nucleotide base identity at the same base position.

Fig. 12 shows an alignment of an SNP sequence of 16S RNA of strain ATCC11828 of *P. acnes* (SEQ ID NO: 290) with SNP sequences isolated from the *P. acnes* strains isolated in Example 1 (PA_001_4_16S, SEQ ID NO: 291; PA_002_18_16S, SEQ ID NO: 292; PA_003_20_16S, SEQ ID NO: 293; PA_005_31_16S, SEQ ID NO: 294; PA_007_33_16S, SEQ ID NO: 295; PA_008_34_16S, SEQ ID NO: 296; PA_009_35_16S, SEQ ID NO: 297; PA_011_39_16S, SEQ ID NO: 298; PA_013_43_16S, SEQ ID NO: 299; PA_014_44_16S, SEQ ID NO: 300; PA_015_45_16S, SEQ ID NO: 301; PA_017_48_16S, SEQ ID NO: 302). Shaded areas indicated nucleotide base identity at the same base position.

Fig. 13 shows a protein expression of human IL-10 (lane 1, IL-10), human growth hormone (lane 2, GH) and human epidermal growth factor (lane 3, EGF) in the growth media of individual genetically modified *P. acnes* strains. The indicated proteins were expressed under the direction of inducible LacZ promoters.

Fig. 14 shows a scheme for construction of growth arrest *P. acnes* mutant strain. The DNA

region of arabinose inducible promoter and regulator was amplified from *B. subtilis* and the fragment of *fts* operon was ligated to the downstream of arabinose inducible promoter. This plasmid was introduced into *P. acnes* for single crossover recombination to construct arabinose regulated growth arrest strain.

Fig. 15 shows growth comparison of *P. acnes* wild type and growth arrest mutant strain. *P. acnes* wild type strain and growth arrest mutant strain were streaked onto the modified reinforced clostridium media with glucose (1%) and arabinose (1.5%) and incubated at 37°C under anaerobic condition. The colony sizes of these strains were checked on the 6th, 10th, and 15th days.

Fig. 16 shows expression of IL-10 (lane 2) in the growth media of a genetically modified *P. acnes* strain induced with 0.1 mM IPTG (isopropyl-beta-D-thiogalactopyranoside) for 1.5 hours. Pre-induced growth media is shown in lane 1.

Detailed Description

[0017] Unless otherwise indicated herein, the materials described in this section are not prior art to the claims in this application and are not admitted to be prior art by inclusion in this section.

[0018] Disclosed herein are genetically modified microbes and compositions comprising genetically modified microbes. Microbes, such as bacteria, can be genetically modified to express and/or secrete biomolecules that are beneficial to mammals. In certain cases genetically modified bacteria are further modified to substantially reduce, or eliminate, expression of harmful virulence factors (e.g., toxins or antigens). In some cases the growth and viability of such genetically modified microbes can be controlled/modulated by introduction of nucleic acids comprising inducible promoters that regulate the expression of proteins that are essential to the growth and/or survival of the modified microbes. In certain cases compositions comprising such genetically modified microbes are formulated for topical delivery to the skin of a mammal.

[0019] Microbes, or microorganisms, as used herein, refer to microscopic organisms, single cell organisms, or multicellular organisms. Examples of microbes include but are not limited to bacteria, archaea, protozoa, and fungi. In certain embodiments a microbe is a bacteria.

[0020] A "genetically modified organism" as used herein, refers to an organism in which the genetic material of the organism has been altered using genetic engineering techniques. The term genetically modified also refers to multiple genetic modifications, e.g. 2, 3, 4, 5, 6, 7, 8, 9, 10 or more genetic modifications, for example, a microbe which has an exogenous gene introduced for expression of a protein, and a modification, such as a gene knockout, which

reduces expression of an endogenous (microbe) gene encoding a pathogenic molecule (e.g., a pathogenic peptide or protein).

[0021] Microbes such as bacteria, eubacteria, yeast, fungi, can be genetically modified in order to produce one or more proteins, produce a biotherapeutic, alter metabolism, prevent overgrowth, and/or prevent expression of a biomolecule. In some cases a genetically modified organism is a genetically modified microbe. In some cases a genetically modified organism is a genetically modified bacteria. In certain embodiments a *P. acnes* bacteria is genetically modified. Those skilled in the art will appreciate that there are multiple ways to produce a genetically modified organism such as by producing a gene knockout, by introducing heterologous nucleic acids and/or by generating mutations.

[0022] In some cases a nucleic acid (e.g., a gene, or portion thereof) is introduced into a microbe using a suitable technique. In some cases a microbe is transformed with a nucleic acid by a suitable technique. Non-limiting examples of suitable techniques for introducing a nucleic acid into a microbe include electroporation, transduction (e.g., injection of a nucleic acid by a bacteriophage), microinjection, by inducing competence (e.g., by addition of alkali cations, cesium, lithium, polyethylene glycol or by osmotic shock), the like or combinations thereof. A nucleic acid can be introduced into a microbe in the form of a linear or circular plasmid, for example. In some cases transformed microbes are selected for integration of a nucleic acid into the genome of the microbe by using a suitable selection method (e.g., a selection marker).

[0023] A gene encodes a specific protein, which after expression via transcription and translation fulfills a specific biochemical function within a living microbe. A gene sometimes includes segments of DNA involved in producing a polypeptide chain and sometimes includes regions preceding and following a coding region (e.g., an open reading frame) involved in the transcription/translation of a gene product and the regulation of the transcription/translation. An essential gene is an endogenous gene (e.g., endogenous to a microbe) that produces a polypeptide (e.g., an essential protein) that is necessary for the growth and/or viability of a microbe. Non-limiting examples of essential proteins of a bacteria include DnaA, FtsA, FtsI, FtsL, FtsK, FtsN, FtsQ, FtsW, FtsZ, ZipA, aroE, atpD, gmk, guaA, lepA, recA, and sodA.

[0024] A "gene knockout" as used herein, refers to a combination of genetic techniques that can render a specific gene inoperable or inactive. In some cases a gene knockout reduces or eliminates expression of a polypeptide from the gene. In certain cases the expression of gene is substantially reduced or eliminated. Substantially reduced means that the expression of a gene is reduced by at least 80%, at least 90%, at least 95% or at least 98% when compared to an endogenous level of expression of a gene. Expression of a gene can be determined by a suitable technique (e.g., by measuring transcript or expressed protein levels). Any suitable technique can be used to generate a gene knockout in a microbe (e.g., a bacteria). Gene knockouts in microbes can be made by transposon mutagenesis, *in vitro* genetic engineering to modify genes contained on plasmids or Bacterial Artificial Chromosomes (BACs) and moving the modified construct to the organism of interest, *in vivo* homologous recombination, and other techniques that are known to those skilled in the art. In certain cases a gene is knocked

out by disabling an endogenous promoter, operon or regulatory element that is essential for transcription or translation of a gene. In some cases a gene is knocked out by introducing one or more mutations that disable the function of a protein expressed from a gene. In certain implementations a gene is partially or completely removed from the genome of a microbe. An endogenous gene can be knocked out by replacing the gene with a different gene (e.g., a heterologous gene, or non-functional gene).

[0025] In some cases, microbes are genetically modified to prevent secretion of a pathogenic molecule (e.g., a pathogenic peptide or protein). A pathogenic molecule can be a toxic molecule (e.g., a toxin). In certain cases a gene encoding a pathogenic molecule and/or a toxic molecule is knocked out. In certain cases a bacteria is genetically modified to substantially reduce or eliminate the expression of a pathogenic molecule or a toxic molecule. Non-limiting examples of toxic molecules include bacterial endotoxins, bacterial exotoxins and bacterial antigens. A bacterial antigen is any bacteria derived molecule (e.g., compound or protein) that induces an immune response in a mammal. In some cases a pathogenic molecule is a Christie-Atkins-Munch-Petersen (CAMP) factor (e.g., a CAMP factor of *P. acnes*). In certain cases one or more CAMP factors (e.g., CAMP1, CAMP2, CAMP3, CAMP4 and/or CAMPS) of *P. acnes* are knocked out. In certain cases a *P. acnes* bacteria is genetically modified to substantially reduce or eliminate the expression of one or more CAMP factors. In some cases a pathogenic molecule is a bacterial glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (e.g., a GAPDH of *P. acnes*). In certain cases one or more GAPDH genes of *P. acnes* are knocked out. In certain embodiments a *P. acnes* bacteria is genetically modified to substantially reduce or eliminate the expression of GAPDH.

[0026] In some cases, microbes are genetically modified to create nutritional auxotrophs. In some cases, microbes are genetically modified to prevent acceptance of genetic material. In some cases, microbes naturally or are genetically modified to prevent donation of genetic material. In some cases, the gene for Competence Protein ComEA is modified to prevent donation of genetic material. In some cases, the gene for Competence Protein ComEA is modified to prevent acceptance of genetic material. In some cases, the gene for Competence Protein ComFA is modified to prevent donation of genetic material. In some cases, the gene for Competence Protein ComFA is modified to prevent acceptance of genetic material. In some cases, the gene for Competence Protein ComA is modified to prevent donation of genetic material. In some cases, the gene for Competence Protein ComA is modified to prevent acceptance of genetic material. In some cases, the gene for Competence Protein ComK is modified to prevent donation of genetic material. In some cases, the gene for Competence Protein ComK is modified to prevent acceptance of genetic material. In certain cases a microbe comprises a CRISPR array, or a CRISPR array is introduced into a microbe (e.g., bacteria) to prevent or reduce acceptance of foreign genetic material from other microbes.

[0027] Genetic modification of the microbes can also be performed to introduce non-functional, and non-detrimental changes to the genome, in which there is no detrimental phenotype to the microbe. A "genetic marker" as described herein, refers to an introduced non-functional sequence in a genome in order to detect the presence of a genetically modified organism or

microbe. Any suitable genetic marker can be incorporated into a genetically modified microbe using a suitable technique. Genetic modification can be performed in order to catalog and distinguish genetically modified microbes from wild type microbes, and in order to determine the presence of a genetically modified microbe in an environment. The genetically modified microbe can be determined in an environment by swabbing the source containing the suspected genetically modified microbe, growing up the microbe in a minimal media culture, obtaining the genetic material, using qPCR with specific primers to the sequence of interest, and sequencing the DNA of the genetically modified microbe. The use of genetic modification to introduce a specific genetic tag can allow one to catalog and determine the presence of genetically modified microbes on a subject of need. Furthermore, the techniques can allow one to determine that the genetically modified microbe is absent from the environment when the microbe is no longer needed. In some cases, microbes are genetically modified to carry a marker for determining the presence or absence of a genetically modified microbe on a host. Markers for determining the propagation of genetically modified bacteria can also be used to analyze the microflora of the subject to ensure that the bacteria are balanced during treatment.

[0028] Genetically modified microbes can be used to secrete biomolecules (e.g., proteins) to treat disorders in a subject in need. Genetically modified microbes can be used to treat subjects in need suffering from a skin disorder. Genetically modified microbes carrying nucleic acid for the controlled expression of peptides can be advantageous as they can move on the skin and propagate deep into the pores and hair follicles allowing absorption of secreted biomolecules. The microbes can be formulated for and used for general cosmesis, for example.

[0029] In several cases described herein, genetically modified microbes can be used to treat subjects suffering from a genetic disorder. In several cases, genetically modified microbes can be used to treat subjects suffering from gastric diseases. In several cases herein, genetically modified microbes can be used to treat subjects suffering from autoimmune diseases. In several cases, genetically modified microbes can be used to treat subjects treated with anticoagulants. In several cases, genetically modified microbes can be used to treat subjects suffering from hemophilia. Genetically modified microbes including nucleic acids for peptides for treatment can be used in conjunction in order to meet the needs of a subject in need. In some cases, a second genetically modified microbe can be used to treat a subject in need. In some cases, a third genetically modified microbe can be used to treat a subject in need. In some cases, more than three genetically modified microbes can be used to treat a subject in need.

[0030] In certain cases a genetically modified microbe comprises a nucleic acid (e.g., a gene) where expression of the gene is regulated by a promoter. A promoter is often introduced at a suitable location relative to a gene of interest. For example, a promoter (e.g., an inducible promoter) is often placed 5' of a transcription start site of a gene of interest. In certain cases a nucleic acid includes a promoter and/or regulatory elements necessary to drive the expression of a gene (e.g., a heterologous gene or an endogenous gene). A promoter can be an endogenous promoter, a heterologous promoter or a combination thereof. In some cases a

promoter is a constitutive promoter (e.g., a T7, SP6, T3, or any suitable constitutive promoter). In some cases a microbe is genetically altered to include a gene (e.g., a gene of interest, an essential gene) under the control of an inducible promoter. An inducible promoter is often a nucleic acid sequence that directs the conditional expression of a gene. An inducible promoter can be an endogenous promoter, a heterologous promoter, or a combination thereof. An inducible promoter can comprise an operon system. An inducible promoter is often configured to regulate the expression of a gene (e.g., a gene of interest, an essential gene). In certain cases an inducible promoter comprises one or more genes, regulatory elements and/or gene products (e.g., a inducible system). In some cases an inducible promoter requires the presence of a certain compound, nutrient, amino acid, sugar, peptide, protein or condition (e.g., light, oxygen, heat, cold) to induce gene activity (e.g., transcription). In certain case an inducible promoter comprises one or more repressor elements. In some cases an inducible promoter (e.g., a promoter comprising a repressor element) requires the absence of a certain compound, nutrient, amino acid, sugar, peptide, protein or condition to induce gene activity (e.g., transcription). Any suitable inducible promoter, system or operon can be used to regulate the expression of a gene (e.g., an essential gene). Non-limiting examples of inducible promoters include lactose regulated systems (e.g., lactose operon systems), sugar regulated systems, metal regulated systems, steroid regulated systems, alcohol regulated systems, IPTG inducible systems, arabinose regulated systems (e.g., arabinose operon systems, e.g., an ARA operon promoter, pBAD, pARA, P_{ARA}E, ARAE, ARA-R-P_{ara}E, portions thereof, combinations thereof and the like), synthetic amino acid regulated systems (e.g., see Rovner AJ, et al., (2015) *Nature* 518(7537):89-93), fructose repressors, a tac promoter/operator (pTac), tryptophan promoters, PhoA promoters, recA promoters, proU promoters, cst-1 promoters, tetA promoters, cadA promoters, nar promoters, P_L promoters, cspA promoters, the like or combinations thereof. In certain cases a promoter comprises a Lac-Z (LacZ) promoter, or portions thereof. In some cases a promoter comprises a Lac operon, or portions thereof. In some cases a inducible promoter comprises an ARA operon promoter, or portions thereof. In certain cases an inducible promoter comprises an arabinose promoter or portions thereof. An arabinose promoter can be obtained from any suitable bacteria. In certain cases an inducible promoter comprises an arabinose operon of *E.coli* or *B.subtilis*. In certain cases an inducible promoter is activated by the presence of a sugar or an analog thereof. Non-limiting examples of sugars and sugar analogs include lactose, arabinose (e.g., L-arabinose), glucose, sucrose, fructose, IPTG, and the like.

[0031] The use of microbes for the production of biomolecules can also be controlled for a measure of safety. In some cases a genetically modified microbe is engineered as a nutritional auxotroph where the growth and/or survival of the microbe depends on the presence of an essential nutrient. In some cases a composition herein comprises such an essential nutrient. Nutritional auxotrophs can be controlled by the depletion of supplied nutrients in order to prevent microbe overgrowth, or to remove the microbe from the environment. For example, for Trp auxotrophs, the supply for tryptophan can be removed or depleted in order to slow the microbe growth or to remove the microbe from the environment completely. In the case of Lys auxotrophs, for example, the supplied lysine can be removed from the environment to slow the growth of the Lys auxotroph or to remove it completely from the environment. In certain cases

a composition comprises an amino acid such as lysine (Lys) or tryptophan (Trp). In some cases, the supplied nutrient can be applied as a compound in a revitalizing topical composition in order to maintain the microbe in the environment as needed.

[0032] Disclosed herein are methods for making a nucleic acid for controlled expression of a peptide for treatment. Gene transcripts for the peptide for treatment can be synthesized through standard molecular cloning techniques known to those skilled in the art and can be used to transform microbes to carry the encoded gene transcript of interest. In certain cases an inducible promoter comprises an operon. In some cases, a nucleic acid includes an operon sequence. An operon can be used to control expression of the gene transcript or the expression of a peptide for treatment at the DNA level. In some cases, the operon is a lac operon. In some cases, the operon is a Trp operon. In some cases, the operon is a repressor operon. Repressor operons as described herein, refers to operons that are controlled by a repressor, or a DNA or RNA binding protein, that can inhibit the expression of one or more genes by binding to the operator, or operon. In a lac operon, the gene is turned off if there is a level of lactose that can bind to the operator and inhibit the RNA polymerase from binding, thus decreasing transcription of the gene of interest. The trp operon is also a repressor operon that works through a negative repressible feedback mechanism. The repressor for the trp operon is tryptophan, which can bind to the operator and prevent transcription of the gene. By controlling the production of the gene of interest by an operon, one can control the level of production of the peptide of treatment by supplying the microbes with the repressor molecule to control the level of secreted biomolecules that are being produced. In some cases, the expression of peptide for treatment can be repressed by adding a repressor molecule. In some cases, the repressor molecule is tryptophan. In some cases, the repressor molecule is lactose.

[0033] Biomolecules as described herein, refer to any type of molecule that is produced by a living organism. The biomolecules can include but are not limited to macromolecules, proteins, sugars, polysaccharides, lipids, nucleic acids, peptides, metabolites, glycolipids, sterols, growth factors, hormones, glycerolipids, vitamins, neurotransmitters, metabolites, enzymes, monomers, oligomers, and polymers. Biomolecules can be produced by microbes. In certain embodiments a gene of interest encodes a biomolecule. In several cases described herein, methods are described in which genetically modified microbes secrete a biomolecule. In several cases, methods are described in which genetically modified microbes carry an enzyme that catalyzes the production of a biomolecule. In several cases, methods are described in which genetically modified microbes carry an enzyme that catalyzes the production hyaluronic acid. In several cases, methods are described in which genetically modified microbes carry an enzyme that catalyzes the production of melanin. Different types of biomolecules can be used to treat subjects in need. In some cases, the subject suffers from skin disorders, genetic disorders, diseases, autoimmune disease, gastric disease, aging damage, and hemophilia. In some cases a subject or a subject in need suffers from acne.

[0034] In certain cases a microbe is genetically modified to express and/or secrete a growth factor. A growth factor can be a mammalian growth factor (*e.g.*, a human growth factor).

[0035] Growth factors are naturally occurring biomolecules that are capable of initiating and stimulating cellular growth, causing cell signaling, proliferation, healing, and the differentiation of cells. Growth factors can be a protein or a peptide. In some cases a growth factor is a hormone (e.g., a mammalian hormone). In certain cases a bacteria is genetically modified by introduction of a nucleic acid that encodes one or more growth factors (e.g., a mammalian growth factors). A nucleic acid that encodes a growth factor may include a suitable promoter and/or regulatory elements that drive the transcription and/or translation (e.g., expression) of the growth factor, an open reading frame that encodes the growth factor and, in some cases, suitable nucleic and/or peptide elements that direct microbial secretion of the growth factor. In certain cases a bacteria is genetically modified by introduction of two or more nucleic acids that encode two or more growth factors (e.g., a mammalian growth factors). In certain cases a bacteria is genetically modified by introduction of a nucleic acid that directs the expression of one or more growth factors (e.g., a mammalian growth factors). In certain cases a bacteria is genetically modified to express and/or secrete a growth factor (e.g., a mammalian growth factor).

[0036] Several examples of growth factors include but are not limited to vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), adrenomedullin (AM), angiopoietin (Ang), autocrine motility factor, bone morphogenetic proteins (BMPs), brain-derived neurotrophic factor (BDNF), epidermal growth factor (EGF), erythropoietin (EPO), fibroblast growth factor (FGF), glial cell line-derived neurotrophic factor (GDNF), granulocyte colony-stimulating factor (G-CSF), granulocyte macrophage colony-stimulating factor (GM-CSF), growth differentiation factor-9 (GDF9), healing factor, hepatocyte growth factor (HGF), hepatoma-derived growth factor (HDGF), insulin-like growth factor (IGF), migration-stimulating factor, myostatin (GDF-8), nerve growth factor (NGF) and other neurotrophins, platelet-derived growth factor (PDGF), thrombopoietin (TPO), transforming growth factor alpha(TGF- α), transforming growth factor beta(TGF- β), tumor necrosis factor-alpha(TNF- α), vascular endothelial growth factor (VEGF), Wnt signaling pathway, placental growth factor (PGF), fetal bovine somatotrophin (FBS), IL-1- Co-factor for IL-3 and IL-6, IL-2- T-cell growth factor, IL-3, IL-4, IL-5, IL-6, and IL-7. In some cases, the genetically modified microbe (e.g., a bacteria) secretes a growth factor.

[0037] Transforming growth factor beta is a hormone that controls proliferation, differentiation and other functions in many cell types. Many cells synthesize TGFB1 and have specific receptors for it. It positively and negatively regulates many other growth factors. It plays an important role in bone remodeling as it is a potent stimulator of osteoblastic bone formation, causing chemotaxis, proliferation and differentiation in committed osteoblasts. In some cases the peptide for treatment includes transforming growth factor beta. TGFB can be used for general cosmesis, aging damage, general aging of tissue to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the transforming growth factor beta includes SEQ ID NO: 63, SEQ ID NO: 64, SEQ ID NO: 65, SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68 or SEQ ID NO: 69.

[0038] Hepatocyte growth factor/scatter factor (HGF/SF) is a hormone for paracrine cellular growth, motility and morphogenic factor. It is secreted by mesenchymal cells and targets and can act primarily on epithelial and endothelial cells, but also acts on hematopoietic progenitor cells. It has been shown to have a major role in embryonic organ development, specifically in myogenesis, in adult organ regeneration and in wound healing.

[0039] For example, receptor tyrosine-protein kinase (MET) can transduce signals from the extracellular matrix into the cytoplasm by binding to hepatocyte growth factor/HGF ligand. This process can regulate many physiological processes including proliferation, scattering, morphogenesis and survival. Ligand binding at the cell surface induces autophosphorylation of MET, on its intracellular domain that provides docking sites for downstream signaling molecules. Following activation by ligand, there are interactions with the PI3-kinase subunit PIK3R1, PLCG1, SRC, GRB2, STAT3 or the adapter GAB 1. Recruitment of these downstream effectors by MET leads to the activation of several signaling cascades including the RAS-ERK, PI3 kinase-AKT, or PLC gamma-PKC cascades. The RAS-ERK activation is associated with the morphogenetic effects, while PI3K/AKT coordinates pro-survival effects. During embryonic development, MET signaling plays a role in gastrulation, development and migration of muscles and neuronal precursors, angiogenesis and kidney formation. In adults, the cascade participates in wound healing, as well as organ regeneration and tissue remodeling.

[0040] Hepatocyte growth factor can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. The process can also promote differentiation and proliferation of hematopoietic cells. In some cases, the peptide for treatment includes hepatocyte growth factor. In some cases, the hepatocyte growth factor includes SEQ ID NO: 70.

[0041] Vascular endothelial growth factor (VEGF) is a signal protein that is produced by cells to stimulate vasculogenesis and angiogenesis. It is part of the system that restores the oxygen supply to tissues when blood circulation is inadequate. VEGF's normal function is to create new blood vessels during embryonic development, new blood vessels after injury, muscle following exercise, and new vessels to bypass blocked vessels. VEGFs can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin.

[0042] VEGF-A is a growth factor active in angiogenesis, vasculogenesis and endothelial cell growth. VEGF-A induces endothelial cell proliferation, promotes cell migration, inhibits apoptosis and induces permeabilization of blood vessels. VEGF-A binds to FLT1/VEGFR1 and KDR/VEGFR2 receptors, heparin sulfate, and heparin. NRP1/Neuropilin-1 binds isoforms VEGF-165 and VEGF-145. Isoform VEGF165B binds to KDR but does not activate downstream signaling pathways, does not activate angiogenesis, and inhibits tumor growth. In some cases, the peptide for treatment includes VEGF- A. VEGF-A can include SEQ ID NO: 71, SEQ ID NO: 72, SEQ ID NO: 73, SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 76, SEQ ID NO: 77, SEQ ID NO: 78, SEQ ID NO: 79, SEQ ID NO: 80, SEQ ID NO: 81, SEQ ID NO: 82, SEQ ID NO: 83, SEQ ID NO: 84, SEQ ID NO: 85, SEQ ID NO: 86, or SEQ ID NO: 87.

[0043] VEGF-B is a growth factor for endothelial cells. VEGF-B167 binds heparin and neuropilin-1 whereas the binding to neuropilin-1 of VEGF-B186 is regulated by proteolysis. In some cases, the peptide for treatment includes VEGF-B. VEGF-B can include SEQ ID NO: 88 or SEQ ID NO: 89.

[0044] VEGF-C is a growth factor active in angiogenesis, and endothelial cell growth, stimulating their proliferation and migration and also has effects on the permeability of blood vessels. VEGF-C can function in angiogenesis of the venous and lymphatic vascular systems during embryogenesis, and also in the maintenance of differentiated lymphatic endothelium in adults. VEGF-C can binds and activate VEGFR-2 (KDR/FLK1) and VEGFR-3 (FLT4) receptors. In some cases, the peptide for treatment includes VEGF-C. VEGF-C can include SEQ ID NO: 90.

[0045] VEGF-D (c-Fos-induced growth factor, or FIGF) is a growth factor active in angiogenesis, lymphangiogenesis and endothelial cell growth, stimulating their proliferation and migration and also has effects on the permeability of blood vessels. VEGF-D can function in the formation of the venous and lymphatic vascular systems during embryogenesis, and also in the maintenance of differentiated lymphatic endothelium in adults. VEGF-D can bind and activate VEGFR-2 (KDR/FLK1) and VEGFR-3 (FLT4) receptors. In some cases, the peptide for treatment includes VEGF-D. VEGF-D can include SEQ ID NO: 91.

[0046] Placental growth factor (PGF) is a member of the VEGF family, and can play a role in angiogenesis and vasculogenesis, during embryogenesis. PGF is active in angiogenesis and endothelial cell growth, stimulating their proliferation and migration. It binds to the receptor FLT1/VEGFR-1. Isoform PlGF-2 binds NRP1/neuropilin-1 and NRP2/neuropilin-2 in a heparin-dependent manner. PGF can also promote cell tumor growth. PGF can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases the peptide for treatment includes PGF. In some cases, PGF includes SEQ ID NO: 92.

[0047] Platelet-derived growth factor subunit A (PDGFA) plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. PDGFA is a potent mitogen for cells of mesenchymal origin. PDGFA is required for normal lung alveolar septum formation during embryogenesis, normal development of the gastrointestinal tract, normal development of Leydig cells and spermatogenesis. PDGFA is required for normal oligodendrocyte development and normal myelination in the spinal cord and cerebellum. PDGFA plays an important role in wound healing. Signaling can also be modulated by the formation of heterodimers with PDGFB. PDGFA can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the peptide for treatment includes PDGFA . PDGFA can include SEQ ID NO: 93 or SEQ ID NO: 94.

[0048] Platelet-derived growth factor subunit B (PDGFB) plays an essential role in the

regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. PDGFB is a potent mitogen for cells of mesenchymal origin. PDGFB is required for normal proliferation and recruitment of pericytes and vascular smooth muscle cells in the central nervous system, skin, lung, heart and placenta. PDGFB is required for normal blood vessel development, and for normal development of kidney glomeruli. PDGFB plays an important role in wound healing. Signaling can also be modulated by the formation of heterodimers of PDGFB with PDGFA. PDGFB can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the peptide for treatment includes PDGFB. PDGFB can include SEQ ID NO: 95 or SEQ ID NO: 96.

[0049] Platelet-derived Growth Factor C (PDGFC) plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. PDGFC is a potent mitogen and chemo-attractant for cells of mesenchymal origin. PDGFC is required for normal skeleton formation during embryonic development, especially for normal development of the craniofacial skeleton and for normal development of the palate. PDGFC is required for normal skin morphogenesis during embryonic development. PDGFC plays an important role in wound healing, where it appears to be involved in three stages: inflammation, proliferation and remodeling. PDGFC plays an important role in angiogenesis and blood vessel development and is involved in fibrotic processes, in which transformation of interstitial fibroblasts into myofibroblasts plus collagen deposition occurs. The CUB domain of PDGFC has a mitogenic activity in coronary artery smooth muscle cells, suggesting a role beyond the maintenance of the latency of the PDGF domain. In the nucleus, PDGFC seems to have additional function. PDGFC can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the peptide for treatment includes PDGFC. PDGFC can include SEQ ID NO: 97, SEQ ID NO: 98, SEQ ID NO: 99, or SEQ ID NO: 100.

[0050] Platelet-derived Growth Factor D (PDGFD) plays an essential role in the regulation of embryonic development, cell proliferation, cell migration, survival and chemotaxis. PDGFD is a potent mitogen for cells of mesenchymal origin. PDGFD plays an important role in wound healing. PDGFD induces macrophage recruitment, increased interstitial pressure, and blood vessel maturation during angiogenesis. PDGFD can initiate events that lead to a mesangial proliferative glomerulonephritis, including influx of monocytes and macrophages and production of extracellular matrix. PDGFD can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the peptide for treatment includes PDGFD. PDGFB can include SEQ ID NO: 101 or SEQ ID NO: 102.

[0051] The gene for Epidermal Growth Factor (EGF) encodes a member of the epidermal growth factor superfamily. The encoded protein is synthesized as a large precursor molecule that is proteolytically cleaved to generate the 53-amino acid epidermal growth factor peptide. This protein acts a potent mitogenic factor that plays an important role in the growth, proliferation and differentiation of numerous cell types. This protein acts by binding the high

affinity cell surface receptor, epidermal growth factor receptor. Defects in this gene are the cause of hypomagnesemia type 4. Dysregulation of this gene has been associated with the growth and progression of certain cancers. Alternate splicing results in multiple transcript variants and isoforms. Pro-EGF can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases, the peptide for treatment includes Pro-EGF. Pro-EGF can have isoforms due to alternative splicing. Pro-EGF can include SEQ ID NO: 103, SEQ ID NO: 104, SEQ ID NO: 105 or SEQ ID NO: 106.

[0052] Fibroblast Growth Factor (FGF) plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. FGF can function as potent mitogen in vitro. FGF can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. There are 22 different isoforms of FGF.

[0053] In some cases, the peptide for treatment includes FGF1. FGF1 can include SEQ ID NO: 107 or SEQ ID NO: 108.

[0054] FGF2 has 4 isoforms produced by alternative initiation. FGF2 plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. FGF2 functions as potent mitogen in vitro. In some cases, the peptide for treatment includes FGF2. FGF2 can include SEQ ID NO: 109, SEQ ID NO: 110, SEQ ID NO: 111, or SEQ ID NO: 112.

[0055] FGF3 has 1 isoform, and plays an important role in the regulation of embryonic development, cell proliferation, and cell differentiation. FGF3 is required for normal ear development. In some cases, the peptide for treatment includes FGF3. FGF3 can include SEQ ID NO: 113.

[0056] FGF4 can have two isoforms due to alternative splicing. FGF4 plays an important role in the regulation of cell survival, cell division, angiogenesis, cell differentiation and cell migration. FGF4 can function as potent mitogen in vitro. In some cases, the peptide for treatment includes FGF4. FGF4 can include SEQ ID NO: 114 or SEQ ID NO: 115.

[0057] FGF5 can have two isoforms due to alternative splicing. FGF5 can play an important role in the regulation of cell proliferation and cell differentiation. FGF5 is required for normal regulation of the hair growth cycle. FGF5 can function as an inhibitor of hair elongation by promoting progression from anagen, the growth phase of the hair follicle, into catagen, the apoptosis-induced regression phase. In some cases, the peptide for treatment includes FGF5. FGF5 can include SEQ ID NO: 116 or SEQ ID NO: 117.

[0058] The FGF6 isoform plays an important role in the regulation of cell proliferation, cell differentiation, angiogenesis and myogenesis, and is required for normal muscle regeneration. In some cases, the peptide for treatment includes FGF6. FGF6 can include SEQ ID NO: 118.

[0059] FGF7 (Keratinocyte Growth Factor, KGF) has one isoform and plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation. FGF7 is required for normal branching morphogenesis. The growth factor is particularly active upon keratinocytes. FGF7 is a possible major paracrine effector of normal epithelial cell proliferation. In some cases, the peptide for treatment includes FGF7. FGF7 can include SEQ ID NO: 119.

[0060] FGF8 has four isoforms due to alternative splicing and plays an important role in the regulation of embryonic development, cell proliferation, cell differentiation and cell migration. FGF8 is required for normal brain, eye, ear, and limb development during embryogenesis. FGF8 is required for normal development of the gonadotropin-releasing hormone (GnRH) neuronal system. In some cases, the peptide for treatment includes FGF8. FGF8 can include , SEQ ID NO: 120, SEQ ID NO: 121, SEQ ID NO: 122, or SEQ ID NO: 123.

[0061] FGF9 has one isoform and plays an important role in the regulation of embryonic development, cell proliferation, cell differentiation and cell migration. FGF9 can have a role in glial cell growth and differentiation during development, gliosis during repair and regeneration of brain tissue after damage, differentiation and survival of neuronal cells, and growth stimulation of glial tumors. In some cases, the peptide for treatment includes FGF9. FGF9 can include SEQ ID NO: 124.

[0062] FGF10 (KGF2) has one isoform, and plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation. FGF10 is required for normal branching morphogenesis. FGF10 can play a role in wound healing. In some cases, the peptide for treatment includes FGF10. FGF10 can include SEQ ID NO: 125.

[0063] FGF11 has one isoform and is involved in nervous system development and function. In some cases, the peptide for treatment includes FGF11. FGF11 can include SEQ ID NO: 126.

[0064] FGF12 has two isoforms due to alternative splicing and is involved in nervous system development and function. In some cases, the peptide for treatment includes FGF12. FGF12 can include SEQ ID NO: 127 or SEQ ID NO: 128.

[0065] FGF13 has 5 isoforms produced by alternative splicing. FGF13 is a microtubule-binding protein which directly binds tubulin and is involved in both polymerization and stabilization of microtubules. Through its action on microtubules, FGF 13 can participate in the refinement of axons by negatively regulating axonal and leading processes branching. FGF13 plays a crucial role in neuron polarization and migration in the cerebral cortex and the hippocampus. In some cases, the peptide for treatment includes FGF13. FGF13 can include or SEQ ID NO: 129, SEQ ID NO: 130, SEQ ID NO: 131, SEQ ID NO: 132, or SEQ ID NO: 133.

[0066] FGF14 has two isoforms produced by alternative splicing and is involved in nervous system development and function. In some cases, the peptide for treatment includes FGF14. FGF14 can include SEQ ID NO: 134 or SEQ ID NO: 135.

[0067] FGF16 has one isoform and plays an important role in the regulation of embryonic development, cell proliferation and cell differentiation, and is required for normal cardiomyocyte proliferation and heart development. In some cases, the peptide for treatment includes FGF16. FGF16 can include SEQ ID NO: 136.

[0068] FGF17 has two isoforms due to alternate splicing and plays an important role in the regulation of embryonic development and as signaling molecule in the induction and patterning of the embryonic brain. FGF17 is required for normal brain development. In some cases, the peptide for treatment includes FGF17. FGF17 can include SEQ ID NO: 137 or SEQ ID NO: 138.

[0069] FGF18 has one isoform and plays an important role in the regulation of cell proliferation, cell differentiation and cell migration. FGF18 is required for normal ossification and bone development. FGF18 stimulates hepatic and intestinal proliferation. In some cases, the peptide for treatment includes FGF18. FGF18 can include SEQ ID NO: 139.

[0070] FGF 19 has one isoform and is involved in the suppression of bile acid biosynthesis through down-regulation of CYP7A1 expression, following positive regulation of the JNK and ERK1/2 cascades. FGF19 stimulates glucose uptake in adipocytes. The activity of FGF19 requires the presence of KLB and FGFR4. In some cases, the peptide for treatment includes FGF 19. FGF19 can include SEQ ID NO: 140.

[0071] FGF20 has one isoform and plays as role as a neurotrophic factor that regulates central nervous development and function. In some cases, the peptide for treatment includes FGF20. In some embodiments, FGF20 includes SEQ ID NO: 141.

[0072] FGF21 has one isoform and stimulates glucose uptake in differentiated adipocytes via the induction of glucose transporter SLC2A1/GLUT1 expression (but not SLC2A4/GLUT4 expression). The activity of FGF21 requires the presence of KLB. In some cases, the peptide for treatment includes FGF21. FGF21 can include SEQ ID NO: 142.

[0073] FGF22 has one isoform and plays a role in the fasting response, glucose homeostasis, lipolysis and lipogenesis. FGF22 can stimulate cell proliferation in vitro, and can be involved in hair development. In some cases, a cell secreting FGF22 can be used to treat alopecia. The peptide for treatment can include FGF22. In some cases, FGF22 includes SEQ ID NO: 143.

[0074] FGF23 has one isoform and is a regulator of phosphate homeostasis. FGF23 inhibits renal tubular phosphate transport by reducing SLC34A1 levels and can upregulate EGR1 expression in the presence of KL. FGF23 acts directly on the parathyroid to decrease PTH secretion. FGF23 is also a regulator of vitamin-D metabolism and can negatively regulates osteoblast differentiation and matrix mineralization. In some cases, the peptide for treatment includes FGF23. FGF23 can include SEQ ID NO: 144.

[0075] Hormones are a class of regulatory biochemicals that are produced in all organisms by

glands, and transported by the circulatory system to distant target organs to coordinate its physiology, function, and behavior. Hormones can serve as a major form of communication between different organs and tissues. Hormones regulate a variety of physiological and behavioral activities, including digestion, metabolism, respiration, tissue function, sensory perception, sleep, perception, stress, growth and development, movement, and reproduction. In some embodiments, the peptide for treatment includes a hormone. Hormones can be used by subjects suffering from disease that is contributed by low hormone production in a subject. In certain cases a bacteria is genetically modified by introduction of a nucleic acid that encodes a hormone (e.g., a mammalian hormone). In certain cases a bacteria is genetically modified to express and/or secrete a hormone (e.g., a mammalian hormone).

In certain cases a hormone is a somatotrophin. In certain cases a hormone is a mammalian somatotrophin. In certain cases a hormone is a bovine or human somatotrophin. In certain cases a somatotrophin is growth hormone (GH).

[0076] Somatotrophin is a hormone that plays an important role in growth control. Its major role in stimulating body growth is to stimulate the liver and other tissues to secrete IGF-1. It stimulates both the differentiation and proliferation of myoblasts. It also stimulates amino acid uptake and protein synthesis in muscle and other tissues. In some cases the peptide for treatment includes somatotrophin. In some cases, the somatotrophin includes SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48 or SEQ ID NO: 49. Somatotrophin can be used by subjects suffering from low human growth hormone (HGH) production or subjects suffering from low testosterone production. In some cases, a cell including a nucleic acid for expressing a peptide includes an amino acid sequence for somatotrophin. As microbes can have the benefit of having the ability to get beneath the horny layer of the dermis and into the pores, the skin would have a better and increased opportunity to absorb these topicals than if the molecules themselves were applied topically by a lotion or a cream. One benefit of delivering a microbe-produced hormone to a subject in need is that such a method reduces the risk of exposure of the hormone to other subjects. As, in some cases, a genetically modified microbe is often engineered to depend on essential nutrients provide only to a subject in need. Therefore, delivery of a microbe-produced hormone to a subject in need can be safer than other methods of providing topical hormones which can often result in unwanted transfer to other subjects.

[0077] Anti-inflammatories are substances or treatments that reduce inflammation. Interleukins (IL) are a group of cytokines that can function as signaling molecules and act as an anti-inflammatory. Anti-inflammatories can be used by subjects in need suffering from an inflammatory disorder, such as acne. Microbes secreting anti-inflammatories have an advantage over creams containing anti-inflammatories, as microbes have the benefit of having the ability to get beneath the horny layer of the dermis and into the pores, increasing the opportunity to absorb these anti-inflammatories.

[0078] In some cases a microbe is genetically modified to express and/or secrete an anti-inflammatory. In certain cases an anti-inflammatory is a cytokine. In some cases, the genetically modified microbe secretes a cytokine. Non-limiting examples of cytokines include

IL-4, IL-6, IL-7, IL-8, IL-10 and IL-13. In certain cases a bacteria is genetically modified by introduction of a nucleic acid that encodes one or more cytokines (e.g., a mammalian cytokines). A nucleic acid that encodes a cytokine may include a suitable promoter and/or regulatory elements that drive the transcription and/or translation (e.g., expression) of the cytokine, an open reading frame that encodes the cytokine and, in some cases, suitable nucleic acid and/or peptide elements that direct microbial secretion of the cytokine. In certain cases a bacteria is genetically modified by introduction of two or more nucleic acids that encode two or more cytokines (e.g., a mammalian cytokines). In certain cases a bacteria is genetically modified by introduction of a nucleic acid that directs the expression of one or more cytokines (e.g., a mammalian cytokines). A bacteria can be genetically modified to express and/or secrete a cytokine (e.g., a mammalian cytokine).

[0079] Interleukin 4 (IL-4) can participate in at least several B-cell activation processes as well as of other cell types. It is a co-stimulator of DNA-synthesis. It induces the expression of class II MHC molecules on resting B-cells. It enhances both secretion and cell surface expression of IgE and IgG1. It also regulates the expression of the low affinity Fc receptor for IgE (CD23) on both lymphocytes and monocytes. In some cases, the peptide for treatment includes interleukin 4 or a portion thereof. The interleukin 4 can include SEQ ID NO: 24.

[0080] Interleukin 10 (IL-10) can inhibit the synthesis of a number of cytokines, including IFN-gamma, IL-2, IL-3, TNF and GM-CSF produced by activated macrophages and by helper T-cells. The peptide for treatment can include interleukin 10 or a portion thereof. The Interleukin 10 can include SEQ ID NO: 25.

[0081] Interleukin 13 (IL-13) is a cytokine that inhibits inflammatory cytokine production. Interleukin 13 synergizes with Interleukin 2 (IL2) in regulating interferon-gamma synthesis. Interleukin 13 can be critical in regulating inflammatory and immune responses. In some cases, the peptide for treatment includes Interleukin 13, or a portion thereof. In some cases, the interleukin 13 includes SEQ ID NO: 26.

[0082] Interleukin-1 receptor type 2 (IL1R2) is a non-signaling receptor for IL1A, IL1B and IL1RN. IL1R2 reduces IL1B activities. In certain embodiments a bacteria is genetically modified to express and/or secrete an IL1R2. IL1R2 can serve as a decoy receptor by competitive binding to IL1B and preventing its binding to IL1R1. IL1R2 can also modulate cellular response through non-signaling association with IL1RAP after binding to IL1B. IL1R2 (membrane and secreted forms) preferentially binds IL1B and poorly IL1A and IL1RN. The secreted IL1R2 recruits secreted IL1RAP with high affinity; this complex formation can be the dominant mechanism for neutralization of IL1B by secreted/soluble receptors. In some cases, the peptide for treatment includes IL1R2, or a portion thereof. In some cases, the interleukin 13 includes SEQ ID NO: 27 or SEQ ID NO: 28.

[0083] Hyaluronic acid or HA/Hyaluronan is a biomolecule that is synthesized by a class of integral membrane proteins called hyaluronan synthases, of which vertebrates have three types: HAS1, HAS2, and HAS3. It is an anionic, glycosaminoglycan distributed widely

throughout connective, epithelial, and neural tissues. Hyaluronic acid is non-sulfated, and forms in the plasma membrane instead of the Golgi, and can be very large, with a molecular weight often reaching the millions (in kDa). One of the chief components of the extracellular matrix, hyaluronan contributes significantly to cell proliferation and migration, and can also be involved in the progression of some malignant tumors.

[0084] HAS1 catalyzes the addition of GlcNAc or GlcUA monosaccharides to a nascent hyaluronan polymer. It is essential in hyaluronan synthesis, since hyaluronic acid is a major component of most extracellular matrices that has a structural role in tissues architectures and regulates cell adhesion, migration and differentiation. HAS1 is one of the isozymes catalyzing the reaction of the addition of GlcNAc or GlcUA monosaccharides to hyaluronan polymers. HAS1 is also able to catalyze the synthesis of chito-oligosaccharide depending on the substrate. HAS2 catalyzes the addition of GlcNAc or GlcUA monosaccharides to a nascent hyaluronan polymer. HAS2 is one of the isozymes that catalyzes this reaction and it is particularly responsible for the synthesis of high molecular mass hyaluronan. HAS 2 is required for the transition of endocardial cushion cells into mesenchymal cells, a process crucial for heart development. HAS2 can also play a role in vasculogenesis. High molecular mass hyaluronan can also play a role in early contact inhibition, a process which stops cell growth when cells come into contact with each other or the extracellular matrix. HAS3 catalyzes the addition of GlcNAc or GlcUA monosaccharides to the nascent hyaluronan polymer.

[0085] As a treatment, dry, scaly skin also known as xerosis such as that caused by atopic dermatitis or eczema can be treated with a prescription skin lotion containing sodium hyaluronate as its active ingredient. In several cases described herein, a nucleic acid encoding hyaluronan synthases or portion thereof is provided. In several cases, a cell can carry a nucleic acid encoding hyaluronan synthases or portion thereof. In some cases, the hyaluron synthases includes HAS1 (SEQ ID NO: 1) HAS2 (SEQ ID NO: 2), HAS3 isoform 1 (SEQ ID NO: 3), or HAS3 isoform 2 (SEQ ID NO: 4). Hyaluronic acid can be used for general cosmesis, plumping of the skin to reduce wrinkles. In some cases, the cell carrying a nucleic acid encoding hyaluronan synthases or portion thereof can lead to production of hyaluronic acid. In several cases, the peptide for treatment can be used for general cosmesis. As the cell can propagate and localize in pores and follicles, the absorption of hyaluronic acid is increased compared to topically applied lotions containing hyaluronic acid. In some cases, a subject in need suffering from xerosis is administered a topical formulation including a cell having a nucleic acid encoding hyaluronan synthases or portion thereof.

[0086] Elastin is a major structural protein of tissues such as aorta and nuchal ligament, which must expand rapidly and recover completely. Elastin is a molecular determinant of late arterial morphogenesis, it can also stabilize arterial structure by regulating proliferation and organization of vascular smooth muscle. Elastin can be used to contribute to skin elasticity as it can be readily absorbed by the skin. In some cases, the peptide for treatment includes elastin or a portion thereof. As microbes can have the benefit of having the ability to get beneath the horny layer of the dermis and into the pores, the skin would have a better and increased opportunity to absorb elastin from bacteria than if the elastin was applied topically through a

lotion or a cream.

[0087] In some cases, the elastin, or portion thereof includes SEQ ID NO: 5, SEQ ID NO: 6, SEQ ID NO: 7, SEQ ID NO: 8, SEQ ID NO: 9, SEQ ID NO: 10, SEQ ID NO: 11, SEQ ID NO: 12, SEQ ID NO: 13, SEQ ID NO: 14, SEQ ID NO: 15, SEQ ID NO: 16, or SEQ ID NO: 17.

[0088] Collagen is a main structural protein of the various connective tissues in animals. Collagen, in the form of elongated fibrils, is can be found in fibrous tissues, tendons, ligaments, skin, and is also abundant in corneas, cartilage, bones, blood vessels, the gut, and intervertebral discs. Collagen is created mostly from the fibroblast. Collagen is composed of a triple helix, consisting of two identical α 1chains and an additional chain that can differ in chemical composition. The amino acid composition of collagen can have high hydroxyproline content. In some cases, the peptide of treatment includes collagen or a portion thereof. Collagen can be absorbed by the skin to add to the extracellular matrix, and the dermal thickness. As microbes can have the benefit of having the ability to get beneath the horny layer of the dermis and into the pores, the skin would have a better and increased opportunity to absorb the collagen than if the molecules themselves were applied topically by a lotion or a cream. In some cases, peptide of treatment including collagen or a portion thereof includes SEQ ID NO: 18, SEQ ID NO: 19, SEQ ID NO: 20, SEQ ID NO: 21, SEQ ID NO: 22 or SEQ ID NO: 23.

[0089] Clotting factors as described herein, refers to chemical and cellular constituents that can cause the blood to coagulate or clot. Blood clotting factors can be used externally by a subject in need suffering from hemophilia, or by a subject under treatment of a blood thinner. Blood thinners can include fish oil, aspirin, anti-platelet drugs, and other types of anticoagulants. Anti-coagulants can include but is not limited to antithrombics, fibrinolytic, and thrombolytics.

[0090] Coagulation Factor VIII is a member of the multi-copper oxidase family. Coagulation Factor VIII is a cofactor for factor IXa which, in the presence of Ca^{+2} and phospholipids, converts factor X to an activated form, Xa. Coagulation Factor VIII is a coagulation cofactor which circulates bound to von Willebrand factor and is part of the intrinsic coagulation pathway. It is a macromolecular complex composed of two separate entities, one of which, when deficient, results in hemophilia A, and the other, when deficient, results in von Willebrand's disease. Hemophilia A is a disorder of blood coagulation characterized by a permanent tendency to hemorrhage. In some cases, the peptide for treatment includes coagulation factor VIII. In some cases, the coagulation factor VIII includes SEQ ID NO: 29 or SEQ ID NO: 30. In some cases, the peptide for treatment includes coagulation factor VIII heavy chain in a 200 kDa isoform. In some cases, the coagulation factor VIII heavy chain in a 200 kDa includes SEQ ID NO: 31. In some cases, the peptide for treatment includes coagulation factor VIII heavy chain in a 92kDa isoform. In some cases, the coagulation factor VIII heavy chain in a 92kDa includes SEQ ID NO: 32. In some cases, the peptide for treatment includes coagulation factor VIII B chain. In some cases, the coagulation factor VIII B chain includes SEQ ID NO: 33. In some cases, the peptide for treatment includes coagulation factor VIIIa light chain. In some

cases, the coagulation factor VIIIa light chain includes SEQ ID NO: 34.

[0091] Factor IX is a vitamin K-dependent plasma protein that participates in the intrinsic pathway of blood coagulation by converting factor X to its active form in the presence of Ca²⁺ ions, phospholipids, and factor VIIIa. In some cases, the peptide for treatment is Factor IX. In some cases, the Factor IV includes SEQ ID NO: 35, SEQ ID NO: 36, SEQ ID NO: 37, SEQ ID NO: 38 or SEQ ID NO: 39.

[0092] Melanin in the skin is produced by melanocytes, found in the basal layer of the epidermis. Although, in general, human beings possess a similar concentration of melanocytes in their skin, the melanocytes in some individuals and ethnic groups more frequently or less frequently express the melanin-producing genes, thereby conferring a greater or lesser concentration of skin melanin.

[0093] Tyrosinase is an oxidase, the rate-limiting enzyme for controlling the production of melanin. Tyrosinase is involved in the hydroxylation of a monophenol and the conversion of an o-diphenol to the corresponding o-quinone. o-Quinone undergoes several reactions to eventually form melanin. In some cases, the peptide for treatment is produced by tyrosinase, or fragment thereof. In some cases, the tyrosinase includes SEQ ID NO: 40, SEQ ID NO: 41, SEQ ID NO: 42, SEQ ID NO: 43 or SEQ ID NO: 44. In some cases, cells can include, for example, a nucleic acid encoding tyrosinase, or fragment thereof. In some cases, the microbes produce tyrosinase for the enzymatic production of melanin. As microbes can have the benefit of having the ability to get beneath the horny layer of the dermis and into the pores, the skin would have a better and increased opportunity to absorb these topicals than if the molecules themselves were applied topically by a lotion or a cream. The production of melanin can be used for "sunless" tanning in order to increase the melanin in a subject.

[0094] Chemokines are a family of small cytokines that are secreted by cells as signaling molecules. Proteins classified as chemokines are small in size (8-10 KDa in size), and have four conserved cysteine residues in conserved locations, forming a conserved 3-dimensional shape among the chemokines. Chemokines can be considered pro-inflammatory and can be induced during an immune response to recruit cells of the immune system to a site of infection, while others are considered homeostatic and are involved in controlling the migration of cells during normal processes of tissue maintenance or development. Chemokines are found in all vertebrates, some viruses and some bacteria, but none have been described for other invertebrates. In some cases, the peptide for treatment includes a chemokine.

[0095] Platelet basic protein (LA-PF4) belongs to the chemokine family. LA-PF4 stimulates DNA synthesis, mitosis, glycolysis, intracellular cAMP accumulation, prostaglandin E2 secretion, and synthesis of hyaluronic acid and sulfated glycosaminoglycan. It also stimulates the formation and secretion of plasminogen activator by human synovial cells. NAP-2 is a ligand for CXCR1 and CXCR2, and NAP-2, NAP-2(73), NAP-2(74), NAP-2(1-66), and most potent NAP-2(1-63) are chemo-attractants and activators for neutrophils. TC-1 and TC-2 are antibacterial proteins, in vitro released from activated platelet alpha-granules. CTAP-III (1-81)

is more potent than CTAP-III desensitize chemokine-induced neutrophil activation. LA-PF4 can be used for general cosmesis, to cause more youthful skin by attracting fibroblasts, and causing them to produce extracellular matrix, and leading to thicker, more youthful skin. In some cases the peptide for treatment includes platelet basic protein. In some cases, the platelet basic protein includes SEQ ID NO: 50, SEQ ID NO: 51, SEQ ID NO: 52, SEQ ID NO: 53, SEQ ID NO: 54, SEQ ID NO: 55, SEQ ID NO: 56, SEQ ID NO: 57, SEQ ID NO: 58, SEQ ID NO: 59, SEQ ID NO: 60, SEQ ID NO: 61 or SEQ ID NO: 62.

[0096] DNA repair enzymes are used for the repair of DNA damaged by reactive oxygen species, replication errors, exogenous damage caused by external agents such as ultraviolet rays, toxins, mutagenic chemicals, DNA intercalating agents, and viruses. There are several types of damage which can be oxidation of bases, alkylation of bases, deamination, depurination, mismatch of bases, monoadduct damage, and diadduct damage. DNA repair enzymes can be used for anti-aging, and reversal of DNA damage via solar damage/radiation.

[0097] Base excision repair (BER) is a mechanism that repairs damaged DNA during the cell cycle, removing small non-helix distorting base lesions from the genome. BER is very important as it removes damaged bases that could lead to mutations by mispairing or lead to breaks in the DNA during replication. The process is initiated by DNA glycosylases, which can recognize and remove damaged or inappropriate bases, forming AP sites. The AP sites are then cleaved by an AP endonuclease resulting in a single strand break that can then be processed by a short or a long patch repair process.

[0098] In some cases, the peptide for treatment includes base excision repair (BER) enzymes. In some cases, BER enzymes includes SEQ ID NO: 145, SEQ ID NO: 146, SEQ ID NO: 147, SEQ ID NO: 148, SEQ ID NO: 149, SEQ ID NO: 150, SEQ ID NO: 151, SEQ ID NO: 152, SEQ ID NO: 153, SEQ ID NO: 154, or SEQ ID NO: 155.

[0099] Direct reversal of DNA damage is another repair mechanism to restore damaged DNA. The formation of pyrimidine dimers, is the major type of damage that is caused by UV light. The damage distorts the DNA double helix and blocks transcription or replication past the damaged site. In some cases, the peptide for treatment includes enzymes for the direct reversal of DNA damage. In some cases, the enzymes for the direct reversal of DNA damage includes SEQ ID NO: 156, SEQ ID NO: 157, or SEQ ID NO: 158.

[0100] DNA mismatch repair proteins are involved in the recognition and repairing of nucleic acid which have erroneous insertions, deletions, and misincorporation of bases that can stem from the processes of DNA replication and recombination, and from DNA damage. In some cases, the peptide for treatment includes enzymes for DNA mismatch repair. In some cases, the enzymes for DNA mismatch repair includes SEQ ID NO: 159, SEQ ID NO: 160, SEQ ID NO: 161, SEQ ID NO: 162, SEQ ID NO: 163, SEQ ID NO: 164, SEQ ID NO: 165, SEQ ID NO: 166, SEQ ID NO: 167, or SEQ ID NO: 168.

[0101] Nucleotide excision repair (NER) is a DNA repair mechanism that removes DNA

damage that is caused by ultraviolet light. The damage of ultraviolet light can lead to DNA adducts which can consist of thymine dimers, and 6,4-photoproducts. The NER proteins recognize the damage leading to the removal of short single-stranded DNA segments that contain the lesion. The undamaged complement is then used as a template by DNA polymerase to synthesize a short complementary sequence which is subsequently ligated by a DNA ligase. In some cases, the peptide for treatment includes enzymes for nucleotide excision repair. In some cases, the enzymes for nucleotide excision repair includes or SEQ ID NO: 169, SEQ ID NO: 170, SEQ ID NO: 171, SEQ ID NO: 172, SEQ ID NO: 173, SEQ ID NO: 174, SEQ ID NO: 175, SEQ ID NO: 176, SEQ ID NO: 177, SEQ ID NO: 178, SEQ ID NO: 179, SEQ ID NO: 180, SEQ ID NO: 181, SEQ ID NO: 182, SEQ ID NO: 183, SEQ ID NO: 184, SEQ ID NO: 185, SEQ ID NO: 186, SEQ ID NO: 187, SEQ ID NO: 188, SEQ ID NO: 189, SEQ ID NO: 190, SEQ ID NO: 191, SEQ ID NO: 192, SEQ ID NO: 193, SEQ ID NO: 194, SEQ ID NO: 195, SEQ ID NO: 196, or SEQ ID NO: 197.

[0102] DNA editing and processing involve the use of several types of nucleases and are involved in DNA replication and repair. For example, DNase IV can remove the 5' overhanging flaps in DNA repair and processes the 5' ends of Okazaki fragments in lagging strand DNA synthesis. Direct physical interaction between this protein and AP endonuclease 1 during long-patch base excision repair provides coordinated loading of the proteins onto the substrate, thus passing the substrate from one enzyme to another. The protein is a member of the XPG/RAD2 endonuclease family and is one of ten proteins essential for cell-free DNA replication.

[0103] MTMR15, also known as myotubularin related protein is a DNA endo-and exonuclease involved in the repair of DNA damage caused by crosslinking agents. FAN1 is recruited to sites of interstrand cross linkage damage by interacting with the protein complex FANCI-FANCD2 complex. Together the proteins promote interstrand crosslink repair in a manner strictly dependent on its ability to accumulate at or near sites of DNA damage and that relies on monoubiquitylation of the FANCI-FANCD2 complex.

[0104] DNase III, or TREX1 is a major nuclear DNA-specific 3'-5' exonuclease that is widely distributed in both proliferating and nonproliferating mammalian tissues. DNase III translocates to the nucleus at S phase after DNA damage by gamma-irradiation or hydroxyurea. DNase III has a preference for single stranded DNA in repair.

[0105] TREX2 encodes for a 3'exonuclease. The encoded protein can participate in double stranded DNA break repair, and can interact with DNA polymerase delta. TREX2 can remove mismatched modified, fragmented, and normal nucleotides in order to generate the 3' termini for subsequent steps in the DNA metabolic pathway.

[0106] EXO1/HEX1 is an 803 amino acid human protein that functions in DNA replication, repair and recombination. EXO1/HEX1 can participate in mismatch-provoked excision directed by strand breaks located either 5-prime or 3-prime to the mispair.

[0107] Aprataxin (APTX) is another protein involved in editing and processing of DNA. APTX plays a role in single stranded DNA repair by removing AMP from DNA ends following ligation attempts of DNA ligase IV during non-homologous end joining.

[0108] SPO11 is an endonuclease of the editing and processing nucleases that functions during meiotic recombination. SPO11 produces double stranded breaks (DSB) in DNA, an important step during the meiotic recombination. In absence of SPO11, there is a failure to initiate the production of DSB which can lead to chromosomes segregating aberrantly which can result in aneuploidy gametes.

[0109] Endonuclease V (ENDOV) is a nuclease of the editing and processing nucleases, and is an endoribonuclease that specifically cleaves inosine-containing RNAs, at the second phosphodiester bond 3' to inosine. ENDOV has a strong preference for single-stranded RNAs (ssRNAs) toward double-stranded RNAs (dsRNAs). ENDOV cleaves mRNAs and tRNAs containing inosine. ENDOV is also able to cleave structure-specific dsRNA substrates containing the specific sites 5'-IIUI-3' and 5'-UIUU-3'. Inosine is present in a number of RNAs following editing; the function of inosine-specific endoribonuclease is still unclear. Inosine could either play a regulatory role in edited RNAs, or it can be involved in antiviral response by removing the hyperedited long viral dsRNA genome that has undergone A-to-I editing.

[0110] In some cases, the peptide for treatment includes editing and processing nucleases. In some cases, the enzymes for editing and processing includes SEQ ID NO: 198, SEQ ID NO: 199, SEQ ID NO: 200, SEQ ID NO: 201, SEQ ID NO: 202, SEQ ID NO: 203, SEQ ID NO: 204, or SEQ ID NO: 205.

[0111] Telomeres are regions at the tips of the chromosomes that get shorter during aging and during cell division, or mitosis. In order to repair the tips of the chromosomes, an enzyme, telomerase, repairs the tips which can have potential to repair age or disease related damage. Telomerase, is a ribonucleoprotein that adds DNA sequence repeats to the 3'end of DNA in the telomeric regions (the ends of eukaryotic chromosomes). In some cases, the peptide for treatment comprises telomerase or a portion thereof. In some cases, the telomerase or portion thereof comprises SEQ ID NO: 206, SEQ ID NO: 207, SEQ ID NO: 208, or SEQ ID NO: 209.

[0112] Protection of telomeres protein 1 is encoded by POT1, a member of the telombin family and is involved in the maintenance of the telomere. POT1 functions by binding to the repeat of telomeres at the end of the eukaryotic chromosome, regulating the telomere length and protecting the chromosome ends from irregular recombination, instability, and abnormal chromosome instability. In several cases, the peptide for treatment comprises protection of telomeres protein 1 or a portion thereof. In some cases, the protection of telomeres protein 1 or portion thereof comprises SEQ ID NO: 210, or SEQ ID NO: 211.

[0113] In some cases, methods are described wherein the peptide for treatment comprises a fusion protein. In some cases, the fusion protein can comprise a first protein sequence comprising an amino acid sequence of elastin, collagen, an anti-inflammatory, clotting factor,

hormone, platelet basic protein, transforming growth factor, hepatocyte growth factor, vascular endothelial growth factor, placental growth factor, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, a DNA repair enzyme, a telomerase, or a protection of telomerase protein 1. In some cases, the fusion protein is fused to a second protein comprising an amino acid sequence of elastin, collagen, an anti-inflammatory, clotting factor, hormone, platelet basic protein, transforming growth factor, hepatocyte growth factor, vascular endothelial growth factor, placental growth factor, platelet derived growth factor, epidermal growth factor, fibroblast growth factor, a DNA repair enzyme, a telomerase, or a protection of telomerase protein 1, wherein the amino acid sequence of the second protein is not the amino acid sequence of the first protein. Fusion proteins can have the added benefit of introducing a second moiety to the peptide of treatment to treat a subject in need.

[0114] In several cases described herein, populations of genetically modified bacteria are created and/or derived from bacterial members from a group consisting of the genus *Propionibacterium*, *Corynebacterium*, *Staphylococcus*, *Streptococcus*, *Lactobacillus*, and *Lactococcus*. In some cases genetically modified bacteria are derived from the genus *Propionibacterium*. Non-limiting examples of species of *Propionibacterium* include *Propionibacterium acidifaciens*, *Propionibacterium acidipropionici*, *Propionibacterium acnes*, *Propionibacterium australiense*, *Propionibacterium avidum*, *Propionibacterium cyclohexanicum*, *Propionibacterium freudenreichii*, *Propionibacterium freudenreichii*, *Propionibacterium granulosum*, *Propionibacterium jensenii*, *Propionibacterium microaerophilum*, *Propionibacterium propionicum* and *Propionibacterium thoenii* and.

[0115] In some embodiments a genetically modified bacteria is derived from the species *Propionibacterium acnes* (*P. acnes*). In some cases, a genetically modified bacteria can be derived from a pathogenic or non-pathogenic strain of *P. acnes*. Recent studies have suggested that there are certain strains of *P. acnes* that are associated with pathogenicity and others that are associated with healthy skin (Fitz-Gibbon, S., et al., (2013) Invest. Dermatol., 133(9):2152-60; Lomholt HB and Kilian M. (2010) PLoS One, 5(8):e12277; McDowell A, et al., (2011) Microbiology 157(Pt 7):1990-2003). Of the types thought to be associated with healthy skin, the Type II, ribotype 6 strain seems to have the lowest association with acne. Type II *P. acnes* contain a CRISPR array that confers immunity to *P. acnes*-specific phages and mobile genetic elements (Bruggemann H, et al., (2012) PLoS ONE 7(3):e34171). This seems to explain why the bacteria may be commensal in nature, as it cannot acquire pathogenic traits from other bacterium.

[0116] A genetically modified bacteria can be derived from any suitable microbiome of *P. acnes*, non-limiting examples of which include microbiomes of type I, type II, type III, type IV and type V. In certain cases a genetically modified bacteria is derived from a strain of *P. acnes* of a phenotype of type I (e.g., clades IA or type IB), type II or type III. A genetically modified bacteria can be derived from any suitable ribotype of *P. acnes*, non-limiting examples of which include ribotypes RT1 through RT30. In certain cases a genetically modified bacteria is derived from a *P. acnes* of ribotype RT1, RT2, RT3, RT4, RT5, RT6, RT7, RT8, RT9 or RT10. In certain cases a genetically modified bacteria is derived from a *P. acnes* of ribotype RT2 or RT6. In

certain embodiments a genetically modified bacteria is derived from a *P. acnes* of strain Type II and ribotype RT2 or RT6.

[0117] A genetically modified bacteria can be derived from a bacteria comprising a CRISPR (*clustered regularly interspaced short palindromic repeat*) locus, sometimes referred to as a CRISPR array (e.g., see Horvath and Barrangou (2010) *Science* 327:167-70; Makarova et al., (2011) *Nat Rev Microbiol* 9:467-77; and Brüggemann H, et al., (2012) *PLoS ONE* 7(3):e34171). Without being limited to theory, CRISPR arrays in bacteria such as *P. acnes* have been shown to confer protective "immunity" against invading genetic elements (e.g., viruses, phage and plasmids). Without being limited to theory, the presence of a CRISPR array can preserve the integrity of the genome of a genetically modified bacteria and prevent introduction of foreign genetic elements from other pathogenic strains of bacteria. Although a CRISPR array is thought to protect bacteria from the introduction of foreign genetic elements from other bacteria, phage and/or virus, bacteria containing a CRISPR array are amenable to transformation, stable integration of transformed DNA and integration of nucleic acid by homologous recombination. In some embodiments a genetically modified bacteria comprises an endogenous CRISPR array. In some embodiments a genetically modified bacteria is derived from an RT2 or RT6 ribotype of *P. acnes*, each of which comprises an endogenous CRISPR array. In some embodiments a genetically modified bacteria comprises an exogenous CRISPR array introduced by genetic manipulation.

[0118] In some cases, the populations of modified bacteria are created from *Propionibacterium acnes*, or a strain thereof. In some cases, the populations of transformed bacteria are created from *Corynebacterium striatum*. In some cases, the populations of transformed bacteria are created from *Staphylococcus epidermis*. In some cases, the populations of transformed bacteria are created from *Streptococcus thermophilus*. In some cases, the populations of transformed bacteria are created from *Lactobacillus acidophilus*. In some cases, the populations of transformed bacteria are created from *Lactococcus lactis*. In some cases, the populations of transformed bacteria are created from the genus *Enterococci*. In some cases, the populations of transformed bacteria are created from the genus *Micrococci*. In some cases, the populations of transformed bacteria are created from the genus *Demodex*. In some cases, the populations of transformed bacteria are created from the genus *Malassezia*. In some cases, the populations of transformed bacteria are created from non-pathogenic *Eschericia coli*. In some cases, the populations of transformed bacteria are created from the genus *Acidovorax*. The populations of transformed bacteria are created from *Acidovorax temperans*. In some cases, the populations of transformed bacteria are created from the genus *Acinetobacter*. In some cases, the populations of transformed bacteria are created from *Acinetobacter haemolyticus*. In some cases, the populations of transformed bacteria are created from *Acinetobacter johnsonii*. In some cases, the populations of transformed bacteria are created from *Acinetobacter junii*. In some cases, the populations of transformed bacteria are created from *Acinetobacter ursingii*. In some cases, the populations of transformed bacteria are created from the genus *Actinomyces*. In some cases, the populations of transformed bacteria are created from *Actinomyces naeslundii*. In some cases, the populations of transformed bacteria are created from *Actinomyces neuii*. In some cases, the populations of

transformed bacteria are created from the genus *Anaerococcus*. In some cases, the populations of transformed bacteria are created from *Anaerococcus prevotii*. In some cases, the populations of transformed bacteria are created from the genus *Atopobium*. In some cases, the populations of transformed bacteria are created from *Atopobium vaginae*. In some cases, the populations of transformed bacteria are created from the genus *Brevibacterium*. In some cases, the populations of transformed bacteria are created from *Brevibacterium paucivorans*. In some cases, the populations of transformed bacteria are created from the genus *Brevundimonas aurantiaca*. In some cases, the populations of transformed bacteria are created from *Brevundimonas aurantiaca*. In some cases, the populations of transformed bacteria are created from *Brevundimonas vesicularis*. In some cases, the populations of transformed bacteria are created from the genus *Candidatus Nostocoida*. In some cases, the populations of transformed bacteria are created from *Candidatus Nostocoida limicola*. In some cases, the populations of transformed bacteria are created from the genus *Corynebacterium*. In some cases, the populations of transformed bacteria are created from *Corynebacterium accolens*. In some cases, the populations of transformed bacteria are created from *Corynebacterium afermentans*. In some cases, the populations of transformed bacteria are created from *Corynebacterium amycolatum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium appendicis*. In some cases, the populations of transformed bacteria are created from *Corynebacterium aurimucosum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium coyleae*. In some cases, the populations of transformed bacteria are created from *Corynebacterium durum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium glaucum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium glucuronolyticum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium imitans*. In some cases, the populations of transformed bacteria are created from *Corynebacterium jeikeium*. In some cases, the populations of transformed bacteria are created from *Corynebacterium kroppenstedtii*. In some cases, the populations of transformed bacteria are created from *Corynebacterium lipophiloflavum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium matruchotii*. In some cases, the populations of transformed bacteria are created from *Corynebacterium minutissimum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium mucifaciens*. In some cases, the populations of transformed bacteria are created from *Corynebacterium pseudodiphthericum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium nigricans*. In some cases, the populations of transformed bacteria are created from *Corynebacterium pseudodiphthericum*. In some cases, the populations of transformed bacteria are created from *Corynebacterium simulans*. In some cases, the populations of transformed bacteria are created from *Corynebacterium singulare*. In some cases, the populations of transformed bacteria are created from *Corynebacterium sundsvallense*. In some cases, the populations of transformed bacteria are created from *Corynebacterium tuberculostearicum*. In some cases, the populations of transformed bacteria are created from the genus *Diaphorobacter*. In some cases, the populations of transformed bacteria are created from *Diaphorobacter nitroreducens*. In some cases, the populations of transformed bacteria are created from the genus *Enhydrobacter*. In some cases, the populations of transformed bacteria are created from

Enhydrobacter aerosaccus. In some cases, the populations of transformed bacteria are created from the genus *Enterobacter*. In some cases, the populations of transformed bacteria are created from *Enterobacter asburiae*. In some cases, the populations of transformed bacteria are created from the genus *Enterococcus*. In some cases, the populations of transformed bacteria are created from *Enterococcus faecalis*. In some cases, the populations of transformed bacteria are created from the genus *Eremococcus*. In some cases, the populations of transformed bacteria are created from *Eremococcus coleocola*. In some cases, the populations of transformed bacteria are created from the genus *Facklamia*. In some cases, the populations of transformed bacteria are created from *Facklamia hominis*. In some cases, the populations of transformed bacteria are created from *Facklamia languida*. In some cases, the populations of transformed bacteria are created from the genus *Gardnerella*. In some cases, the populations of transformed bacteria are created from *Gardnerella vaginalis*. In some cases, the populations of transformed bacteria are created from the genus *Gemella*. In some cases, the populations of transformed bacteria are created from *Gemella haemolysans*. In some cases, the populations of transformed bacteria are created from *Gemella morbillorum*. In some cases, the populations of transformed bacteria are created from *Gemella sanguinis*. In some cases, the populations of transformed bacteria are created from the genus *Gordonia*. In some cases, the populations of transformed bacteria are created from *Gordonia. Bronchialis*. In some cases, the populations of transformed bacteria are created from *Gordonia. sputi*. In some cases, the populations of transformed bacteria are created from *Gordonia. terrae*. In some cases, the populations of transformed bacteria are created from the genus *Granulicatella*. In some cases, the populations of transformed bacteria are created from *Granulicatella elegans*. In some cases, the populations of transformed bacteria are created from the genus *Hyphomicrobium*. In some cases, the populations of transformed bacteria are created from *Hyphomicrobium facile*. In some cases, the populations of transformed bacteria are created from the genus *Janibacter*. In some cases, the populations of transformed bacteria are created from *Janibacter melonis*. In some cases, the populations of transformed bacteria are created from the genus *Kocuria*. In some cases, the populations of transformed bacteria are created from *Kocuria marina*. In some cases, the populations of transformed bacteria are created from *Kocuria palustris*. In some cases, the populations of transformed bacteria are created from *Kocuria rhizophila*. In some cases, the populations of transformed bacteria are created from the genus *Lactobacillus*. In some cases, the populations of transformed bacteria are created from *Lactobacillus crispatus*. In some cases, the populations of transformed bacteria are created from *Lactobacillus jensenii*. In some cases, the populations of transformed bacteria are created from the genus *Leuconostoc*. In some cases, the populations of transformed bacteria are created from *Leuconostoc argentinum*. In some cases, the populations of transformed bacteria are created from the genus *Methylobacterium*. In some cases, the populations of transformed bacteria are created from *Methylobacterium extorquens*. In some cases, the populations of transformed bacteria are created from *Methylobacterium mesophilicum*. In some cases, the populations of transformed bacteria are created from the genus *Micrococcus*. In some cases, the populations of transformed bacteria are created from *Micrococcus luteus*. In some cases, the populations of transformed bacteria are created from the genus *Microlunatus*. In some cases, the populations of transformed bacteria are created from *Microlunatus phosphovorius*. In some cases, the populations of transformed bacteria are

created from the genus *Mobiluncus curtisii*. In some cases, the populations of transformed bacteria are created from *Mobiluncus curtisii subsp. holmesii*. In some cases, the populations of transformed bacteria are created from the genus *Mycobacterium*. In some cases, the populations of transformed bacteria are created from *Mycobacterium chlorophenolicum*. In some cases, the populations of transformed bacteria are created from *Mycobacterium obuense*. In some cases, the populations of transformed bacteria are created from the genus *Nakamurella*. In some cases, the populations of transformed bacteria are created from *Nakamurella multipartita*. In some cases, the populations of transformed bacteria are created from the genus *Pedomicrobium*. In some cases, the populations of transformed bacteria are created from *Pedomicrobium australicum*. In some cases, the populations of transformed bacteria are created from the genus *Peptoniphilus*. In some cases, the populations of transformed bacteria are created from *Peptoniphilus harei*. In some cases, the populations of transformed bacteria are created from the genus *Peptostreptococcus*. In some cases, the populations of transformed bacteria are created from *Peptostreptococcus anaerobius*. In some cases, the populations of transformed bacteria are created from the genus *Prevotella*. In some cases, the populations of transformed bacteria are created from *Prevotella bivia*. In some cases, the populations of transformed bacteria are created from *Prevotella corporis*. In some cases, the populations of transformed bacteria are created from *Prevotella disiens*. In some cases, the populations of transformed bacteria are created from *Prevotella melaninogenica*. In some cases, the populations of transformed bacteria are created from the genus *Propionibacterium*. In some cases, the populations of transformed bacteria are created from *Propionibacterium acnes*. In some cases, the populations of transformed bacteria are created from *Propionibacterium granulosum*. In some cases, the populations of transformed bacteria are created from the genus *Pseudomonas*. In some cases, the populations of transformed bacteria are created from *Pseudomonas aeruginosa*. In some cases, the populations of transformed bacteria are created from *Pseudomonas saccharophila*. In some cases, the populations of transformed bacteria are created from *Pseudomonas stutzeri*. In some cases, the populations of transformed bacteria are created from *Pseudomonas tremiae*. In some cases, the populations of transformed bacteria are created from the genus *Rhodococcus*. In some cases, the populations of transformed bacteria are created from *Rhodococcus corynebacterioides*. In some cases, the populations of transformed bacteria are created from *Rhodococcus erythropolis*. In some cases, the populations of transformed bacteria are created from the genus *Rothia*. In some cases, the populations of transformed bacteria are created from *Rothia aeria*. In some cases, the populations of transformed bacteria are created from *Rothia dentocariosa*. In some cases, the populations of transformed bacteria are created from *Rothia mucilaginosa*. In some cases, the populations of transformed bacteria are created from *Rothia nasimurium*. In some cases, the populations of transformed bacteria are created from the genus *Serratia*. In some cases, the populations of transformed bacteria are created from *Serratia liquefaciens*. In some cases, the populations of transformed bacteria are created from *Serratia marcescens subsp. Sakuensis*. In some cases, the populations of transformed bacteria are created from the genus *Sphingobium*. In some cases, the populations of transformed bacteria are created from *Sphingobium amiens*. In some cases, the populations of transformed bacteria are created from the genus *Staphylococcus*. In some cases, the populations of transformed bacteria are created from *Staphylococcus capitis*. In some cases,

the populations of transformed bacteria are created from *Staphylococcus caprae*. In some cases, the populations of transformed bacteria are created from *Staphylococcus cohnii*. In some cases, the populations of transformed bacteria are created from *Staphylococcus epidermidis*. In some cases, the populations of transformed bacteria are created from *Staphylococcus haemolyticus*. In some cases, the populations of transformed bacteria are created from *Staphylococcus hominis*. In some cases, the populations of transformed bacteria are created from *Staphylococcus saccharolyticus*. In some cases, the populations of transformed bacteria are created from *Staphylococcus warneri*. In some cases, the populations of transformed bacteria are created from the genus *Stenotrophomonas*. In some cases, the populations of transformed bacteria are created from *Stenotrophomonas maltophilia*. In some cases, the populations of transformed bacteria are created from the genus *Streptococcus*. In some cases, the populations of transformed bacteria are created from *Streptococcus agalactiae*. In some cases, the populations of transformed bacteria are created from *Streptococcus cristatus*. In some cases, the populations of transformed bacteria are created from *Streptococcus gordonii*. In some cases, the populations of transformed bacteria are created from *Streptococcus infantis*. In some cases, the populations of transformed bacteria are created from *Streptococcus intermedius*. In some cases, the populations of transformed bacteria are created from *Streptococcus mitis*. In some cases, the populations of transformed bacteria are created from *Streptococcus parasanguinis*. In some cases, the populations of transformed bacteria are created from *Streptococcus parasanguinis*. In some cases, the populations of transformed bacteria are created from *Streptococcus salivarius*. In some cases, the populations of transformed bacteria are created from *Streptococcus sanguinis*. In some cases, the populations of transformed bacteria are created from the genus *Tetrasphaera*. In some cases, the populations of transformed bacteria are created from *Tetrasphaera elongata*. In some cases, the populations of transformed bacteria are created from the genus *Tsukamurella*. In some cases, the populations of transformed bacteria are created from *Tsukamurella tyrosinosolvens*. In some cases, the populations of transformed bacteria are created from the genus *Tsukamurella*. In some cases, the populations of transformed bacteria are created from the genus *Veillonella*. In some cases, the populations of transformed bacteria are created from *Veillonella parvula*. In some cases, the populations of transformed bacteria are created from *Veillonella parvula*. In some cases, the populations of transformed bacteria are created from *Bradyrhizobiaceae U8776*. In some cases, the populations of transformed bacteria are created from *Carnobacterium AJ427446*. In some cases, the populations of transformed bacteria are created from *Corynebacterium AY581888*. In some cases, the populations of transformed bacteria are created from *Corynebacterium AF543288*. In some cases, the populations of transformed bacteria are created from *Corynebacterium X81872*. In some cases, the populations of transformed bacteria are created from *Corynebacterium X84253*. In some cases, the populations of transformed bacteria are created from *Dermacoccus AF409025*. In some cases, the populations of transformed bacteria are created from *Finegoldia AB109769*. In some cases, the populations of transformed bacteria are created from *Haemophilus AF224309*. In some cases, the populations of transformed bacteria are created from *Methylobacterium AY741717*. *Neisseria DQ409137*.

[0119] Bacterial strains can be modified to avoid undesired reactions, inflammation, for

methods to easily remove the bacteria, attenuate the bacteria, and for preventing the release of unwanted bacterial biomolecules that can be detrimental to the host.

[0120] In some bacteria there are toxic proteins that can be involved with lysis and inflammation. Bacteria for example can secrete toxins such as endotoxins and exotoxins. Endotoxins are cell associated substances that are structural components of the bacteria and are released from bacteria or from bacterial cells that are a result of lysis from the host defense mechanisms. Exotoxins are secreted by bacteria and can be small biomolecules, proteins, and can also be minimal peptides that act enzymatically. Several examples of bacterial toxins include but are not limited to hemolysin (*E. coli*), alpha toxin (*S. aureus*), leukocidin (*S. aureus*), CAMP factor (*Propionibacterium*), hyaluronidase (*Propionibacterium*), neuraminidase (*Propionibacterium*), enterotoxin B (*S. aureus*), verotoxin (*E. coli*). For example *Propionibacterium acnes* naturally make the biomolecule CAMP Factor, which has a co-hemolytic activity with sphingomyelinase that can confer cytotoxicity to keratinocytes and macrophages. The CAMP Factor together with the acid sphingomyelinase from host cells can lead to lysis and inflammation in the host. In another example, hemolysis is a mechanism that is employed by many bacterial pathogens that work to degrade, invade host cells and to resist the hosts' immune system. *P. acnes* carries within its genome 5 different CAMP homologs (CAMP1, CAMP2, CAMP3, CAMP4 and CAMP5). Many other types of bacteria can also secrete cytotoxins. *Staphylococcus aureus*, for example, can produce a wide variety of virulence factors that include but are not limited to enzymes, toxins, superantigens, exfoliative toxins, alpha toxin, beta toxin, delta toxin, and several types of bicomponent toxins.

[0121] In some bacteria, lipase can be used for a beneficial effect. In some microbes, genes for lipases are not knocked out as lipases can be used to break down oils in subjects suffering from axillary odor or plantar odor. In some cases, *Propionibacterium* is engineered to release lipases to reduce sebaceous secretions.

[0122] In order to prevent detrimental effects from virulence factors, modifications can be performed to knock out specific genes of interest that are involved in the secretion of toxic factors of the bacteria to create conditional mutants. Conditional mutants can be described as having a wild type phenotype under certain permissive environmental conditions and a mutant phenotype under other restrictive conditions. In some cases, genes encoding toxic proteins can be mutated or knocked out to prevent nosocomial infections or disease symptoms in a host. In some cases, genes encoding enzymes that cause inflammation in a host can be mutated or knocked out to prevent manifestation of disease in a host. In some cases, genes encoding proteins involved in the synthesis of host viral factors can be mutated or knocked out in the bacteria. In some cases, the genes are for CAMP factor. In some cases the genes are for lipases. However, in some microbes, genes for lipases are not knocked out as lipases can be used to break down oils in subjects suffering from axillary odor or plantar odor. In some cases, *Propionibacterium* is engineered to release lipases to reduce sebaceous secretions. In some cases, the genes are for alpha toxin. In some cases, the genes are for beta toxin. In some cases the genes are for delta toxin. In some cases, the genes are for types of bicomponent toxins. In some cases, the genes are for hemolysins.

[0123] Several microbes do not require any growth factors and can synthesize essential purines, pyrimidines, amino acids and vitamins, in which they can start with a carbon source as part of their own metabolism. However, some types of microbes will require purines, pyrimidines, amino acids and vitamins in order to grow, and must be added in culture media to grow these microbes. By genetically modifying microbes so that they require a growth factor not needed by a wild type, a person skilled in the art can produce genetically modified "auxotrophs" or a mutant organism that has a nutritional requirement not shared by the parent organism.

[0124] Genetically modified microbes can be modified so that they are nutritional auxotrophs. In order to create a dependency on their surroundings for survival, one can control the amount of microbes are in a microenvironment, or one can eliminate a population of microbes by depleting the environment of a necessary nutrient. In several cases, the microbe comprises a genome with a mutation or knock out in a gene that codes for glutamine synthetase. In several cases, the microbe comprises a genome with a mutation or knock out in a gene that codes for asparagine synthetase. In several cases, the microbe comprises a genome with a mutation or knock out in a gene that codes for aspartokinase. In several cases, the microbe comprises a genome with a mutation or knock out in a gene that codes for aspartate semialdehyde dehydrogenase.

[0125] Toxins are also secreted by many fungi, which allow their successful colonization and infection of hosts under predisposing conditions. Secreted proteins can be involved in the virulence of several fungi species. For example, hydrolytic enzyme production, which also plays a role in the pathogenicity of bacteria and yeasts are commonly associated with virulence. Several toxins by fungi include but are not limited to secreted aspartyl proteinases (Sap) (*C. albicans*), phospholipase B enzymes (*C. albicans*), lipases (*C. albicans*). In order to prevent infection by fungi, specific genes can be mutated or knocked out to prevent virulent factors from causing disease in a host.

[0126] Fungal cells can be genetically engineered to secrete biomolecules. In several cases described herein, populations of transformed fungal cells are created from members from a group consisting of the genus. In several cases described herein, populations of transformed bacteria is created from bacterial members from a group consisting of the genus *Candida*. In several cases described herein, populations of transformed bacteria is created from bacterial members from a group consisting of *Candida albicans*, *Candida glabrata*, *Candida tropicalis*, *Candida parapsilosis*, and *Candida krusei*.

[0127] In certain cases a composition comprises one or more genetically modified microbes. In some cases a composition comprises at least 1, at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, or at least 10 genetically modified microbes. In some cases, a composition comprises 2, 3, 4, 5, 6, 7, 8, 9 or 10 or more genetically modified microbes. In certain cases a genetically modified microbe in a composition is configured to express one or more genes of interest. In certain cases a composition comprises a microbe

that is genetically modified so that the expression of a pathogenic molecule (e.g., a pathogenic protein endogenous to the microbe) is substantially reduced or eliminated. In some cases a composition comprises a genetically modified microbe where one or more genes (e.g., endogenous essential genes) of the microbe are knocked out. In some cases a composition comprises a genetically modified microbe configured to express one or more essential genes under the direction of an inducible promoter.

[0128] In certain cases a composition comprises a pharmaceutical acceptable excipient or carrier. Pharmaceutical acceptable excipient or carriers contemplated for use herein are often not toxic to a genetically modified microbe. Pharmaceutical compositions can be formulated in a suitable manner using one or more physiologically acceptable carriers comprising excipients and auxiliaries, which can be used pharmaceutically. Proper formulation can depend upon the route of administration chosen. In particular, a suitable formulation, ingredient, excipient, the like or combinations thereof as listed in "Remington's Pharmaceutical Sciences," Mack Publishing Co., Easton, PA, 18th edition, 1990. can be used with a composition described herein. Compositions herein can be incorporated into or used with a suitable material described in Remington's.

[0129] As used herein the term "pharmaceutically acceptable" and "physiologically acceptable" mean a biologically acceptable formulation, gaseous, liquid or solid, or mixture thereof, which is suitable for one or more routes of administration, *in vivo* delivery or contact. Such formulations include solvents (aqueous or non-aqueous), solutions (aqueous or non-aqueous), emulsions (e.g., oil-in-water or water-in-oil), suspensions, syrups, elixirs, dispersion and suspension media, coatings, isotonic and absorption promoting or delaying agents, compatible with pharmaceutical administration or *in vivo* contact or delivery. Aqueous and non-aqueous solvents, solutions and suspensions may include suspending agents and thickening agents. Such pharmaceutically acceptable carriers include tablets (coated or uncoated), capsules (hard or soft), microbeads, powder, granules and crystals. Supplementary active compounds (e.g., preservatives, antibacterial, antiviral and antifungal agents) can also be incorporated into the compositions.

[0130] Genetically engineered microbes can be placed within excipients that are optimal for the survival of the bacteria or the fungus. In several cases described herein, compositions are described which comprise vehicles or excipients that help maintain the integrity and viability of the bacteria. Vehicles as described herein can refer to a substance of no therapeutic value that is used to convey an active medicine for administration. Pharmaceutical vehicle as described herein can refer to a carrier or inert medium used as a solvent in which the medicinally active agent is formulated and or administered. Vehicles can include polymeric micelles, liposomes, lipoprotein-based carriers, nano-particle carriers, dendrimers, and other vehicles for bacteria that are known to one skilled in the art. An ideal vehicle can be non-toxic, biocompatible, non-immunogenic, biodegradable, and can avoid recognition by the host's defense mechanisms. In several cases described herein, compositions are described which comprise vehicles or excipients that help maintain the integrity of the fungus. In some cases, the vehicles are pharmaceutical vehicles. In some cases, the pharmaceutical vehicles include pharmaceutical

compositions. In some cases, the vehicle is polymeric micelles. In some cases, the vehicle is liposomes. In some cases, the vehicle is lipoprotein-based carriers. In some cases, the vehicle is nano-particle carriers. In some cases, the vehicle is dendrimers.

[0131] Pharmaceutical compositions can be formulated to be compatible with a particular route of administration. Thus, pharmaceutical compositions include carriers, diluents, or excipients suitable for administration by various routes.

[0132] In some embodiments, a composition is formulated as a topical (e.g., dermal) formulation. In some embodiments, a composition is formulated for topical administration to a mammal. A topical formulation may include a formulation such as a gel formulation, a cream formulation, a lotion formulation, a paste formulation, an ointment formulation, an oil formulation, and a foam formulation. The composition further may include an absorption emollient.

[0133] Additional examples of a composition can optionally be formulated to be delivered to the mucosum, or by inhalation, respiration, intranasal, oral, buccal, or sublingual.

[0134] Salts may be added. Non-limiting examples of salts include acetate, benzoate, besylate, bitartate, bromide, carbonate, chloride, citrate, edetate, edisylate, estolate, fumarate, gluceptate, gluconate, hydrobromide, hydrochloride, iodide, lactate, lactobionate, malate, maleate, mandelate, mesylate, methyl bromide, methyl sulphate, mucate, napsylate, nitrate, pamoate (embonate), phosphate, diphosphate, salicylate and disalicylate, stearate, succinate, sulphate, tartrate, tosylate, triethiodide, valerate, aluminium, benzathine, calcium, ethylene diamine, lysine, magnesium, meglumine, potassium, procaine, sodium, tromethamine or zinc.

[0135] Chelating agents may be added. Non-limiting examples of chelating agents include ethylenediamine, ethylene glycol tetraacetic acid, 1,2-bis(o-aminophenoxy)ethane- N,N,N',N'-tetraacetic acid, Penicillamine, Deferasirox, Deferiprone, Deferoxamine, 2,3-Disulfanylpropan-1-ol, Dexrazoxane, Iron(II,III) hexacyanoferrate(II,III), (R)-5-(1,2-dithiolan-3-yl)pentanoic acid, 2,3-Dimercapto-1-propanesulfonic acid, Dimercaptosuccinic acid, or diethylene triamine pentaacetic acid.

[0136] Buffering agents may be added to. Non-limiting examples of buffering agents include phosphate, citrate, acetate, borate, TAPS, bicine, tris, tricine, TAPSO, HEPES, TES, MOPS, PIPES, cacodylate, SSC, MES or succinic acid.

[0137] Cosolvents may be added. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters. Non-limiting examples of cosolvents contain hydroxyl groups or other polar groups, for example, alcohols, such as isopropyl alcohol; glycols, such as propylene glycol, polyethyleneglycol, polypropylene glycol, glycol ether; glycerol; polyoxyethylene alcohols and polyoxyethylene fatty acid esters.

[0138] Supplementary compounds (e.g., preservatives, antioxidants, antimicrobial agents including biocides and biostats such as antibacterial, antiviral and antifungal agents) can also be added. Pharmaceutical compositions may therefore include preservatives, antioxidants and antimicrobial agents.

[0139] Preservatives can be used to inhibit undesired microbial growth or increase stability of ingredients thereby prolonging the shelf life. Suitable preservatives are known in the art and include, for example, EDTA, EGTA, benzalkonium chloride or benzoic acid or benzoates, such as sodium benzoate. Antioxidants include, for example, ascorbic acid, vitamin A, vitamin E, tocopherols, and similar vitamins or provitamins.

[0140] In several cases herein, methods are described to preserve the genetically engineered bacteria cryogenically for future use. In several cases herein, methods are described to preserve the bacteria by freeze drying the genetically engineered bacteria for future use. In several cases herein, methods are described to preserve the genetically engineered fungus cryogenically for future use. In several cases herein, methods are described to preserve the genetically engineered fungus by freeze drying the bacteria for future use.

[0141] Particular non-limiting classes of anti-virals include reverse transcriptase inhibitors; protease inhibitors; thymidine kinase inhibitors; sugar or glycoprotein synthesis inhibitors; structural protein synthesis inhibitors; nucleoside analogues; and viral maturation inhibitors. Specific non-limiting examples of anti-virals include nevirapine, delavirdine, efavirenz, saquinavir, ritonavir, indinavir, nelfinavir, amprenavir, zidovudine (AZT), stavudine (d4T), larnivudine (3TC), didanosine (DDI), zalcitabine (ddC), abacavir, acyclovir, penciclovir, ribavirin, valacyclovir, ganciclovir, 1,-D-ribofuranosyl-1,2,4-triazole-3 carboxamide, 9->2-hydroxy-ethoxy methylguanine, adamantanamine, 5-iodo-2'-deoxyuridine, trifluorothymidine, interferon and adenine arabinoside.

[0142] Pharmaceutical formulations and delivery systems appropriate for the compositions and methods of the invention are known in the art (see, e.g., Remington: The Science and Practice of Pharmacy (2003) 20th ed., Mack Publishing Co., Easton, PA; Remington's Pharmaceutical Sciences (1990) 18th ed., Mack Publishing Co., Easton, PA; The Merck Index (1996) 12th ed., Merck Publishing Group, Whitehouse, NJ; Pharmaceutical Principles of Solid Dosage Forms (1993), Technomic Publishing Co., Inc., Lancaster, Pa.; Ansel and Soklosa, Pharmaceutical Calculations (2001) 11th ed., Lippincott Williams & Wilkins, Baltimore, MD; and Poznansky et al., Drug Delivery Systems (1980), R. L. Juliano, ed., Oxford, N.Y., pp. 253-315).

[0143] In certain cases a composition comprises a molecule configured to engage, either directly or indirectly, an inducible promoter. In some cases a molecule configured to engage an inducible promoter is configured to activate the inducible promoter (e.g., activate gene transcription/translation). In some cases a molecule configured to engage an inducible promoter is configured to repress activation of gene expression by the inducible promoter. In some cases a molecule configured to engage an inducible promoter comprises a compound,

alcohol, nutrient, metal, amino acid (*e.g.*, an amino acid such as a synthetic amino acid), sugar or sugar analog (*e.g.*, lactose, arabinose, IPTG), peptide, or protein. In certain cases a composition comprises lactose, arabinose, IPTG, tryptophan, or tetracycline.

[0144] In certain cases herein, methods of making a nucleic acid for transformation or integration into a microbe genome are described. Methods can include providing a nucleic acid sequence encoding a peptide, and joining said nucleic acid sequence to a nucleic acid sequence encoding a regulatory element or a nucleic acid encoding a secretory peptide. In some cases, a nucleic acid comprises a sequence encoding a peptide wherein the peptide comprises an amino acid sequence of a secretory peptide. In some cases, a nucleic acid comprises signal sequence for secretion. In some cases, a nucleic acid comprises a sequence, or encodes a polypeptide sequence that is recognized by a cells secretory pathway. In some cases, a nucleic acid encodes a non-secreted protein (*e.g.*, an enzyme) for production of a biomolecule of interest. In some cases, a signal sequence for secretion comprises the sequence SEQ ID NO: 212, SEQ ID NO: 213, SEQ ID NO: 214, SEQ ID NO: 215, or SEQ ID NO: 216.

[0145] Those skilled in the art will appreciate that gene expression levels are dependent on many factors, such as promoter sequences and regulatory elements. Another factor for maximal protein selection is adaptation of codons of the transcript gene to the typical codon usage of a host. As noted for most bacteria, a small subset of codons are recognized by tRNA species leading to translational selection and is important in the limitations of protein expression. In this aspect, many synthetic genes can be designed to increase their protein expression level. The design process of codon optimization can be to alter rare codons to codons known for protein expression efficiency. In some cases, codon selection is described, wherein codon selection can be performed by using algorithms that are known to those skilled in the art to create synthetic genetic transcripts optimized for high protein yield. Programs containing algorithms for codon optimization are known to those skilled in the art. Programs can include, for example, OptimumGene™, GeneGPS® algorithms, etc. Additionally synthetic codon optimized sequences can be obtained commercially for example from Integrated DNA Technologies and other commercially available DNA sequencing services. In some cases, peptides for secretion are described wherein the genes for the peptides for secretion are codon optimized for expression in humans. In some cases, peptides for secretion are described, wherein the genes for the complete gene transcript are codon optimized for expression bacteria, which can include gene transcripts a secretory peptide and other peptides that are known to increase the level of expression. In some cases, peptides are described, wherein genes for the peptide for secretion are optimized to have selected codons specifically for protein expression in bacterial and fungal cells.

[0146] The term "subject" includes but is not limited to a subject having or at risk of having a disorder which would benefit from contact with or administration of a genetically modified microbe as set forth herein. Subjects include mammalian animals (mammals), such as humans, a non-human primate (apes, gibbons, gorillas, chimpanzees, orangutans, macaques), a domestic or companion animal (dogs and cats), a farm animal (poultry such as

chickens and ducks, horses, cows, goats, sheep, pigs), and experimental animals (mouse, rat, rabbit, guinea pig). Subjects include veterinary animals, as well as animal disease models, for example, mouse and other animal models of a skin disorder.

[0147] Invention genetically modified microbes and compositions comprising genetically modified microbes can be employed in various methods and uses and medicaments. Such methods and uses and medicaments include, for example, administration *ex vivo* and *in vivo*. In various cases, methods and uses and medicaments provided include methods and uses and medicaments for treatment of skin disorders.

[0148] Skin disorders can arise from genetic predisposition to skin disorders, as well as the disturbance of the natural flora or natural bacteria that can reside on the skin. Several types of skin disorders exist for example but are not limited to acne, actinic keratosis, alopecia areata, athlete's foot, onychomycosis, atopic dermatitis, osmidrosis, eczema, fungal infection of the nails, psoriasis, rosacea, slow wound healing, folliculitis, keratosis pilaris, perioral dermatitis, angiofibromas, cutaneous inflammation, cosmesis, aging damage, dyschromia, premature greying hair, and seborrhea. In some embodiments described herein, methods to treat skin disorders are described. In several cases, methods to treat rosacea are described. In several cases, methods to treat alopecia are described. In several cases, methods to treat onychomycosis are described. In several cases, methods to treat osmidrosis are described. In several cases, methods to treat slow wound healing are described. In several cases, methods to treat premature greying hair are described. In several cases, methods to treat cutaneous inflammation are described. In several cases, methods to treat dyschromia are described.

[0149] Acne or acne vulgaris, is a common skin disease that can affect the face, neck, chest, and back. It is characterized by the regions on the skin that has seborrhea. Regions of the skin that have acne, can also have comedones, papules, nodules, cysts, boils, cystic acne and pimples. Acne can be caused by hormones, such as testosterone and can be affected by associated sebaceous glands. Causes for acne can be from hormonal, genetic and infectious effects. Management for acne can be the use of topical medications such as benzoyl peroxide, salicylic acid, and hormones. More common can be the use of antibiotics to treat acne, however, with the ability to develop bacterial resistance, many types of antibiotics are becoming less effective.

[0150] *Propionibacterium acnes* (*P. acnes*) is an anaerobic bacterial species that is widely concluded to cause acne. *Staphylococcus aureus* a natural floral bacteria of the skin is also believed to be an opportunistic bacteria to infect the skin even though it is seen in both healthy and infected skin. However it has been shown in studies that *P. acnes* as well as *S. aureus* have been developing antibiotic resistance, thereby increasing the need to develop a new treatment for skin disorders that are normally treated with antibiotics. In several cases described herein, genetically modified bacteria that secrete biomolecule(s) are used to treat acne. In several cases, described herein, genetically modified fungus that secretes biomolecules are used to treat acne.

[0151] Rosacea as described herein, refer to a chronic condition characterized by facial erythema and sometimes pimples. Rosacea has four subtypes, three affecting the skin and the fourth affecting the eyes (ocular type). Earlier treatment was the use of topical steroids, however, treatment in the form of topical steroids can aggravate the condition with longtime usage. Therefore new methods of treatment have been currently researched. Rosacea affects both sexes, but is almost three times more common in women.

[0152] Rosacea begins as redness on the central face across the cheeks, nose, or forehead, but can also less commonly affect the neck, chest, ears, and scalp. In some cases, additional symptoms, such as semi-permanent redness, telangiectasia (dilation of superficial blood vessels on the face), red domed papules (small bumps) and pustules, red gritty eyes, burning and stinging sensations, and in some advanced cases, a red lobulated nose, thinophyma, may develop. Triggers that cause episodes of flushing and blushing play a part in the development of rosacea. Exposure to temperature extremes can cause the face to become flushed as well as strenuous exercise, heat from sunlight, severe sunburn, stress, anxiety, cold wind, and moving to a warm or hot environment from a cold one such as heated shops and offices during the winter. There are also some food and drinks that can trigger flushing, including alcohol, food and beverages containing caffeine (especially, hot tea and coffee), foods high in histamines and spicy food.

[0153] Certain medications and topical irritants can quickly trigger rosacea. Some acne and wrinkle treatments that have been reported to cause rosacea include microdermabrasion and chemical peels, as well as high dosages of isotretinoin, benzoyl peroxide, and tretinoin. *Steroid induced rosacea* is the term given to rosacea caused by the use of topical or nasal steroids. These steroids are often prescribed for seborrheic dermatitis. Dosage should be slowly decreased and not immediately stopped to avoid a flare up. Intestinal flora can play a role in causing the disease.

[0154] Oral tetracycline antibiotics (tetracycline, doxycycline, minocycline) and topical antibiotics such as metronidazole are usually the first line of defense prescribed by doctors to relieve papules, pustules, inflammation and some redness.

[0155] Oral antibiotics can help to relieve symptoms of ocular rosacea. If papules and pustules persist, then sometimes isotretinoin can be prescribed. Isotretinoin has many side effects and is normally used to treat severe acne but in low dosages is proven to be effective against papulopustular and phymatous rosacea. Some individuals respond well to the topical application of sandalwood oil on the affected area, particularly in reducing the prevalence of pustules and erythema. The oral antibiotics can be a first line of defense against bacteria on the skin that can trigger the rosacea, however due to the rise of antibiotic resistance, new developments have been sought in order to treat both bacterial ailments of the skin as well as rosacea. In several cases, described herein, genetically modified microbes are used for treatment of rosacea.

[0156] Alopecia areata as described herein refer to a condition in which hair is lost from some

or all areas of the body, usually from the scalp. Because it causes bald spots on the scalp, especially in the first stages, it is sometimes called spot baldness. In 1-2% of cases, the condition can spread to the entire scalp (alopecia totalis) or to the entire epidermis (alopecia universalis). Conditions resembling AA, and having a similar cause, occur also in other species.

[0157] The condition is thought to be a systemic autoimmune disorder in which the body attacks its own antigen hair follicles and suppresses or stops hair growth. T cell lymphocytes cluster around affected follicles, causing inflammation and subsequent hair loss. A few cases of babies being born with congenital AA have been reported, but these are not cases of autoimmune disease, because an infant is born without a definitely developed immune system. Endogenous retinoids metabolic defect is a key part of the pathogenesis of the AA. Also, some evidence indicates AA affects the part of the hair follicle associated with hair color. Hair that has turned gray cannot be affected. In some cases, genetically modified microbes are used for treatment of an immune disorder.

[0158] Onychomycosis refers to a fungal infection of the nail. The infection can be caused by pathogens of onychomycosis include dermatophytes, *Candida*, and nondermatophytic molds. Dermatophytes are the fungi most commonly responsible for onychomycosis in the temperate western countries; while *Candida* and nondermatophytic molds are more frequently involved in the tropics and subtropics with a hot and humid climate. Pathogens can include *Candida* and nondermatophytic molds, in particular members of the mold generation *Scytalidium* (*Neoscytalidium*), *Scopulariopsis*, and *Aspergillus*. *Candida* spp. mainly cause fingernail onychomycosis in people whose hands are often submerged in water. *Scytalidium* mainly affects people in the tropics, though it persists if they later move to areas of temperate climate. Treatments mostly include topical or antifungal medications such as terbinafine, itraconazole, and fluconazole.

[0159] Osmidrosis refers to body odor, in which sebaceous and apocrine glands can play a role. Medical conditions can be referred to as bromhidrosis, apocrine bromhidrosis, osmidrosis, ozochrotia, fetid sweat, and malodorous sweating. Osmidrosis or bromhidrosis is defined by a foul odor due to a water rich environment that is supportive of bacterial growth which is also caused by an abnormal increase in perspiration. In some cases, methods are used for the treatment of osmidrosis.

[0160] "Poor wound healing" can be caused by a poor immune system, diabetes mellitus, low human growth hormone, rheumatoid arthritis, poor circulation from vascular or arterial diseases, zinc deficiency, vitamin deficiency, lupus, etc. In several cases, poor wound healing can lead to opportunistic infections by the normal flora. In several cases, treatment for wound healing using genetically modified microbes are described.

[0161] "Cutaneous inflammation" and "Chronic cutaneous inflammation" can be hallmarked by macrophage infiltration of the dermal area. During an infection, the macrophage response is to mediate a chronic inflammation, which is seen in several inflammatory dermatoses which includes but is not limited to psoriasis, atopic dermatitis, and chronic contact dermatitis. In

order to decrease the inflammatory response, treatments have been described in which elimination of the macrophage at the site of the inflammation is sought. One way is to block the activity of the effector molecules or the cytokines that are produced by the macrophage. Another way is to target the macrophage for apoptotic elimination locally during the inflammation by engineering an immunotoxin. Treatment can be for other skin disorders caused by cutaneous inflammation or chronic cutaneous inflammation such as cutaneous graft-versus-host disease, lichenoid, sclerodermatous, granuloma annulare, sarcoid, chronic contact dermatitis, psoriasis, UV skin injuries, atopic dermatitis, and cutaneous T-Cell lymphoma. However, most ways to eliminate the local response is time consuming. In this respect, having an engineered microbe population to eliminate the macrophage locally at the site of chronic cutaneous inflammation can provide a fast treatment for either cutaneous inflammation, or chronic cutaneous inflammation.

[0162] In some implementations kits may be provided that include genetically modified microbes, such as a bacteria of the genus *Propionibacterium*, compositions and pharmaceutical formulations thereof, packaged into suitable packaging material. Kits, which are not part of the present invention, are used in various *in vitro*, *ex vivo* and *in vivo* methods and uses, for example a treatment method or use as disclosed herein.

[0163] A kit typically includes a label or packaging insert including a description of the components or instructions for use *in vitro*, *in vivo*, or *ex vivo*, of the components therein. A kit can contain a collection of such components, e.g., a genetically modified microbe, such as a bacteria of the genus *Propionibacterium* alone, or in combination with another therapeutically useful composition (e.g., an anti-inflammatory drug).

[0164] The term "packaging material" refers to a physical structure housing the components of the kit. The packaging material can maintain the components sterilely, and can be made of material commonly used for such purposes (e.g., paper, corrugated fiber, glass, plastic, foil, ampules, vials, tubes, etc.).

[0165] Kits of the invention can include labels or inserts. Labels or inserts include "printed matter," e.g., paper or cardboard, or separate or affixed to a component, a kit or packing material (e.g., a box), or attached to an ampule, tube or vial containing a kit component. Labels or inserts can additionally include a computer readable medium, such as a disk (e.g., hard disk), optical disk such as CD- or DVD-ROM/RAM, DVD, MP3, magnetic tape, or an electrical storage media such as RAM and ROM or hybrids of these such as magnetic/optical storage media, FLASH media or memory type cards.

[0166] Labels or inserts can include identifying information of one or more components therein, dose amounts, clinical pharmacology of the active ingredient(s) including mechanism of action, pharmacokinetics and pharmacodynamics. Labels or inserts can include information identifying manufacturer information, lot numbers, manufacturer location and date.

[0167] Labels or inserts can include information on a condition, disorder, disease or symptom

for which a kit component may be used. Labels or inserts can include instructions for the clinician or for a subject for using one or more of the kit components in a method, use, treatment protocol or therapeutic regimen. Instructions can include dosage amounts, frequency or duration, and instructions for practicing any of the methods and uses, treatment protocols or therapeutic regimes set forth herein.

[0168] Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Although methods and materials similar or equivalent to those described herein can be used in the practice or testing of the present invention, suitable methods and materials are described herein.

[0169] The examples set forth below illustrate certain embodiments and do not limit the technology.

Examples

Example 1. Isolation of the RT6 *P. acnes* strain

[0170] E-swabs (Fisher) were used to collect bacteria from the human skin as follows: volunteer subjects were asked to wash their faces with soap and water and clean it with ethanol wipes before they applied the E-swab over their cheeks and forehead. The applicator was next transferred to a tube with the transfer medium. The tube was vortexed to release the bacteria into the medium. A 100-fold dilution of the transfer medium was plated onto RIC plates. After a week of anaerobic growth, colonies were observed on each plate. These colonies were grown in the BHI medium until they reached the appropriate density for DNA isolation. Genomic DNA was prepared using a commercial kit (Epicentre) and was PCR amplified using *P. acnes*-specific 16S rDNA primers Pas9/Pas11 (Table 1). The PCR fragments were gel purified and sequenced using the primer 16SFseq. The sequences were aligned to the 16S rDNA gene (NC_017550, SEQ ID NO:290) from ATCC 11828 (type II) and ATCC 6919 (type I). The colonies that showed a C to T conversion at nucleotide 1315 were assigned as type II RT6. Colonies were also tested for the RecA gene to confirm they belonged to a type II strain. After PCR amplification with primers RecAF/RecAR, the fragments were gel purified and sequenced with primers RecAFseq and RecARseq. A combination of sequences obtained with both primers covered over 90% of the RecA gene. The sequenced RecA fragments were aligned to the RecA gene from ATCC 11828 and ATCC 6919 strains for a reference sequence.

Table 1. Primers used for isolation of the RT6 *P. acnes* strain

Name	Sequence	Comment
PAS9F	CCCTGCTTTTGTGGGGTG (SEQ ID NO: 217)	16SrDNA upstream
PAS11R	CGACCCCAAAGAGGGAC (SEQ ID NO: 218)	16SrDNA downstream

Name	Sequence	Comment
16SFseq	ATCGCGTCGGAAGTGTAATC (SEQ ID NO: 219)	Sequencing primer
RecAF	AGCTCGGTGGGGTTCTCTCATC (SEQ ID NO: 220)	<i>RecA</i> upstream
RecAR	GCTTCCTCATACCACTGGTCATC (SEQ ID NO: 221)	<i>RecA</i> downstream
RecAF seq	GGTACCACTGCCATCTTCATTA (SEQ ID NO: 222)	Sequencing upstream
RecARseq	CACCAGCAGGGAATCTGTATC (SEQ ID NO: 223)	Sequencing downstream

Example 2. Molecular cloning.

[0171] PCR amplification was performed using Q5 high fidelity DNA polymerase (New England Biolab) and PCR machine (Eppendorf) following manufacturer's recommendations. For the gene assembly reactions, Gibson assembly method (NEBuilder HiFi DNA Assembly Cloning Kit, New England Biolab) and Golden Gate assembly method (NEB Golden Gate assembly Kit, New England Biolab) were used following manufacturer's recommendations.

Example 3. Isolation of Genomic DNA.

[0172] Total genomic DNA was isolated from the bacterial strains by phenolchloroform extraction according to the procedure described by Rhee (2011) with minor changes. *P. acnes* strains were cultured in brain heart infusion broth (BHI) at 37 °C until late log phase. After harvest the cells, the cell pellet was resuspended with 10 mM Tris-Cl pH 8.0, 10 mM Na₂EDTA and lysozyme (final concentration = 1 mg/mL) was added. After incubation at 37 °C for 20 min., 10 % of SDS was added (final concentration = 1.4 %) and incubated on ice for 10 min. The lysate was extracted 3 times with equal volume of phenolchloroform mixture (25:24:1 of phenol, chloroform and isoamyl alcohol), and then the DNA was precipitated with ethanol and dried.

Example 4. Electroporation of *P. acnes*.

[0173] *P. acnes* was transformed according to the procedure described by Cheong (2008) and Rhee *et al.* (2007). Cells were grown in 9 mL of BHI with Oxyrase for broth (Oxyrase) in a 13 × 100 screw cap tube until the OD 600 nm reached about 0.5. Cells were collected by centrifugation (4 °C; 4300 g; 10 min) and washed with ice-cold SG medium (glycerol, 10%;

sucrose, 0.5 M) three times. Cells were resuspended in SG medium (about 5×10^9 – 10^{10} CFU/ml). These electro-competent cells were used immediately. Seventy-five microliters of cell suspension was mixed with DNA and transferred to chilled electroporation cuvette (1 mm gap). The electroporation conditions using a Bio-Rad electroporator were 1.5 KV, 25 μ F and 600 Ω . The time constant was set between 8.5 and 10 ms. After electroporation, cells were transferred to 2 ml of pre-warmed (37 °C) BHI with Oxrase for broth. These cells were incubated at 37 °C for 10 hrs. Cells were collected by centrifugation (2000 x g, 10 min.) at room temperature and spread on reinforced clostridial agar plate with antibiotics. Plates were incubated at 37 °C at anaerobic condition.

Example 5. Construction of the growth arrest strain of *Bacillus subtilis*.

[0174] The truncated *dnaA* gene of *B. subtilis* was amplified by PCR with the genome DNA of *B. subtilis* strain 168 as a template and the primers (d-B_F:aggaaggaTCCATGGAAAATATATTAGACCTG (SEQ ID NO: 224), d-B_R:ctatgaccatgattacgCGCATGAATAACGGTCGTATG (SEQ ID NO: 225)). The DNA region with chloramphenicol gene and IPTG inducible promoter was amplified by PCR with primers (125_F:gcctgcaggtcgactTTAAGTTATTGGTATGACTGGTTTAAAG (SEQ ID NO: 226), 125_R:tecatggaTCCTTCCTCCTTTAATTGG (SEQ ID NO: 227)). These PCR products were assembled by NEBuilder® HiFi DNA Assembly Cloning Kit. The product was digested by HindIII and the 3,257 bp fragment was self-ligated. The self-ligated DNA was transformed into *B. subtilis* strain and selected the transformants on LB with Cm (5 μ g/mL) and the different concentration of IPTG (0, 0.05, 0.1 and 0.25 mM).

Example 6. Construction of the growth arrest strains of *P. acnes*.

[0175] Arabinose inducible *fts* operon. The arabinose inducible promoter and regulator of *B. subtilis* was truncated was amplified by PCR with the genome DNA of *B. subtilis* strain 168 as a template and the primers (araRE F :

TGCAGTTCTAGACGACACAGGCTGACGAAATTA (SEQ ID NO: 228), araRE R : CGTAGCGAATTCCATTTCCCTGCCCTCCCGAA (SEQ ID NO: 229)). The 1468 bp of PCR product was ligated into pUC18 hydrolyzed by XbaI and EcoRI. The truncated *fts* operon of *P. acnes* was amplified by PCR with the genome DNA of *P. acnes* strain ATCC 11828 as a template and the primers (ftzope F :

CTGACTGAATTGCTTCCCAACGGGGCCGTTT (SEQ ID NO: 230), ftzope R : GACTGCGAATTCCTGAAGAGCCGTCACCGACA (SEQ ID NO: 231)). The 1332 bp of PCR product hydrolyzed by EcoRI, was ligated into pUC18 with arabinose promoter also hydrolyzed by EcoRI. After insertion of the 1236 bp of DNA with erythromycin gene, this plasmid was introduced into *P. acnes* strain and transformants were selected on modified reinforced clostridial medium with L-arabinose (1.5 % w/v).

Example 7. Lactose inducible dnaA gene.

[0176] The β -galactose promoter region and truncated dnaA gene of *P. acnes* were amplified by PCR with the genome DNA of *P. acnes* strain ATCC11828 as a template and the primers (0410-1 F : GGCTTCTGGTCTCGAGTGTTGTGGAACGACAACA (SEQ ID NO: 232), 0410-1 R : GGCTTCTGGTCTCGATGGCACCAACCTTAGAGAG (SEQ ID NO: 233), 0410-2 F : GGCTTCTGGTCTCGCCATGTCCGACACACCGTTC (SEQ ID NO: 234), 0410-2 R : GGCTTCTGGTCTCGGGTAGAGACTGGGTAGAGACG (SEQ ID NO: 235)). These PCR products and DNA fragment with antibiotic genes, erythromycin and chloramphenicol genes were ligated into pUC18 by Golden Gate assembly method (NEB Golden Gate assembly Kit, New England Biolab). This plasmid was introduced into *P. acnes* strain and transformants were selected on modified reinforced clostridial medium with lactose (1.0 % w/v).

Table 2. List of primers

Target gene	Name	Sequences (5' - 3')
IL10	I10 Ec F	CGTCACCATATGCACTCCTCCGCTCTGCT (SEQ ID NO: 236)
	I10 Ec R	GTAGCTGGATCCTCAGTTGCGGATCTTCATGG (SEQ ID NO: 237)
EGF	EgcEc F	CGATGCCATATGAACTCCGACTCCGAGTGT (SEQ ID NO: 238)
	EgfEc R	GTTCACGGATCCTCAGCGCAGCTCCCACCACT (SEQ ID NO: 239)
GH	GHEc F	CGTGACCATATGTTCCCGACCATCCCGCT (SEQ ID NO: 240)
	GHEc R	GTGACTGGATCCTCAGAAACCGCAGGAGCCCT (SEQ ID NO: 241)

Example 8. Construction of CAMPII mutant *P. acnes* strain.

[0177] From the start codon of CAMPII gene of *P. acnes*, 400 bp of truncated ORF was amplified by PCR with genome DNA of *P. acnes* ATCC 11828 strain as templates and the primers (0422NB-1 F : AGTAACTTGGTCTGACATGAAGAAGACCCATCTTG (SEQ ID NO: 242), 0422NB-1 R : TATATATATTTATTATCCGATGGACCTTGTTTTGGAGAG (SEQ ID NO: 243)). The 438 bp of PCR product and DNA fragment with erythromycin and chloramphenicol resistance genes were ligated with pUC18 by NEBuilder HiFi DNA Assembly Cloning Kit and transformants of this ligation product were selected on LB with amp (100 μ g/mL) and cm (20mg/mL) plate. After

transformation into *E. coli* dcm⁻ dam⁻ strain, the plasmid was introduced into *P. acnes* and selected anaerobically on reinforced clostridial medium with erm (5µg/mL) or cm (5µg/mL) plates. The CAMPII mutant colonies were checked by PCR.

Example 9. Construction of markerless mutant *P. acnes* strain (Fig. 9).

[0178] The upstream and downstream regions of target gene were amplified by PCR. These regions should be bigger than 500 bp. These DNA fragments were ligated with the plasmid that had antibiotic marker and didn't have replication origin for *P. acnes*. This suicide vector was introduced into *P. acnes* and the transformants were selected by antibiotics. After confirmation of these, cells were cultured in reinforced clostridial medium without antibiotics to allow the second homologous recombination. After streak on the plate, the markerless mutant colonies were screened by PCR.

Example 10. Construction of lactose inducible IL10 secretion *P. acnes* strain.

[0179] The 1037 bp of β -galactose promoter region of *P. acnes* was amplified by PCR with the genome DNA of *P. acnes* strain ATCC11828 as a template and the primers (0505-1_F : TAAACTTGGTCTGACAGTGCGCACCGATGAGCGGCAGA (SEQ ID NO: 244), 0505-1_R : TTGCAAACATGGCACCAACCTTAGAGAGTCATG (SEQ ID NO: 245)). For secretion of IL10, the 188 bp of the signal peptide region was by PCR with the genome DNA of *B. subtilis* strain 168 as a template and the primers (0505-2_F : GGTTGGTGCCATGTTTGCAAAACGATTCAAAAC (SEQ ID NO: 246), 0505-2_R : AGGAGTG CATATGATAAATAGACATGGTTCCG (SEQ ID NO: 247)). The 568 bp of human IL10 ORF was amplified by PCR (0505-3_F : CTATTTATCATATGCACTCCTCCGCTCTG (SEQ ID NO: 248), 0505-3_R : TTATCCGATTCATGAGACTGTGAGTTGCGGATCTTCATGG (SEQ ID NO: 249)). These PCR products and DNA fragment with erythromycin and chloramphenicol resistance genes were ligated with pUC18 by NEBuilder HiFi DNA Assembly Cloning Kit and transformants of this ligation product were selected on LB with amp (100µg/mL) and cm (20 mg/mL) plate. After transformation into *E. coli* dcm⁻ dam⁻ strain, the plasmid was introduced into *P. acnes* and selected anaerobically on reinforced clostridial medium with erm (5 µg/mL) or cm (5 µg/mL) plates. After check the transformant by PCR, induction by lactose and secretion of IL10 was analyzed by ELISA.

Example 11. MC/9 Cell Culture.

[0180] The murine MC/9 cells were obtained from ATCC (CRL-8306) and were propagated in DMEM supplemented with 10% FBS (Gibco), 10% Rat T-STIM (BD), 2mM glutamate, 0.05 mM 2-mercaptoethanol and pen/strep. Cells were maintained at a density of 2×10^5 cells/ml in a 5%

CO₂ incubator at 37 °C.

Example 12. Functional assays for IL-10.

[0181] The purified recombinant human IL-10 was quantified using a commercial ELISA for human IL-10 (Biolegend). The biological activity of the expressed IL-10 was tested by using a dose-dependent co-stimulation (with human IL-4) of MC/9 cell proliferation. Briefly, cells were spun down and washed twice with RPMI1640 (Gibco) to remove all traces of the Rat-T-STIM in the growth medium. Next, cells were re-suspended in DMEM supplemented with 10% FBS, 2 mM glutamate, pen/strep, 2-mercaptoethanol and 200 pg/ml human IL-4 (Peprotech) and were plated at a density of 20,000 cells per well in a 96-well plate. A commercial recombinant human IL-10 (Peprotech) was used to generate a standard curve. Cells were grown for 82 hours and cell proliferation was determined using an XTT assay (Biotium).

Example 13. Isolation of the R6 Type II *P. acnes*.

[0182] Isolated *P. acnes* strains were checked to determine what ribotypes they were, with the goal of isolating a Type II, RT6 *P. acnes*. From an alignment of the RecA gene from a type II and type I strains (Fig. 10), we can see 10 nucleotide differences. These variations are used to identify sub-types of *P. acnes*. The ATCC strain 11828 was used as a positive control for a type II *P. acnes*.

[0183] The RecA gene sequenced from the putative RT6 clones aligns with the region 720-1191 of the RecA gene from ATCC11828 strain (Fig. 11). The alignment with the type I strain, NCTC 737, shows 5 bp variations indicating that the colonies are of type II strains.

[0184] Additionally, the RecA gene sequenced from the putative RT6 clones aligns with the region 64-325 of the RecA gene from ATCC11828 strain (SEQ ID NO: 276). The alignment with the type I strain, NCTC 737 (SEQ ID NO: 277), shows 3 bp variations indicating that the colonies are of type II strains.

[0185] SNP sequences for ATCC11828 aligned with RT6 SNP sequence from literature and 9 isolates (C→T at 1315)(Fig. 12). Nine of the isolated strains from our studies match the RT6 SNP sequence as published (e.g., Fitz-Gibbon, S., et al., (2013) Invest. Dermatol., 133(9):2152-60).

Example 14. Knock-In of the Human IL-10, EGF and HGH genes into RT6 *P. acnes* strain.

[0186] The genes for human IL-10, human EGF and human GH were all cloned into separate *P. acnes* strains under inducible LacZ promoters. Upon incubation of the cells with 0.1 mM

IPTG we began to see expression of the respective genes and secretion into the growth media (Fig. 13).

Example 15. Growth arrest of *P. acnes* by introduction of inducible promoters to housekeeping genes.

[0187] Growth of the *P. acnes* was only achievable upon induction by IPTG (Table 3). The same constructs are being made with an arabinose inducible promoter so that the human gene secretion and growth arrest can be controlled by separate nutrients.

Table 3.

IPTG (mM)	Incubation time		
	1 day	2 day	5 day
0	-	-	-
0.05	-	-	-
0.10	+/-*	-	++*
0.25	+/-*	+/-*	++*

Example 16. Construction of CAMP II mutant and IL-10 expression strain of *P. acnes*

[0188] The 1100 bp upstream region from CAMP II ORF of *P. acnes* (Primers Up-C F and Up-C R), and 400 bp of truncated CAMP II ORF (Primers CAMP II F and CAMP II R) were amplified by PCR with genome DNA of *P. acnes* ATCC 11828 strain. For secretion of IL10, the 168 bp of the signal peptide region was by PCR with the genome DNA of *B. subtilis* strain 168 as a template (Primers Sig-C F and Sig-C R). The 568 bp of human IL10 ORF was amplified by PCR (Primers I10-C F and I10-C R). These PCR products and DNA fragment with erythromycin resistance genes were ligated with pUC19 by NEBuilder HiFi DNA Assembly Cloning Kit and transformants of this ligation product were selected on LB with amp (100 µg/mL) plate. After transformation into *E. coli* dcm⁻ dam⁻ strain, the plasmid was introduced into *P. acnes* and selected anaerobically on reinforced clostridial medium (RCM) with erm (5 µg/mL) plates. The transformant having IL-10 gene with CAMP II promoter and signal peptide was checked by PCR. For construction of CAMP II mutant strain, this transformant was cultured in RCM without erythromycin to allow the second homologous recombination. After streak on the plate, the CAMP II mutant colonies were screened by PCR.

Table 4. List of primers

Name	Sequences (5' - 3')
19-C F	GAATTTCGAGCTCGGTACC (SEQ ID NO: 250)
19-C R	ACTGGCCGTCGTTTTACAAC (SEQ ID NO: 251)
Up-C F	GTTGTAAAACGACGGCCAGTGAAGGCACCCATGAGCCT (SEQ ID

Name	Sequences (5' - 3')
	NO: 252)
Up-C R	TTGCAAACATAAAGGTTCTCCGTTTATTGGTTG (SEQ ID NO: 253)
Sig-C F	GAGAACCTTTATGTTTGCAAACGATTCAAAC (SEQ ID NO: 254)
Sig-C R	AGGAGTGCATATGATAAATAGACATGGTTCCG (SEQ ID NO: 255)
I10-C F	CTATTATCATATGCACTCCTCCGCTCTG (SEQ ID NO: 256)
I10-C R	GGGTCTTCTTCATTCAGTTGCGGATCTTCATGG (SEQ ID NO: 257)
CAMP II F	CCGCAACTGAATGAAGAAGACCCATCTTG (SEQ ID NO: 258)
CAMP II R	TAGAGACTGGGGACCTTGTTTTGGAGAG (SEQ ID NO: 259)
Erm-C F	AACAAGGTCCCCAGTCTCTAGAATCGGATAATAAATATATATAAAC (SEQ ID NO: 260)
Erm-C R	CGGGTACCGAGCTCGAATTCCGATTATCTAGACAGCTCC (SEQ ID NO: 261)

Example 17. Construction of GAPDH mutant and IL-10 expression strain of *P. acnes*.

[0189] The 1000 bp upstream region from GAPDH ORF of *P. acnes* (Primers Up-G F and Up-G R), and 400 bp of truncated GAPDH ORF (Primers Gapdh F and gapdh R) were amplified by PCR with genome DNA of *P. acnes* ATCC 11828 strain. For secretion of IL10, the 168 bp of the signal peptide region was by PCR with the genome DNA of *B. subtilis* strain 168 as a template (Primers Sig-G F and Sig-G R). The 568 bp of human IL10 ORF was amplified by PCR (Primers I10-G F and I10-G R). These PCR products and DNA fragment with erythromycin resistance genes were ligated with pUC19 by NEBuilder HiFi DNA Assembly Cloning Kit and transformants of this ligation product were selected on LB with amp (100µg/mL) plate. After transformation into *E. coli* dcm⁻ dam⁻ strain, the plasmid was introduced into *P. acnes* and selected anaerobically on reinforced clostridial medium (RCM) with erm (5µg/mL) plates. The transformant having IL-10 gene with GAPDH promoter and signal peptide was checked by PCR. For construction of GAPDH mutant strain, this transformant was cultured in modified RCM (m-RCM) with lactate and without erythromycin to allow the second homologous recombination. After streak on the m-RCM with lactate and RCM plates, colonies that grew only on the m-RCM with lactate were picked and these mutants were checked by PCR.

Table 5. List of primers

Name	Sequences (5' - 3')
19-G F	GAATTCGAGCTCGGTACC (SEQ ID NO: 262)
19-G R	ACTGGCCGTCGTTTTACAAC (SEQ ID NO: 263)
Up-G F	GTTGTAAAACGACGGCCAGTTCGTCAATGGCGCTGGAC (SEQ ID NO: 264)

Name	Sequences (5' - 3')
Up-G R	TTGCAAACATTAAGGGATCTCCTCCAAATGAG (SEQ ID NO: 265)
Sig-G F	AGATCCCTTAATGTTTGCAAAACGATTCAAAAC (SEQ ID NO: 266)
Sig-G R	AGGAGTGCATATGATAAATAGACATGGTTCCG (SEQ ID NO: 267)
I10-G F	CTATTTATCATATGCACTCCTCCGCTCTG (SEQ ID NO: 268)
I10-G R	TGACGGTCATTTCAGTTGCGGATCTTCATGG (SEQ ID NO: 269)
Gapdh F	CCGCAACTGAATGACCGTCAAGGTTGGTATC (SEQ ID NO: 270)
Gapdh R	TAGAGACTGGCCATAACGAAGGTGCCGTC (SEQ ID NO: 271)
Erm-G F	TTCGTTATGGCCAGTCTCTAGAATCGGATAATAAATATATAAAC (SEQ ID NO: 272)
Erm-G R	CGGGTACCGAGCTCGAATTCCGATTATCTAGACAGCTCC (SEQ ID NO: 273)

Example 18. References

[0190]

Sörensen M, M. T. (2010). Mutagenesis of *Propionibacterium acnes* and analysis of two CAMP factor knock-out mutants. *J Microbiol Methods*, 211-216.

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Patentkrav

1. Sammensætning formuleret til topisk eller mukosal administration til et pattedyr, hvor sammensætningen omfatter en genetisk modificeret *Propionibacterium acnes* (*P. acnes*) type II, ribotype 6-stamme omfattende en nukleinsyre, der koder for en pattedyrsvækstfaktor eller et pattedyrscytokin, hvor nukleinsyren omfatter et regulatorisk element, der driver ekspressionen af pattedyrsvækstfaktoren eller pattedyrscytokinet i *P. acnes*-stammen, *P. acnes*-stammen omfatter et endogent CRISPR-array, og nukleinsyren og det regulatoriske element er integreret i *P. acnes*-stammens genom.

2. Sammensætning ifølge krav 1, hvor pattedyrsvækstfaktoren er

- (a) et hormon;
- (b) et humant eller bovint hormon;
- (c) et somatotrophin, hvor somatotrophinet fortrinsvis omfatter SEQ ID NO: 45, SEQ ID NO: 46, SEQ ID NO: 47, SEQ ID NO: 48 eller SEQ ID NO: 49;
- (d) udskilt af *P. acnes*-stammen;
- (e) en menneskelig vækstfaktor;
- (f) udvalgt fra gruppen bestående af transformerende vækstfaktor beta (TGF- β), vaskulær endotelvækstfaktor (VEGF), hepatocytvækstfaktor (HGF), fibroblastvækstfaktor (FGF), insulinlignende vækstfaktor-1 (IGF1), blodpladeafledt vækstfaktor (PDGF), granulocytmonocytkolonistimulerende faktor (GM-CSF) og epidermal vækstfaktor (EGF);
- (g) en transformerende vækstfaktor omfattende en hvilken som helst af SEQ ID NO: 63 til SEQ ID NO: 69 eller en hepatocytvækstfaktor omfattende SEQ ID NO: 70;
- (h) en vaskulær endotelvækstfaktor (VEGF) omfattende en hvilken som helst af SEQ ID NO: 71 til SEQ ID NO: 91;
- (i) en blodpladeafledt vækstfaktor (PDGF) omfattende en hvilken som helst af SEQ ID NO: 93 til SEQ ID NO: 102;
- (j) en epidermal vækstfaktor (EGF), omfattende en hvilken som helst af SEQ ID NO: 103 til SEQ ID NO: 106, eller
- (k) en fibroblastvækstfaktor (FGF) omfattende en hvilken som helst af SEQ ID NO: 107 til SEQ ID NO: 144.

3. Sammensætning ifølge krav 1, hvor

- (a) *P. acnes*-stammen udskiller pattedyrscytokinet;

- (b) pattedyrscytokinet er et immunsuppressivt cytokin;
- (c) pattedyrscytokinet er et humant cytokin;
- (d) cytokinet vælges fra gruppen bestående af interleukin-10 (IL-10), interleukin-6 (IL-6), interleukin-7 (IL-7) og interleukin-8 (IL-8), hvor cytokinet fortrinsvis vælges fra gruppen bestående af SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, SEQ ID NO: 27 og SEQ ID NO: 28; eller
- (e) pattedyrscytokinet omfatter IL-10, hvor IL-10 fortrinsvis omfatter SEQ ID NO: 25.

4. Sammensætning ifølge krav 1, hvor nukleinsyren omfatter en inducerbar promotor konfigureret til at regulere ekspressionen af pattedyrsvækstfaktoren eller pattedyrscytokinet.

5. Sammensætning ifølge krav 1, hvor nukleinsyren

- (a) er under styring af en endogen promotor, der styrer ekspressionen af pattedyrsvækstfaktoren eller pattedyrscytokinet; eller
- (b) omfatter en endogen promotor, der er konfigureret til at styre ekspressionen af pattedyrsvækstfaktoren eller pattedyrscytokinet; hvor den inducerbare promotor eller endogene promotor fortrinsvis omfatter en lacZ-promotor eller lacZ-operon eller en ara-operonpromotor.

6. Sammensætning ifølge krav 1, hvor nukleinsyren, der koder for pattedyrsvækstfaktoren eller pattedyrscytokinet, indsættes i hele eller en del af et endogent gen, der koder for et patogent protein.

7. Sammensætning ifølge krav 1, hvor ekspression af et endogent protein er væsentligt reduceret eller elimineret.

8. Sammensætning ifølge krav 1, hvor ekspression af et patogent protein er væsentligt reduceret eller elimineret, hvor det patogene protein fortrinsvis omfatter et endotoksin og/eller et eksotoksin, eller det endogene patogene protein omfatter et glyceraldehyd 3-phosphat-dehydrogenase- (GADPH) protein eller et CAMP-protein.

9. Sammensætning ifølge krav 1, og som omfatter en inducerbar promotor, der regulerer ekspression af et essentielt protein, hvor fortrinsvis

- (a) det essentielle protein er valgt fra gruppen bestående af kromosomreplikationsinitiatorproteinet DnaA, FtsA, FtsI, FtsL, FtsK, FtsN, FtsQ, FtsW, FtsZ,

ZipA, aroE, atpD, gmk, guaA, lepA, recA og sodA;

(b) den inducerbare promotor kan induceres af et sukker, fortrinsvis lactose eller arabinose;

(c) den inducerbare promotor kan induceres af en aminosyre; eller

(d) den inducerbare promotor kan induceres af en aminosyre.

10. Sammensætning ifølge krav 1, hvor *P. acnes* *P. acnes*-stammen har flere genetiske modifikationer, hvor *P. acnes*-stammen har en modifikation, hvori 1) ekspresion af et endogent protein i er væsentligt reduceret eller elimineret; og 2) en inducerbar promotor regulerer ekspresion af et essentielt protein.

11. Sammensætning, der omfatter en flerhed af genetisk modificerede *P. acnes*-stammer ifølge krav 1, hvor hver af *P. acnes*-stammerne har flere genetiske modifikationer, og hvor *P. acnes*-stammen har en modifikation, hvori 1) ekspresion af et endogent protein er væsentligt reduceret eller elimineret; og 2) en inducerbar promotor regulerer ekspresion af et essentielt protein.

12. Genetisk modificeret *Propionibacterium acnes* (*P. acnes*) type II, ribotype 6-stamme, der omfatter:

a) en pattedyrsvækstfaktor eller et humant IL-10, hvor henholdsvis pattedyrsvækstfaktoren eller IL-10 udskilles, og

b) en inducerbar promotor, der styrer ekspresion af et essentielt protein valgt fra gruppen bestående af et kromosomreplikationsinitiatorprotein DnaA, FtsA, FtsI, FtsL, FtsK, FtsN, FtsQ, FtsW, FtsZ, ZipA, aroE, atpD, gmk, guaA, lepA, recA og sodA;

hvor ekspresionen af et endogent CAMP2- og/eller endogent GADPH-protein er væsentligt reduceret eller elimineret i den genetisk modificerede *P. acnes*-stamme.

13. Sammensætning omfattende den genetisk modificerede *P. acnes* type II, ribotype 6-stamme ifølge krav 12, hvor sammensætningen fortrinsvis er

(a) konfigureret til topisk eller mukosal administration til et pattedyr, eller

(b) omfatter et sukker eller en aminosyre, hvor den inducerbare promotor induceres eller stimuleres ved tilstedeværelsen af sukkeret eller aminosyren.

14. Genetisk modificeret *P. acnes*-stamme eller -sammensætning ifølge et hvilket som helst af kravene 1-13 til anvendelse i en fremgangsmåde til behandling af en hud- eller neglelidelse.

DRAWINGS

Drawing

Fig. 1

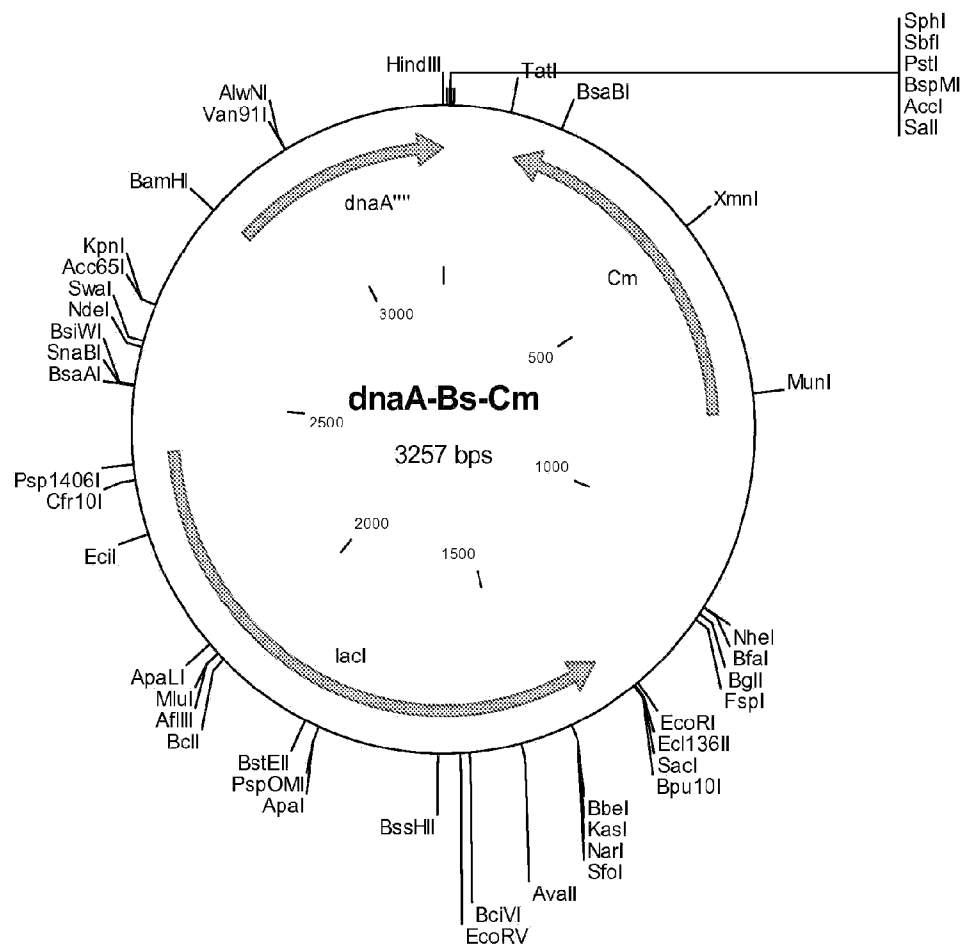


Fig. 2

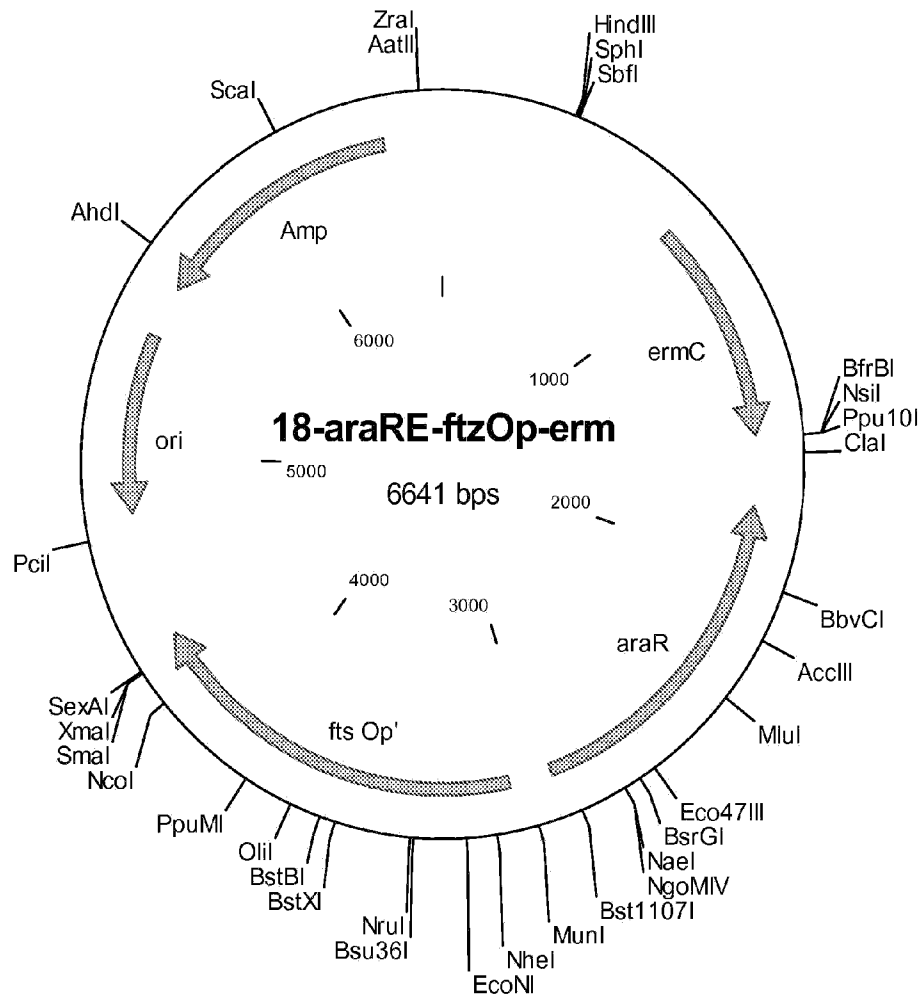


Fig. 3

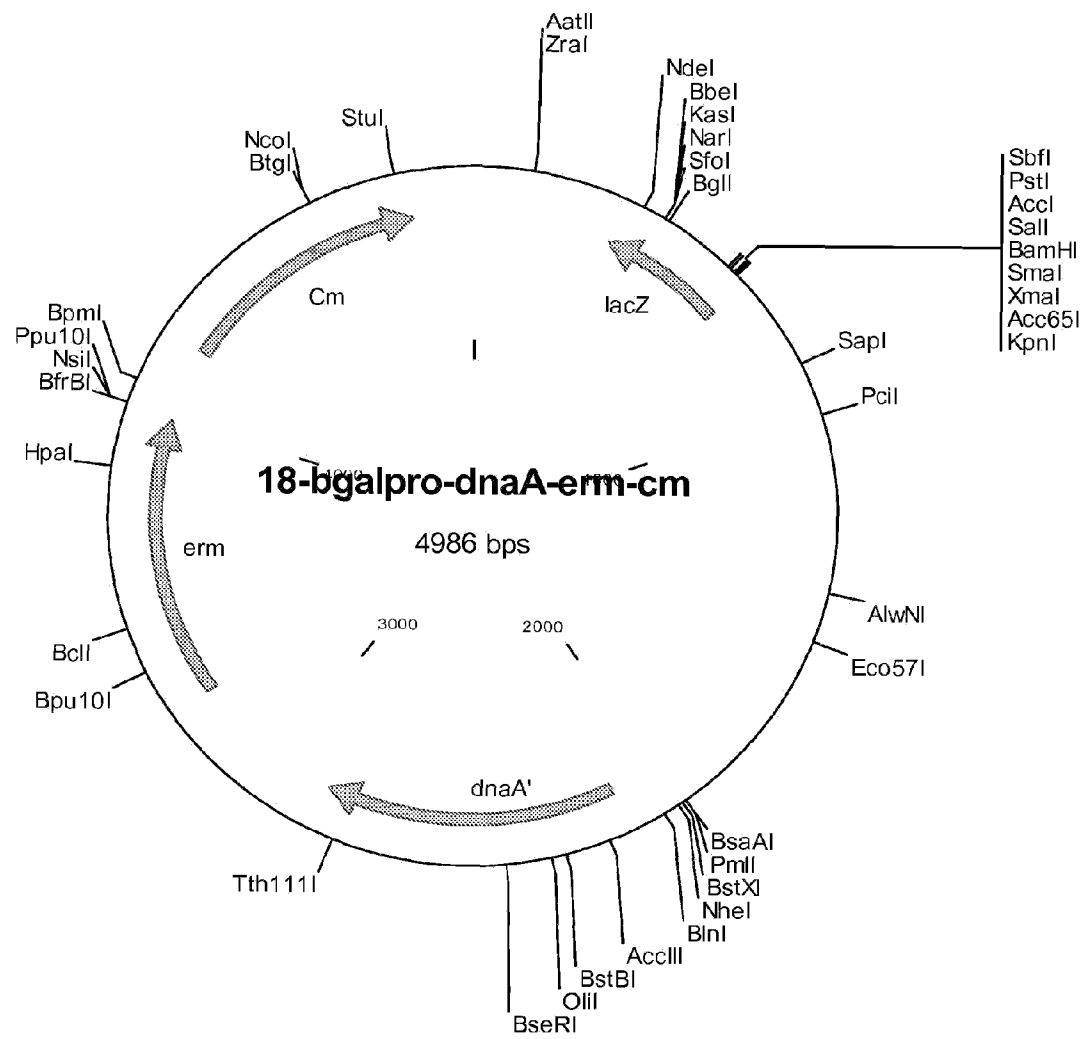


Fig. 4A

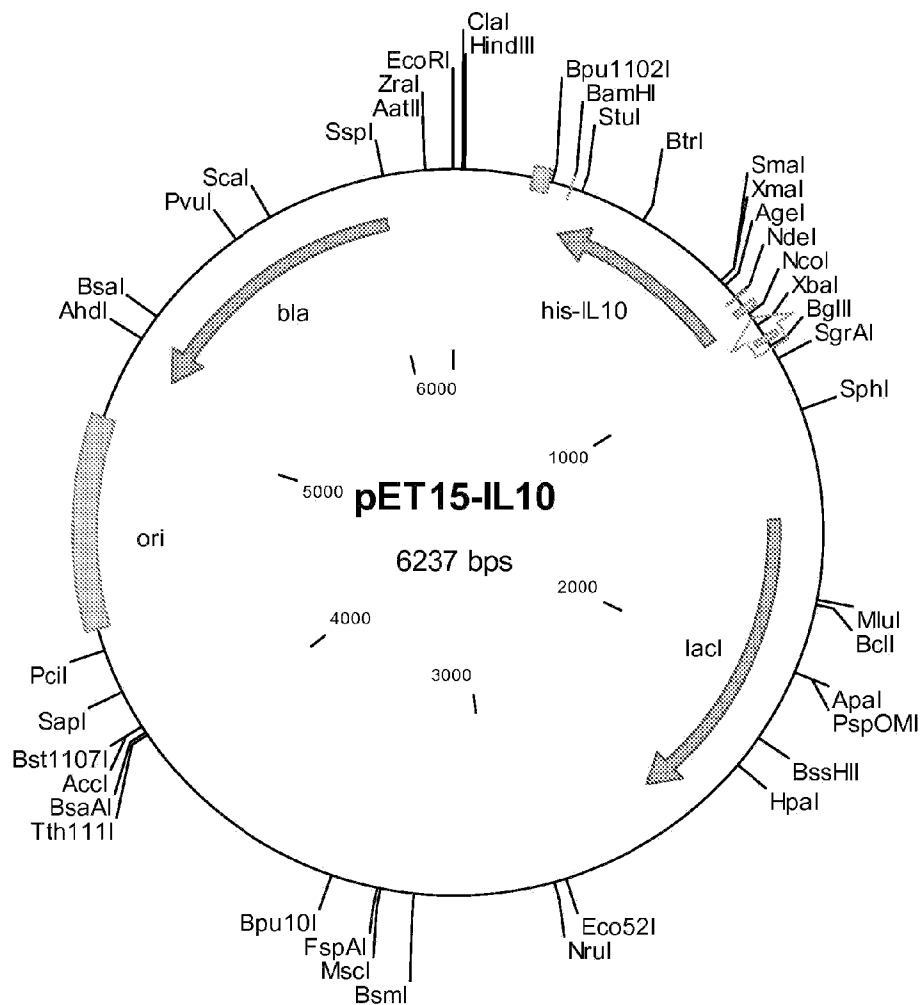


Fig. 4B

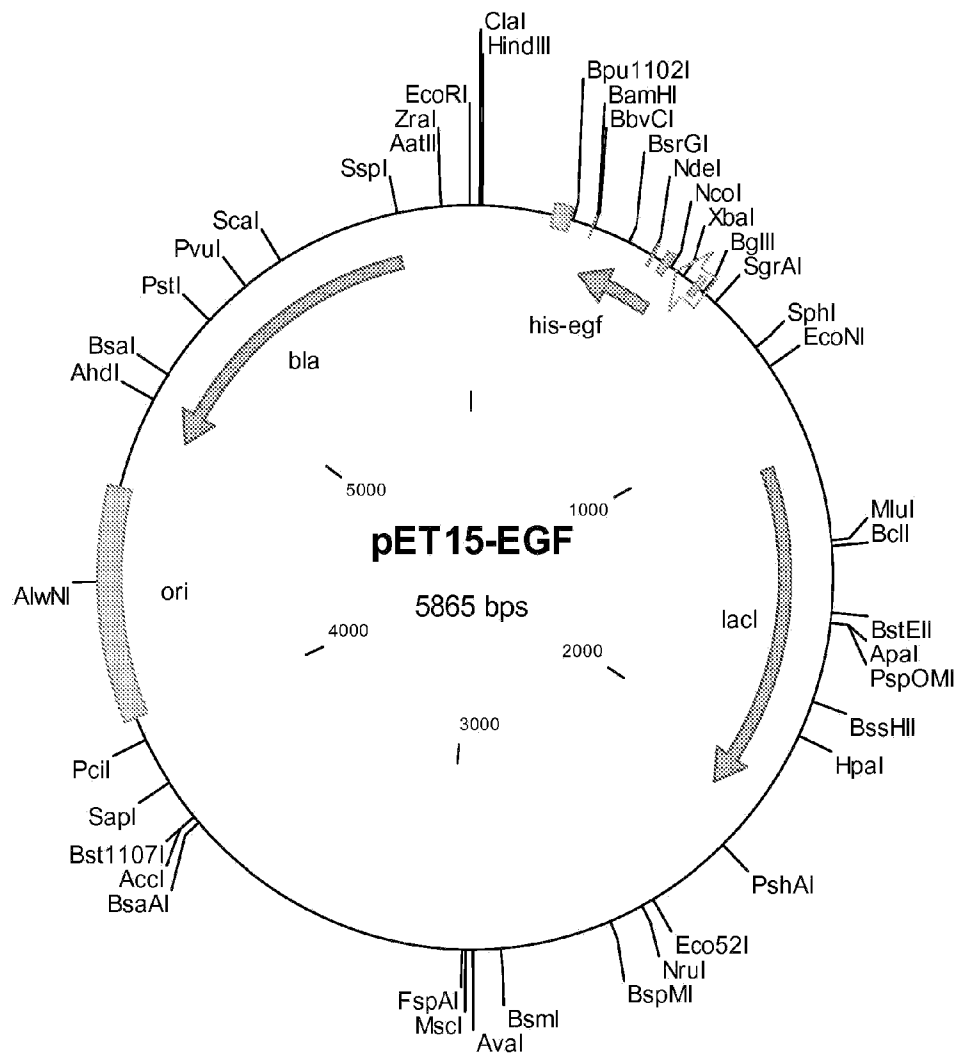


Fig. 4C

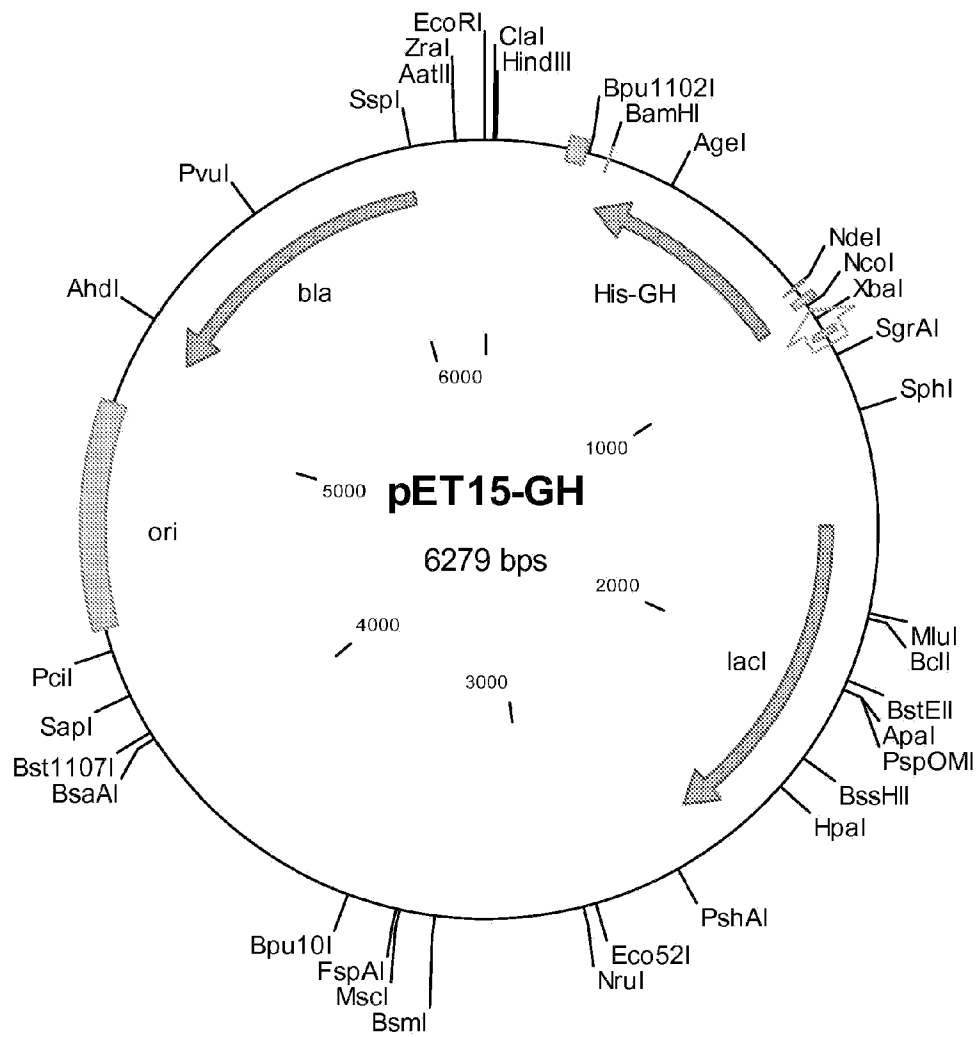


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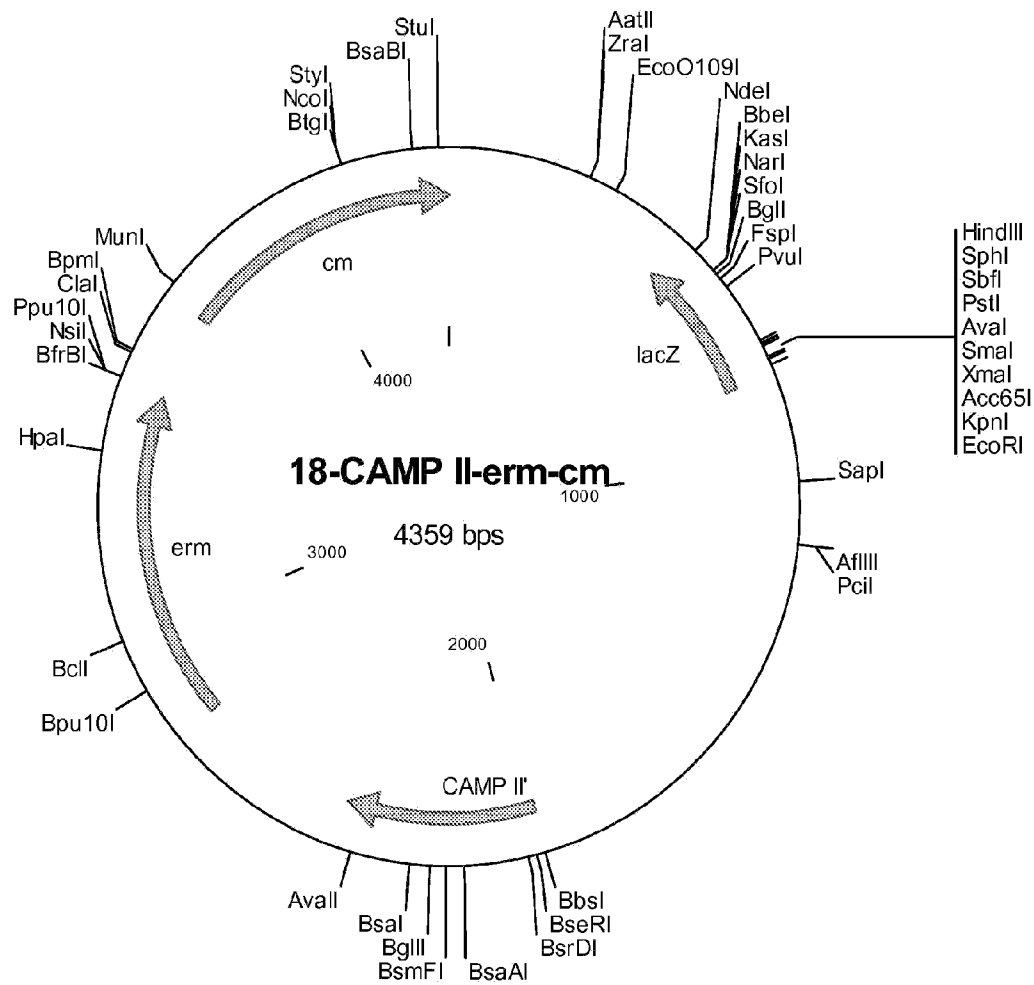


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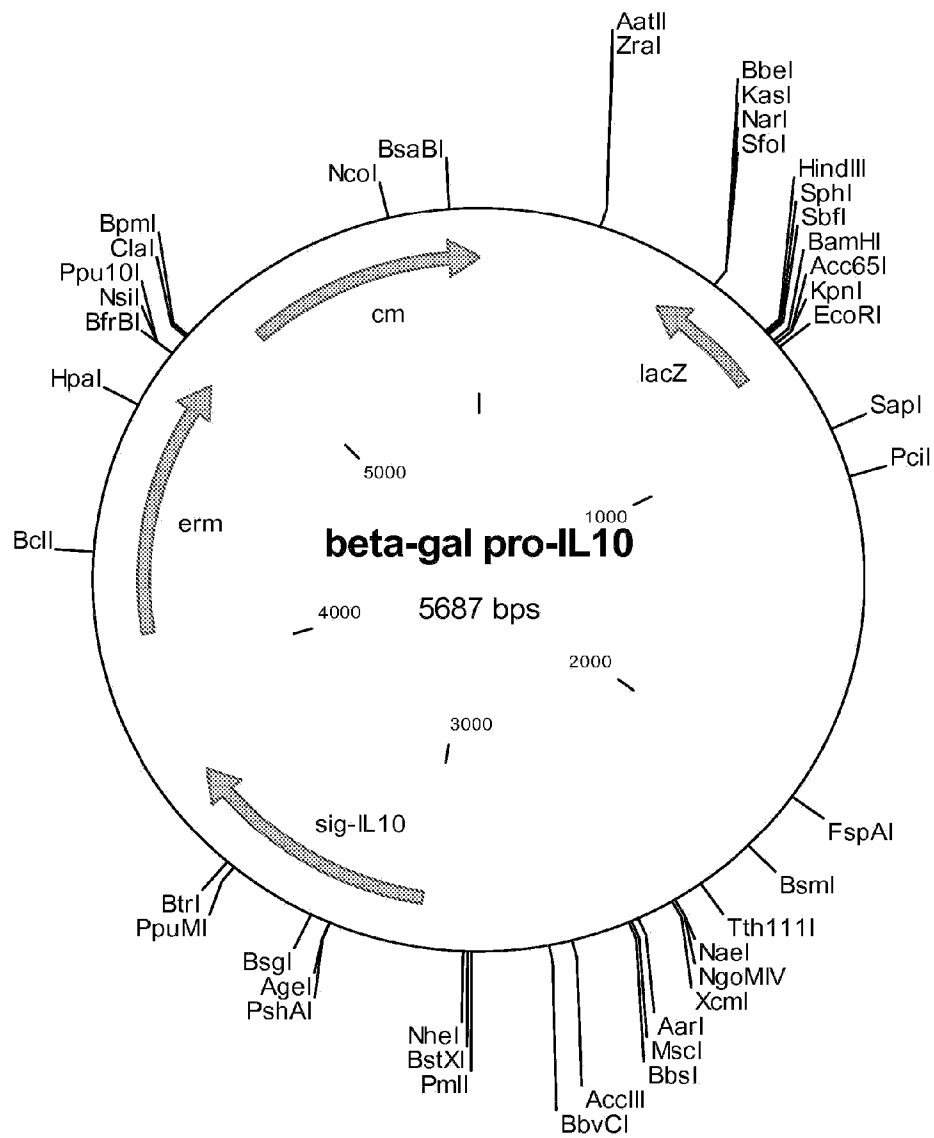


Fig. 7

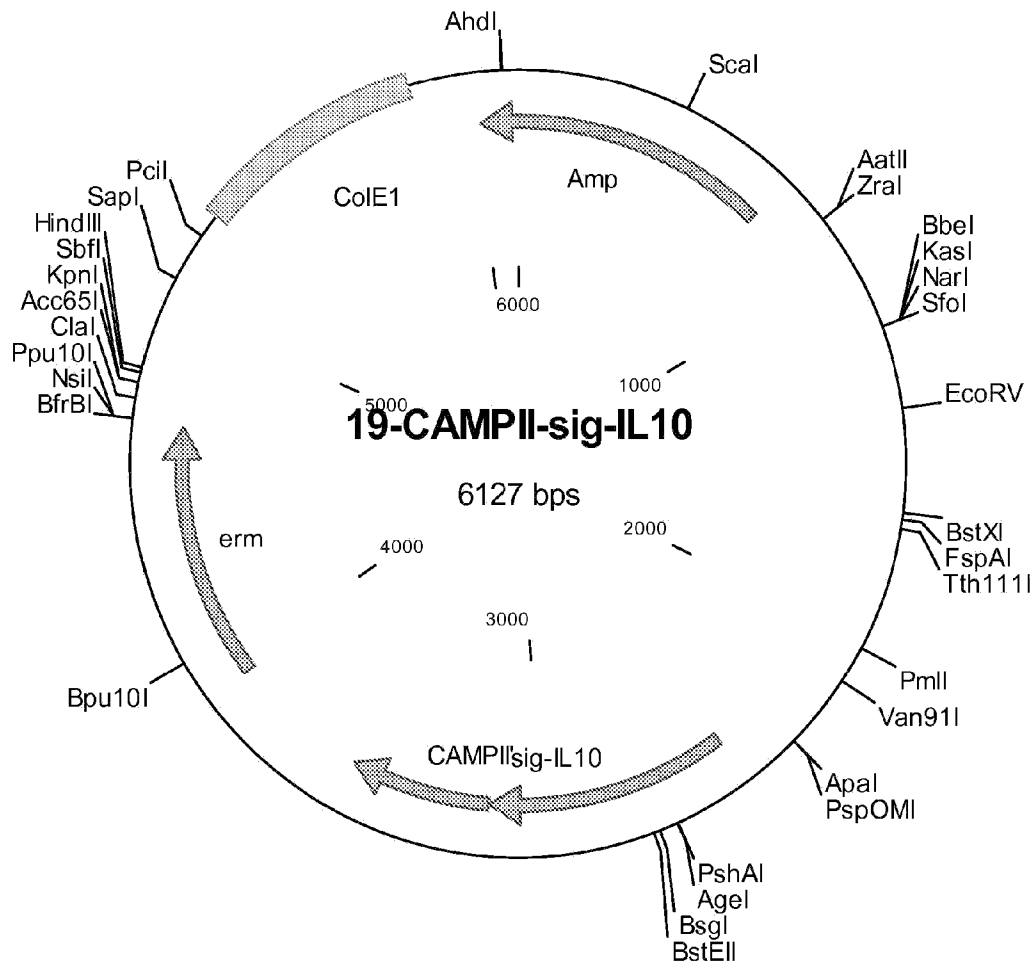


Fig. 8

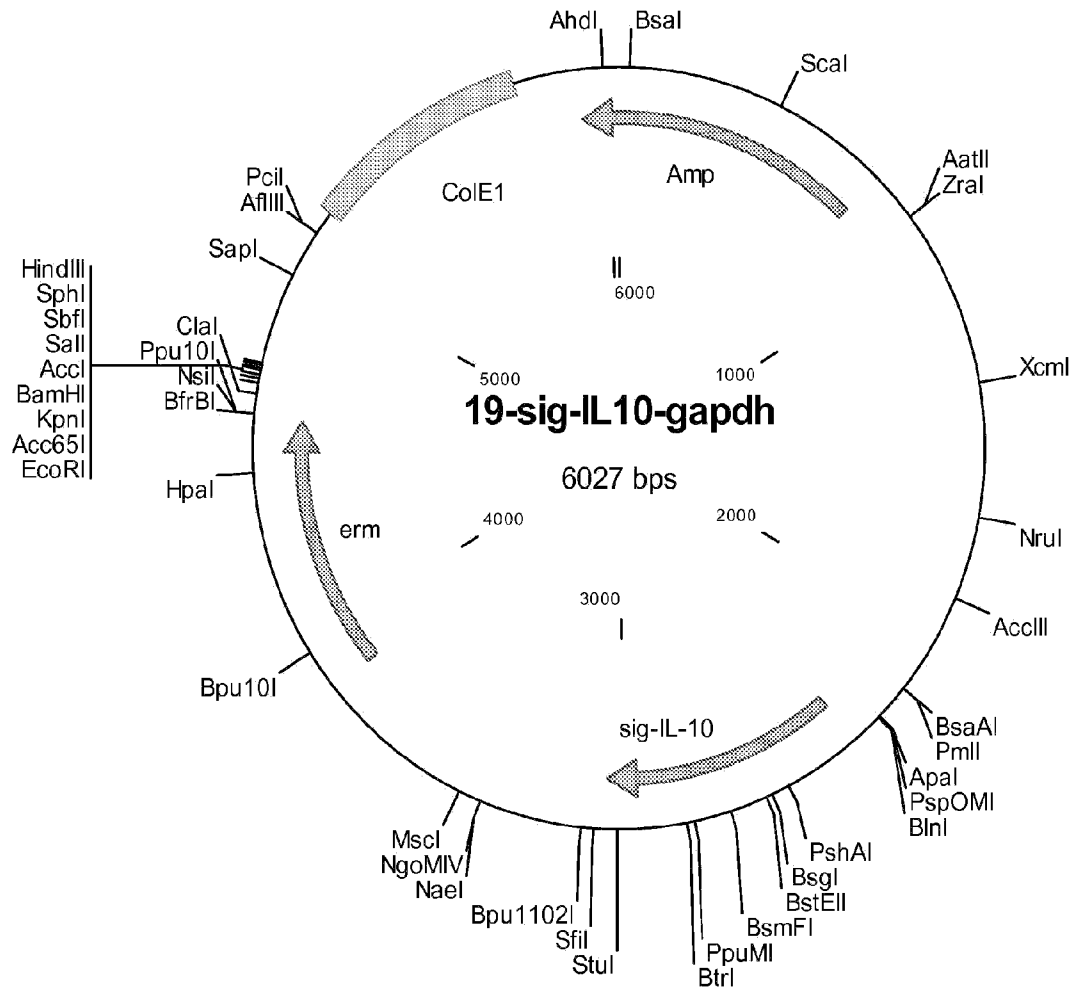


Fig. 9

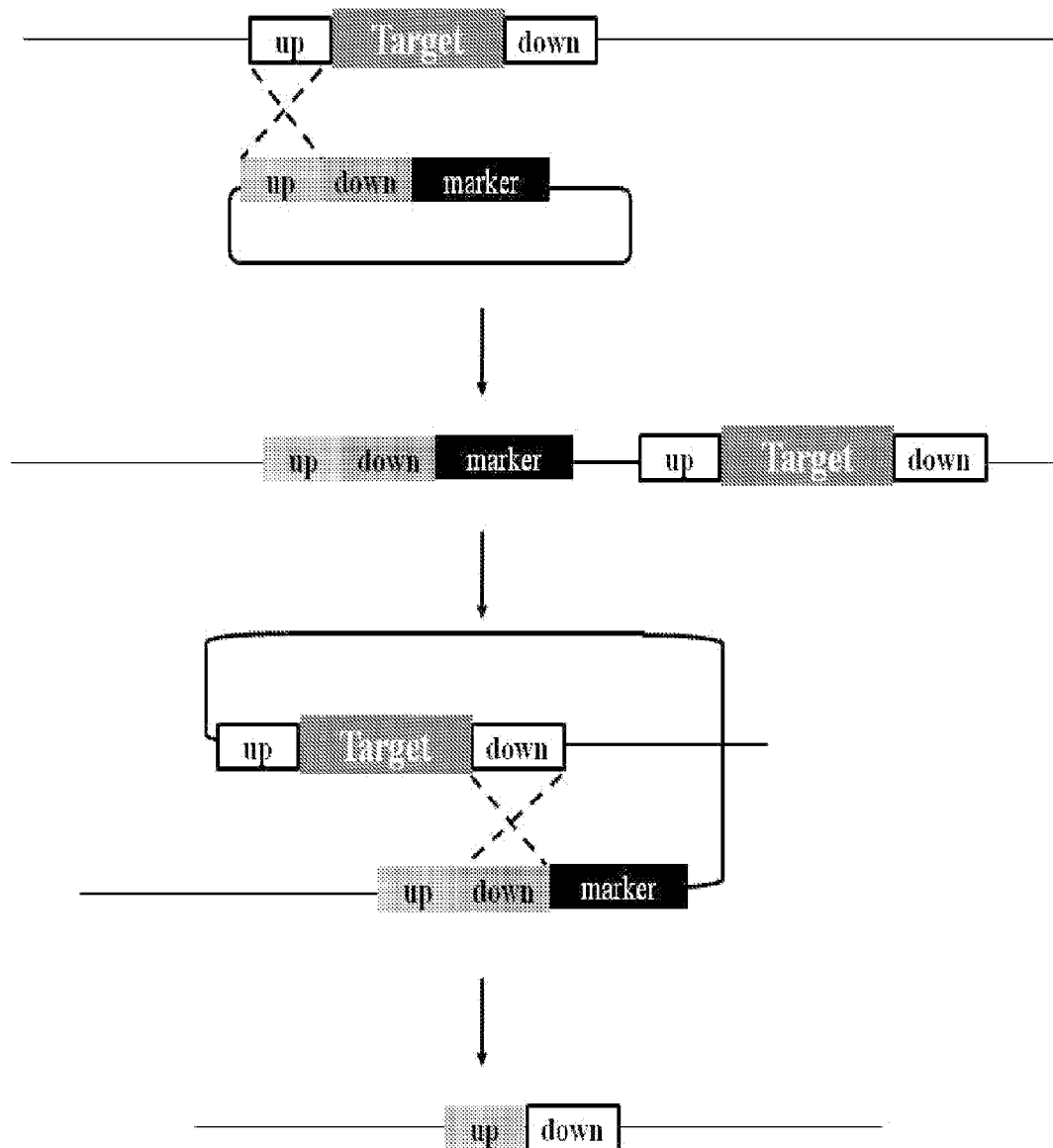


Fig. 10

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Fig. 11

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Fig. 12

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Fig. 13

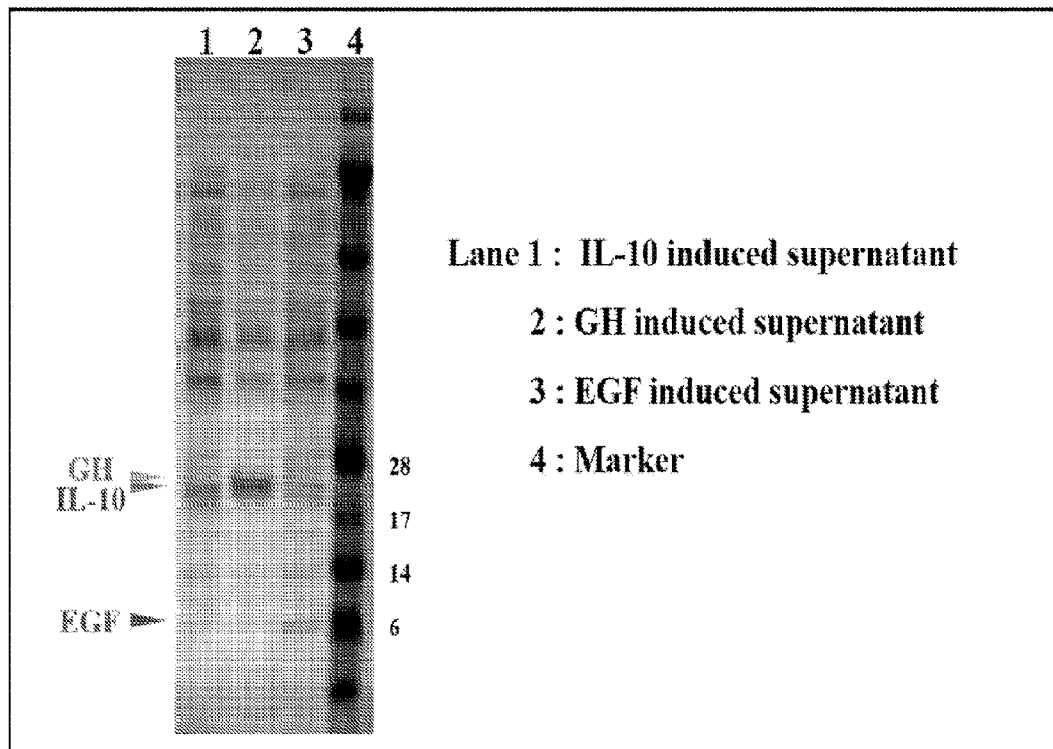


Fig. 14

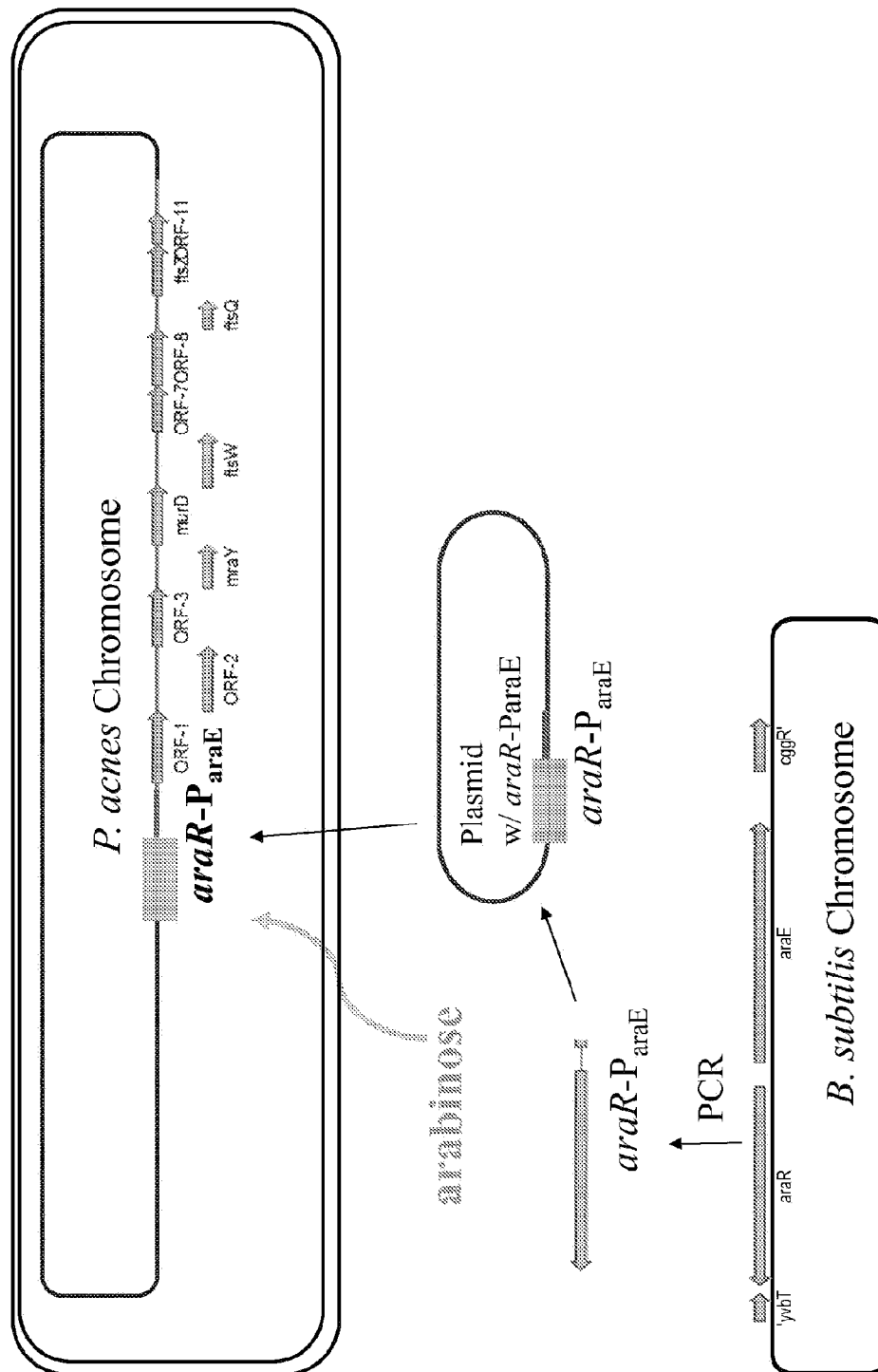


Fig. 15

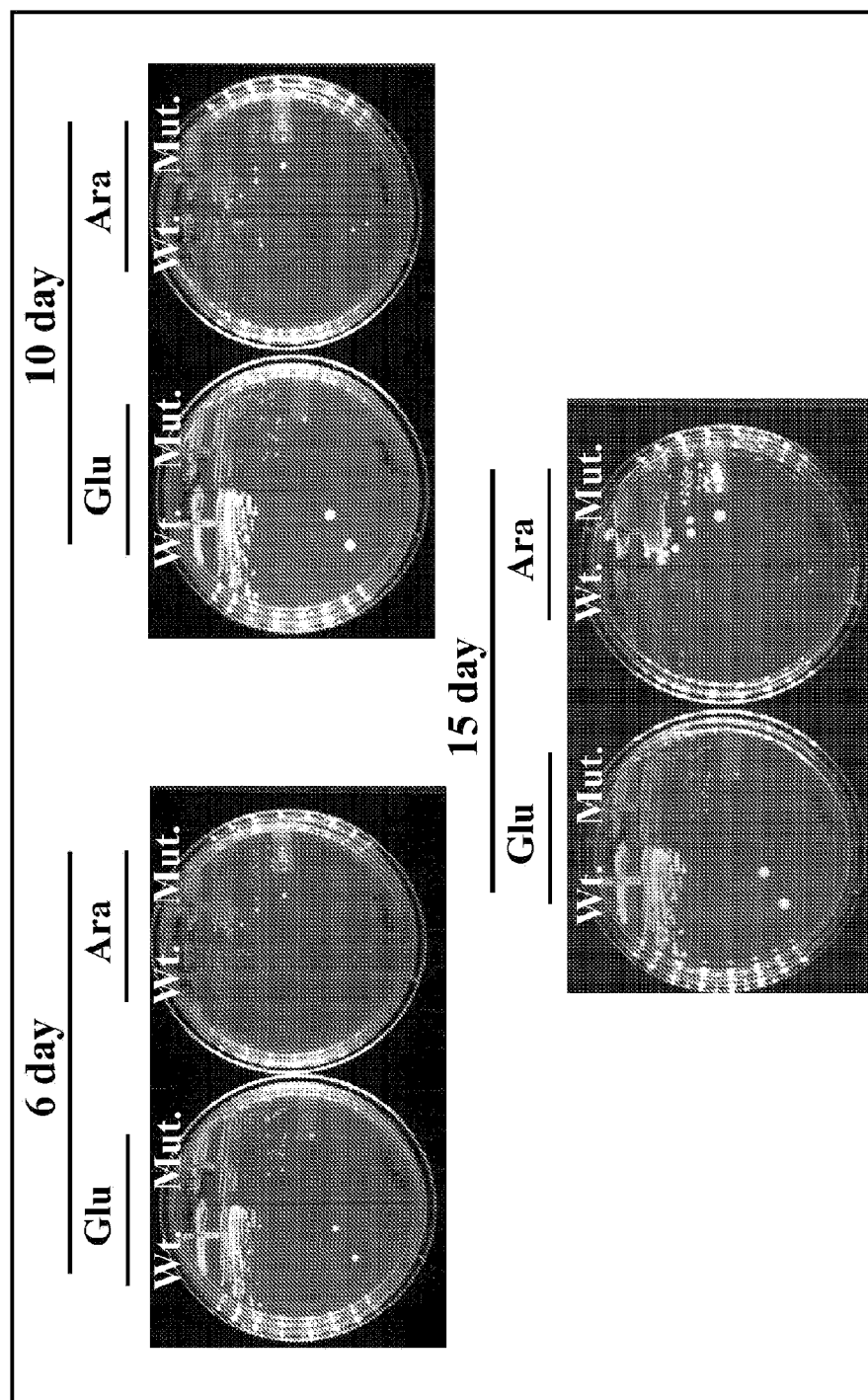
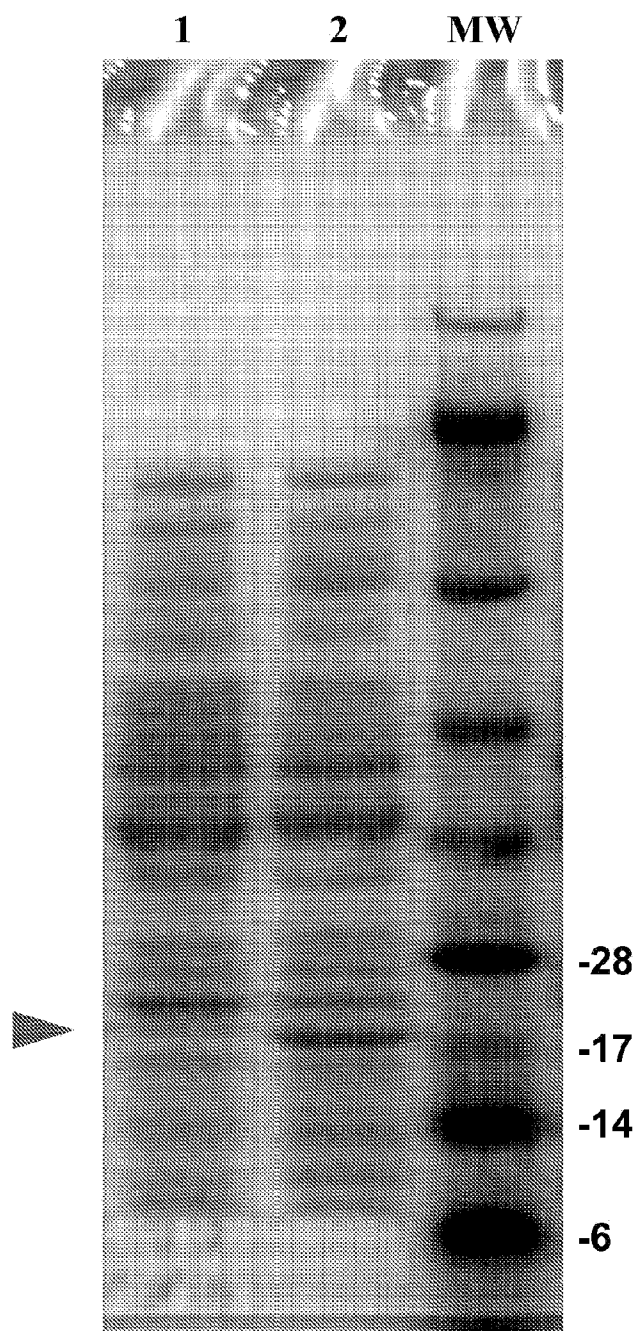


Fig. 16



SEKVENSLISTE

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