(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
5 April 2007 (05.04.2007)

PCT

(10) International Publication Number
WO 2007/036706 A1

(51) International Patent Classification:
C07K 14/32 (2006.01) *A61K 38/16* (2006.01)
C12P 21/02 (2006.01)

(21) International Application Number:
PCT/GB2006/003570

(22) International Filing Date:
26 September 2006 (26.09.2006)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
60/720,464 27 September 2005 (27.09.2005) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available)*: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available)*: ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declaration under Rule 4.17:
— *of inventorship (Rule 4.17(iv))*

Published:
— *with international search report*
— *before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments*

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

(54) **Title:** VARIANTS OF THE LANTIBIOTIC MERSACIDIN AND THEIR USE

(57) **Abstract:** This invention relates to variants of the lantibiotic, mersacidin, and to methods of their manufacture and use.

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VARIANTS OF THE LANTIBIOTIC MERSACIDIN AND THEIR USE

FIELD OF THE INVENTION

This invention relates to variants of the lantibiotic mersacidin and their use.

BACKGROUND OF THE INVENTION

Mersacidin belongs to a group of bactericidal peptides that are called lantibiotics. The name signifies that these peptides contain the amino acids lanthionine and/or 3-methyllanthionine. Mersacidin has activity against methicillin-resistant *Staphylococcus aureus* (MRSA) and is therefore of considerable interest in medicine.

Mersacidin is produced by a specific species of the genus *Bacillus*, which has been designated HIL Y-85,54728 ("HIL"). The cloning of the mersacidin gene is disclosed by Bierbaum et al, 1995.

Mersacidin is produced by processing of a small protein of 68 amino acids. The N-terminal 48 amino acids of the protein form a leader sequence, and the C-terminal 20 amino acids are a propeptide sequence which is processed by modifying enzymes to produce mersacidin. The sequence of the mersacidin gene, *mrsA*, is provided as SEQ ID NO:1 and its translation as SEQ ID NO:2. The sequence of the mature peptide, which includes post-translationally modified residues, is shown in Figure 1 (SEQ ID NO:3).

The *mrsA* gene forms part of the *mrs* gene cluster of about 12.3 kb (Altena et al, 2000). The gene cluster includes regulatory genes which control the production of mersacidin by regulating the expression of the *mrsA* gene and/or its modifying enzymes. The *mrsA* gene is expressed in early stationary phase of the growth of the Bacillus HIL strain.

Variants of naturally occurring antibiotics can be useful in medicine. Variants can be produced synthetically, semi-synthetically (e.g. by chemical modification of fermented products) or by genetic changes to the organisms which produce them. Potentially, mersacidin could be varied by all three routes, with the latter two being of particular interest. For example, modification of amino acids could be used to produce variants which have altered activity profiles, as well as properties such as bioavailability, biodistribution or the ability to overcome resistance mechanisms to

mersacidin itself. Altered amino acids may also be useful to introduce reactive side-chains allowing modification of the peptide by chemical means.

Szekat *et al.* (2003) Appl. Env. Microbiol. **69**, 3777-3783 describe the construction of an expression system for generation of variant mersacidins. Modified *mrsA* genes were generated by site-directed mutagenesis and introduced into the *mrs* gene cluster by homologous recombination with the native *mrsA* gene. Three variants of mersacidin were produced; F3L, S16I and E17A (where the numbers refer to the numbering of the mature mersacidin peptide sequence and the letters are the 1-letter amino acid code).

Of these three variants, both the S16I and E17A were essentially inactive (about 1,000-fold greater Minimum Inhibitory Concentration (MIC) measured against *M. luteus*) while the F3L peptide was weakly active (MIC of 12.5 mg/l against *M. luteus*, compared to wild-type of 0.195). The data of Szekat *et al* thus suggest that mersacidin is very sensitive to alterations and variation of the primary sequence is likely to be deleterious.

SUMMARY OF THE INVENTION

We have identified a number of mersacidin variants which have activity against a range of bacteria, including two strains of methicillin resistant *S. aureus* (MRSA). Thus unlike the variants described by Szekat *et al*, the variants of the present invention have anti-bacterial activity which in many cases is of a comparable or even better level than that of mersacidin itself. The invention thus provides novel antibiotic compounds, genes encoding such compounds, methods of making such compounds and their use in the treatment of human or animal subjects, particularly in conditions requiring anti-bacterial therapy. These and other aspects of the invention are described herein below.

DESCRIPTION OF THE DRAWINGS

Figure 1 shows the structure of mersacidin. The compound is produced from 20 amino acids, 8 of which, including the C-terminal cysteine, are involved in lanthionine bridge formation. The compound includes the non-naturally occurring amino acids dehydroalanine (Dha) and [2-aminobutyric acid] (Abu), which are produced by post-translational modification of serine and threonine respectively. The 2-aminobutyric acid moieties are further combined with cysteine moieties to form thioether crosslinks known as methyllanthionines.

DETAILED DESCRIPTION OF THE INVENTION

Mersacidin Variants

In one aspect, the invention provides a mersacidin variant wherein the variant comprises a modification to position 3, 5, 6, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 1 below:

Table 1

F3	L5	P6	G7	G8	G9	G10	V11	L14	Dha16
F3W	L5A	P6H	G7A	G8A	G9A	G10A	V11L	L14V	Dha16G
F3R	L5I	P6A	G7N	G8C	G9S	G10V	V11I	L14I	Dha16A
F3D	L5M	P6N	G7Q	G8N	G9T	G10S	V11M	L14M	Dha16Dhb
F3I	L5N	P6Q	G7W	G8Q	G9N	G10Dha	V11K		Dha16H
F3P	L5H	P6V	G7S	G8H	G9R	G10M	V11C		
F3S		P6M	G7T	G8E	G9Y	G10Y			
F3C		P6F	G7M	G8I	G9H	G10W			
F3M		P6Y	G7I	G8S	G9Q	G10I			
F3N		P6G	G7H	G8P	G9L	G10Dhb			
F3H		P6L	G7F			G10R			
		P6I				G10K			
		P6D				G10H			
		P6E				G10N			

Where Dha is dehydroalanine and Dhb is dehydrobutyrine. When these modified amino acid residues are present, this is due to post-translational modification of serine and threonine residues respectively, brought about by the expression of other genes of the *mrsA* gene cluster.

In a preferred aspect, the variant comprises a modification to position 3, 6, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 2 below:

Table 2

F3	P6	G7	G8	G9	G10	V11	L14	Dha16
F3W	P6H	G7A	G8A	G9A	G10A	V11L	L14V	Dha16G
F3R	P6A	G7N	G8C	G9T	G10V	V11I	L14I	Dha16A
F3D	P6N	G7Q	G8N	G9R	G10S	V11M	L14M	Dha16Dhb
	P6Q	G7W	G8Q	G9H	G10Dha	V11K		Dha16H
	P6V	G7T	G8H		G10M	V11C		
	P6M	G7M			G10Y			
	P6Y				G10W			

In a more preferred aspect, the variant comprises a modification to position 3, 6, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 3 below:

Table 3

F3	P6	G7	G8	G9	G10	V11	L14	Dha16
F3W	P6H	G7A	G8A	G9A	G10A	V11L	L14V	Dha16G
F3D	P6A	G7N	G8N	G9T	G10V	V11I	L14I	Dha16A
	P6N	G7Q	G8Q	G9R	G10S	V11M	L14M	Dha16Dhb
		G7T	G8H	G9H	G10Dha	V11K		Dha16H
		G7M			G10M			
					G10Y			

In an even more preferred aspect, the variant comprises a modification to position 3, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 4 below:

Table 4

F3	G7	G8	G9	G10	V11	L14	Dha16
F3W	G7N	G8A	G9A	G10V	V11I	L14V	Dha16G
		G8N	G9H	G10Y	V11M	L14M	Dha16Dhb
					V11L	L14I	Dha16H
							Dha16A

Variants which comprise a modification selected from the group F3W, G8A, G9A, G9H, V11I, V11L, L14I, L14M, L14V, Dha16G and Dha16Dhb are particularly preferred.

In one aspect, the mersacidin variants may comprise a combination of two or more of the above modifications, for example from 1 to 4, such as 2 or 3 of the modifications (with the remaining residues being that of the wild-type mersacidin sequence). Thus in one aspect, a variant comprising any one of the above-mentioned modifications may be a variant consisting of two, three or four changes in combination, or just consisting of a single positional change.

In one aspect, we have found the change F3W to provide a mersacidin variant ("mersacidin F3W") which has activity against a range of microoganisms which is more potent than mersacidin itself. Thus in one aspect, the mersacidin variant may comprise F3W together with one, two or three other changes. Such mersacidins include mersacidin F3W G8A, mersacidin F3W G9A, mersacidin F3W G9H, mersacidin F3W V11I, mersacidin F3W V11L, mersacidin F3W L14I, mersacidin F3W L14M, mersacidin F3W L14V, mersacidin F3W Dha16G and mersacidin F3W Dha16Dhb.

In another aspect, the mersacidins include mersacidin G8A G9A, mersacidin G8A G9H, mersacidin G8A V11I, mersacidin G8A V11L, mersacidin G8A L14I, mersacidin G8A L14M, mersacidin G8A L14V, mersacidin G8A Dha16G and mersacidin G8A Dha16Dhb.

In another aspect, the mersacidins include mersacidin G9A V11I, mersacidin G9H V11I, mersacidin V11I L14I, mersacidin V11I L14M, mersacidin V11I L14V, mersacidin V11I Dha16G and mersacidin V11I Dha16Dhb.

In another aspect, the mersacidins include mersacidin G9A L14I, mersacidin G9H L14I, mersacidin V11L L14I, mersacidin L14I Dha16G and mersacidin L14I Dha16Dhb.

In another aspect, the mersacidins include Dha16Dhb, mersacidin G9A Dha16Dhb, mersacidin G9H Dha16Dhb, mersacidin V11L Dha16Dhb, mersacidin L14M Dha16Dhb, and mersacidin L14V Dha16Dhb.

Formulations and Compositions

The mersacidin variants of the invention may be provided in substantially isolated form, e.g. free or substantially free of material with which they are associated with in a host cell used for their production.

The mersacidin variant may be in the form of a salt, particularly a pharmaceutically acceptable salt. These include basic salts, such as an alkali or alkaline earth metal salt, e.g. a sodium, potassium, calcium or magnesium salt. The salt may also be an acid addition salt such as those formed with hydrochloric acid, hydrobromic acid, sulfuric acid, nitric acid, phosphoric acid and the like, and organic acids such as acetic acid, propionic acid, glycolic acid, pyruvic acid, oxalic acid, malic acid, malonic acid, succinic acid, maleic acid, fumaric acid, tartaric acid, citric acid, benzoic acid, cinnamic acid, mandelic acid, methanesulfonic acid, ethanesulfonic acid, p-toluenesulfonic acid, salicylic acid and the like. A potassium salt is preferred. The preparation of a potassium salt is described in US-A-5,112,806.

The mersacidin variant may be prepared in the form of a pharmaceutical composition, The composition may be in the form of a liquid, gel or solid.

Pharmaceutically acceptable carriers or diluents include those used in formulations suitable for oral, rectal, nasal, topical (including buccal and sublingual), vaginal or parenteral (including subcutaneous, intramuscular, intravenous, intradermal, intrathecal and epidural) administration. Oral, nasal and topical administration may include administration by way of aerosols.

Topical formulations may also be present in the form of creams, ointments or gels, depending upon the site of intended use. Topical compositions of the invention may be in any pharmaceutical form normally used for topical application, in particular in the form of an aqueous, aqueous-alcoholic or oily solution, an oil-in-water or water-in-oil or multiple emulsion, an aqueous or oily gel, a liquid, pasty or solid anhydrous product. The composition may also contain the usual adjuvants in the cosmetics and dermatological fields, such as one or more of a hydrophilic or lipophilic gelling agent, hydrophilic or lipophilic active agent, preserving agent and antioxidant. When the composition of the invention is an emulsion, the proportion of the fatty phase can range from 5 to 80% by weight, and preferably from 5 to 50% by weight, relative to the total weight of the composition. The oils, the emulsifiers and the co-emulsifiers used in the composition in emulsion form are chosen from those used conventionally in the field considered. The emulsifier and the co-emulsifier are present in the composition in a proportion ranging from 0.3 to 30% by weight, and preferably from 0.5 to 20% by weight, relative to the total weight of the composition.

Oils which can be used include mineral oils (liquid petroleum jelly), oils of plant origin (avocado oil, soybean oil), oils of animal origin (lanolin), synthetic oils (perhydrosqualene), silicone oils (cyclomethicone) and fluoro oils (perfluoropolyethers). Fatty alcohols (cetyl alcohol) fatty acids and waxes (carnauba wax, ozokerite) can also be used as fatty substances.

Emulsifiers and co-emulsifiers which can be used include, for example, of fatty acid esters of polyethylene glycol, such as PEG 20 stearate, and fatty acid esters of glycerol, such as glyceryl stearate.

The formulations may conveniently be presented in unit dosage form and may be prepared by any of the methods well known in the art of pharmacy. Such methods include the step of bringing into association the active ingredient with the carrier which constitutes one or more accessory ingredients. In general the formulations are prepared by uniformly and intimately bringing into

association the active ingredient with liquid carriers or finely divided solid carriers or both, and then, if necessary, shaping the product.

For solid compositions, conventional non-toxic solid carriers include, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium carbonate, and the like may be used. The active compound as defined above may be formulated as suppositories using, for example, polyalkylene glycols, acetylated triglycerides and the like, as the carrier. Liquid pharmaceutically administrable compositions can, for example, be prepared by dissolving, dispersing, etc, an active compound as defined above and optional pharmaceutical adjuvants in a carrier, such as, for example, water, saline aqueous dextrose, glycerol, ethanol, and the like, to thereby form a solution or suspension. If desired, the pharmaceutical composition to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying agents, pH buffering agents and the like, for example, sodium acetate, sorbitan monolaurate, triethanolamine sodium acetate, sorbitan monolaurate, triethanolamine oleate, etc. Actual methods of preparing such dosage forms are known, or will be apparent, to those skilled in this art; for example, see "Remington: The Science and Practice of Pharmacy", 20th Edition, 2000, pub. Lippincott, Williams & Wilkins. The composition or formulation to be administered will, in any event, contain a quantity of the active compound(s) in an amount effective to alleviate the symptoms of the subject being treated.

For oral administration, a pharmaceutically acceptable non-toxic composition is formed by the incorporation of any of the normally employed excipients, such as, for example, pharmaceutical grades of mannitol, lactose, cellulose, cellulose derivatives, sodium crosscarmellose, starch, magnesium stearate, sodium saccharin, talcum, glucose, sucrose, magnesium, carbonate, and the like. Such compositions take the form of solutions, suspensions, tablets, pills, capsules, powders, sustained release formulations and the like.

Parenteral administration is generally characterized by injection, either subcutaneously, intramuscularly or intravenously. Injectables can be prepared in conventional forms, either as liquid solutions or suspensions, solid forms suitable for solution or suspension in liquid prior to injection, or as emulsions. Suitable excipients are, for example, water, saline, dextrose, glycerol, ethanol or the like. In addition, if desired, the pharmaceutical compositions to be administered may also contain minor amounts of non-toxic auxiliary substances such as wetting or emulsifying

agents, pH buffering agents and the like, such as for example, sodium acetate, sorbitan monolaurate, triethanolamine oleate, triethanolamine sodium acetate, etc.

Another approach for parenteral administration employs the implantation of a slow-release or sustained-release system, such that a constant level of dosage is maintained. See, e.g., US Patent No. 3,710,795.

Dosage forms or compositions containing active ingredient in the range of 0.1 to 95% with the balance made up from non-toxic carrier may be prepared. Preferably, percentages of active ingredient of 0.1% to 50% in solution are employable.

Combined Preparations

Compositions of a mersacidin variant of the invention may also comprise a second active agent, including a different mersacidin variant including those described herein, a different antibacterial agent, or another agent intended to treat a second symptom or cause of a condition to be treated.

Various antibacterial agents can be used in conjunction with the mersacidin variants of the present invention. These include quinolones, tetracyclines, glycopeptides, aminoglycosides, β-lactams, rifamycins, coumermycins, macrolides, ketolides, azalides, and chloramphenicol. In particular embodiments an antibiotic of the above classes can be, for example, one of the following:

β-Lactam Antibiotics: imipenem, meropenem, biapenem, cefaclor, cefadroxil, cefamandole, cefatrizine, cefazedone, cefazolin, cefixime, cefinenoxime, cefodizime, cefonicid, cefoperazone, ceforanide, cefotaxime, cefotiam, cefpimizole, cefpiramide, cefpodoxime, cefsulodin, ceftazidime, cefteram, ceftezole, ceftibuten, ceftizoxime, ceftriaxone, cefuroxime, cefuzonam, cephaacetrile, cephalexin, cephaloglycin, cephaloridine, cephalothin, cephapirin, cephradine, cefinetazole, cefoxitin, cefotetan, azthreonam, carumonam, flomoxef, moxalactam, amidinocillin, amoxicillin, ampicillin, azlocillin, carbenicillin, benzylpenicillin, carfecillin, cloxacillin, dicloxacillin, methicillin, mezlocillin, nafcillin, oxacillin, penicillin G, piperacillin, sulbenicillin, temocillin, ticarcillin, cefditoren, SC004, KY-020, cefdinir, ceftibuten, FK-312, S-1090, CP-0467, BK-218, FK-037, DQ-2556, FK-518, cefozopran, ME1228, KP-736, CP-6232, Ro 09-1227, OPC-20000 and LY206763.

Macrolides: azithromycin, clarithromycin, erythromycin, oleandomycin, rokitamycin, rosaramicin, roxithromycin, and troleandomycin.

Ketolides: ABT-773, Telithromycin (HMR 3647), HMR3562, HMR3004, HMR3787, ABT-773, CP-654,743, C2-fluoro ketolide, A1957730, and TE802.

Quinolones: amifloxacin, cinoxacin, ciprofloxacin, enoxacin, fleroxacin, flumequine, lomefloxacin, nalidixic acid, norfloxacin, ofloxacin, levofloxacin, oxolinic acid, pefloxacin, rosoxacin, temafloxacin, tosufloxacin, sparfloxacin, clinafloxacin, PD131628, PD138312, PD140248, Q-35, AM-1155, NM394, T-3761, rufloxacin, OPC-17116, DU-6859a, and DV-7751a.

Tetracyclines: chlortetracycline, demeclocycline, doxycycline, lymecycline, methacycline, minocycline, oxytetracycline, and tetracycline.

Glycopeptides: vancomycin and derivatives thereof.

Aminoglycosides: amikacin, arbekacin, butirosin, dibekacin, fortimicins, gentamicin, kanamycin, meomycin, netilmicin, ribostamycin, sisomicin, spectinomycin, streptomycin, tobramycin, clindamycin, and lincomycin.

Rifamycins: rifamycin SV, rifamycin O, rifabutin, rifampicin, rifampin, and rifalizil.

Instead of a second antibacterial agent, the composition may comprise a second agent intended to treat a further symptom or cause of a condition to be treated by the mersacidin variant. For example, the composition may comprise an analgesic agent, e.g. a non-steroidal anti-inflammatory compound. Particularly where the composition is for the treatment of skin infections, the composition may comprise a dermatological agent such as a steroid, for treatment of inflammation of the skin. Other agents which may be useful in dermatological applications include retinoids, bactericidal agents such as benzoyl peroxide and anti-fungal agents.

In these aspects of the invention, the mersacidin variant to be combined with a second active agent may be any one of the variants mentioned above, including mersacidin F3W, mersacidin G8A, and mersacidin F3W G8A.

Uses of Mersacidin Variants

Mersacidin variants of the invention (including compositions thereof as described above) may be administered to a human or animal subject in methods of treatment, for example in the treatment of bacterial infection, particularly MRSA (methicillin resistant staphylococcus aureus) infection. Such treatment may comprise the step of administering to a subject in need of treatment an effective amount of said mersacidin variant or composition thereof.

Thus the invention also provides a mersacidin variant or composition thereof for use in a method of treatment or prophylaxis of the human or animal body. The invention also provides a mersacidin variant or composition thereof for use in a specific method of treatment or prophylaxis of the human or animal body, the specific method including those described herein below. The invention also provides the use of a mersacidin variant or composition thereof for the manufacture of a medicament for use in a specific method of treatment or prophylaxis of the human or animal body, the specific method including those described herein below.

Thus the variants or compositions thereof of the invention may be used for the treatment of bacterial infections, including systemic bacterial infections, caused by bacteria including *Clostridium difficile, Staphylococcus spp., Streptococcus spp*, *Enterococcus spp, Propionibacterium acnes*, and *Helicobacter pylori.*

The *Staphylococcus spp.* may be coagulase-negative staphylococci. The *Staphylococcus spp* may be in particular *Staphylococcus. epidermidis.* The *Staphylococcus spp* may be *Staphylococcus aureus* including drug-resistant species, such as MRSA, VISA (Vancomycin Intermediate *Staph. aureus*), VRSA (Vancomycin Resistant *Staph. aureus*), GISA (glycopeptide-intermediate *Staph. aureus*), LRSA (linezolid-resistant *Staph. aureus*), or mupirocin-resistant *Staph. aureus.* The *Streptococcus spp.* may be *Streptococcus pyogenes, Streptococcus agalactiae*, or *Streptococcus pneumoniae. Enterococcus spp. include Enterococcus faecium, Enterococcus. faecalis.*

The variants and composition may be used for systemic treatment of bacteraemia (including catheter related bacteraemia), pneumonia, skin and skin structure infections (including surgical site infections), endocarditis and osteomyelitis. The variants or compositions may also be used for topical treatment of skin infections including acne ie. *Propionibacterium acnes.* The variants and

compositions thereof may also be used in the treatment of eye infections, such as conjunctivitis, and for oral treatment for gut super-infection, such as that caused by *Clostridium difficile* including multiply-resistant *C. difficile* (pseudomembranous colitis), or gut infections associated with *Helicobacter pylori*.

The variants may also be used in the treatment or prevention of infection of the skin in wounds or burns. In addition, the variants and compositions thereof may be used in prophylactic methods, such as for the clearance of the nares to prevent transmission of MRSA. This may be practiced on subjects at risk of infection (e.g. patients entering a hospital) or on health professionals or other carers at risk of being carriers of such infections. Prophylactic clearance of gut flora ahead of abdominal surgery is also contemplated.

The effective amount of the mersacidin variant to be administered will ultimately be at the discretion of the physician, taking into account the severity of the disease in a particular subject (e.g. a human patient or animal model) and the overall condition of the subject. Suitable dose ranges will typically be in the range of from 1 to 50 mg/kg, e.g. from 5 to 25 mg/kg, with doses typically being administered in twice daily, daily or every other day as the physician finds appropriate.

Nucleic Acids

In another aspect, the invention provides a nucleic acid, generally a DNA, coding for a peptide precursor of a mersacidin variant of the invention. By "precursor", it is meant coding for the naturally occurring amino acids which are post-translationally modified by other elements of the mrsA gene cluster to produce mersacidin. Thus for example mersacidin G10Dha may be encoded by a sequence at which codon 10 is for serine.

The nucleic acid may be fused in-frame to nucleic acid encoding the N-terminal 48 amino acids of the mrsA protein leader sequence. The nucleic acid, or its fusion may be present in a replicable vector. The vector, e.g. a plasmid vector, may contain an origin of replication (e.g. a replication origin functional in a bacterial host cell, particularly a *Bacillus* host cell), together with other elements such as an antibiotic marker gene. One or more other genes of the mrsA gene cluster may be present in the vector. For example, the mrsR1 gene may be present in the vector.

The nucleic acid sequence may also form part of the mersacidin biosynthesis gene cluster, in which it has replaced the *mrsA* wild-type gene. Such a replacement may be achieved by homologous recombination.

Nucleic acids of the invention may be made by any standard methodology known as such in the art. Typically, the nucleic acids are made by oligonucleotide mutagenesis of the *mrsA* gene, as described by Szekat *et al*, though any other suitable method may be used.

Host Cells

The nucleic acids of the invention may be present in a host cell, particularly a bacterial host cell such as a Bacillus host cell (e.g. *Bacillus* sp. HIL Y-85,54728 or a derivative thereof). Where the nucleic acid is in the form of a vector, the host cell may comprise a mrsA gene cluster in which the *mrsA* gene is inactive, e.g. due to mutation of the gene sequence such that no transcription occurs, or due to the presence of a mutation which results in an inactive gene product (e.g. mersacidin E17A).

A restriction map of the mrs gene cluster is shown in Altena et al, 2000. The sequence of this cluster is available as GenBank accession number: AJ250862. Using the deposited HIL strain as a source of DNA, the overlapping restriction fragments illustrated in Altena et al may be obtained by, for example, PCR amplification based on primers derived from AJ250862. These fragments are assembled using standard cloning procedures and the mrs gene cluster cloned into a suitable cloning vector. Such a vector may be pTRKH2 (O'Sullivan and Klaenhammer 1993).

The vector may be transformed into a laboratory strain of *B. subtilis* such as *B. subtilis* 168 in order to replicate, and plasmid DNA isolated from this host. The plasmid may be integrated into this host, or recovered and introduced into other host cells, particularly low-GC Gram positive host cells. These include *Bacillus* species, particularly *B. subtilis*, as well as for example *S. carnosus.*

Accordingly the present invention provides a bacterial host cell which carries a vector comprising the mrs gene cluster and one of a vector of the present invention or wherein the mrs gene cluster has been modified to produce a mersacidin variant of the present invention. The invention also provides a bacterial host cell in which the mrs gene cluster has been integrated into the genome, wherein said cell produces a mersacidin variant of the present invention.

In a preferred aspect, the host cell is *Bacillus* sp. HIL Y-85,54728. In another aspect, the invention may be a *SigH* deficient *Bacillus* sp. HIL Y-85,54728 ("Δ*SigH* HIL Y-85,54728"), or a *Bacillus* species carrying the mrsA gene cluster in which the *mrsA* gene codes for a variant mersacidin of the present invention. We have found that the use of Δ*SigH* HIL Y-85,54728 can provide certain advantages for improved production of mersacidin and its variants, as discussed herein below.

Sigma H is the product of the *sigH* (or *spo0H*) gene. It is essential for transcription of genes that function in the transition from exponential to stationary phase and in the induction of sporulation. Mutants deficient in SigH do not sporulate. SigmaH activates transcription of a number of other regulatory proteins e.g. *spo0A*, *spo0F*, *kinA*, *spo0M*, *spoVG*, *spoVS* and the *spoIIA* family as well as the *phr* family of secreted peptide pheromones. For further details see Britton *et al.* J Bacteriol. 184, 4881-90; 2002.

ΔSigH HIL strains of the invention may be made utilising the HIL strain deposited as NCIMB Accession Number NCIMB 41211, deposited 19th March 2004. In order to make the ΔSigH derivative, the *SigH* gene in the HIL strain may be inactivated in accordance with standard techniques available in the art, including for example homologous recombination. Such techniques are described further in WO2005/093069, the contents of which are incorporated herein by reference.

In its simplest form, a construct such as a plasmid which contains part of a *Bacillus SigH* coding sequence is introduced into the HIL strain, e.g. by protoplast transformation. The vector contains a selectable marker such as a chloramphenicol acetyl transferase gene, and the transformed cells are selected for integration of the marker into the chromosome. The *SigH* coding sequence is widely available in the art, and is also available in databases, such as GenBank, accession no. NC_000964.

An alternative approach is to carry out a double homologous recombination (gene replacement). With this approach only a single defect is needed. When the second recombination event occurs it can either restore the wild-type sigH or generate the mutant.

In another aspect, the ΔSigH HIL may also be an HIL derivative in which the mrsA gene product is inactive, either because the *mrsA* gene is transcriptionally inactive, or because the gene product is a mutant which does not show antibacterial activity against bacteria which are normally killed by mersacidin. Such bacteria include *Micrococcus luteus*, such as *M. luteus* ATCC 4498.

A ΔMrsA HIL in which the *mrsA* gene is inactivated by insertion into the *mrsA* gene of an erythromycin resistance gene is disclosed in Altena et al, 2000. Another ΔMrsA HIL is the E17A HIL disclosed by Szekat et al, 2003. A further ΔMrsA HIL is one in which the *mrsA* gene is altered to include a stop codon resulting in a truncated and inactive gene product. All these and other ΔMrsA HIL strains may be used to produce ΔMrsA ΔSigH HIL strains for use in the invention.

Production of Mersacidin Variants

The invention also provides a method of making a mersacidin variant which method comprises culturing a host cell of the invention in a culture medium and recovering the mersacidin variant from the medium.

The mersacidin variant may be recovered from the medium by standard techniques in the art, such as separation from other components of the culture medium by chromatographic means. Such means include the use of hydrophobic resins, reversed phase chromatography, ion exchange chromatography and HPLC. The recovery of mersacidin is illustrated in US-A-5,112,806.

One process which may be used is to bind the mersacidin variant from the culture supernatant onto a hydrophobic resin such as HP20, then elute with acetonitrile-water or methanol-water. This is followed by dilution with water so as to allow binding onto a hydrophobic column such as a C18 reversed phase resin. The mersacidin is then eluted with acetonitrile or methanol and the eluate evaporated to reduce volume. The pH is then adjusted to about pH 2.5 with phosphate buffer and the solution bound onto a strong cation exchanger such Varian SCX, followed by elution with 50% methanol, 250mM phosphate buffer pH7. The eluate is desalted on another C18 column, eluted with methanol, then lyophilised.

Where variants have a different charge from mersacidin, alterations to the process may be introduced. For example, the ion exchange step may be altered or omitted if the charge is

different and hplc might be utilised. If the mersacidin variant is partly bound to the bacteria in which it is produced the product may be released by treatment with methanol, acetonitrile or similar solvents.

Reference herein to "recovery" or "recovering" includes the purification of the mersacidin or variant thereof to a degree such that it will be suitable for pharmaceutical use. Thus generally recovery will include the steps of removal of the microorganism (e.g. by centrifugation or filtration), separating the lantibiotic from other bacterial components present in the culture medium, and optionally if desired components of the culture medium. Thus the mersacidin variant will be in substantially isolated form.

The mersacidin variant may be recovered in a solution, such as a buffer required to elute the mersacidin variant from a chromatography column, or it may be recovered in the form of a lyophilized fraction.

Deposit Information

Bacillus sp. HIL Y-85,54728 was deposited on 19th March 2004 with NCIMB, Accession No. NCIMB 41211 in the name of Novacta Biosystems Limited.

Reference to Earlier Application.

Mersacidin F3W, mersacidin G8A and mersacidin F3W G8A are described in WO2005/093069 (PCT/GB2005/001055) filed 21 March 2005 and claiming priority from 0406870.6 filed 26 March 2004, the disclosures of which are incorporated herein by reference in their entirety. To the extent that such compounds and matter relating to their manufacture or use are described in PCT/GB2005/001055 and such compounds and matter relating form part of the state of the art effective against any application arising from the present disclosure, such compounds or matter relating are disclaimed to the extent that such compounds or matter relating are claimed or disclosed, whichever is applicable, in the earlier application and that such a disclaimer is effective under national law of the country in which the relevant application is made.

Having generally described this invention, the following examples are provided to further describe this invention and fully enable those skilled in the art to make and use this invention, including its best mode. However, the scope of this invention should not be interpreted as limited to the

specifics of these examples, but rather, for that purpose, reference should be made to the appended claims and equivalents thereof.

Example 1: Production of Mersacidin Variants.

Bioactive Mersacidin Variants

Site-directed mutagenesis of mersacidin may be performed using methods known per se in the art, e.g. as disclosed by Szekat *et al, ibid.* Mersacidin variants having antibacterial activity as determined by a bioassay using agar plates containing *Micrococcus luteus* ATCC 4698 as indicator strain were made. These variants are set out in Table 1:

Table 1

F3	L5	P6	G7	G8	G9	G10	V11	L14	Dha16
F3D	L5A	P6H	G7A	G8A	G9A	G10A	V11L	L14V	Dha16G
F3R	L5I	P6A	G7N	G8C	G9S	G10V	V11I	L14I	Dha16A
F3W	L5M	P6N	G7Q	G8N	G9T	G10S	V11M	L14M	Dha16Dhb
F3I	L5N	P6Q	G7W	G8Q	G9N	G10Dha	V11K		Dha16H
F3P	L5H	P6V	G7S	G8H	G9R	G10M	V11C		
F3S		P6M	G7T	G8E	G9Y	G10Y			
F3C		P6F	G7M	G8I	G9H	G10W			
F3M		P6Y	G7I	G8S	G9Q	G10I			
F3N		P6G	G7H	G8P	G9L	G10Dhb			
F3H		P6L	G7F			G10R			
		P6I				G10K			
		P6D				G10H			
		P6E				G10N			

Expression and Analysis of Variants in Bacillus HIL ΔmrsA:

After four days incubation at 30°C, four random colonies obtained from each transformation were tested for production of the respective mersacidin variant. Seed cultures were carried out by growing the colonies in a 15 ml centrifuge tube (Falcon) containing 3 ml of Tryptic Soy Broth supplemented with chloramphenicol (25 mg/L). After 24 hours incubation at 30°C and 250 rpm, 0.5 ml of the seed culture was used to inoculate 10 ml of 2xBPM supplemented with chloramphenicol (25 mg/L) in a 50 ml conical flask. Mersacidin variants production was assessed after 5 days incubation at 30°C and 250 rpm. Fermentation samples were spun down at 4000 rpm for 10 min in 15 ml centrifuge tubes. The supernatants were transferred to 50 ml centrifuge tubes containing100 mg of conditioned resin Diaion HP-20 (Supelco). After incubation at room temperature for 6 hours with shaking, the supernatants were discarded and the resin containing the mersacidin variant was washed with 2 x 10 ml of water. A second washing step was carried

out with 2 x 10 ml of methanol:water (1:1). Mersacidin variants were eluted from the resins with 1 ml of 100% methanol. The eluates were evaporated to dryness and resuspended in 0.250 ml of methanol:water (1:1) and analysed by LC-MS, HPLC and bioassay.

Fermentation broth samples and/or the concentrated resin eluates were transferred to HPLC vials and 20 µl of each sample was analysed by LC-MS using the HPLC gradient conditions listed in Table 5 and the mass spectrometry conditions listed in Table 6.

Prior to bioassay of the components in broth samples and fermentation concentrate, samples were fractionated using an analytical HPLC coupled to a 96 well microtitre plate fraction collector. In general, 0.2 ml of broth sample or fermentation concentrate was loaded onto the column and the components were resolved and collected as described in Table 7. The fractions in the 96 well microtitre plates were evaporated to dryness and the resulting residues were dissolved in 50 µl of methanol:water (1:1). For each variant the resuspended residues from fractions 36 to 43 were loaded onto bioassay agar plates containing *Micrococcus luteus* ATCC 4698 as indicator strain. The bioassay plates containing the mersacidin variant samples were left at room temperature for 1 hour to allow diffusion of the sample into the agar prior to incubation at 30 °C overnight.

Table 5:HPLC conditions used in the analysis of broth samples and fermentation concentrate samples by LC-MS

Column	Phenomenex Luna HPLC column (5 µ, C18(C2), 150 × 4.6 mm)		
Mobile Phase A	10 % Acetonitrile / 0.1 % Formic Acid		
Mobile Phase B	90 % Acetonitrile / 0.1 % Formic Acid		
Flow Rate	1 ml/min		
Gradient	Time 0 minutes	100 % A	0 % B
	Time 10 minutes	0 % A	100 % B
	Time 11 minutes	0 % A	100 % B
	Time 11.1 minutes	100 % A	0 % B
	Time 15 minutes	100 % A	0 % B
	Cycle time 15 minutes		

Table 6: Mass Spectrometer Parameters used in the analysis of broth samples and fermentation concentrate by LC-MS

Ionisation	Electrospray +ve mode
Mass Range	250 to 1500 m/z
Capillary Voltage	3.10 KV
Cone Voltage	40 V

Skimmer Lens Offset	5 V
Ion Energy	1.4

Table 7:Analytical HPLC conditions used to fractionate broth samples and fermentation concentrate:

Column	Phenomenex Luna HPLC column (3 µ, C18(C2), 150 x 4.6 mm)		
Mobile Phase A	30 % Acetonitrile		
Mobile Phase B	65 % Acetonitrile		
Flow Rate	1 ml/min		
Gradient	Time 0 minutes	100 % A	0 % B
	Time 10 minutes	0 % A	100 % B
	Time 11 minutes	0 % A	100 % B
	Time 11.2 minutes	100 % A	0 % B
	Time 15 minutes	100 % A	0 % B
	Cycle time 15 minutes		
Injection Volume	200 µl		
Detection	254 and 210 nm		
Fraction Collection	0.2 min/fraction		
Fractions Collected	60 fractions		

Example 2: MIC Data for isolated Mersacidin variants

A selection of the variants produced in Example 1 above were tested further for activity against a range of bacteria. Minimum inhibitory concentrations (MICs) for all organisms with the exception of *Streptococcus pneumoniae* were determined by two-fold serial antibiotic dilutions in Mueller-Hinton broth (MHB) supplemented with 50 µg/ml calcium as calcium chloride dihydrate. Minimum inhibitory concentrations (MICs) for *S. pneumoniae* were determined by two-fold serial antibiotic dilutions in Brain Heart Infusion (BHI) broth supplemented with 50 µg/ml calcium as calcium chloride dihydrate. Antimicrobial agent stock solutions were prepared and stored according to NCCLS standard M7-A6.

Actively growing broth cultures were diluted to contain 10^5 to 10^6 CFU/ml by adjusting to an absorbance of 0.2 – 0.3 at 600nm, equivalent to the McFarland 0.5 standard. They were then diluted a further 1:100 in broth. The assays were performed in duplicate in sterile 96-well microtitre plates in a total volume of 200 µl (160 µl broth, 20 µl antibiotic, 20 µl inoculum) in a concentration range from 64 µg/ml to 0.06 µg/ml. The 12^{th} well of the microtitre plate contained no antimicrobial agent. Vancomycin was used as a reference antibiotic for quality control. Plates were incubated aerobically, shaking, for 18 – 20 hours at 37°C with the MIC defined as the lowest concentration of drug that produced no visible growth.

The results for the variants F3W, G7N, G8N, G8Q, G9H, G9A, G9S, G10V, G10Y, V11I, V11L, V11M, L14M, L14V, S16G, Dha16Dhb, Dha16A, L14I, G10A, G10N, G9R, G9N, P6H and G7A are set out in Tables 8A and 8B below (figures in µg/ml):

Table 8A:

	E. faecium 7131121	*E. faecium* 19579	*E. faecalis* 29212	*S. aureus* R33	*S. aureus* SH1000
Compound					
G7N		64,64	>64, >64	32, 16	64,64
G8N		>64, >64	>64, >64	16, 32	>64, >64
G9H		64, 64	>64, >64	16, 8	32, 32
G9A		64, 64	>64, >64	8, 8	32, 32
G10V		>64, >64	>64, >64	>64, >64	>64, >64
G10Y		64, 64	32, 32	64, 64	>64, >64
Dha16G	64, 64	32, 32	64,64	16, 16	32, 32
V11M	64, 64	32, 32	64, 64	32, 32	64, 64
L14M	64, 64	64, 64	64, 64	16, 16	32, 32
L14V	>64, >64	64, 64	64, 64	16, 16	32, 32
Dha16Dhb	64, 64		32, 32	8, 8	32, 32
V11I	16, 16		16, 16	16, 16	32, 32
F3W	34, 34	8.5, 8.5	8.5, 17	8.5, 8.5	17, 34
G9S	>64, >64	>64, >64	>64, >64	>64, >64	>64, >64
G8Q	>64, >64	>64, >64	>64, >64	32, 32	>64, >64
V11L	32, 32	32, 32	64, 32	16, 16	64, 64
Dha16A	64, 64	32, 32	64, 64	32, 16	32, 32
L14I	32, 64	16, 16	32, 32	8, 8	32, 32
G10A	>64, >64	>64, >64	>64, >64	>64, >64	>64, >64
G10N	>64, >64	>64, >64	>64, >64	>64, >64	>64, >64
G9R	>64, >64	>64, >64	>64, >64	>64, >64	>64, >64
G9N	>64, >64	>64, >64	>64, >64	64, >64	>64, >64
P6H	>64, >64	>64, >64	>64, >64	32, 64	64, 64
G7A	>64, >64	64, 64	>64, >64	32, 64	>64, >64
Mersacidin	64, 32	32, 16	32, 32	16, 16	32, 32

Table 8B:

	S. epidermidis 11047	*M. luteus* 4698	*S. pneumoniae* R6	*E. coli* SM1411	*E. faecium* 11.4103A
Compound					
G7N	32, 32	<4, <4	16, 16		32, 64
G8N	64, 64	8, 8	>64, >64		64, 64
G9H	32, 32	<4, <4	16, 16		32,16
G9A	32, 32	<4, <4	8, 8		16, 16
G10V	64, 64	<4, <4	16, 16		>64, >64
G10Y	16, 8	<4, <4	<4, <4		64, 64
Dha16G	16, 16	2, 4	2, 2		
V11M	32, 16	16, 16	32, 32		
L14M	32, 16	2, 2	4, 4		
L14V	16, 16	8, 4	4, 4		
Dha16Dhb	16, 16	1, 1	32, 32		
V11I	16, 16	.5, .5	16, 16		
F3W	8.5, 8.5	4.25, 2.13	4.25, 2.13		
G9S	>64, >64	64, 64	>64, >64		
G8Q	32, 64	32, 64	NYD		
V11L	16, 16	8, 4	4, 4		
Dha16A	16, 16	8, 4	4, 4		
L14I	16, 8	1, 2			
G10A	>64, 64	8, 8			
G10N	>64, >64	8, 8	>64, >64		
G9R	>64, >64	16, 16	>64, >64		
G9N	64, 64	64, >64	>64, >64		
P6H	64, 32	16, 16	>64, 64		
G7A	64, 64	64, 64	>64, >64		
Mersacidin	16, 8		<4, <4	>64, >64	16, 16

Example 3: Further MIC Data.

MIC tests were performed with some of the variants of Example 2 on a range of other organisms. The results are shown in Tables 9 and 10 (figures in μg/ml):

Table 9

	S. pyogenes 16205	*S. aureus* CS	*S.pyogenes* 13608	*S.aureus* SG511	
G8A	1	8	1	8	
P6A	8	32	4	8	
F3W	0.125	4	0.125	2	
Dha16H	4	64	4	8	
Mersacidin	0.5	4	0.25	2	
	S.aureus COL MRSA	*S.aureus* 1012-13 MRSA	*M.luteus* 4698	*E.faecium* 4147 VRE	*E.faecium* 4147-1
G8A	32	16	4	64-16h, 128-24h	128
P6A	64	32	4	128-16h, 256-24h	256
F3W	16	4	0.5	32	32-16h, 64-23h
Dha16H	64	64	4	128	128-16h, 256-23h
Mersacidin	16	8	0.5	64	64

Table 10

	S. pyogenes 16205	*S. aureus* Mu50	*Streptococcus* G 017882	*S.aureus* SG511	*S.aureus* COL	*S.aureus* LT 1012-13	*M.luteus* 4698	*E.faecium* BM 4147
G9A	16, 8	>64, >64	32, 32	8, 16	64, >64	>64, >64	8, 8	>64, >64
G9H	16, 8	>64, >64	16, 16	8, 16	>64, >64	>64, >64	8,4	>64, >64
G8N	64, 64	>64, >64	64, 64	32, 64	>64, >64	>64, >64	32, 32	>64, >64
P6H	8, 8	>64, >64	16, 8	8, 16	>64, >64	>64, >64	4, 2	>64, >64
P6N	8, 16	>32, >32	16, 8	8, 8	>32, >32	>32, >32	8, 4	>32, >32

Example 4: Activity of G8H.

The G8H variant was tested as described in Example 2. The MIC (in μg/ml) against eight of the Example 2 strains was as set out in Table 11:

Table 11:

	E.faecium 7131121	*E.faecium* 19579	*E.faecalis* 29212	*S.aureus* R33
G8H	>64, >64	>64, >64	>64, >64	64, 64
	S.aureus	*S.epidermidis*	*M.luteus*	*S.pneumoniae*
G8H	SH1000	11047	4698	R6
	64, >64	64, 64	8, 8	16, 16

Example 5: Activity against fusidic acid resistant *Staphylococcus aureus.*

MICs (in μg/ml) were determined, as described in Example 2, for three variants against fusidic acid resistant strains of *Staphylococcus aureus*. The results are shown in Table 12:

Table 12:

	mersacidin	**F3W**	**Dha16Dhb**	**Dha16G**
S. aureus 8325-4	8, 8	4, 4	8, 8	16, 16
S. aureus CS1116	4, 2	4, 2	2, 2	4, 4
S. aureus CS957	8, 8	4, 4	16, 16	16, 16
S. aureus CS767	16, 16	8, 8	16, 16	16, 16
S. aureus CS858	16, 16	8, 8	16, 16	32, 16
S. aureus CS741	8, 8	8, 8	8, 8	16, 16
S. aureus CS1145	4, 4	4, 4	16, 16	16, 16
S. aureus CS872	8, 8	4, 4	8, 8	16, 16
S. aureus CS866	8, 8	4, 4	16, 16	16, 16
S. aureus CS607	16, 16	8, 8	16, 16	16, 16
S. aureus CS22	8, 8	4, 4	16, 16	16, 16

Example 6: Activity against mupirocin resistant *Staphylococcus aureus.*

MICs were determined, as described in Example 2, for three variants against mupirocin resistant strains of *Staphylococcus aureus.* Results are shown in Table 13 in μg/ml:

Table 13:

	mersacidin	**F3W**	**Dha16Dhb**	**Dha16G**
S. aureus 8325-4	8, 8	4, 4	8, 8	16, 16
S. aureus GISA-2	16, 16	4, 4	8, 8	16, 16
S. aureus LZ6	8, 8	8, 8	16, 16	16, 16
S. aureus LZ8	8, 8	8, 8	16, 16	16, 16
S. aureus LZ9	16, 16	16, 16	32, 32	16, 16
S. aureus LZ10	16, 16	16, 16	16, 16	16, 16
S. aureus 420	8, 8	4, 4	8, 16	16, 16
S. aureus 1205	16, 16	16, 16	32, 32	32, 32
S. aureus 1120	16, 16	8, 8	16, 16	16, 16
S. aureus 1454	8, 16	8, 8	16, 16	16, 16
S. aureus 1086	16, 16	16, 16	16, 16	16, 16

Example 7: Activity against *Streptococcus pyogenes.*

MICs were determined, as described in Example 2, for five variants against strains of *Streptococcus pyogenes.* Results are shown in Table 14 in μg/ml:

Table 14:

	mersacidin	**F3W**	**V11I**	**L14I**	**Dha16Dhb**	**Dha16G**
S. pyogenes 7755441	2, 2	1, 1	2, 2	2, 2	2, 2	8, 8
S. pyogenes 7713283	2, 1	1, 1	1, 1	2, 4	2, 2	8, 8
S. pyogenes 7865844	2, 1	1, 2	1, 1	2, 2	2, 2	8, 8
S. pyogenes 7753040	2, 2	1, 1	1, 1	2, 2	2, 2	8, 8
S. pyogenes 7755255	1, 1	1, 1	1, 2	2, 2	2, 2	8, 8
S. pyogenes 7756725	1, 1	0.5, 1	1, 1	1, 1	1, 2	4, 4
S. pyogenes 7757080	1, 1	1, 1	0.5, 1	1, 2	2, 2	8, 4
S. pyogenes GRL05045	1, 1	1, 1	2, 1	2, 2	2, 2	8, 8
S. pyogenes 7865253	0.5, 1	0.5, 1	0.5, 1	1, 2	1, 2	4, 4
S. pyogenes GRL05046	1, 2	0.5, 1	1, 2	1, 2	2, 4	4, 8

Example 8: Activity against viridans *Streptococcus.*

MICs were determined, as described in Example 2, for five variants against strains of viridans *Streptococcus.* Results are shown in Table 15 in μg/ml:

Table 15:

	mersacidin	**F3W**	**V11I**	**L14I**	**Dha16Dhb**	**Dha16G**
S. salivarius GRL05064	1, 1	0.5, 0.5	1, 1	1, 1	2, 2	4, 8
S. mitis 1 7722543	1, 2	1, 1	2, 2	4, 4	4, 4	8, 8
S. mitis 1 7863547	2, 2	1, 1	2, 2	4, 4	2, 2	8, 8
Aerococcus viridans 2 BC6008.2	4, 2	2, 2	4, 4	8, 8	4, 4	8, 16
S. oralis 5823	4, 4	2, 4	4, 4	8, 8	8, 8	16, 16
S. salivarius GRL05063	4, 8	1, 2	4, 8	4, 4	4, 4	8, 8
S. constellatus GRL05065	8, 8	4, 4	8, 8	16, 16	16, 16	16, 16
S. oralis GRL05066	4, 4	2, 2	2, 2	4, 4	8, 4	16, 8
S. oralis GRL05069	32, 32	16, 16	16, 16	16, 16	32, 32	32, 32

Example 9: Activity against *Propionibacterium acnes.*

Test organisms were selected from 3-7 day growth on Wilkens-Chalgren agar (WCA) supplemented with furazolidone (1-2 μg/ml). Fresh Wilkens-Chalgren broth (WCB) was inoculated by direct colony suspension with single colonies of *P. acnes* and adjusted to a density equivalent to the McFarland 0.5 standard (1 x 10^8 CFU/ml), then further diluted in sterile WCB, supplemented with 50μg/ml Ca^{2+} (as calcium chloride dihydrate), for a final inoculum in sterile 96-well microtitre plates of approximately 10^5 CFU/ml. Two-fold serial antibiotic dilutions were performed in sterile water with stock solutions prepared and stored according to NCCLS standards (M11-A5, 2001). The assays were performed in duplicate with Vancomycin and Clindamycin used as reference antibiotics for quality control. Plates were incubated anaerobically for 48-72 hours at 37°C with the MIC defined as the concentration of drug where a marked reduction occurred in the appearance of growth on the test plate compared to growth on the control plate. All manipulations were performed in duplicate in ambient atmosphere in pre-reduced media with only brief exposure to air.

MICs (in µg/ml) of three variants against strains of *P. acnes* was as shown in Table 16:

Table 16:

	mersacidin	F3W	Dha16Dhb	Dha16G
P. acnes P37	2, 2	1, 1	4, 8	4, 4
P. acnes AT1	2, 2	1, 1	4, 4	4, 4
P. acnes AT26	2, 2	1, 1	2, 2	2, 2
P. acnes 101897d	2, 2	1, 0.5	2, 2	1, 1
P. acnes PF284	2, 2	1, 1	2, 2	2, 2
P. acnes PF286	2, 2	1, 1	2, 2	2, 2
P. acnes PF289	2, 2	1, 2	8, 8	4, 4
P. acnes PF290	4, 2	0.5, 1	1, 2	2, 2
P. acnes PF291	4, 2	0.5, 0.5	0.5, 1	2, 2
P. acnes 1348	2, 2	2, 2	4, 4	8, 8
P. acnes 1431	4, 4	2, 2	4, 4	2, 2

Example 10: Activity against *Clostridium difficile.*

Minimum inhibitory concentrations (MICs) for *C. difficile* were determined and antimicrobial agent stock solutions were prepared and stored according to the NCCLS reference agar dilution method for anaerobic bacteria (M11-A5, 2001). Two-fold serial antibiotic dilutions were prepared in Wilkens-Chalgren agar (WCA). Test organisms were selected from 48 hour growth on Braziers (C.C.E.Y.) agar, subcultured in Schaedler broth to a density equivalent to a McFarland 0.5 standard (1 x 10^8 CFU/ml), with a final inoculum onto WCA plates supplemented with 50µg/ml Ca^{2+} (as calcium chloride dehydrate) of approximately 10^5 CFU/spot. *Bacteroides fragilis* ATCC 25285 was included as a reference control strain and Metronidazole was used as a reference antibiotic for quality control. All manipulations were performed in duplicate in ambient atmosphere in pre-reduced media with only brief exposure to air. Plates were incubated anaerobically for 48 hours at 37°C with the MIC defined as the concentration of drug where a marked reduction occurred in the appearance of growth on the test plate compared to growth on the control plate.

MICs of the F3W and F3W-L14I variants are shown in Table 17 in µg/ml:

Table 17:

	mersacidin	F3W	F3W-L14I
C. difficile ATCC 43594	2, 4	2, 2	2, 2
C. difficile ATCC 43255	2, 4	2, 2	2, 2

Example 11: Activity of Double Variants

MICs of double variants were determined as in Example 2 against eight of the example 2 strains. The MICs are shown in Table 18 in µg/ml:

Table 18:

	E.faecium	*E.faecium*	*E.faecalis*	*S.aureus*
	7131121	**19579**	**29212**	**R33**
L14I Dha16G	32,32	32,32	16,8	8,8
L14I Dha16Dhb	>64,>64	32,64	>64,>64	8,8
V11I Dha16G	64,64	32,32	64,64	16,16
F3W L14I	8,8	8,8	8,8	2,2
V11I L14I	16,16	16,8	8,16	4,4
	S.aureus	***S.epidermidis***	***M.luteus***	***S.pneumoniae***
	SH1000	**11047**	**4698**	**R6**
L14I Dha16G	16,32	16,16	16,8	32,32
L14I Dha16Dhb	32,64	32,64	1,1	4,4
V11I Dha16G	32,32	32,32	0.5,0.5	4,4
F3W L14I	8,8	4,4	0.125,0.125	0.5,0.25
V11I L14I	16,16	4,8	0.25,0.25	0.5,0.5

Summary of Sequences

The following is a summary of sequences described herein.

SEQ ID NO:1

MrsA gene sequence of the MrsA encoding sequence including the leader sequence and the propeptide region. The propeptide encoding region is shown underlined:

```
       atgagtca agaagctatc attcgttcat ggaaagatcc tttttcccgt gaaaattcta
5161   cacaaaatcc agctggtaac ccattcagtg agctgaaaga agcacaaatg gataagttag
5221   taggtgcggg agacatggaa gcagcatgta cttttacatt gcctggtggc ggcggtgttt
5281   gtactctaac ttctgaatgt atttgttaa
```

SEQ ID NO:2 – Translation of SEQ ID NO:1. The propeptide region is underlined.

MSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAGDMEAACTFTLPGGGGVCTLTS ECIC

5

Claims

1. A mersacidin variant wherein the variant comprises a modification to position 3, 5, 6, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 1:

Table 1

F3	L5	P6	G7	G8	G9	G10	V11	L14	Dha16
F3W	L5A	P6H	G7A	G8A	G9A	G10A	V11L	L14V	Dha16G
F3R	L5I	P6A	G7N	G8C	G9S	G10V	V11I	L14I	Dha16A
F3D	L5M	P6N	G7Q	G8N	G9T	G10S	V11M	L14M	Dha16Dhb
F3I	L5N	P6Q	G7W	G8Q	G9N	G10Dha	V11K		Dha16H
F3P	L5H	P6V	G7S	G8H	G9R	G10M	V11C		
F3S		P6M	G7T	G8E	G9Y	G10Y			
F3C		P6F	G7M	G8I	G9H	G10W			
F3M		P6Y	G7I	G8S	G9Q	G10I			
F3N		P6G	G7H	G8P	G9L	G10Dhb			
F3H		P6L	G7F			G10R			
		P6I				G10K			
		P6D				G10H			
		P6E				G10N			

2. A variant according to claim 1 which comprises a modification selected from the group F3W, G8A, G9A, G9H, V11I, V11L, L14I, L14M, L14V, Dha16G and Dha16Dhb.

3. A variant according to claim 1 consisting of a modification to position 3, 5, 6, 7, 8, 9, 10, 11, 14 or 16 of mersacidin as set out in Table 1.

4. A variant according to claim 1 comprising the modification F3W and a second modification at one of position 5, 6, 7, 8, 9, 10, 11, 14 or 16 set out in Table 1.

5. A variant according to claim 4 selected from the group mersacidin F3W G9A, mersacidin F3W G9H, mersacidin F3W V11I, mersacidin F3W V11L, mersacidin F3W L14I, mersacidin F3W L14M, mersacidin F3W L14V, mersacidin F3W Dha16G and mersacidin F3W Dha16Dhb.

6. A nucleic acid encoding a variant according to any one of claims 1 to 5.

7. An expression vector comprising the nucleic acid of claim 6.

8. A host cell comprising the nucleic acid of claim 6 or vector of claim 7.

9. A host cell according to claim 8 wherein said host cell is *Bacillus* sp. HIL Y-85,54728 or a variant thereof in which the *mrsA* gene encodes the variant of any one of claims 1 to 5.

10. A composition comprising a mersacidin variant according to any one of claims 1 to 6 together with a pharmaceutically acceptable carrier.

11. A composition according to claim 10 wherein said carrier is in the form of an aqueous, aqueous-alcoholic or oily solution, an oil-in-water or water-in-oil or multiple emulsion, an aqueous or oily gel, a liquid, pasty or solid anhydrous product suitable for topical administration.

12. A variant according to any one of claims 1 to 6, or composition of claim 10 or 11 for use in a method of treatment or prophylaxis of the human or animal body.

13. A variant according to any one of claims 1 to 6, or composition of claim 10 or 11 for use in a method of treatment or prophylaxis of the human or animal body, wherein said treatment or prophylaxis is selected from: treatment of systemic bacterial infections; systemic treatment of bacteraemia; treatment of pneumonia; treatment of skin and skin structure infections; treatment of endocarditis, treatment of osteomyelitis; treatment of acne; treatment of an eye infection; treatment of a gut super-infection; treatment or prevention of infection of the skin in wounds or burns.

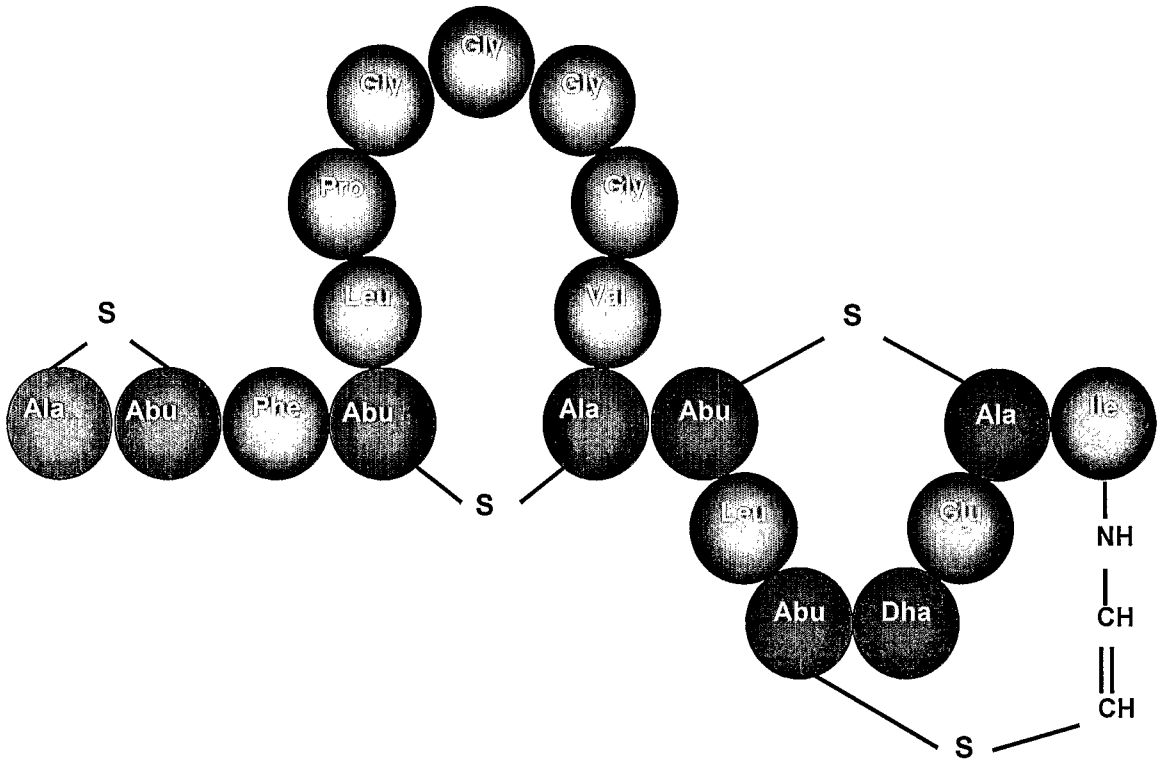
14. A variant according to any one of claims 1 to 6, or composition of claim 10 or 11 for use in a method of treatment or prophylaxis of the human or animal body wherein the treatment is of a bacterial infection selected from an infection caused by *Clostridium difficile, Streptococcus spp, Enterococcus spp., Staphylococcus spp., Propionibacterium acnes*, and *Helicobacter pylori.*

15. A variant or composition for use according to claim 14 wherein said *Staphylococcus spp.* are coagulase-negative staphylococci including *Staphylococcus epidermidis*, or said *Staphylococcus spp.* is *S. aureus or* a drug-resistant species selected from MRSA, VISA , VRSA, GISA, LRSA, and mupirocin-resistant *Staph. aureus.*

16. A variant or composition for use according to claim 14 wherein said *Streptococcus spp.* are selected from the group *Streptococcus pyogenes*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*.

17. A variant or composition for use according to claim 14 wherein said *Enterococcus spp* is *E. faecium* or *E. faecalis*.

FIGURE 1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SEQUENCE LISTING

<110> Novacta Biosystems Limited
Dawson, Michael John
Cortes Bargallo, Jesus
Appleyard, Antony Nicholas

<120> Variants of the Lantibiotic Mersacidin and their Use

<130> AHBCP6403232

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<151> 2005-09-27

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20 25 30

Glu Ala Gln Met Asp Lys Leu Val Gly Ala Gly Asp Met Glu Ala Ala
35 40 45

Cys Thr Phe Thr Leu Pro Gly Gly Gly Gly Val Cys Thr Leu Thr Ser
50 55 60

Glu Cys Ile Cys
65

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/003570

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K14/32 C12P21/02 A61K38/16

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

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
C07K C12P A61K

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

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)
EPO-Internal, BIOSIS, EMBASE, WPI Data, CHEM ABS Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	SZEKAT C ET AL: "CONSTRUCTION OF AN EXPRESSION SYSTEM FOR SITE-DIRECTED MUTAGENESIS OF THE LANTIBIOTIC MERSACIDIN" APPLIED AND ENVIRONMENTAL MICROBIOLOGY, WASHINGTON,DC, US, vol. 69, no. 7, July 2003 (2003-07), pages 3777-3783, XP001206733 ISSN: 0099-2240 abstract	1-17
A	EP 0 700 998 A1 (HOECHST AG [DE] AVENTIS PHARMA GMBH [DE]) 13 March 1996 (1996-03-13) the whole document	1-17

-/--

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

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"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
27 February 2007	06/03/2007

Name and mailing address of the ISA/	Authorized officer
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Form PCT/ISA/210 (second sheet) (April 2005)

INTERNATIONAL SEARCH REPORT

International application No
PCT/GB2006/003570

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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Information on patent family members

International application No
PCT/GB2006/003570

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