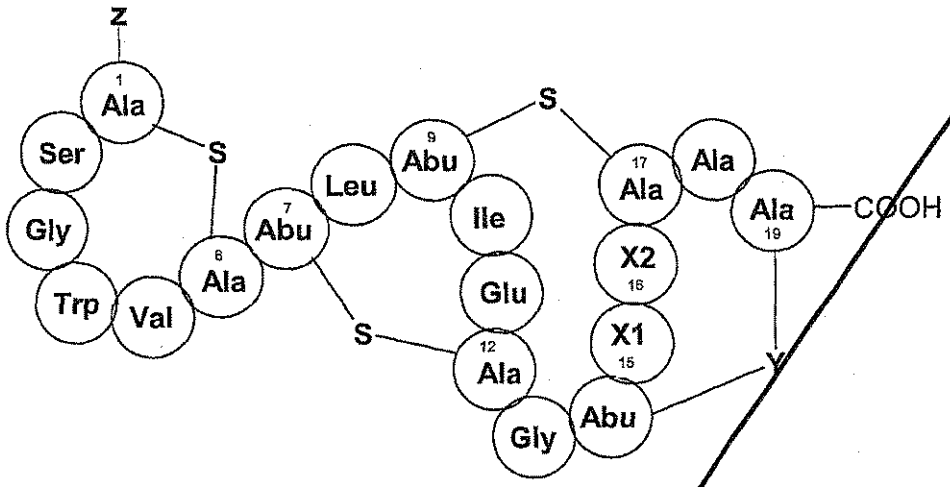
Claims

1. A compound of the formula:



wherein:

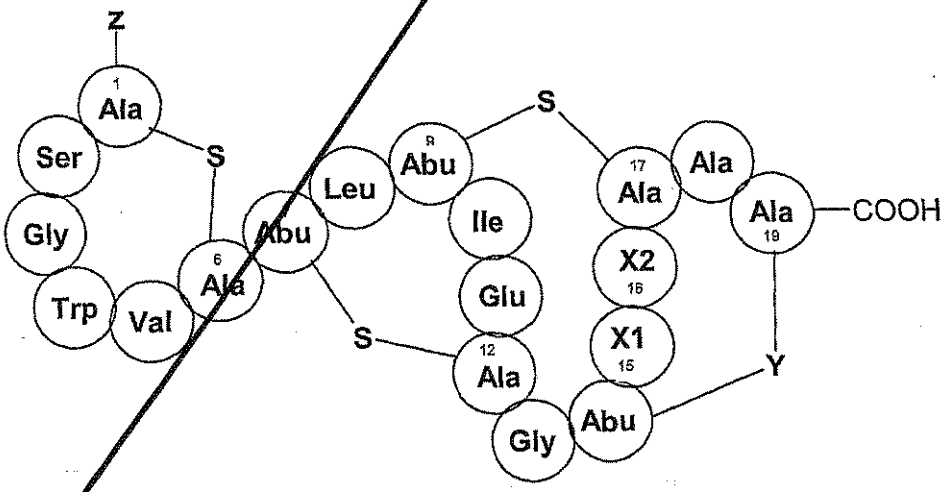
-X1-X2- represent -Leu-Val-; -Val-Val- or -Leu-Ile-;

Y is -S- or -S(O)-; and

Z is either NH_2 or Ala-,

or a pharmaceutically acceptable salt thereof.

2. A compound of the formula:



wherein:

-X1-X2- represent -Leu-Val-; -Val-Val- or -Leu-Ile-;

Y is -S- or -S(O)-; and

Z is either NH_2 or Ala-,
or a biologically active variant thereof, or a derivative of said compound or variant, or a pharmaceutically acceptable salt of said compound, variant or derivative.

3. A compound according to claim 2 wherein the variant is a compound in which Z is an amino acid.

4. A compound according to claim 3 wherein the amino acid is selected from the group Ala, Ile-, Lys-, Phe-, Val-, Glu-, Asp-, His-, Leu-, Arg-, Ser- and Trp and said amino acids are in the D- or L- configuration.

5. A compound according to claim 3 wherein the variant is a compound in which Z is an amino acid selected from the group Ile-, Lys-, Phe-, Val-, Glu-, Asp-, His-, Leu-, Arg- and Ser-.

6. A compound according to any one of claims 2 to 5 wherein said derivative is a compound which comprises a C-terminal of the formula -COR, in which R represents the group $-NR^1R^2$, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula $-(CH_2)_n-NR^3R^4$, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or (C_1 -C_4) alkyl or R^3 and R^4 taken together represent a group $-(CH_2)_3-$, $-(CH_2)_4-$, $(CH_2)_2-O-(CH_2)_2-$, $-(CH_2)_2-S-(CH_2)_2-$ or $-(CH_2)_5-$;
or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted in position 4 with a substituent selected from:

(a) (C_1 -C_4)alkyl;

(b) (C_5 -C_7)-cycloalkyl,

(c) pyridyl,

(d) $-(CH_2)_p-NR^5R^6$ in which p represents an integer from 1 to 8 and R^5 and R^6 independently represent hydrogen or (C_1-C_4) alkyl;

(e) piperidinyl;

(f) substituted piperidinyl, wherein the substituted piperidinyl bears a N-substituent which is (C_{1-4})alkyl;

(g) benzyl; and

(h) substituted benzyl, wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, (C_1 -C_4)alkyl and (C_1 -C_4)alkoxy.

7. A compound according to any one of claims 2 to 6 wherein said derivative is a compound which comprises a C-terminal of the formula -COR, in which R represents the

group -NR^1R^2, wherein R^1 and R^2 independently represent hydrogen, a group of formula -$(CH_2)_n$- NR^3R^4, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or (C_1 -C_4) alkyl or R^3 and R^4 taken together represent a group -$(CH_2)_3$-, -$(CH_2)_4$-, $(CH_2)_2$-O-$(CH_2)_2$-, -$(CH_2)_2$-S-$(CH_2)_2$- or -$(CH_2)_5$-, or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted in position 4 with a substituent selected from (C_1 -C_4)alkyl, (C_5 -C_7)-cycloalkyl, pyridyl, benzyl, and substituted benzyl wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, (C_1 -C_4)alkyl and (C_1 -C_4)alkoxy.

8. A compound according to any one of claims 2 to 7 in which the compound comprises a modification to a carboxy function of a side chain of an internal residue such that said function is modified from -COOH to a group -$COOR^5$ in which R^5 represents hydrogen, (C_1 -C_4)alkyl or (C1 -C_4)alkoxy (C_2 -C_4)alkyl.

9. A compound according to any one of claims 2 to 8 in which the compound comprises a modification to the N-terminal amino group such that this group -NH_2 is instead a group -NHR^6 wherein R^6 represents C_{1-4}alkyl.

10. An isolated nucleic acid encoding a polypeptide selected from any one of the group of SEQ ID NOs:1-4, SEQ ID NOs:11-14, SEQ ID NOs:212, 22, 23 and 119.

11. An isolated nucleic acid encoding a polypeptide selected from any one of the group consisting of SEQ ID NOs:1-4, SEQ ID NOs:11-14, SEQ ID NOs: 212, 22, 23 and 119 or a biologically active variant thereof.

12. An isolated polypeptide comprising a sequence selected from any one of SEQ ID NOs:101-132, or a variant having at least 90% sequence identity thereto.

13. An isolated polypeptide comprising a sequence selected from any one of SEQ ID NOs:201-231, or a variant having at least 90% sequence identity thereto.

14. An expression construct comprising the nucleic acid of claim 10 or 11.

15. A set of expression constructs comprising a first nucleic acid according to claim 10 or 11 and a second nucleic acid encoding a *LanM* polypeptide comprising SEQ ID NO:120 or SEQ ID NO:213 or a variant having at least 90% identity to SEQ ID NO:120 or SEQ ID NO:213.

16. A set of expression constructs comprising a first nucleic acid according to claim 10 or 11, or a first and second nucleic acid according to claim 7, and a third nucleic acid encoding a *LanR* polypeptide comprising SEQ ID NO:122 or SEQ ID NO:216 or a variant having at least 90% identity to SEQ ID NO:122 or SEQ ID NO:216.

17. A set of expression constructs according to claim 15 or 16 and further comprising a nucleic acid encoding a *LanT* polypeptide of SEQ ID NO:123 or a variant thereof, or SEQ ID NO:214 or a variant thereof.

18. A set of expression constructs according to claim 15, 16 or 17 further comprising a nucleic acid encoding a *lanO* polypeptide of SEQ ID NO:122 or a variant thereof, or SEQ ID NO:215 or a variant thereof.

19. A recombinant vector or vectors comprising the expression constructs of any one of claims 14 to 17.

20. A host cell transformed with the vector or vectors of claim 19.

21. The host cell of claim 20 which is an actinomycete.

22. The actinomycete of claim 21 which is a non-producer of a lantibiotic polypeptide.

23. The actinomycete of claim 21 or 22 which is selected from the group consisting of *Streptomyces lividans, S. coelicolor* and *S. cinnamoneus.*

24. A method of making a compound according to any one of claims 1 to 9 comprising expressing a nucleic acid encoding a polypeptide comprising a sequence selected from the group of SEQ ID NOs:1, 2, 3, 11, 12, 13, 212, 22 and 23, or a variant of any of said sequences in a lantibiotic-producing host cell.

25. The method of claim 24 wherein said host cell has a mutation in its endogenous *LanA* gene such that the gene is not expressed or the gene product is inactive.

26. The method of claim 24 or 25 wherein the host cell is *A. garbadinensis* or *A. liguriae.*

27. The method of any one of claims 24 to 26 wherein the host cell is *A. liguriae* NCIMB 41362.

28. The method of claim 24 wherein the host cell is an actinomycete which does not produce a lantibiotic and wherein the host cell is transformed with SEQ ID NO:100 or SEQ ID NO:200, or a fragment or variant of said sequences, wherein the fragment or variant confers on the cell the ability to produce a lantibiotic.

29. The method of claim 28 wherein the host cell is transformed with nucleic acid encoding SEQ ID NOs:116-127, or a variant thereof wherein said nucleic acid encodes polypeptides having at least 90% identity to each of SEQ ID NOs:116-127.

30. The method of claim 28 wherein the host cell is transformed with nucleic acid encoding SEQ ID NOs:206-220, or a variant thereof wherein said nucleic acid encodes polypeptides having at least 90% identity to each of SEQ ID NOs:206-220.

31. *A. liguriae* NCIMB 41362.

32. A derivative of *A. liguriae* NCIMB 41362 comprising a mutation in its *LigA* gene such that the gene is not expressed or the gene product is inactive.

33. A recombinant DNA cassette which comprises a nucleotide sequence encoding the lantibiotic LigA pre-pro-peptide of SEQ ID NO:212, wherein said sequence comprises

a first restriction site at or adjacent the N-terminal encoding region of the encoding sequence;

optionally a second restriction site downstream of the first restriction site and within the encoding sequence; and

a third restriction site at or adjacent the C-terminal encoding region of the encoding sequence,

wherein at least one of said restriction sites does not occur within the ligA coding sequence of SEQ ID NO:200.

34. A recombinant DNA cassette which comprises a nucleotide sequence encoding the lantibiotic LanA pre-pro-peptide of SEQ ID NO:119, wherein said sequence comprises

a first restriction site at or adjacent the N-terminal encoding region of the encoding sequence;

optionally a second restriction site downstream of the first restriction site and within the encoding sequence; and

a third restriction site at or adjacent the C-terminal encoding region of the encoding sequence,

wherein at least one of said restriction sites does not occur within the lanA coding sequence of SEQ ID NO:100.

35. A method of making a library of lantibiotic-encoding gene sequences which comprises:

providing an expression cassette according to claim 33 or 34

introducing into the cassette, between the first and second restriction sites, the first and third restriction sites, or the second and third restriction sites, a multiplicity of sequences each of which corresponds to the corresponding lantibiotic-encoding sequence apart from having from 1 to 15 nucleotide changes; and

recovering an expression library encoding a multiplicity of lantibiotic-variant sequences.

36. A method which comprises:

providing an expression library produced according to claim 35;

introducing one or more members of said library into host cells capable of expression of a lantibiotic; and

screening said host cells for lantibiotic activity.

37. The method of claim 36 which further comprises recovering the lantibiotic.

38. The method of claim 36 or 37 which further comprises isolating a host cell comprising a member of the library.

39. An isolated nucleic acid comprising SEQ ID NO:100 or SEQ ID NO:200, or a fragment or variant of said sequences, wherein the fragment or variant confers on an actinomycete host cell the ability to produce a lantibiotic.

40. The isolated nucleic acid of claim 39 wherein the nucleic acid encodes SEQ ID NOs:116-127, or a variant thereof wherein said nucleic acid encodes polypeptides having at least 90% identity to each of SEQ ID NOs:116-127.

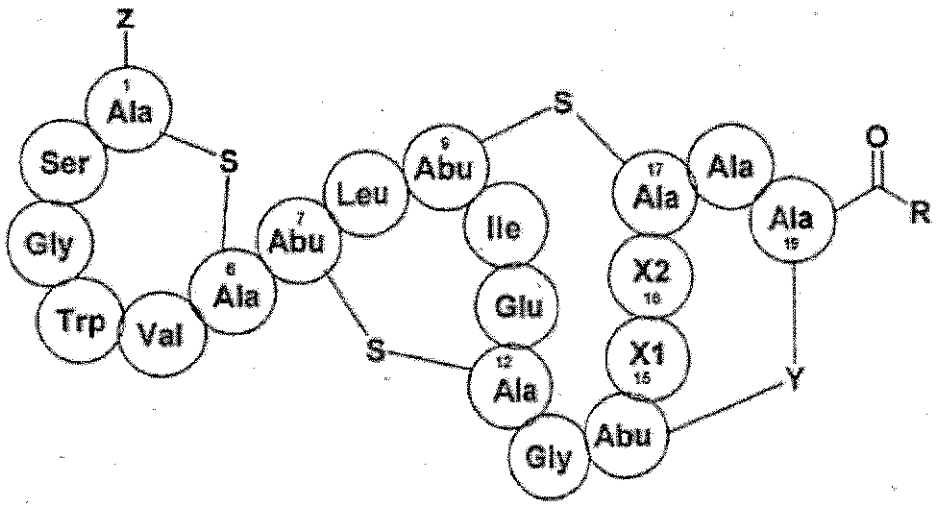
41. The isolated nucleic acid of claim 39 wherein the host cell is transformed with nucleic acid encoding SEQ ID NOs:206-220, or a variant thereof wherein said nucleic acid encodes polypeptides having at least 90% identity to each of SEQ ID NOs:206-220.

42. A pharmaceutical composition comprising a compound according to any one of claims 1 to 9 together with a pharmaceutically acceptable carrier or diluent.

43. A method treatment or prophylaxis of a bacterial infection in a subject, which method comprises administering to said subject a compound according to any one of claims 1 to 9, or a composition according to claim 42.

Claims

1. A compound of the formula:



wherein:

-X1-X2- represents -Leu-Val- or -Leu-Ile-;

-Y- is -S- or -S(O)-;

Z is either an amino or $-NH_2$ wherein the latter represents the N-terminus of the Ala at position 1;

R represents the group —OH or $—NR^1 R^2$, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula $-(CH_2)_n-NR^3R^4$, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or C_{1-4}alkyl, or R^3 and R^4 taken together represents a group $-(CH_2)_3-$, $-(CH_2)_4-$, $(CH_2)_2-O-(CH_2)_2$, $-(CH_2)_2-S-(CH_2)_2$ or $-(CH_2)_5-$; or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted at position 4 with a substituent selected from:

(a) C_{1-4}alkyl;

(b) C_{5-7}cycloalkyl;

(c) pyridyl,

(d) $-(CH_2)_p-NR^5R^6$ in which p represents an integer from 1 to 8 and R_5 and R^6

independently represent hydrogen or C_{1-4}alkyl;

(e) piperidinyl;

(f) substitute piperidinyl, wherein the substituted piperindinyl bears a N-substituent which is C_{1-4}alkyl;

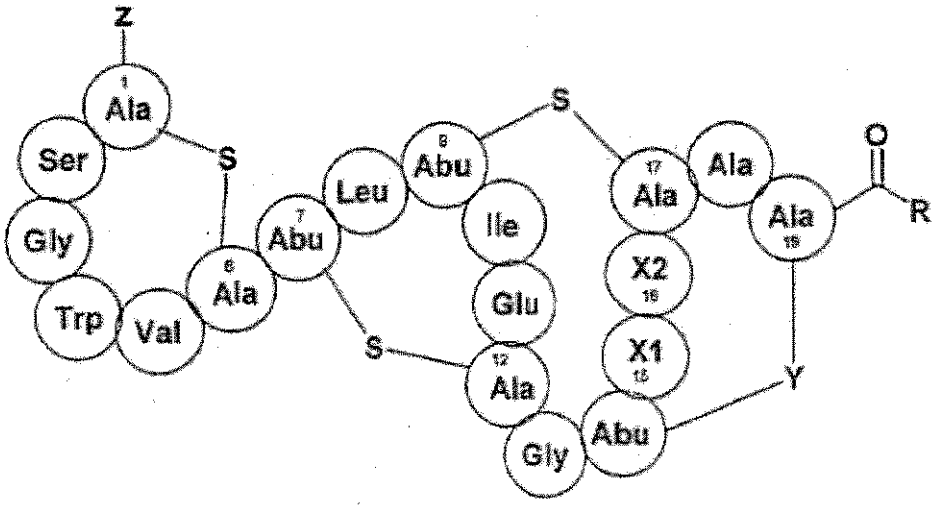
(g) benzyl; and

(h) substituted benzyl, wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, C_{1-4}alkyl and C_{1-4}alkoxy,

or a biologically active variant thereof, or a pharmaceutically acceptable salt of said compound or variant,

wherein the biologically active variant comprises 1, 2, 3 or 4 amino acids substituted at positions selected from positions 2, 3, 4, 5, 8, 10, 11, 13, 15, 16 and 18 of the compound„ with the proviso that -X1-X2- is -Leu-Val- or -Leu-Ile-.

2. A compound of the formula



wherein:

-X1-X2- represents -Leu-Val- or -Leu-Ile-;

-Y- is -S- or -S(O)-;

Z is either an amino or $-NH_2$ wherein the latter represents the N-terminus of the Ala at position 1;

R represents the group —OH or $—NR^1R^2$, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula $-(CH_2)_n-NR^3R^4$, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or C_{1-4}alkyl, or R^3 and R^4 taken together represents a group $-(CH_2)_3-$, $-(CH_2)_4-$, $(CH_2)_2-O-(CH_2)_2$, $-(CH_2)_2-S-(CH_2)_2$ or $-(CH_2)_5-$; or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted at position 4 with a substituent selected from:

(i) C_{1-4}alkyl;

(j) C_{5-7}cycloalkyl;

(k) pyridyl,

(l) $-(CH_2)_p-NR^5R^6$ in which p represents an integer from 1 to 8 and R^5 and R^6 independently represent hydrogen or C_{1-4}alkyl;

(m) piperidinyl;

(n) substitute piperidinyl, wherein the substituted piperindinyl bears a N-substituent which is C_{1-4}alkyl;

(o) benzyl; and

(p) substituted benzyl, wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, C_{1-4}alkyl and C_{1-4}alkoxy,

or a pharmaceutically acceptable salt of said compound, with the proviso that -X1-X2- is -Leu-Val- or -Leu-Ile-.

3. A compound according to claim 1 or claim 2, wherein Z is an amino acid.

4. A compound according to claim 3, wherein the amino acid is selected from the group Ala, Ile-, Lys-, Phe-, Val-, Glu-, Asp-, His-, Leu-, Arg-, Ser- and Trp and said amino acids are in the D- or L- configuration.

5. A compound according to claim 3, wherein Z is an amino acid selected from the group Ile-, Lys-, Phe-, Val-, Glu-, Asp-, His-, Leu-, Arg- and Ser-.

6. A compound according to claim 3, wherein the amino acid is Ala.

7. A compound according to any one of claims 1 to 6, wherein R represents the group $-NR^1R^2$.

8. A compound according to any one of claims 1 to 7, wherein R represents the

group -NR^1 R^2 , wherein R^1 and R^2 independently represent hydrogen, a group of formula -$(CH_2)_n$-NR^3R^4 , in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or (C_1-C_4) alkyl or R^3 and R^4 taken together represent a group -$(CH_2)_3$-, -$(CH_2)_4$-, -$(CH_2$-O-$(CH_2)_2$-, -$(CH_2)_2$-S-$(CH_2)_2$- or -$(CH_2)_5$-, or R^1 and R^2 taken together with the adjacent nitrogen atom represent a piperazine moiety which may be substituted in position 4 with a substituent selected from (C_1-C_4) alkyl, (C_5-C_7)-cycloalkyl, pyridyl, benzyl, and substituted benzyl wherein the phenyl moiety bears 1 or 2 substituents selected from chloro, bromo, nitro, (C_1-C_4)alkyl and (C_1-C_4)alkoxy.

9. A compound according to claim 8, wherein R^1 and R^2 independently represent:

(i) hydrogen;

(ii) a group of formula -$(CH_2)_n$-NR^3R^4, in which n represents an integer from 2 to 8 and R^3 and R^4 independently represent hydrogen or C_{1-4}alkyl.

10. A compound of claim 1, wherein the compound is selected from the group consisting of:

deoxyactagardine B N-[3-dimethylaminopropyl]monocarboxamide;

deoxyactagardine B N41-(1-methyl-4-piperidinyl)piperazine]monocarboxamide;

deoxyactagardine B [1-(3-dimethylaminopropyl)piperazine]monocarboxamide;

deoxyactagardine B;

D-Ala(0)deoxyactagardine B;

L-11e(0)deoxyactagardine B;

L-Val(0)deoxyactagardine B;

L-Phe(0)deoxyactagardine B;

L-Lys(0)deoxyactagardine B; and

L-Trp(0)deoxyactagardine B.

11. A compound according to any one of claims 1 to 8 in which the compound comprises a modification to a carboxy function of a side chain of an internal residue such that said function is modified from -COOH to a group –$COOR^5$ in which R^5 represents hydrogen, (C_1-C_4)alkyl or (C_1-C_4)alkoxy(C_2-C_4)alkyl.

12. A compound according to any one of claims 1 to 9 in which the compound comprises a modification to the N-terminal amino group such that this group -NH_2 is

instead a group -NHR^6 wherein R^6 represents C_{1-4}aalkyl.

13. A pharmaceutical composition comprising a compound according to any one of claims 1 to 12 together with a pharmaceutically acceptable carrier or diluent.

14. The use of a compound according to any one of claims 1 to 12, or a composition according to claim 13 in the manufacture of a medicament for the treatment of a bacterial infection.

15. The use according to claim 14, wherein said infection is a gut super-infection caused by *Clostridium difficile.*

16. A method for producing a host cell suitable for expressing a type B lantibiotic peptide deoxyactagardine, actagardine or a variant thereof, the method comprising the step of transforming a cell by introducing:

a *lanA* gene, said gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same wherein:

1 to 5 amino acids in the sequence is/are substituted by another amino acid, or

the amino acid sequence is at least 90% identical thereto,

optionally together with one or more associated cluster genes suitable for conversion of a precursor peptide into said peptide,

thereby generating a host cell comprising a polynucleotide sequence encoding said peptide and suitable for expressing same, wherein the type B lantibiotic peptide has antimicrobial activity.

17. A method according to claim 16, where the cell is transformed with a *lanA* gene and one or more cluster genes.

18. A method of according to claim 17, wherein the associated gene cluster comprises a gene encoding a LanR polypeptide or a variant thereof 90% identical thereto, wherein said polypeptide is a factor regulating the production of the LanA polypeptide.

19. A method according to claim 17 or claim 18, wherein the gene cluster further comprises a gene encoding a LanM polypeptide or a variant thereof at least 90% identical thereto, wherein said polypeptide is derived from the lantibiotic gene cluster and is a modification factor required for conversion of the precursor polypeptide to a lantibiotic compound.

20. A method according to claim 19, wherein a LanM polypeptide has the amino acid sequence shown in SEQ ID 120 or 213.

21. A method according to any one of claims 17 to 20, wherein the gene cluster further comprises a gene encoding a LanO polypeptide or variant at least 90% identical thereto, wherein said polypeptide is derived from the lantibiotic gene cluster and is a factor involved in the oxidation of deoxy-forms to -S(O)- analogue thereof.

22. A method according to claim 21, wherein the LanO polypeptide has the amino acid sequence of SEQ ID No 122 or 215.

23. A method according to any one of claims 19 to 22, wherein the associated gene cluster further comprises a gene encoding a LanT polypeptide or a variant thereof at least 90% identical thereto, wherein said polypeptide is derived from the lantibiotic gene cluster and is a transport factor.

24. A method according to claim 23, wherein the LanT polypeptide has the amino acid sequence shown in SEQ ID No 123 or 214.

25. A method according to any one of claims 16 to 24, wherein the host cell is a lantibiotic producing host cell.

26. A method according to claim 25, wherein the host cell comprises a gene encoding a LanO polypeptide and said gene is inactivated.

27. A method according to claim 26, wherein the gene encoding a LanO polypeptide is inactivated by mutation or deletion.

28. A method according to any one of claims 16 to 27, which further comprises the step of culturing the host cell and expressing a lantibiotic peptide encoded therein.

29. A method according to claim 28, which further comprises the step of recovering the lantibiotic.

30. A method according to claim 28 or 29, which further comprises the step of derivatising the carboxy function of the C-terminal to provide a monoamide derivative.

31. A cell obtainable from the method of any one of claims 16 to 27, wherein said cell is selected from a lantibiotic producing host cell with a mutated endogenous *lanA* gene and a non-producer capable of expressing a *lanA* gene, wherein the cell comprises a *lanA* gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same, wherein 1 to 5 amino acids in the LanA polypeptide sequence is/are substituted by another amino acid, or the amino acid sequence is at least 90% identical thereto.

32. A vector suitable for transforming a host cell in the method defined in any one of claims 16 to 30, said vector comprising an expression construct comprising a gene encoding a LanA peptide selected from actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or a variant of any one of same wherein:

1 to 5 amino acids in the peptide are substituted by another amino acid, or

the amino acid sequence is at least 90% identical thereto,

optionally together with one or more associated cluster genes required for conversion of a precursor peptide into a product peptide, and wherein the LanA peptide has antimicrobial activity.

33. A vector according to claim 32, wherein the expression construct further comprises a *lanA* promoter sequence.

34. A lantibiotic producing host cell suitable for expressing a type B lantibiotic peptide, the host cell comprising a mutation in its endogenous *lanA* gene, said gene encoding a LanA polypeptide comprising a precursor of actagardine, actagardine B, ala(0)-actagardine, ala(0)-actagardine B or an amino acid variant of any one of the same,

wherein 1 to 5 amino acids in the LanA polypeptide sequence is/are substituted by another amino acid, or the amino acid sequence is at least 90% identical thereto, such that the gene is not expressed or the gene product is inactive.

35. Use of a host cell for the expression of actagardine, a variant thereof, deoxyactagardine or a variant thereof, wherein the cell is a lantibiotic producing cell with a gene encoding a LanO polypeptide and said polypeptide is derived from the lantibiotic gene cluster and is a factor involved in the oxidation of deoxy-forms to -S(O)- analogue thereof and:

said gene is inactivated, or

the expression of said gene is increased.

36. Use of a host cell according to claim 35, wherein the gene encoding a LanO polypeptide is inactivated by mutation or deletion.

37. Use of a host cell comprising a gene encoding a LanA peptide, a precursor of a lantibiotic peptide for the production of actagardine, a variant thereof, deoxyactagardine or a variant thereof, with the proviso that wherein the host is a !antibiotic producing cell comprising an endogenous gene encoding a LanA peptide, the latter is mutated such that the endogenous gene is not expressed or the endogenous gene product is inactive or the endogenous gene is deleted.