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Export and modification of (poly)peptides in the lantibiotic way

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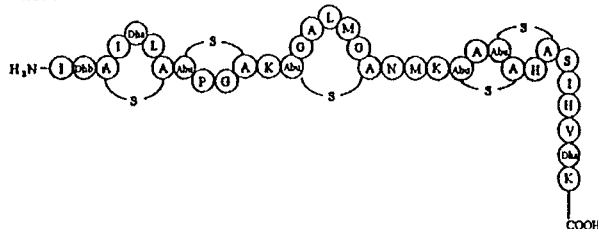
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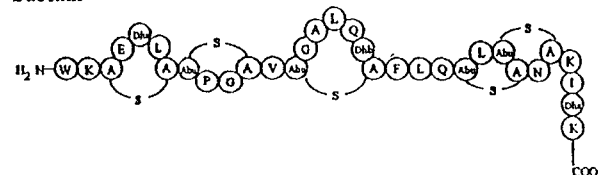
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(54) Title: EXPORT AND MODIFICATION OF (POLY)PEPTIDES IN THE LANTIBIOTIC WAY

Nisin



Subtilin



(57) Abstract: The invention includes a method for harvesting a polypeptide produced by a host cell, wherein the polypeptide has not undergone intra-cellular post-translational modification, such as dehydration of a serine or a threonine, and/or thioether bridge formation. The invention also includes a method for producing thioether containing peptides and dehydroalanine/dehydrobutyrine-containing peptides, wherein extracellularly thioether rings may be formed.



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Title: Export and modification of (poly)peptides in the lantibiotic way.

5 The invention is related to the field of lantibiotics and to the field of post-translational modifications of (poly)peptides.

Lantibiotics form a group of unique ribosomally synthesised and post-translationally modified antibiotic peptides that are produced by, and primarily act on, Gram-positive bacteria (for review see McAuliffe et al., FEMS Microbiol. Rev. 25, :285-308 (2001). Because by definition they contain intramolecular thioether bridges or rings formed by the thioether amino acids *lanthionine* (Lan) and 3-methyl*lanthionine* (MeLan) and they all are peptide *antibiotics* with moderate to strong bactericidal activity, they take their name from these most eye-catching properties.

15 Thioether rings protect peptides against proteolytic degradation. For instance the lantibiotic nisin remains active after trypsin treatment. Thioether rings are essential for some lantibiotic activities. For instance opening of ring A or C in nisin causes deletion of the membrane permeabilization capacity. Ring A of nisin is necessary for its capacity to autoinduce its own synthesis and for nisin's capacity to block the peptidoglycan synthesis by interacting with lipid II. It is essential to have thioether rings and not disulfide rings since replacement of thioether rings by disulfide bridges leads to loss of antimicrobial activity. They do not spoil the environment and are not toxic for animals or man and find, or may find, applications as biopreservatives in the preparation of food and beverages, but also as bactericidal agent in cosmetics and veterinary and medical products. Because the growing number of multidrug resistant pathogenic micro-organisms has created the threat of another "pre-antibiotic era" for many bacterial diseases, it is expected that lantibiotics also may serve as new lead compound to remedy this alarming problem. Mainly for these reasons, in the past decade, lantibiotics have experienced a marked increase in basic and applied research activities, leading to an extraordinary increase in our knowledge of their structural and functional properties, their mechanisms of action and of the genes and protein components involved in their biosynthesis and secretion. For example, lantibiotics have now become subject to "protein engineering" projects, with the aim of altering, via site-directed mutagenesis, their activity, stability

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and spectrum of susceptible target cells. In this description we focus upon the linear (type A) lantibiotics since at present very little specific information is available for the circular (type B) lantibiotics.

The lantibiotics subtilin and nisin belong to and are representative for the peptide antibiotics or lantibiotics of type A. They contain the rare amino acids dehydroalanine, (Dha) dehydrobutyrine (Dhb), *meso*-lanthionine, and 3-methylanthionine and the characterising thioether bridges. Nisin is the most prominent lantibiotic and is used as a food preservative due to its high potency against certain Gram-positive bacteria. It is produced by *Lactococcus lactis* strains belonging to serological group N. The potent bactericidal activities of nisin and many other lantibiotics are based on their capacity to permeabilize the cytoplasmic membrane of target bacteria. Breakdown of the membrane potential is initiated by the formation of pores through which molecules of low molecular weight are released. In addition, nisin inhibits cell wall synthesis by binding to lipid II, a precursor of peptidoglycan synthesis, modulates the activity of autolytic enzymes and inhibits the outgrowth of spores (see also Breukink and de Kruijf, Biochem. Biophys. Acta 1462:223-234, 1999).

In several countries nisin is used to prevent the growth of clostridia in cheese and canned food. The nisin peptide structure was first described by Gross & Morell (J. Am. Chem. Soc 93:4634-4635, 1971), and its structural gene was isolated in 1988 (Buchmann et al., J. Biol. Chem. 263:16260-16266, 1988). Nisin has two natural variants, nisin A and nisin Z, which differ in a single amino acid residue at position 27 (histidin in nisin A is replaced by asparagin in nisin Z).

Subtilin is produced by *Bacillus subtilis* ATCC 6633. Its chemical structure was first unravelled by Gross & Kiltz (Biochem. Biophys. Res. Commun. 50: 559-565, 1973) and its structural gene was isolated in 1988 (Banerjee & Hansen, J. Biol. Chem. 263:9508-9514, 1988). Subtilin shares strong similarities to nisin with an identical organization of the lanthionine ring structures (Fig. 1), and both lantibiotics possess similar antibiotic activities.

Due to its easy genetic analysis *B. subtilis* became a very suitable model organism for the identification and characterization of genes and proteins involved in lantibiotic biosynthesis. The pathway by which nisin is produced is very similar to that of subtilin, and the proteins involved share significant homologies over the entire proteins (for review see also De Vos et al., Mol. Microbiol. 17:427-437, 1995).

Another well known and studied lantibiotic, produced by *Staphylococcus epidermis* 5, is Pep 5, which contains three ring structures (one MeLan and two Lan), an N-terminal oxobutyryl residue, and two Dhb residues (Kellner et al., *Angew. Chemie Int. Ed. Engl.* 28:616-619, 1989)

5 The respective posttranslationally acting genes have been identified adjacent to the structural genes, and together they are organized in operon-like structures (Fig. 2). These genes are thought to be responsible for post-translation modification, transport of the modified prepeptide, proteolytic cleavage, and immunity which prevents toxic effects on the producing bacterium. In addition to
10 this, biosynthesis of subtilin and nisin is strongly regulated by a two-component regulatory system which consists of a histidin kinase and a response regulator protein.

According to a present model (Fig. 3) it is assumed that an extracellular growth phase-dependent signal may activate the membrane localized histidine
15 kinase. The nature of this signal may be different for subtilin and nisin biosynthesis. Whereas in nisin biosynthesis, nisin itself has an inducing function, it was shown for subtilin biosynthesis that its biosynthesis is sporulation dependent.

According to the model, after its auto-phosphorylation the SpaK and NisK
20 histidine kinase transfer the phosphate residue to the response regulator which in turn activates the genes necessary for subtilin and nisin biosynthesis. Thereafter, the prepeptide is modified at a membrane localized modification complex (lantionine synthetase) consisting of the intracellular SpaB/SpaC and the NisB/NisC proteins, respectively. According to the model, these proteins are
25 also associated with the SpaT and the NisT transporter, respectively.

As in any lantibiotic, the presubtilin or prenisin molecule consists of a leader segment and a mature segment, and the leader segment is thought to play several roles in the biosynthetic pathway. It is thought not to be just a translocation signal sequence, but thought to provide recognition signals for the
30 modification enzymes and to suppress antimicrobial activity until the mature peptide is released from the cell. As also postulated by Qiao and Saris, (*Fems Microbiol. Let.* 144:89-93(1996), the modified prepeptide is in the case of some lantibiotics proteolytically cleaved after its transport through the cellular membrane, but in the case of other lantibiotics cleavage of the leader from the
35 modified peptide occurs inside the cell before secretion. In the case of nisin,

cleavage is performed by NisP, whereas in the case of subtilin no specific protease has been found within the operon-like structure. However, *B. subtilis* is rich in extra-cellular proteases and possibly subtilisin, which also recognizes proline at position-2, could cleave the modified pre-subtilin.

5 The gene clusters flanking the structural genes for various linear (type A) lantibiotics have recently been characterized (for review see Siezen et al., Antonie van Leeuwenhoek 69:171-184, 1996). The best studied representatives are those of nisin (*nis*), subtilin (*spa*), epidermin (*epi*), Pep5 (*pep*), cytolysin (*cyl*), lactocin S (*las*) and lacticin 481 (*lct*). Comparison of the lantibiotic gene clusters shows that
10 they contain conserved genes that probably encode similar functions. The *nis*, *spa*, *epi* and *pep* clusters contain *lanB* and *lanC* genes that are presumed to code for two types of enzymes that have been implicated in the modification reactions characteristic of all lantibiotics, i.e. dehydration and thio-ether ring formation. The *cyl*, *las* and *lct* gene clusters have no homologue of the *lanB* gene,
15 but they do contain a much larger *lanM* gene that is the *lanC* gene homologue. Most lantibiotic gene clusters contain a *lanP* gene encoding a serine protease that is presumably involved in the proteolytic processing of the prelantibiotics. All clusters contain a *lanT* gene encoding an ATP-binding cassette (ABC)-like transporter spanning the plasma membrane of a cell and likely to be involved in
20 the export of (precursors of) the lantibiotics from the cell. The *lanE*, *lanF* and *lanG* genes in the *nis*, *spa* and *epi* clusters encode another transport system that is possibly involved in self-protection. In the nisin and subtilin gene clusters two tandem genes, *lanR* and *lanK*, have been located that code for a two-component regulatory system.

25 Finally, non-homologous genes are found in some lantibiotic gene clusters. The *nisL* and *spaI* genes encode lipoproteins that are involved in immunity, the *pepL* gene encodes a membrane-located immunity protein, and *epiD* encodes an enzyme involved in a post-translational modification found only in the C-terminus of epidermin. Several genes of unknown function are also found in the
30 *lan* gene cluster. Commonly, a host organism or cell carrying one or more of said genes (here for example *lanT*, *lanI*, *lanA*, *lanP*, *lanB* and *lanC*) in said cluster are identified with a shorthand notation such as *lanTIAPBC*. The above identified genes are clearly different from genes encoding the secretion apparatus for the non-lantibiotic lactococcins that is composed of two membrane proteins LcnC and

LcnD, as for example discussed in Franke et al., J. Biol. Chem 274:8484-8490, (1999).

A database has been assembled for all putative gene products of type A lantibiotic gene clusters. Database searches, multiple sequence alignment and secondary structure prediction have been used to identify conserved sequence segments in the LanB, LanC, LanE, LanF, LanG, LanK, LanP, LanM, LanR and LanT gene products that may be essential for structure and function (Siezen et al., *ibid*). This database allows for a rapid screening of newly determined sequences in lantibiotic gene clusters.

10 However, despite all above cited recent knowledge obtained in the field, attempts to engineer novel lantibiotic-like peptides comprising newly synthesised non-naturally occurring thioether bridges have been scarce, if not rather unsuccessful. In US 5,861,275, nisin-subtilin chimeras have been produced in the Gram-positive *Bacillus subtilis*, that however, do not comprise thioether bridges other than naturally occurring in either nisin or subtilin. In a different application (US 2002/0019518), *Bacillus subtilis* was used to produce a chimeric polypeptide comprising a lantibiotic peptide and a subtilin leader segment, a lantibody, that remains associated within the cell wall.

A novel thioether bridge in lantibiotic Pep5 has been engineered by Bierbaum et al., Appl. Env. Microbiol. 62:385-392, 1996 by modifying the Gram-positive bacterium *Staphylococcus epidermis* 5 by depleting the host organism of the gene cluster *pepTIAPBC* and replacing it with a gene cluster *pepIAPBC*, wherein *pepA* was or was not replaced with mutated structural genes encoding for a Pep 5 peptide wherein amino acids were substituted; genes coding for peptides with substitutions C27A (Cysteine to Alanine at position 27), C33A, A19C, Dhb16A, Dhb20A, K18Dha were generated. Only the A19C substitution resulted in novel thioether ring formation. The clone corresponding to the A19C substitution produced a rather small amount of a peptide that showed only little activity. It was thought that prolonged exposure of the peptide to intracellular protease of the producing transformed cell was causal to this disappointing result.

The K18Dha substitution in Pep 5 resulted in a clone that produced incompletely dehydrated serine at position 18. Kuipers et al., (J. Biol. Chem. 267:24340-24346, 1992) engineered a new Dhb residue into nisin Z by substituting M17Q/G18T in said lantibiotic, but also obtained only incomplete

dehydration of the resulting threonine and no additional ring formation. The incomplete dehydration is generally thought to be a result of questionable substrate specificity of the dehydrating enzyme LanB in the transformed cell.

In short, no large measure of success has yet been achieved in providing novel thioether bridges to lantibiotics in Gram-positive organisms, let alone that engineered thioether bridge formation has been provided to polypeptides of non-lantibiotic descent or by organisms other than Gram-positive bacteria.

Paul, Leena K et al (FEMS Microbiol. Lett. 176:45-50, 1999) recently studied the subtilin leader peptide as a translocation signal in the Gram-negative *E. coli*, by default devoid of a specific lantibiotic transporter system, and provided a fusion-protein comprising the subtilin leader peptide and part of the mature subtilin attached to *E. coli* alkaline phosphatase (AP) to study said possible translocation. Although the fusion protein was translocated to the periplasmic side of the cytoplasmic membrane, it remained associated with that membrane. In earlier work, (Izaguirre & Hansen, Appl. Environ. Microbiol 63:3965-3971, 1997) the same fusion protein was expressed in the Gram-positive *Bacillus subtilis*, where it was cleaved off from said membrane after successful translocation, but where no dehydration of serines or threonines of the AP polypeptide, let alone thioether bridge formation was observed. Novak J et al., ASM general meeting 96:217 (1999), recently provided an *E. coli* host cell with an ORF (ORF1) encoding an ABC transporter of 341 amino acids, which is thought to be involved in the translocation of the lantibiotic mutacin II in *Streptococcus mutans*. However, an intact gene product of said ORF1 was not produced in *E. coli*, whereas a truncated protein of unknown identity or functionality was observed.

For the purpose of protein engineering of lantibiotics (for an extensive review see Kuipers et al., Antonie van Leeuwenhoek 69:161-170, 1996) or for the purpose of engineering newly designed (poly)peptides with lantibiotic-type posttranslational modifications, for example for pharmaceutical use, much attention has recently (see for example Entian & de Vos, Antonie van Leeuwenhoek 69:109-117, 1996; Siegers et al., J. Biol. Chem. 271:1294-12301, 1996 ;Kiesau et al., J. Bacter. 179:1475-1481, 1997) been given to understanding the role of the LanB, LanC (or LanM) and LanT complex, the enzymes thought to be involved (in that order) in dehydration, thioether ring formation and transportation of the lantibiotic out of the cell.

The present invention shows that the unmodified peptide, being coupled to its leader peptide, can be transported out of the cell without prior modification, and LanB and LanC (or LanM), when acting at all, may act not in, but also
5 outside of the cell (see also fig. 4).

Where it was earlier commonly thought that LanB and LanC act in concert to modify the peptide only before it is translocated, it is herein furthermore provided that after transportation, the as yet unmodified peptide
10 extracellularly may undergo its specific posttranslational modification leading to dehydration and thioether bridge formation, bringing the role of the transporter protein to central stage to modify a (poly)peptide in the lantibiotic way, it being a prerequisite to present the unmodified peptide to the modification machinery.

Thus, the invention provides the insight that dehydration of a serine or
15 threonine of a (poly)peptide and subsequent thioether bridge formation can satisfactorily occur when a pre(poly)peptide has been transported out of the host cell wherein it was produced by translation, preferably by a transporter protein such as an ABC transporter, preferably at least functionally corresponding to a transporter commonly identifiable as LanT. Dehydration (and optionally
20 thioether bridge formation) is then only enzymatically catalysed by an enzyme or enzymes that are at least functionally corresponding to LanB and/or LanC. Said transporter transports the to-be-modified (poly)peptide through the membrane of the host cell where it is positioned in working proximity to extra-cellular located LanB for dehydration.

25 In a further preferred embodiment, the invention provides a method according to the invention further comprising harvesting said desired (poly)peptide after detecting the presence of said leader peptide in the culture medium or supernatant of said cell (see also fig. 5). For detecting said presence, it is preferred that said medium contains only few nutrients, i.e is a so-called minimal
30 medium. Also, it is preferred that said presence is detected by harvesting the supernatant by aspirating and dispensing the supernatant into and out of a pipet tip (or other harvesting device) that contains a microvolume bed of affinity chromatography media fixed at the end of the tip that is preferably without dead volume. This procedure is herein also called "ziptipping" and allows, after

subsequent elution, for relatively pure presentation of desired (poly)peptide for further analyses.

Preferably by using the combination of growth in minimal medium and ziptipping the supernatant of this culture, samples of sufficient purity can be obtained that are well suited for detection or analyses by high resolution MALDI-TOFMS. This allows most significant measurement of leader peptide and thus prediction of desired (poly)peptide content. Detection of said leader peptide therefore can be used to ascertain the export of (poly)peptide coupled to this lantibiotic leader, especially in those cases where the leader peptidase acts extracellularly.

In a further preferred embodiment, the invention provides for a method wherein the host cell producing the desired (poly)peptide is essentially devoid of leader peptidase (LanP) activity, thereby allowing the production and extracellular harvest -by using anti-leader antibodies- of desired (poly)peptide that is essentially still coupled to its leader peptide. Also, in this way, potential intracellular toxic effects of desired (poly)peptide provided with thioether bridges are reduced. It of course also suffices to design a leader peptide that cannot be cleaved by the leader peptidase of the host cell used. In both cases, the desired (poly)peptide can later be obtained free from the leader peptide, for example by specific proteolytic cleavage, using added LanP, or by another suitable protease capable of cleaving the leader peptide from the desired (poly)peptide.

Furthermore, it is herein provided that in a desired (poly)peptide, a serine or serines, that are N-terminally located from cysteines are dehydrated and coupled to more C-terminally located cysteines. As further exemplified herein in the detailed description, a (poly)peptide sequence with a serine and a cysteine "S - C" contains (preferably after thioether bridge formation) alanines in said positions of the serine and cysteine: "A - A". These alanines are coupled by - aside from the peptide backbone- a thioether bridge.

Generally a method as provided herein allows a high detection level for measuring levels of (lantibiotic) (poly)peptides directly from the culture supernatant, considering that the ratio leader peptide versus desired (poly)peptide is essentially 1:1. Such guidance allows for efficient culture methods to produce the desired polypeptide, and allows for determining appropriate or optimal time-points at which said culture may be harvested.

In a preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of peptide hormones or fragments of these hormones or analogues from these hormones originating from hypophysis and/or peptide hormones with similar actions such as vasopressin, terlipressin, desmopressin, cispressin, oxytocin, adrenocorticotrophic hormone and human growth hormone.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of peptide hormones or fragments of these hormones or analogues from these hormones originating from hypothalamus, and/or peptide hormones with similar actions such as gonadoliberinII, luteinizing hormone releasing hormone, leuprolide, and other synthetic analogues of LHRH such as gonadoreline, gosereline, busereline, leuproreline, nafareline, triptoreline, and cetrotorelix, somatostatin, analogues of somatostatin such as octreotide, somatostatin, corticotropin inhibiting peptide, corticotropin-release factor, urocortin, urotensin II and growth hormone release factor.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of peptide hormones or fragments of these hormones or analogues from these hormones originating from adrenocortex, adrenal medulla, kidney and heart and/or peptide hormones with similar actions such as adrenomedullin, angiotensin I, atrial natriuretic factor, bradykinin, brain natriuretic peptide, C-type natriuretic peptide and vasonatrin peptide.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of peptide hormones or fragments of these hormones or analogues from these hormones originating from other endocrine/exocrine organs such as the pancreas, thyroid and parathyroid and/or peptide hormones with similar actions such as calcitonin, osteocalcin, glucagon, insulin, insulin-like growth factor-I or II, parathormone, and cholecystokinin.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of peptide hormones or fragments of these hormones or (synthetic) analogues from these hormones with antibiotic (-like) activity and/or peptide hormones with similar actions such as dermaseptin, defensin I, bombinin-like peptide, histatin-5, indolicidin, magainin-1 and ceratotoxin A.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of biological active peptides or fragments of these peptides and/or hormones or analogues from these peptides and/or peptides with similar actions such as exendin-3, secretin, human pancreatic polypeptide, peptide YY, gastric inhibitory polypeptide, big gastrin-I, pentagastrin, gastrin releasing peptide, motilin, neuropeptide Y, galanin, alpha-neurokinin, deltorphin, alpha-endorphin, beta-endorphin, leu-enkephalin, met-enkephalin, allatostatin I, anthopleurin-A, anti-inflammatory peptide 1, delta sleep inducing peptide, alpha-dendrotoxin, eledoisin, echistatin, small cardioactive peptide A or B, cerebellin, charybdotoxin, conopressin G, conotoxin EI, corazonin, experimental allergic encephalitogenic peptide, experimental autoimmune encephalomyelitis complementary peptide, tocinoic acid / pressinoic acid, brain derived acidic fibroblast growth factor (1-11), brain derived acidic fibroblast growth factor (102-111), brain derived basic fibroblast growth factor (1-24), fibrinogen binding inhibitor peptide, fibroblast growth factor inhibitory peptide and transforming growth factor alpha.

In another preferred embodiment, the invention provides a method according to the invention, and a (poly)peptide according to the invention wherein said desired (poly)peptide is selected from the group of biological active peptides or fragments of these peptides and/or hormones or analogues from these peptides and/or peptides with similar actions such as guanylin, helospectin I, hepatitis B surface antigen fragment, intercellular adhesion molecule, tachyplesin I, HIV (gp 120) antigenic peptide fragment, HIV (gp 41) antigenic peptide I fragment, HIV (gp41) antigenic peptide 5, HIV protease inhibitors, IGF II 69-84, interleukin-8 fragment, interleukin-2 fragment(60-70), leucokinin I, leukopyrokinin, mastoparan, melanin concentrating hormone, melittin, and ras oncogene related peptides.

Considering that lanthionine formation between for example dehydrobutyrine and cysteine is energetically possible at room temperature and can also occur spontaneously the transported (poly)peptide can form thioether bridges spontaneously or where it is positioned in working proximity to extra-cellular located LanC for subsequent enzymatically induced thioether bridge formation. Alternatively, said transporter transports the to-be-modified polypeptide through the membrane of the host cell where it is positioned in working proximity to extra-cellular located LanM for dehydration and subsequent thioether bridge formation.

With this insight, the invention provides a method from which several fields can benefit. In short, the invention provides use of lantibiotic exporters (LanT) for export of peptides or proteins which optionally may have been converted by lantibiotic enzyme(s), in particular enabling extracellular formation of lanthionines and other rings. Amino acids are able to form short sequences (peptides) and longer sequences (proteins). Peptides and proteins [herein also referred to as (poly)peptides] are both important classes of biomolecules, both e.g., for nutrition, for pest control and for fighting disease. Their importance is illustrated by the number and range of therapies based on them recently created by the biochemical and pharmaceutical industries. There is also a large number of protein and peptide based pharmaceuticals and it should also be understood that the use of therapeutic pharmaceuticals is not limited to humans but also extends to animal, plant and other biosystems. However, the manufacture of many present and potential protein or peptide pharmaceuticals has limitations: in particular many are prepared in living cells (*in vivo*) but these cells must be ruptured or lysed (killed), and the contents extracted, separated, and purified, in order to provide a given quantity of the peptide or protein. This is a complex process, and also the amount of any desired peptide or protein in any cell at any time is limited.

The present invention bypasses this problem by introducing into the living cells a factor which allows the cells to continuously transport proteins or peptides and export them through the cell wall, so that the product produced intercellularly may be collected extracellularly and the cells may remain vital and continuing to produce materials. It is evident that this permits both substantially easier and higher rate production of the desired products. Now that is known that said Lan T transporter or functional equivalent thereof acts on

unmodified (poly)peptide to which the leader is still attached, one such field relates to the expression and production of recombinant (poly)peptides of other than bacterial descent and relates to expression and/or production of a (poly)peptide of eukaryotic (be it of plant, animal or fungal origin) or viral descent as well. Such peptides are these days widely produced by recombinant means for use in the production of pharmaceuticals, for example as active compound such as a (poly)peptide hormone, or cytokine, or antibody fragment, or biopesticide agent, or as antigen for a vaccine or immunogenic composition. Surprisingly, it is now possible to use a lantibiotic-type transporter system to export peptides of eukaryotic or viral, and not only of bacterial (prokaryotic) descent. In a first embodiment, the invention provides a method allowing for extra-cellular harvest of a desired (poly)peptide--which can be out of the realm of bacterial lantibiotics or even be of eukaryotic or viral descent--produced by a recombinant host cell, said method comprising the steps of: a) selecting a recombinant host cell comprising or provided with a first recombinant nucleic acid having a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding said desired (poly)peptide (useful examples of which are given in table 1), whereby said first and second fragment are within the same open reading frame of said first nucleic acid and said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic (useful examples of such a leader peptide are given in table 2), and selecting said host cell for the presence of a lantibiotic transporter protein commonly known as LanT, (such a host cell can be a Gram-positive or Gram-negative prokaryote or an eukaryote provided with such a transporter) and allowing for the translation of said first nucleic acid. As said, it is preferred that said cell is essentially devoid of leader peptidase activity, or comprises leader peptidase that cannot cleave the specific leader peptide used. Such a host cell is for example obtained by, at least functionally, deleting the *lan P* gene.

In a preferred embodiment, the invention provides a method allowing for extra-cellular harvest of a desired (poly)peptide which has not undergone intracellular post-translational modification comprising dehydration of a serine or a threonine and/or thioether bridge formation. In the detailed description herein, it is for example demonstrated how to obtain nisin prepeptide (i.e. nisin leader and unmodified nisin) extracellularly. The nisin prepeptide was obtained using a host cell selected for the presence of two plasmids, one encoding the nisin

prepeptide, and one encoding NisT, whereby said host cell was further characterized by at least the functional absence of at least one of the other gene products derived from the *Nis*-gene cluster, such as NisB, NisC, or NisP.

The invention thus provides a (poly)peptide harvestable after the (poly)peptide has been transported from the producing host cell, obviating the need to lyse or disrupt the host cells to proceed to harvest. However, if one wishes to do so, the desired polypeptide can of course be harvested from within the cell as well. Cultures of cells provided with said transporter protein can now be kept alive and in use, whereby the desired (poly)peptide can be harvested from for example the supernatant of spun-down host cells. These host cells need not be of Gram-positive descent per se, now that Gram-negative prokaryotes or even eukaryotes can be provided with such a properly placed transporter that greatly enhances the gamut of expression systems that can be used to express and produce a desired (poly)peptide.

Furthermore, the invention provides a method allowing for extra-cellular modification of a desired (poly)peptide produced by a recombinant host cell said method comprising the steps of: a) selecting a recombinant host cell comprising a first nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding said desired (poly)peptide, whereby said first and second fragment are within the same open reading frame of said first nucleic acid and said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a wild-type lantibiotic, and b) selecting said host cell for the presence of a transporter protein commonly known as LanT or a functional equivalent thereof and c) selecting said host cell for the presence of an essentially extra-cellular protein (such as LanB, LanC or LanM) capable of providing post-translational modification, and d) allowing for the translation of said first nucleic acid. In a preferred embodiment, the invention provides a method allowing for extra-cellular modification of a desired (poly)peptide which has not undergone intra-cellular post-translational modification comprising dehydration of a serine or a threonine and/or thioether bridge formation. It is preferred that said essentially extra-cellular enzyme is capable of dehydrating a serine or a threonine, or is capable of providing for thioether bridge formation. Herewith the invention provides a method for lantibiotic-type modification of non-lantibiotic polypeptides, surprisingly even when said (poly)peptide is of essentially eukaryotic or viral descent. This is very

useful for altering various characteristics of such products, especially for example related to stability or pharmacological profiles of useful polypeptides, such as selectable from Table 1. It is of course useful to use a leader peptide as selected from Table 2 or functional equivalents thereof.

5 Furthermore, the invention provides a method allowing for extra-cellular modification of a desired (poly)peptide produced by a recombinant host cell, wherein said modification comprises thioether bridge formation. Preferably, the location of serines, threonines or cysteines in the desired (poly)peptide is selected such that thioether ring formation by the enzyme system selected follows
10 naturally. For example, serine and threonine dehydration followed by thioether ring formation by coupling to cysteines preferably is performed as follows. In the case of lantibiotic enzymes belonging to the so-called type B lantibiotics, ring formation occurs from dehydrated serines/threonines to more C-terminally or to more N-terminally located cysteines. In the case of lantibiotic enzymes belonging
15 to so-called type A lantibiotics ring formation occurs only from dehydrated serines/threonines to more C-terminally located cysteines. Conversion by enzymes belonging to type A lantibiotics occurs in time from N to C-terminal direction from dehydrated serines/threonines to the nearest more C-terminally located available cysteine. In the case of enzymes belonging to type A lantibiotics
20 at a preferential distance of one to four amino acids to available cysteines, lanthionines are formed. It is more preferred that 2 to 3 amino acids are between a dehydrated serine/threonine on the one hand and a cysteine on the other hand. The optimal distance is two amino acids. From Table 1 peptides with above preferred distances for optimal thioetherbridge formation may be selected. At
25 distances between four and thirteen amino acids lanthionine formation can occur but becomes less efficient. At these distances also dehydration of serines and threonines without subsequent lanthionine formation next to absence of dehydration of serine/threonine occurs. It is preferred to have flanking regions of serines and threonines that allow activity of the dehydrating enzyme. To help
30 achieve this, it is preferred that at least the six to eight amino acids (three to four on each side) surrounding dehydrated serines/threonines are mostly hydrophobic. At each of these positions in 40-80% of the cases the amino acid is preferably hydrophobic, in 20-40% hydrophilic, of which in 5-15% are positively charged. It is preferred that negatively charged amino acids hardly occur. The
35 composition of the flanking regions on the desired (poly)peptide preferably differs

from the one of serine and threonine in lantibiotic-type leader peptides. In leader peptides serines and threonines occur but are never dehydrated, whereas cysteines do not occur. The six to eight positions most closely to leader serines/threonines contain less hydrophobic amino acids and more negatively charged amino acid than in positions around propeptide serine/threonine; per position in only 20-40% of the cases the amino acid is hydrophobic and in around 20% of the cases a negatively charged amino acid is preferred.

With respect to the peptidase cleavage site at least two types of leader peptides exist from which guidance can be obtained to design better cleavable peptides or proteins. One class needs the subtilisin-like serine protease LanP for cleavage, which occurs after Pro-Gln, Pro-Arg, Ala-Asp, Ala-Glu. In the case of nisin a positively charged residue at position -1 and a hydrophobic residue at position -4 seem necessary for interaction with NisP. This subtilisin-like serine protease LanP acts on the prepeptides of for instance Pep5, Epilancin K7, Nisin A, Nisin-Z, Epidermin, Gallidermin.

In the other class the leader peptides are cleaved after Gly-Gly, Gly-Ala or Gly-Ser sequences. The latter holds for many other non lantibiotic bacteriocin leader peptides. The subtilisin like proteases are not known to cleave these sequences, hence a different type of protease is cleaving these leader peptides. It has been shown that in some bacteriocins -both lantibiotic and non lantibiotic- this second protease is a domain of the transport system LanT. This type of leader peptidase acts for instance on prepeptides of Lacticin-481, Variacin, Mutacin-II, Streptococcin-A-FF22, Salivaricin-A and Sublancin.

In addition a two component lantibiotic, Cytolysin-LL / Cytolysin LS, exists of which each component is cleaved twice, once by the 'double glycine type' and thereafter by the subtilisin-like peptidase.

The invention furthermore provides a method for the modification of a desired polypeptide according to the invention wherein said host cell is a Gram-negative prokaryote or an eukaryote. Furthermore, the invention provides a (poly)peptide modified with a method according to the invention.

Also, the invention provides a host cell, such as a Gram-negative prokaryote or an eukaryote, provided with a recombinant nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding a desired (poly)peptide, whereby said first and second fragment are within the same open reading frame of said first nucleic acid and said leader

peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic. In a preferred embodiment, a host cell according to the invention is provided wherein said desired (poly)peptide is of essentially eukaryotic or viral descent, for example selected from Table 1 and/or
5 wherein said leader peptide is selected from Table 2.

Furthermore, the invention provides a host cell according to the invention said host cell provided with or selected for the presence of at least a LanT protein or functional equivalent thereof wherein said host cell is further characterized by at least the functional absence of at least one of the other gene products
10 derived from the *Lan*-gene cluster, such as LanB, LanC, (or a functional part from LanM) or LanP. In a preferred embodiment, said host cell comprises a Gram-negative prokaryote or an eukaryote.

Such a host cell as provided herein finds a specific use in a method of producing a (poly)peptide for harvest or modification, as provided herein above.
15 For the purpose of harvest it is especially preferred that LanT is present but that LanB and/or Lan C (or LanM), but preferably both, are absent, at least functionally absent in that they are hampered in binding to or interfering with the polypeptide to be harvested. For the purpose of modification, it is especially preferred that LanT and an essentially extra-cellular protein allowing extra-
20 cellular modification, such as LanB, Lan C or LanM, or instead of LanB, a (preferably N-terminal) LanM fragment having LanB function or instead of LanC, a (preferably C-terminal) LanM fragment having LanC function is present, whereas a further extended or even complete lantibiotic gene-product cluster is preferably not, at least not functionally present.

25 Another embodiment of the invention is a host cell which is provided with genes coding for LanB, or the equivalent N-terminal part of LanM, with or without a gene coding for LanT, which is capable of exporting dehydrated lantibiotic prepeptides, which have mutations such that chemically or proteolytically fragments can be liberated that are provided with dehydro alanine
30 and/or dehydrobutyrine. Such fragments can inhibit an enzyme, specifically a protease, such as cysteine protease or aspartyl protease.

Furthermore, the invention provides a recombinant nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding a desired (poly)peptide, whereby said first and
35 second fragment are within the same open reading frame of said first nucleic acid

and said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic, and wherein said desired (poly)peptide is of essentially eukaryotic or viral descent. Furthermore, the invention provides an proteinaceous substance comprising a polypeptide encoded
5 by a nucleic acid according to the invention. Such a proteinaceous substance can be harvested, or modified according to a method as provided herein. Use of a host cell or nucleic acid or proteinaceous substance according to the invention for the production of a desired (poly)peptide, and its use in producing a pharmaceutical composition is herein also provided. In particular, the invention provides a
10 (poly)peptide of Gram-negative prokaryotic, viral or eukaryotic descent (examples can be found in Table 1) wherein a serine or threonine has been dehydrated or which has been provided with a thioether bridge. The advantage of such a polypeptide for example lays in the creation of variants of known peptide or protein based drugs, where for example the dose or frequency of
15 administration can be reduced, thus lowering treatment cost, treatment time, and patient inconvenience; the creation of variants of new protein or peptide based drugs where the drug may not have been effective or admitted for use in an unstabilized form; and the creation of new therapeutic entities *per se*.
The invention is further explained in the detailed description.

Figure legends

Fig. 1 Peptide structure of mature nisin and subtilin

5 Fig. 2 Genomic organisation of genes involved in subtilin and nisin biosyntheses

Fig. 3 Model for nisin biosynthesis wherein modification occurs intra-cellularly.

Fig. 4 Model for nisin biosynthesis wherein modification occurs extra-cellularly.

10

Nisin prepeptide is exported by NisT ("T"), dehydrated by extracellular NisB (B) and subjected to thioether ring closure by extracellular NisC (C).

Extracellular leader peptidase, NisP (P) cleaves of the leader peptide. Nisin interacts with a membrane bound histidine kinase NisK (K) which

15

phosphorylates a response regulator NisR (R), which in its turn switches on transcription of the *nis*-genes (+, +). The producer cells are protected against nisin by the concerted action of the lipopeptide NisI and the transport system NisEFG.

20 Fig. 5

Detection of lantibiotic leader peptide directly from the culture medium by MALDI-TOFMS.

25

By using the combination of growth in minimal medium and ziptipping the supernatant of this culture, samples of sufficient purity were obtained for high resolution MALDI-TOFMS. This allowed most significant measurement of nisin leader peptide. This has to our knowledge never been reported. The detection of lantibiotic leader peptide therefore can be used to ascertain the export of

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(poly)peptide coupled to this lantibiotic leader, i.e. in those cases where the leader peptidase acts extracellularly. Generally this method allows a high detection level for measuring (lantibiotic) (poly)peptides directly from the culture supernatant.

35 Fig 6.

Transport of unmodified nisin prepeptide via the nisin transporter NisT.

(Example 1)

Fig. 7.

- 5 Transport via NisT of an angiotensin1-7 variant fused to the C-terminus of the nisin leader. (Example 2)

Fig. 8.

- 10 Transport via NisT of a vasopressin variant fused to the C-terminus of the nisin leader. (Example 3)

Fig. 9.

Transport and dehydration by NisBT of nisin prepeptide. (Example 4)

- 15 Fig. 10AB

Transport, dehydration and ring formation in nisin prepeptide by *Lactococcus lactis* cells having plasmid pNGnisABTC.

Fig. 10A: no induction, Fig. 10B: induction (Example 6)

- 20 Fig. 11

Transport via NisT of unmodified nisin prepeptide, C-terminally extended with an enkephalin variant. (Example 13).

25

- Overnight cultures of nisin producing *Lactococcus lactis* NZ9700 grown in M17 broth supplemented with 0.5% glucose were diluted 1/100. At optical density at 660 nm equal to 0.4, cells were centrifuged and the medium was replaced by
30 minimal medium (Jensen and Hammer, 1993. Appl. Environ. Microbiol. 59: 4363-4366) containing 1/1000 of 0.4 mm pore filtered overnight *Lactococcus lactis* NZ9700 supernatant. After overnight incubation the medium was ziptipped using C18 ziptips (Millipore). As matrix for MALDI-TOFMS analysis a cyano cinnamic acid was used.

The picture shows a peak at 2349.6 corresponding to the nisin leader peptide (theoretical value of 2351.2). Two subpeaks, of 2372.5 and of 2388, correspond to sodium and potassium adducts respectively. One peak of 2482.2 corresponds to the nisin leader peptide with the first methionine still attached (theoretical value is identical: 2482.2). At higher mass the peaks 3352.5, 3372.6 and 3390.1 correspond to nisin, a sodium adduct and a potassium adduct respectively.

10

Detailed description.

Lantibiotic enzymes are special. There is no strong homology if any at all with other enzymes. DNA and amino acid sequences of many lantibiotic enzymes are known, but no structures have been determined. The genes involved in the biosynthesis, processing and export of lantibiotics are present in a *lanA B C/M (D) P R K T F E G I* cluster. There is no uniform order or orientation of the genes in the different clusters indicating that rearrangements have occurred in the evolution. Lanthionines are the most typical post-translationally formed residues in antibiotics. They are formed via two steps. First propeptide serines and threonines are dehydrated giving rise to dehydro-alanines and dehydrobutyrines respectively. LanB has been proposed to play a role in dehydration, since it has a weak homology to IIVa, a threonine dehydratase from *E.coli*. Moreover it has been shown that overexpression of NisB increases the occurrence of dehydration of serine 33 in nisin A, from 10% in the normal situation up to 50% in the case of overexpressed NisB. The LanB protein consists of about 1000 residues. LanB are membrane associated proteins. LanC is thought to be responsible for the subsequent addition of cysteine SH groups to the dehydro amino acids, which results in the thioether rings. In the case of PepC experimental data support this idea. The presently known LanC proteins are composed of about 400 residues. In type A lantibiotics the N-terminal part of lanthionine and methylanthionine residues are formed by the dehydroalanine or dehydrobutyrine residues, whereas the C-terminal half is formed by the cysteine residues.

Dehydroalanines and dehydrobutyrines are essential in various (poly)peptides for the activity of the specific (poly)peptide. Dehydroresidues are for instance essential for the nisin-mediated inhibition of the outgrowth of bacterial spores (Liu and Hansen 1996. Appl Environ. Microbiol. 59:648-651), for a neurokinin receptor antagonist (Lombardi et al 1998. Bioorganic and Medicinal Chemistry Letters 8 : 1153-1156), for phenylalanine ammonia lyase (Schuster and Rétey 1995 PNAS 92:8433-8437) for an inhibitor of tripeptidyl peptidase II (Tomkinson et al., 1994. Archives of Biochemistry and Biophysics 314: 276-279), for a peptide inhibitor of HIV-1 protease (Siddiqui et al. 2001. Indian Journal of Biochemistry and Biophysics. 38: 90-95), for activity of peptides against Gram-negative bacteria (Ferrari et al 1996 2:4- Journal of Antibiotics) and for activity of antifungal peptides (Kulanthaivel et al WO 2000063240). (Poly)peptides containing dehydroresidues can be produced by cells having LanT and LanB or LanT and the N-terminal part of lanM which is equivalent to LanB.

The above mentioned activities can be of significant economic importance. For instance inhibition of tripeptidyl peptidase II is of relevance in the battle against obesity. This is evident from the fact that tripeptidyl peptidase II degrades octapeptide cholecystokinin-8, an endogenous satiety agent.

Lanthionine formation between dehydrobutyrine and cysteine is energetically possible at room temperature and can also occur spontaneously.

Lantibiotic maturation and secretion is thought to occur at membrane-associated multimeric lanthionine synthetase complex consisting of proteins LanB, LanC and the ABC transporter molecules LanT. At least two molecules of LanC and two molecules of LanT are part of the modification and transport complex. Some lantibiotics do not have the *lanB* gene, but have a much larger *lanM* gene, whose product has C-terminally some homology with the *lanC* gene product. Since no *lanB* homologue is present in LanM producing clusters the N-terminal part of the LanM protein might fulfil the dehydration reaction typically performed by LanB. The chemical synthesis of lantibiotics is possible but extremely costly and time consuming. Several mutant lantibiotics that contain amino acid substitutions have been obtained by genetic engineering. However, despite many studies, until now only in the lantibiotic Pep5 one lanthionine ring in a new position has been obtained.

As said, the lantibiotic export systems, LanT, (whose sequences are already known) are in general thought to be dedicated for the transport of the fully

modified lantibiotic. Indeed the two enzymes involved in the lanthionine formation in nisin (NisB and NisC) have been reported to be located intracellularly in a NisBCT membrane associated complex (Siegers et al., 1996). Such intracellular localization suggests that the prepeptides are dehydrated by LanB whereafter rings are formed by LanC followed by export by LanT. Furthermore if the thioether ring forming enzymes, NisB (responsible for dehydration, which is the first step in ring formation) or NisC (responsible for ring formation between dehydro residues and cysteines) are inactivated by in frame deletion of 61 aa or by plasmid insertion respectively, no peptide is exported any more. The latter suggests that absence of (methyl)lanthionines prevent export.

However, it has now surprisingly been measured that prepeptide, such as nisin prepeptide can be transported through the nisin transporter. This result was obtained using a strain with two plasmids, one coding for the nisin prepeptide and one coding for the nisin transporter. No prepeptide production was observed in a control experiment in which a strain with only the plasmid coding for the prepeptide was used. Some lantibiotics contain dehydrated serines / threonines that do not participate in thioether ring formation. From the latter, in combination with the observation that unmodified peptide is exported, it may be theorized that also translocation or prepeptide without thioether rings but with dehydro residues is possible. It is known that the second step in lanthionine ring formation is less difficult to achieve since it can also occur spontaneously at room temperature. Therefore after production of prepeptides with dehydro residues lanthionine rings can be formed extracellularly.

In order to avoid cellular incompatibilities with newly formed thioether and or dehydroresidue containing (poly)peptides, *in vitro* synthesis of thioether (poly)peptides can be performed. Inside out membrane vesicles with LanB or LanBC or LanBT or LanBCT or LanMT, obtained by french pressing cells, can be mixed with a cell extract, obtained by sonicating a cell pellet and centrifugation, with ATP and an ATP generating system, with protease inhibitors and with a leader-(poly)peptide fusion with serines/threonines and cysteines in adequate positions. In the case of LanC or LanCT containing vesicles peptides with dehydro residues can be closed by LanC to form stereospecifically thioether rings. After vortexing with one volume of chloroform, and centrifugation the supernatant contains the fusion peptide with thioether rings and or

dehydroresidues as shown by Maldi TOF analysis of the ziptipped supernatant. The formation of rings follows the observed mass after peroxydation since peroxydation gives an addition of three oxygens to free cysteines occurs, whereas only 1 or 2 oxygen atoms to thioether bridges.

5 For the *in vitro* activities of LanB, LanC and lanM, instead of using membrane vesicles obtained by french pressing also isolated lantibiotic enzymes produced by bacterial or eukaryote organisms can be reconstituted in membrane vesicles or liposomes.

LanC (or C-terminal LanM) (–containing vesicles or proteoliposomes) can also be used in *in vitro* assays for generating stereospecific lanthionines in
10 chemical lanthionine forming procedures, which in the absence of lanC yield diastereomers (Galande and Spatola 2002. Letters in Peptide Science 8:247-251).

The present finding provides the possibility to make new lantibiotics and thus to stabilize peptides / proteins by thioether rings, D-alanines or other
15 residues formed by lantibiotic enzymes. Before (methyl) lanthionine formation, typically the distance of dehydro residues to cysteines is 2-5 residues but also much larger distances are possible. (Methyl)lanthionines can be formed from dehydro residues either to more N-terminally located or to more C-terminally located cysteines. In addition the lantibiotic transport system can be used for the
20 export of other proteins by inserting the sequence coding for the leader peptide in front of the protein DNA sequence.

Short (poly)peptides with dehydroresidues and/or thioether rings can also be obtained by embedding them in a DNA lantibiotic sequence. For instance into a specific eukaryotic peptide of 10 amino acids a thioether ring can be engineered
25 as follows. Based on a lantibiotic of 20 amino acids with a thioether ring between position 13 and 16 a DNA sequence can be designed coding for the first 10 amino acids of the lantibiotic followed by the 10 amino acids of the eukaryotic peptide with a serine in position 13 and a cysteine in position 16. By genetically introducing a (chemical) cleavage site the resulting hybrid peptide, exported in
30 the medium, can be cleaved and the eukaryotic peptide with thioether ring is liberated.

It has been described that a (poly)peptide can be genetically appended behind a lantibiotic and exported (Hansen, US2002019518: Construction of a strain of bacillus subtilis 168 that displays the sublancin lantibiotic on the
35 surface of the cell. However it is also possible to append polypeptides genetically

behind a lantibiotic sequence and have the resulting fusion (poly)peptide without modification exported via LanT, or exported via LanBT with just dehydration of serines and threonines in the lantibiotic and in the appended (poly)peptide without ring formation or exported via LanBTC/LanMT with dehydration and ring formation in both the lantibiotic and in the appended (poly)peptide.

It is also possible to generate a lanthionine and/or dehydroresidue containing (poly)peptide by omitting a C-terminal fragment of a lantibiotic sequence and adding a longer fragment to the remaining lantibiotic sequence. For instance the sequence of the 34 amino acid lantibiotic nisin can be replaced by the first 30 N-terminal amino acids, and C-terminally extended by 6 amino acids: KYSGFC. After dehydration and thioether ring formation by cells with NisBTC, and extracellular trypsin treatment, a lanthionine variant of enkephalin is produced. In this case the nisin Ser33 residue is taking part of the newly formed enkephalin molecule after dehydration and lanthionine formation. Trypsin liberates the enkephalin by cleaving behind the lysine residue that replaces the histidine in nisin. Active lanthionine variants of enkephalin are known (Svenssen et al 2003. Journal of Pharmacology and Experimental Therapeutics 304: 827-832).

Several uses are already foreseen. For instance peptide / protein drugs that are rapidly degraded in the blood plasma can be protected against proteolysis by thioether rings. Also, new lantibiotics can be used as antibiotics especially against Gram-positive bacteria. This is useful since there is a growing and spreading resistance against classical antibiotics. Also, new lantibiotics can be used as (food) additives to prevent bacterial growth and increase the shelf life of (food) products. Mastering the enzymatic synthesis of thioether rings further furnishes the possibility of synthesizing a broad variety of new antimicrobial peptides, which gives many possibilities to circumvent resistance. Lantibiotics have a variety of antimicrobial activities: membrane permeabilization, inhibition of cell wall synthesis, modulation of enzyme activities, inhibition of outgrowth of spores. New lantibiotic-type peptides or proteins are more stable (i.e. less prone to proteolytic cleavage) and can have modulated activity or a different spectrum of activity. A selection of such peptides or proteins is herein provided in the examples given below. Dehydro-peptides can be engineered which can be used to block enzymatic activity, especially protease activity.

EXAMPLE 1

The NisT transporter can transport unmodified nisin prepeptide

This example involves a *Lactococcus lactis* strain that lacks the entire
5 chromosomal nisin gene cluster, but produces simultaneously plasmid encoded
NisT and the NisA prepeptide. Unmodified NisA can be found in the culture
supernatant, which demonstrates that NisT is sufficient for the transport of
unmodified prepeptides to the exterior of the cell.

10 Materials and Methods:

Use for the nisin inducible expression of *nisT* in *Lactococcus lactis* a pNZ8048
(Kuipers et al. 1997. Tibtech. 15: 135-140) derived plasmid. Amplify the *nisT*
gene using primers NisT.fw (5'-CGG TCT CCC ATG GAT GAA GTG AAA GAA
TTC ACA TCA AAA C) and NisT.rev (5'-CGG TCT CTC TAG ATT ATT CAT CAT
15 TAT CCT CAT ATT GCT CTG) with chromosomal DNA of NZ9700 (a nisin
producing *L. lactis* strain; Kuipers et al. 1997. Tibtech. 15: 135-140) as template.
Use as PCR conditions: 5 min 94 °C, 30 times [30s 94 °C, 30s 50°C, 3 min 72°C],
10 min 72°C. Purify the PCR product with the Roche PCR-isolation kit. Digest
the expression vector with *NcoI/XbaI* and the PCR fragment with *Eco31I*
20 (underlined in the primers, the sticky ends it generates are indicated in italics
and are compatible with *NcoI* and *XbaI*) and ligate subsequently the fragments
using T4 ligase (Roche). Designate the resulting plasmid pNG-*nisT*. This plasmid
contains a chloramphenicol (Cm) resistance gene as selection marker.

Use for the nisin inducible production of the NisA prepeptide in *L. lactis* a
25 variant of pNZ8048 that contains an erythromycin (Em) resistance selection
marker instead of a Cm marker. Amplify the *nisA* gene using primers
NisA.fw (5'-CGG TCT CTC ATG AGT ACA AAA GAT TTT AAC TTG GAT TTG
G) and NisA.rev (5'-TAT ATG GAT CCT TTG CTT ACG TGA ATA CTA CAA
TGA CAA G) and chromosomal DNA of strain NZ9700 as template under the
30 same conditions as described above. Purify the PCR product with the Roche PCR-
isolation kit. Digest the expression vector with *NcoI/BamHI* and the PCR
fragment with *Eco31I* and *BamHI* (underlined in the primers, the sticky ends it
generates are indicated in italics and are compatible with *NcoI* and *BamHI*) and
ligate subsequently the fragments using T4 ligase (Roche). Designate the
35 resulting plasmid pNG-*nisA*.

Grow *L. lactis* strains NZ9000 or PA1001 (a NZ9000 derivative lacking AcmA activity to abolish cell lysis (Buist et al. 1995. J. Bacteriol. 177: 1554-1563) and lacking HtrA to diminish extracellular proteolytic activity (Poquet et al. 2000. Mol. Microbiol. 35: 1042-1051) with both pNG-*nisT* (Cm) and pNG-*nisA* (Em) in M17-based medium (Terzaghi and Sandine. 1975. Appl. Microbiol. 29: 807-813) to an OD₆₀₀ of 0.4. Collect the cells by centrifugation and resuspend in the same volume of Minimal Medium (Jensen and Hammer, 1993. Appl. Environ. Microbiol. 59: 4363-4366) and induce for expression of NisT and NisA prepeptide by addition of nisin as described before (Kuipers et al. 1997. Tibtech. 15: 135-140). After overnight induction and subsequent centrifugation, pipet the culture supernatants up and down in C18 ziptips (Millipore): two times 10 µl 50% acetonitril followed by two times 10 µl demineralized water followed by eight times 10 µl supernatant, followed by two times washing with 10 µl demineralized water, followed by elution by using 2 times 10 ul 50% acetonitril containing 0.1% TFA. Vacuum dry the final eluent and store at -20 °C until analysis by mass spectrometry. Prior to analysis resuspend the dry material in 2.5 µl of 50% acetonitril containing 0.1% TFA and apply 1 µl to the target. After drying, apply 1 µl of matrix (10 mg/ml alpha-cyano-4-hydroxycinnamic acid completely dissolved (by mildly heating and vortexing) in 50% acetonitril containing 0.1% TFA) to the target. Use the following MALDI-TOFMS (linear mode) laser settings: 100% coarse energy, 50% fine, source 20KV, extra 19800, force 15000, suppression 500, pulse time 40, pulse voltage 2200, sampling rate 500 MHz, sensitivity 50mV, shots 15.

25 Results

Analyse culture-supernatants of the following induced cultures and analyse by MALDI-TOFMS:

NZ9000 (or PA1001)

NZ9000[pNG-*nisA*] (or PA1001[pNG-*nisA*])

30 NZ9000[pNG-*nisA* + pNG-*nisT*] or (PA1001[pNG-*nisA* + pNG-*nisT*])

Observe no peaks in samples derived from cultures A and B. Measure in sample C two main peaks: Figure 6. The first one close to or identical to 5832.8 Da corresponding to the unmodified nisin prepeptide: (5831.8 plus 1 proton). The second with about 130 Da higher mass than the first one, which might

35 correspond to the nisin prepeptide with two zinc atoms (Bierbaum, G. 1999. Ed.

J. W. Kelly. Amino acids, Peptides, Porphyrins, Alkaloids 4, 275-301. Elsevier. Comprehensive Natural Products Chemistry. Eds. D. Barton, K. Nakanishi & O. Meth-Cohn) or –more likely- to the nisin prepeptide with the methionine in position 1 still present. This result is consistent with unmodified nisin prepeptide being transported by the nisin transporter NisT. This result demonstrates 5 unequivocally that NisT is sufficient for the transport of the nisin prepeptide and that modification prior to transport is not required.

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EXAMPLE 2

Transport via NisT of a fusion peptide of the nisin leader and an angiotensin variant

5 This example describes the transport out of *Lactococcus lactis* via the nisin transporter NisT of a variant of angiotensin¹⁻⁷ which is preceded by the nisin leader. It is shown once more that the nisin transporter is not specific for nisin, but that many (poly)peptides can be transported provided that they are fused to the nisin leader.

10

Materials and Methods.

As in example 3, precise genetic fusion of a eukaryote peptide, in this example angiotensin 1-7, behind the nisin leader is obtained.

Obtain the gene of the angiotensin1-7 variant by annealing two phosphorylated

15 oligo's: ang1: 5' ACGCAATCGTTCTTATATTTGTCCTTAAG 3'

and ang2: 5' GATCCTTAAGGACAAATATAAGAACGATT 3'

The annealed fragment includes a stopcodon and has at its 5'-end the ACGC overhang and at the 3'-end a *Bam*HI compatible sticky end. Ligate the annealed gene fragment into *Eco*31I and *Bam*HI digested pLP1 and designate the

20 resulting plasmid pLP1ang.

Induce strain PA1001 carrying pNGnisT (example 1) and pLP1ang for expression as described in example 1. Perform purification of the secreted peptide and analyses by MALDI-TOFMS (linear mode) essentially as described in example 1.

25 Result

Figure 7: Samples derived from the ziptipped supernant of PA1001 + pNGnisT + pLP1ang show a Maldi TOF peak coresponding to the nisin leader fused to the angiotensin 1-7 variant. This peak is absent in samples derived from cells with just pNGnisT or with just pLPang1. These data prove that the leader-angiotensin

30 fusion peptide can be transported out of the cell via NisT.

EXAMPLE 3

Transport via NisT of a fusion peptide of the nisin leader and a vasopressin variant.

5 This examples shows export out of *Lactococcus lactis* PA1001 via NisT of a fusion of the nisin leader C-terminally extended with a vasopressin variant. Vasopressin is a 9 amino acid (aa) peptide antidiuretic hormone. It has cysteines in position 1 and 6: CYFQNCPRG that form an internal disulfide bond. This example involves a C1S vasopressin variant. This example involves precise fusion
 10 of serine altered vasopressin (SerVaso) to the NisA leaderpeptide again by genetic modification.

Materials and methods

15 To obtain a precise and in frame fusion of SerVaso with the NisA leaderpeptide, convert first a pNZ8048 expression vector derived plasmid pNG-*nisI-SC3*, that contains SC3 behind the nisin leader into a general NisA leaderpeptide secretion vector. Introduce suitable restriction sites and remove the c-myc-SC3 sequences by PCR amplification of the entire plasmid using primers: pLP.1 (5'-CGG TCT
 20 CAG CGT GGT GAT GCA CCT GAA TC) and pLP.2 (5'-CCA CGC TGA GAC
CGC AGC TGG GAT CCG GCT TGA AAC GTT CAA TTG AAA TGG). Cut the PCR product with Eco31I (underlined sequences in the primers) resulting in sticky ends (in italics in the primer sequences) that are compatible for self-
 25 ligation of the plasmid. After self-ligation the resulting plasmid, pLP1, can be used for precise fusion of peptides and proteins after the ...GASPR aa sequence of the NisA leaderpeptide by making use of the *Eco31I* restriction site. DNA fragments to be inserted at this position should then contain a 5'- ACGC sticky end to allow ligation. At the 3'-end the DNA fragment should contain a sticky end that is compatible with *Bam*HI (site introduced by primer pLP.2, indicated in
 30 bold).

Obtain the SerVaso gene by annealing two oligo's: VP.1: 5'-ACG CTC ATA TTT TCA AAA TTG TCC TCG TGG TTA AG and VP.2: 5'-GAT CCT TAA CCA CGA GGA CAA TTT TGA AAA TAT GA. The annealed fragment includes a stopcodon and has at its 5'-end the ACGC overhang and at the 3'-end a *Bam*HI compatible
 35 sticky end. Ligate the SerVaso gene fragment into *Eco31I* and *Bam*HI digested pLP1 and designate the resulting plasmid pLP1vp.

Induce strain PA1001 carrying pNGnisT (example 1) and pLPvp for expression as described in example 1. Perform purification of the secreted peptide and analyses by MALDI-TOFMS (linear mode) essentially as described in example 1.

5 Result

Fig. 8. A MALDI-TOFMS mass consistent with export of the fusion peptide of nisin leader C-terminally extended by C1S vasopressin. This result demonstrates that a eukaryote peptide can be fused to a lantibiotic leader peptide and exported via a lantibiotic transporter as such.

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EXAMPLE 4

Export of nisin prepeptide via the nisin transporter NisT and modification by the nisin dehydrating enzyme NisB without subsequent enzymatic thioether bridge formation.

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Materials and methods

Clone *NisBT* as in example 1 using the primers nisB fw, (5'-CGG TCT CGC ATG ATA AAA AGT TCA TTT AAA GCT CAA CCG TTT TTA GTA AG) and nisT rev (5'-CGG TCT CTC TAG ATT ATT CAT CAT TAT CCT CAT ATT GCT CTG).

10 Transform NZ9000 + pNG-*nisBT* (Cm) with pNG-*nisA* (Em) (constructed as in example 1). Grow in minimal medium NZ9000 + pNG-*nisBT* + pNG-*nisA* cells and induce as in example 1. Ziptip the supernatant and analyse by MALDI-TOFMS as in example 1.

15 Results

Fig. 9. A MALDI-TOFMS a peak around 5690 Da in the sample derived from the supernatant from NZ9000 + pNG-*nisBT* + pNG-*nisA* cells. Absence of this peak in samples derived from the supernatant of NZ9000 + pNG-*nisT* + pNG-*nisA* (example 1). Consistence with dehydration of most serines (probably Ser33

20 remains untouched as in nisine itself) and all threonines.

EXAMPLE 5

Export of nisin prepeptide via the nisin transporter NisT and modification by the nisin dehydrating enzyme NisB without subsequent enzymatic thioether bridge formation.

5

Materials and Methods

Construct the plasmid pNG-*nisABT* similar to the organisation of these genes in the wild type nisin producer NZ9700, which is with an inverted repeat between the *nisA* and the *nisBT* genes. In short: PCR on Chromosomal DNA with primers

10 *nisA*fw: 5' CGG TCT CTC ATG AGT ACA AAA GAT TTT AAC TTG GAT TTG G
3' and *nisT*rev: 5' CGG TCT CTC TAG ATT ATT CAT CAT TAT CCT CAT ATT
GCT CTG 3'. Clone the PCR product into pGEM-T (A-T ligation). Digest pGEM-
T*nisABT* with BsaI. Ligate *nisABT* (BSAI) with pNG8048E(*ncoI/XbaI*).

Transform this plasmid to NZ9000, grow and induce. In this case after induction
15 *nisBT* are transcribed only in low quantity by limited readthrough. Subject the
supernatant to ziptipping and MALDI-TOFMS as in example 1.

Results

A MALDI-TOFMS peak around 5690 consistent with export of the nisin
20 prepeptide and dehydration of propeptide serines (most) and threonines (all).

EXAMPLE 6

Export of nisin prepeptide via the nisin transporter NisT and modification by the nisin dehydrating enzyme NisB followed by NisC-mediated thioether bridge formation, involving a pNG-*nisABTC* plasmid.

5

Materials and Methods

Construct the plasmid pNG-*nisABTC* similar to the organisation of these genes in the wild type nisin producer NZ9700, which is with an inverted repeat between the *nisA* and the *nisBTC* genes. This construction can be performed analogous to the construction of pNG_{nisABT} described in example 5, using - instead of *nisT* rev- *nisC* rev: 5' CGG TCT CTC TAG ATC ATT TCC TCT TCC CTC CTT TCA AAA AAT C 3'.

Alternatively, and this is the construction of the *nisABTC*-containing plasmid with which the presented data were obtained, clone the *nisABTC* genes on a gateway plasmid. Restrict pNG8048E with HindIII. Remove the Em-r containing fragment by isolating the vector fragment (3kb) from gel. Self-ligate the vector fragment and designate the resulting vector pBMDL1. PCR pBMDL1 with to loose the Cm-r and introduce a PstI site and a XbaI site. Isolate this fragment isolated out of gel and restrict with PstI and XbaI. Cut pNG8048E also with PstI and XbaI to obtain the Em-r fragment. Isolate this 1 kb fragment out of gel and ligate with the former PCR-product. Term the new plasmid pBMDL2. Restrict pBMDL2 with SmaI to linearize it and to obtain blunt ends. Insert a Gateway Vector Conversion Cassette (RfA) (1.7 kb) by blunt-end ligation. Thus obtain a vector to be termed pBMDL3. To prepare a vector that contains the *nisABTC* genes, introduce Gateway's attB-sites by means of PCR. The 6.4 kb PCR-fragment was cleaned over a Zymoclean DNA Clean & Concentrator Kit column. A BP-reaction was performed with this PCR-product and pDONR201 (Invitrogen) during ON incubation at 25C. Obtain the entry vector pBMDL4 in *E.coli* DH5alpha cells via chemical transformation. Perform with this entry vector pBMDL4 and the already created pBMDL3 a LR-reaction. Obtain the vector pBMDL5 (with *nisABTC*) in *E.coli* EC1000

(contains RepA on chromosome) via electroporation. Isolate pBMDL5 (containing *nisABTC*) and transform into PA1001 (electroporation).

Grow NZ9000 + pBMDL5 (or pNGnisABTC) and compare induced and uninduced samples. Analyse the supernatant as in example 1. Next to this, subject trypsin treated supernatant to a growth inhibition assay of nisin sensitive, erythromycin resistant *Lactococcus lactis*. In addition test the trypsinated supernatant for its capacity to induce the nisin promoter with the Gus assay (Kuipers et al. 1995. J. Biol. Chem. 270:27299-27304)

10 Results

In the uninduced sample a small peak is visible of modified prepeptide (dehydration and lanthionine formation) and a larger peak of unmodified prepeptide (Fig 10A). In the induced sample a large peak is visible of modified prepeptide (dehydration and lanthionine ring formation) and a small peak which might correspond to modified prepeptide with methionine 1 (Fig 10B).

Trypsinated samples of both induced and uninduced supernatants are able to induce the nisin promoter as measured by the gus assay. Induced and trypsinated supernatants have growth inhibitory capacity comparable to the supernatant of the wild type nisin producer NZ9700. Apparently the nisin

promoter is leaky. This result on the uninduced sample confirms again the result of example 1 that unmodified prepeptide can be exported.

These results of the induced samples are consistent with export of the nisin prepeptide which has undergone all lanthionine bridge formations. Trypsin cleaves of the leader liberating active nisin with antimicrobial activity and inducing capacity. NisBTC are therefore sufficient for lanthionine formation.

EXAMPLE 7

EpilancinBC-mediated synthesis by *Staphylococcus epidermis* (prokaryote, Gram-positive) of epilancin leader (table 2) coupled to glucagon (table 1) with thioether rings.

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The [C5, S24, C29]-sequence of glucagon is HSQGCFTSDYSKYLDSRRAQDFVSWLMNC (table 1). This sequence allows the epilancin K7 enzymes to form thioether rings between S2-C5 and S24-C29.

10 Materials and methods

Clone a construct leader-epilancin K7 followed in an open reading frame by mutant glucagon, followed by epilancin BTC. Transform the above plasmid to *Staphylococcus epidermis*. Induce transcription. Continue overnight cell growth in minimal medium, centrifuge, perform ziptipping of the supernatant and

15 MALDI-TOFMS analysis (linear mode).

Result

A MALDI-TOFMS peak consistent with production of glucagon with dehydrated serines and threonines and thioether rings as indicated in table 1. Maldi TOF
20 analysis of peroxydated samples indicate thioether bridge formation, since to a cysteines 3 oxygens can be added whereas to thioether bridges one or two oxygens are be added.

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EXAMPLE 8

Production by *Streptococcus salivarius* (prokaryote Gram positive) of [S3, S12] tachyplesin I (table 1) following export via SalT.

5 Materials and methods

Clone a construct salivaricin-leader (table 2) followed in an open reading frame by mutant tachyplesin (table 1). Clone *salivaricinT* on a second plasmid with different antibiotic marker. Transform both plasmids to a *Streptococcus salivarius* strain devoid of salivaricin genes. Induce transcription of both
10 plasmids, during 2-4 hours of continued growth. Add every 30 min 0.2 mM pmsf (protease inhibitor). Perform ziptipping as in example 1 and analyse by MALDI-TOFMS (linear mode).

Result

15 A mass spectrometry peak corresponding to tachyplesin.

EXAMPLE 9

Production by *Streptococcus salivarius* (prokaryote Gram positive) of [S3, S12] tachyplesin I (table 1) with salivaricinB-dehydrated serine-3 and serine-12 without subsequent enzymatic thioether ring formation.

5

Tachyplesin has the following [S3,S12]-sequence: KWSFRVCYRGISYRRCR

Materials and methods

10 Clone a construct salivaricin leader (table 2) followed in an open reading frame by mutant tachyplesin (table 1). Clone *salivaricinBT* on a second plasmid with different antibiotic marker. Transform this plasmid to a *Streptococcus salivarius* strain devoid of salivaricin genes. Induce transcription of both plasmids, during 2-4 hours of continued growth. Perform ziptipping as in example 1 and analyse by MALDI-TOFMS (linear mode).

15

Result

A mass spectrometry peak corresponding to tachyplesin with dehydrated serines.

20

EXAMPLE 10

Production by *Streptococcus salivarius* (prokaryote Gram positive) [S3, S12] tachyplesin I (table1) with salivaricinB-dehydrated S3 and S12 without subsequent enzymatic thioether ring formation.

5

Tachyplesin has the following [S3, S12]-sequence: KWSFRVCYRGISYRRCR

Materials and methods

10 Clone a construct salivaricin-leader (table 2) followed in an open reading frame by mutant tachyplesin (table 1) and thereafter *salivaricinBT*. Transform this plasmid to a *Streptococcus salivarius* strain devoid of salivaricin genes. Induce transcription, during 2-4 hours of continued growth. Perform ziptipping as in example 1 and analyse by MALDI-TOFMS (linear mode).

15 Result

A mass spectrometry peak corresponding to tachyplesin with dehydrated serines.

EXAMPLE 11

Production by *Lactococcus lactis* (prokaryote, Gram-positive) via lacticinT of vasonatrin (table 1) without modifications.

5 Lacticin 481-T has leader peptidase activity and therefore in this particular example in the supernatant of the cell culture vasonatrin is found without leader. Vasonatrin is amongst others involved in vaso relaxation. Its sequence is:
GLSKGCFGLKLDRIGSMSGLGCNSFRY.

10 Materials and methods

Clone a construct lacticin 481-leader (table 2) followed in an open reading frame by vasonatrin (table 1). Clone *lacticin 481-T* on a second plasmid with different antibiotic marker. Transform both plasmids to a *L. Lactis* strain devoid of lacticin 481 genes. Induce transcription of both plasmids during overnight growth in
15 minimal medium. Perform ziptipping as in example 1 and analyse by MALDI-TOFMS (linear mode).

Result

A mass spectrometry peak corresponding to vasonatrin.

20

EXAMPLE 12

Production by *Lactococcus lactis* (prokaryote, Gram-positive) via lacticinT of vasonatrin (table 1), with lacticin M mediated thioether rings

5 Vasonatrin is amongst others involved in vaso relaxation. It has an amino acid sequence, that without mutations permits the formation of two lanthionine rings. Its sequence is: GLSKGCFGLKLDRIQSMSGGLGCNSFRY. Lanthionine rings can be formed from S3-C6 and from S16-C22.

10 Materials and methods

Clone a construct lacticin 481-leader (table 2) followed by vasonatrin (table 1) and lacticinM coding sequences. Transform the plasmid to *Lactococcus lactis* PA1001. Induce transcription of the plasmid, during overnight growth in minimal medium. Perform ziptipping as in example 1 and analyse by MALDI-TOFMS
15 (reflectron mode). Analyse peroxydized (in the case of a thioether bridge one or two oxygens add; in the case of cysteines three oxygens are added) and non-peroxydized samples.

Result

20 Mass spectrometry peaks consistent with lacticinM leader coupled to vasonatrin with two thioether rings and two more dehydrated serines.

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5 EXAMPLE 13

Transport via NisT of nisin prepeptide C-terminally fused to an enkephalin variant.

10 This example shows that unmodified nisin prepeptide with a C-terminal extension can be exported via NisT. It has been described by others that mature lantibiotics can be exported via LanBTC / TM with a C-terminal extension, but it is not yet known that also unmodified prepeptide with a C-terminal extension can be exported via only the transporter LanT. This example involves a *Lactococcus lactis* strain that lacks the entire chromosomal nisin gene cluster, but produces
15 simultaneously plasmid encoded NisT and a fusion of NisA prepeptide and an enkephalin variant, YTGFC, (the enkephalin genetically fused to the C-terminus of nisin prepeptide). Unmodified fusion prepeptide can be found in the culture supernatant, which demonstrates that NisT is sufficient for the transport of prepeptide with C-terminal extension to the exterior of the cell.

20

Materials and methods.

Lactococcus lactis, PA1001 (see example 1), was transformed with pNGnisT, which was constructed as described in example 1. pNGnisA-enkT and pNGnisA-enkS were constructed as follows. pNGnisA was PCRed with the primer couple
25 5'-GCACGTGTTGCTTTGATTGATAGC-3' and
5'-CTGGATCCTTAACAAAAACCTGTGTATTTGCTTACGTGAATACTAC-
'3 (*Bam*H1 site underlined), which leads to C-terminal fusion of the enkephalin variant YTGFC to nisin A (enkT). The PCR product was ligated and transformed to *Lactococcus lactis* PA1001 +
30 pNGnisT. The resulting strain, PA1001 + pNGnisT + pNZenkT was grown in MG17 medium to OD600 = 0.4, pelleted and resuspended in minimal medium supplemented with 1/1000 filtered supernatant of the wild type nisin producer *Lactococcus lactis* NZ9700. After overnight incubation cells were pelleted and the supernatant was ziptipped and subjected to maldi TOF analysis.

35

Result:

Figure 11. Peaks close to 6400.05 (nisine-prepeptide without methionine1 C-terminally extended with YTGFC) were observed by maldi TOF. Hence the nisin prepeptide genetically fused to an enkephalin variant can be exported via the nisin transporter. Therefore it can be concluded that lantibiotic transporters can also be used for the transport of unmodified lantibiotic prepeptides that are C-terminally extended by a fused peptide.

Table 1, selected (poly)peptides

Table 1: (poly)peptides of which the coding DNA is preceded by lantibiotic leader coding DNA in an open reading frame.

5 Mutation possibilities allowing posttranslational thioether ring(s) formation given for example for vasopressin applies also to other sequences, including those which have already one ring, within other (poly)peptides in Table 1, taking into account the description of thioether ring formation mentioned in the text.

10 Table 1: (poly)peptides of which the coding DNA is preceded by lantibiotic leader coding DNA in an open reading frame. Mutation possibilities allowing posttranslational thioether ring(s) formation given, for example, for vasopressin applies also to other sequences, including those which have already one ring, within other (poly)peptides in Table 1, taking into account the description of thioether ring
15 formation mentioned in the text.

Table 1A:

Vasopressin: Function : as an antidiuretic hormone:

(A1, S2, R8)-sequence: ASFQNCPRG ; lanthionine ring S2-C6

20 (A1, S2, C3, R8)-sequence: ASCQNCPRG ; lanthionine ring S2-C3

(A1, S2, C4, R8)-sequence: ASFCNCPRG ; lanthionine ring S2-C4

(A1, S2, C5, R8)-sequence: ASFQCCPRG (SEQ ID NO:); lanthionine ring S2-C5

(A1, S2, A6, C7, R8)-sequence: ASFQNACRG ; lanthionine ring S2-C7

(A1, S2, A6, C8)-sequence: ASFQNAPCG ; lanthionine ring S2-C8

25 (A1, S2, A6, R8, C9)-sequence: ASFQNAPRC ; lanthionine ring S2-C9

(A1, S3, R8)-sequence: AYSQNCPRG ; lanthionine ring S3-C6

(A1, S3, C4, R8)-sequence: AYSCNCPRG ; lanthionine ring S3-C4

(A1, S3, C5, R8)-sequence: AYSQCCPRG ; lanthionine ring S3-C5

(A1, S3, A6, C7, R8)-sequence: AYSQNACRG; lanthionine ring S3-C7

30 (A1, S3, A6, C8)-sequence: AYSQNAPCG ; lanthionine ring S3-C8

(A1, S3, A6, R8, C9)-sequence: AYSQNAPRC ; lanthionine ring S3-C9

(A1, S4, R8)-sequence: AYFSNCPRG ; lanthionine ring S4-C6

(A1, S4, C5, R8)-sequence: AYFSCCPRG ; lanthionine ring S4-C5

(A1, S4, A6, C7, R8)-sequence: AYFSNACRG ; lanthionine ring S4-C7

(A1, S4, A6, C8)-sequence: AYFSNAPCG ; lanthionine ring S4-C8

(A1, S4, A6, R8, C9)-sequence: AYFSNAPRC ; lanthionine ring S4-C9

(A1, S5, R8)-sequence: AYFQSCPRG ; lanthionine ring S5-C6

(A1, S5, A6, C7, R8)-sequence: AYFQSACRG ; lanthionine ring S5-C7

5 (A1, S5, A6, C8)-sequence: AYFQSAPCG ; lanthionine ring S5-C8

(A1, S5, A6, R8, C9)-sequence: AYFQSAPRC ; lanthionine ring S5-C9

(A1, S6, C7, R8)-sequence: AYFQNSCRG ; lanthionine ring S6-C7

(A1, S6, C8)-sequence: AYFQNSPCG ; lanthionine ring S6-C8

(A1, S6, R8, C9)-sequence: AYFQNSPCG ; lanthionine ring S6-C9

10 (A1, S7, C8)-sequence: AYFQNCSCG ; lanthionine ring S7-C8

(A1, S7, R8, C9)-sequence: AYFQNCSRC ; lanthionine ring S7-C9

(A1, S7, C9)-sequence: AYFQNCPCG ; lanthionine ring S8-C9.

Terlipressin (antidiuretic hormone):

15 S4-Sequence: GGGSYFQNCPKG

Posttranslational lanthionine: S4-C9.

Cispressin (antidiuretic hormone):

S4-Sequence: GGGSYFNCPKG

20 Posttranslational lanthionine ring: S4-C8.

Adrenomedullin Hypotensive peptide, may function as a hormone in circulation control

A13,S16-Sequence:

YRQSMNNFQGLRAFGSRFGTCTVQKLAHQIYQFTDKDKDN

25 VAPRSKISPQGY

Posttranslational lanthionine: S16-C21.

Allatostatin I (neuropeptide inhibitor of juvenile hormone synthesis)

C6-Sequence: APSGACRLYGFGFL

Posttranslational lanthionine: S3-C6.

30

Angiotensin I

S7,C10-Sequence: DRVYIHSFHC

Posttranslational lanthionine S7-C10

Function: In response to lowered pressure, the enzyme renin cleaves angiotensin I, from angiotensinogen, then removes a dipeptide to yield the physiologically active angiotensin II, the most potent pressor substance known, which helps regulate volume and mineral balance of body fluids.

5

Anthopleurin-A (neuropeptide)

A4-Sequence:

GVSALCSDGPSVRGNTLSGTLTYPSGCPGWHNCKAHGPTI
G WCKKQ

10

Posttranslational lanthionine rings: S3-C6, S27-C31, S33-C38, T44-C48.

Anti-inflammatory peptide 1 (anti-inflammation)

S1,C6-Sequence: SQMKKCLDS

Posttranslational lanthionine: S1-C6

Dermaseptin (antimicrobial peptide)

15

C10-Sequence:

ALWKTMLKKCGTMALHAGKAALGAAADTISQGTQ

Posttranslational lanthionine: T5-C10.

Bombinin-like peptide (antimicrobial peptide)

C8-Sequence: GIGASILCAGKSALKGLAKGLAEHFAN

20

Posttranslational lanthionine: S5-C8.

Histatin-5 (antimicrobial salivary peptide)

S4,C7-Sequence: DSHSKRCHGYKRKFHDKHHSRGRY

Posttranslational lanthionine: S4-C7.

Indolicidin (antimicrobial peptide)

25

S2,C5-Sequence: ISPWCWPWWPWR

Posttranslational lanthionine: S2-C5.

Magainin-1 (antimicrobial peptide)

C13-Sequence: GIGKFLHSAGKFCKAFVGEIMKS

Posttranslational lanthionine: S8-C13.

30

Atrial Natriuretic Factor (potent vasoactive substance with a key role in cardiovascular homeostasis and cGMP-stimulating activity).

Sequence: SLRRSSCFGGRMDRIGAQSGLGCNSFRY

Posttranslational lanthionines: S1-C7, S19-C23.

Bradykinin (important role in renal physiology and behavior).

C9-Sequence: RPPGFSPFC

Posttranslational lanthionine: S6-C9.

Brain Natriuretic Peptide (acts as a cardiac hormone involved in natriuresis, diuresis,

5 vasorelaxation, inhibition of renin and aldosteron secretion, improves heart function)

S16,C19-Sequence: SPKMVQGSQCFGRKMSRICSSSSGLGCKVLRH

Posttranslational lanthionine: S8-C10, S16-C19

C-type Natriuretic peptide (exhibits natriuretic and vasodepressor activity)

Sequence:

10 DLRVDTKSRAAWARLLQEHPNARKYKGANCKGLSKGCFGLK
LDRIG SMSGLGC

Posttranslational lanthionine ring: S34-C37, S47-C53.

Vasonatrin peptide (vasorelaxation)

Sequence: GLSKGCFGLKLDLDRIGSMSGLGCNSFRY

15 Posttranslational lanthionine ring: S3-C6,S17-C22.

Delta sleep inducing peptide (delta sleep induction)

S2,C6-Sequence: WSGGNCSGE

Posttranslational lanthionine ring: S2-C6.

Alpha-dendrotoxin

20 S11,S26-Sequence:

PRRKLCLHRSPGRCYDKIPAFYYNSKKKQCERFDWSGC
GGNSNRFKTIEECRRTCIG

Posttranslational lanthionine: S11-C15, S26-C31

Function: affects potassium channels.

25 Eledoisin

C4-Sequence: PSKCAFIGLM Posttranslational lanthionine ring: S2-C4

Function: neuron excitation, causing behavioral responses, vasodilators,
secretagogues, causing contraction of smooth muscles.

Echistatin

30 Sequence:

ECESGPCCRNCKFLKEGTICKRARGDDMDDYCNGKTCDCPRNPHK
GPAT

Posttranslational lanthionine rings: S4-C7, T18-C20, T36-C37

Function: Inhibitor of fibrinogen-dependent platelet aggregation.

alpha-endorphin

S2,C6-Sequence: YSGFMCSEKSQTPLVT

Posttranslational lanthionine ring: S2-C6

Function: opioid.

5 beta-endorphin

S21,C26-Sequence: YGGFMTSEKSQTPLVTLFKNSIIKNCYKKGE

Posttranslational lanthionine ring: S21-C26

Function: opioid.

Defensin I

10 S2,S12-Sequence: ASYCRIPACIAGSRRYGTCTYQGRLWAFCC

Posttranslational lanthionine rings: S2-C4, S13-C19

Function: antimicrobial peptide.

Secretin

S23,C26-Sequence: HSDGTFTSELSRLREFARLQRLSQGCV

15 Posttranslational lanthionine ring:S23-C26

Function: pH regulation in the stomach.

Urocortin

C19-Sequence: DNPSLSIDLTFHLLRLLCLARTQSQRERAEQNRIIFDSV

20 Posttranslational lanthionine ring: T16-C19

Function: stimulates ACTH secretion.

Urotensin II

S5-Sequence: AGTASCFWKYCV

Posttranslational lanthionine rings: T3-C6, S5-C11

25 Function: osmoregulation and corticotropinrelease factor.

Small Cardioactive Peptide A

S4,C7-Sequence: ARPSYLCFPRM

Posttranslational lanthionine:S4-C7

Function: inhibits acetylcholine release

30 Small Cardioactive peptide B

S4,C7-Sequence: MNYSAFCRM

Posttranslational lanthionine: S4-C7

Function: stimulates contraction in the gut, increases amplitude of the heart beat.

Ceratotxin A

C9-Sequence: SIGSALKKCLPVAKKIGKIALPIAKAALP Posttranslational
lanthionine: S4-C9

Function: antimicrobial, hemolytic peptide with activity against Gram-
5 positive and Gram-negative bacteria, stable at 100 degrees Celsius.

Cerebellin

C7-Sequence: SGSAKVCFSAIRSTNH

Posttranslational lanthionine: S3-C7

Function: neuromodulation, stimulation of norepinephrine release, enhances
10 indirectly adrenocortical secretion.

Charybdotoxin

S33-Sequence: FTNVSCTTSKECWSVCQRLHNTSRGKCMNKKSRCYS

Posttranslational (methyl)lanthionine: T3-C7, T8-C13, S15-C17, T23-C28,
15 S33-C35

Function: inhibitor calcium - and voltage activated potassium channels.

Cholecystokinin

C8-Sequence: KAPSGRMCIVKNLQLDPSHRISDRYMGWMDF

Posttranslational lanthionine: S4-C8

Function: Gall bladder contraction and release of pancreatic enzymes in the
20 gut.

Conopressin G

S1-Sequence: SFIRNCPKG

Posttranslational lanthionine: S1-C6

Function: behavioral control.
25

alpha-Conotoxin EI

S2,S5-Sequence: RSHCSYHPTCNMSNPQIC

Posttranslational lanthionine: S2-C4, S5-C10, S13-C18

Function: blocking nicotinic acetylcholine receptors.

30 Corazonin

C9-Sequence: TFQYSRGWCN

Posttranslational lanthionine: S5-C9

Function: Regulation heart beat.

Leu-enkephalin

S2,C3-Sequence: YSCFL

Posttranslational lanthionine ring: S2-C3

Function: opioid

5 Met-enkephalin

S2,C3-Sequence: YSCFM Posttranslational lanthionine ring: S2-C3

Function: opioid.

Oxytocin

10 (S1)-Sequence: SYIQNCPLG Posttranslational lanthionine ring S1-C6

Function: Oxytocin stimulates uterine contraction and lactation; increases Na⁺ secretion; stimulates myometrial GTPase and phospholipase C.

Exendin-3

C35-Sequence:

15 HSDGTFTSDLSKQMEEEEAVRLFIEWLKNGGPSSGCPPPS

Posttranslational lanthionine ring: S32-C35

Function: secretin-like.

Experimental Allergic Encephalitogenic peptide

C5-Sequence: FSWGCEGQR Posttranslational lanthionine ring: S2-C5

20 Function: myelin membrane stabilization.

Experimental Autoimmune Encephalomyelitis Complementary peptide

S4,C7-Sequence: VFISGPCRLLG Posttranslational lanthionine ring: S4-C7

Effect: having a role in autoimmune encephalomyelitis.

GonadoliberinII

25 (C9)-sequence: QHWSHGWYCG Posttranslational lanthionine ring: S4-C9

Function: stimulates the secretion of gonadotropins; it stimulates the secretion of both luteinizing and follicle stimulating hormones.

Tocinoic acid / pressinoic acid

(S1,I3)-Sequence: SYIQNC posttranslational lanthionine ring S1-C6

30 Function: Tocinoic acid is an oxytocin inhibitor, induces maternal behavior.

Leuprolide

Sequences:

XHWSYGCRPX

Posttranslational thioether ring: S4-C7

XHWSYXCRX

Posttranslational thioether ring: S4-C7

Function: LHRH agonist.

Calcitonin

5 Accession number: P01258

(S1)-Sequence: SGNLSTCMLGTYTQDFNKFHTFPQTAIGVGAP

Posttranslational thioether ring S1-C7

Function: CaPi incorporation in bones.

ACTH, Adrenocorticotropic hormone

10 (Q5,C6)-Sequence:

SYSMQCFRWGKPVGKKRRPVKVYPNGAEDESAAEAFPLEF

posttranslational lanthionine ring S1-C6

ACTH-fragment-sequence:

SYSMECFRWG

15 Posttranslational ring: S2-C6

Function: ACTH stimulates synthesis and secretion of glucocorticoids by adrenal cortex.

Hepatitis B surface antigen fragment

C6-Sequence: MGTNLCVPNPLGFFPDHQLDP

20 Posttranslational modification: T3-C6 lanthionine ring

Function: surface antigen

Corticotropin inhibiting peptide

S4,C8-Sequence: FRWSKPVCKKRRPVKVYPNGAEDSAAEAFPLE

Posttranslational lanthionine: S4-C8

25 Function: inhibition ACTH.

Corticotropin-Release Factor

S30,C33-Seq:

SEPPISLDLTFHLLREVLEMARAEQLAQSAHCNRKLMEII

Posttranslational lanthionine: S30-C33

30 Function: release of corticotrophin.

Somatostatin

(S3)-Sequence:AGSKNFFWKFTFTSC

posttranslational lanthionine ring S3-C14

Function:somatotropin release inhibition factor, growth hormone release inhibiting factor.

Human pancreatic polypeptide

(S18, C21)-Sequence:

5 APLEPVYPGDNATPEQMSQYCADLRRUINMLTRPRY,

Posttranslational lanthionine ring S18-C21

Function: Agonist at Y4 neuropeptide receptors.

Peptide YY

10 (S22,C25,T29,C32)-Sequence:

YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY

Posttranslational (methyl)lanthionine rings S22-C25, T29-C32

Function: Gut hormone that inhibits both secretin- and cholecystokinin-stimulated pancreatic secretion.

15 Glucagon

(C5,S24,C29)-Sequence: HSQGCFTSDYSKYLDSRRAQDFVSWLMNC

Posttranslational lanthionine rings S1-C5, S24-C29

Function: restoring blood glucose level when too low.

alpha-neurokinin

20 (C9)-sequence: HKTDSFVGCM

posttranslational lanthionine ring S5-C9

function: tachykinin antagonist.

LHRH1, Luteinizing Hormone Releasing Hormone

Function: regulates secretion of gonadotropins, luteinizing hormone and sex
25 steroids.

(Q1, C7)-Sequence: QHWSYGCRPG

Posttranslational lanthionine ring S4-C7

(S1, C4)-Sequence: SHWCYGLRPG posttr. ring: S1-C4

(S1, A4, C5)-Sequence: SHWACGLRPG posttr. ring: S1-C5

30 (S1, A4, C6)-Sequence: SHWAYCLRPG posttr. ring: S1-C6

(Q1, S2, A4, C5)-Sequence: QSWACGLRPG posttr. ring: S2-C5

(Q1, S2, A4, C6)-sequence: QSWAYCLRPG posttr. ring: S2-C6

(Q1, S2, A4, C7)-sequence: QSWAYGCRPG posttr. ring: S2-C7

(Q1, S3, A4, C6)-sequence: QHSAYCLRPG posttr. ring: S3-C6

	(Q1, S3, A4, C7)-sequence:	QHSAYGCRPG	posttr. ring: S3-C7
	(Q1, S3, A4, C8)-sequence:	QHSAYGLCPG	posttr. ring: S3-C8
	(Q1, C8)-sequence:	QHWSYGLCPG	posttr. ring: S4-C8
	(Q1, C9)-sequence:	QHWSYGLRCG	posttr. ring: S4-C9
5	(Q1, A4, S5, C8)-sequence:	QHWASGLCPG	posttr. ring: S5-C8
	(Q1, A4, S5, C9)-sequence:	QHWASGLRCG	posttr. ring: S5-C9
	(Q1, A4, S5, C10)-sequence:	QHWASGLRPC	posttr. ring: S5-C10
	(Q1, A4, S6, C9)-sequence:	QHWAYSLRCG	posttr. ring: S6-C9
	(Q1, A4, S6, C10)-sequence:	QHWAYSLRPC	posttr. ring: S6-C10
10	(Q1, A4, S7, C10)-sequence:	QHWAYGSRPC	posttr. ring: S7-C10.

LHRH2, Luteinizing Hormone Releasing Hormone fragment

Function: regulates secretion of gonadotropins, luteinizing hormone and sex steroids.

(Q1, C7)-Sequence: QHWSHGCYPG

15 Posttranslational lanthionine ring S4-C7

	(S1, C4)-Sequence:	SHWCHGWYPG	posttr. ring: S1-C4
	(S1, A4, C5)-Sequence:	SHWACGWYPG	posttr. ring: S1-C5
	(S1, A4, C6)-Sequence:	SHWAHCWYPG	posttr. ring: S1-C6
	(Q1, S2, A4, C5)-Sequence:	QSWACGWYPG	posttr. ring: S2-C5
20	(Q1, S2, A4, C6)-sequence:	QSWAHCWYPG	posttr. ring: S2-C6
	(Q1, S2, A4, C7)-sequence:	QSWAHGCYPG	posttr. ring: S2-C7
	(Q1, S3, A4, C6)-sequence:	QHSAHCWYPG	posttr. ring: S3-C6
	(Q1, S3, A4, C7)-sequence:	QHSAHGCYPG	posttr. ring: S3-C7
	(Q1, S3, A4, C8)-sequence:	QHSAHGWCPG	posttr. ring: S3-C8
25	(Q1, C8)-sequence:	QHWSHGWCPG	posttr. ring: S4-C8
	(Q1, C9)-sequence:	QHWSHGWYCG	posttr. ring: S4-C9
	(Q1, A4, S5, C8)-sequence:	QHWASGWCPG	posttr. ring: S5-C8
	(Q1, A4, S5, C9)-sequence:	QHWASGWYCG	posttr. ring: S5-C9
	(Q1, A4, S5, C10)-sequence:	QHWASGWYPC	posttr. ring: S5-C10
30	(Q1, A4, S6, C9)-sequence:	QHWAHSWYCG	posttr. ring: S6-C9
	(Q1, A4, S6, C10)-sequence:	QHWAHSWYPC	posttr. ring: S6-C10
	(Q1, A4, S7, C10)-sequence:	QHWAHGSYPC	posttr. ring: S7-C10.

Brain derived acidic fibroblast growth factor (102-111)

(S103,C109)-Sequence: HSQKHWFCGL

Posttranslational lanthionine ring S103-C109

Function: growth factor.

Brain derived basic fibroblast growth factor (1-24)

5 Sequence: PALPEDGGSGAFPPCHFVKDPKRLY

Posttranslational lanthionine ring S11-C17

Function: growth factor.

Insulin

Sequences:

10 alpha-chain: GIVEQCCASVCSLYQLENYCN (SEQ ID NO:)

(S9-C14, T27-C30)-beta chain:

FVNQHLCGSHLVECLYLVCGERGFFYTPKC (SEQ ID NO:)

Posttranslational (methyl)lanthionine rings S9-C14, T27-C30

disulfide bonds: alpha 6 - 11 alpha 7 - beta 7, alpha 20 - beta 19

15 function: diabetes treatment.

Parathormone:

(S36-C39, T79-C82)-Sequence:

SVSEIELMHNLGKHLNSMERVEWLRKKLQDVHNFVSLGCPLAPRDAG

SERPRKKEDNVLVESHEKSLGEADKADVNVLTAKACSE (SEQ ID

20 NO:)

Posttranslational (methyl)lanthionine rings S36-C39, T79-C82

Function: modulation of serum calcium content affecting the mineral and bone physiology.

Fibrinogen Binding Inhibitor peptide

25 S6,C9-Sequence: HHLGGSKQCGDV

Posttranslational lanthionine: S6-C9.

Fibroblast growth factor inhibitory peptide

S1,C3-Sequence: SPCGHYKG

Posttranslational lanthionine ring: S1-C3

30 Effect: inhibition fibroblast growth factor.

Galanin

C10-Sequence: GWTLNSAGYCLGPHAVGNHRSFSDKNGLTS

Posttranslational lanthionine ring: S6-C10

Function: contracts smooth muscle of the gastrointestinal and genitourinary tract, regulates growth hormone release, modulates insulin release.

Gastric Inhibitory Polypeptide

5 S28,C31-Sequence:

YAEGTFISDYSIAMDKIHQQDFVNWLLSQKCKKNDWKHNITQ

Function: potent stimulation of insulin secretion and relatively poor inhibitor of gastric acid secretion.

Big Gastrin-I

10 S8,C11-Sequence: LGPQGPPSLVCDPSKKQGPWLEEEEEAYGW MDF

Posttranslational lanthionine ring: S8-C11

Function: stimulates gastric HCl secretion, pancreatic enzyme secretion, smooth muscle contraction and increases blood circulation and water secretion in the stomach and intestine.

15 Pentagastrin

S1,C4-Sequence: SWMCF

Posttranslational lanthionine ring: S1-C4

Gastrin Releasing Peptide

S9,C12-Sequence: VLPAGGGSVLCKMYPRGNHWAVGHLM

20 Posttranslational lanthionine ring: S9-C12

Function: gastrin release.

Transforming growth factor alpha

Sequence:

VVSHFNDCPDSHTQFCFHGTCRFLVQEDKPACVCHSGYVGAR

25 CEHA DLLA

Posttranslational (methyl)lanthionine ring: S3-C8, T21-C22, S37-C44

Function: TGF alpha is a mitogenic polypeptide that is able to bind to the egf receptor and act synergistically with TGF beta to promote anchorage-independent cell proliferation in soft agar.

30 Human growth hormone

C7-Sequence:

FPTIPLCRLFDNAMLRAHRLHQLAFDTYQEFEEAYIPKEQKYS

Posttranslational methylanthionine ring: T3-C7

Function: growth hormone, stimulates amongst others protein synthesis and amino acid uptake.

Growth hormone release factor

C22-Sequence:

5 YADAIFTNSYRKVLGQLSARKCLQDIMSRQQGESNQERGARAR
L

Posttranslational lanthionine ring: S18-C22

Function: release of growth hormone.

Guanylin

10 Sequence: PGTCEICAYAACTGC

Posttranslational lanthionine ring: T3-C4, T13-C15

Function: activator of guanylate cyclase.

Helospectin I

S15,C18-Sequence:

15 HSDATFTA EYSKLLSKLCLQKYLE SILGSSTSPRPPSS

Posttranslational lanthionine ring: S15-C18

Hepatitis B surface antigen fragment

C6-Sequence: MGTNLCVPNPLGFFPDHQLDP

Posttranslational methyllanthionine ring: T3-C6

20 Function: exendin-1: secretin-like.

Intercellular adhesion molecule

Sequence: NAQTSVSPSKVILPRGGSVLVTC

Posttranslational lanthionine ring: S18-C23

Function: anti-hiv.

25

Tachyplesin I

(S3,S12)-Sequence: KWSFRVCYRGISYRRCR

Posttranslational lanthionine rings S3-C7, S12-C16

Function Hiv cell fusion inhibitor, anti tumor peptide, antimicrobial peptide.

30 HIV (gp 120) antigenic peptide fragment

(S10,C14)-Sequence: CGKIEPLGVSPCKCKRRVVQREKR

Posttranslational lanthionine ring S10-C14.

HIV (gp 41) antigenic peptide I fragment

(S2)-Sequence: GSSGKLICTTAVPWNAS

Posttranslational lanthionine S2-C8.

HIV (gp41) antigenic peptide 5

(S20)-Sequence: RVT AIEKYLQDQARLNSWGSAFRQVCHTTVPWVND S

Posttranslational lanthionine ring S20-C26.

5 HIV protease inhibitors

Sequence: TVSF CF

Posttranslational lanthionine ring T1-C5

Function: inhibitor HIV protease.

Insulin-like growth factor-I analog

10 S1,C4-Sequence: SYACPLKPAKSC

Posttranslational lanthionine rings: S1-C4, S11-C12.

IGF II 69-84:

(C7)-Sequence: DVSTPPCVLPDNFPRY (SEQ ID NO:)

Posttranslational lanthionine ring S3-C7.

15 Interleukin-8 fragment:

(S6, C10)-Sequence: AVLPRSAKEC (SEQ ID NO:)

Posttranslational lanthionine ring S6-C10

Function: attraction neutrophils, basophils and T-cells, but not monocytes. It is involved in neutrophil activation and is released from several cell-types in response to inflammation.

20

Interleukin-2 fragment(60-70) (T-cell growth factor)

Sequence: LTFKFYMSKKC (SEQ ID NO:)

Posttranslational lanthionine ring S67-C70.

25 Leucokinin I (neuroactive peptide)

C8-Sequence: DPAFNSWC (SEQ ID NO:)

Posttranslational lanthionine ring: S6-C8.

Leukopyrokinin

C4-Sequence: TSFCPRL (SEQ ID NO:)

30 Posttranslational lanthionine ring: T1-C4

Function: mediates visceral muscle contractile activity.

Mastoparan

S5,C8-Sequence: INLKSLACLAKKIL (SEQ ID NO:)

Posttranslational lanthionine ring: S5-C8

Function: Wasp venom membrane-active toxin.

Melanin concentrating hormone

S11-Sequence: DFDMLRCMLGSVYRPCWQV (SEQ ID NO:)

Posttranslational lanthionine ring: S11-C16

- 5 Function: possible neurotransmitter, involved in the regulation of goal directed behavior.

Melittin

C14-Sequence: GIGAVLKVLTGLPCLISWIKRKRQQ (SEQ ID NO:)

Posttranslational lanthionine ring: T10-C14

- 10 Function: Bee venom membrane-active peptide.

Motilin

C9-Sequence: FVPIFTYGCLQRMQEKERNKGQ (SEQ ID NO:)

Posttranslational lanthionine ring: T6-C9

Function: regulation of interdigestive gastrointestinal motility.

- 15 Neuropeptide Y

C26-Sequence: YPSKPDNPGEDAPAEDMARYYSALRCYINLITRNR
(SEQ ID NO:)

Posttranslational lanthionine ring S22-C26

Function: control of feeding and secretion of gonadotropin-release hormone.

- 20 Osteocalcin

S4,C8-Sequence: YLYSWLGCPVPYPDPDELADHIGFQEA YRRFYGPV
(SEQ ID NO:)

Posttranslational lanthionine ring: S4-C8

- 25 Function: constitutes 1-2% of the total bone protein, it binds strongly to apatite and calcium.

(N-acetyl-)beta-endorphin 1-27

(C21)-Sequence: YGGFMTSEKSQTPLVTLFKNCIIKNAY (SEQ ID NO:)

Posttranslational methyllanthionine T16-C21

Functions: analgesia, behavioral changes, growth hormone release.

- 30 Ras oncogene related peptide

HU-ras^{ha}

(S2, C5)-Sequence: GSGGCGKS (SEQ ID NO:)

Posttranslational lanthionine ring S2-C5.

Ras oncogene related peptide

Hu-ras^{T24}

(S2, C5)-Sequence: GSVGCGKS (SEQ ID NO:)

Posttranslational lanthionine ring S2-C5.

5 Ras oncogene related peptide

Hu-(Hu-ras^{t24})-Lys

(S3, C6)-Sequence: YGSVGCGKSK (SEQ ID NO:)

Posttranslational lanthionine ring S3-C6.

10 Table 1B:

Albumin

Accession number: P02768

Sequence: DTHKSE IAHRFKDLGE EHFKGLVLIA FSQYLQQCPF
 DEHVKLVNEL TEFAKTCVAD ESHAGCEKSL HTLFGDELCK VASLRETYGD
 15 MADCCEKQEP ERNECFLSHK DDSPDLPKPK PDPNTLCDEF KADEKKFWGK
 YLYEIARRHP YFYAPELLYY ANKYNGVFQE CCQAEDKGAC LLPKIETMRE
 KVLASSARQR LRCASIQKFG ERAKAWVA RLSQKFPKAE FVEVTKLVTD
 LTKVHKECCH GDLLECADDR ADLAKYICDN QDTISSKLKE CCDKPLLEKS
 HCIAEVEKDA IPENLPPLTA DFAEDKDVCK NYQEAKDAFL GSFLYEYSRR
 20 HPEYAVSVLL RLAKEYEATL EECCAADDPH ACYSTVFDKL KHLVDEPQNL
 IKQNCDOFEK LGEYGFQNAL IVRYTRKVPQ VSTPTLVEVS RSLGKVGTRC
 CTKPESERMP CTEDYLSLIL NRLCVLHEKT PVSEKVTKCC TESLVNRRPC
 FSALTPDETY VPKAFDEKLF TFHADICTLP DTEKQIKKQT ALVELLKHKP
 KATEEQLKTV MENFVAFVDK CCAADDKEAC FAVEGPKLVV STQTALA

25 Disulfide bonds: 77-86;99-115;114-125;148-193;192-201;224-270;269-277;289-
 303;302-313;340-385;384-393;416-462;461-472;485-501;500-511;538-
 583;582-591 (numbers correspond to the precursor protein which contains 24
 amino acids more, N-terminally)

Function: regulation colloidal osmotic pressure of the blood plasma, binding blood
 30 plasma molecules.

Alglucerase

Accession number: P04062

Sequence: A RPCIPKSFY SSVVCVCNAT YCDSFDPPTF PALGTFSRYE
 STRSGRRMEL SMGPIQANHT GTGLLLTLQP EQKFQKVKGF GGAMTDAAAL
 NILALSPPAQ NLLKSYFSE EGIGYNIIRV PMASCDFSIR TYTYADTPDD
 FQLHNFSLPE EDTKLIPLI HRALQLAQRV VLLASPWTS PTWLKTNGAV
 5 NGKGSLSKGP GDIYHQTWAR YFVKFLDAYA EHKLQFWAVT
 AENEPSAGLL SGYPFQCLGF TPEHQRFIA RDLGPTLANS THHNVRLML
 DDQRLLLPWH AKVVLTDPEA AKYVHGIAVH WYLDLFLAPAK
 ATLGETHRLF PNTMLFASEA CVGSKFWEQS VRLGSWDRGM QYSHSIITNL
 LYHVVGWTDW NLALNPEGGP NWVRNFVDSP IVDITKDTF YKQPMFYHLG
 10 HFSKFIPEGS QRVGLVASQK NDLDAVALMH PDGSAVVVVL NRSSKDVPLT
 IKDPAVGFLE TISPGYSIHT YLWHRQ

Function: glucosylceramidase.

Alpha-galactosidase

15 Accession number: P06280

Sequence: LDNGLARTP TMGWLHWERF MCNLDCQEEP DSCISEKLFM
 EMAELMVSEG WKDAGYEYLC IDDCWMAPQR DSEGRLQADP
 QRFPHGIRQL ANYVHSKGLK LGIYADVGNK TCAGFPGSFG YYDIDAQTFA
 DWGVDLLKFD GCYCDSLENL ADGYKHMSLA LNRTGRSIVY
 20 SCEWPLYMWP FQKPNYTEIR QYCNHWRNFA DIDDSWKSIIK SILDWTSFNQ
 ERIVDVAGPG GWNDPDMLVI GNFGLSWNQQ VTQMALWAIM
 AAPLFMSNDL RHISPAKAL LQDKDVIAIN QDPLGKQGYQ LRQGDNFVW
 ERPLSGLAWA VAMINRQEIG GPRSYTIAVA SLGKGVACNP ACFITQLLPV
 KRKLGFEYEWTSRLRSHINPT GTVLLQLENT MQMSLKDLL

25 Function: galactosidase.

Alteplase

Accession number: P00750

Sequence: SYQVI CRDEKTQMIY QHQSWLRPV LRSNRVEYCW
 CNSGRAQCHS VPKSCSEPR CFNGGTCQQA LYFSDFVCQC PEGFAGKCCE
 30 IDTRATCYED QGISYRGTWS TAESGAECTN WNSSALAQKP YSGRRPDAIR
 LGLGNHNYCR NPDRDSKPWC YVFKAGKYSS EFCSTPACSE GNSDCYFGNG
 SAYRGTHSLT ESGASCLPWN SMILIGKVYT AQNPSAQALG LGKHNYCRNP
 DGDAKPWCHV LKNRRLTWEY CDVPCSTCG LRQYSQPQFR IKGGLFADIA
 SHPWQAAIFA KHRRSPGERF LCGGILISSC WILSAAHCFQ ERFPPHHLTV

ILGRTYRVVP GEEEQKFEVE KYIVHKEFDD DTYDNDIALL QLKSDSSRCA
 QESSVVRTVC LPPADLQLPD WTECELSGYG KHEALSPFYS ERLKEAHVRL
 YPSSRCTSQH LLNRTVTDNM LCAGDTRSGG PQANLHDACQ GDSGGPLVCL
 NDGRMTLVGI ISWGLGCGQK DVPGVYTKVT NYLDWIRDNM RP

5

Disulfide: 41-71; 69-78; 86- 97; 91-108;110-119; 127-208; 148 -190; 179-203; 215-
 296;236-278; 267-291; 299-430; 342-358; 350-419; 444-519; 476-492;509-
 537 (counted with 35 additional N-terminal aa)

Function: cleaves plasminogen to form plasmin

10

Antithrombin III

Accession number: P01008

Sequence: HGSPVDIC TAKPRDIPMN PMCIYRSPEK KATEDEGSEQ
 KIPEATNRRV WELSKANSRF ATTFYQHLAD SKNDNDNIFL SPLSISTAFA
 15 MTKLGACNDT LQQLMEVFKF DTISEKTSQ IHHFFAKLNC RLYRKANKSS
 KLVSANRLFG DKSLTFNETY QDISELVYGA KLQPLDFKEN AEQSRAAINK
 WVSNKTEGRI TDVIPSEAIN ELTVLVLVNT IYFKGLWWSK FSPENTRKEL
 FYKADGESCS ASMMYQEGKF RYRRVAEGTQ VLELPFKGDD ITMVLILPKP
 EKSLAKVEKE LTPEVLQEWL DELEEMMLVV HMPRFRIEDG FSLKEQLQDM
 20 GLVDLFSPEK SKLPGIVAEG RDDLYVSDAF HKAFLEVNEE GSEAAASTAV
 VIAGRSLNPN RVTFKANRPF LVFIREVPLN TIIFMGRVAN PCVK

Disulfide: 40-160;53-127; 279-462 (counted with 32 aa signal sequence)

Function: inhibition coagulation.

Aprotinin

25

Accession number: P00974
 Sequence: RPDFC LEPPYTGPK ARIIRYFYNA KAGLCQTFVY
 GGCRAKRNNF KSAEDCMRTC GGA

Disulfide: 40-90; 49-73; 65-86 (counting with 35 aa N-terminal)

Function: Inhibits trypsin, kallikrein, chymotrypsin and plasmin.

30

Asparaginase

Accession number: P20933

Sequence:
 alpha-chain: SPLPLV VNTWPFKNAT EAAWRALASG GSALDAVESG
 CAMCEREQCD GSVGFGGSPD ELGETTLDAM IMDGTTMDVG

AVGDLRRIKN AIGVARKVLE HTHTLLVGE SATTFAQSMG FINEDLSTSA
 SQALHSDWLA RNCQPNYWRN VIPDPSKYCG PYKPPGILKQ DIPIHKETED
 DRGHD

beta-chain: TIGMV VIHKTGHIAA GTSTNGIKFK IHGRVGDSP
 5 PGAGAYADDT AGAAAATGNG DILMRFLPSY QAVEYMRRGE DPTIACQKVI
 SRIQKHFPEF FGAVICANVT GSYGAACNKL STFTQFSFMV YNSEKNQPTE
 EKVDCI

Disulfide: 64-69; 163-179; 286-306; 317-345 (counted with 23 extra N-terminal aa)

Function: Cleaving glycoproteins.

10 Becaplermin

Accession number: P01127

Sequence: SLGSLTIAE PAMIAECKTR TEVFEISRRL IDRTNANFLV
 WPPCVEVQRC SGCCNNRNVQ CRPTQVQLRP VQVRKIEIVR KKPIFKKATV
 TLEDHLACKC ETVAAARPVT RSPGGSQEQR AKTPQTRVTI RTVRVRRPPK
 15 GKHRKFKHTH DKTALKETLG

Disulfide: 97-141; 130-178; 134-180;124-124 INTERCHAIN; 133-133
 INTERCHAIN. (counting with 81 aa N-terminal)

Function: growth factor from platelet.

Bone morphogenic protein 7

20 Accession number: P34819

Sequence: GKHNSAPMFM LDLYNAMAVE EGGGPAGQGF SYPYKAVFST
 QGPPLASLQD SHFLTDADMV MSFVNLVEHD KEFFHPRYHH REFRFDLSKI
 PEGEAVTAAE FRIYKDYIRE RFDNETFRIS VYQVLQEHLG RESDLFLLDS
 RTLWASEEGW LVFDITATSN HWVVNPRHNL GLQLCVETLD GQSINPK

25 Function: induces bone formation, involved in Ca regulation.

Catalase

Accession number: P04040

Sequence: ADSRDPASDQ MQHWKEQRAA QKADVLTGGA GNPVGDKLVN
 ITVGPRGPLL VQDVVFTDEM AHFDRERIPE RVVHAKGAGA FGYFEVTHDI
 30 TKYSKAKVFE HIGKKTPIAV RFSTVAGESG SADTVRDPRG FAVKFYTEDG
 NWDLVGNNTN IFFIRDPILF PSFIHSQKRN PQTHLKDPDM VWDFWSLRPE
 SLHQVSFLFS DRGIPDGHRH MNGYGSHTFK LVNANGEAVY CKFHYKTDQG
 IKNLSVEDAA RLSQEDPDYG IRDLFNAIAT GKYPSTWTFYI QVMTFNQAET
 FPFNPFDLTK VVPHKDYPLI PVGKLVNLRN PVNYFAEVEQ IAFDPSNMPP

GIEASPKML QGRLFAYPDT HRHRLGPNYL HIPVNCPYRA RVANYQRDGP
MCMQDNQGGGA PNYYPNSFGA PEQQPSALEH SIQYSGEVRR FNTANDDNVT
QVRAFVNVNL NEEQRKRLCE NIAGHLKDAQ IFIQKKAVKN FTEVHPDYGS
HIQALLDKYN AEKPKNAIHT FVQSGSHLAA REKANL

5 Function: protection against H₂O₂.

Cecropin B

Accession number: P01508

Sequence: KWKV FKKIEKMGRN IRNGIVKAGP AIAVLGEAKA LG

Function: Antibacterial.

10 Cellulase

Accession number: P23548

Sequence: MKKKGLKKTFFVIASLVMGF TLYGYTPVSA DAASVKGYYH
TQGNKIVDES GKEAAFNGLN WFGLETPNYT LHGLWSRSMD
DMLDQVKKEG YNLIRLPYSN QLFDSSSRPD SIDYHKNPDL VGLNPIQIMD

15 KLIKAGQRG IQILDRHRP GSGGQSELWY TSQYPESRWI SDWKMLADRY
KNNPTVIGAD LHNEPHGQAS WGTGNASTDW RLAAQRAGNA ILSVNPNWLI
LVEGVDHNVQ GNNSQYWWGG NLTG VANYPV VLDV PNRVVY
SPHDYGPVGS SQPWFNDPAF PSNLP AIWDQ TWGYISKQNI APVLVGEFVG
RNVDLSCPEG KWQNALVHYI GANNLYFTYW SLNPNSGDTG
20 GLLDDWTTW NRPKQDMLGR IMKPVVSVAQ QAEAAAE

Function: hydrolysis cellulose.

Choriogonadotropin alpha

Accession number: P01215

Sequence: APDVQD CPECTLQENP FFSQPGAPIL QCMGCCFSRA

25 YPTPLRSKKT MLVQKNVTSE STCCVAKSYN RVTVMGGFKV ENHTACHCST
CYYHKS

Function: A heterodimer of a common alpha chain and a unique beta chain confers
biological specificity to thyrotropin, lutropin, follitropin and gonadotropin.

Choriogonadotropin beta

30 Accession number: P01233

Sequence: SKEPLRPRCR PINATLAVEK EGCPVCITVN TTICAGYCPT
MTRVLQGVLP ALPQVVCNYR DVRFESIRLP GCPRGVNPVV SYAVALSCQC
ALCRRSTTDC GGPKDHPLTC DDP RFQDSSS SKAPPSLPS PSRLPGPSDT
PILPQ

Disulfide: 29-77; 43-92; 46-130; 54-108; 58-110; 113-120

Function: stimulates steroid production.

Chymopapain

Accession number: P14080

5 PQSID WRAKGAVTPV KNQGACGSCW AFSTIATVEG INKIVTGNLL
 ELSEQELVDC DKHSYGCKGG YQTTSLQYVA NNGVHTSKVY
 PYQAKQYKCR ATDKPGPKVK ITGYKRVPSN CETSFLGALA NQPLSVLVEA
 GGKPFQLYKS GVFDGPCGTK LDHAVTAVGY GTSDGKNYII IKNSWGPNWG
 EKGYMRLKRQ SGNSQGTCGV YKSSYYPFKG FA

10 Disulfide: 156-197; 190-229; 287-338 (counting with 134 aa N-terminal)

Function: Thiol protease.

Chymotrypsin

Accession number: P54414

15 MTTSAARKGL RTRGSACPRA TRSASSISSR AQVIVAGPIT DKLAQRTVAH
 LLALAEDSDE PINMLISSPG GHVESGDMIH DVIKfirPTV RTIGLAWVAS
 AGALIFVGAD KENRYCLPNT RFLIHQPSVG IGGTSTDMMI QAEQVRLMRD
 RLNQIFAEAT GQPVERIEKD TQRDFWLNTQ EALDYGLLGK VIRSVDELK

Function: serine protease.

Big Endothelin

20 Sequence: CSCSSLMDKECVYFCHLDIIWVNTPEHVVPYGLGSPRS

Function: endothelins are endothelium derived vasoconstrictor peptides.

Clostridium botulinum toxin type A

Accession number: Q45894

Sequence A-light chain: PFVnkQFNyK DPVNGVDIAY IKIPNAGQMQ
 25 PVKAFKIHnk IWVIPERDTF TNPEEGDLNP PPEAKQVPVS YYDSTYLSTD
 NEKDNYLkgV TKLferiYST DLGRMLLTSI VRGIPFWGGS TIDTELKVID
 TNCINVIQPD GSYRSEELNL VIIGPSADII QFECKSFGHD VLNLTRNGYG
 STQYIRFSPD FTFGFEEsLE VDTNPLLgAG KfatDPAVTL AHELihAEHR
 LYGIainPNR VFkVNTNAYY EMSGLEVSFE ELRTFGGHDA KfIDSLQENE
 30 FRlyYYnkFK DVASTLNKAK SIIGTTASLQ YMKNVFKEKY LLEDTSgKF
 SVDKlKFDKL YKMLTEI YTE DNFVNFFKVI NRKTYLNFDK AVFRINIVPD
 ENYTIKdGFN LKGANLSTNF NGQNTeINSR NFTRLKNFTG LFEfYKLLCV
 RGIIPFKTKS LDEgYNK

Sequence A-heavy chain: ALN DLCIKVNNWD LFFSPSEDNF TNDLDKVEEI
 TADTNIEAAE ENISLDLIQQ YYLTFDFDNE PENISIENLS SDIIGQLEPM
 PNIERFPNGK KYELDKYTMF HYLRAQEFEH GDSRIILTNS ABEALLKPNV
 AYTFFSSKYV KKINKAVEAF MFLNWAEEELV YDFTDETNEV TTMDKIADIT
 5 IVPYIGPAL NIGNMLSKGE FVEAIIFTGV VAMLEFIPEY ALPVFGTFAI
 VSYIANKVLT VQTINNALS K RNEKWDEVYK YTVTNWLAKV NTQIDLIREK
 MKKALENQAE ATKAIINYQY NQYTEEEKNN INFNIDDLSS KLNESINSAM
 ININKFLDQC SVSYLMNSMI PYAVKRLKDF DASVRDVLLK YIYDNRGTLV
 LQVDRLKDEV NNTLSADIPF QLSKYVDNKK LLSTFTEYIK NIVNTSILSI
 10 VYKKDDLIDL SRYGAKINIG DRVYYDSIDK NQIKLINLES STIEVILKNA
 IVYNSMYENF STSFWIKIPK YFSKINLNNE YTIINCIENN SGWKVSLNYG
 EIIWTLQDNK QNIQRVVFYK SQMVNISDYI NRWIFVTITN NRLTKSKIYI
 NGRLLIDQKPI SNLGNIHASN KIMFKLDGCR DPRRYIMIKY FNLFDKELNE
 KEIKDLYDSQ SNSGILKDFW GNYLQYDKPY YMLNLDFPNK YVDVNNIGIR
 15 GYMYLKGPRG SVVTTNIYLN STLYEGTKFI IKKYASGNED NIVRNNDRVY
 INVVVKNKEY RLATNASQAG VEKILSALEI PDVGNLSQVV VMKSKDDQGI
 RNKCKMNLQD NNGNDIGFIG FHLYDNIACL VASNWYNRQV GKASRTFGCS
 WEFIPVDDGW GESSL

Disulfide: 429-453 INTERCHAIN (BY SIMILARITY); 1234-1279

20 Function: blocking neurotransmitter release by hydrolysis of snap25.

Clostridium botulinum toxin type B

Accession number: P10844

Sequence: PVTINNFNYN DPIDNNNIIM MEPPFARGTG RYYKAFKITD
 RIWIIPERYT FGYKPEDFNK SSGIFNRDVC EYYDPDYLNT NDKKNIFLQT
 25 MIKLFNRIKS KPLGEKLLEM IINGIPYLGD RRVPLEEFNT NIASVTVNKL
 ISNPGEVERK KGIFANLIIF GPGPVLNENE TIDIGIQNHF ASREGFGGIM
 QMKFCPEYVS VFNNVQENKG ASIFNRRGYF SDPALILMHE LIHVLHGLYG
 IKVDDLPIVP NEKKFFMQST DAIQAEELYT FGGQDPSIIT PSTDKSIYDK
 VLQNFGRGIVD RLNKVLVCIS DPNININIYK NKFKDKYKQV EDSEGKYSID
 30 VESFDKLYKS LMGFTETNI AENYKIKTRA SYFSDSLPPV KIKNLLDNEI
 YTIEEGFNIS DKDMEKEYRG QNKAINQAY EEISKEHLAV YKIQMCKSVK
 APGICIDVDN EDLFFIADKN SFSDDLKNE RIEYNTQSNY IENDFPINEL
 ILDTDLISKI ELPSNTESL TDFNVDVPVY EKQPAIKKIF TDENTIFQYL
 YSQTFLDIR DISLTSSFDD ALLFSNKVYS FFSMDYIKTA NKVVEAGLFA

GWVKQIVNDF VIEANKSNTM DKIADISLIV PYIGLALNVG NETAKGNFEN
 AFEIAGASIL LEFPELLIP VVGAFLLSEY IDNKNKIKT IDNALTKRNE
 KWSDMYGLIV AQWLSTVNTQ FYTIKEGMYK ALNYQAQALE EIIKYRYNIY
 SEKEKSNINI DFNDINSKLN EGINQAIDNI NNFINGCSVS YLMKKMIPLA
 5 VEKLLDFDNT LKKNLLNYID ENKLYLIGSA EYEKSKVNKY LKTIMPFDLN
 IYTNDTILIE MFNKYNSEIL NNILNLRYK DNNLIDLSGY GAKVEVYDGV
 ELNDKNQFKL TSSANSKIRV TQNQNIIFNS VFLDFSVSFW IRIPKYKNDG
 IQNYIHNEYT IINCMKNNSG WKISIRGNRI IWTLIDINGK TKSVEFFEYNI
 REDISEYINR WFFVTITNNL NNAKIYINGK LESNTDIKDI REVIANGEII
 10 FKLDGDDIRT QFIWMKYFSI FNTELSQSNI EERYKIQSYS EYLKDFWGNP
 LMYNKEYYMF NAGNKNSYIK LKKDSPVGEI LTRSKYNQNS KYINYRDLYI
 GEKFIIRKKS NSQSINDDIV RKEDYIYLDL FNLNQEWRVY TYKYFKKEEE
 KLFLAPISDS DEFYNTIQIK EYDEQPTYSC QLLFKKDEES TDEIGLIGIH
 RFYESGIVFE EYKDYFCISK WYLKEVKRKP YNLKLGCNWQ FIPKDEGWTE
 15 Disulfide: 436-445 INTERCHAIN (PROBABLE).

Function: endopeptidase that cleaves synaptobrevin-2 and thus blocks neurotransmission.

Collagen

Accession number: P30754

20 Sequence: YRAGPRYIQA QVGPIGPRGP PGPPGSPGQQ GYQGLRGEPG
 DSGPMGPIGK RGPPGPAGIA GKSGDDGRDG EPGPRGGIGP MGPRGAGGMP
 GMPGPXGHRG FRGLSGSXGE QGKSGNQGPD GGPGPAGPSG PIGPRGQTGE
 RGRDGKSGLP GLRGVDGLAG PPGPPPIGS TGSPGFPGTP GSKGDRGQSG
 IXGAQGLQGP VGLSGQPGVA GENHHPGMPG MDGANGEPGA SGESGLPGPS
 25 GFPGPRGMPG TAGSPGQAGA XGDGGPTGEQ GRPGAPGVXG
 SSGPPGDVGA PGHAGEAGKR GSPGSPGAG SPGPQGDRGL PGSRGLPGMT
 GASGAMGIPG EKGPSGEPGA KGPTGDTGRQ GNQGTPGIAG LPGNPGSDGR
 PGKDGRPGIR GKDGKQGEQG PQGPQGLAGL QGRAGPPGAR
 GEPGKNGAPG EPGAHQEQGD AGKDGETGAA GPPGAAGPTG
 30 ARGPPGPRGQ QGFQGLAGA QTPGEAGKTG ERGAVGATGP SGPAGPGER
 GAPGDRGNVG PRGMGERGA TGPAGPTGSP GVAGAKGQGG
 PPGPAGLVGL PGERGPKGVG GSXGSRGDIG PRGKAGERGK DGERGERGEN
 GLPGPSGLAA SXGERGDMGS PGERGSPGPA GERGPAGSQG IQGQPGPPGD
 AGPAGTXGDI GFPGERGTRG ATGKQGARGP RGLAGKRGLR GAGGSRGETG

AQGEIGLPGS PGQPGLPGPS GQPGPSGPAG TAGKQGVXGA RGSPGLVGKQ
 GDRGSDGEPG RDGTXGERGE DGPPGVSGPT GAPGQQGERG
 MPGMVGLRGE TGPMGGQGMX GDGGPPGPSG DRGERGNAGP
 QGPTGPSGQA GAPGQEGAPG KDGLPGLAGR PGERGEPGVA
 5 GRAGSQLAG LMGQRGLPGA AGPPGDRGER GEPGGQGVQG
 PVGAPGSQGP AGIMGMXGEA GGKGAXGDKG WTGLPGLQGL
 QGTPGHSGES GPPGAPGPRG ARGEAGGRGS QGPPGKDGQP GPSGRVGP
 PSRDDGRSGP PGPPGPPGPP GNSDYGA

Function: fibril formation

10 Collagenase

Accession number: P08897

Sequence: IINGYEAYTG LFPYQAGLDI TLQDQRRVWC GGSLIDNKWI
 LTAAHCVHDA VSVVVYLGSA VQYEGEAVVN SERIISHSMF NPDTYLNDVA
 LIKIPHVEYT DNIQPIRLPS GEELNNKFEN IWATVSGWGQ SNTDTVILQY
 15 TYNLVIDNDR CAQEYPPGII VESTICGDTG DGKSPCFGDS GPFVLSDKN
 LLIGVVSFVS GAGCESGKPV GFSRVTSYMD WIQQNTGIIF

Disulfide: 60-76; 181-196; 206-234

Function: Serine protease.

Corticotropin, ACTH

20 Accession number: P01189

Sequence: WCLE SSQCQDLTTE SNLLECIRAC KPDLAETPM FPGNGDEQPL
 TENPRKYVMG HFRWDRFGRR NSSSSGSSGA GQKREDVSAG EDCGPLPEGG
 PEPRSDGAKP GPREGKRSYS MEHFRWGKPV GKKRRPVKVY PNGAEDESAE
 AFPLEFKREL TGQRLREGDG PDGPADDGAG AQADLEHSLV VAAEKKDEGP
 25 YRMEHFRWGS PPKDKRYGGF MTSEKSQTPL VTLFKNAIIK NAYKKKGE

Disulfide: 28-50 (counting with 26 aa signal)

Function: melanocyte stimulation.

Dornase alfa

Accession number: P24855

30 LKIAAFNI QTFGETKMSN ATLVSYIVQI LSRVDIALVQ EVRDSHLTAV
 GKLLDNLNQG APDTYHYVVS EPLGRNSYKE RYLFVYRPDQ
 VSAVDSYYYD DGCEPCGNDT FNREPAIVRF FSRFTEVREF AIVPLHAAPG
 DAVAEIDALY DVYLDVQEKW GLEDVMLMGD FNAGCSYVRP

SQWSSIRLWT SPTFQWLIPD SADTTATPTH CAYDRIVVAG MLLRGAVVPD
SALPFNFQAA YGLSDQLAQA ISDHYPVEVM LK

Disulfide: 123-126; 195-231 (counted with 21 aa extra N-terminal)

Function: endonucleolytic, binds G-actin.

5 Eptacog alpha (factor VII)

Accession number: P08709

Sequence: NAFLEELRP GSLERECKEE QCSFEEAREI FKDAERTKLF
WISYSDGDQC ASSPCQNGGS CKDQLQSYIC FCLPAFEGRN CETHKDDQLI
CVNENGGCEQ YCSDHTGTKR SCRCHEGYSL LADGVSTPT VEYPCGKIPI
10 LEKRNASKPQ GRIVGGKVCP KGECPWQVLL LVNGAQLCGG TLINTIWWVS
AAHCFDKIKN WRNLI AVLGE HDLSEHDGDE QSRRVAQVII PSTYVPGTTN
HDIALRLHQ PVVLT DHVVP LCLPERTFSE RTLAFVRFSL VSGWGQLLDR
GATALELMVL NVPRLMTQDC LQQRKVGDS PNITEYMFCA GYSDGSKDSC
KGDSGGPHAT HYRGTWYLTG IVSWGQGCAT VGHFGVYTRV

15 SQYIEWLQKL MRSEPRPGVL LRAPFP

Disulfide: 77-82; 110-121; 115-130; 132-141; 151-162; 158-172; 174-187; 195-322;
219-224; 238-254; 370-389; 400-428 (counted with 61 aa N-terminally)

Function: coagulation.

Etanercept

20 Accession number: P20333

Sequence: LPAQVAFT PYAPEPGSTC RLREYYDQTA QMCCSKCSPG
QHAKVFCTKT SDTVCDSCED STYTQLWNWV PECLSCGSRC SSDQVETQAC
TREQNRICTC RPGWYCALS K QEGCRLCAPL RKC RPFGVA R PGTETSDVV
CKPCAPGTFS NTSSTDICR PHQICNVVAI PGNASRDAVC TSTSPTRSMA
25 PGAVHLPQPV STRSQHTQPT PEPSTAPSTS FLLPMGPSPP AEGSTGDFAL
PVGLIVGVTA LGLLIIGVVN CVIMTQVKKK PLCLQREAKV PHLPADKARG
TQGPEQQHLL ITAPSSSSSS LESSASALDR RAPTRNQPQA PGVEASGAGE
ARASTGSSDS SPGGHGTQVN VTCIVNVCSS SDHSSQCSSQ ASSTMGDTDS
SPSESPKDEQ VPFSKEECAAF RSQLETPETL LGSTEEKPLP LGVPDAGMKP S

30 Disulfide: 40-53; 54-67; 57-75; 78-93; 96-110; 100-118; 120-126; 134-143; 137-161;
164 179 (counted with 22 aa extra N-terminally)

Function: receptor TNF-alpha.

Erythropoietin

Accession number: P01588

Sequence: APP RLICDSRVLE RYLLEAKEAE NITGCAEHC SLNENITVPD
TKVNFYAWKR MEVGQQAQVEV WQGLALLSEA VLRGQALLVN
SSQPWEPLQL HVDKAVSGLR SLTLLRALG AQKEAISPPD AASAAPLRTI
TADTFRKLFR VYSNFLRGKL KLYTGEACRT GDR

5 Disulfide: 34-188; disulfide: 56-60 (counted with 27 aa N-terminally)

Erythropoietin fragment:

Sequence:

YASHFGPLGWVCK

Posttranslational lanthionine ring: S3-C12

10 Erythropoietin fragment2:

Sequence:

YASHFGPLTWVCK

Posttranslational lanthionine ring: S3-C12

Function: erythroipoiese.

15 Exendin-4

Accession number: P26349

Sequence: MPVESGL SSEDSSASSES FASKIKRHGE GTFTSDLSKQ
MEEEAURLFI EWLKNGGPSS GAPPPSG

20 86 AMIDATION (G-87 PROVIDE AMIDE GROUP). (counted with 23 aa signal N-terminally)

Function: secretin-like.

Factor VIII

Accession number: P00451

Sequence: A TRRYYLGAVE LSWDYMQSDL GELPVDARFP PRVPKSFPEF
25 TSVVYKKTFL VEFTDHLFNI AKPRPPWMGL LGPTIQAEVY DTVVITLKNM
ASHPVSLHAV GVSYWKASEG AEYDDQTSQR EKEDDKVFPG
GSHTYVWQVL KENGPMSADP LCLTYSYLSH VDLVKDLNSG LIGALLVCRE
GSLAKEKTQT LHKFILLFAV FDEGKSWHSE TKNSLMQDRD
AASARAWPKM HTVNGYVNRS LPGLIGCHRK SVYWHVIGMG TTPEVHSIFL
30 EGHTFLVRNH RQASLEISPI TFLTAQTLLM DLGQFLLFCH ISSHQHDGME
AYVKVDSCPE EPQLRMKNNE EAEDYDDDLT DSEMDVVRFD DDNSPSFIQI
RSVAKKHPKT WWHYIAAEEE DWDYAPLVLA PDDRSYKSQY LNNGPQRIGR
KYKKVRFMAY TDETFKTREA IQHESGILGP LLYGEVGDTL LIFKNQASR
PYNIYPHGIT DVRPLYSRRL PKGVKHLKDF PILPGEIFKY KWTVTVEDGP

TKSDPRCLTR YYSSFVNMER DLASGLIGPL LICYKESVDQ RGNQIMSDKR
NVILFSVFDE NRSWYL TENI QRFLPNPAGV QLEDPEFQAS NIMHSINGYV
FDSLQLSVCL HEVAYWYILS IGAQTDFLSV FFSGYTFKHK MVYEDTLTLF
PFSGETVFMS MENPGLWILG CHNSDFRNRG MTALLKVSSC DKNTGDYYED
5 SYEDISAYLL SKNNAIEPRS FSQNSRHPST RQKQFNATTI PENDIEKTD
WFAHRTMPK IQNVSSDLL MLLRQSPTPH GLSLSDLQEA KYETFSDDPS
PGAIDSNNSL SEMTHFRPQL HHSGDMVFTP ESGLQLRLNE KLGTTAATEL
KKLDFKVSST SNNLISTIPS DNLAAGTDNT SSLGPPSMPV HYDSQLDTTL
FGKKSSPLTE SGGPLSLSEE NNDSKLLESG LMNSQESSWG KNVSSSTESGR
10 LFKGKRAHGP ALLTKDNALF KVSISLLKTN KTSNNSATNR KTHIDGPSLL
IENSPSVWQN ILES DTEFKK VTPLIHDRML MDKNATALRL NHMSNKTTSS
KNMEMVQQKK EGPIPPDAQN PDMSFFKMLF LPESARWIQR THGKNSLNSG
QGSPKQLVS LGPEKSV EQ NFLSEKNKV VVGKGEFTKDV GLKEMVFPSS
RNLFLTNDN LHENNTHNQE KKIQEEIEKK ETLIQENVVL PQIHTVTGTK
15 NFMKNLFLS TRQNVESYD GAYAPVLQDF RSLNDSTNRT KKHTAHFSKK
GEEENLEGLG NQTKQIVEKY ACTTRISPNT SQQNFVTQRS KRALKQFRP
LEETELEKRI IVDDTSTQWS KNMKHLTPST LTQIDYNEKE KGAITQSPLS
DCLTRSHSIP QANRSPLPIA KVSSFPSIRP IYLTRVLFQD NSSHLPAASY
RKKDSGVQES SHFLQGAKKN NLSLAILTLE MTGDQREVG S LGTSATNSVT
20 YKKVENTVLP KPDLPKTS GK VELLPKVHIY QKDLFPTETS NGSPGHLDLV
EGSLLQGTEG AIKWNEANRP GKVPFLRVAT ESSAKTPSKL LDPLAWDNHY
GTQIPKEEWK S QEK SPEKTA FKKKDTILSL NACESNHAIA AINEGQNKPE
IEVTWAKQGR TERLCSQNPP VLKRHQREIT RTTLQSDQEE IDYDDTISVE
MKKEDFDIYD EDENQSPRSF QKKTRHYFIA AVERLWDYGM SSSPHVLRNR
25 AQS GSVPQFK KVV FQEFTDG SFTQPLYRGE LNEHLGLLGP YIRAEVEDNI
MVTFRNQASR PYSFYSSLIS YEEDQRQGA E PRKNFVKPNE TKTYFWKVQH
HMAPTKDEFD CKAWAYFSDV DLEKDVHSGL IGPLL VCHTN
TLNPAHGRQV TVQE FALFFT IFDETKSWYF TENMERN CRA PCNIQMEDPT
FKENYRFHAI NGYIMDTLPG LVMAQDQRIR WYLLSMGSNE NIHSIHFSGH
30 VFTVRKKEEY KMALYNLYPG VFETVEM LPS KAGIWRVECL IGEHLHAGMS
TLFLVYSNKC QTPLGMASGH IRDFQITASG QYGQWAPKLA RLHYS GSINA
WSTKEPFSWI KVDLLAPMII HGIKTQGARQ KFSSLYISQF IIMYSLDGKK
WQTYRGNSTG TLMVFFGNVD SSGIKHNIFN PPIARYIRL HPTHYSIRST
LRMELMGCDL NSCSMPLGME SKAISDAQIT ASSYFTNMFA TWSPSKARLH

LQGRSNAWRP QVNNPKEWLQ VDFQKTMKVT GVTTQGVKSL
 LTSMYVKEFL ISSSQDGHQW TLFFQNGKVK VFQGNQDSFT PVVNSLDPPL
 LTRYLRIHPQ SWVHQIALRM EVLGCEAQDL Y

Disulfide: 172-198;547-573; 1851-1877; 2040-2188; 2193-2345 (counted with 19 aa

5 extra N-terminally)

Function: coagulation.

Factor IX

Accession number: P00740

Sequence:

10 *Light chain:* NSG KLEEFVQGNL ERECMEEKCS FEEAREVFEN
 TERTTEFWKQ YVDGDQCESN PCLNGGCKD DINSYECWCP FGFEGKNCEL
 DVTCNIKNGR CEQFCKNSAD NKVVCSCTEG YRLAENQKSC EPAVPFPCGR
 VSVSQTSKLT R

Heavy chain: AEAVFPDVD YVNSTEAETI LDNITQSTQS FNDFTRVVG
 15 EDAKPGQFPW QVVLNGKVDA FCGGSIVNEK WIVTAAHCVE
 TGVKITVVAG EHNIEETEHT EQKRVIRII PHHNYNAAIN KYNHDIALLE
 LDEPLVLNSY VTPICIADKE YTNIFLKFGS GYVSGWGRVF HKGRSALVLQ
 YLRVPLVDRA TCLRSTKFTI YNNMFCAGFH EGGRDSCQGD SGGPHVTEVE
 GTSFLTGIIS WGEECAMK GK YGIYTKVSRV VNWIKKTKL T

20 Disulfide: 64-69; 97-108; 102-117; 119-128; 134-145; 141-155; 157-170 (counted in
 the precursor)

Function: coagulation.

Factor X

25 Accession number: P00742

Sequence:

Light chain: ANSFLEEMKK GHLERECMEE TCSYEEAREV FEDSDKTNEF
 WNKYKDGQDQC ETSPCQNQGK CKDGLGEYTC TCLEGFEGKN CELFTRKLC
 LDNGDCDQFC HEEQNSVVCS CARGYTLADN GKACIPTGPY PCGKQTLER

30 *Heavy chain:* R KRSVAQATSS SGEAPDSITW KPYDAADLDP TENPFDLLDF
 NQTQPERGDN NLTRIVGGQE CKDGECPWQA LLINEENEGF CGGTILSEFY
 ILTAAHCLYQ AKRFKVRVGD RNTEQEEGGE AVHEVEVVIK HNRFTKETYD
 FDI AVLRLKT PITFRMNVAP ACLPERDWAE STLMTQKTGI VSGFGRTHEK
 GRQSTRKML EVPYVDRNSC KLSSSFITQ NMFCAGYDTK QEDACQGDSG

GPHVTRFKDT YFVTGIVSWG EGCARKGKYG IYTKVTAFLK WIDRSMKTRG
LPKAKSHAPE VITSSPLK

Disulfide: 90-101; 95-110; 112-121; 129-140; 136-149; 151-164; 172-342; 241-246;
261 277; 390-404; 415-443 (counted with 40 aa signal sequence)

- 5 Function: coagulation, factor Xa (part of factor X-heavy chain) is a vitamin K-dependent glycoprotein that converts prothrombin into thrombin in the presence of amongst others anionic phospholipid.

Factor XIII

Accession number: P00488

- 10 Sequence: VN LQEFLNVT SV HLFKERWDTN KVDHHTDKYE NNKLIVRRGQ
SFYVQIDFSR PYDPRRDLFR VEYVIGRYPQ ENKGTYPVP IVSELQSGKW
GAKIVMREDR SVRLSIQSSP KCTVGGKFRMY VAVWTPYGV L RTSRNPETDT
YILFNPWCED DAVYLDNEKE REEYVLNDIG VIFYGEVNDI KTRSWSYGQF
EDGILDTCLY VMDRAQMDLS GRGNPIKVS R VGSAMVNAKD
15 DEGVLVGSWD NIYAYGVPPS AWTGSVDILL EYRSEN PVR YGQCWVFAGV
FNTFLRCLGI PARIVTNYFS AHDNDANLQM DIFLEEDGNV NSKLTKDSVW
NYHCWNEAWM TRPDLPVGFG GWQAVDSTPQ ENSDGM YRCG
PASVQAIKHG HVC FQFDAPF VFAEVNSDLI YITAKKDGTH VVENVDATHI
GKLI VTKQIG GDGMMDITDT YKFQEGQEEE RLALETALMY GAKKPLNTEG
20 VMKSR SNVDM DFEVENAVLG KDFKLSITFR NNSHNRYTIT AYLSANITFY
TGVPKAEFKK ETFDVTLEPL SFKKEAVLIQ AGEYMGQ LLE QASLHFFVTA
RINETRDVLA KQKSTVLTIP EIIKVRGTQ VVGSDMTVTV QFTNPLKETL
RNVVHLDGP GVTRPMKKMF REIRPNSTVQ WEEVCRPWVS
GHRKLIASMS SDSL RHVYGE LDVQIQRRPS M

- 25 Function: coagulation, indirectly stabilizing fibrin chains.

Fibronectin

Accession number: P02751

- Sequence: QAQQMVQPQ SPVAVSQSKP GCYDNGKHYQ INQQWERTYL
GNALVCTCYG GSRGFNCESK PEAEETCFDK YTGNTYRVGD TYERP KDSMI
30 WDCTCIGAGR GRISCTIANR CHEGGQSYKI GDTWRRPHET GGYMLECVCL
GNGKGEWTCK PIAEKCFDHA AGTSYVVGET WEKPYQG WMM
VDCTCLGEGS GRITCTSRNR CNDQDTRTSY RIGDTWSKKD NRGNLLQCIC
TGNGRGEWKC ERHTSVQTTS SGSGPFTDVR AAVYQPQPHP QPPPYGHCVT
DSGVVYSVGM QWLKTQGNKQ MLCTCLGNGV SCQETA VTQT

YGGNSNGEPC VLPFTYNGRT FYSC TTEGRQ DGHLWCSTTS NYEQDQKYSF
 CTDHTVLVQT QGGNSNGALC HFPFLYNNHN YTDCTSEGRR
 DNMKWC GTTQ NYDADQKFGF CPMAAHEEIC TTNEGV MYRI
 GDQWDKQHDM GHMMRCTCVG NGRGEWTCIA YSQLRDQCIV
 5 DDITYNVNDT FHKRHEEGHM LNCTCFGQGR GRWKCDPVDQ
 CQDSETGTFY QIGDSWEKYV HGVRYQCYCY GRGIGEWHCQ PLQTYPSSSG
 PVEVFITETP SQPN SHPIQW NAPQPSHISK YILRWRPKNS VGRWKEATIP
 GHLNSYTIKG LKPGVVYEGQ LISIQQYGHQ EVTRFDFTTT STSTPVTSNT
 VTGETTPFSP LVATSESVTE ITASSFVVS W VSASDTVSGF RVEYELSEEG
 10 DEPQYLDLPS TATSVNIPDL LPGRKYIVNV YQISEDGEQS LILSTSQT TA
 PDAPPDPTVD QVDDTSIVVR WSRPQAPITG YRIVYSPSVE GSSTELNLPE
 TANSVTLSDL QPGVQYNITI YAVEENQEST PVVIQQUETT G TPRS DTVPS P
 RDLQFVEVTD VKVTIMWTPP ESAVTGYRVD VIPVNLPGEH GQRLPISRNT
 FAEVTGLSPG VTY YFKVFAV SHGRESKPLT AQTTKLDAP TNLQFVNETD
 15 STVLVRWTPP RAQITGYRLT VGLTRRGQPR QYNVGPSVSK YPLRNLQPAS
 EYTVSLVAIK GNQESPKATG VFTTLQPGSS IPPYNTEVTE TTIVITWTPA
 PRIGFKLGVR PSQGGEAPRE VTSDSGSIVV SGLTPGVEYV YTIQVLRDGG
 ERDAPIVNVK V TPLSPPTNL HLEANPDTGV LTVSWERSTT PDITGYRITT
 TPTNGQQGNS LEEVVHADQS SCTFDNLSPG LEYNVSVYTV KDDKESVPIS
 20 DTIIPAVPPP TDLRFTNIGP DTMRV TWAPP PSIDL TNFLV RYSPVKNEED
 VAELSISPSD NAVVLTNLLP GTEYVVS VSS VYEQHESTPL RGRQKTGLDS
 PTGIDFSDIT ANSFTVHWIA PRATITGYRI RHHPEHFSGR PREDRVPHSR
 NSITLTNLTP GTEYVVSIVA LNGREESPLL IGQQSTVSDV PRDLEVVAAT
 PTSLLISWDA PAVTVRY YRI TYGETGGNSP VQEFTVPGSK STATISGLKP
 25 GVDYTITVYA VTGRGDSPAS SKPISINYRT EIDKPSQM QV TDVQDNSISV
 KWL PSSSPVT GYRVTTTPKN GPGPTKTKTA GPDQTEMTIE GLQPTVEYVV
 SVY AQNPSGE SQPLVQTAVT NIDRPKGLAF TDVDVDSIKI AWESPQGQVS
 RYRVTYSSPE DGIHELFPAP DGEEDT AELQ GLRPGSEYTV SVVALHDDME
 SQPLIGTQST AIPAPTDLKF TQVTPTSLSA QWTPPNVQLT GYRVRVTPKE
 30 KTGPMKEINL APDSSSVVVS GLMVATKYEV SVYALKDTLT SRPAQGVVTT
 LENVSPPRRA RVTDATETTI TISWRKTET ITGFQVDAVP ANGQTPIQRT
 IKPDVRSYTI TGLQPGTDYK IYLYTLNDNA RSSPVVIDAS TAIDAPSNLR
 FLATTPNSLL VSWQPPRARI TGYI IKYEKP GSPPREV VPR PRPGVTEATI
 TGLEPGTEYT IYVIALKNNQ KSEPLIGRKK TDELPQLVTL PHPNLHGPEI

LDVPSTVQKT PFVTHPGYDT GNGIQLPGTS GQQPSVGQQM IFEEHGFRRR
 TPPTTATPIR HRPRPYPPNV GEEIQIGHIP REDVDYHLYP HGPGLNPNAS
 TGQEALSQTT ISWAPFQDTS EYIISCHPVG TDEEPLQFRV PGTSTSATLT
 GLTRGATYNI IVEALKDQQR HKVREEVTV GNSVNEGLNQ PTDDSCFDPY
 5 TVSHYAVGDE WERMSESGFK LLCQCLGFGS GHFRCDSRW
 CHDNGVNYKI GEKWDRQGEN GQMMSCTCLG NGKGEFKCDP
 HEATCYDDGK TYHVGEQWQK EYLGAICSCT CFGGQRGWRC
 DNCRRPGGEP SPEGTTGQSY NQYSQRYHQR TNTNVNCPIC CFMPLDVQAD
 REDSRE

10 Disulfide: 52-78; 76-87; 97-125; 123-135; 141-169; 167-179; 186-215; 213-225;
 231-260; 258-270; 308-335; 333-342; 360-386; 374-401; 420-446; 434-461;
 470-498; 496-508; 518 545; 543-555; 561-589; 587-599; 2206-2235; 2233-
 2245; 2251-2278; 2276-2288; 2295-2319; 2317-2333; 2367-2367; 2371-2371
 INTERCHAIN (WITH 2367 OF OTHER CHAIN). (counted with 31 aa extra
 15 N-terminally)

Function: wound healing, cell shape.

Fibrinogen

Accession number: P02671

Sequence: GPRVV ERHQSACKDS DWPFCSDEDW NYKCPSGCRM
 20 KGLIDEVNQD FTNRINKLKN SLFEYQKNNK DSHSLTTNIM EILRGDFSSA
 NNRDNTYNRV SEDLRSRIEV LKRKVIEKVQ HIQLLQKNVR AQLVDMKRLE
 VDIDIKIRSC RGSCSRALAR EVDLKDIEDQ QKQLEQVIAK DLLPSRDRQH
 LPLIKMKPVP DLVPGNFKSQ LQKVPPEWKA LTDMPQMRME LERPGGNEIT
 RGGSTSYGTG SETESPRNPS SAGSWNSGSS GPGSTGNRNP GSSGTGGTAT
 25 WKPGSSGPGS TGSWNSGSSG TGSTGNQNP SPRPGSTGTW NPGSSERGSA
 GHWTSESSVS GSTGQWHSES GSFRPDSPGS GNARPNNPDW GTFEEVSGNV
 SPGTRREYHT EKLVTSGDK ELRTGKEKVT SGSTTTTRS CSKTVTKTVI
 GPDGHKEVTK EVVTSEDGSD CPEAMDGLTL SGIGTLDGFR HRHPDEAAFF
 DTASTGKTFP GFFSPMLGEF VSETESRGSE SGIFTNTKES SSHHPGIAEF
 30 PSRGKSSSYS KQFTSSTSYN RGDSTFESKS YKMADEAGSE ADHEGTHSTK
 RGHAKSRPVR DCDDVLQTHP SGTQSGIFNI KLPGSSKIFS VYCDQETSLG
 GWLLIQQRMD GSLNFNRTWQ DYKRGFGSLN DEGEGEFWLG
 NDYLHLLTQR GSVLRVELED WAGNEAYAEY HFRVGSEAEG
 YALQVSSYEG TAGDALIEGS VEEGAEYTSH NNMQFSTFDR DADQWEENCA

EVYGGGWYWN NCQAANLNGI YYPGGSYDPR NNSPYEIENG
VWVVSFRGAD YSLRAVRMKI RPLVTQ

Disulfide: 47-47 INTERCHAIN (WITH C-47'); 55-55 INTERCHAIN (WITH C-95
IN BETA); 64 -64 INTERCHAIN (WITH C-49 IN GAMMA); 68-68
5 INTERCHAIN (WITH C-106 IN BETA); 180 -180 INTERCHAIN (WITH C-
165 IN GAMMA); 184-184 INTERCHAIN (WITH C-223 IN BETA); 461-
491

Function: fibrin formation, platelet aggregation.

Filgrastim

10 Accession number: P09919

Sequence: TPLGPASSLP QSFLKCLEQ VRKIQGDGAA LQEKLVSCEA
TYKLCHPEEL VLLGHSLGIP WAPLSSCPSQ ALQLAGCLSQ LHSGLFLYQG
LLQALEGISP ELGPTLDTLQ LDVADFATTI WQQMEELGMA PALQPTQGAM
PAFASAFQRR AGGVLVASHL QSFLEVSYRV LRHLAQP

15 Disulfide: 69-75; 97-107 (counted with 30 aa N-terminally)

Function: granulocyte stimulation.

Follitropin alpha

Accession number: P37036

Sequence: FPBGZFTMZG CPZCKLKZBK YFSKLGAPIY ZCMGCCFSRA
20 YPTPARSKKT MLVPKNITSZ ATCCVAKAFT KATVMGBARV ZNHTZCHCST
CYYHKS

Disulfide: 11-35; 14-64; 32-86; 36-88; 63-91

Function: follicle stimulation.

Follitropin beta

25 Accession numbers: P01225

Sequence: S CELTNITIAI EKEECRFCIS INTTWCAGYC YTRDLVYKDP
ARPKIQKTCT FKELVYETVR VPGCAHHADS LYTYPVATQC HCGKCDSDST
DCTVRGLGPS YCSFGEMKE

Disulfide: 21-69; 35-84; 38-122; 46-100; 50-102; 105-112 (counted with 18 aa N-
30 terminally)

Function: follicle stimulation.

Growth hormone releasing hormone

Accession number: P48144

Sequence: HADGLLDR ALRDILVQLS ARKYLHSLTA VRVGEEEEEDE
EDSEPLS

Function: growth hormone release.

Pituitary adenylate cyclase activating polypeptide

5 Accession number: P48144

Sequence: H SDGIFTDSYS RYRKQMAVKK YLAAVLGRRY RQRFRN
(amidation of last residue)

Function: see name.

Hyaluronidase

10 Accession number: P38567

Sequence: LNFRA PPVIPNVPFL WAWNAPSEFC LGKFDEPLDM SLFSFIGSPR
INATGQGVTI FYVDRLGYYP YIDSITGVTV NGGIPQKISL QDHLDKAKKD
ITFYMPVDNL GMAVIDWEEW RPTWARNWKP KDVYKNRSIE
LVQQQNVQLS LTEATEKAKQ EFEKAGKDFL VETIKLGKLL RPNHLWGYYL
15 FPDCYNHHYK KPGYNGSCFN VEIKRNDLDS WLWNESTALY PSIYLNTQQS
PVAATLYVRN RVREAIRVSK IPDAKSPLPV FAYTRIVFTD QVLKFLSQDE
LVYTFGETVA LGASGIVTWG TLSIMRSMKS CLLLDNYMET ILNPYIINVT
LAAKMCSQVL CQEQGVCIRK NWNSSDYHL NPDNFQIQLK KGGKFTVRGK
PTLEDLEQFS EKFCYSCYST LSCKEKADV KDTDAVDVCIA DGVCIDAFK

20 PPMETEEPQI FYNASPSTLS ATMFIVSILF LISSVASL

Function: glycosyl hydrolase.

Hirudin II

Accession number: P28504

Sequence: ITYTDCTESG QDLCLCEGSN VCGKGNKCIL GSNNGEENQCV
25 TGEGTPKPQS HNDGDFEELP EAYLQ

Disulfide: 6-14; 16-28; 22-39

Function: thrombin inhibitor.

Imiglucerase

Accession number: P04062

30 Sequence: A RPCIPKSFY SSVVCVCNAT YCDSFDPTF PALGTFSTRYE
STRSGRRMEL SMGPIQANHT GTGLLLTLQP EQKFQKVKGF GGAMTDAAAL
NILALSPPAQ NLLKSYFSE EGIGYNIIRV PMASCDFSIR TYTYADTPDD
FQLHNFSLPE EDTKLIKPLI HRALQLAQRV VLLASPWTS PTWLKTNGAV
NGKGSLLKQPP GDIYHQTWAR YFVKFLDAYA EHKLQFWAVT

AENEPSAGLL SGYPFQCLGF TPEHQRFIA RDLGPTLANS THHNVRLML
 DDQRLLLPHW AKVVLTDPEA AKYVHGIAVH WYLDFLAPAK
 ATLGETHRLF PNTMLFASEA CVGSKFWEQS VRLGSWDRGM QYSHSIITNL
 LYHVVGWTDW NLALNPEGGP NWVRNFVDSF IVDITKDTF YKQPMFYHLG
 5 HFSKFIPEGS QRVGLVASQK NDLDAVALMH PDGSAVVVVL NRSSKDVPLT
 IKDPAVGFLF TISPGYSIHT YLWHRQ

Function: Glucohydrolase.

Interleukin 2

Accession number: P01585

10 Sequence: APTSSSTKKT QLQLEHLLLD LQMILNGINN YKNPKLTRML
 TFKFYMPKKA TELKHLQCLE EELKPLEEVL NLAQSKNFHL RPRDLISNIN
 VIVLELKGSE TTFMCEYADE TATIVEFLNR WITFCQSIIS TLT

Disulfide: 78-125 (counted with 20 aa signal N-terminally)

Function: growth factor.

15 Interferon alpha-4

Accession numbers: P01562

Sequence: CDLPETH SLDNRRTLML LAQMSRISPS SCLMDRHDFG
 FPQEEFDGNQ FQKAPASVL HELIQQIFNL FTTKDSSAAW DEDLLDKFCT
 ELYQQLNDLE ACVMQEERVG ETPLMNADSI LAVKKYFRRI TLYLTEKKYS
 20 PCAWEVVRAE IMRSLSLSTN LQERLRRKE

Disulfide: 24-122; 52-162 (counted with 23 aa N-terminally)

Function: Antiviral, interferon stimulates the production of two enzymes, a protein
 kinase and an oligoadenylate synthetase.

Interferon-beta

25 Accession numbers: P01575

Sequence: INYKQLQLQ ERTNIRKQCE LLEQLNGKIN LTYRADFKIP
 MEMTEKMQKS YTAFAIQEML QNVFLVFRNN FSSTGWNETH VVRLLDDELHQ
 QTVFLKTVLE EKQEERLTWE MSSTALHLKS YYWRVQRYLK
 LMKYNSYAWM VVRAEIFRNF LIIRRLTRNF QN

30 Function: antiviral, antibacterial and anticancer.

Intrinsic factor

Accession number: P27352

Sequence: ST QTQSSCSVPS AQEPLVNGIQ VLMENSVTSS AYPNPSILIA
 MNLAGAYNLK AQKLLTYQLM SSDNNDLTIG HLGLTIMALT SSCRDPGDKV

SILQRQMENW APSSPNAEAS AFYGPSLAIL ALCQKNSEAT LPIAVRFAKT
 LLANSSPFNV DTGAMATLAL TCMYNKIPVG SEEGYRSLFG QVLKDIVEKI
 SMKIKDNGII GDIYSTGLAM QALSVTPEPS KKEWNCKKTT DMILNEIKQG
 KFHNPMISIAQ ILPSLKGKTY LDVPQVTCSP DHEVQPTLPS NPGPGPTSAS
 5 NITVIYTINN QLRGVLELFFN ETINVSVKSG SVLLVVLEEA QRKNPMFKFE
 TTMTSWGLVV SSINNIAENV NHKTYWQFLS GVTPLNEGVA DYIPFNHEHI
 TANFTQY

Disulfide: 26-246; 103-288; 143-182 (counted with 18 aa N-terminally extra)

Function: cobalamin endocytosis.

10 Invertase

Accession number: Q60115

Sequence: MFNFNASRWT RAQAMKVNKF DLTTSMPEIG TDFPIMRDDL
 WLWDTWPLRD INGNPVSFKG WNVIFSLVAD RNIPWNRHS HARIGYFYSK
 DGKSWVYGGH LLQESANTRT AEWSSGGTIMA PGSRNQVETF FTSTLFDKNG
 15 VREAVAAVTK GRIYADSEGV WFKGFDQSTD LFQADGLFYQ
 NYAENNLWNF RDPHVFINPE DGETYALFEA NVATVRGEDD IGEDEIGPVP
 ANTVVPKIDAN LCSASIGIAR CLSPDRTEWE LLPPLLTAFG VNDQMERPHV
 IFQNGLTLYF TISHDSTYAD GLTGSDGLYG FVSENGIFGP YEPLNGSGLV
 LGGPASQPTE AYAHYIMNNG LVESFINEII DPKSGKVIAG GSLAPTVRVE
 20 LQGHETFATE VFDYGYIPAS YAWPVWPFPPD RRK

Function: sucrase.

Lepirudin

Accession number: P01050

Sequence: VVYTDCTESG QNLCLCEGSN VCGQGKNCIL GSDGEKNQCV
 25 TGEGTPKPKQS HNDGDFEIP EEYLQ

Disulfides: 6-14; 16-28

Function: thrombin inhibitor.

Lutropin beta

Accession number: P01229

30 Sequence: SREPLRPWCH PINAILAVEK EGCPVCITVN TTICAGYCPT
 MMRVLQAVLP PLPQVVCTYR DVRFESIRLP GCPRGVDPVV SFPVALSCRC
 GPCRRSTSDC GGPKDHPLTC DHPQLSGLLF L

Disulfide: 29-77; 43-92; 46-130; 54-108; 58-110; 113-120

Function: stimulates synthesis of steroids.

Lysozyme

Accession number: P21270

Sequence: MDPRLREEVV RLIIALTSDN GASLSKRLQS RVSALEKTSQ
 IHSDTILRIT QGLDDANKRI IALEQSRDDL VASVSDAQLA ISRLESSIGA
 5 LQTVVNGLDS SVTQLGARVG QLETGLADV RVDHDLNVARV DTAERNIGSL
 TTELSTLTLR VTSIQADFES RISTLERTAV TSAGAPLSIR NNRITMGLND
 GLTLSGNNLA IRLPGNTGLN IQNGGLQFRF NTDQFQIVNN NLTLKTTVFD
 SINSRIGATE QSYVASAVTP LRLNSSTKVL DMLIDMSTLE INSSGQLTVR
 STSPNLRYPI ADVSGGIGMS PNYRFR

10 Function: hydrolysis peptidoglycan.

Metalloproteinase inhibitor

Accession number: P16035

Sequence: CSCS PVHPQQAFCN ADVVIRAKAV SEKEVDSGND IYGNPIKRIQ
 YEIKQIKMFK GPEKDIEFIY TAPSSAVCGV SLDVGGKKEY LIAGKAEGDG
 15 KMHITLCDFI VPWDTLSTTQ KKSLNHRYQM GCECKITRCP MIPCYISSPD
 ECLWMDWVTE KNINGHQAKF FACIKRSDGS CAWYRGAAPP KQEFLDIEDP
 Disulfides: 27-98; 29-127; 39-152; 154-201; 159-164; 172-193 (counted with 26 aa

N-terminally)

Function: inactivation protease.

20 Neurophysin

Accession number: P01185

Sequence: AMSDLELRQ CLPCGPGGKG RCFGPSICCA DELGCFVGT
 EALRCQEENY LPSPCQSGQK ACGSGRCAA FGVCCNDESC VTEPECREGF
 HRRA

25 Disulfide: 41-85; 44-58; 52-75; 59-65; 92-104; 98-116; 105-110

Function: Neurophysin binds vasopressin.

Papain

Accession number: P00784

30 Sequence: VY MGLSFGDFSI VGYSQNDLTS TERLIQLFES WMLKHNKIYK
 NIDEKIYRFE IFKDNLKYID ETNKKNNSYW LGLNVFADMS NDEFKEKYTG
 SIAGNYTTTE LSYEEVLNDG DVNIPEYVDW RQKGAVTPVK NQGSCGSCWA
 FSAVVTIEGI IKIRTGNLNE YSEQELDCD RRSYGCNGGY PWSALQLVAQ
 YGIHYRNTYP YEGVQRYCRS REKGPYAAKT DGVRQVQPYN EGALLYSIAN

QPVSVVLEAA GKDFQLYRGG IFVGPCGNKV DHAVAAVGYG PNYILIKNSW
GTGWGGENGYI RIKRGTGNSY GVCGLYTSSF YPVKN

Disulfide: 155-196; 189-228; 286-333 (counted with 18 aa N-terminally)

Function: Proteinase.

5 Pepsin

Accession number: P00790

Sequence: VDEQPLEN YLDMEYFGTI GIGTPAQDFT VVFDTGSSNL
WVPSVYCSSL ACTNHNRFNP EDSSTYQSTS ETVSITYGTG SMTGILGYDT
VQVGGISDTN QIFGLSETEP GSFLYYAPFD GILGLAYPSI SSSGATPVFD
10 NIWNQGLVSQ DLFSVYLSAD DQSGSVVIFG GIDSSYYTGS LNWVPVTVEG
YWQITVDSIT MNGEAIACAE GCQAIVDGTG SLLTGPTSPI ANIQSDIGAS
ENSDGDMVVS CSAISSLPDI VFTINGVQYP VPPSAYILQS EGSCISGFQG
MNLPTESGEL WILGDVFIHQ YFTVFDLANN QVGLAPVA

Disulfide: 107-112; 268-272; 311-344 (counted with 62 aa N-terminally)

15 Function: Peptidase.

Plasminogen

Accession number: P00747

Sequence: E PLDDYVNTQG ASLFSVTKKQ LGAGSIEECA AKCEEDEEFT
CRAFQYHSKE QQCVIMAENR KSSIIIRMRD VVLFEEKVYL SECKTGNGKN
20 YRGTMSTKN GITCQKWSST SPHRPRFSPA THPSEGLEEN YCRNPDNDPQ
GPWCYTDDPE KRYDYCDILE CEEECMHCSG ENYDGKISKI MSGLECAWD
SQSPHAHGYI PSKFPNKNLK KNYCRNPDRE LRPWCFTTDP NKRWELCDIP
RCTTPPPSSG PTYQCLKGTG ENYRGNVAVT VSGHTCQHWS AQTPTHNRT
PENFPCKNLD ENYCRNPDGK RAPWCHTTNS QVRWEYCKIP SCDSSPVSTE
25 QLAPTAPPEL TPVVQDCYHG DGQSYRGTS TTTTGKKCQS WSSMTPHRHQ
KTPENYPNAG LTMNYCRNPD ADKGPWCFTT DPSVRWEYCN
LKKCSGTEAS VVAPPVVLL PDVETPSEED CMFGNGKGYR GKRATTVTGT
PCQDWAAQEP HRHSIFTPET NPRAGLEKNY CRNPDGDVGG PWCYTTNPRK
LYDYCDVPC AAPSFDCKP QVEPKKCPGR VVGGCVAHPH
30 SWPWQVSLRT RFGMHFCGGT LISPEWVLT AHCLEKSPRP SSKVILGAH
QEVNLEPHVQ EIEVSRLFLE PTRKDIALK LSSPAVITDK VIPACLPSPN
YVVADRTECF ITGWGETQGT FGAGLLKEAQ LPVIENKVCN RYEFNLGRVQ
STELCAGHLA GGTDSCQGDS GGPLVCFEKD KYILQGVTSW GLGCARPKNK
GVYVRVSRFV TWIEGVMRNN

Disulfide: 49-73; 53-61; 103-181; 124-164; 152-176; 185-262; 188-316; 206-245;
 234-257; 275- 352; 296-335; 324-347; 377-454; 398-437; 426-449; 481-560;
 502-543; 531-555; 567-685; 577- 585; 607-623; 699-766; 729-745; 756-784

Function: Protease.

5 Protamine

Accession number: P04554

Sequence: MVRYRVRSL S ERSHEVYRQQ LHGQEQGHHG QEEQGLSPEH
 VEVYERTHGQ SHYRRRHCSR RRLHRIHRRQ HRSCRRRKRR SCRHRRRHRR
 GCRTRKRTCR RH

10 Function: histon substitution.

Prothrombin

Accession number: P12259

Sequence: AQ LRQFYVAAQG ISWSYRPEPT NSSLNLSVTS FKKIVYREYE
 PYFKKEKPQS TISGLLGPTL YAEVGDIIKV HFKNKADKPL SIHPQGIRYS
 15 KLSEGASYLD HTFPAEKMDD AVAPGREYTY EWSISEDSPG THDDPPCLTH
 IYSHENLIE DFNSGLIGPL LICKKGTLTE GGTQKTFDKQ IVLLFAVFDE
 SKSWSQSSSL MYTVNGYVNG TMPDITVCAH DHISWHLLGM SSGPELFSIH
 FNGQVLEQNH HKVSAITLVS ATSTTANMTV GPEGKWIISS LTPKHLQAGM
 QAYIDIKNCP KKTRNLKKIT REQRRHMKRW EYFIAAEEVI WDYAPVIPAN
 20 MDKKYRSQHL DNFSNQIGKH YKKVMYTQYE DESFTKHTVN
 PNMKEDGILG PIIRAQVRDT LKIVFKNMAS RPYSIYPHGV TFSPYEDEVN
 SSFTSGRNNT MIRAVQPGET YTYKWNILEF DEPTENDAQC LTRPYSDVD
 IMRDIASGLI GLLICKSRS LDRRGIQRAA DIEQQAVFAV FDENKSWYLE
 DNINKFCENP DEVKRDDPKF YESNIMSTIN GYVPESITTL GFCFDDTVQW
 25 HFCSVGTQNE ILTIHFTGHS FIYGKRHEDT LTLFPMRGES VTVTMDNVGT
 WMLTSMNSSP RSKKLRLKFR DVKCI PDDDE DSYEIFEPPE STVMATRKMH
 DRLEPEDEES DADYDYQNRL AAALGIRSFR NSSLNQEEEE FNLTALALEN
 GTEFVSSNTD IIVGSNYSSP SNISKFTVNN LAEPQKAPSH QQATTAGSPL
 RHLIGKNSVL NSSTAETHSSP YSEDPIEDPL QPDVTGIRLL SLGAGEFKSQ
 30 EHAKHKGPKV ERDQAAKHRF SWMKLLAHKV GRHLSQDTGS
 PSGMRPWEDL PSQDTGSPSR MRPWKDPPSD LLLLKQSNSS KILVGRWHLA
 SEKGSYEIIQ DTDED TAVNN WLISPQNASR AWGESTPLAN KPGKQSGHPK
 FPRVRHKS LQ VRQDGGKSRL KKSQFLIKTR KKKKEKHTHH APLSPRTFHP
 LRSEAYNTFS ERRLKHSLVL HKSNETSLPT DLNQTLPSMD FGWIASLPDH

NQNSSNDTGQ ASCPPGLYQT VPPEEHYQTF PIQDPDQMHS TSDPSHRSSS
 PELSEMLEYD RSHKSFPTDI SQMSPSSEHE VWQTVISPD L SQVTLSPELS
 QTNLSPDL SH TTLSPELIQR NLSPALGQMP ISPDLSHTTL SPDL SHTTLS
 LDLSQTNLSP ELSQTNLSPA LGQMPLSPDL SHTTLSLDFS QTNLSPELSH
 5 MTLSPELSQT NLSPALGQMP ISPDLSHTTL SLDFSQTNLS PELSQTNLSP
 ALGQMPLSPD PSHTTLSLDL SQTNLSPELS QTNLSPDLSE MPLFADLSQI
 PLTPDL DQMT LSPDLGETDL SPNFGQMSLS PDL SQVTLSP DISDTLLPD
 LSQISPPPDL DQIFYPSESS QSLLLQEFNE SFPY PDLGQM PSPSSPTLND
 TFLSKEFNPL VIVGLSKDGT DYIEIIPKEE VQSEDDYAE IDYVPYDDPY
 10 KTDVRTNINS SRDPDNIAAW YLRSNNGNRR NYIAAEEIS WDYSEFVQRE
 TDIEDSDDIP EDTTYKKVVF RKYLDSTFTK RDPRGEYEEH LGILGPIIRA
 EVDDVIQVRF KNLASRPYSL HAHGLSYEKS SEGKTYEDDS PEWFKEDNAV
 QPNSSYTYVW HATERSGPES PGSACRAWAY YSAVNPEKDI HSGLIGPLLI
 CQKGILHKDS NMPVDMREFV LLFMTFDEKK SWYYEKKSRS
 15 SWRLTSSEM K SHEFHAI NG MIYSLPGLKM YEQEWVRLHL LNIGGSQDIH
 VVHFHGQTLL ENGNKQHQLG VWPLLPGSFK TLEMKASKPG
 WWLLNTEVGE NQRAGMQTPF LIMDRDCRMP MGLSTGIISD SQIKASEFLG
 YWEPRLARLN NGGSYNAWSV EKLAAEFASK PWIQVDMQKE VIITGIQTQG
 AKHYLKSCYT TEFYVAYSSN QINWQIFKGN STRNVMYFNG NSDASTIKEN
 20 QFDPPIVARY IRISPTRAYN RPTLRLELQG CEVNGCSTPL GMENGKIENK
 QITASSFKKS WWGDYWEPFR ARLNAQGRVN AWQAKANNNK
 QWLEIDLLKI KKITAITQG CKSL SSEM YV KSYTIHYSEQ GVEWKP YRLK
 SSMVDKIFEG NTNTKGHVKN FFPPIISRF IRVIPKTWNQ SITLRLELFG
 CDIY

25 Disulfide: 167-193; 500-526; 1725-1751; 1907-2061; 2066-2221 (counted with 28 N-terminal aa)

Function: Coagulation.

Protirelin

Accession number: P20396

30 Sequence: QPEAAQ QEAVTAAEHP GLDDFLRQVE RLLFLRENIQ
 RLQGDQGEHS ASQIFQSDWL SKRQHPGKRE EEEEEGV EEEEGGAVGP
 HKRQHPGRRE DEASWSVDVT QHKRQHPGRR SPWLAYAVPK
 RQHPGRRLAD PKAQRSWEEE EEEEEEREEDL MPEKRQHPGK RALGGPCGPQ
 GAYGQAGLLL GLLDDLRSQ GAEEKRQHPG RRAAWVREPL EE

Function: thyrotropin release.

SC3

Accession number: P16933

Sequence:

5 GGHPGT TTPPVTTT VTTPPSTTTI AAGGTCTTGS LSCCNQVQSA
 SSSPV TALLG LLGIVLSDLN VLVGISC SPL TVIGVGGSGC SAQTVCCENT
 QFNGLINIGC TPINIL

Function: hydrophobin.

Sermorelin

10 Accession number: P01286

Sequence: YADAI FTNS YRKVLGQLSA RKL LQDIMSR QQGESNQERG
 ARARL

Function: growth hormone release.

15 Streptodornase

Accession number: P26295

IPPYHH NTVLAKTVSV NQTYGEYKDY YTVIGESNID QSAFPKIYKT
 TERVYKGQGT SEKRVTVSDV VYNPLDGYKR STGAYGVVTK
 DMIDMSKGYR EKWETNPEPS GWFRFYNRAD NEEISEKEYD SRRTKSYKVT
 20 NNVPVVL TTL KGKKYN SHLF VASHLFADSL GGKSIRKNAI TGTQM QNVGT
 RKGGMQYIEK KVLSHITKNP DVYVFYSAIP EYQGAELLAR SVLVSALSSD
 GVINETVRVF NTADGFNINY EKGGLLTESP VSEIDNIEDS TTDEIENSVD
 DSEEIVYNDT TTEEEEN

Function: DNase.

25 Streptokinase

Accession number: P00779

Sequence: IAGP EWLLDRPSVN NSQLVVS VAG TVEGTNQDIS LKFFEIDLTS
 RPAHGGKTEQ GLSPKSKPFA TDSGAMSHKL EKADLLKAIQ EQLIANVHSN
 DDYFEVIDFA SDATITDRNG KVFYFADKDGS VTLPTQPVQE FLLSGHVRVR
 30 PYKEKPIQNQ AKSVDVEYTV QFTPLNPDDD FRPGLKDTKL LKTLAIGDTI
 TSQELLAQAQ SILNKNHPGY TIYERDSSIV THDNDIFRTI LPMDQEFTYR
 VKNREQAYRI NKKSGLNEEI NNTDLISEKY YVLKKGEKPY DPFDRSHLKL
 FTIKYVDVDT NELLKSEQLL TASERNLDFR DLYDPRDKAK LLYNNLDAFG

IMDYTLTGKV EDNHDDTNRI ITVYMGKRPE GENASYHLAY DKDRYTEEER
 EVYSYLRYTG TPIPDNPND

Function: activating plasminogen.

Thyroglobulin

5 Accession number: P01266

Sequence: N IFEYQVDAQP LRPCQLQRET AFLKQADYVP QCAEDGSFQT
 VQCQNDGRSC WCVGANGSEV LGSRQPGRPV ACLSFCQLQK QQILLSGYIN
 STDTSYLPQC QDSGDYAPVQ CDVQQVQCWC VDAEGMEVYG

10 TRQLGRPKRC PRSCEIRNRR LLHGVGDKSP PQCSAEGEFM PVQCKFVNTT
 DMMIFDLVHS YNRFPAFVT FSSFQRRFPE VSGYCHCADS QGRELAETGL
 ELLLDEIYDT IFAGLDLPST FTETTLRYIL QRRFLAVQSV ISGRFRCPTK
 CEVERFTATS FGHPYVPSR RNGDYQAVQC QTEGPCWCVD

AQGKEMHGTR QQGEPPSCAE GQSCASERQQ ALSRLYFGTS GYFSQHDLFS
 SPEKRWASPR VARFATSCPP TIKELFVDSG LLRPMVEGQS QQFSVSENLL

15 KEAIRAIFPS RGLARLALQF TTNPKRLQQN LFGGKFLVNV GQFNLSGALG
 TRGTFNFSQF FQQLGLASFL NGGRQEDLAK PLSVGLDSNS STGTPEAAKK
 DGTMNKPTVG SFGFEINLQE NQNALKFLAS LLELPEFLLF LQHAISVPED
 VARDLGDVME TVLSSQTCEQ TPERLFVPSC TTEGSYEDVQ CFSGECWCVN
 SWGKELPGSR VRGGQPRCPT DCEKQRARMQ SLMGSQPAGS TLFVPACTSE

20 GHFLPVQCFN SECYCVDAEG QAIPGTRSAI GKPKKCPTPC QLQSEQAFLR
 TVQALLSNSS MLPTLSDTYI PQCSTDGQWR QVQCNGPPEQ VFELYQRWEA
 QNKGQDLTPA KLLVKIMSYR EAASGNFSLF IQSLYEAGQQ DVFPVLSQYP
 SLQDVPLAAL EGKRPQPREN ILLEPYLFWQ ILNGQLSQYP GSYSDFSTPL
 AHFDLRNCWC VDEAGQELEG MRSEPSKLPT CPGSCEEAKL RVLQFIRETE

25 EIVSASNSSR FPLGESFLVA KGIRLRNEDL GLPPLFPPRE AFAEQFLRGS
 DYAIRLAAQS TLSFYQRRRF SPDDSAGASA LLRSGPYMPQ CDAFGSWEPV
 QCHAGTGHCW CVDEKGGFIP GSLTARSLQI PQCPTTCEKS RTSGLLSSWK
 QARSQENPSP KDLFVPACLE TGEYARLQAS GAGTWCVDPA SGEELRPGSS
 SSAQCPSLCN VLKSGVLSRR VSPGYVPACR AEDGGFSPVQ CDQAQGSCWC

30 VMDSGEEVPG TRVTGGQPAC ESPRCPLPFN ASEVVGGTIL CETISGPTGS
 AMQQCQLLCR QGSWSVFPPG PLICSLESGR WESQLQPRA CQRPQLWQTI
 QTQGHFQLQL PPGKMCSADY AGLLQTFQVF ILDELTARGF CQIQVKTFGT
 LVSIPVCNNS SVQVGCLTRE RLGVNVTWKS RLEDIPVASL PDLHDIERAL
 VGKDLLGRFT DLIQSGSFQL HLDSKTFPAE TIRFLQGDHF GTSPTWFGC

SEGFYQVLTS EASQDGLGCV KCPEGSYSQD EECIPCPVGF YQEAGSLAC
 VPCPVGRRTTI SAGAFSQTHC VTDCQRNEAG LQCDQNGQYR ASQKDRGSGK
 AFCVDGEGRR LPWWETEAPL EDSQCLMMQK FEKVPEKVI FDANAPVAVR
 SKVPDSEFPV MQCLTDCTED EACSFFTST TEPEISCDFY AWTSDNVACM
 5 TSDQKRDALG NSKATSFGL RCQVKVRS HG QDSPAVYLKK GQGSTTTLQK
 RFEPTGFQNM LSGLYNPIVF SASGANLTD HLFCLLACDR DLCCDGFVLT
 QVQGGAIICG LLSSPSVLLC NVKDWMDPSE AWANATCPGV TYDQESHQVI
 LRLGDQEFIK SLTPLEGTQD TFTNFQQVYL WKDSDMGSRP ESMGCRKDTV
 PRPASPTAEG LTTELFSPVD LNQVIVNGNQ SLSSQKHWLF KHLFSAQQAN
 10 LWCLSRCVQE HSFCQLAEIT ESASLYFTCT LYPEAQVCD IMESNAQGCR
 LILPQMPKAL FRKKVILEDK VKNFYTRLPF QKLMGISIRN KVPMSEKSI
 NGFFECERRC DADPCCTGFG FLNVSQKGG EVTCLTLNSL GIQMCSEENG
 GAWRILDCGS PDIEVHTYPF GWYQKPIAQN NAPSFCPLVV LPSLTEKVSL
 DSWQSLALSS VVVDPSIRHF DVAHVSTAAT SNFSAVRDLC LSECSQHEAC
 15 LITTLQTQPG AVRCMFYADT QSCTHSLQGQ NCRLLLREEA THYRKPGIS
 LLSYEASVPS VPISTHGRLL GRSQAIQVGT SWKQVDQFLG VPYAAPPLAE
 RRFQAPEPLN WTGSWDASKP RASCWQPGTR TSTSPGVSED CLYLVNFIQ
 NVAPNASVLV FFHNTMDREE SEGWPAIDGS FLAAVGNLIV VTASYRVGVF
 GFLSSGSGEV SGNWGLLDQV AALTWVQTHI RGFGGDP RR
 20 SLAADRGGAD VASIHLLTAR ATNSQLFRR VLMGGSALSP AAVISHERAQ
 QQAIALAKEV SCPMSSSQEV VSCLRQKPAN VLNDAQTKLL AVSGPFHYWG
 PVIDGHFLRE PPARALKRSL WVEVDLLIGS SQDDGLINRA KAVKQFEESR
 GRTSSKTAFY QALQNSLGGE DSDARVEAAA TWYYSLEHST DDYASFSRAL
 ENATRDYFII CPIIDMASAW AKRARGNVFM YHAPENYGHG SLELLADVQF
 25 ALGLPFYPAY EGQFSLEEK LSLKIMQYFS HFIRSGNPNY PYEFSRKVPT
 FATPWPDFVP RAGGENYKEF SELLPNRQGL KKADCSFWSK YISSLKTSAD
 GAKGGQSAES EEEELTAGSG LREDLLSLQE PGSKTYSK

Function: precursor thyroid hormone.

Urokinase,

30 accession: P00749

Sequence:

MRALLARLLLCVLVVSDSKGSNELHQVPSNCDCLNGGTCVSNKYF FT
 SNIHWCNCPKKFGGQHCEIDKSKTCYEGNGHFYRGKASTDTMGRPCLPWNS
 ATV LQQTYFTHAHRSDALQLGLGKHNYCRNPDNRRRPWCYVQVGLKPLVQ

ECMVHDCADGKKPSSPPEEFTLKFQCGQKTLRPRFKIIGGEFTTIENQPWFAAI
YRRHRGGSVITYVCGGSLISPCWVISAFTTHCFIDYPPKKEDYIVYLGRSRLNSN
TQGEMKFEVENLILHKDYSADTLAHHNDIALLKIFTRSKEGRCAQPSRTIQTIC
LPSMYNDPQFGTSCEITGFGKENSTDYLYPEQLKMTVVKLIFTSHRECQPHY
5 YGSEVTTKMLCAADPQWKT DSCQGD SGGPLVCSLQGRMTLTGIVSWGRG
FT CALKDKPGVYTRVSHFLPWIRSHTK EENGLAL

Function: plasminogen activation.

Table 2, leader peptides

Table 2

5	Epicidin-280	MENKKDLFDLEIKKDNMENNNELEAQ
	Pep-5	MKNNKNLFDLEIKKETSQNTDELEPQ
	Epilancin-K7	MNNSLFDLNLNKGVETQKSDLSPQ
	Nisin-A/Z	MSTKDFNLDLVSVSKKDSGASPR
	Subtilin	MSKFDDFDLDVVKVSKQDSKITPQ
10	Epidermin	MEAVKEKNDLFDLVDKVNAKESNDSGAEPR
	Gallidermin	MEAVKEKNELFDLVDKVNAKESNDSGAEPR
	Mutacin-1140/III	
15		MSNTQLLEVLGTETFDVQEDLFAFD
		TTDTTIVASNDDPD TR
	Lacticin-481	MKEQNSFNLLQEVTESELDLILGA
	Variacin	MTNAFQALDEVTDAILGG
	Mutacin-II	MNKLNSNAVVSLENEVSDSELDLILGG
20	Streptococcin-A-FF22	MEKNNEVINSIQEVSLEELDQIIGA
	Salivaricin-A	MNAMKNSKDILNNAIEEVSEKELMEVAGG
	Sublancin	MEKLFKEVKLEELLENQKGS
	Lactocin-S	MKTEKKVLDELHSAKMGARDVESSMNAD
25	Ruminococcin A	MRNDVLTLTNPMEEKELEQILGG
	Butyrivibriocin OR79A	MNKELNALTNPIDEKELEQILGG
	Streptococcin A-M49	MTKEHEIINSIQEVSLEELDQIIGA
	Bacteriocin J46	MKEQNSFNLLQEVTESELDLILGA
	Salivaricin A1	
30		MKNSKDILTNAIEEVSEKELMEVAGG
	Streptin	MNNTIKDFDLKTNKKDTATPY
	Plantaricin-W alpha	MKISKIEAQARKDFFKKIDTNSNLLNVNGA
	Lacticin-3147A1	MNKNEIETQPVTWLEEVSDQNFDEDVFGA

Staphylococcin-C55 alpha

MKSSFLEKDIEEQVTWFEEVSEQEFDDDDIFGA

Plantaricin-W beta

MTKTSRRKNAIANYLEPVDEKSINESFGAGDPEAR

5 Lacticin-3147A2

MKEKNMKKNDTIELQLGKYLEDDMIELAEGDESHGG

Staphylococcin-C55 beta

MKNELGKFLLENELELGKFSESDMLEITDDEVYAA

Cytolysin-LL

10 MENLSVVPSFEELSVEEMEAIQGSGDVQAE

Cytolysin-LS

MLNKENQENYYSNKLELVGPSFEE

LSLEEMEAIQGSGDV QAE

Cinnamycin

15 MTASILQQSVVDADFRAALLENPAAFGASAAALPTPVEAQD

QASLDFWTKDIAATEAFA

Mersacidin

MSQEAIIRSWKDPFSRENSTQNPAGNPFSELKEAQMDKLVGAG

DNEAA

20

Claims

1. A method allowing for harvest of a desired (poly)peptide produced by a recombinant host cell comprising the steps of:

5

a) selecting a recombinant host cell comprising a first nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding said desired (poly)peptide, whereby said first and second fragment are within the same open reading frame of said first nucleic acid and said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic, and

10

b) selecting said host cell for the presence of a transporter protein commonly known as LanT or a functional equivalent thereof, and

15

c) allowing for the translation of said first nucleic acid; and

d) harvesting said desired (poly)peptide.

20

2. A method according to claim 1 further comprising harvesting said desired (poly)peptide after detecting the presence of said leader peptide in the culture medium of said cell.

25

3. A method according to claim 1 or 2 wherein said desired (poly)peptide is of essentially eukaryotic or viral descent.

4. A method according to claim 1 or 2 wherein said (poly)peptide is selected from Table 1.

30

5. A method according to anyone of claims 1 to 4 wherein said leader peptide is selected from Table 2.

6. A method according to anyone of claims 1 to 5 wherein said host cell is a Gram-negative prokaryote or an eukaryote.

35

7. A method according to anyone of claims 1 to 6 wherein said (poly)peptide has not undergone intra-cellular post-translational modification comprising dehydration of a serine or a threonine and/or thioether bridge formation.

5 8. A (poly)peptide harvested with a method according to anyone of claims 1 to 7.

9. A method allowing for modification of a desired (poly)peptide produced by a recombinant host cell, said method comprising steps a, b and c of claim 1 and
10 further comprising selecting said host cell for the presence of an enzyme capable of providing post-translational modification.

10. A method according to claim 9 allowing for extra-cellular modification of said desired (poly)peptide said method further comprising selecting said host cell
15 for the presence of an essentially extra-cellular enzyme capable of providing post-translational modification.

11. A method according to claim 9 or 10 wherein said enzyme is capable of dehydrating a serine or a threonine.

20

12. A method according to claim 9 or 10 wherein said enzyme is capable of providing for thioether bridge formation.

13. A method according to anyone of claims 9 to 12 wherein said desired
25 (poly)peptide is of essentially eukaryotic or viral descent.

14. A method according to anyone of claims 9 to 12 wherein said (poly)peptide is selected from Table 1.

30 15. A method according to anyone of claims 8 to 14 wherein said leader peptide is selected from Table 2.

16. A method according to anyone of claims 8 to 15 wherein said modification
35 comprises dehydration of a serine or a threonine and/or thioether bridge formation.

17. A method according to anyone of claims 8 to 16 wherein said host cell is a Gram-negative prokaryote or an eukaryote.

5 18. A method according to anyone of claims 8 to 17 wherein said (poly)peptide has not undergone intra-cellular post-translational modification comprising dehydration of a serine or a threonine and/or thioether bridge formation.

10 19. A method for the production of (poly)peptides comprising dehydro alanines and/or dehydro butyric acid residues comprising;

a) selecting a recombinant host cell comprising a nucleic acid coding for a peptide of interest and a nucleic acid coding for LanB or the N-terminal part of LanM and optionally a nucleic acid coding for LanT;

15

b) allowing for the translation of said nucleic acids; and

c) optionally lysing said host cells; and

20 d) harvesting said desired (poly)peptide.

20. A (poly)peptide modified with a method according to anyone of claims 8 to 19.

25 21. A (poly)peptide according to claim 20, comprising one or more dehydroalanine and/or dehydro butyric acid residues.

22. A (poly)peptide according to claim 20-21 comprising one or more thioether rings.

30

23. A host cell provided with a recombinant nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding a desired (poly)peptide, whereby said first and second fragment are within the same open reading frame of said first nucleic acid and

said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic.

24. A host cell according to claim 23 wherein said desired (poly)peptide is of
5 essentially eukaryotic or viral descent.

25. A host cell according to claim 24 wherein said (poly)peptide is selected
from Table 1.

10 26. A host cell according to anyone of claims 23 to 25 wherein said leader
peptide is selected from Table 2.

27. A host cell according to anyone of claims 23 to 26 comprising a Gram-
negative prokaryote or an eukaryote.

15

28. A host cell provided with a LanT protein and not provided with a LanB
protein or functional equivalent thereof.

29. A host cell according to anyone of claims 23 to 27 provided with a LanT
20 protein and not provided with a LanB protein or functional equivalent thereof.

30. A host cell provided with a LanT protein or functional equivalent thereof
and not provided with a LanC protein or functional equivalent thereof.

25 31. A host cell according to anyone of claims 23 to 27 provided with a LanT
protein and not provided with a LanC protein or functional equivalent thereof.

32. A host cell according to claim 30 or 31 provided with a LanB protein or
functional equivalent thereof.

30

33. A host cell of Gram-negative or eukaryotic origin provided with a LanT,
LanB, LanC, and/or LanM protein or functional equivalent thereof.

34. A host cell according to claim 27 provided with a LanT, LanB, LanC,
35 and/or LanM protein or functional equivalent thereof.

35. A recombinant nucleic acid comprising a first nucleic acid fragment encoding a leader peptide and a second nucleic acid fragment encoding a desired (poly)peptide, whereby said first and second fragment are within the same open reading frame of said first nucleic acid and said leader peptide is at least functionally equivalent to a N-terminal leader peptide found with the prepeptide of a lantibiotic, and wherein said desired (poly)peptide is of essentially eukaryotic or viral descent.
- 10 36. A nucleic acid according to claim 35 wherein said (poly)peptide is selected from Table 1.
37. A nucleic acid according to claim 35 or 36 wherein said leader peptide is selected from Table 2.

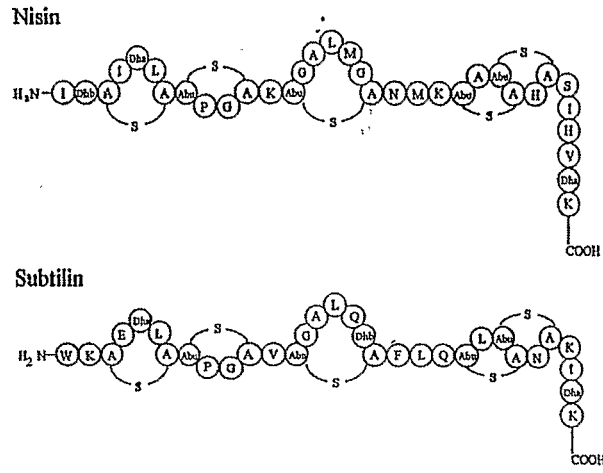


Figure 1

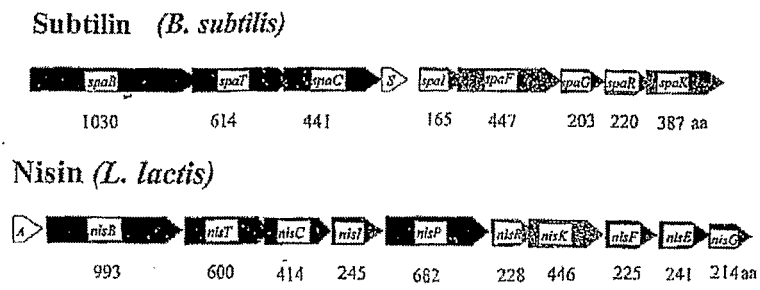


Figure 2

Nisin biosynthesis

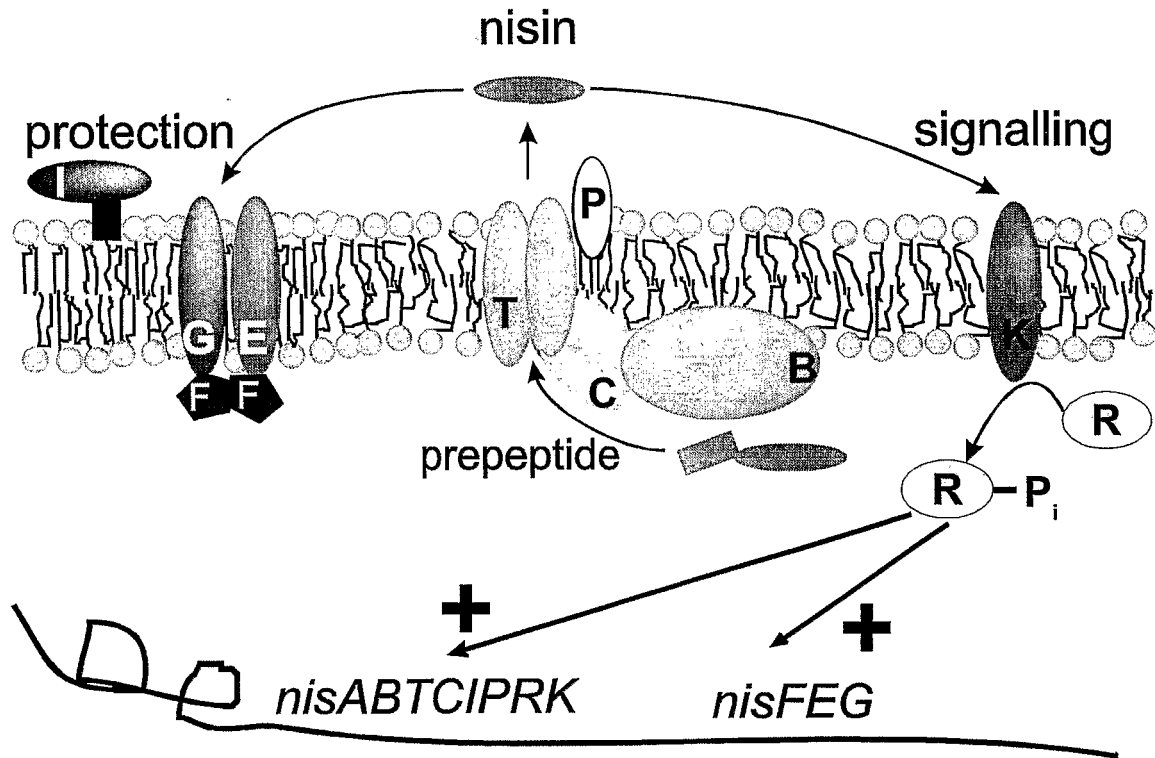


Figure 3

Nisin biosynthesis

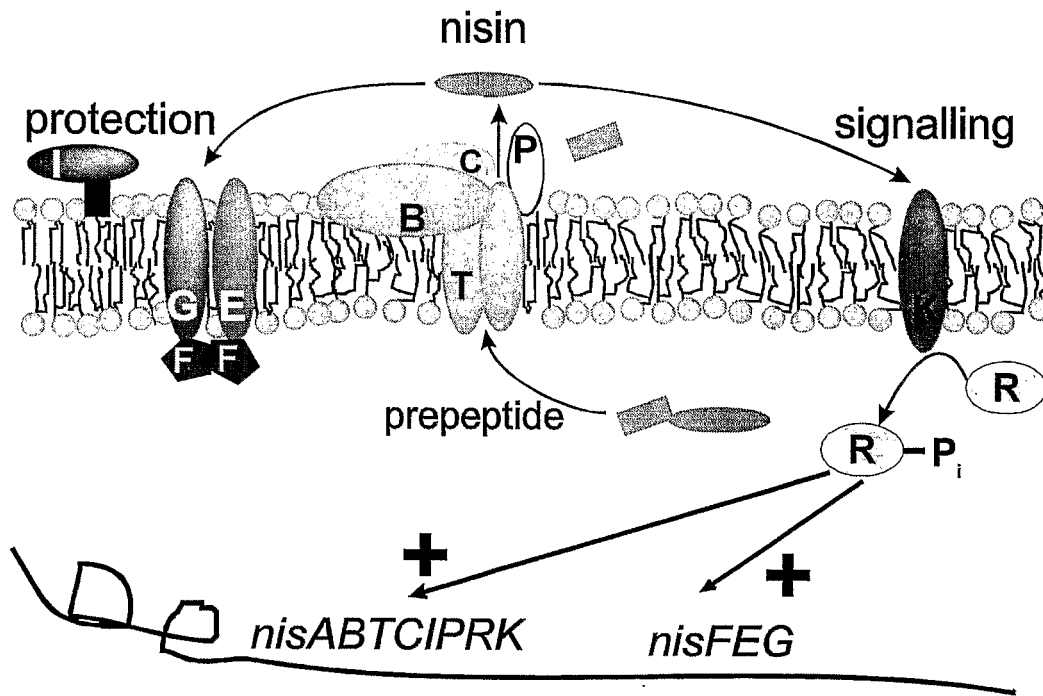


Figure 4.

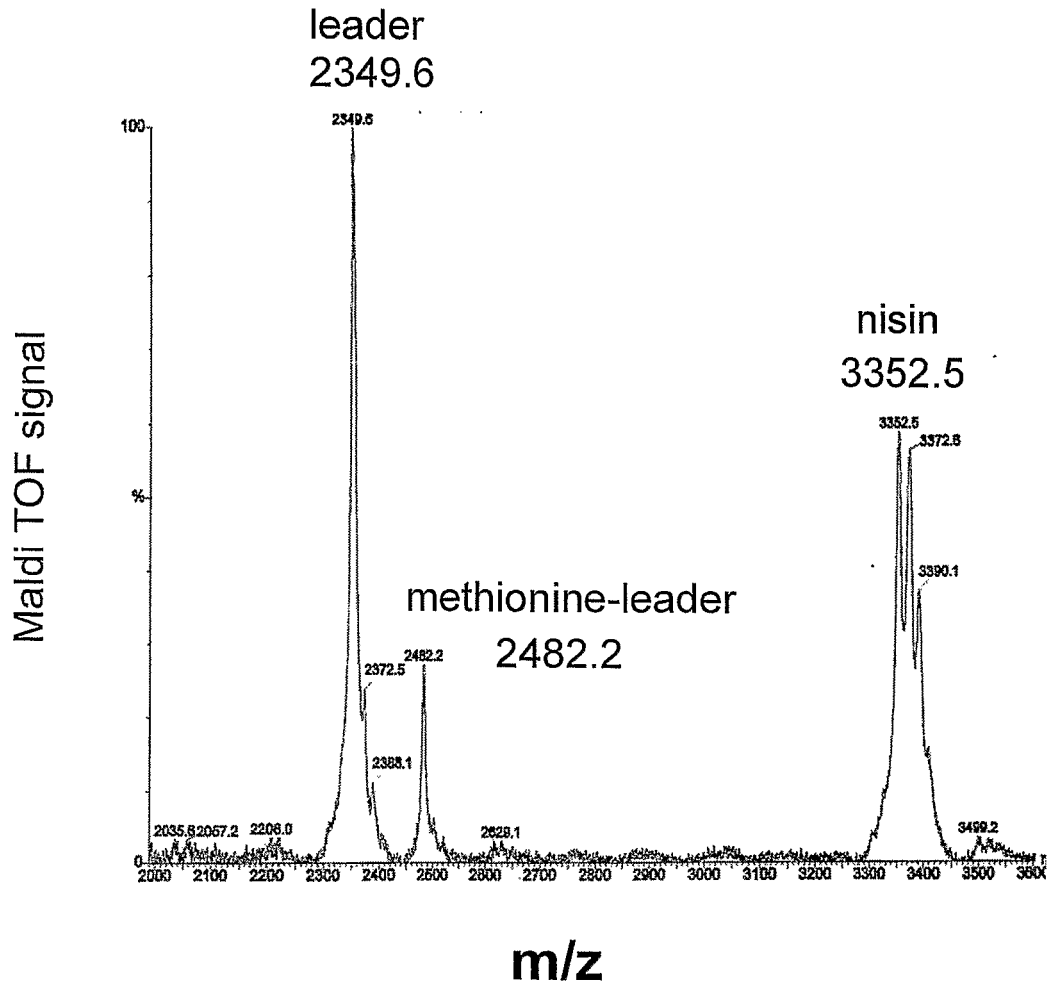


Figure 5.

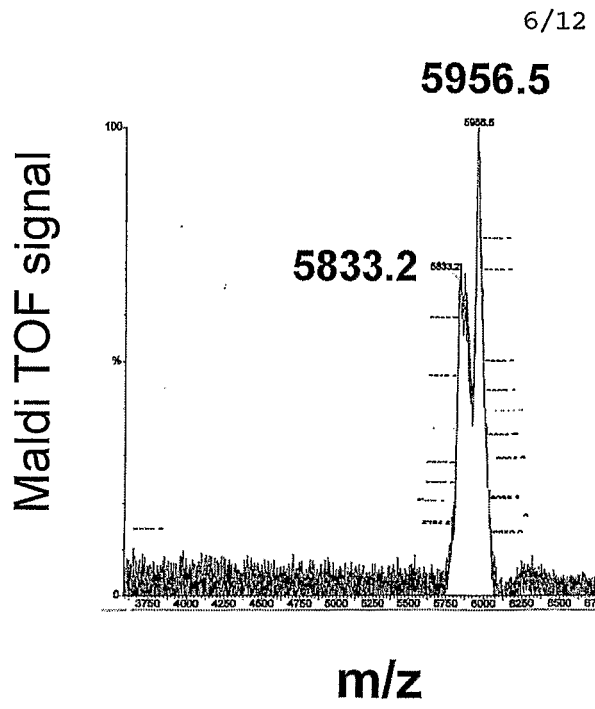


Figure 6

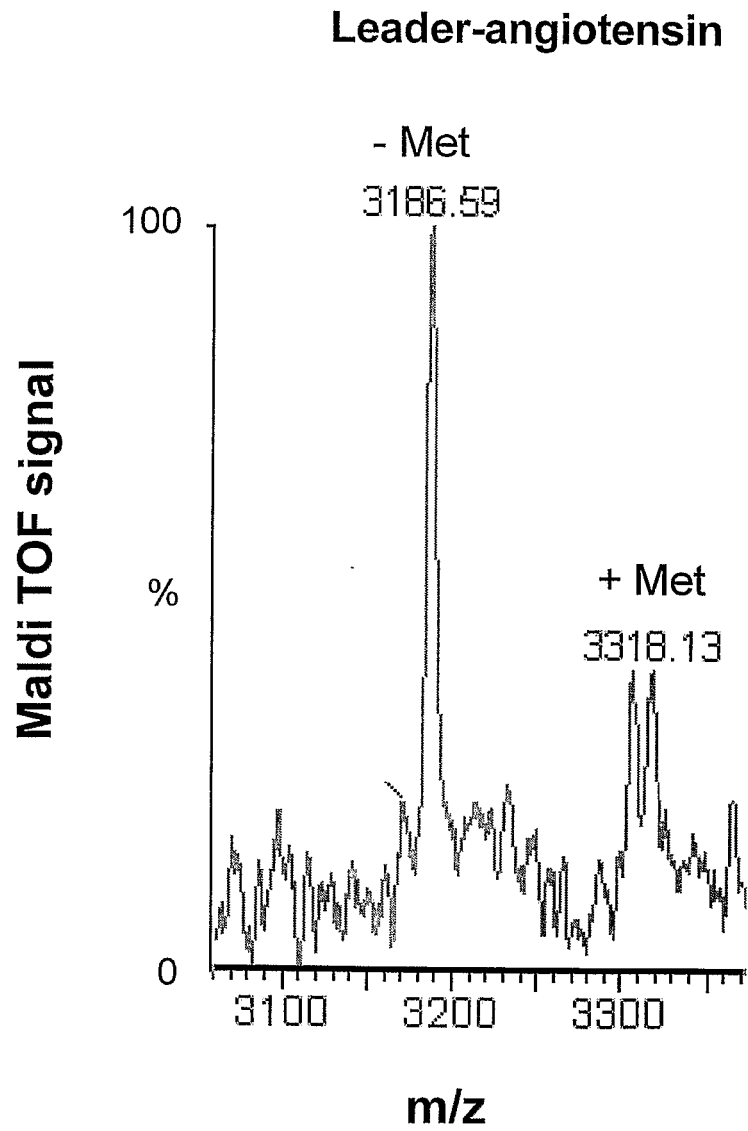


Figure 7

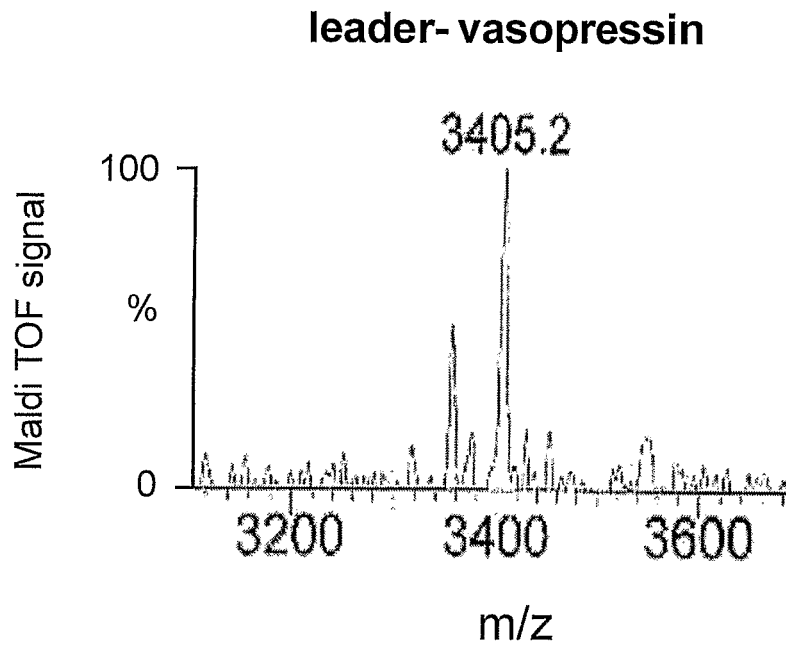


Figure 8

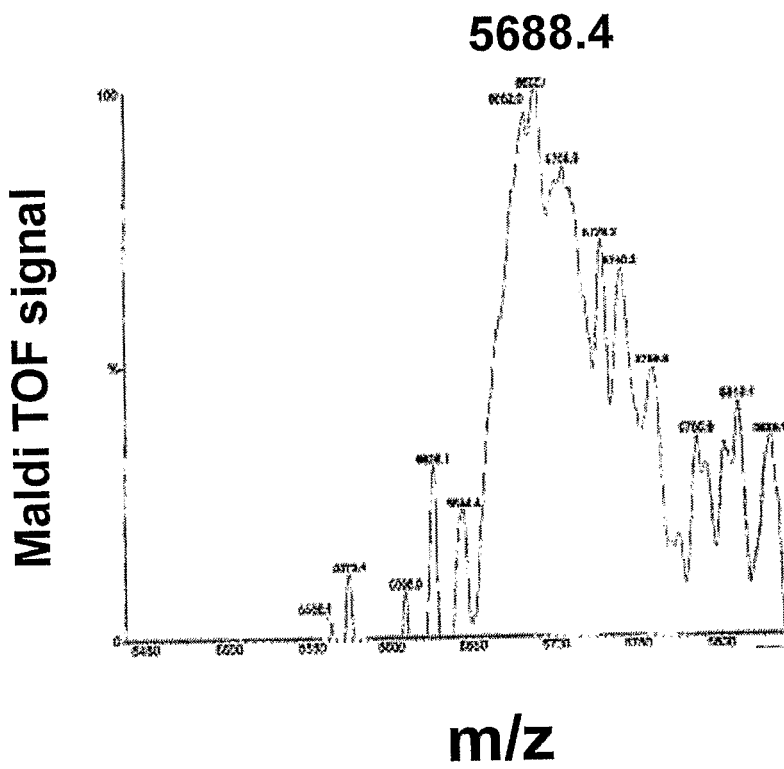


Figure 9



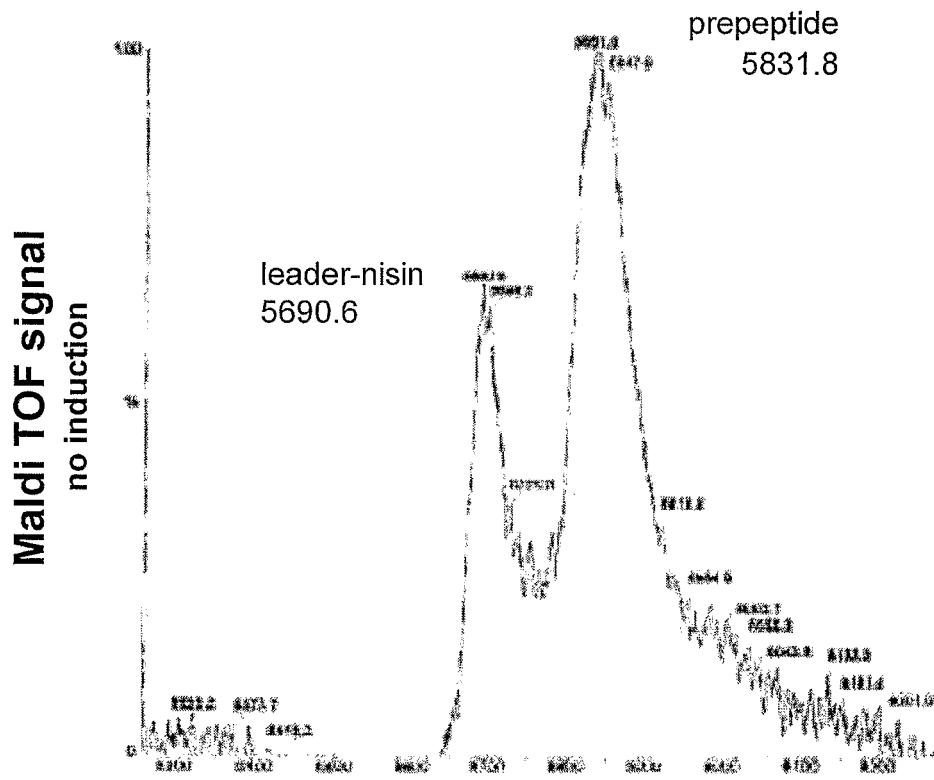


Figure 10A

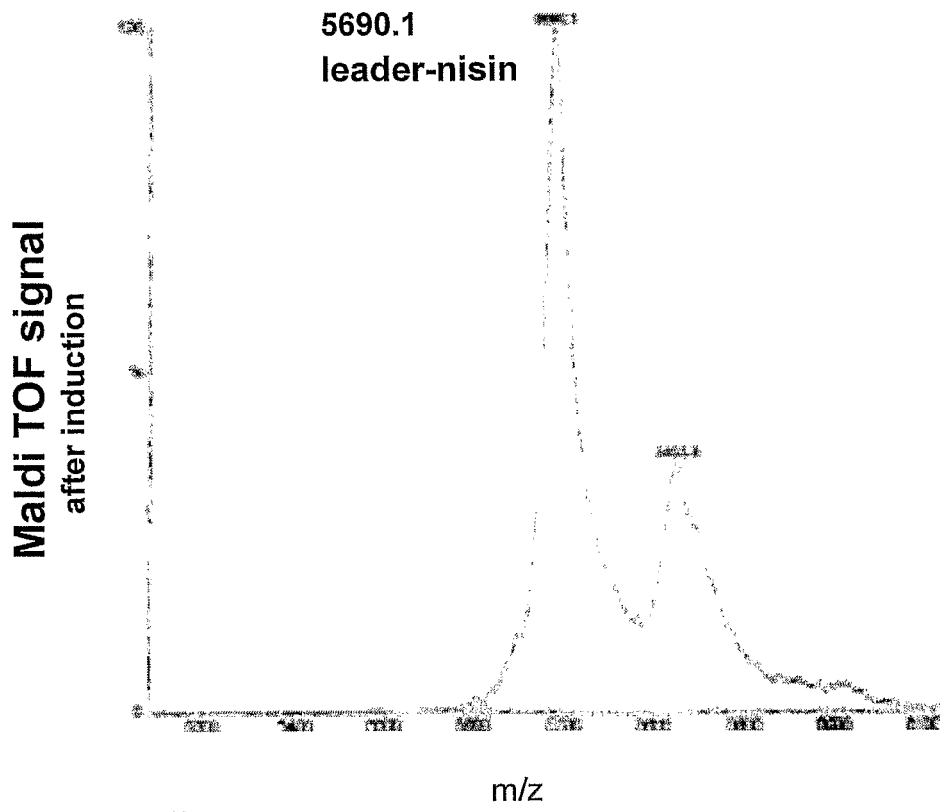


Figure 10B

nisin prepeptide-enkephalinT

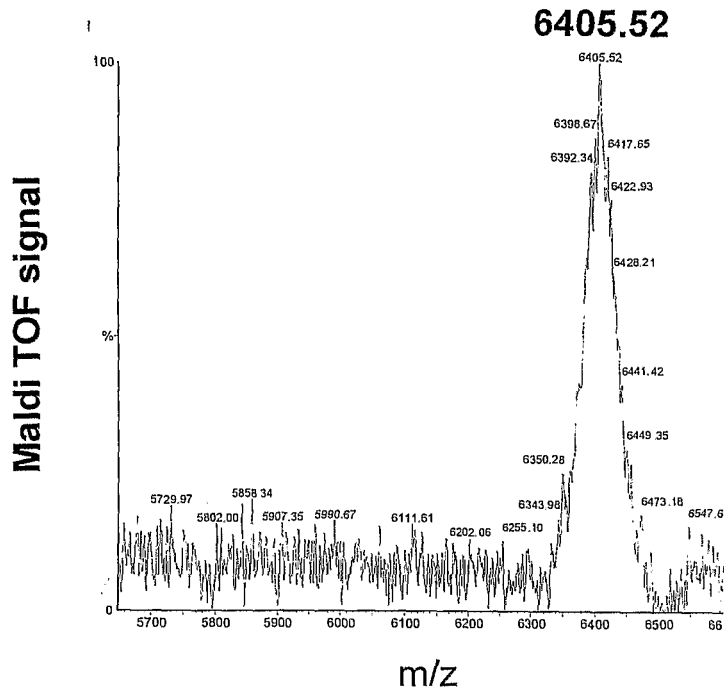


Figure 11

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/NL 03/00389

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K14/315

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)
IPC 7 C07K

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, EMBASE, SCISEARCH, CHEM ABS Data, MEDLINE, BIOSIS, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	<p>IZAGUIRRE GONZALO ET AL: "Use of alkaline phosphatase as a reporter polypeptide to study the role of the subtilin leader segment and the SpaT transporter in the posttranslational modifications and secretion of subtilin in Bacillus subtilis 168." APPLIED AND ENVIRONMENTAL MICROBIOLOGY, vol. 63, no. 10, 1997, pages 3965-3971, XP002217349 ISSN: 0099-2240 the whole document</p> <p style="text-align: center;">--- -/--</p>	1-34

Further documents are listed in the continuation of box C.

Patent family members are listed in annex.

° Special categories of cited documents :

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- *Z* document member of the same patent family

Date of the actual completion of the international search

8 September 2003

Date of mailing of the international search report

01/10/2003

Name and mailing address of the ISA

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Authorized officer

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INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/INL 03/00389

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	PAUL LEENA K ET AL: "Studies of the subtilin leader peptide as a translocation signal in Escherichia coli K12." FEMS MICROBIOLOGY LETTERS, vol. 176, no. 1, 1 July 1999 (1999-07-01), pages 45-50, XP002217350 ISSN: 0378-1097 the whole document ---	1-34
X	NOVAK J ET AL: "CLONING, SEQUENCING AND EXPRESSION OF AN ABC TRANSPORTER INVOLVED IN THE PRODUCTION OF THE LANTIBIOTIC MUTACIN II IN STEPTOCOCCUS MUTANS" ABSTRACTS OF THE GENERAL MEETING OF THE AMERICAN SOCIETY FOR MICROBIOLOGY, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, US, vol. 96, 1996, page 217 XP000892159 ISSN: 0067-2777 the whole document ---	1-34
X	FRANKE CHRISTIAN M ET AL: "Membrane topology of the lactococcal bacteriocin ATP-binding cassette transporter protein LcnC: Involvement of LcnC in lactococcin a maturation." JOURNAL OF BIOLOGICAL CHEMISTRY, vol. 274, no. 13, 26 March 1999 (1999-03-26), pages 8484-8490, XP002217351 ISSN: 0021-9258 the whole document ---	1-34
Y	US 5 861 275 A (HANSEN J NORMAN) 19 January 1999 (1999-01-19) the whole document ---	1-34
Y	US 2002/019518 A1 (HANSEN J NORMAN) 14 February 2002 (2002-02-14) the whole document ---	1-34
Y	QIAO MINGQIANG ET AL: "Evidence for a role of NisT in transport of the lantibiotic nisin produced by Lactococcus lactis N8." FEMS MICROBIOLOGY LETTERS, vol. 144, no. 1, 1996, pages 89-93, XP001117575 ISSN: 0378-1097 the whole document ---	1-34
	-/--	

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Internat Application No

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C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	<p>BREUKINK E ET AL: "The lantibiotic nisin, a special case or not?" BIOCHIMICA ET BIOPHYSICA ACTA. BIOMEMBRANES, AMSTERDAM, NL, vol. 1462, no. 1-2, 15 December 1999 (1999-12-15), pages 223-234, XP004273118 ISSN: 0005-2736 the whole document</p>	1-34
Y	<p>SIEZEN R J ET AL: "COMPARISON OF LANTIBIOTIC GENE CLUSTERS AND ENCODED PROTEINS" ANTONIE VAN LEEUWENHOEK, DORDRECHT, NL, vol. 2, no. 69, February 1996 (1996-02), pages 171-184, XP001095134 the whole document</p>	1-34
A	<p>MCAULIFFE OLIVIA ET AL: "Lantibiotics: Structure, biosynthesis and mode of action" FEMS MICROBIOLOGY REVIEWS, ELSEVIER, AMSTERDAM, NL, vol. 25, no. 3, May 2001 (2001-05), pages 285-308, XP002209342 ISSN: 0168-6445</p>	
A	<p>FATH M J ET AL: "ABC TRANSPORTERS: BACTERIAL EXPORTERS" MICROBIOLOGICAL REVIEWS, AMERICAN SOCIETY FOR MICROBIOLOGY, WASHINGTON, DC, US, vol. 57, no. 4, 1 December 1993 (1993-12-01), pages 995-1017, XP002050868 ISSN: 0146-0749</p>	

INTERNATIONAL SEARCH REPORT

Internat Application No

PCT/NL 03/00389

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
US 5861275	A	19-01-1999	AU 720529 B2	01-06-2000
			AU 7164196 A	17-04-1997
			CA 2230933 A1	03-04-1997
			EP 0852502 A1	15-07-1998
			JP 2001517923 T	09-10-2001
			NZ 319425 A	23-12-1998
			WO 9711713 A1	03-04-1997
			US 6153405 A	28-11-2000
			ZA 9608160 A	02-05-1997
<hr/>				
US 2002019518	A1	14-02-2002	US 2002052005 A1	02-05-2002
			US 2002165139 A1	07-11-2002
<hr/>				