ANTIMICROBIAL AGENTS PRODUCED BY XENORHABDUS GRIFFINIAE STRAIN XN45

This invention relates to antimicrobial compositions and methods of making and using such antimicrobial compositions. Components of the antimicrobial compositions are obtained from the whole broth product of fermentation of cultures of Xenorhabdus griffiniae XN45, or are derived from materials so obtained. In an aspect, compositions and methods for controlling antibiotic resistant bacteria are provided.
Antimicrobial agents produced by *Xenorhabdus griffiniae* strain XN45

Cross-Reference to Related Applications

This application claims priority to Kenya application KE/P/2014/002066, filed 16 May 2014, the contents of which are incorporated herein by reference.

Introduction

*Xenorhabdus* is a bacterial genera of the family Enterobacteriaceae. They are gut symbionts of nematodes of the entomopathogenic family Steinernematidae. They form a mutualistic relationship that largely contributes to the entomopathogenicity as well as fecundity of the nematode.

*Xenorhabdus* are known for the production of antimicrobial agents. The genus comprises over 20 described species with each *Xenorhabdus* species bearing its own antibiotic profile. Some of the antimicrobial compounds derived from various *Xenorhabdus* species have documented efficacy against antibiotic resistant bacteria. Nemaucin is a known antimicrobial agent effective against a broad range of antibiotic resistant bacteria. It is produced by *Xenorhabdus cabalinasii*. Susceptible organisms include both Methicillin resistant *Staphylococcus aureus* (MRSA) and Vancomycin resistant *Enterococcus* (VRE).

The qualitative and quantitative increase in antibiotic resistant bacteria warrants the need for development of antibiotics. Methicillin resistant *Staphylococcus aureus*, beta-lactam resistant *Klebsiella pneumoniae* and *Enterococcus spp.* are becoming widespread. An increasing prevalence of hospital acquired MRSA has also been revealed. These findings highlight the need to expedite antibiotic development.

Relevant art: WO 2012/085177.

Summary

In an aspect is a biologically pure culture of the microorganism *Xenorhabdus griffiniae* strain XN45 having antimicrobial activity. In embodiments:

- the antimicrobial activity is antibiotic activity against antibiotic resistant bacteria;
- the antimicrobial activity is antibiotic activity against antibiotic resistant bacteria,
and wherein the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*;

the antimicrobial activity is antibiotic activity against livestock diseases;

the antimicrobial activity is antibiotic activity against mastitis and bacteria

causing mastitis in cows;

the antimicrobial activity is antibiotic activity against mastitis in cows, wherein
the bacteria causing the mastitis is an antibiotic resistant bacteria;

the biologically pure culture is cell free and sterilized; and

the biologically pure culture is in liquid or solid form.

In another aspect is an antimicrobial agent comprising the
microorganism *Xenorhabdus griffiniae* strain XN45 or a substance selected from the

group consisting of a whole broth culture of the microorganism *Xenorhabdus griffiniae*
strain XN45, an extract of a supernatant of a whole broth culture of the

microorganism *Xenorhabdus griffiniae* strain XN45, and spores of the

microorganism *Xenorhabdus griffiniae* strain XN45 as an effective ingredient. In

embodiments:

the antimicrobial agent has antibiotic activity against antibiotic resistant bacteria;

the antimicrobial activity is antibiotic activity against livestock diseases;

the antimicrobial activity is antibiotic activity against mastitis and bacteria

causing mastitis in cows;

the antimicrobial activity is antibiotic activity against mastitis in cows, wherein
the bacteria causing the mastitis is an antibiotic resistant bacteria;

the antimicrobial agent has antibiotic activity against antibiotic resistant bacteria, and

wherein the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*;

the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*;

the substance is the whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45;

the substance is an extract of a supernatant of a whole broth culture of the

microorganism *Xenorhabdus griffiniae* strain XN45; and

the substance is spores of the microorganism *Xenorhabdus griffiniae* strain XN45.
In an aspect is a method for producing the biologically pure culture as above, the method comprising growing *Xenorhabdus griffiniae* strain XN45 in a liquid culture medium and extracting the liquid culture medium.

In an aspect is a medicament for topical administration in the treatment of infections by antibiotic resistant bacteria, the medicament comprising the biologically pure culture as above.

In an aspect aspect is an antibacterial composition produced by the bacteria *Xenorhabdus griffiniae* strain XN45 that specifically inhibits growth of antibiotic resistant bacteria. In embodiments:

- the composition is isolated from *Steinernema* sp. Scarpo nematodes deposited at Kenya Agricultural and Livestock Research Organisation- Horticulture Research Institute, Entomopathogenic Nematology Lab (KALRO-HRI,EPN LAB);
- the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*;
- the composition comprises mursamacin;
- the composition is the whole broth extract of cultures of *Xenorhabdus griffiniae* strain XN45;
- the composition is cell free and comprises sterilized extracts;
- the composition is in liquid form or a solid compound obtained by processes such as lyophilization of the liquid broth extract;
- the composition is a whole broth extract as above that possess antibiotic activity use of the composition as above as a medicament, and use of the topical medicament as above as a wound dressing therapy in the treatment of infections by antibiotic resistant bacteria.

In an aspect is the use of an antimicrobial agent comprising: the microorganism *Xenorhabdus griffiniae* strain XN45 or a substance selected from the group consisting of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45, an extract of a supernatant of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45, and spores of the microorganism *Xenorhabdus griffiniae* strain XN45 as an effective ingredient, to treat a bacterial infection. In embodiments:

- the bacterial infection is by an antibiotic resistant bacteria;
the bacterial infection is mastitis in livestock;
the bacterial infection is an infection in a human;
the bacterial infection is of the antibiotic resistant bacteria Methicillin resistant
Staphylococcus aureus;

the antimicrobial agent is present in an antimicrobial amount (i.e., an amount
effective to cause antimicrobial activity); and

wherein the use comprises administration of the antimicrobial agent as part of a
formulation.

These and other aspects of the invention will be apparent to one of skill in the art
based on the disclosure provided herein.

Technical field of the invention
The Technical Field of the invention is pharmaceutically active compositions and
methods of making and using the same such as, in aspects, antimicrobial compositions.

Detailed Description of Various Embodiments
The present invention relates to the bacteria Xenorhabdus griffiniiae strain XN45.
The bacteria was isolated from Steinernema sp. scarpo currently deposited at Kenya
Agricultural and Livestock Research Organisation- Horticulture Research Institute,
Entomopathogenic nematology Lab (KALRO-HRI,EPN LAB). The isolated bacteria were
identified to be Xenorhabdus griffiniiae and designated strain XN45.

In an aspect is a biologically pure culture of the microorganism Xenorhabdus
griffiniiae strain XN45 having antimicrobial activity.

In another aspect is an antimicrobial agent comprising the
microorganism Xenorhabdus griffiniiae strain XN45 or a substance selected from the
group consisting of a whole broth culture of the microorganism Xenorhabdus griffiniiae
strain XN45, an extract of a supernatant of a whole broth culture of the
microorganism Xenorhabdus griffiniiae strain XN45, and spores of the
microorganism Xenorhabdus griffiniiae strain XN45 as an effective ingredient.

In an aspect is a method for producing the biologically pure culture as above and
herein, the method comprising growing Xenorhabdus griffiniiae strain XN45 in a liquid
culture medium and extracting the liquid culture medium.

In an aspect is a medicament for topical administration in the treatment of infections by antibiotic resistant bacteria, the medicament comprising the biologically pure culture as above. By “antibiotic resistant bacteria” as used herein is meant bacteria that is resistant to treatment by traditional treatment methods, compounds, formulations, and the like (i.e., other than the methods and materials described herein).

In an aspect is an antibacterial composition produced by the bacteria *Xenorhabdus griffiniae* strain XN45 that specifically inhibits growth of antibiotic resistant bacteria.
The antimicrobial compositions described herein are isolated from *Xenorhabdus griffiniae* strain XN45, or are derived from compounds and compositions isolated from *Xenorhabdus griffiniae* strain XN45.

In embodiments, the antimicrobial compositions have antibiotic activity, such as antibiotic activity including antibiotic activity for antibiotic resistant bacteria. Examples of bacteria and antibiotic resistant bacteria include methicillin resistant *Staphylococcus aureus* (MRSA), *Escherichia coli*, and *Enterococcus faecalis*. Examples further include hospital-acquired (i.e., nosocomial) infections of bacteria and antibiotic resistant bacteria, such as hospital-acquired MRSA. Further examples include nosocomial infections of the open wound variety. Further examples include the control of livestock diseases. For example the antibiotic activity of the compositions herein may be used to control mastitis skin infections of dairy cattle or the like.

In embodiments, the antimicrobial compositions are compositions with one or more antibiotic agents, such agents being obtained from *Xenorhabdus griffiniae* strain XN45.

The antimicrobial compositions are selected from: the whole broth product of fermentation of cultures of *Xenorhabdus griffiniae* strain XN45; compositions containing compounds isolated from the whole broth product of fermentation of cultures of *Xenorhabdus griffiniae* strain XN45, or compounds derived from such isolated compounds or the whole broth product; an extract of a supernatant of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45; spores of the microorganism *Xenorhabdus griffiniae* strain XN45; and the microorganism *Xenorhabdus griffiniae* strain XN45.

In embodiments, the term “mursamacin” is used to refer to an extract of a supernatant of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45.

Compounds can be isolated from the whole broth product via any suitable method, such as by solvent extraction, lyophilization, or the like. Further antimicrobial compounds may be derived from compounds in the whole broth (either after isolation from the whole broth or without isolation). By “derived from” is meant a compound that is the result of one or more chemical transformation on the original compound, and is
meant to include prodrugs, compounds with protecting groups, metabolites, and the like.

In embodiments, the methods of production of the antimicrobial compositions include growing the microorganism *Xenorhabdus griffiniæ* strain XN45 in a liquid culture medium. During such growth, the growing bacteria secretes antimicrobial compounds into the whole broth, thereby imparting antimicrobial properties to the whole broth. The whole broth may be used without further purification, or may be further processed.

Further processing of the whole broth may involve purification such as by sterilization, removal of cells, removal of one or more solvents, solvent extraction of one or more compounds, and the like.

By “antimicrobial activity” is meant that a compound or composition, when used to treat a medium containing microbes (e.g., bacteria) has a bacteriostatic and/or bactericidal effect, i.e., it does one or more of the following: inhibits the growth of new microbes (e.g., reduces the rate of growth by 10, 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, 99.9, or 99.99%, or eliminates new growth altogether), and/or reduces the population of microbes. Reduction in the population can include partial reduction (e.g., 20, 30, 40, 50, 60, 70, 80, 90, 95, 99, 99.9, or 99.99%) and can also include complete elimination of the population of microbes. Antimicrobial activity may also include prophylactic activity — i.e., in embodiments the compound or composition can be used to inhibit the initial growth of microbes and the initiation/development of a colony thereof.

Such antimicrobial activity requires that the compound or composition is used in an antimicrobial amount — e.g., a bacteriostatic concentration, a bactericidal concentration, etc. (such concentrations may be the same or different). Such amount may be dependent upon a number of variables (e.g., location and environment of the microbes, type of microbe, etc.) but will be readily determined by routine experimentation. For example, determination of IC₅₀ values for a composition requires only routine experimentation and is guided by the disclosure herein. Dosage and regimen data will likewise be readily determined by routine experimentation as guided by the disclosure herein.

Antimicrobial compositions described herein comprise the active agent and may comprise one or more additional components as necessary for proper storage and
administration of the formulation. Examples of additional components include known and later developed carriers, preservatives, stabilizers, colorants, flavoring agents, pH control agents, solvents, and the like.

Antimicrobial compositions described herein comprise the active agent and may comprise one or more additional active agents. Examples of additional agents include known and later developed antibacterial agents (other than those described herein), anti-inflammatories, and the like.

The antimicrobial compositions described herein may be administered in any convenient manner and may take any convenient dosage form. Such dosage methods include injection, oral dosage, inhalation, transmembrane (e.g., via a patch), and the like.

It is to be understood that while the invention has been described in conjunction with examples of specific embodiments thereof, that the foregoing description and the examples that follow are intended to illustrate and not limit the scope of the invention. It will be understood by those skilled in the art that various changes may be made and equivalents may be substituted without departing from the scope of the invention, and further that other aspects, advantages and modifications will be apparent to those skilled in the art to which the invention pertains.
EXAMPLES

Materials and Methods

Isolation of the producing bacteria

Steinernema sp. scarpo infective juvenile nematodes were obtained from Kenya Agricultural and Livestock Research Organisation- Horticulture Research Institute, Entomopathogenic Nematology Lab, Thika, Kenya (KALRO-HRI,EPN LAB).

A culture suspension of these nematodes in sterile water was used to infect last instar larvae of Galleria mellonella. Whatman filter paper 1 was lined to the lid of a 90 millimeter (mm) petri dish. With the use of sterile injection needles, 2ml of the distilled water suspension of the nematodes was inoculated onto the filter paper. Five last instar larvae were then placed onto the bottom of the petri dish. This was then inverted over the lid.

The petri dish was sealed with parafilm and incubated at room temperature in the dark for 72 hours. Mortality rate of 100% was observed after 31 hours.

A differential media, NBTA (Nutrient agar (HiMedia) 28g/L supplemented with 25mg/L 2,3,5 triphenyltetrazolium chloride(Sigma-Aldrich) and 40mg/l bromothymol blue (Fluka Analytical ), used in the identification of Xenorhabdus bacteria, was prepared.

The cadavers were obtained from incubation. They were surface sterilized under aseptic conditions in 70% isopropanol. A second surface sterilization was done by immersion in 90% isopropanol. Lastly, flame sterilization was done by igniting the cadavers over an open flame then quickly dipping into sterile water.

Dissection of the cadaver was done to obtain insect haemolympth. It was a clear translucent liquid. This was streaked onto NBTA and incubated at 30 °C for 72 hours.

Blue distinct colonies with irregular margins were observed. These were sub-cultured onto NBTA plates and incubated at 28°C. Single colonies were selected and inoculated into 5ml of Luria Bertani medium (LB) (10 g/L Tryptone 5 g/L Bacto-Yeast Extract (Difco) 10 g/l Sodium Chloride). This was incubated at 33 °C at 150 revolutions per minute (rpm) for 5 hours followed by 28 °C for 24 hours yielding 31 hours incubation
time. The culture (900 µl) was transferred to a sterile 1.5 ml cryogenic storage tube. It was topped up with 300 µl of LB that had been premixed with 300 µl of glycerol to yield a final concentration of 20% (v/v) glycerol. These stocks were frozen overnight at -30 °C then transferred to a -80 °C refrigerator for long term storage. Stab cultures (LB with 8g/L agar) of the isolates were made and stored at room temperature in the dark.

Molecular characterization of the bacteria

DNA was extracted from plates cultures of the isolate using a Fast DNA®SPIN Kit for Soil (MP Biomedicals, Santa Ana, CA). Concentration of extracted DNA was determined via UV-vis spectroscopy (Shimadzu®1800) yielding 305 ng/µl. Enzymatic amplification of a partial gene fragment of the 16s rRNA gene of Xenorhabdus was carried via the polymerase chain reaction (PCR). PCR conditions were as follows: Taq polymerase (Genscript®) 0.3µl, 10x Taq polymerase buffer (Genscript®) 2µl, 10mM dNTPs (New England Biolabs®) 0.2µl 10mM each forward (27f-

AGAGTTTGATCATGGCTCAG) and Reverse (1392r-ACGGGCGGTGTGGC) primers 1µl, nuclease free water 13.5 µl. Cycle conditions were 94 °C for 5 minutes, 94 °C for 30 seconds, 47 °C for 15 seconds, 72 °C for 1 minute 30 seconds with a final extension was 72 °C for 7 minutes. A total of 40 cycles were carried out in a programmable thermocycler (Thermo Scientific Arktik Thermal Cycler). Visualization of amplified gene products was done on an agarose gel (12g/L low melt agarose in TAE buffer) that had been supplemented with Ethidium bromide at final concentration of 0.5ug/ml. The gel was run at 5 V/cm for 1 hour 12 minutes. Visualization was done atop an ultra violet radiation emitting surface. The 1500 base pair band was cut out using a sterile scalpel. Purification of PCR products from was done with a Genscript® Gel excision kit. The purified PCR products were sequenced (Macrogen™, Amsterdam, Netherlands) via a Sanger platform.

Obtained chromatograms of the 16s rRNA sequences were trimmed and visually assessed for quality using BioEdit® software. Trimmed 16s rRNA gene sequences were then assessed for homology to the nearest described species against database sequences.

Fermentation of antibiotics by Xenorhabdus sp.
A single red colony with a complete and slightly rough margin was inoculated into 5ml LB and incubated at 150 rpm at 33 °C for 3 hours. This culture served as a 1% (v/v) inoculum for fermentation procedures.

Sterile Media for fermentation procedures LB (10g/L tryptone, 5g/L Bactoyeast extract, 10 g/L Sodium Chloride) prepared. The LB (500ml) was dispensed into a sterile 1 liter Erlenmeyer flask.

The starter culture (5ml) was then inoculated into the flask. This was incubated at 150rpm at 33°C for 180 hours. LB with no inoculum was also incubated to serve as a sterility control.

Purification of the whole broth extract

The broth culture was obtained. Centrifugation of the culture was done at 6,000 g for 10 minutes at 4 °C. The cell free supernatant was decanted and subsequently filter sterilized through a 0.4 µm filter membrane and then a 0.2 µm filter membrane. This resulted in the sterile whole broth extract, referred to herein as “mursamacin”. This was then subsequently used to assess for inhibitory effects against bacteria.

Extraction of compounds from the whole broth extract

The whole broth extract (275ml) was mixed with chloroform (2:1) and stirred with a magnetic stirrer (30 minutes). The mixture was distributed into 40ml high density polypropylene tubes and centrifuged at 20,000g for 20 minutes at 25°C. A yellow top layer, and clear bottom layer inter-phased by a white precipitate was obtained. The top yellow layer, termed as the aqueous layer, was decanted and pooled. The bottom layer, termed as the organic phase, was pooled into a chrome-vanadium pan and left in a chemical hood to allow for evaporation of chloroform. After 72 hours, a lipid like layer was observed at the bottom of the pan. This was dissolved in 70 ml methanol (100%) that was subsequently diluted to form a 90% methanol extract. The methanol was removed by rotary evaporation yielding a yellow lipid like substance.

This was dissolved in 3.9 ml of dimethyl sulfoxide (100%) yielding 70x concentrate of the organic derivative of mursamacin. This was used in inhibition assays.
Inhibition assays

The inhibitory effect of mursamacin and extracts derived from it, was tested against isolates of *Escherichia coli*, *Enterococcus faecalis* (Government Chemist, Kenya) and Methicillin resistant *Staphylococcus aureus* (Kenya Medical Research Institute, Center for Respiratory Disease research).

Two assays were used. A broth dilution assay of mursamacin and a plate inhibition assay of the organic derivative of mursamacin.

For the broth dilution assay, a dilution range was made representing varying percentages of mursamacin in 2X LB medium (20g/L Tryptone 10g/L Bactoyeast Extract (Difco) 20 g/l NaCl). Mursamacin amounts of 0ml, 0.5ml, 1ml, 1.5ml, 2ml, 2.5ml, 3ml, 3.5ml, 4ml, 4.5ml, 4.9ml were mixed with 4.9ml, 4.4ml, 3.9ml, 3.4ml, 2.9ml, 2.4ml, 1.9ml, 1.4ml, 0.9ml, 0.4ml of 2X LB respectively. This yielded mursamacin concentrations of 0% 10% 20% 30% 40% 50% 60% 70% 80% 90% 100% respectively. Test bacteria (100ul) was then inoculated into each of the concentrations and incubated for 21 hours at 37°C without agitation. Average incubating inoculum was ca. 254,000 colony forming units (cfu) per ml.

Turbidity of the broth was used as a measure of inhibition. This was done by detecting the amount absorbance of broth cultures by a 600nm UV light beam across 1 cm light path (A600nm) using a uv-vis spectrometer (Beckman DU-640 series).

Percentage growth inhibition was then calculated with this formula ((1-A600nm culture with mursamacin)/A600nm culture without mursamacin)*100. The obtained value was then subtracted by ((1-A600nm culture without mursamacin)/A600nm culture without mursamacin)*100, to correct for inhibition due to the culture medium.

The plate inhibition assay was conducted as follows. Methicillin resistant *Staphylococcus aureus* cultures were plated onto Muller-Hinton (Oxoid) agar plates and incubated overnight at 37 °C. Inocula were prepared from these cultures by diluting colonies in physiological saline (PS) (0.9% (w/v) NaCl solution) and turbidity adjusted to a 0.5 McFarland standard. Plating was done by soaking sterile cotton pieces in PS and applying it over the plates. The plates were left briefly to dry. Sterile 6mm Whatman 1 filter papers were then placed onto the plates. The organic derivative (50μl) of mursamacin dissolved in dimethyl sulfoxide was pipetted onto the filter paper to serve as
the test antimicrobial.

An equal amount of sterile 100% (v/v) dimethyl sulfoxide was similarly inoculated to serve as a negative control. Plates were sealed with parafilm and incubated at 37 °C overnight.

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Results

Isolation and characterisation of the bacteria.

Bacteria was isolated and the following morphological characteristics assessed.

<table>
<thead>
<tr>
<th>Table 1: Morphological characteristics of <em>Xenorhabdus</em> isolates.</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Morphological characteristics of <em>Xenorhabdus griffiniiae</em> XN45</strong></td>
</tr>
<tr>
<td>Colony color^</td>
</tr>
<tr>
<td>Swarming motility*</td>
</tr>
<tr>
<td>Swimming motility**</td>
</tr>
<tr>
<td>Growth at &gt; 40°C</td>
</tr>
<tr>
<td>^NBTA. <em>NBTA (0.8%agar)</em>* (NBTA&lt; 0.4% agar)</td>
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</tbody>
</table>

These morphological characteristics confirmed the isolated bacteria to be of phase one variation. Molecular characterization was performed on the isolated 16s rRNA gene. It had 1305 base pairs. It was outlined as sequence one and named partial16s rRNA gene of *Xenorhabdus griffiniiae* XN45.

Homology searches against database sequences yielded the nearest described species as *Xenorhabdus griffiniiae*.

Inhibition assays

For the inhibition assay of mursamacin against *E.coli*, a clear broth with no visible turbidity was observed at 100% mursamacin concentrations indicating complete inhibition. Percentage growth inhibitions were also calculated.
Table 2:
Concentrations of mursamacin that inhibits 20% growth, 68% and complete inhibition in *E. coli* after overnight incubation at 37°C

<table>
<thead>
<tr>
<th>20% growth inhibition</th>
<th>68% growth inhibition</th>
<th>Complete inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>60% mursamacin</td>
<td>80% mursamacin</td>
<td>100% mursamacin</td>
</tr>
</tbody>
</table>

Inhibition of growth by the organic derivative of mursamacin against MRSA showed complete inhibition at less than 12.5% concentrations indicating high potency as a drug.

Table 3:
Concentrations of the organic derivative of mursamacin completely inhibiting Methicillin resistant *Staphylococcus aureus*

<table>
<thead>
<tr>
<th>100% mursamacin</th>
<th>50% mursamacin</th>
<th>25% mursamacin</th>
<th>12.5% mursamacin</th>
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<tbody>
<tr>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

Plate inhibition assay

The organic derivative of mursamacin was used as the test antimicrobial in a plate inhibition assay against MRSA.

After overnight incubation at 37 °C, a zone of inhibition of diameter 9 mm was observed indicating an inhibitory effect against MRSA.

Genetic analysis

The bacteria *Xenorhabdus griffiniae* XN45 has a gene sequence that is > 98% identical to SEQ ID NO 1 (> partial 16s rRNA gene sequence of Xenorhabdus griffine strain XN45).
What is claimed is:

1. A biologically pure culture of the microorganism *Xenorhabdus griffiniae* strain XN45 having antimicrobial activity.

2. An antimicrobial agent comprising the microorganism *Xenorhabdus griffiniae* strain XN45 or a substance selected from the group consisting of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45, an extract of a supernatant of a whole broth culture of the microorganism *Xenorhabdus griffiniae* strain XN45, and spores of the microorganism *Xenorhabdus griffiniae* strain XN45 as an effective ingredient.

3. The biologically pure culture of claim 1, wherein the antimicrobial activity is antibiotic activity against antibiotic resistant bacteria.

4. The antimicrobial agent of claim 2, wherein the antimicrobial agent has antibiotic activity against antibiotic resistant bacteria.

5. The biologically pure culture of claim 1, wherein the antimicrobial activity is antibiotic activity against antibiotic resistant bacteria, and wherein the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*.

6. The antimicrobial agent of claim 2, wherein the antimicrobial agent has antibiotic activity against antibiotic resistant bacteria, and wherein the antibiotic resistant bacteria is Methicillin resistant *Staphylococcus aureus*.

7. The biologically pure culture of claim 1, wherein the biologically pure culture is cell free and sterilized.

8. The biologically pure culture of claim 1, wherein the biologically pure culture is in
liquid or solid form.

9. A method for producing the biologically pure culture of claim 1, the method comprising growing *Xenorhabdus griffiniæ* strain XN45 in a liquid culture medium and extracting the liquid culture medium.

10. A medicament for topical administration in the treatment of infections by antibiotic resistant bacteria, the medicament comprising the biologically pure culture of claim 1.

11. The use of an antimicrobial agent to treat a bacterial infection, the antimicrobial agent comprising: the microorganism *Xenorhabdus griffiniæ* strain XN45 or a substance selected from the group consisting of a whole broth culture of the microorganism *Xenorhabdus griffiniæ* strain XN45, an extract of a supernatant of a whole broth culture of the microorganism *Xenorhabdus griffiniæ* strain XN45, and spores of the microorganism *Xenorhabdus griffiniæ* strain XN45.

12. The use of claim 11, wherein the bacterial infection is by an antibiotic resistant bacteria.

13. The use of claim 11, wherein the bacterial infection is selected from mastitis in livestock or an infection in a human.

14. The use of claim 11, wherein the bacterial infection is of the antibiotic resistant bacteria Methicillin resistant *Staphylococcus aureus*.

15. The use of claim 11, wherein the antimicrobial agent is present in an antimicrobial amount and wherein the use comprises administration of the antimicrobial agent as part of a formulation.
**INTERNATIONAL SEARCH REPORT**

**A. CLASSIFICATION OF SUBJECT MATTER**

INV. A61K35/74
ADD.

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)

A61K

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

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

EPO-Internal, WPI Data, BIOSIS, EMBL, FSTA

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

<table>
<thead>
<tr>
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<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
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<td>2,4,6, 11-15</td>
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**Further documents are listed in the continuation of Box C.**

**See patent family annex.**

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

**Date of the actual completion of the international search**

2 September 2015

**Date of mailing of the international search report**

10/09/2015

Name and mailing address of the ISA/
European Patent Office, P.B. 5818 Patentlaan 2
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Fax. (+31-70) 340-3016

Authorized officer

Obel, Nicolai
<table>
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<th>Relevant to claim No.</th>
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</table>
| A        | Stewart J Hinchcliffe ET AL:
"Insecticidal Toxins from the Photorhabdus and Xenorhabdus Bacteria",
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page 101, column 2, lines 1-8 | 1-15 |
| A        | EP 2 030 623 A1 (NESTEC SA [CH])
4 March 2009 (2009-03-04)
paragraphs [0005] - [0013] | 1-15 |
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