Study of Nisin and Sublancin:

A Strategy for Protection of the United States Food Supply from Pathogenic Bacterial Spores Introduced through Bio-terrorism

JIFSAN Project

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The United States remains woefully unprepared to protect the public against terrorists wielding biological agents.

The consequences of a big biological strike could be epically catastrophic, and rapid advances in science are placing the creation of these weapons within the reach of even graduate students, they said.

Anthrax bacteria remain among the easiest microbes to manufacture and weaponize. Deepening alarm is the prospect of new genetically engineered pathogens that could be both more deadly and more difficult to detect and treat. A 2003 CIA study described the effects of these genetically altered strains as potentially "worse than any disease known to man."

To counteract the attack that officials are nearly certain will come one day, the nation needs long lists of new biowarfare antidotes.
Anthrax and Botulism

- Air-borne
- Water-borne
- Food-borne

\{ Pathogens \}

High fatality rates
No cure once infection established
Life Cycle of *Bacillus anthracis*

- Vegetative cell
- Sporulation (restricted nutrients)
- Forespore
- Sporulating cell
- Spore maturation
- Spore released
- Germination (germination stimulants)
- Germinated spore
- Mature dormant spore
- Inhibitors

Inhibitors prevent spore outgrowth and germination.
Non-toxic Inhibitors of Spore Germination and Outgrowth

Nitrite
- Common chemical food additive that inhibits *B. anthracis* and *C. botulinum* spores

Nisin
- Sophisticated antimicrobial peptide that inhibits spores and kills vegetative cells
- World-wide use as a versatile food preservative
- Member of a family of antimicrobial peptides called “Lantibiotics”
The Lantibiotic Family of Antimicrobial Peptides

Gene-encoded peptides
- Produced by Gram-positive bacteria
- Structures can be altered by genetic engineering

Contain unusual amino acid residues
- Introduced by post-translational modification
- Non-standard amino acids possess unique chemical and biological properties
Many Lantibiotics are Known

<table>
<thead>
<tr>
<th>Name of Lantibiotic</th>
<th>Mr</th>
<th>Producer Organism</th>
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<tbody>
<tr>
<td>Nisin</td>
<td>3353</td>
<td>Lactococcus lactis</td>
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<tr>
<td>Subtilin</td>
<td>3317</td>
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<td>Epidermin</td>
<td>2164</td>
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<td>4635</td>
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<td>Streptococcus mutans</td>
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<td>Cytolysin</td>
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<td>Enterococcus faecalis</td>
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</table>
Nisin & Sublancin

Nisin

1928, Rogers & Whittier

Sublancin

1997, Paik & Hansen
Inhibition of Pathogenesis of *Bacillus anthracis*

Novel Activity !!!

dormant spore

Germination

*Nisin & Sublancin*

germinated spore

Outgrowth

*Nisin or Sublancin*

vegetative cell

Nisin

lysed cells
Mechanism of Antimicrobial Action

- Molecular target of Nisin/Sublancin action
- Mechanism of interaction of cellular target
  - Covalent attachment of nisin/sublancin to target?
  - Involvement of dehydro residues?
Covalent Attachment of Dehydro Residues
Cysteine Addition to Nisin

Reaction at pH 7

\[ k_1 = 0.097 \quad k_{11} = 0.000 \]

\[ k_2 = 0.097 \quad k_{22} = 0.000 \]

\[ k_3 = 0.025 \quad k_{33} = 0.010 \]
Labeled Probes

Nisin — Biotin

Nisin — Fluorescein
Bacillus cereus T Spore
Uninhibited Outgrowing Spore

Spore Coat

emerging cell
Nisin-Fluorescein Inhibited Spores
Nisin-Fluorescein Labeled Spore

Fluorescence Microscopy
Electron Microscopy

Immunogold Nisin-biotin Spore
Nisin-Biotin Labeled Spores

Immunogold Detection
SDS-PAGE of *B. cereus* Cells Labeled with Nisin-Biotin

(+) Lysate of cells treated with biotinylated nisin

(-) Lysate of untreated cells
Can Lantibiotics Respond to Mutagenized Pathogens?

- New Pathogens introduced
  - Genetically-engineered *B. anthracis*
  - Molecular target modified

- Genetically Engineer the Lantibiotic
  - System for mutagenesis
  - System for selection of biologically-active mutants.
Lantibiotic Libraries

- *B. subtilis* 168 as expression host
- Express mutagenized Lantibiotic in a form that is displayed on the exterior of the producer cell
- Use cell-target ligands to identify biologically-useful Lantibiotic analogs
- Determine Lantibiotic structure by sequence analysis of mutant gene
**Lantibiotic Library**

\[ \text{Mutagenized Lantibiotic} \]

\[ \text{Variable Region} \]

\[ \text{Constant Region} \]

\[ \text{C-terminal attachment polypeptide} \]

\[ \text{NH}_2 \]

\[ \text{Cell Wall} \]

\[ \text{Membrane} \]

\[ \text{Genome} \]

\[ \text{B. subtilis 168} \]
Amino Acid sequence of Engineered Display Peptide

Figure 8. Lantibody Display Peptide as expressed from 168. Consists of mature sublancin segment a 20-residue poly Gly sequence (38-57), and the subtilin leader segment (58-81).
Conclusions

The Lantibiotic family of antimicrobial peptides are natural inhibitors of pathogenic bacterial spores.

Exploitation of their natural properties provide a short-term response to spore pathogens in the food supply.

The development of Lantibiotic libraries offers a means to adapt to new forms of bioterror agents, such as genetically-engineered anthrax and other weaponized pathogens.
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