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
November, 2004

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washingtonpost.com

U.S. Unprepared Despite Progress, Experts Say

By John Mintz and Joby Warrick
Washington Post Staff Writers
Monday, November 8, 2004; Page A01

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.

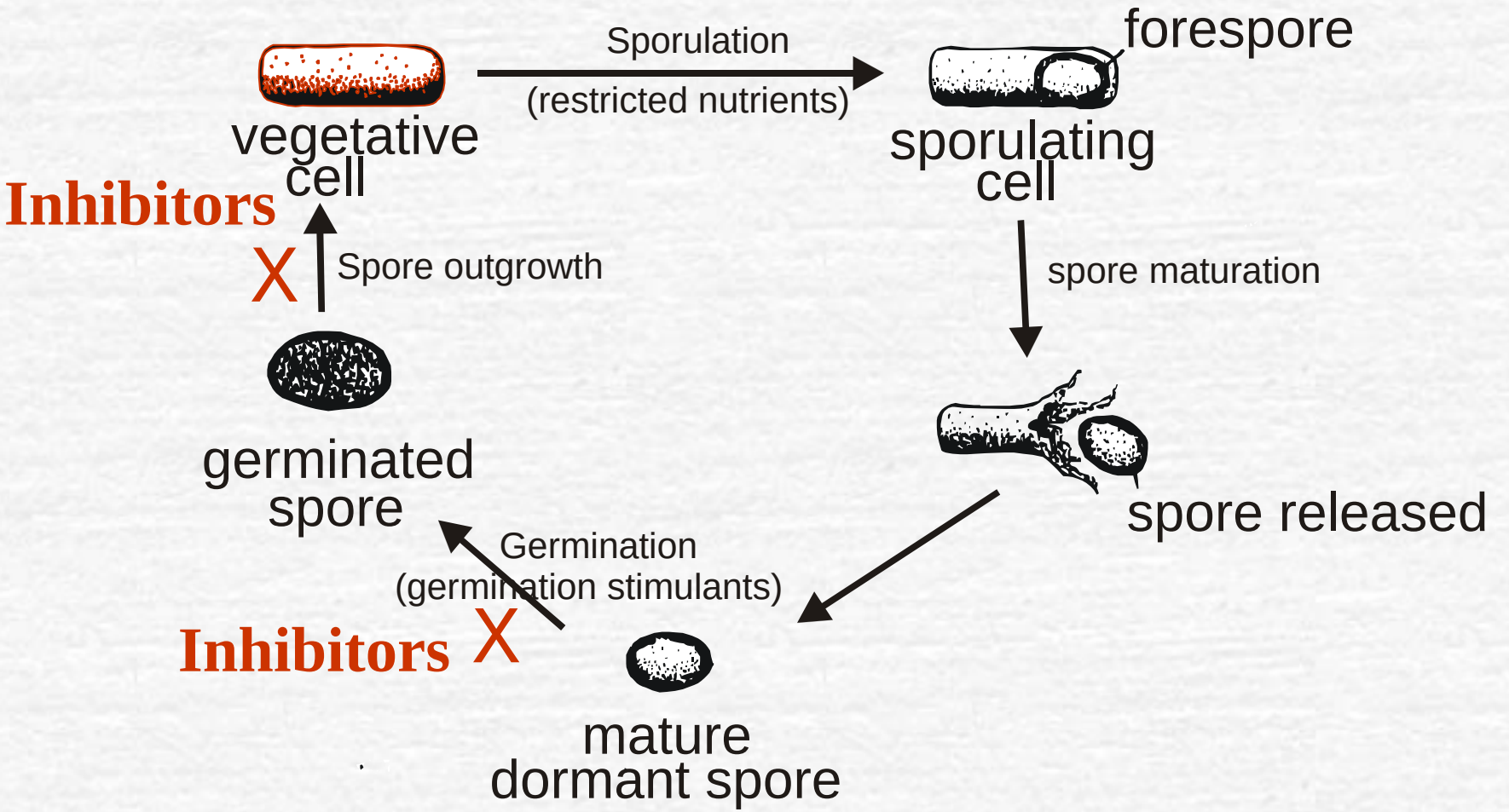
Anthrax and Botulism

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

} Pathogens

High fatality rates
No cure once infection established

Life Cycle of *Bacillus anthracis*



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

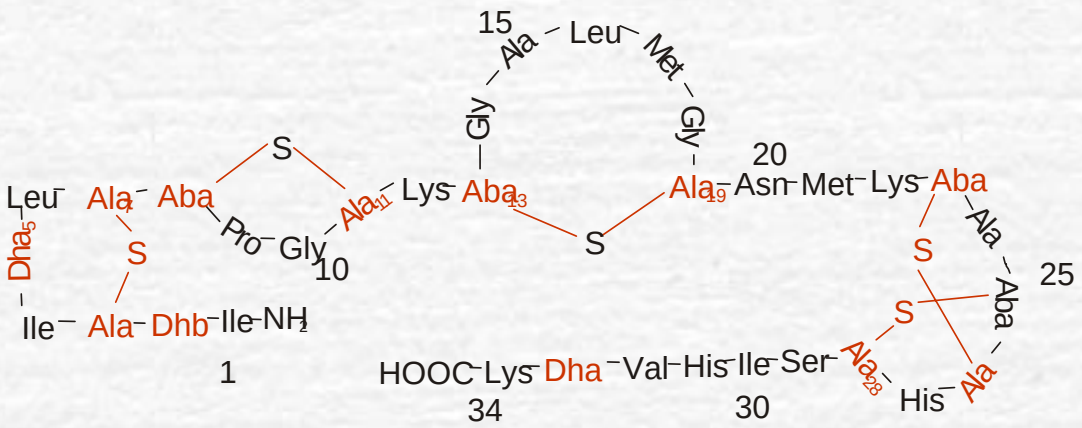
- Gene-encoded peptides
  - Produced by Gram-positive bacteria
  - Structures can be altered by genetic engineering
- Contain unusual amino acid residues
  - Introduced by post-translational modification
  - Non-standard amino acids possess unique chemical and biological properties

Many Lantibiotics are Known

Name of Lantibiotic	M_r	Producer Organism
Nisin	**3353**	***Lactococcus lactis***
Subtilin	**3317**	***Bacillus subtilis***
Epidermin	**2164**	***Staphylococcus epidermidis***
Pep5	**3488**	***Staphylococcus epidermidis***
Duramycin A	**2012**	***Streptomyces cinnamoneus***
Duramycin B		***Streptomyces cinnamoneus***
Duramycin C		***Streptomyces cinnamoneus***
Cinnamycin	**2041**	***Streptomyces cinnamoneus***
Ancovenin	**1959**	***Streptomyces sp.***
Mersacidin	**1825**	***Bacillus subtilis***
Actagardine	**1890**	***Actinoplanes sp.***
Lacticin 481	**2901**	***Lactococcus lactis***
Streptococcin AFF 22	**2795**	***Streptococcus pyrogenes***
Salivaricin A	**2315**	***Streptococcus salilvarius***
Lactocin S	**3769**	***Lactobacillus sake***
Carnocin IU 49	**4635**	***Carnobacterium piscicola***
Mutacin	**3245**	***Streptococcus mutans***
Cytolysin		***Enterococcus faecalis***

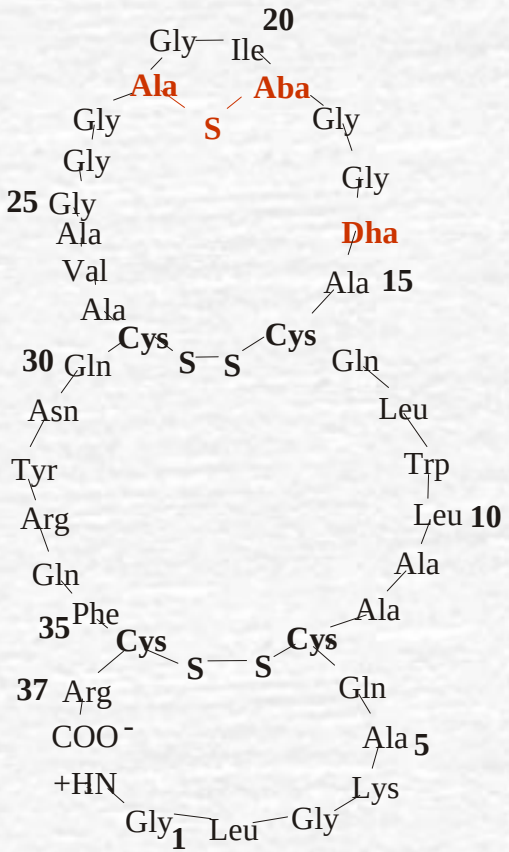
Nisin & Sublancin

Nisin



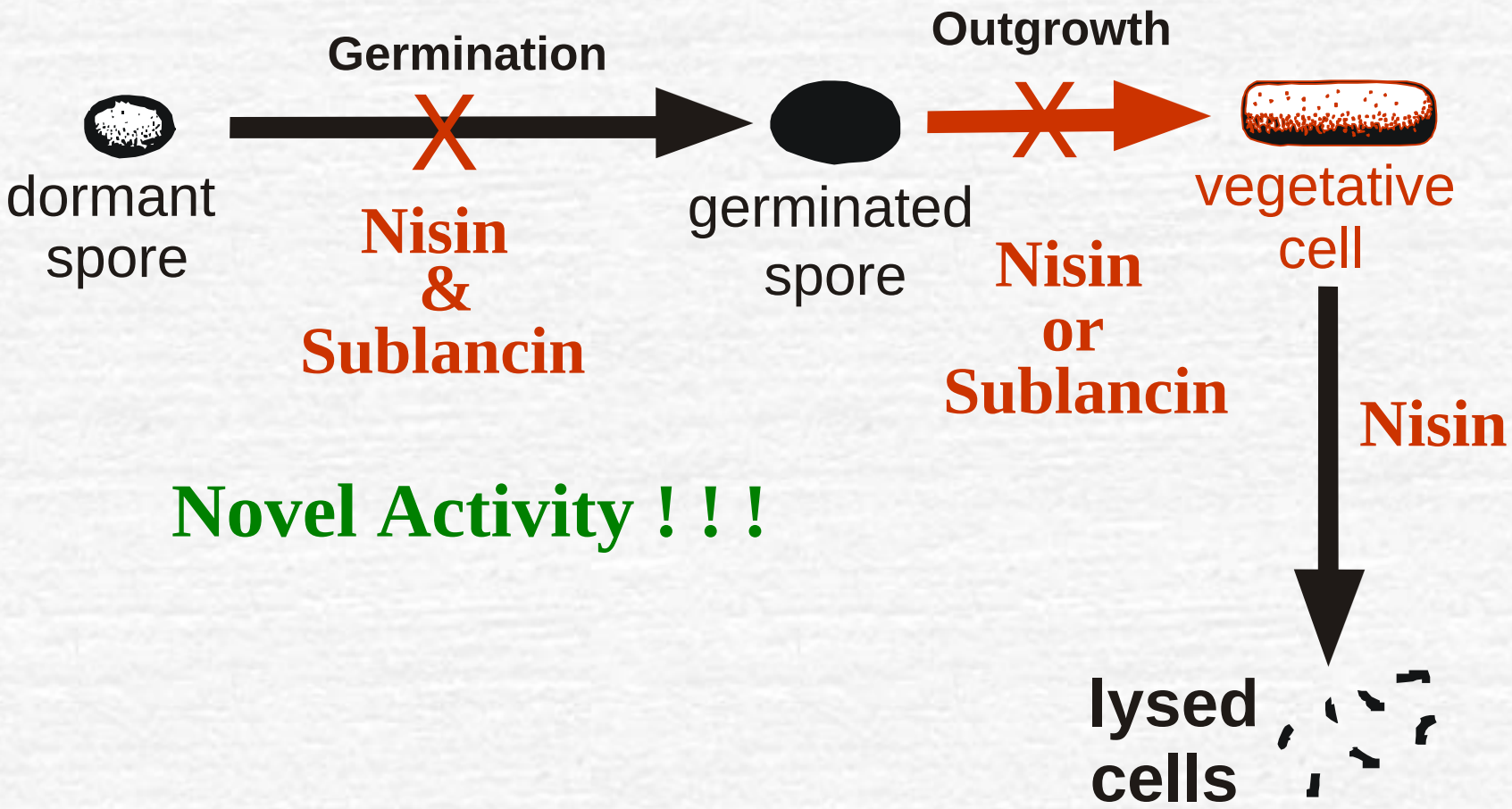
1928, Rogers & Whittier

Sublancin



1997, Paik & Hansen

Inhibition of Pathogenesis of *Bacillus anthracis*

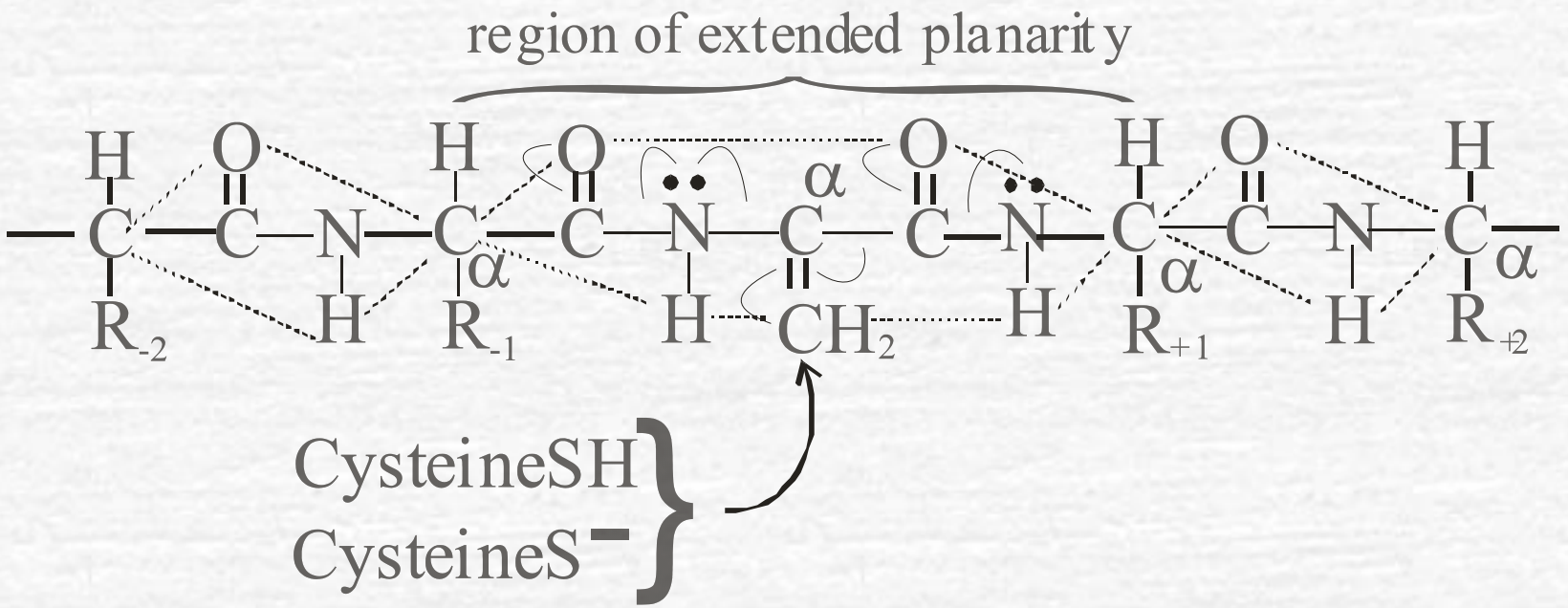


Novel Activity ! ! !

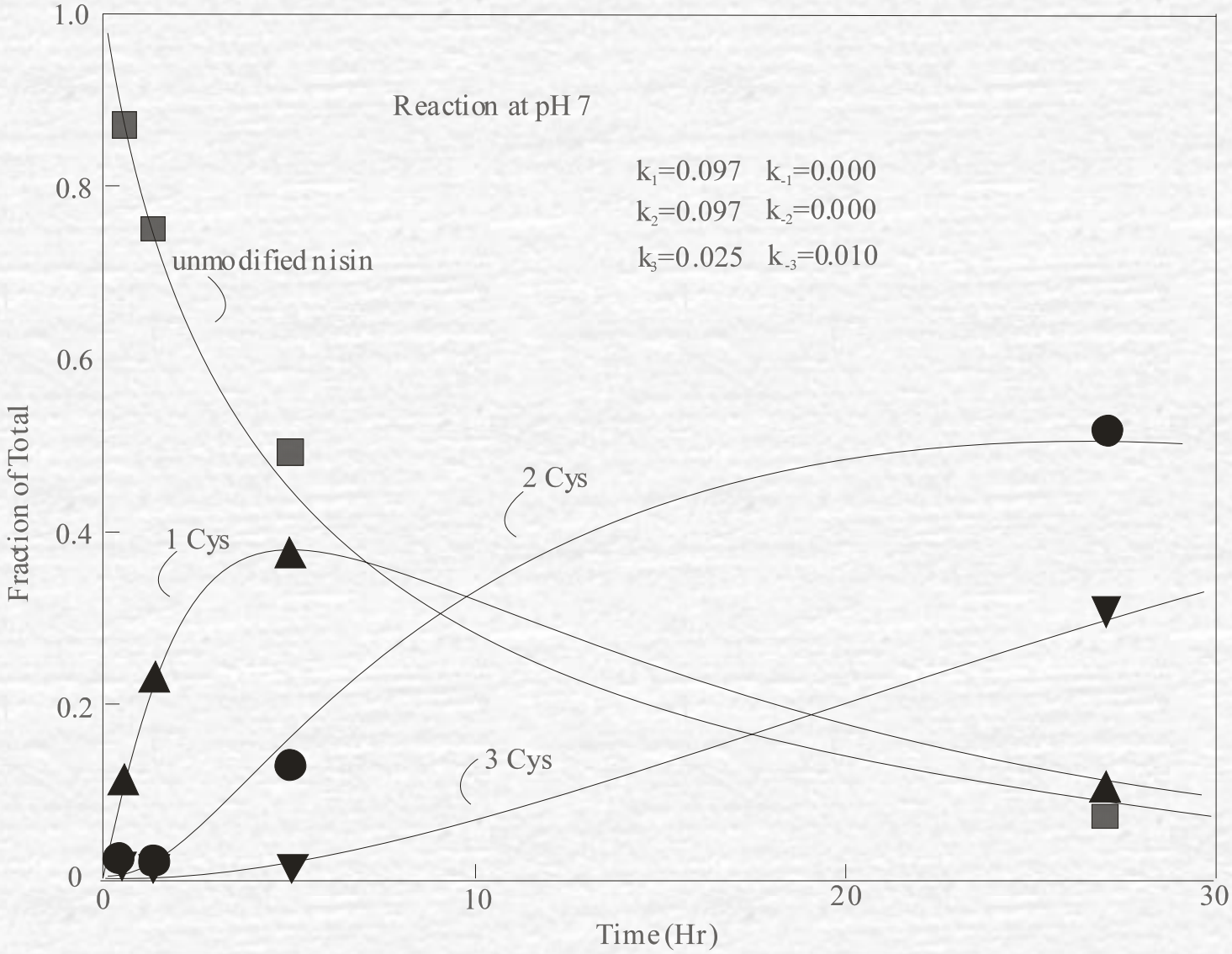
Mechanism of Antimicrobial Action

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

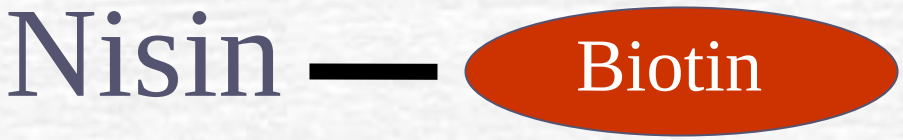
Covalent Attachment of Dehydro Residues

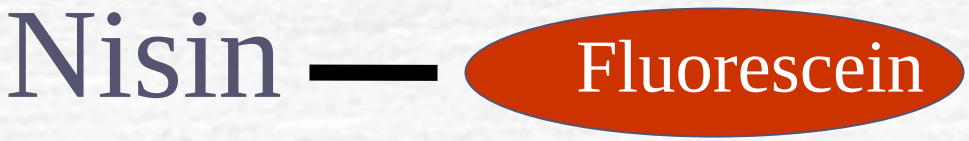


Cysteine Addition to Nisin

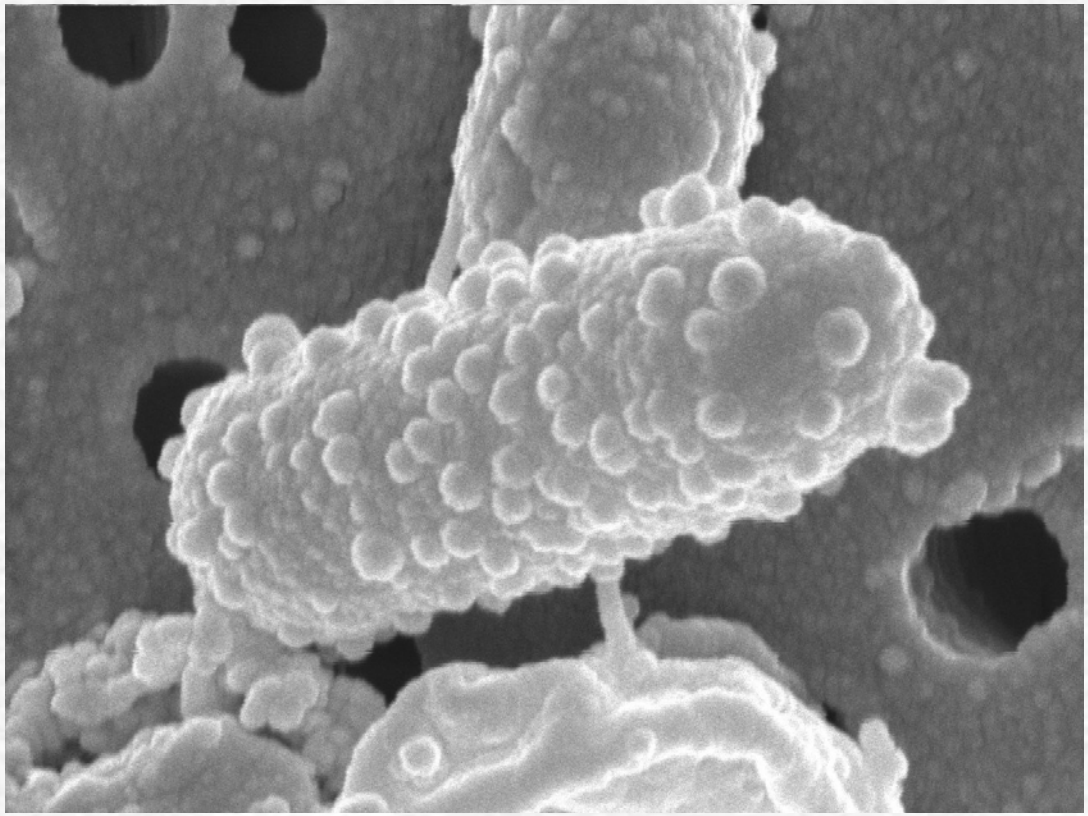


Labeled Probes

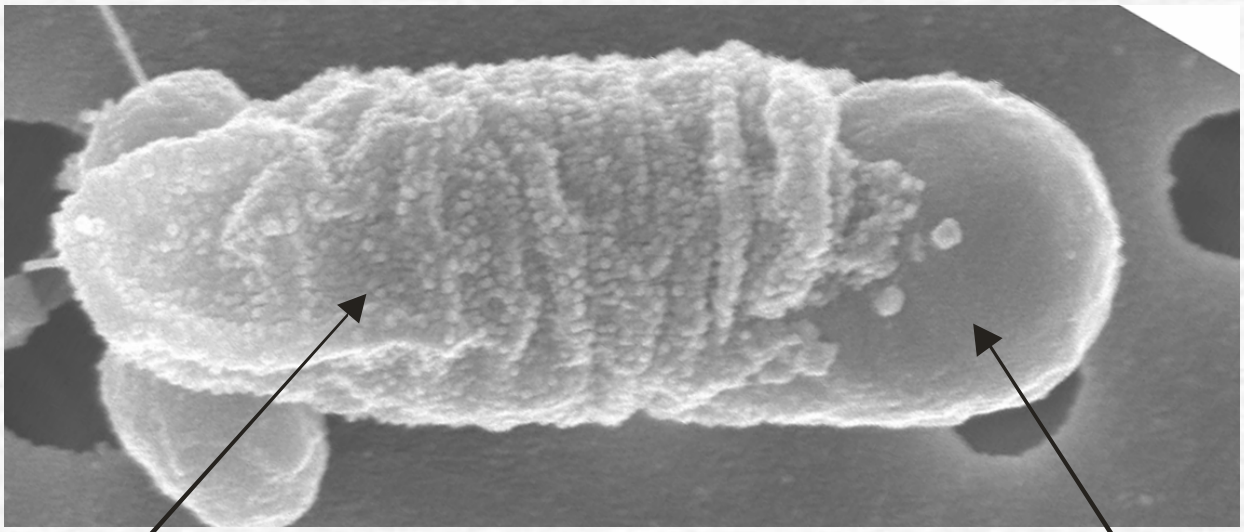




Bacillus cereus T Spore



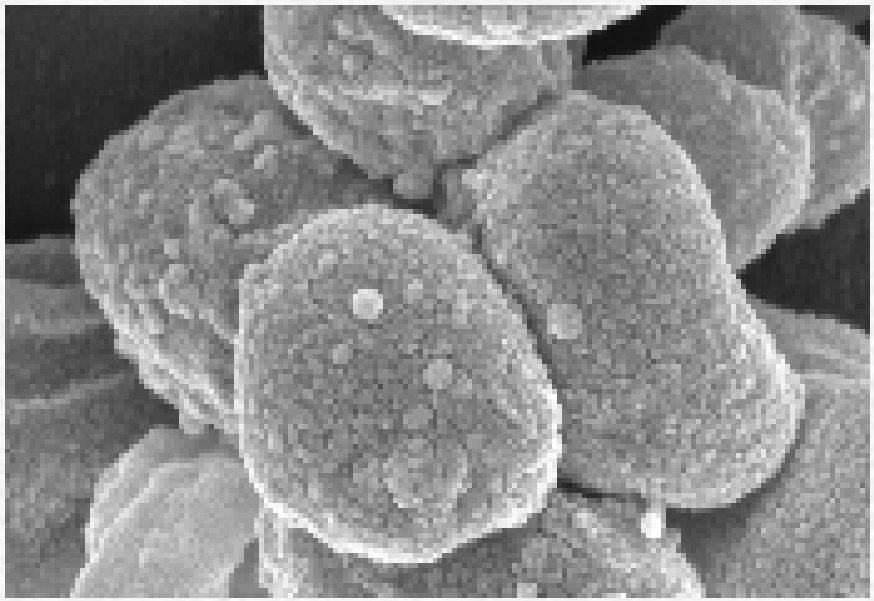
Uninhibited Outgrowing Spore



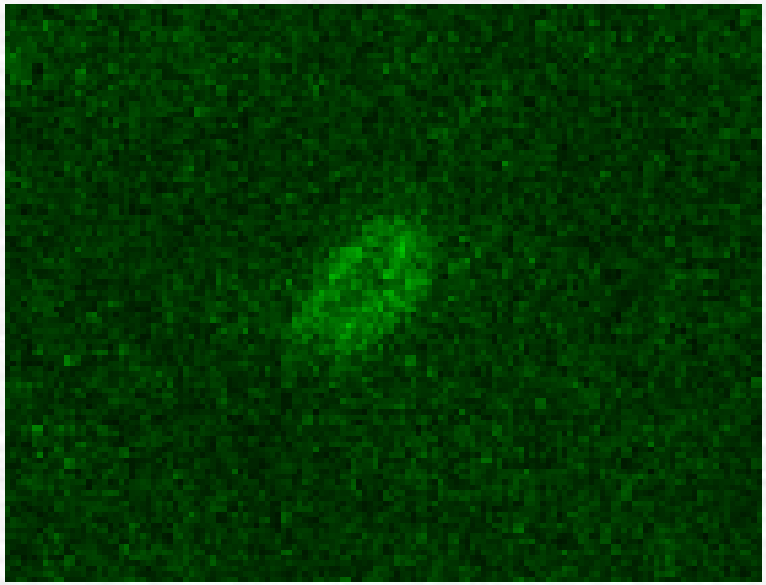
Spore Coat

emerging cell

Nisin-Fluorescein Inhibited Spores



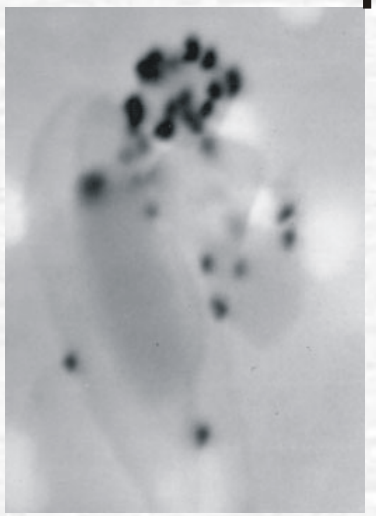
Nisin-Fluorescein Labeled Spore



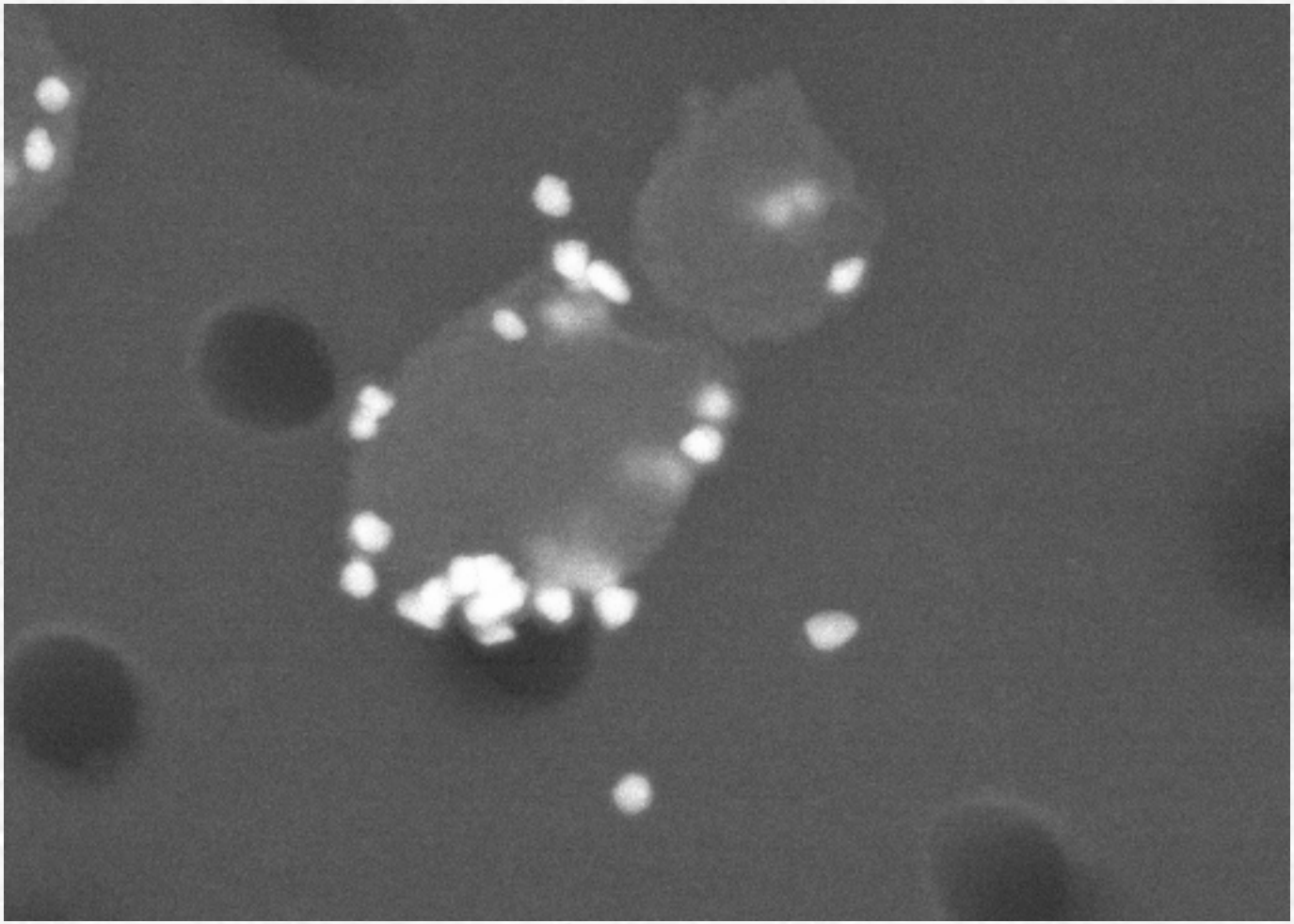
Fluorescence Microscopy

Electron Microscopy

Immunogold
Nisin-biotin Spore

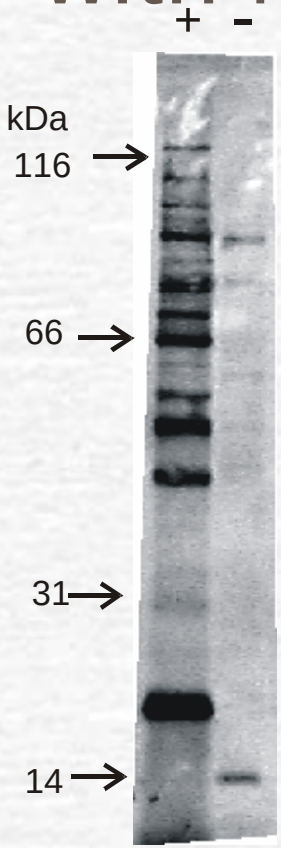


Nisin-Biotin Labeled Spores



Immunogold Detection

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



(+) Lysate of cells treated with biotinylated nisin

(-) Lysate of untreated cells

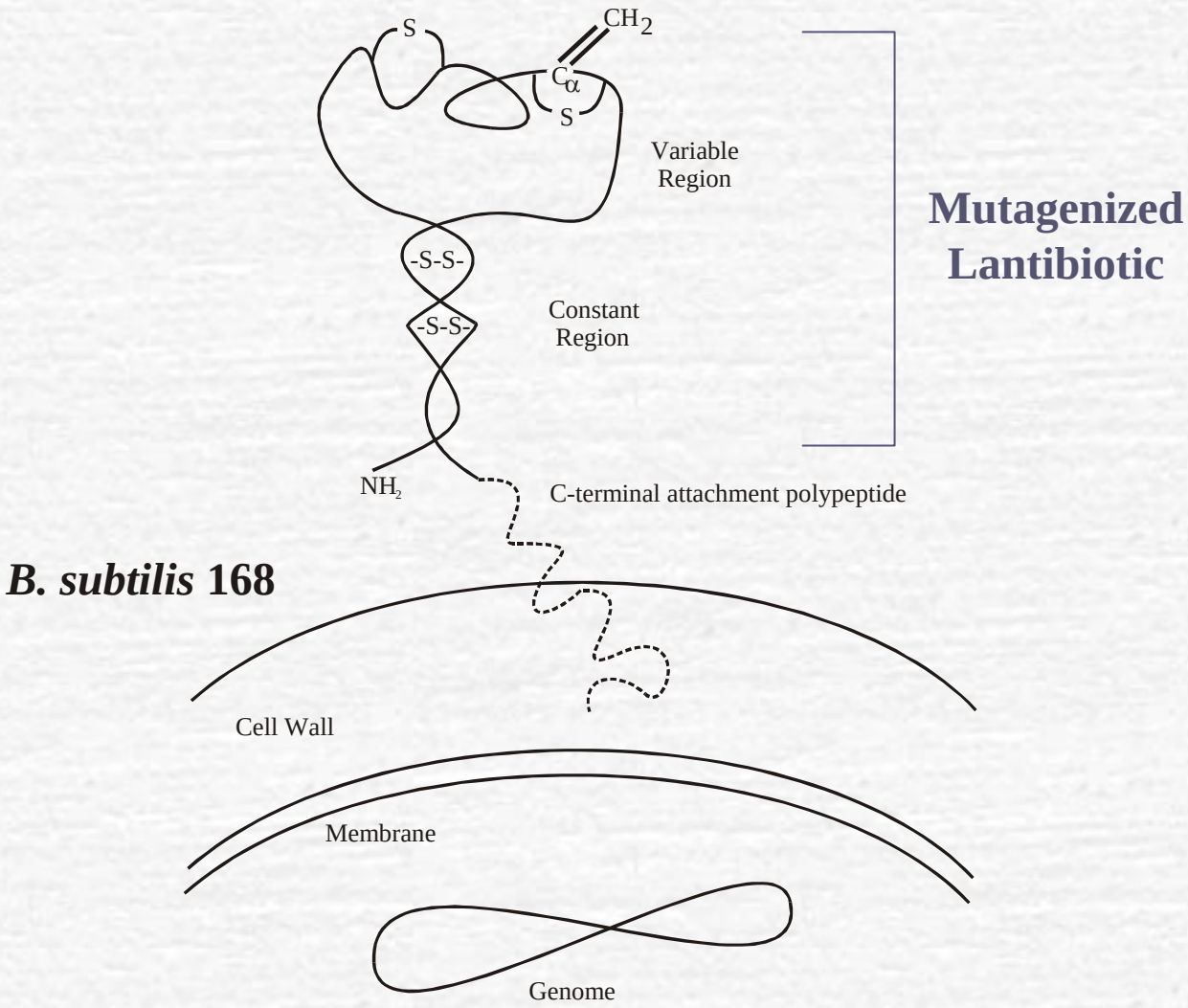
Can Lantibiotics Respond to Mutagenized Pathogens?

- 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



Amino Acid sequence of Engineered Display Peptide

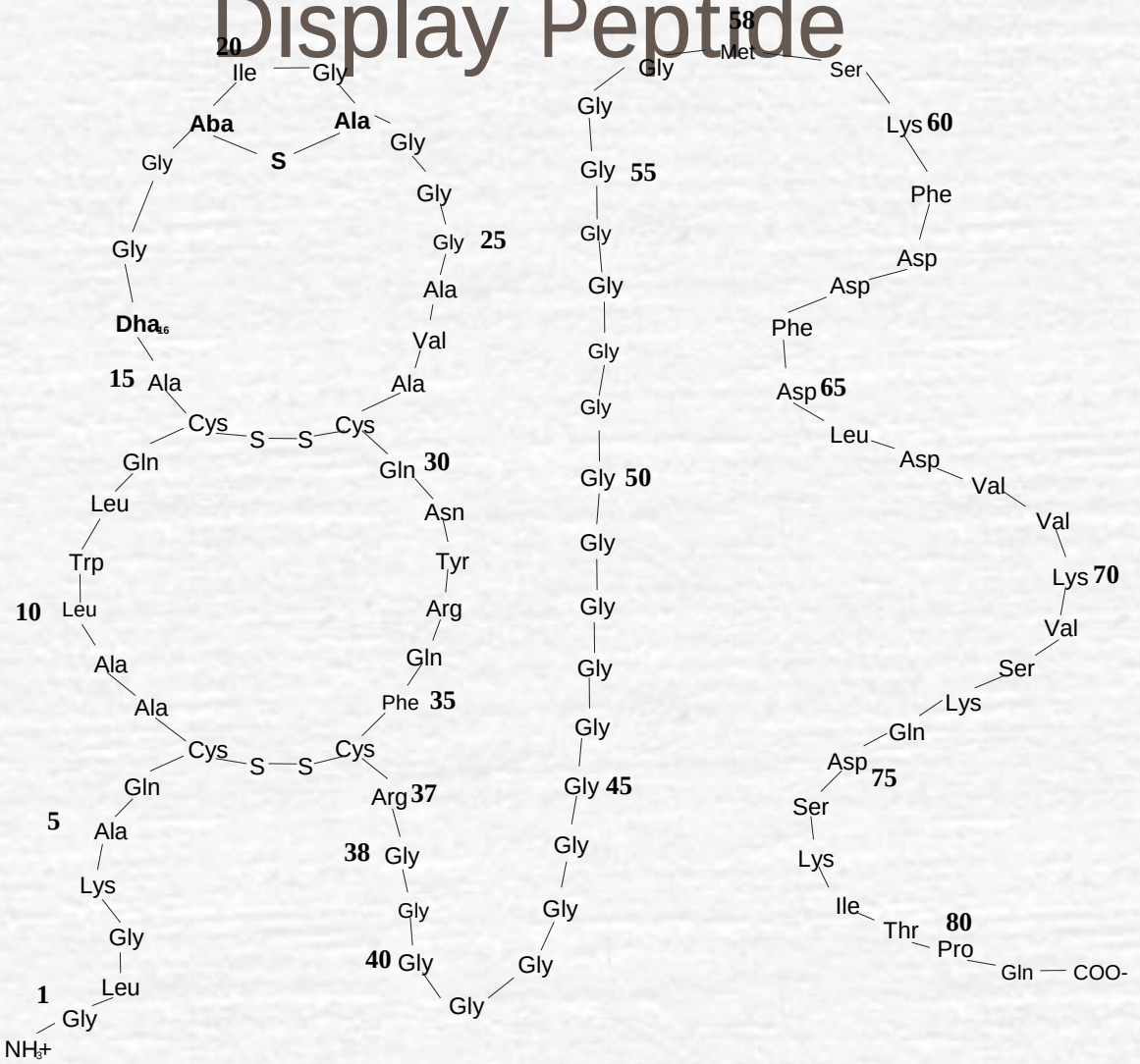


Figure 8. Lantibody Display Peptide as expressed from 168. Consists of mature subtilin segment (1-37), a 20-residue poly Gly sequence (38-57), and the subtilin leader segment (58-81).

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.

Acknowledgments

- Srilatha Kuntumalla
- Monica Sharma
- Amer Villaruz
- Sahru Yuksel
- Gaobo Zhou
- Brian Balgley