



# **Life Science for Sustainability: Plants as Production Factories for Recombinant Proteins**

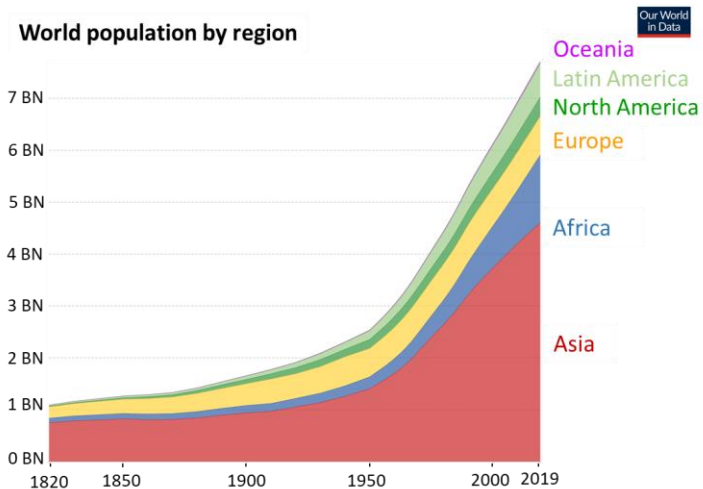
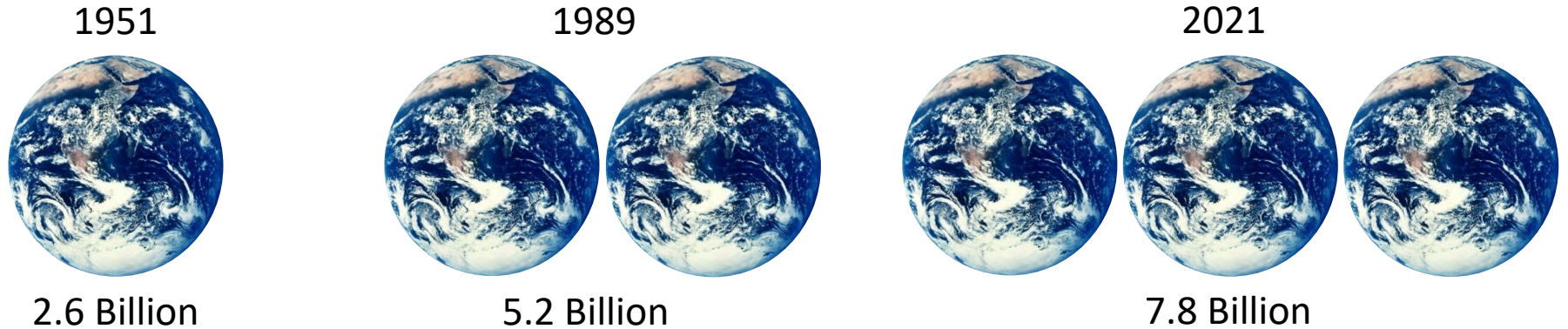
Anatoli Giritch

Nomad Bioscience GmbH, Halle (Saale), Germany

Fifth International Jean Monnet Summer School  
National University of Food Technologies, Kyiv, Ukraine  
June 10, 2021

# Global Population and Sustainable Development

## Needs and challenges

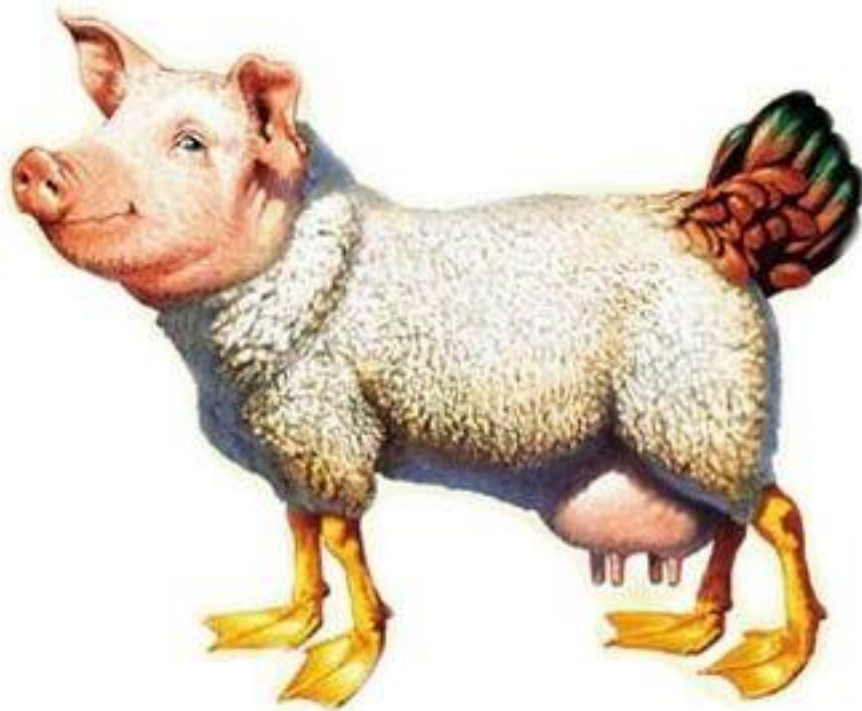


Sustainable development: balancing local and global efforts to meet human needs without destroying or degrading the natural environment.

- ✓ Political
- ✓ Cultural
- ✓ Technological



# Biotechnology in the Modern World



[https://warriorsofmyth.fandom.com/wiki/Wollmilchsau?file=Eierlegende\\_wollmilchsau.jpg](https://warriorsofmyth.fandom.com/wiki/Wollmilchsau?file=Eierlegende_wollmilchsau.jpg)

Die eierlegende Wollmilchsau  
(egg-laying wool-milk-sow)

**Biotechnology** is technology that utilizes biological systems, living organisms or parts of this to create various products or for environmental management

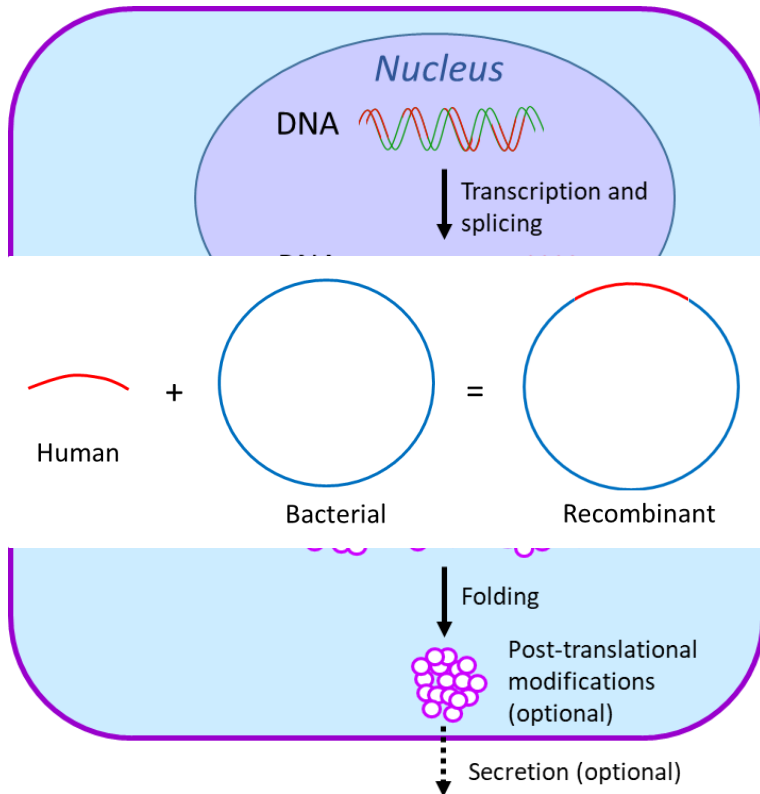
- ✓ Health
- ✓ Food
- ✓ Agriculture
- ✓ Industry
- ✓ Environment



# Native vs Recombinant Proteins

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- Proteins (polypeptides) = natural biopolymers composed of amino acids, 16% human body
- Cellular functions: structural, enzymatic, transport, regulatory, defence etc.
- Use: nutritional, medicine (vaccines, drugs, diagnostics), food industry, fuel industry etc.



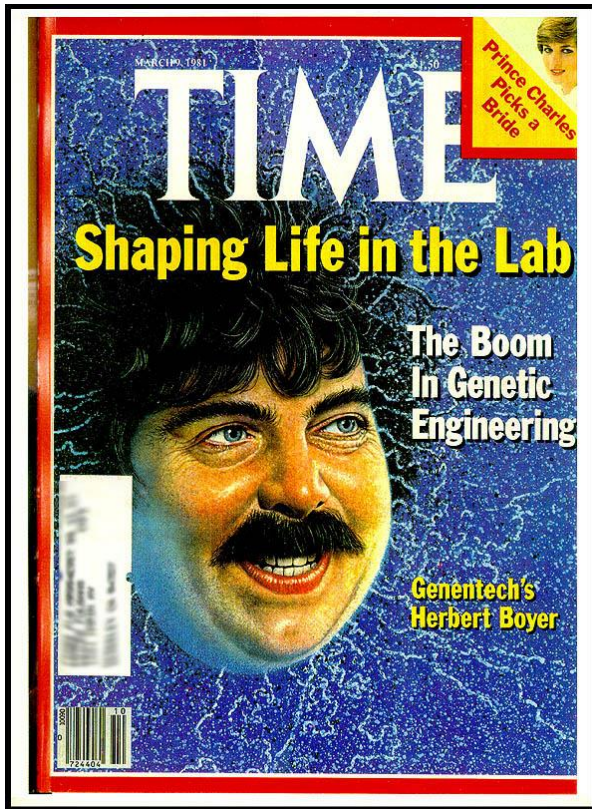
- Recombinant proteins are produced from recombinant DNA using biotechnological methods
- Recombinant DNA is a DNA molecule created in laboratory using methods of genetic recombination which combines genetic material from different sources
- Recombinant proteins can be the copies of native proteins or novel molecules not existing in the nature
- Various production hosts: bacteria, fungi, insect cells, mammalian cells, plants



## Back to the History

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- 1972 - The idea for the gene cloning technique first arose at a scientific meeting on plasmids in Honolulu
- 1973 and 1974 - Stanley Cohen, Herbert Boyer, and colleagues at Stanford and UC San Francisco published first three landmark papers on recombinant DNA



Time Magazine-March 9, 1981

- 1976 - Genentech, incorporated by Herbert Boyer and venture capitalist Robert Swanson, was the first company founded on the basis of recombinant DNA technology
- Genentech produced recombinant human insulin in *E. coli*
- Humulin (recombinant human insulin) was approved by FDA in 1982
- Humulin was the first recombinant pharmaceutical approved for use
- Production and commercialization by Eli Lilly up to day





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# Production platforms

minus  $\longrightarrow$  plus

SWOT Analysis

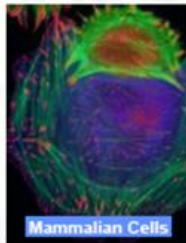
Speed						
R&D speed						
Operating cost						
Capital cost						
Glycosylation						
Safety						
Regulatory risk						



Animals



Yeasts



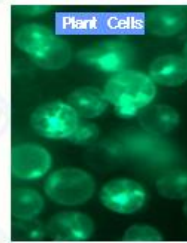
CHO cells



Plants



E. coli



Plant cells

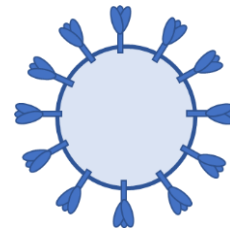
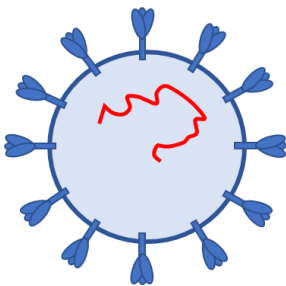


# Plant-Made Recombinant Proteins: Approved & in Development

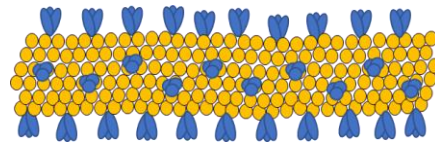
## Several products advanced:

- Glucocerebrosidase/Protalix, approved
- Anti-carries Mab/Planet, approved
- Fabry Disease Therapy/Protalix, clinical studies completed
- Cystic Fibrosis Therapy/Protalix, Phase II
- Lactoferrin/Ventria, Phase II completed
- Anti-HIV Mab PharmaPlanta, Phase I
- Antimicrobial Proteins/Nomad, GRAS approved
- Thaumatin/Nomad, GRAS approved
- NHL Vaccine/Icon-Bayer, Phase I completed
- Influenza Vaccine/Medicago, approved
- Anti-Ebola Mab, Mapp, Phase I-II
- COVID-19 Vaccine/Kentucky BioProcessing, Phase II
- COVID-19 Vaccine/Medicago, Phase III

*SARS-Cov-2  
Virus*



Viris-Like Particle (VLP vaccine),  
Medicago



Epitope vaccine  
(TMV scaffold),  
Kentucky BioProcessing



# Nomad Bioscience GmbH



Yuri Gleba

- Plant Biotech Company, founded in 2008 in Halle (Saale), Germany
- Subsidiary: Nomads UAB, Vilnius, Lithuania
- Formerly known as Icon Genetics GmbH (since 1999)
- Research & Development (R&D)
- Developed plant-based expression systems for recombinant proteins: magnICON<sup>®</sup> and NOMADIC<sup>®</sup>
- Main focus: pharmaceuticals, food safety, agronomic traits, biomaterials



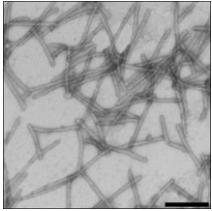




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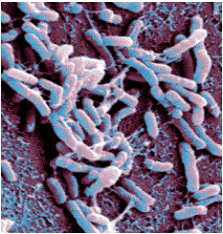
# magnICON® Expression Technology

1



**Virus**  
(TMV or PVX)

2



**Agrobacterium tumefaciens**

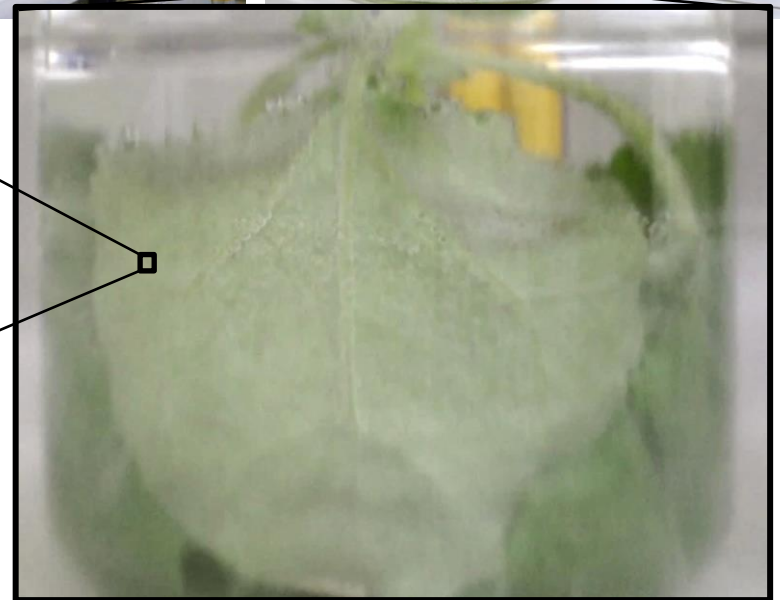
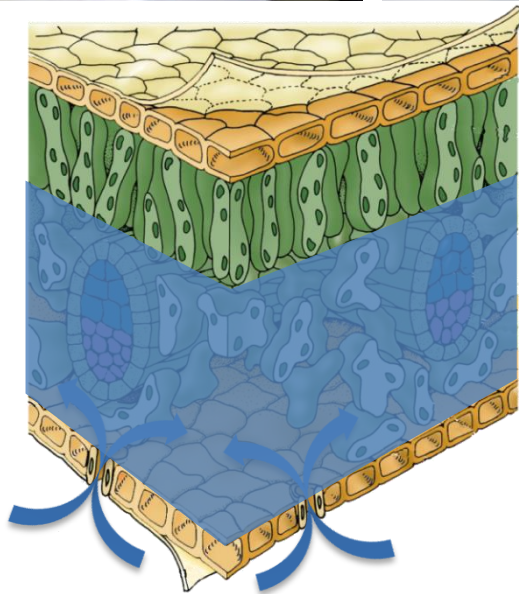
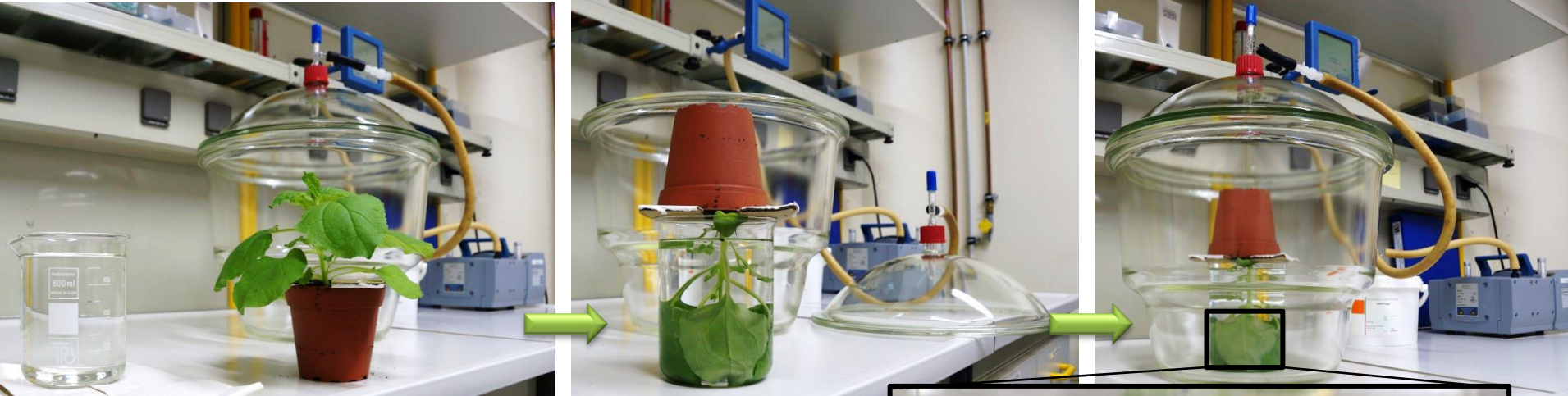
3



**Production host**  
*Nicotiana benthamiana*



# magnICON® Expression System Vacuum Infiltration





# magnICON® & NOMADIC® Expression Technologies



magnICON®

transfection using vacuum infiltration  
ideal for high-cost products,  
e.g. **biopharmaceuticals, vaccines**



NOMADIC®

transfection using spraying  
ideal for low-cost products,  
e.g. **industrial enzymes, agronomic traits**



magnICON®  
transgenic

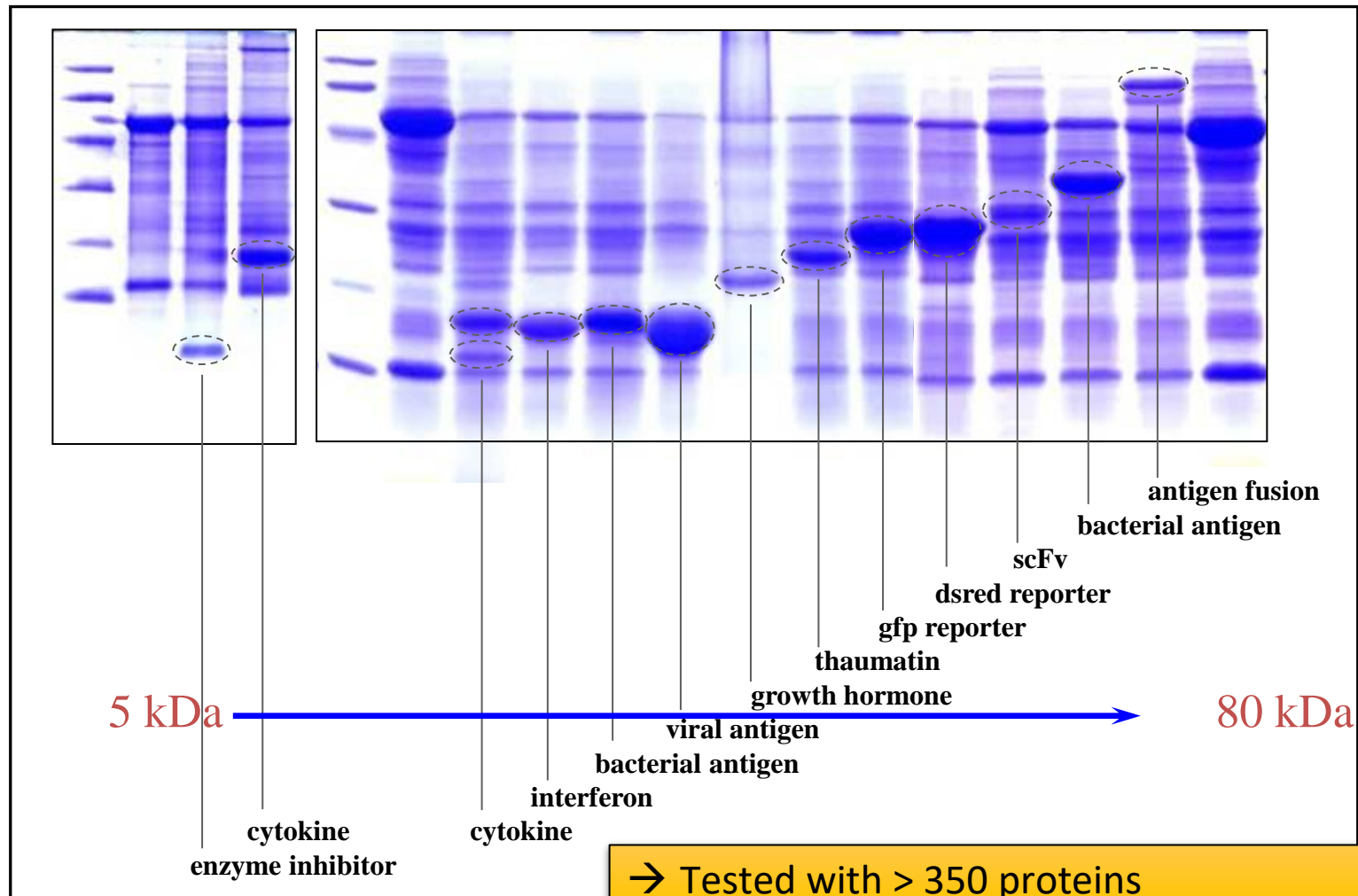
ethanol-inducible amplification  
ideal for high-volume products,  
e.g. **biomaterials, antimicrobials**



# cGMP Certified Facility in Halle, Germany



# magnICON<sup>®</sup>: Platform Versatility



→ Tested with > 350 proteins  
 → Expression level up to 5-6 g/kg fresh weight



# Origin of Virology

- Dmitri Iosifovich Ivanovsky (1864–1920) son of an impoverished landowner from Kherson region (Ukraine)
  - 1886 - Adolf Mayer described mosaic disease of tobacco
  - 1887- Ivanovsky studies at St. Petersburg University, investigates tobacco disease ('ryabucha') in South Ukraine and Bessarabia
  - 1890 – The same studies in Crimea
  - 1892 - Ivanovsky published the paper describing extremely small pathogen passing through porcelain filters that retain bacteria
  - 1892 - Defended his thesis at St. Volodymyr University of Kyiv
  - 1897 - Martinus Beijerinck named the new pathogen *virus*



Dmitri Ivanovsky in his young years  
(from Lechevalier H., Dmitri Iosifovich  
Ivanovski, Bacteriol Rev. 1972, 36: 2, 135–  
145).



Tobacco Mosaic Disease  
(<http://ephytia.inra.fr/en/C/10885/Tobacco-Main-Symptoms>)

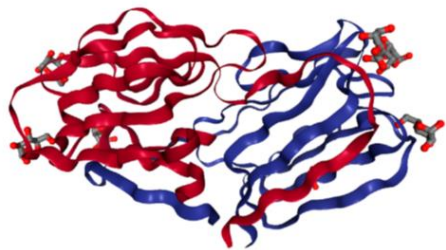


Taras Shevchenko National University of Kyiv,  
former St. Volodymyr University of Kyiv

# Griffithsin against SARS-CoV-2

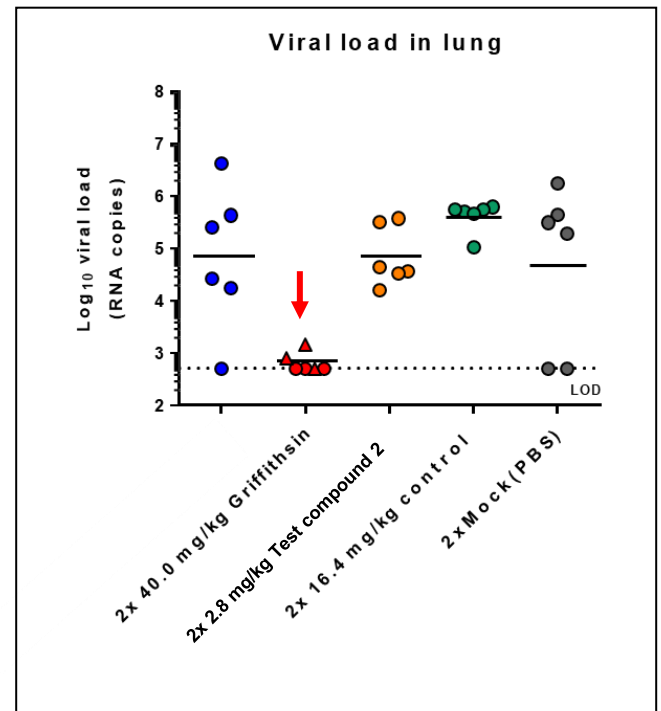
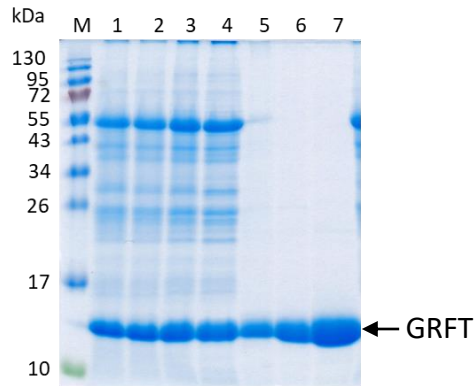
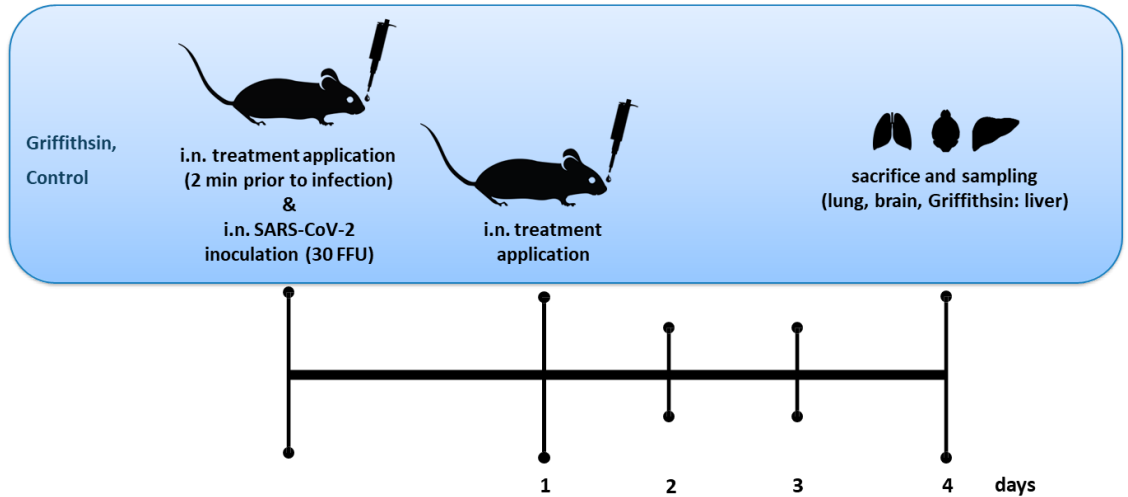


*Griffithsia*



*Lectin Griffithsin*

Monomer: 122 aa; 12.8 kDa  
Functional as dimer



# Mapp Biopharmaceutical: Ebola Immunotherapy



Nancy Writebol, Samaritan's Purse aid worker





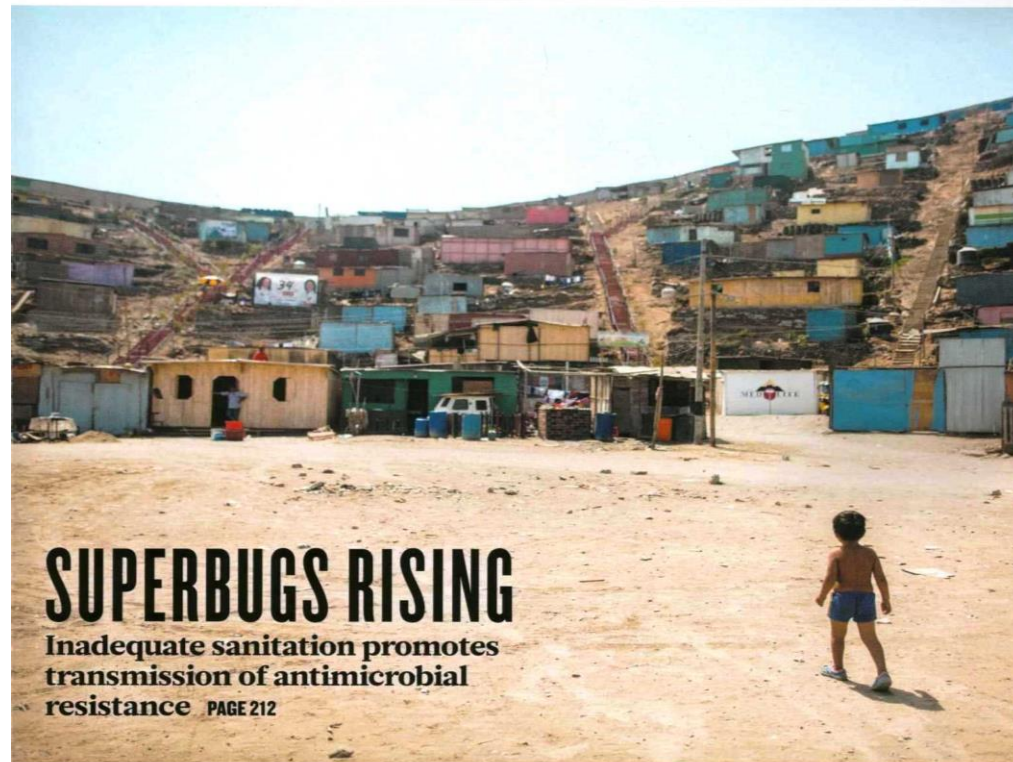
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# Pan-Drug Resistant Bacteria?

OUTLOOK  
Open Access content

# nature

THE INTERNATIONAL WEEKLY JOURNAL OF SCIENCE



## SUPERBUGS RISING

**Inadequate sanitation promotes transmission of antimicrobial resistance** **PAGE 212**

CYBERSECURITY

**PASSWORD PSYCHOLOGY**  
*The human fallibility factor in cybercrime*  
**PAGE 164**

HUMAN EMBRYOLOGY

**THE 14-DAY QUESTION**  
*Are research guidelines being overtaken by events?*  
**PAGES 169, 182 & 251**

ATMOSPHERIC SCIENCE

**ECHO OF AN ANCIENT AIR**  
*Micrometeorites record high-level Archaean oxygen*  
**PAGES 184 & 235**

NATURE.COM/NATURE

12 May 2016 £10

Vol. 533, No. 7602

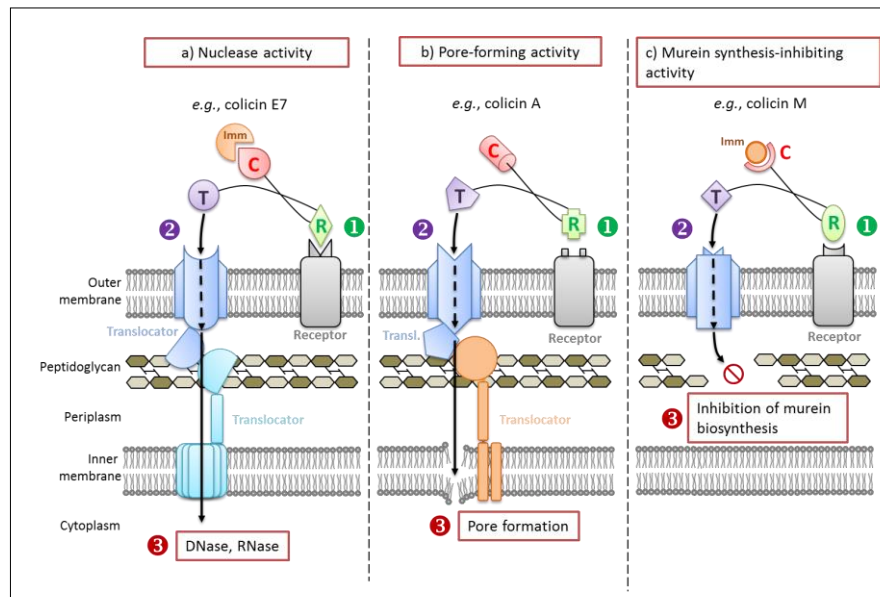
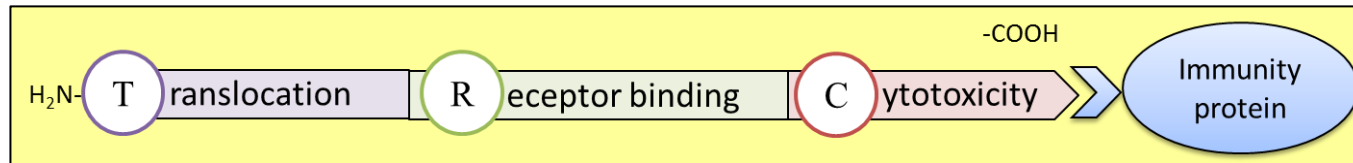


# Bacteriocins: Antimicrobials for Gram-negatives

**Bacteriocins** – ribosomally synthesized antimicrobial protein produced by bacteria to inhibit growth of similar or closely related bacterial strains

**Colicins** – bacteriocins from *E. coli*

**Salmocins** – bacteriocins from *Salmonella*



Klein *et al.*, *Biochem. J.*, 2016  
473 (18) 2799-2812





# Antimicrobial Proteins vs. Antibiotics

Compounds	Antibiotics	Bacteriocins	Phage endolysins
<b>Features</b>			
Identity	Small molecule	Protein/peptide	Protein
Synthesis	Secondary metabolite	Ribosomal	Ribosomal
Activity	Varying spectrum	Narrow spectrum	Narrow spectrum
Mode of action	Intracellular targets and cell membrane	Pore formation, DNase/RNase activity, inhibition of cell wall synthesis	Cell wall degradation
Environmental Stability	Stable	Easily degradable	Easily degradable



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# Foodborne Pathogens

## Bacterial pathogens:

### Gram-negative

*Escherichia coli*

*Salmonella spp.*

*Klebsiella spp.*

*Shigella spp.*

*Campylobacter spp.*

*Yersinia spp.*

*Vibrio spp.*

### Gram-positive

*Clostridium spp.*

*Listeria monocytogenes*

*Bacillus cereus*

*Staphylococcus aureus*

## Viral pathogens:

Norovirus

Hepatitis A

SARS

Rotaviruses

Newly emerging

viruses

## Parasitic pathogens:

Nematodes

-*Ascaris*

-*Trichinella*

Platyhelminths

Protozoa

-*Cryptosporidia*

-*Toxoplasma*

## Diarrheal diseases, USA, 2000-2008

### Infections:

Norovirus (58%)

*Salmonella spp.* (11%)

*Clostridium perfringens* (10%)

*Campylobacter spp.* (9%)

*Escherichia coli* (1%)

### Hospitalizations:

*Salmonella spp.* (35%)

Norovirus (26%)

*Campylobacter spp.* (15%)

*Toxoplasma gondii* (8%)

*Escherichia coli* (2%)

### Deaths:

*Salmonella spp.* (28%)

*Toxoplasma gondii* (24%)

*Listeria monocytogenes* (19%)

Norovirus (11%)

*Escherichia coli* (1%)

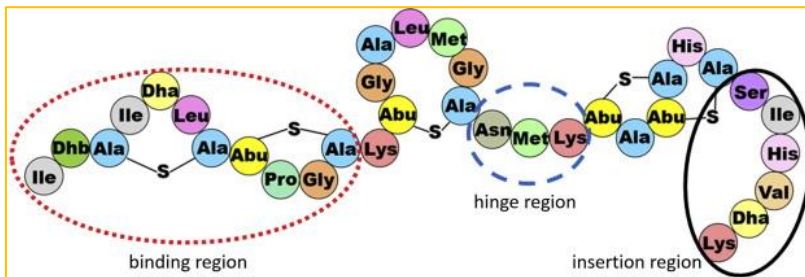
Scallan E et al 2011 Emerg Infect Dis

Newell DG et al 2010 Int J Food Microb 139

# Bacteriocin Nisin as a Food Preservative

**NISIN:** polycyclic antibacterial peptide produced by *Lactococcus lactis*

- 34 aa peptide
- Nisin A and Nisin Z (more soluble)
- Non-toxic, heat stable, sensitive to digestive proteases
- Produced since 1950s
- approved by FDA and EFSA as a food preservative
- GRAS status in USA, EU food additive list: E234
- Gram+ bacteria: LAB, *Listeria*, *Staphylococcus*, *Clostridium*



Nisin A. MW=3.4 kDa, 34 aa

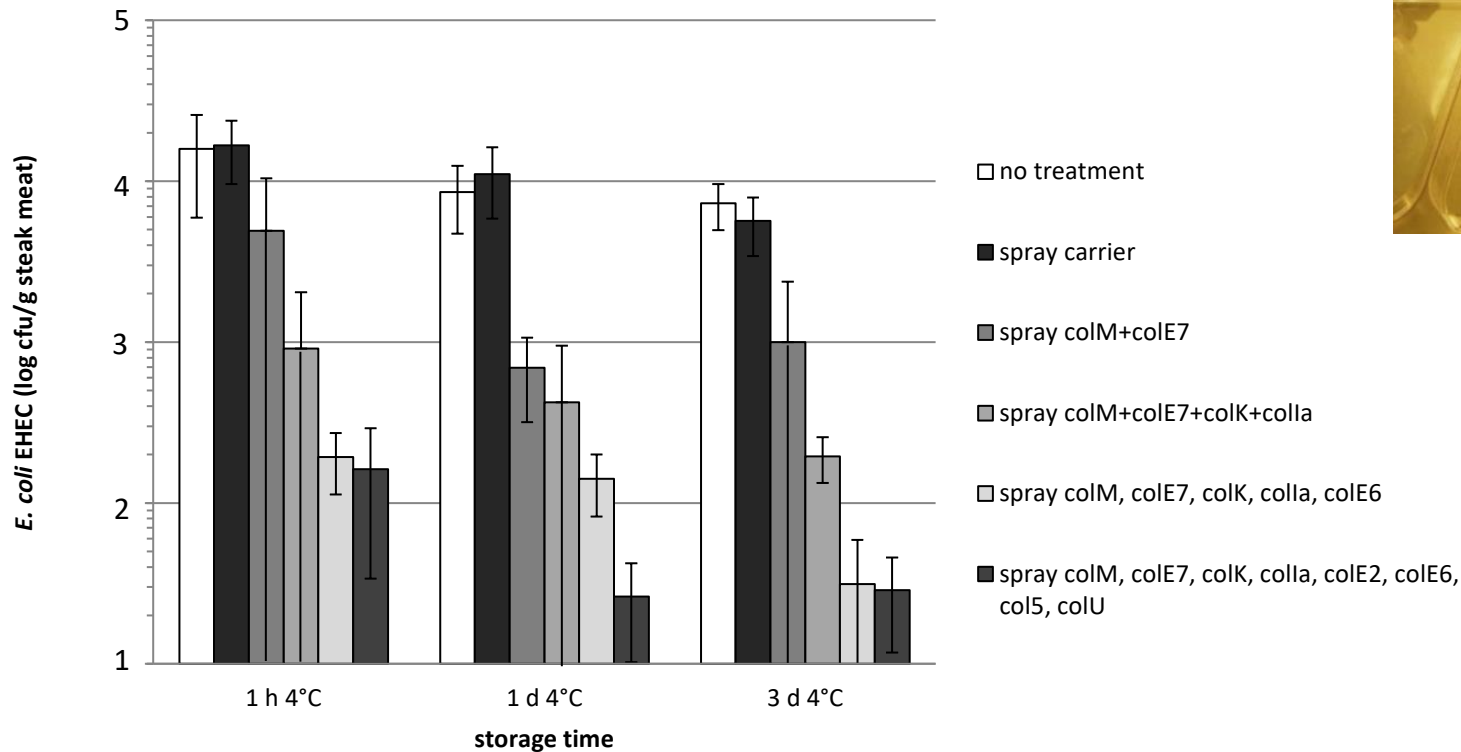
Dha, dehydroalanine; Dhb, dehydrobutyrine; Ala-S-Ala, mesolanthionine; Abu-S-Ala,  $\beta$ -methyllanthionine.

Han al. (2017) Acta Biomaterialia 53, 242-249



# Effect of Colicin Cocktail on *E. coli* ('Big Seven') on Fresh Pork Steak Meat

effect of colicin treatment on bacterial populations of *E. coli* EHEC strains (Big 7) on fresh steak meat

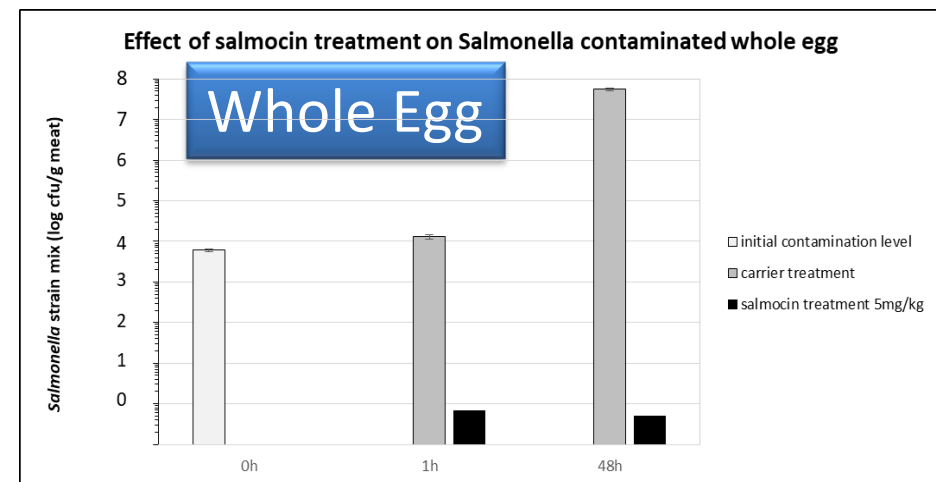
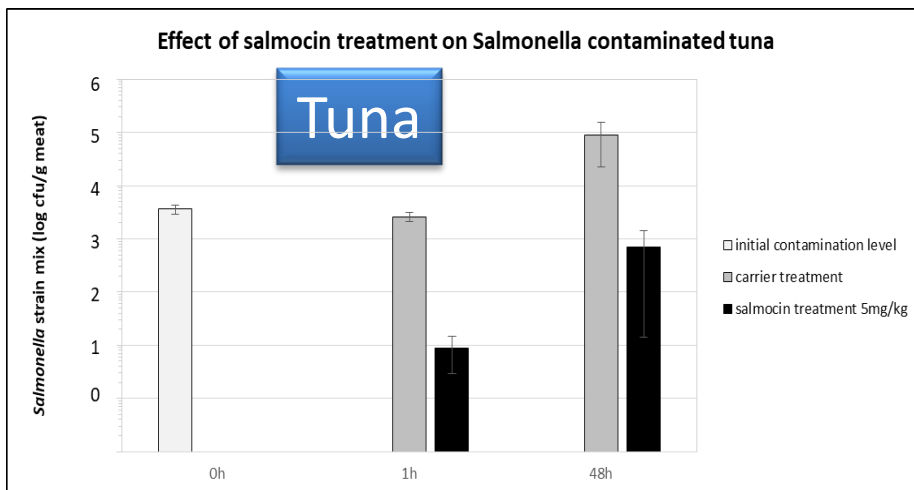
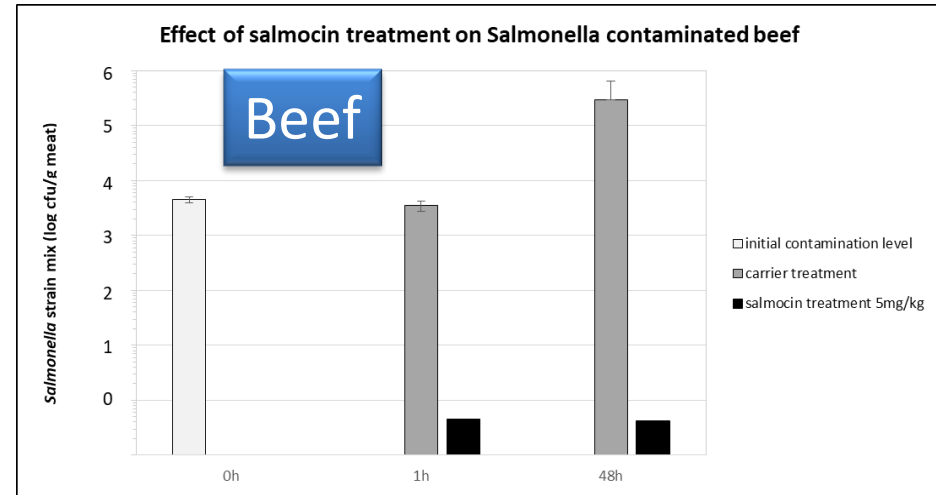


reduction of <i>E. coli</i> EHEC strains cells upon incubation:	1 h 4°C	1 d 4°C	3 d 4°C
comparison carrier /colicin treatment (3+1 mg/kg):	0.53 logs	1.2 logs	0.75 logs
comparison carrier /colicin treatment (3+1+1+1 mg/kg):	1.26 logs	1.42 logs	1.46 logs
comparison carrier /colicin treatment (3+1+1+1+1 mg/kg):	1.94 logs	1.89 logs	2.26 logs
comparison carrier /colicin treatment (3+1+1+1+1+1+1+1 mg/kg):	2.01 logs	2.64 logs	2.29 logs



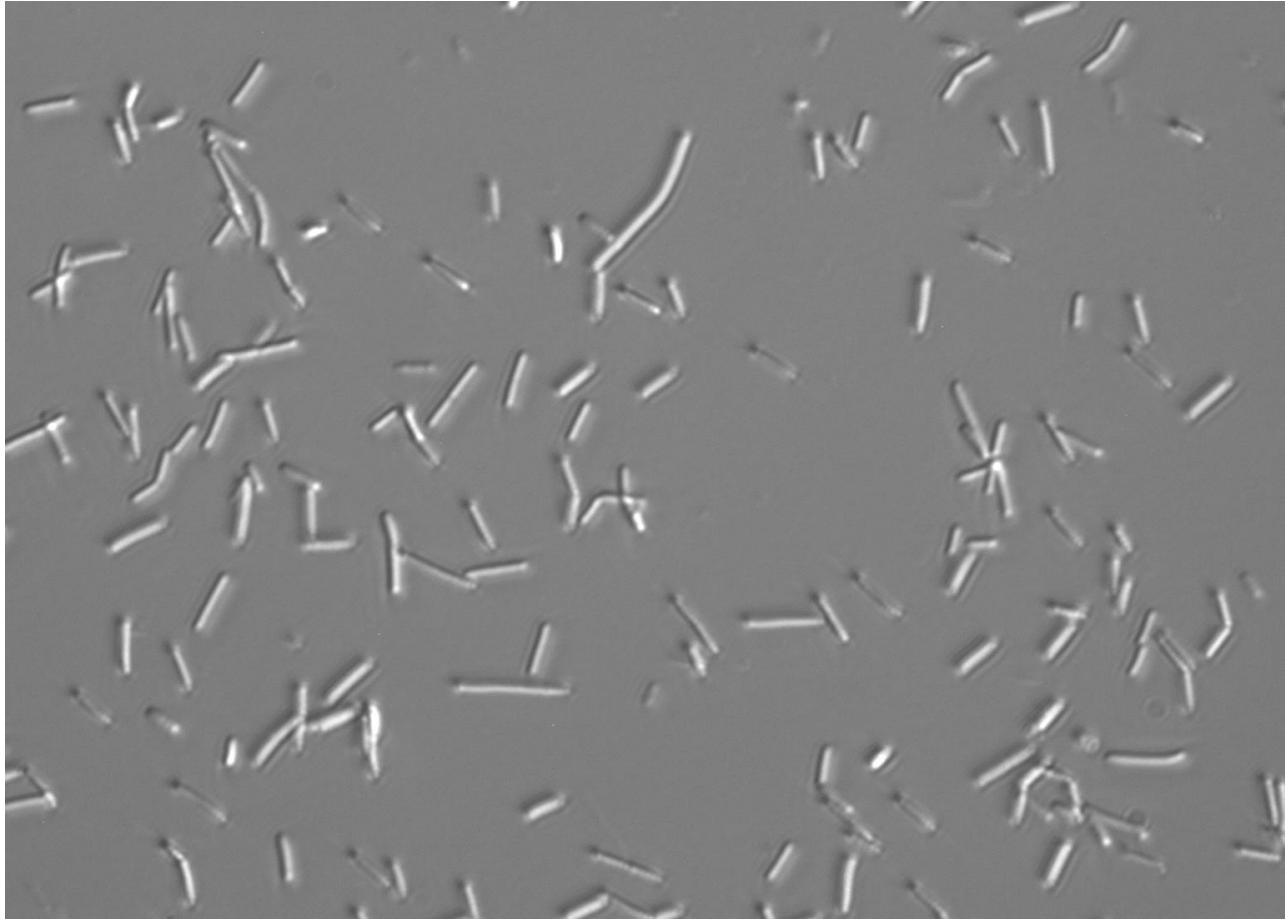
# Activity of Salmocin SalE1b on Beef, Tuna and Whole Egg

5 mg/g SalE1b





# Lysis of *Clostridium perfringens* cells with plant-made bacteriophage lysin

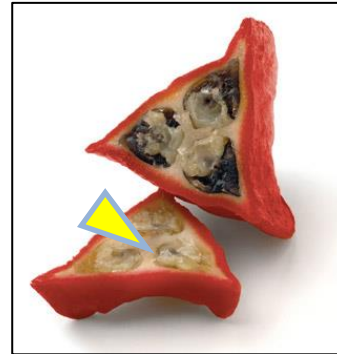


*Clostridium perfringens* NCTC8237 cells treated with plant-made bacteriophage lysin Psm (3-18 min post treatment). Video by Dr. Vaiva Kazanaviciute, UAB Nomads.



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# Natural Thaumatin



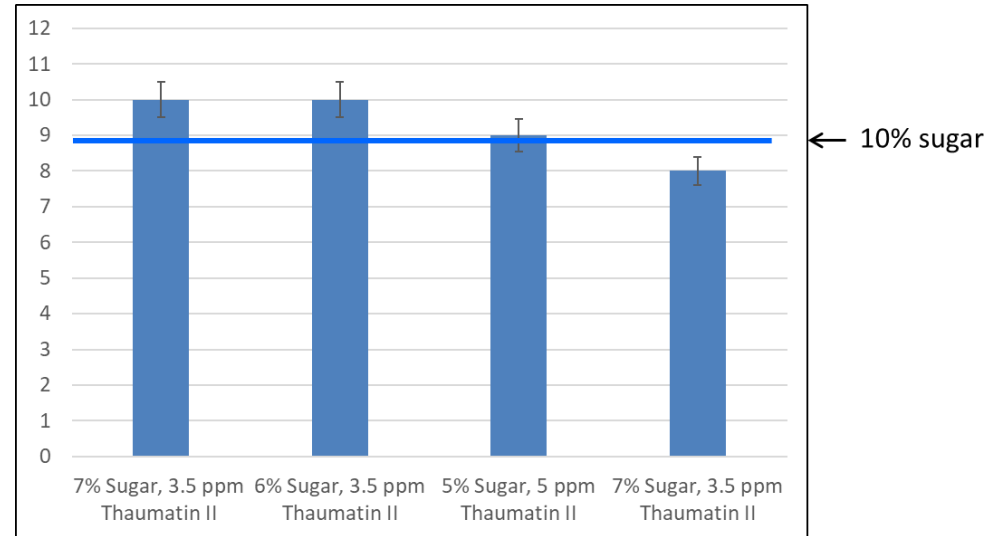
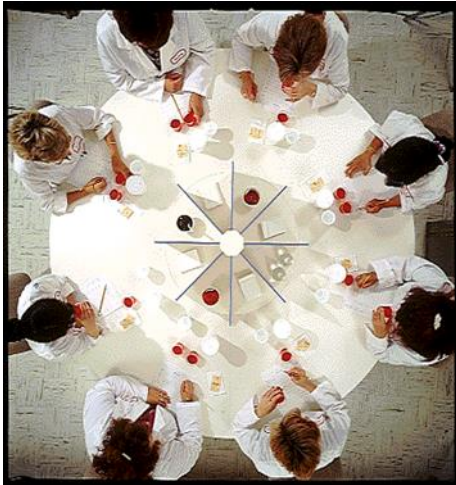
- Natural source: fruit arils of *Thaumatococcus daniellii*
- Natural source: only rainforests of Africa
- Natural source: mixture of Thaumatin I, II, a and b
- 22 kDa protein; 207 amino acids; 8 disulphide bonds; not glycosylated; water soluble; resistant to heating; stable under acidic pH; easily purified
- 2000-3000 times sweeter than sucrose on w/w basis
- Sweetness with slow onset, lingering sweetness, licorice aftertaste in the natural product
- Introduced in early 70ies by Tate & Lyle
- Approved in EU (E957), in Japan, Israel as sweetener, as flavour modifier in USA (FEMA GRAS 3732)





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# Thaumatococcus II: 50% Sugar Reduction Case



At 5 ppm, 5 mg of Thaumatin II is equivalent of 50 g sugar meaning that at this conc  
At \$2-5 per gram COGs, the 1 kg sugar entration,  
Thaumatin II is 10,000 times sweeter than sugar  
equivalent will cost \$0.2—0.5, similar to or lower than the cost of sugar



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# Regulatory Status

## Nomad`s bacteriocins - GRAS submissions/acceptances\*

Product/Origin	GRAS GRN	Submission date	Response date
<a href="#">Colicins/Escherichia coli</a>	000593	07.2015	12.2015/FDA
<a href="#">Colicins/E.coli</a>	000676	11.2016	05.2017/FDA 01.2017/USDA
<a href="#">Salmocins/ Salmonella enterica</a>	000824	11.2018	21.10 2019/FDA
<a href="#">Endolysins, Clostridium phages</a>	000802	07.2018	04.2019/FDA
<a href="#">Nicotiana as a GRAS host</a>	000775	04.2018	10.2018/FDA

\*All bacteriocins are 'food processing aids'.  
Colicins are also listed in USDA/FSIS Directive 7120.1

- GRAS ('Generally Recognized As Safe') is regulatory approval path for food additives in USA
- Nomad has received seven GRAS regulatory approvals, with one submission pending, in the USA
- All bacteriocins approved as food processing aids (no need for labelling)

## Nomad`s thaumatins - GRAS submissions/acceptances

Product	GRAS GRN	Submission date	Response date
Thaumatins sweeteners	000738	10.2017	04.2018/FDA
Thaumatins 2 Sweetener	000910	02.2020	09.2020
Thaumatins 2 taste modifier	000920	04.2020	pending



# Thank you for attention!

