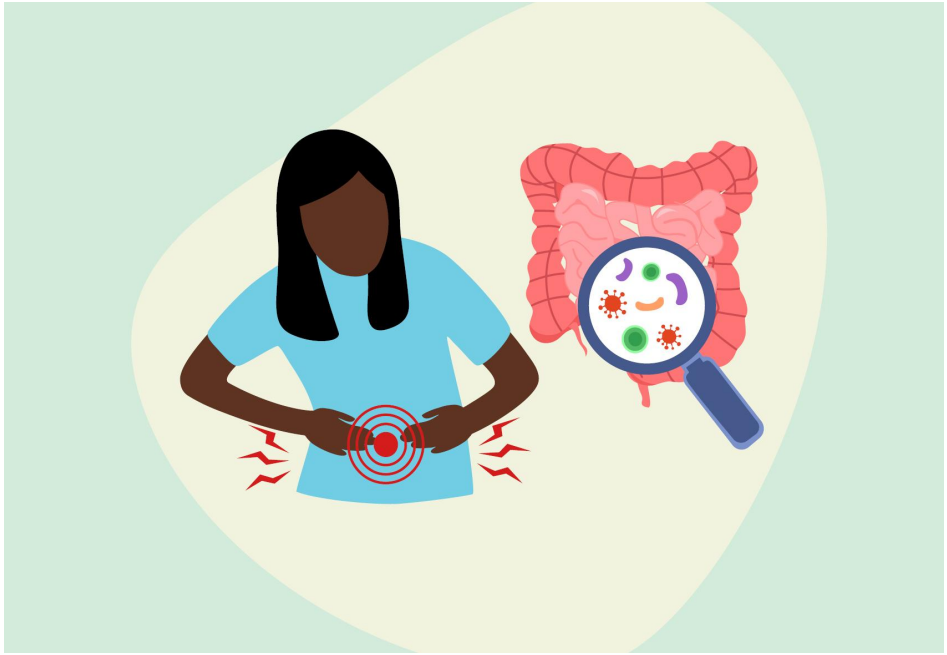
Probiotic fighting gastro-intestinal infections

Targeted therapy against mild and severe Salmonella infections

Adrien Faure, Georges Barnikol, Hien Tran Thi, Isika Ram, Pitt Meyer

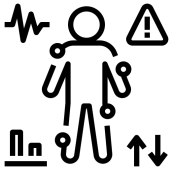
École polytechnique fédérale de Lausanne

Gastro-intestinal pathogens



Salmonella, *Clostridium perfringens, Campylobacter …*

Cholera toxin, thermolabile and thermostable toxins, Shiga toxin …



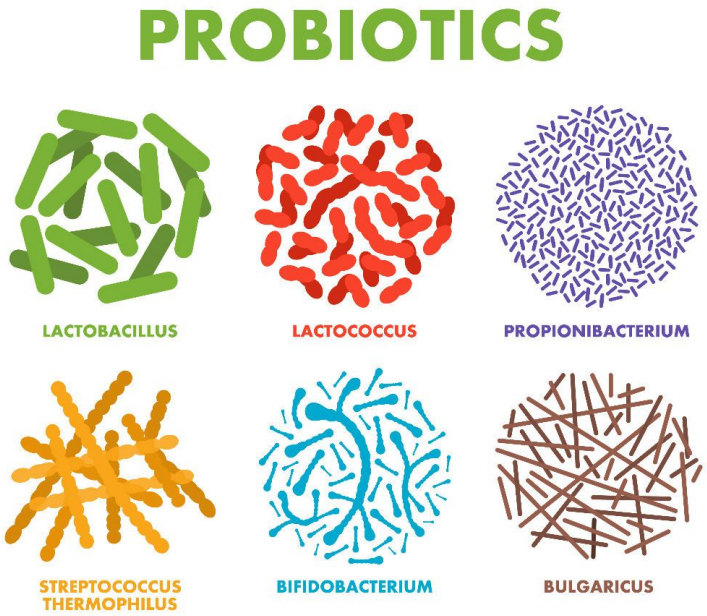
Fever, abdominal pain, nausea, diarrhea, vomiting …

Incidence and economic burden

- **600 million cases / year**
- 420 thousand deaths / year
- Medical cost of **48 million USD / year** in the US
- Increasing **antimicrobial resistance**

Victim of the May 2018 Salmonella *outbreak in the US East Coast*

Probiotics in the context of gastrointestinal infections

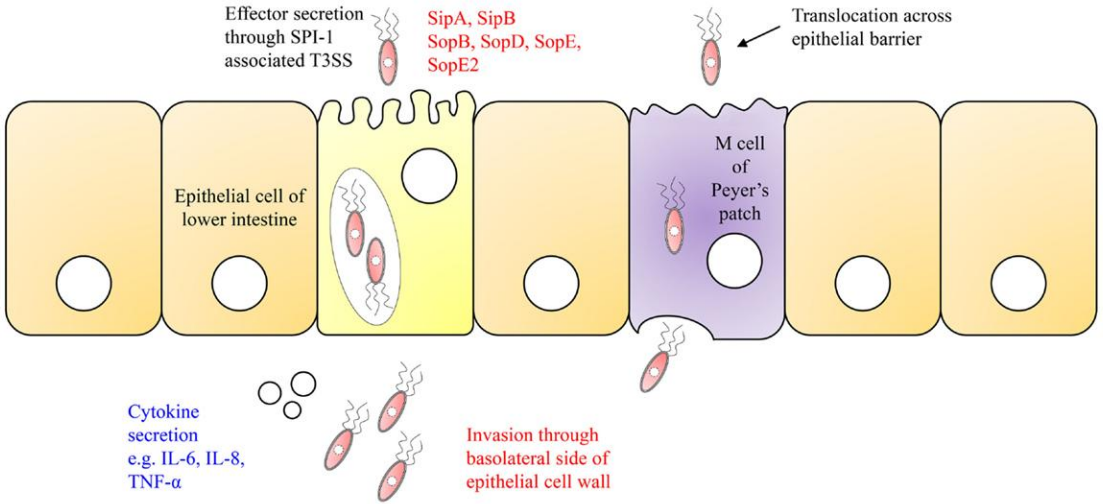


- Live beneficial microorganisms
- Efficacious to treat or prevent dysbiosis
- Compete with pathogenic strains
- Neutralise toxins

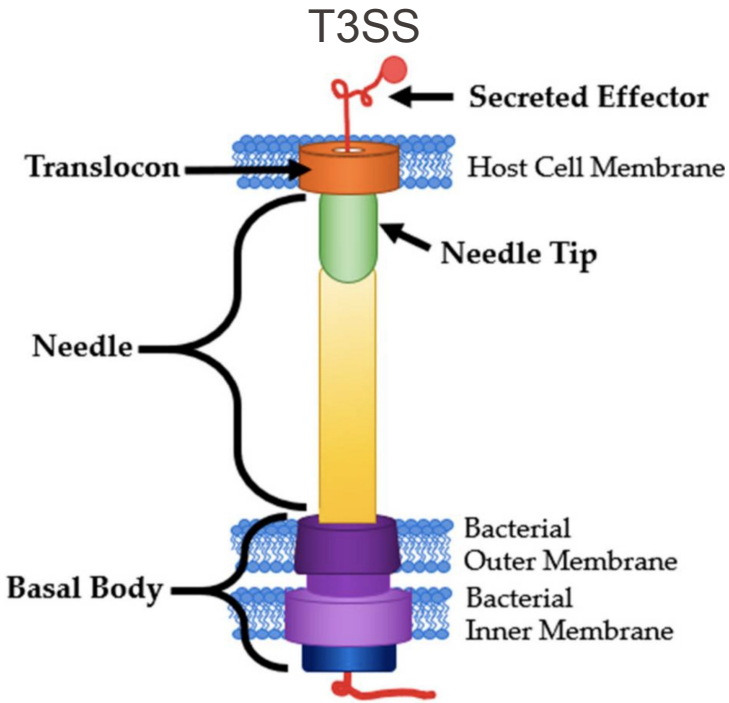
Can we make them better ?

Infection mechanism of the host

- Type 3 secretion systems deliver virulence factors
- Factors mediate host cell penetration
- Once inside systemic infection possible



Hurley D. et al., Front. Immunol., 2014

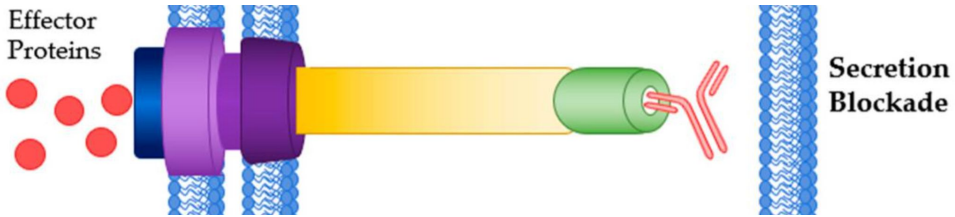


Hotinger J. A. et al., *Antibodies*, 2020

Inhibition of infection

Inhibition of invasion

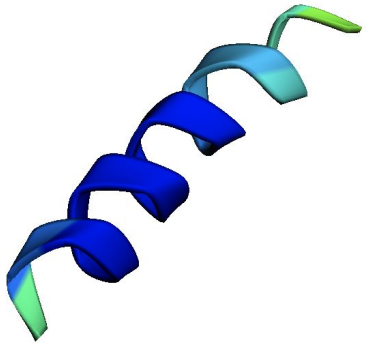
- Develop a binder for the T3SS of salmonella



Hotinger J. A. et al., *Antibodies*, 2020

Elimination from the gut

- Express antimicrobial peptide JH-3 specific for salmonella



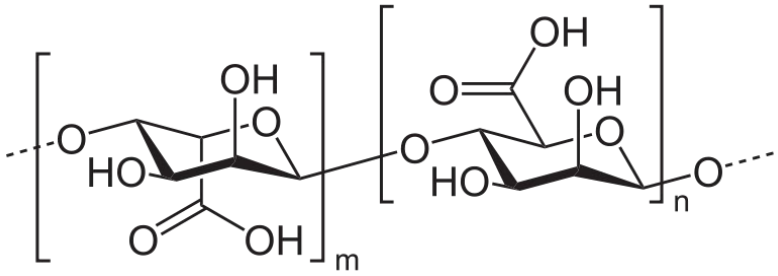
Wang L. et al., *Probiotics and Antimicrobial Proteins*, 2019

Mirdita M. et al., *Nature Methods*, 2022

Formulation

- E. coli Nissle 1917 as host strain
  - Probiotic strain
  - Insertion of genes of interest into the genome
  - Preservation by freeze-drying
- Capsule of alginate (polysaccharide polymer)
  - Resistant to low pH (stomach)
  - Swell above pH 6.6 (found in intestine)
  - Release of bacteria in intestine

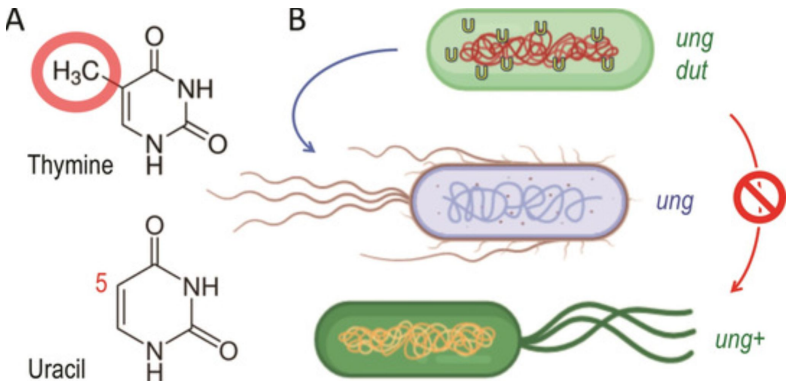
Abra-Khajouei R. *et al.* Mar. Drugs, 2022



Alginate

https://www.nanalyze.com/2022/09/omnicell-stock-automated-pharmacy/

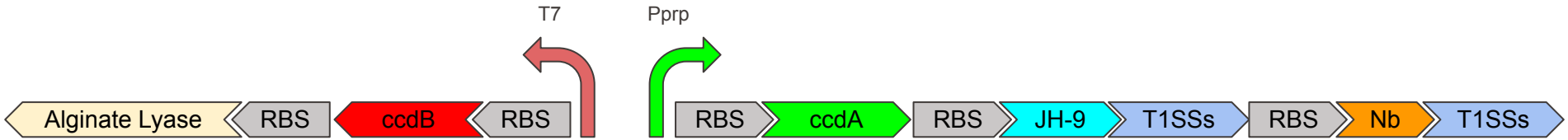
Safety



Algar E. et al., *ChemBioChem*, 2020

- No horizontal gene transfer
  - Integration into genome
  - Uracilation of genome due to dut/ung mutations
- Bacteria die after therapy and also in the environment
  - ccdB toxin constitutively expressed
  - Inhibitor ccdA of the toxin ccdB produced in presence of the food additive propionate
  - Propionate delivered with lyophilized bacteria in alginate capsules

GMO debated but several studies up to Phase 2 trials

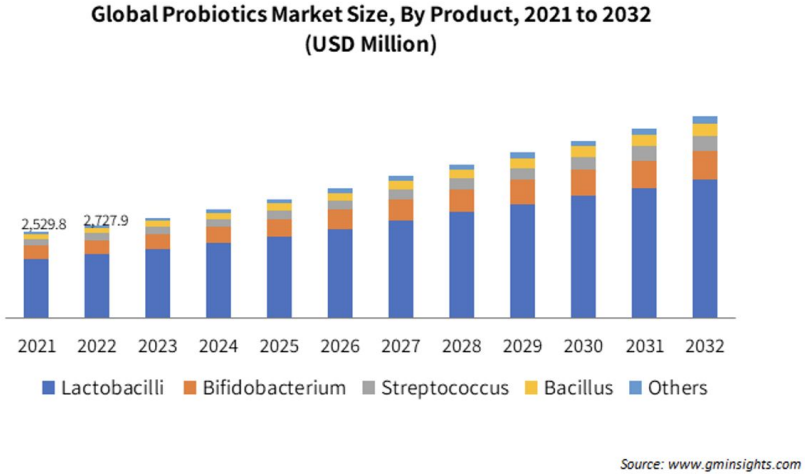


Unique advantages

- More selective than antibiotics
- Less resistance development of pathogens
- Targeted to intestine
- Platform for treatment of many different gastrointestinally pathogenic bacteria

Business Size

- Global probiotic market size valued USD 2.5 billion in 2022 and projected to witness over 8% compound annual growth rate from 2023 to 2032 [1]



Source: www.gminsights.com

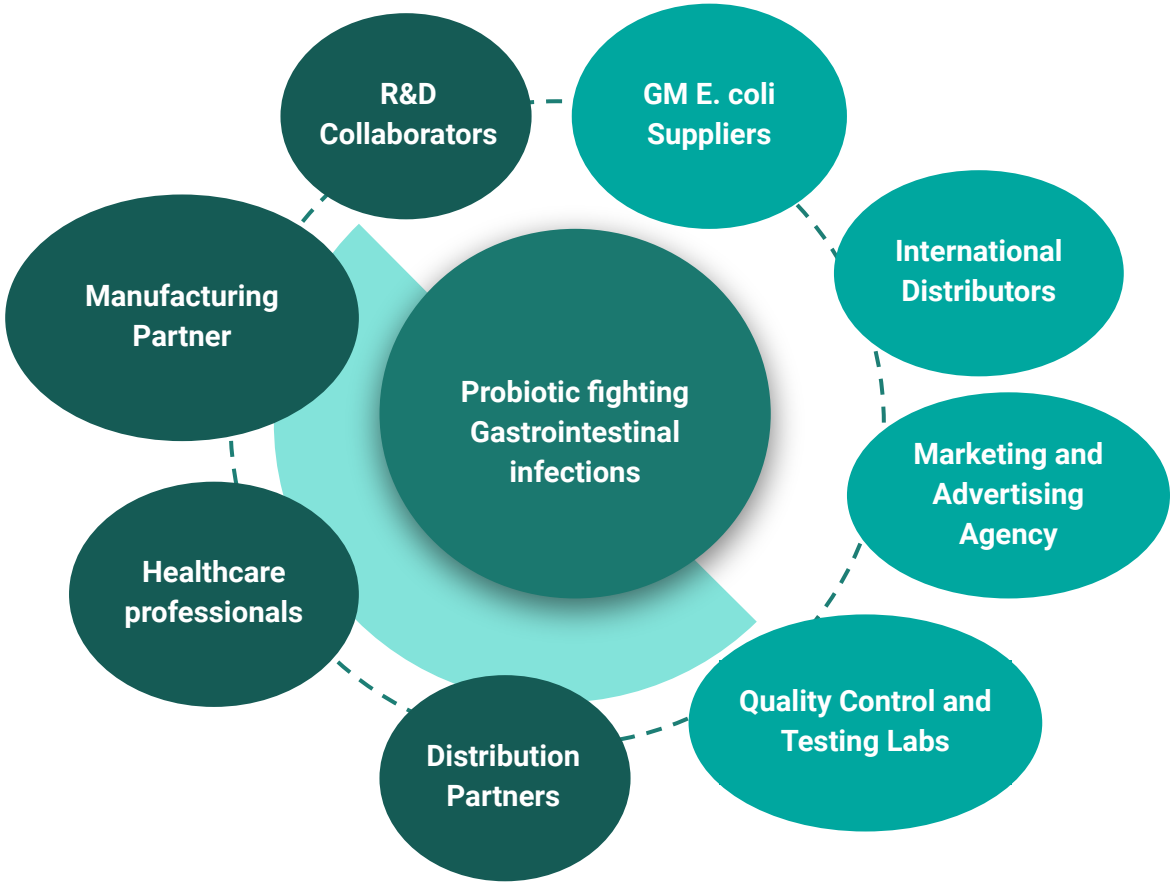
[1] “Probiotics Market Size Statistics, Global Report 2023-2032.”

Business Model

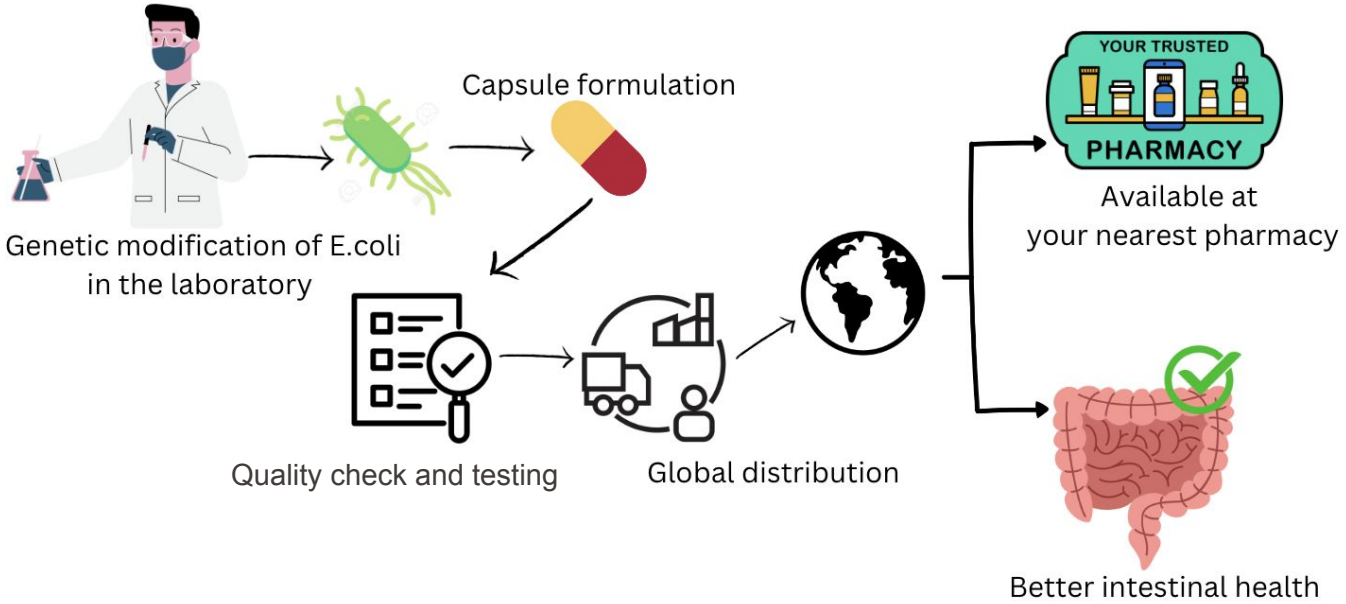
- Probiotic available over the counter
  - E.g. in pharmacies
- Emergency option for home
  - Can be used when feeling to become sick/Can also be preventive
- Can be used by adults seeking immune support and working in high-risk environment
- Cheap production and storage
- Product name: Salmokilla^{TM}

https://engoo.com/app/daily-news/article/at-the-pharmacy/YbTj4AcgEeivIvczY5XRdg

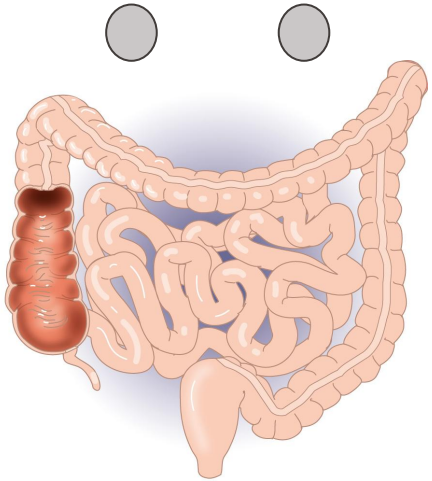
Business Partners



Summary


Capsule formulation
YOUR TRUSTED
PHARMACY
Available at
your nearest pharmacy
Genetic modification of E.coli
in the laboratory
Quality check and testing
Global distribution
Better intestinal health

Thank you very much for your attention!



https://anatomytool.org/content/slagter-drawing-large-intestine-opened-caecum-no-labels

Additional information

Concerns

Rearrangements of the microbiome

Reconstitutive pill at the end of the treatment

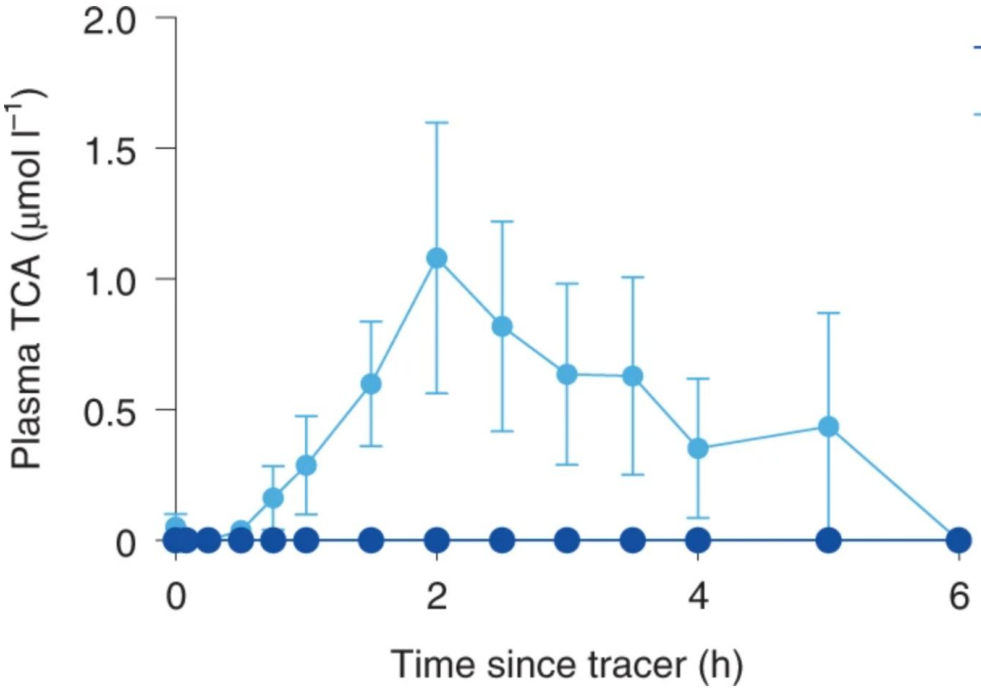
Alternative Bacterial Strain

Bacteroides xylanisolvens AY11-1

- Degrade Alginate
- Upon swelling of capsule can degrade alginate (Nutriment boost)
- Can be used as probiotic

Fu T. *et al.*, Nutrients, 2023

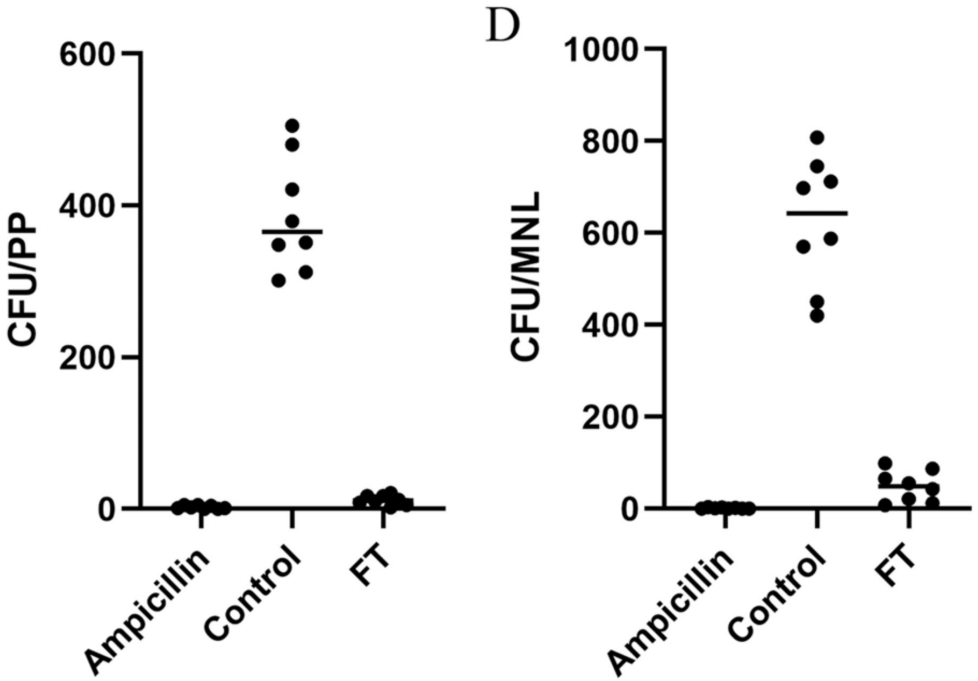
E. coli Nissle for the treatment of Phenylketonuria



- Genomic insertion of constructs
- In Phase 1/2a study promising
- Conversion of Phenylalanine into trans-cinnamate (TCA)

Puurunen M. K. et al., *Nature Metabolism*, 2021

Inhibition of T3SS inhibits salmonella infection



- Small molecular compound

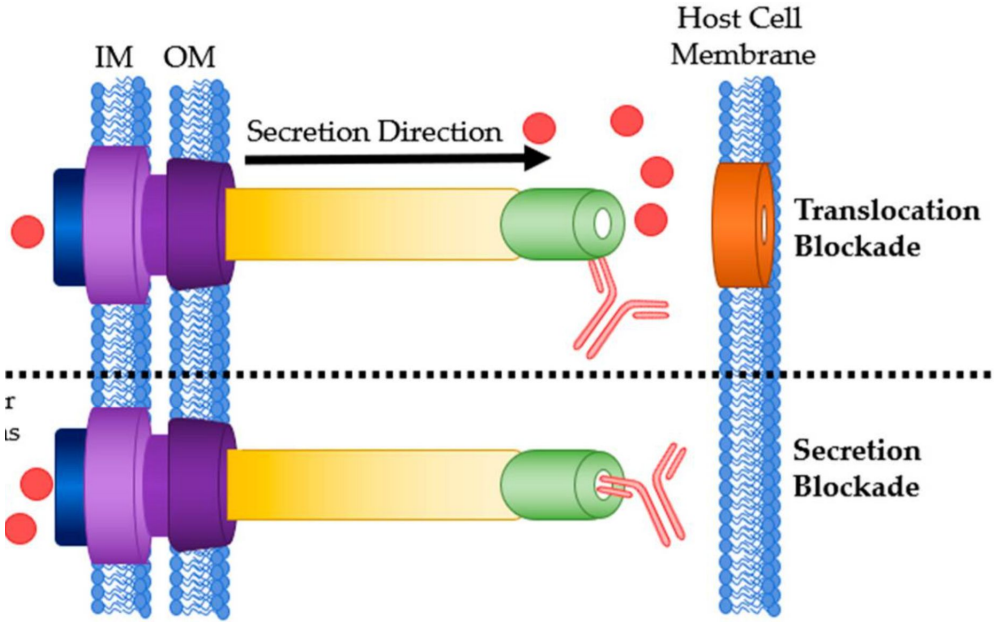
  Fluorothiazinon (FT)
- Tested on Salmonella enterica serovar Typhimurium

PP = Payer's patches, MNL = Mesenchymal lymph nodes

Zigangirova N. A. et al., *Nature Journal of antibiotics*, 2021

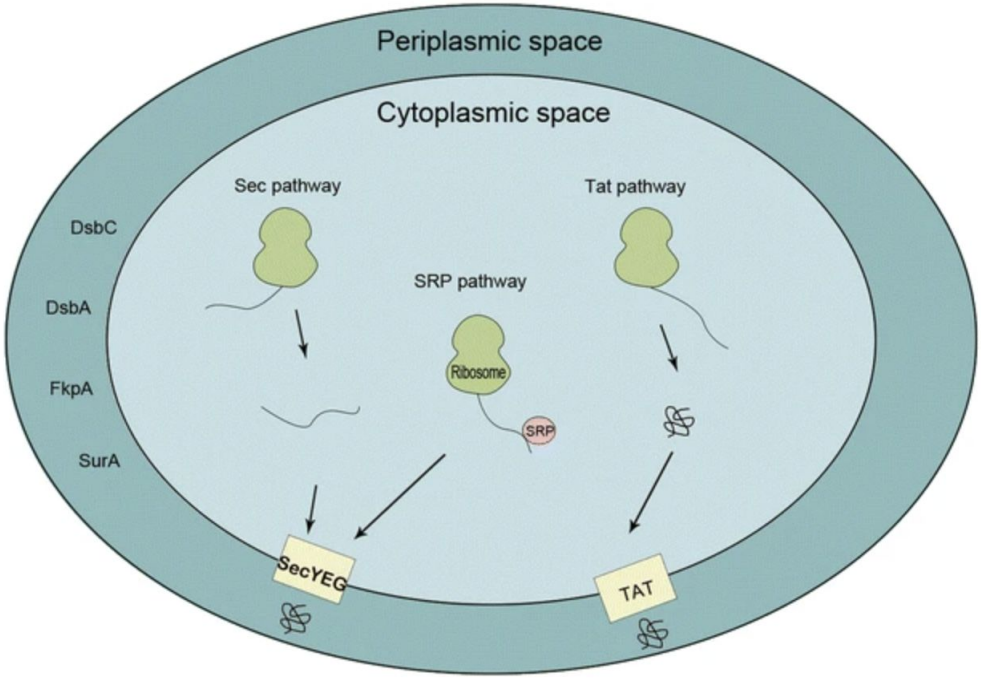
Inhibition of T3SS by antibodies/nanobodies

- Inhibition of translocation to target cell
- Blockage of secretion



Hotinger J. A. et al., *Antibodies*, 2020

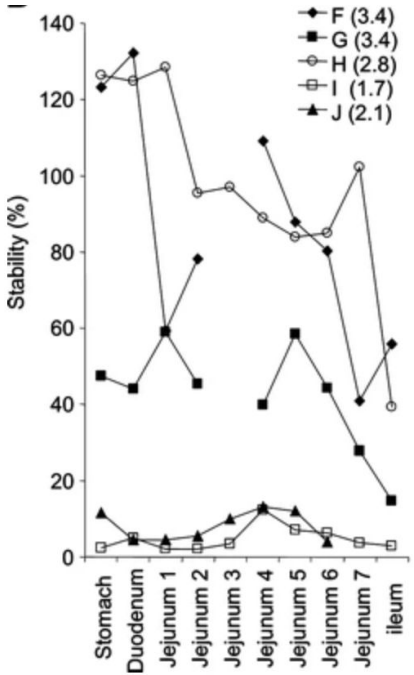
Expression of nanobodies in bacteria



- Expression possible in the periplasmic space

Liu Y. et al., *Applied Microbiology and Biotechnology*, 2017

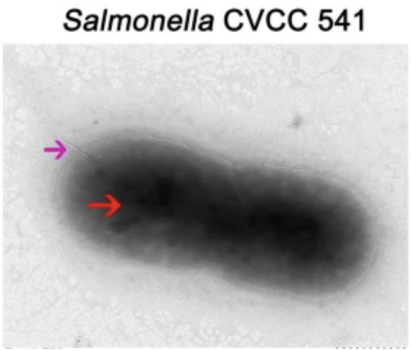
Stability of nanobodies in the gut

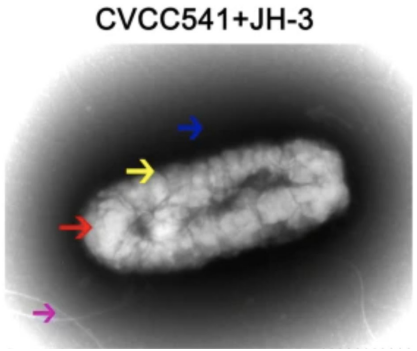


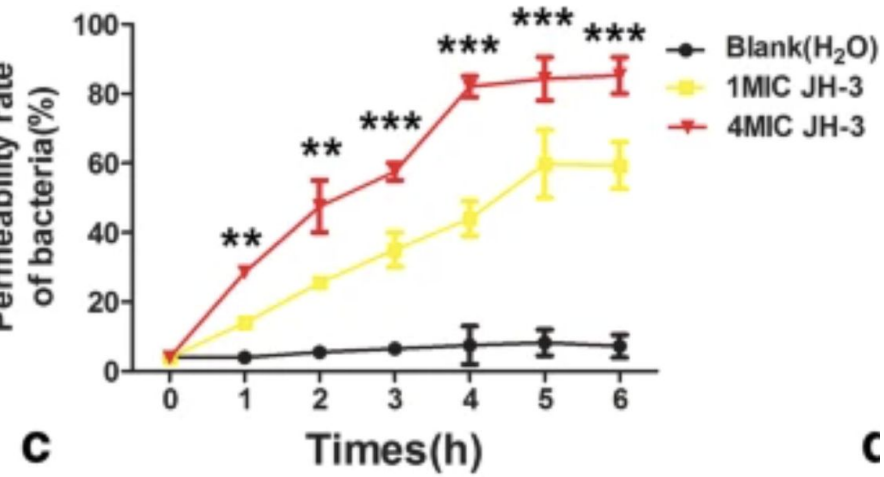
- Stability can be greatly increased by optimization with specific mutations
- Data from stability of nanobodies in the intestine of piglets

Harmsen M. M. et al., *Applied Genetics and Molecular Biotechnology*, 2006

Mechanism of action of JH-3 in Salmonella







- JH-3 creates pores in the cellular membrane of the bacteria
- Leakage of the cytoplasm occurs

Wang L. et al., *Probiotics and Antimicrobial Proteins*, 2019

What are the weak points of salmonella?

- Heat sensitivity:165°F (73.8°C)
- Disinfection:concentrations of 70–80% ethanol, Virkon S
- pH Levels: acidic conditions as low as pH 4.0-5.0
- Hygiene and sanitation: Contamination can occur through various means
- Proper food handling: Salmonella can spread through multiple avenues

Business Model?

- **Market Analysis:** By collecting data of patients(no., their frequency of visits, cure rate)
- **People above age?** → **Adults** (seeking immune support to prevent, work in high-risk environment)
- **At which state of the disease it could be used?** When to use? When to stop?
  - When feeling to become sick/Can also be preventive

- **Additional therapy with the antibiotics for longer period of disease. And for better digestive system.**
- **Effective:** till the use of propionate supplement is given.
- **Application: One single pill/ Two,** How to use? → Together with our bacteria in the capsule
- **Cost:** Not too expensive as it could be produced by the GMO
- **Storage:** Temp conditions? For long term storage → Lyophilized (RT)
- **Key Partners:** GM E.coli supplier, Manufacturing Partners, R&D collaborators, Healthcare Professionals, Distribution Partners, Quality Control and Testing Labs, Marketing and Advertising Agencies, International Distributors.
- **Availability:** In Pharmacies, online platforms for in future emergency stock.