The new antibiotics discovered from unculturable bacterium

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Outline

01 Nonculturable bacterium

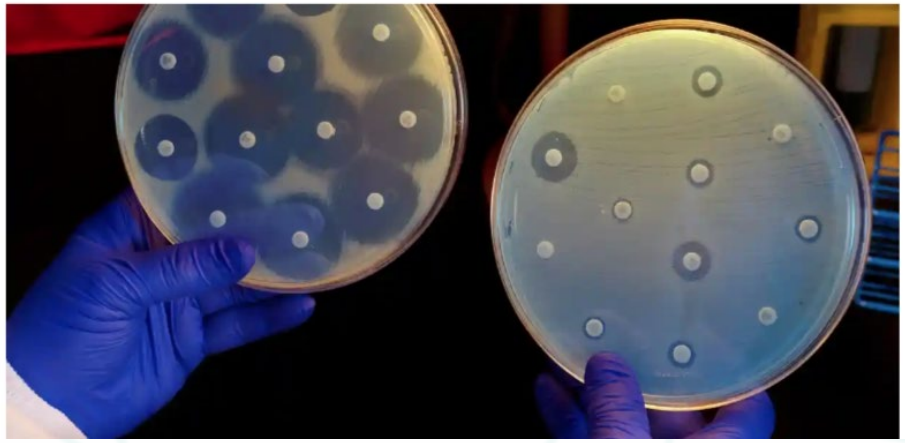
02 Nonculturable bacterium cultivation

03 Antibiotic discovery from unculturable bacterium

04 “Resistance-resistant” mechanism

Antimicrobial resistance now a leading cause of death worldwide, study finds

Lancet analysis highlights need for urgent action to address antibiotic-resistant bacterial infections



Report signals increasing resistance to antibiotics in bacterial infections in humans and need for better data

9 December 2022 | News release | Geneva |Reading time: 3 min (697 words)

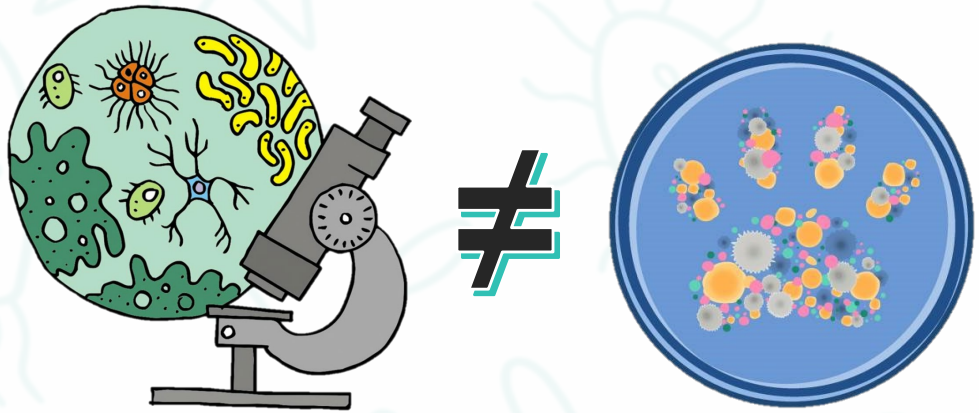
A new World Health Organization (WHO) report reveals high levels of resistance in bacteria, causing life-threatening bloodstream infections, as well as increasing resistance to treatment in several bacteria causing common infections in the community based on data reported by 87 countries in 2020.

- The spread of resistant organisms is producing a human health crisis, as we are witnessing the emergence of pathogens resistant to all available antibiotics.
- Overmining of soil-dwelling bacteria, traditional screening sources, ended the golden era of antibiotic discovery in the 60s, because they tend to yield previously known compounds.
- Most bacterial species, over 99%, are uncultured, and methods to grow these organisms have been developed.

01 Nonculturable bacterium

Nonculturable bacterium

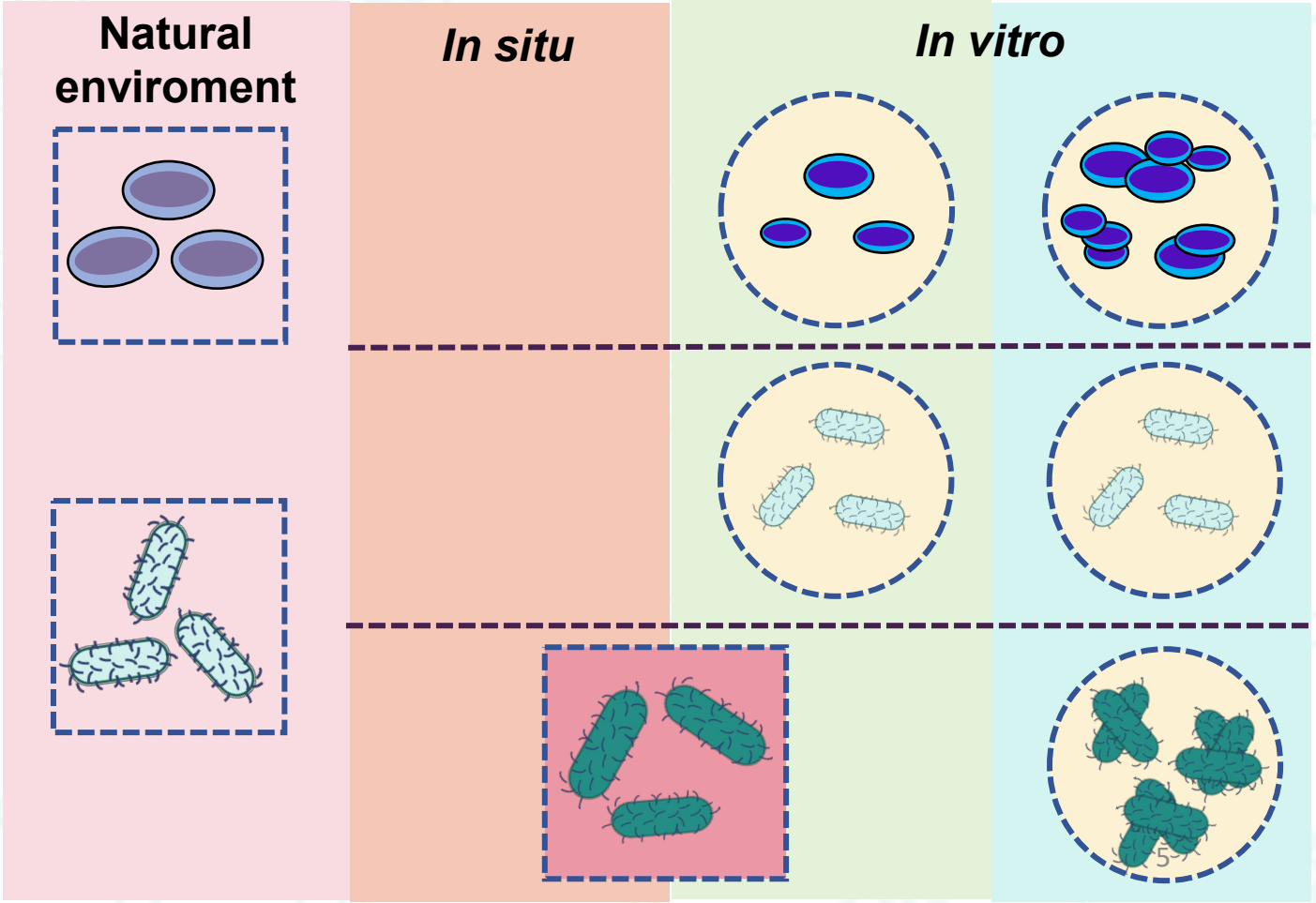
- In 1898, Heinrich Winterberg found that the number of microbial cells in his samples did not match the number of colonies formed on nutrient media.
- '**Viable but nonculturable**', bacteria cells maintain their viability but unable to grow/form colonies on routinely-used laboratory media(Xu et al. 1982);



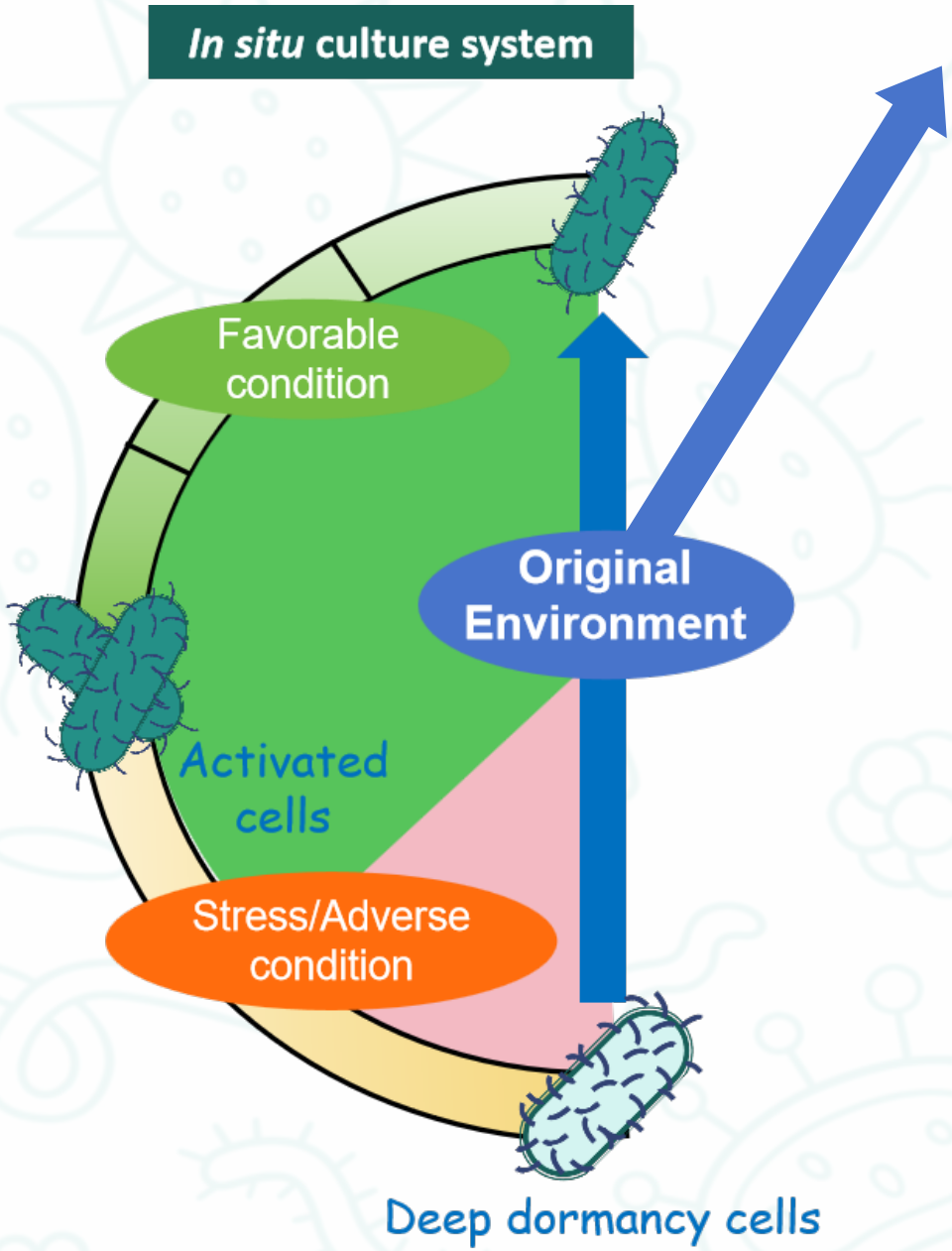
Microbial uncultivability cannot simply be explained by the unfitness of specifc strains to certain culture conditions.

(A Bodor et al., Rev Environ Sci Biotechnol, 2020)

In situ incubation, microbes are induced from a non-growing state by unknown growth factors from the environment



Nonculturable bacterium resuscitation



Microbial interaction turn “nonculturable” into “culturable”

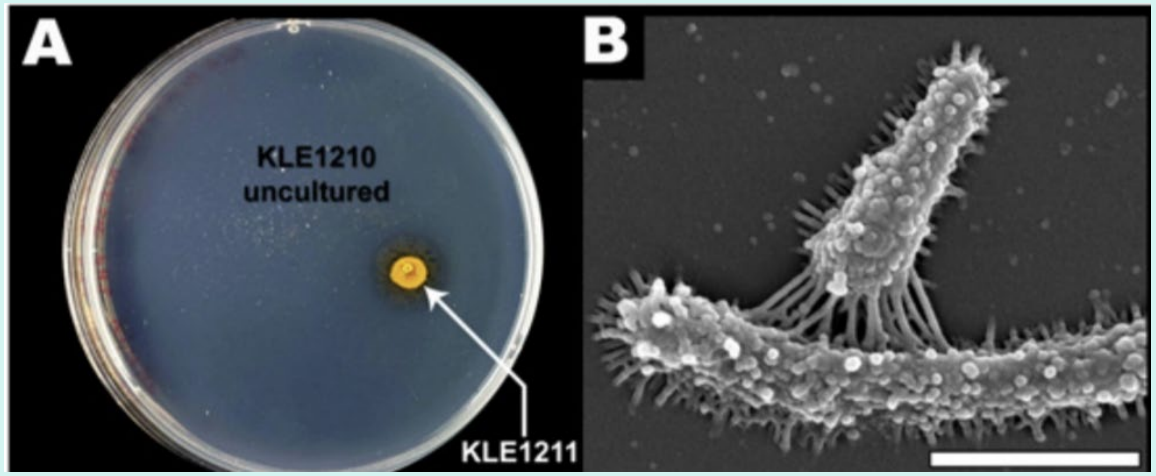
- **Autoinducers**

Date of sampling	No. of samples tested	(A) LB	(B) LB + CAI-1
June 2012	7	0	4
November 2012	15	0	5
November 2012	10	1	7
Total	32	1	16
% of total	100	3.1	50

The resuscitation of *Vibrio cholerae* from surface waters is dramatically enhanced by using enrichment media containing autoinducers CAI-1 & AI-2(S Nayeemul Bari et al. 2013).

Nonculturable bacterium resuscitation

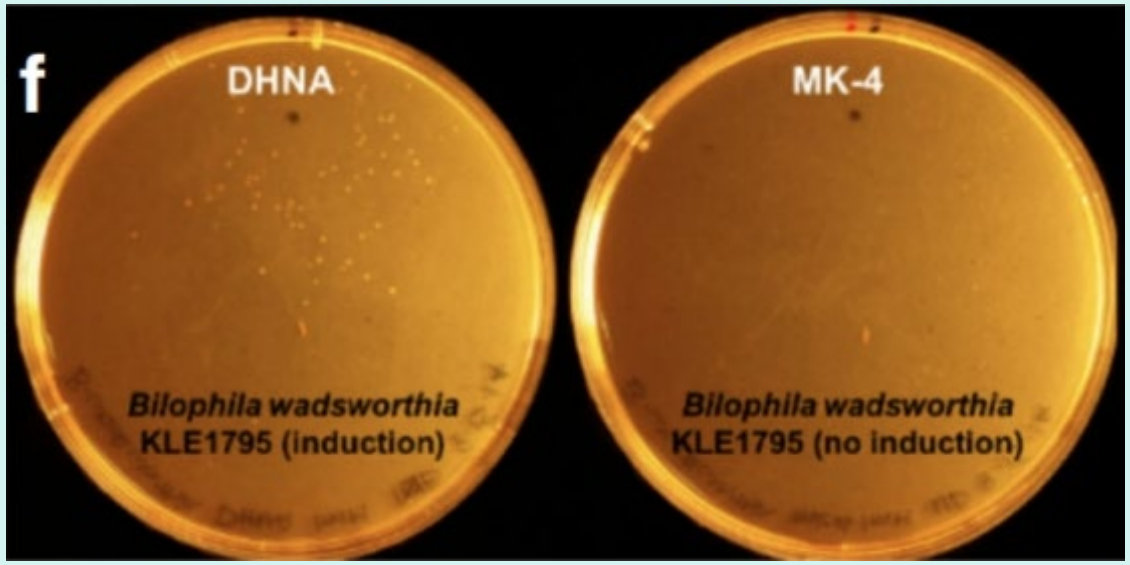
- **Siderophores**



(A Onofrio et al., Chemistry & Biology, 2010)

Previously uncultured bacteria cannot produce siderophores autonomously, they are chemically dependent on other members in the natural environment.

- **Resuscitation promoting factors**



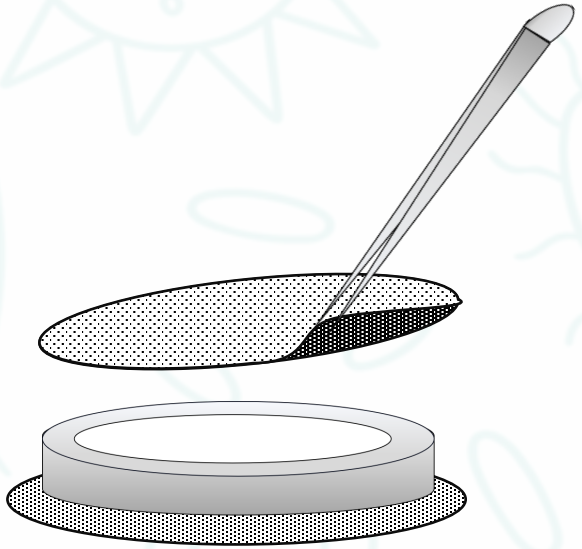
(K Fenn et al., Microbiome, 2017)

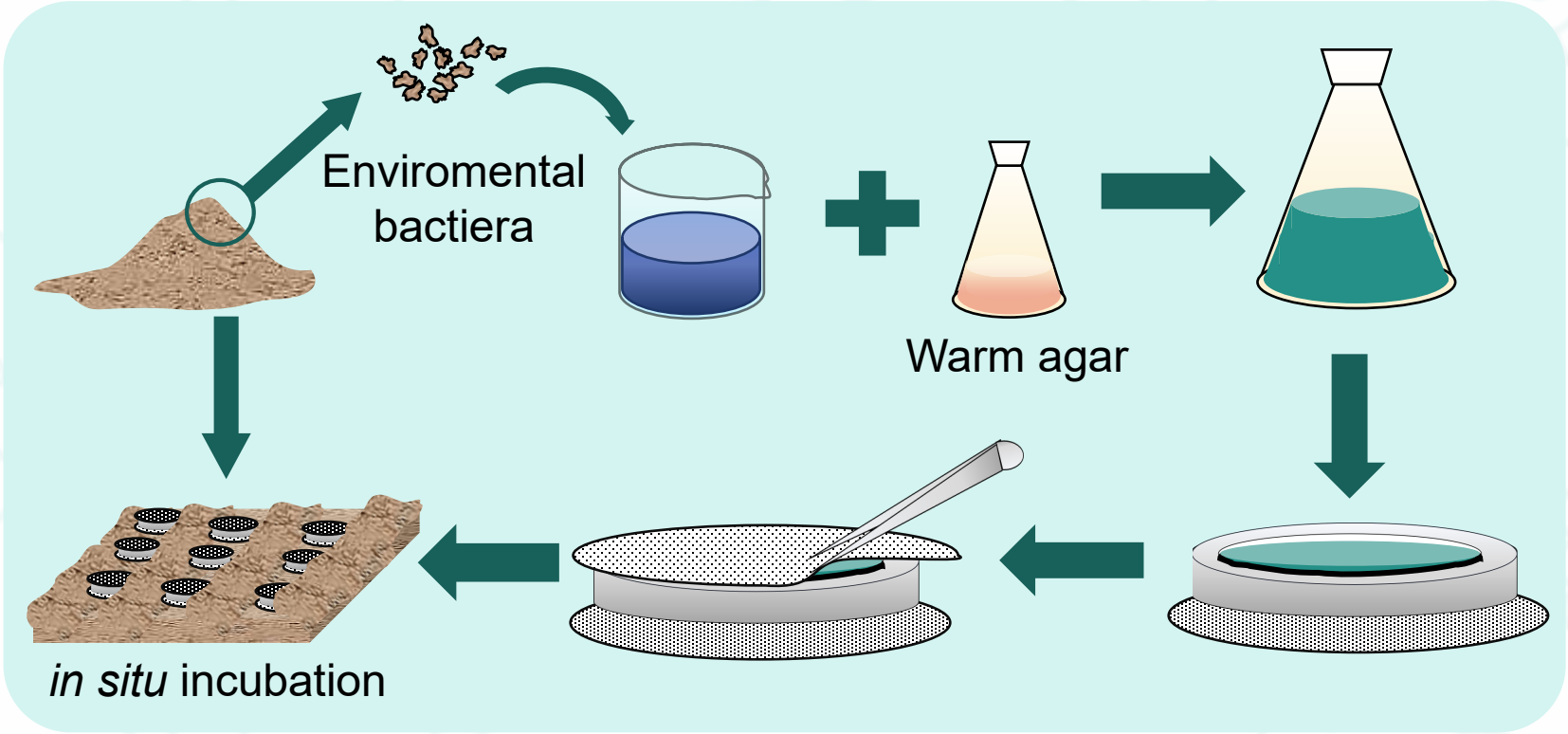
Quinones can be used to improve existing bacterial growth media or modulate human gut microbiota by encouraging the growth of important symbionts.

02 Nonculturable bacterium cultivation

- Isolation?
- Incubation?
- Fermentation?

1. Diffusion Chamber

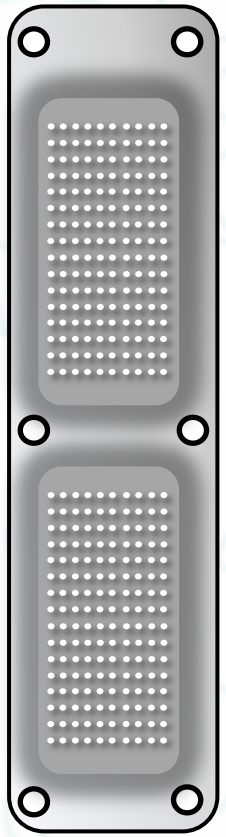




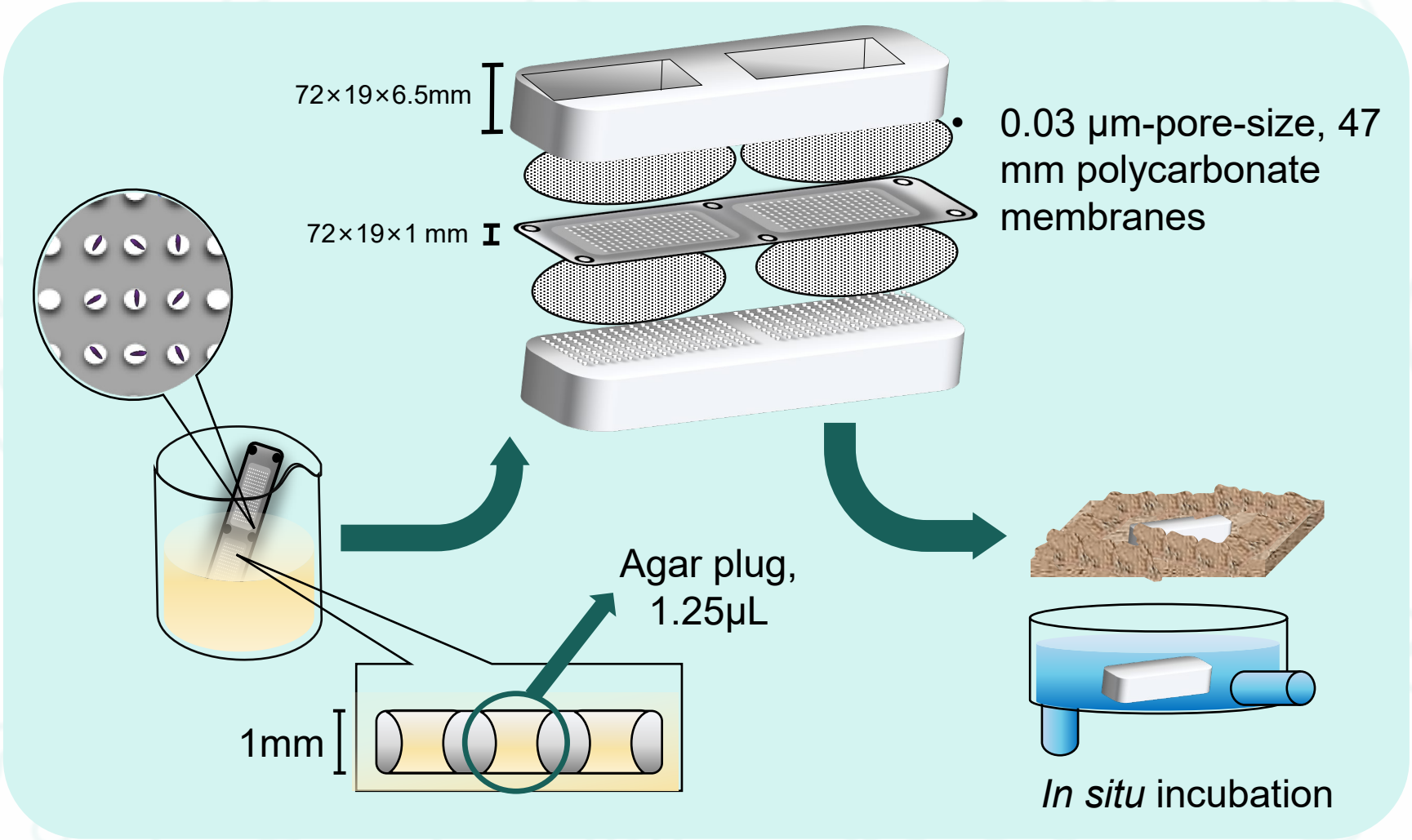
- A washer sandwiched between two 0.03 μm-pore-size polycarbonate membranes

(T Kaeberlein et al., Science, 2002)

2. Isolation Chip



- Hydrophobic plastic polyoxymethylene
- 192 through-holes per array, 1 mm in diameter



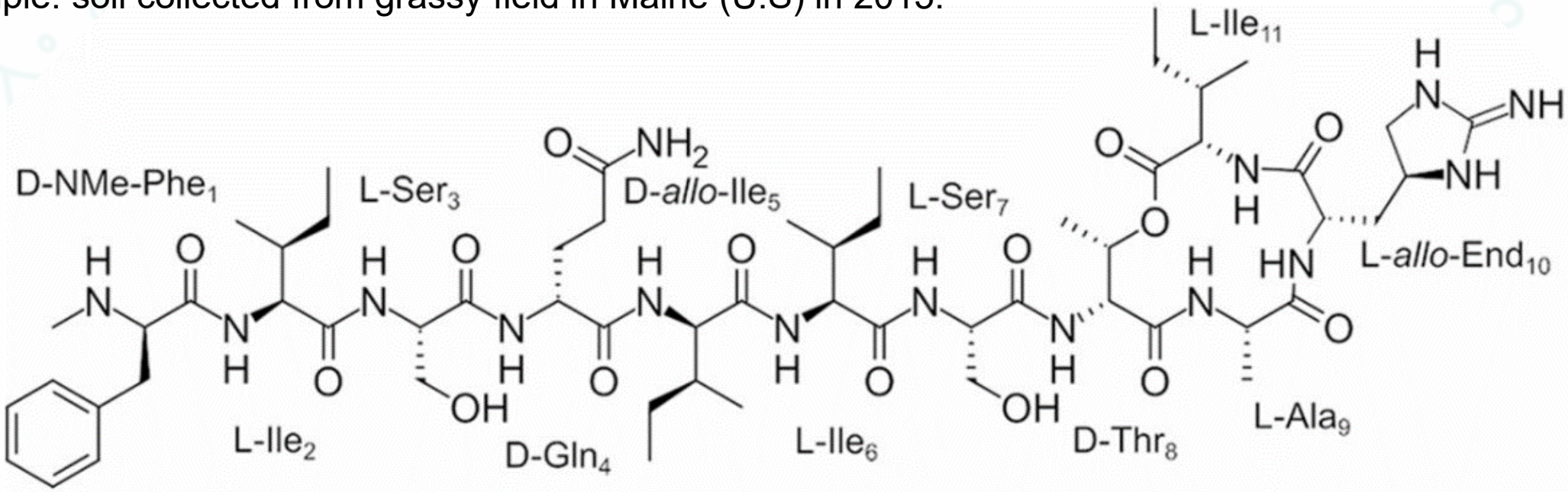
(D Nichols et al., Applied and Environmental Microbiology, 2010)

03 Antibiotics discovery

- Teixobactin
- Clovibactin

Teixobactin: the antibiotic from unculturable bacterium

1. Isolate strain: from uncultured bacteria ***Eleftheria terrae;***
2. Isolation technique: **iChip** method;
3. Sample: soil collected from grassy field in Maine (U.S) in 2015.



- A molecular mass of 1,242 Da;
- *Elefitheria terrae* belongs to a new genus related to Aquabacteria, **which was not known to produce antibiotics**.

(L Ling et al., Nature,2015)

Potent antimicrobial activity against Gram-positive pathogens

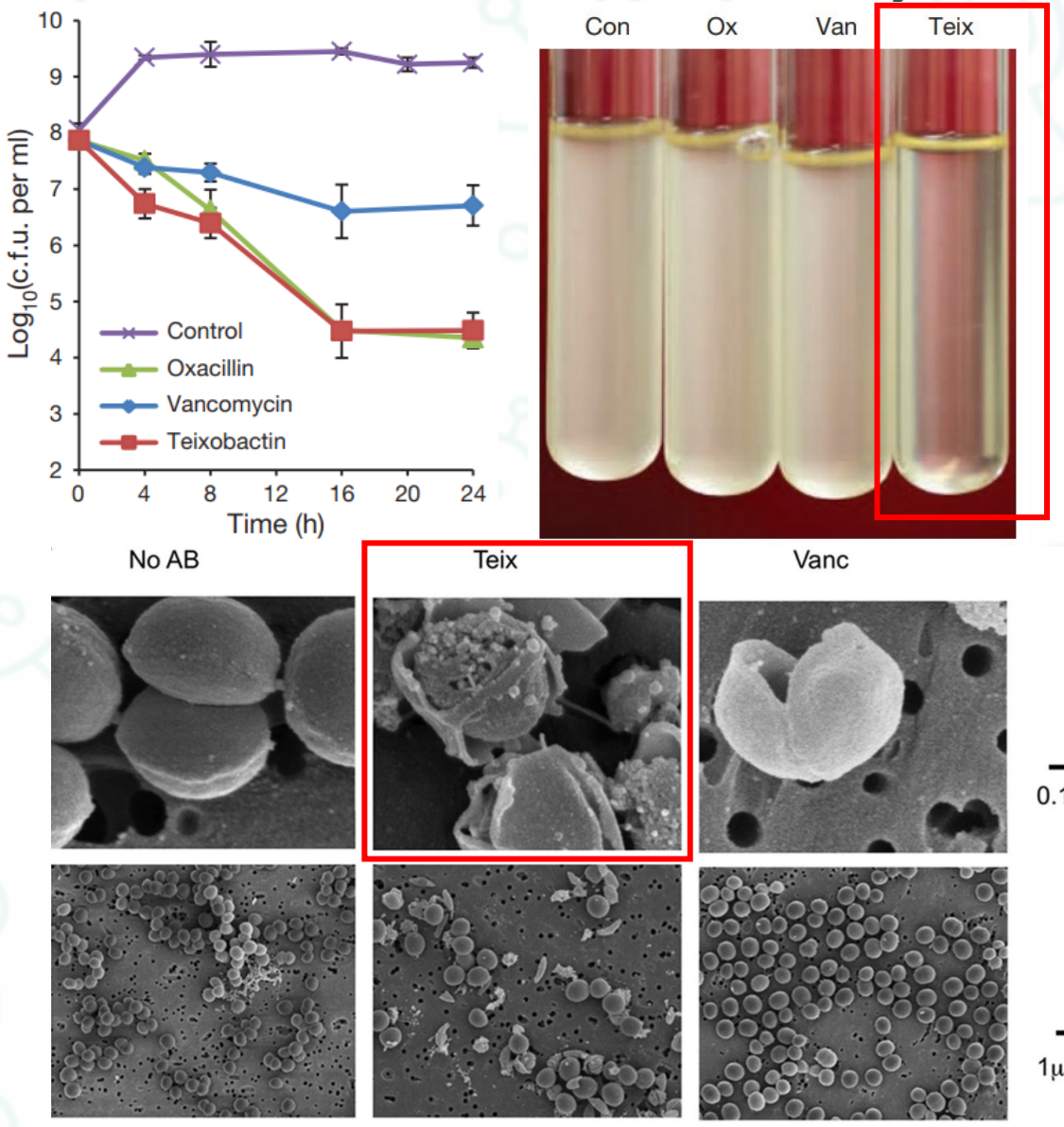
Activity of Teixobactin against pathogenic microorganisms

Organism and genotype	Teixobactin MIC (μg ml^{-1})
S. aureus (MSSA)	0.25
S. aureus + 10% serum	0.25
S. aureus (MRSA)	0.25
Enterococcus faecalis (VRE)	0.5
Enterococcus faecium (VRE)	0.5
Streptococcus pneumoniae (penicillin^{R})	≤0.03
Streptococcus pyogenes	0.06
Streptococcus agalactiae	0.12
Viridans group streptococci	0.12
B. anthracis	≤0.06
Clostridium difficile	0.005
Propionibacterium acnes	0.08
M. tuberculosis H37Rv	0.125
Haemophilus influenzae	4
Moraxella catarrhalis	2
Escherichia coli	25
Escherichia coli (*asmB1*)	2.5
Pseudomonas aeruginosa	>32
Klebsiella pneumoniae	>32

The MIC was determined by broth microdilution. MSSA, methicillin-sensitive *S. aureus*; VRE, vancomycin-resistant enterococci.

- Superior to vancomycin in certain populations
- Ineffective against most Gram-negative bacteria
- Exposure to Teixobactin results in collapse of the cell wall

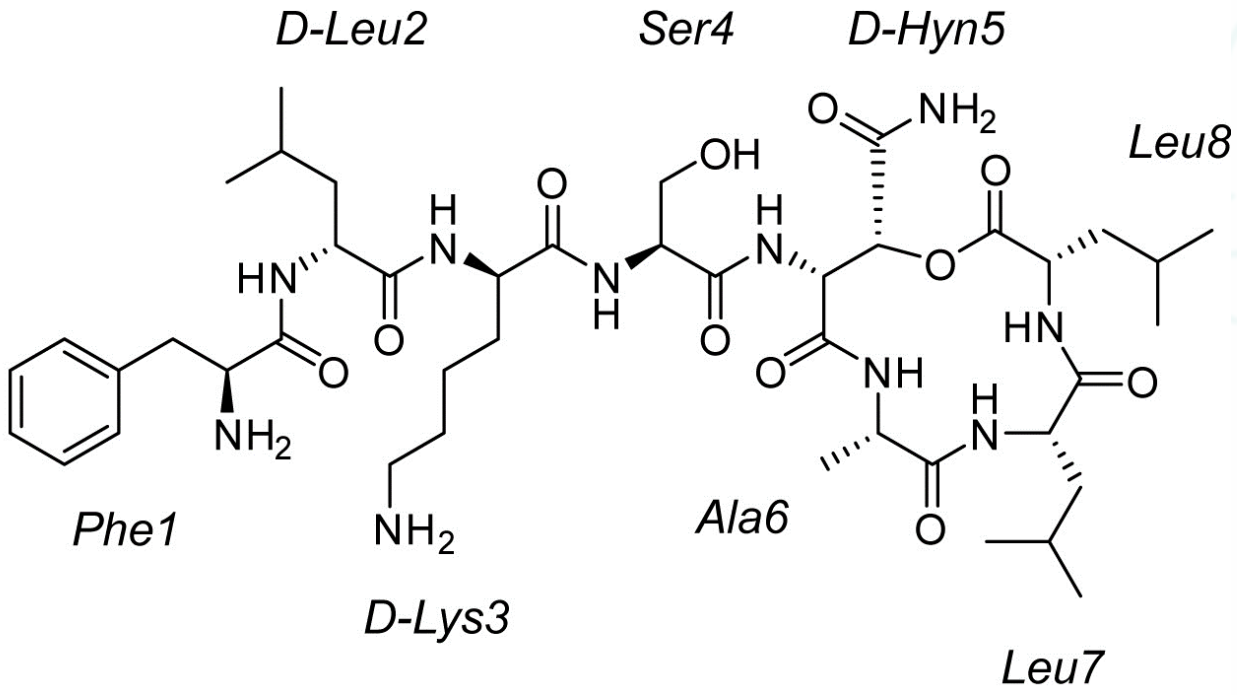
Teixobactin treatment resulted in *S. aureus* lysis



(L Ling et al., Nature, 2015; T Homma et al., Antimicro Agents and Chemo, 2016)

Clovibactin: a recent discovered antibiotic from unculturable bacterium

1. Clovibactin was screened and isolated within prolonged incubation (18 weeks) in 96-well plates from a sandy soil collected in North Carolina;
2. The antibiotic-producing isolate, based on 16S rDNA sequence, is 99% identical to ***Eleftheria terrae*.**



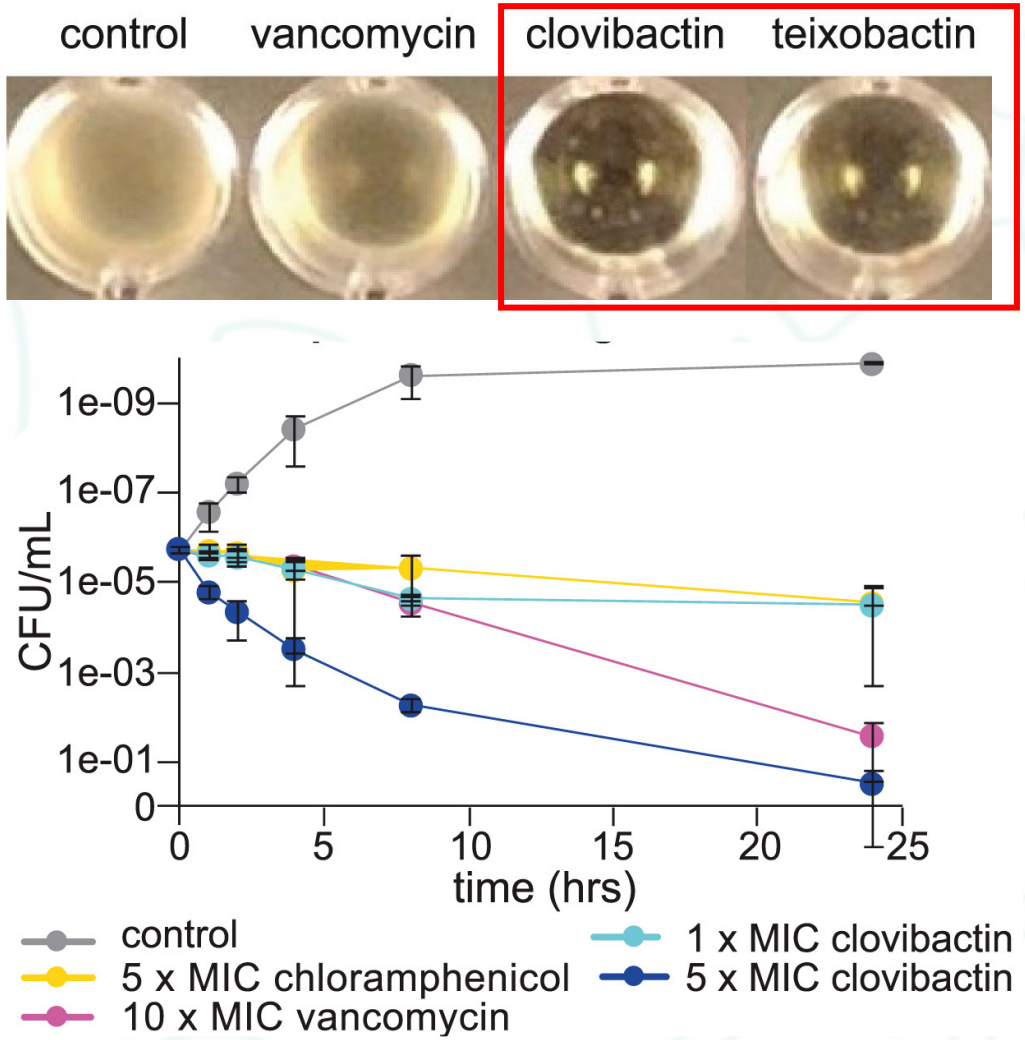
Antimicrobial activity of Clovibactin

Strain	MIC (μg/mL)
Staphylococcus aureus	
NCTC 8325–4 (MSSA)	0.5–1
ATCC 29213 (MSSA)	0.5–1
ATCC 700699 (GISA)	1–2
NRS71 (epidemic MRSA)	1
NRS108 (MRSA, also synercid^{R})	1
ATCC 33591 (MRSA)	1–2
Mu50 (VISA)	2
SG511	0.125
HG001	2
Staphylococcus epidermidis	
ATCC 35982 (*mecA* positive)	0.5
NRS8 (*mecA* positive)	0.5
Staphylococcus haemolyticus	
NRS9 (*mecA* positive)	1
NRS69 (*mecA* positive)	0.5
Gram-negative	
Haemophilus influenzae SJ7	2
Escherichia coli K12	64
E. coli WO153 (AB1157:*asmB1 ΔtolC:kan*)	1–2
Pseudomonas aeruginosa PA-01	>128

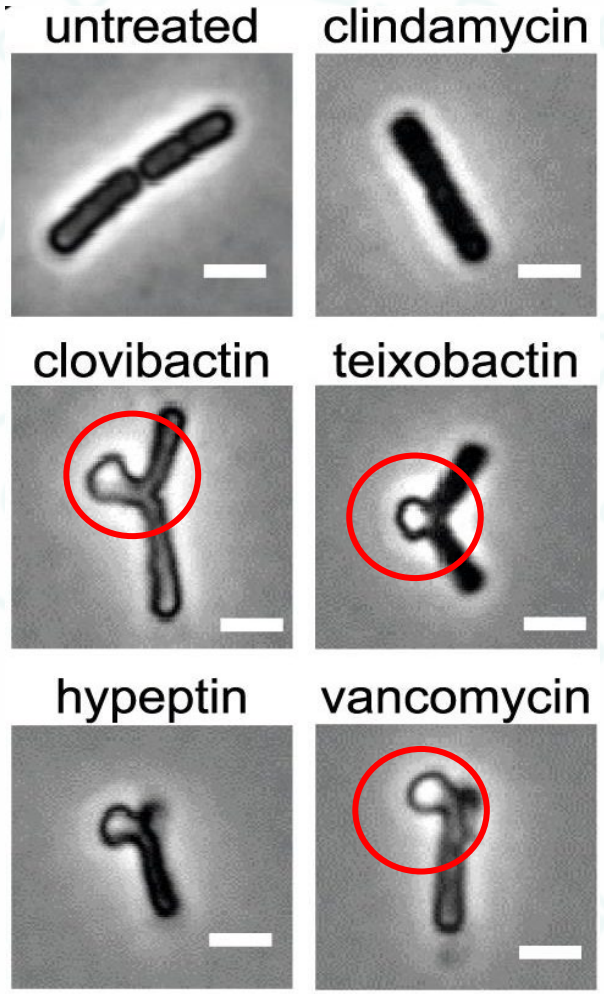
(R Shukla et al., Cell, 2023)

Clovibactin exhibits analogous effect as Teixobactin in bactericidal activity

Clovibactin treatment resulted in *S. aureus* lysis



Clovibactin treatment results in cell-shape deformations in *B. subtilis*

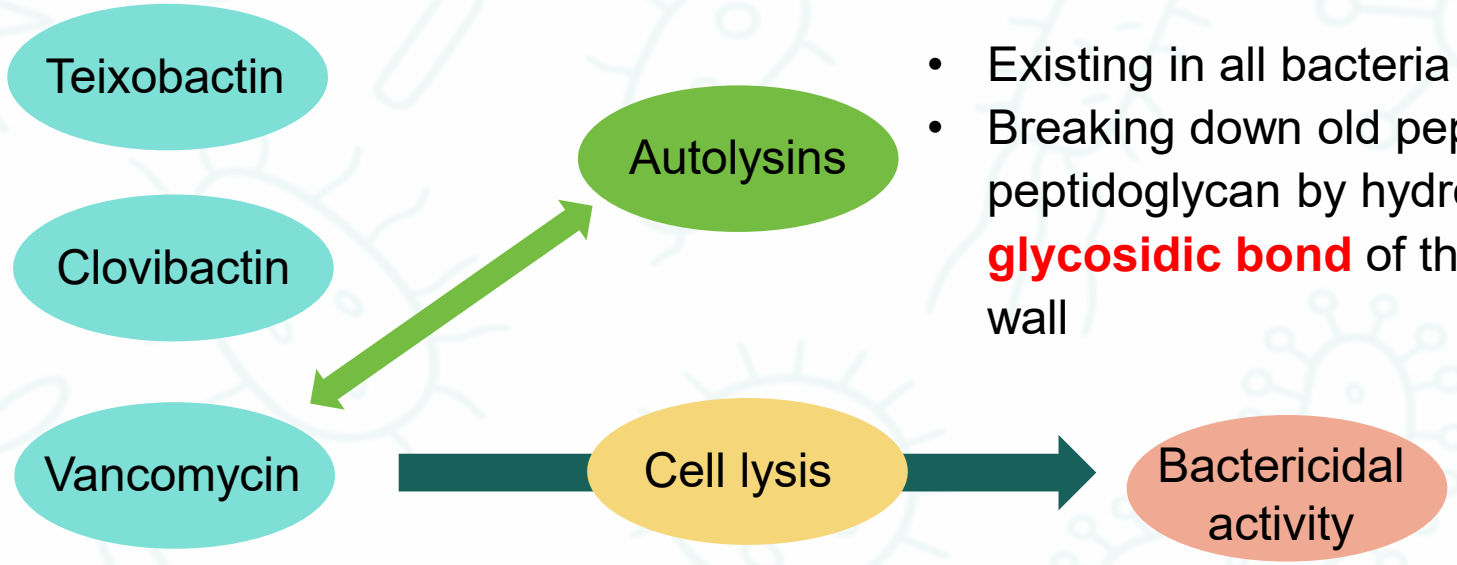


- Antibacterial activity against a broad range of Gram-positive pathogens
- More effective in killing *S. aureus* compared with vancomycin
- Induced strong *S. aureus* lysis

(R Shukla et al., Cell, 2023)

Cell-wall formation related antimicrobial target

NO resistant mutants to Teixobactin/Clovibactin even when plating on media with a low dose (4×MIC)



- Existing in all bacteria
- Breaking down old peptidoglycan to form new peptidoglycan by hydrolyzing the **β-1,4-glycosidic bond** of the **peptidoglycan** cell wall

Cell wall-acting antibiotic

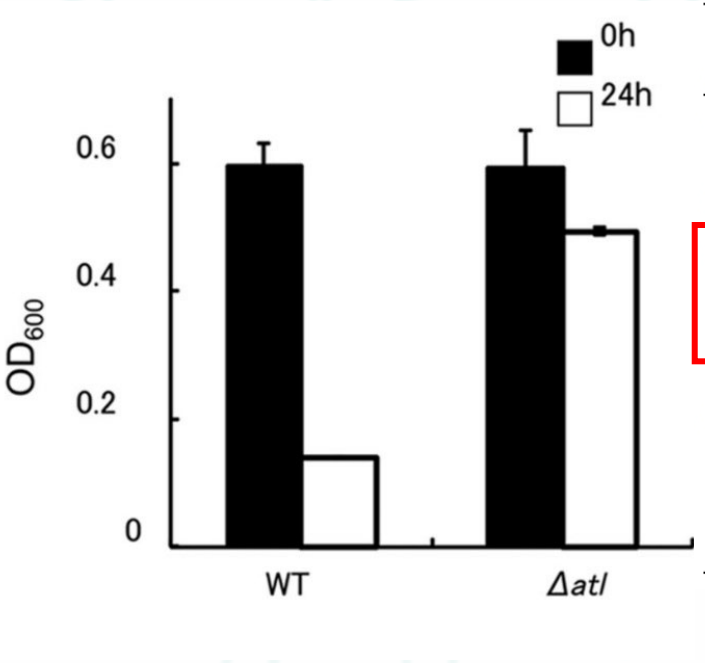
- Forming **hydrogen bonds** with the D-alanyl-D-alanine (D-Ala-D-Ala) peptide of the peptidoglycan precursor
- Enhancing **cell autolysis**

Teixobactin & Clovibactin → autolysis/cell wall formation?

(T Homma et al., Antimicro Agents and Chemo, 2016; R Shukla et al., Cell, 2023)

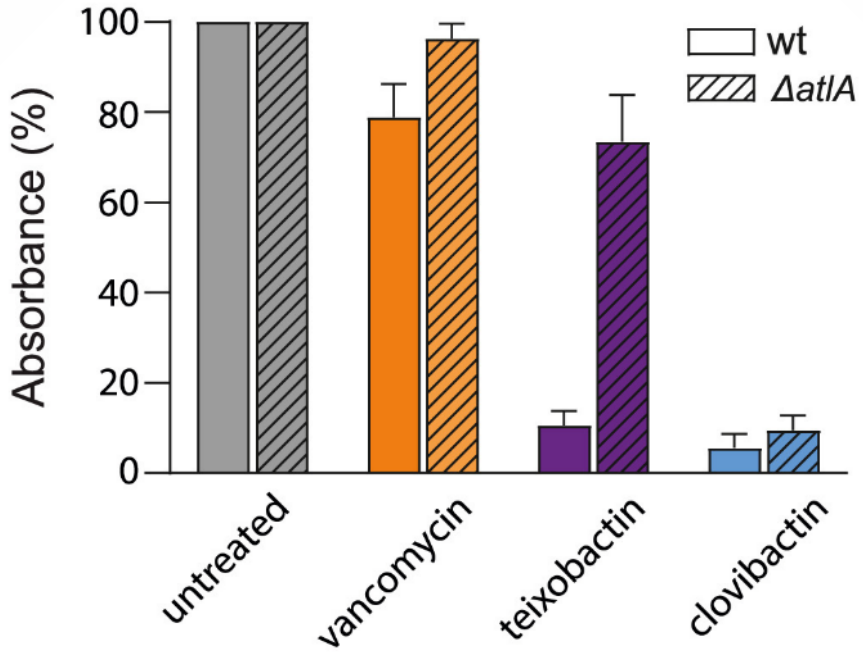
Autolysis induced by Teixobactin & Clovibactin

Teixobactin-induced lysis in *S. aureus* and its atl mutant.



Strain	MIC(□g/ml) Teix
HG003	0.5
UAMS-1	0.5
USA300	
WT	0.5
Δatl	0.5
ΔsaeS	0.031
SA113	
WT	0.5
ΔtarO	0.5
ΔdltA	0.031

Clovibactin-induced lysis in *S. aureus* and its atlA mutant

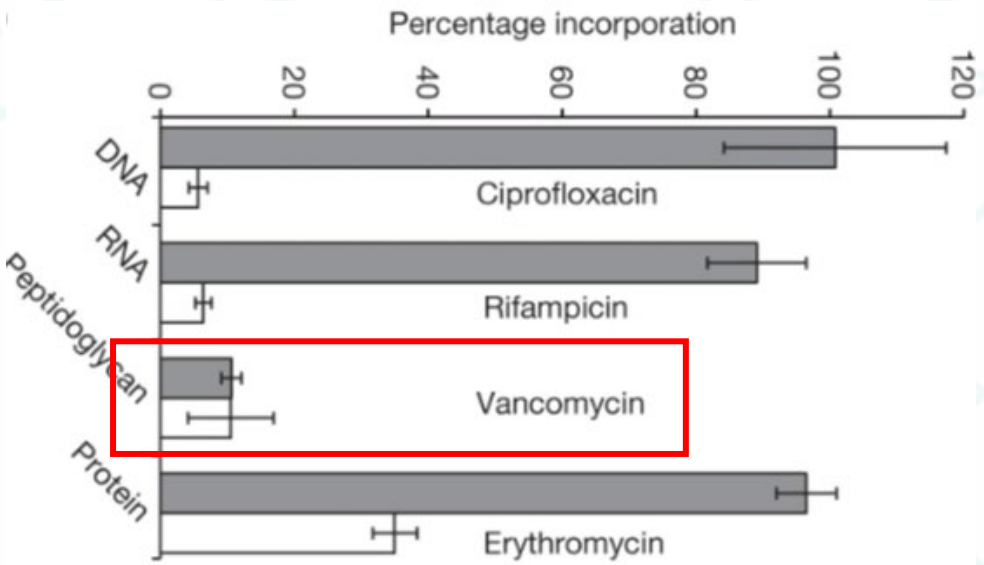


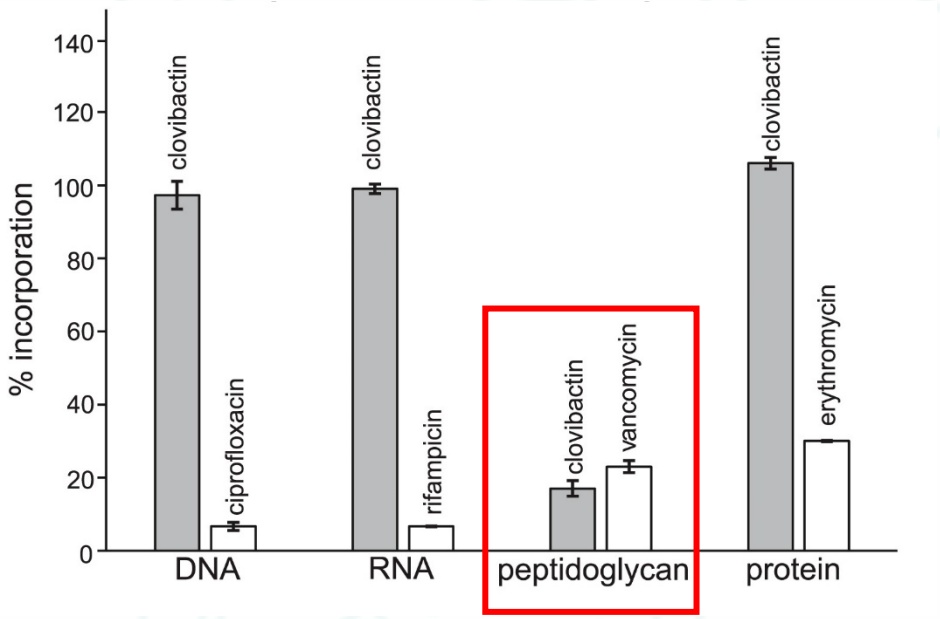
- Although Teixobactin-induced lysis is dependent on the autolysin, the MIC of Teixobactin was not affected by mutation of atl
- Clovibactin-induced lysis does not primarily rely on AtlA activity. Moreover, killing of *S. aureus* by Clovibactin was almost unaffected in both wild type and in the ΔtlA mutant, in contrast to Teixobactin

(T Homma et al., Antimicro Agents and Chemo, 2016; R Shukla et al., Cell, 2023)

04 “Resistance-resistant” bactericidal mechanism

Teixobactin & Clovibactin inhibit cell-wall related macromolecular biosynthesis





Peptidoglycan consists of linear chains of two alternating amino-sugars, N-acetylglucosamine (GlcNAc) and N-acetylmuramic acid (MurNAc), which are covalently linked and decorated with a pentapeptide chain.

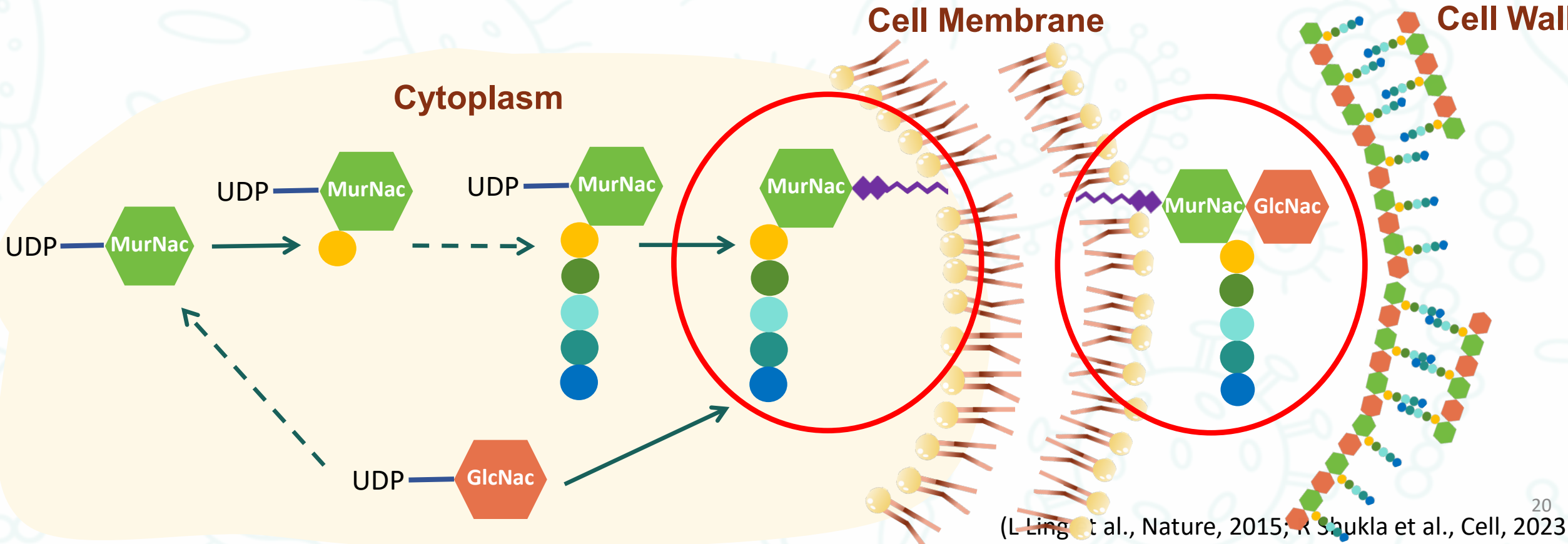
(L Ling et al., Nature, 2015; R Shukla et al., Cell, 2023)

Schematic of peptidoglycan synthesis

① Uridine diphosphate N-acetylmuramate (UDP-MurNAc) + pentapeptide segment = UDP-MurNAc-pp

② Undecaprenyl pyrophosphate moiety ($C_{55}PP$, C55-isoprenyl pyrophosphate) + UDP-MurNAc-pp = **lipid I**

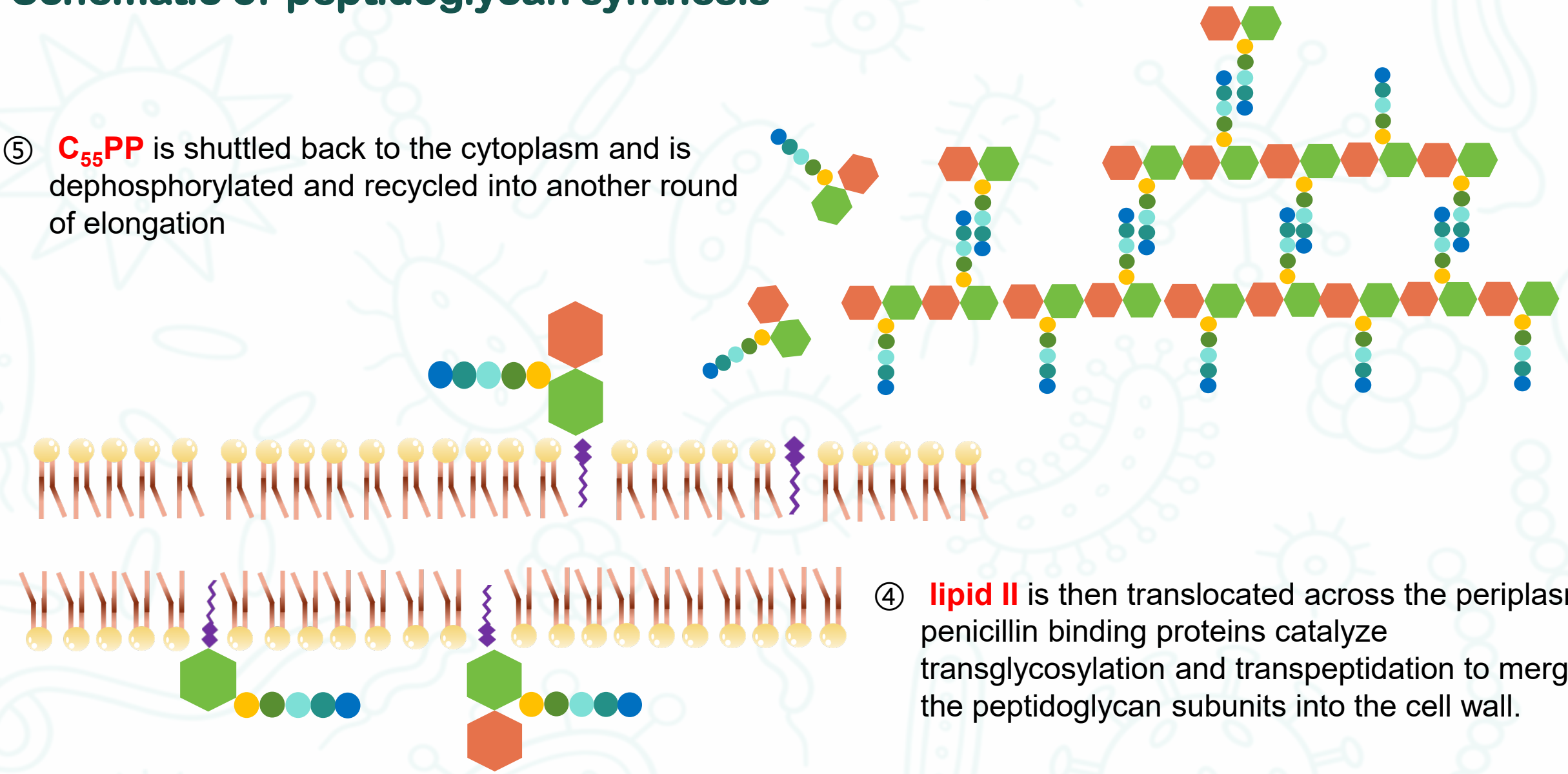
③ Uridine diphosphate N-acetylglucosamine (UDP-GlcNAc) + lipid I = **lipid II**



(L Ling et al., Nature, 2015; R Shukla et al., Cell, 2023)

Schematic of peptidoglycan synthesis

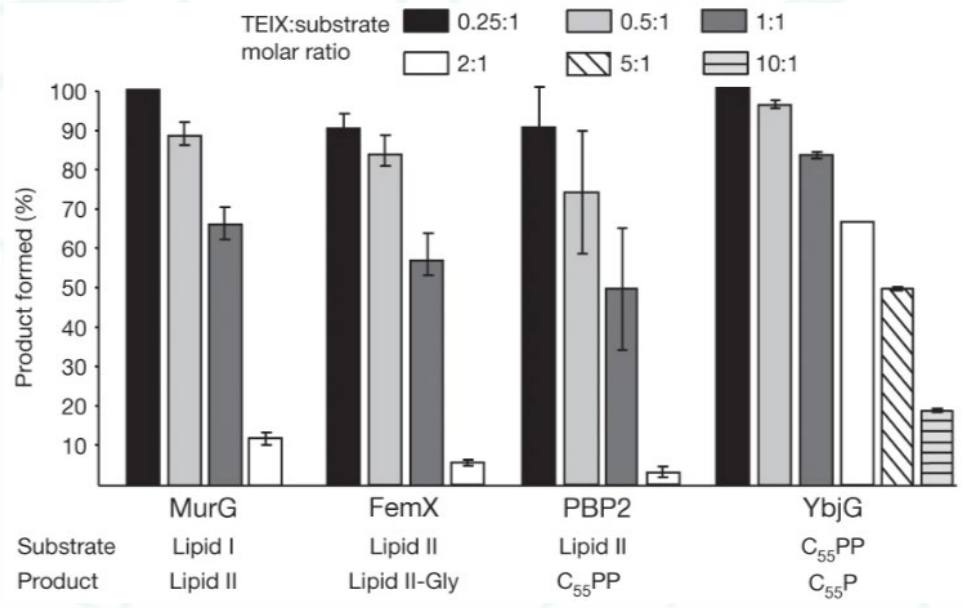
⑤ **$C_{55}PP$** is shuttled back to the cytoplasm and is dephosphorylated and recycled into another round of elongation

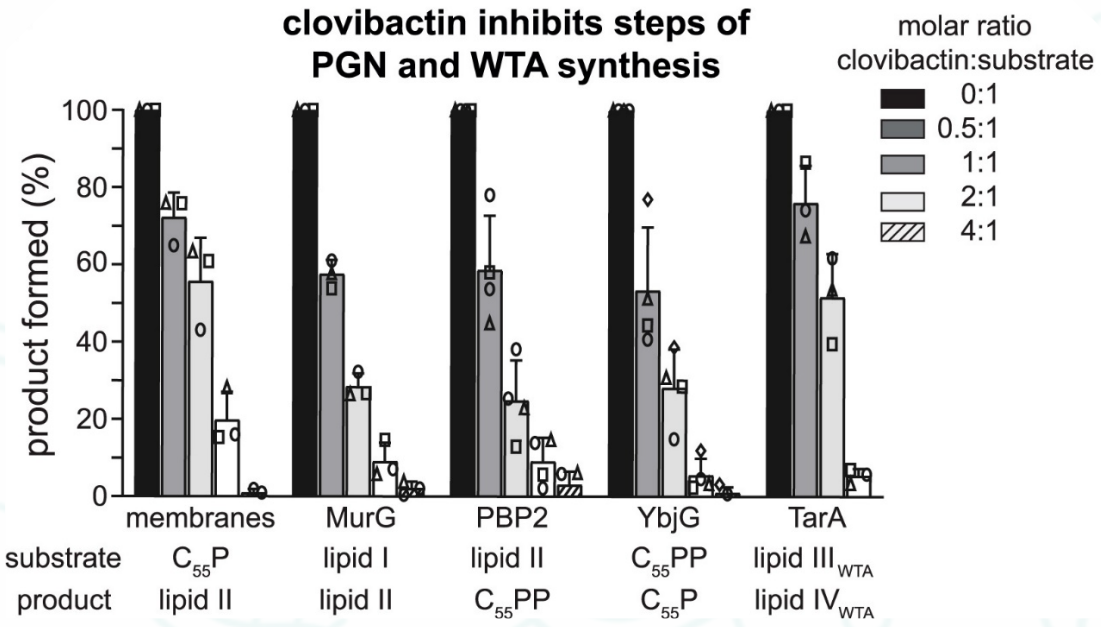


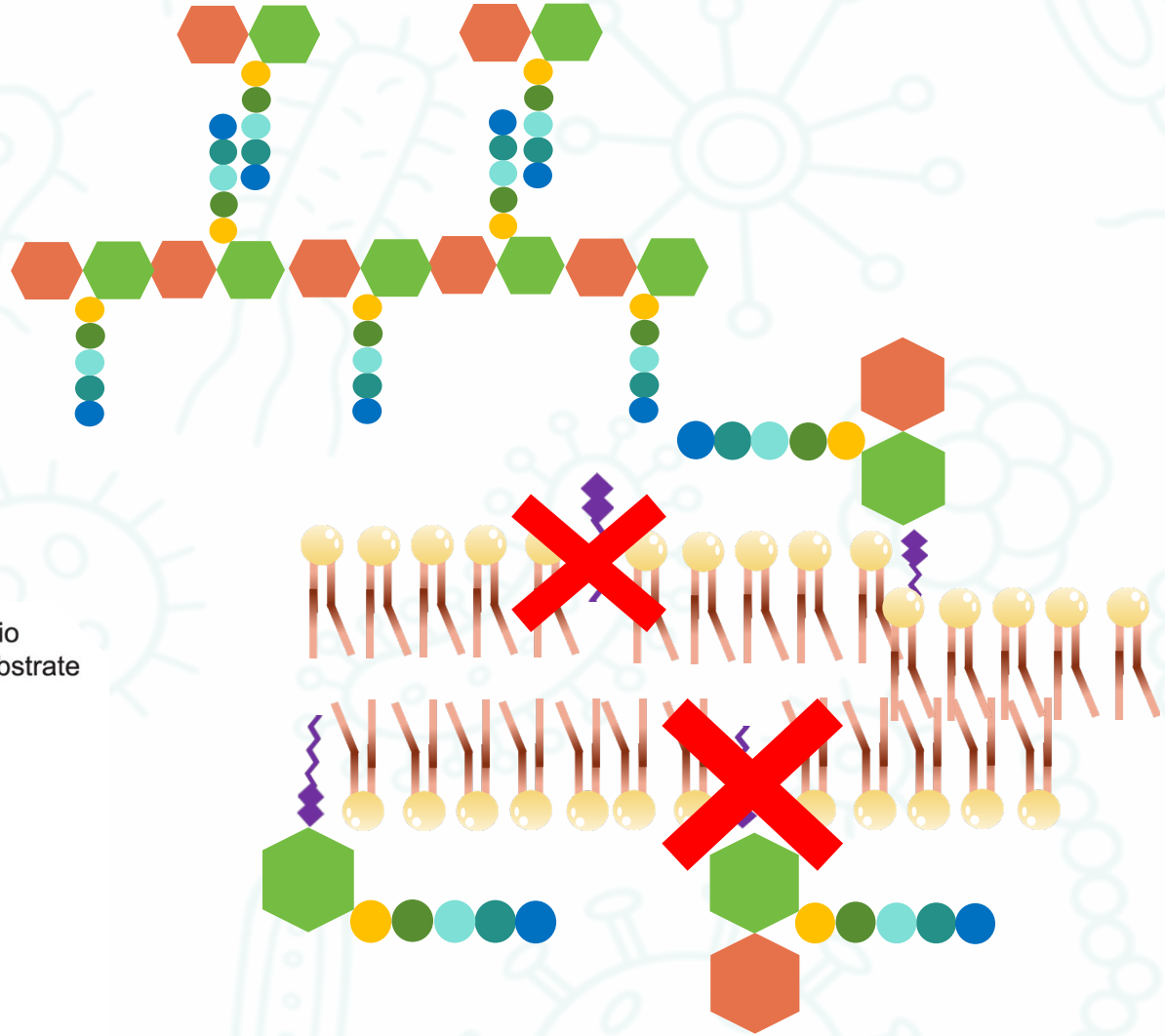
④ **lipid II** is then translocated across the periplasm, penicillin binding proteins catalyze transglycosylation and transpeptidation to merge the peptidoglycan subunits into the cell wall.

(L Ling et al., Nature, 2015; R Shukla et al., Cell, 2023)

Teixobactin & Clovibactin dramatically inhibit the lipid II/C_{55}PP





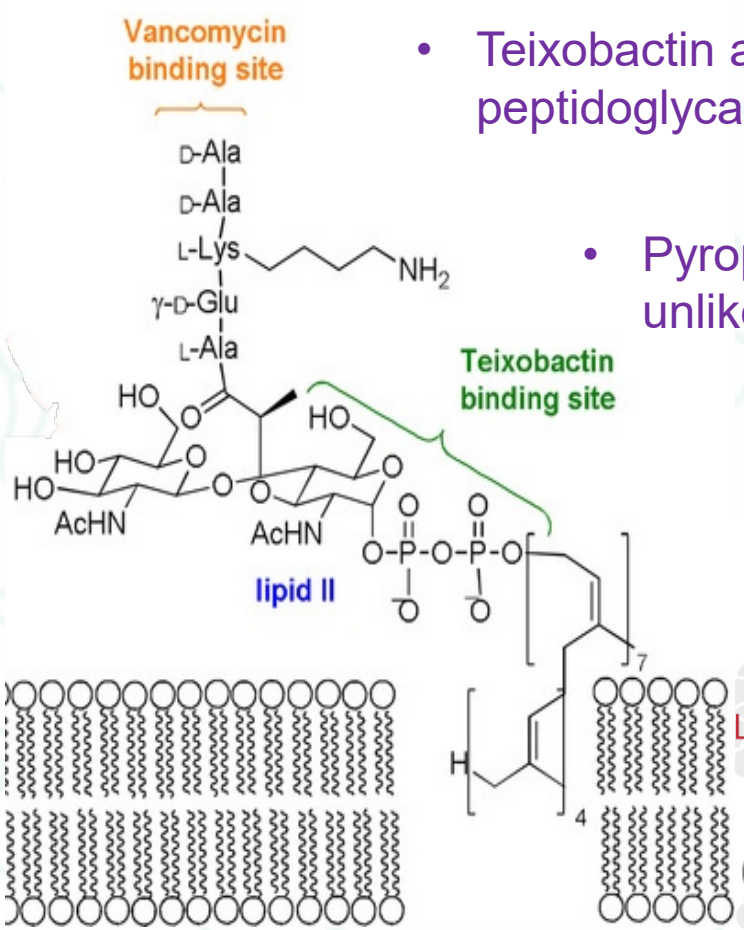


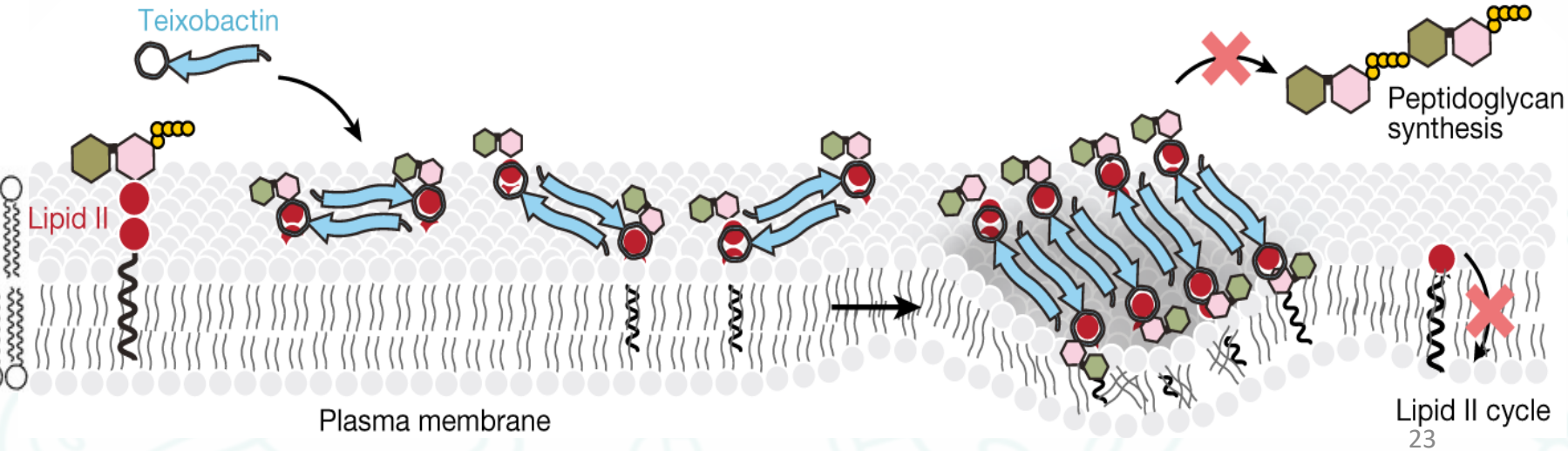
(L Ling et al., Nature, 2015; R Shukla et al., Cell, 2023)

Teixobactin “resistance-resistant” binding scheme

NO resistant mutants to Teixobactin/Clovibactin even when plating on media with a low dose (4×MIC)

- Teixobactin appears to bind to the pyrophosphate moiety of lipid II but not to mature peptidoglycan, this could account for the lack of cross-resistance with vancomycin.
  - Pyrophosphate is both an essential and immutable moiety of cell wall lipid intermediates, unlike other portions of the molecules.



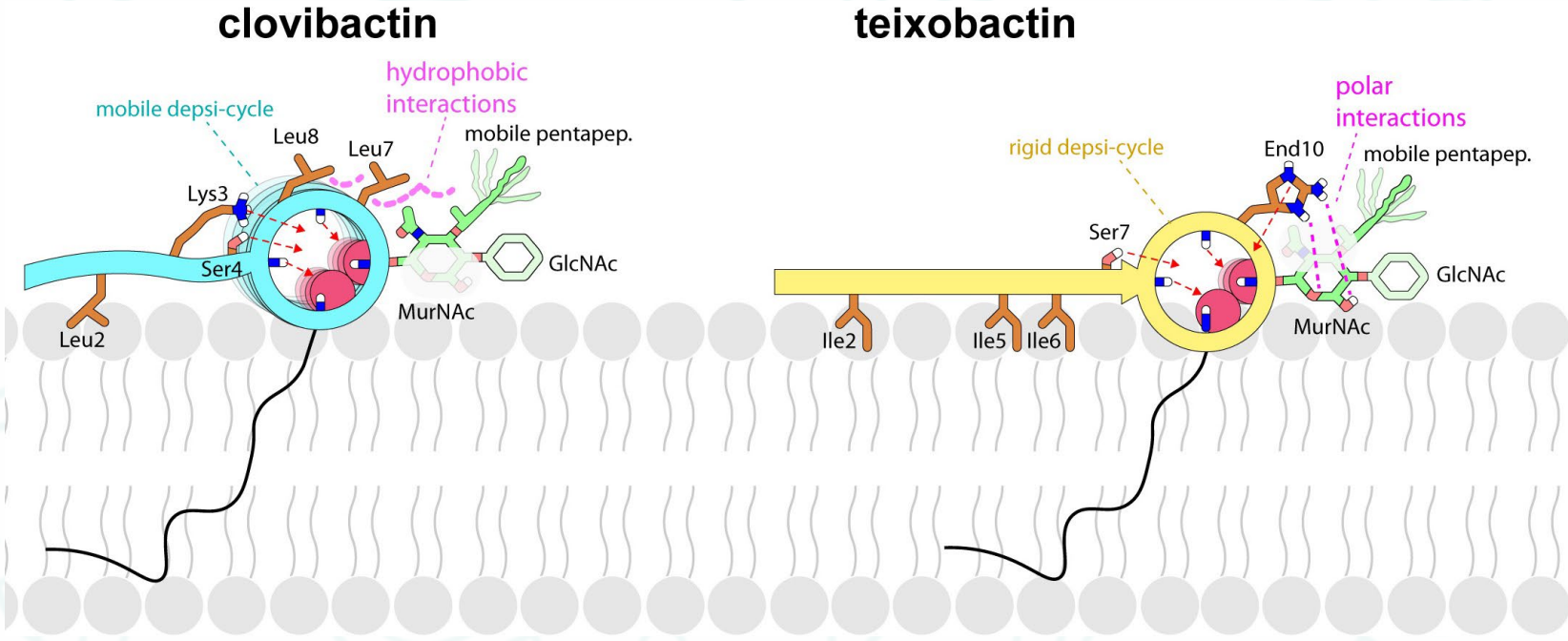


(R Shukla et al., Nature, 2022; G Chu et al., Chemistry 2018)

Clovibactin “resistance-resistant” binding scheme

- Clovibactin’s N-terminal domain is only loosely anchored into the membrane by **a single long hydrophobic** residue.
- It targets the pyrophosphate group of lipid II using backbone amino protons of its depsi-cycle.
- The side chains of the depsi-cycle that face the lipid II sugars are exclusively **hydrophobic**, which interact with the hydrophobic side of the MurNAc sugar.

- Teixobactin is firmly anchored into the membrane by **three long hydrophobic residues**, which rigidifies the teixobactin supramolecular fibrils.
- It specifically binds the pyrophosphate group of lipid II using backbone amino protons of its depsi-cycle.
- Binding of the anionic pyrophosphate group is presumably supported by **polar interactions** with the hydroxyl-group of Ser7.



(R Shukla et al., Cell, 2023)

Conclusion

- Unculturable bacteria provide a large source for antimicrobial screening and are another natural library of antibiotics.
- Unculturable bacteria cultivation techniques are well developed with higher resuscitating rate and less time-consuming.
- Teixobactin and Clovibactin both provide novel targeting sites against MRSA with promising bactericidal activity and non-mutagenic capacity, contributing to another therapeutic alternative with vancomycin against multi-drug resistant Gram-positive pathogens.
- The unculturable bacterium, *Eleftheria terrae*, has not been intensively studied as a potential antibiotic-producing strain.

Major concerns

Vancomycin is unable to bind with the pentapeptide moiety when bacteria remain in the deep dormancy status.
How about Teixobactin or Clovibactin?

The varied cell lytic activity induced by Teixobactin and Clovibactin.

THANKS

Q&A

Presenter: Hu Haitao

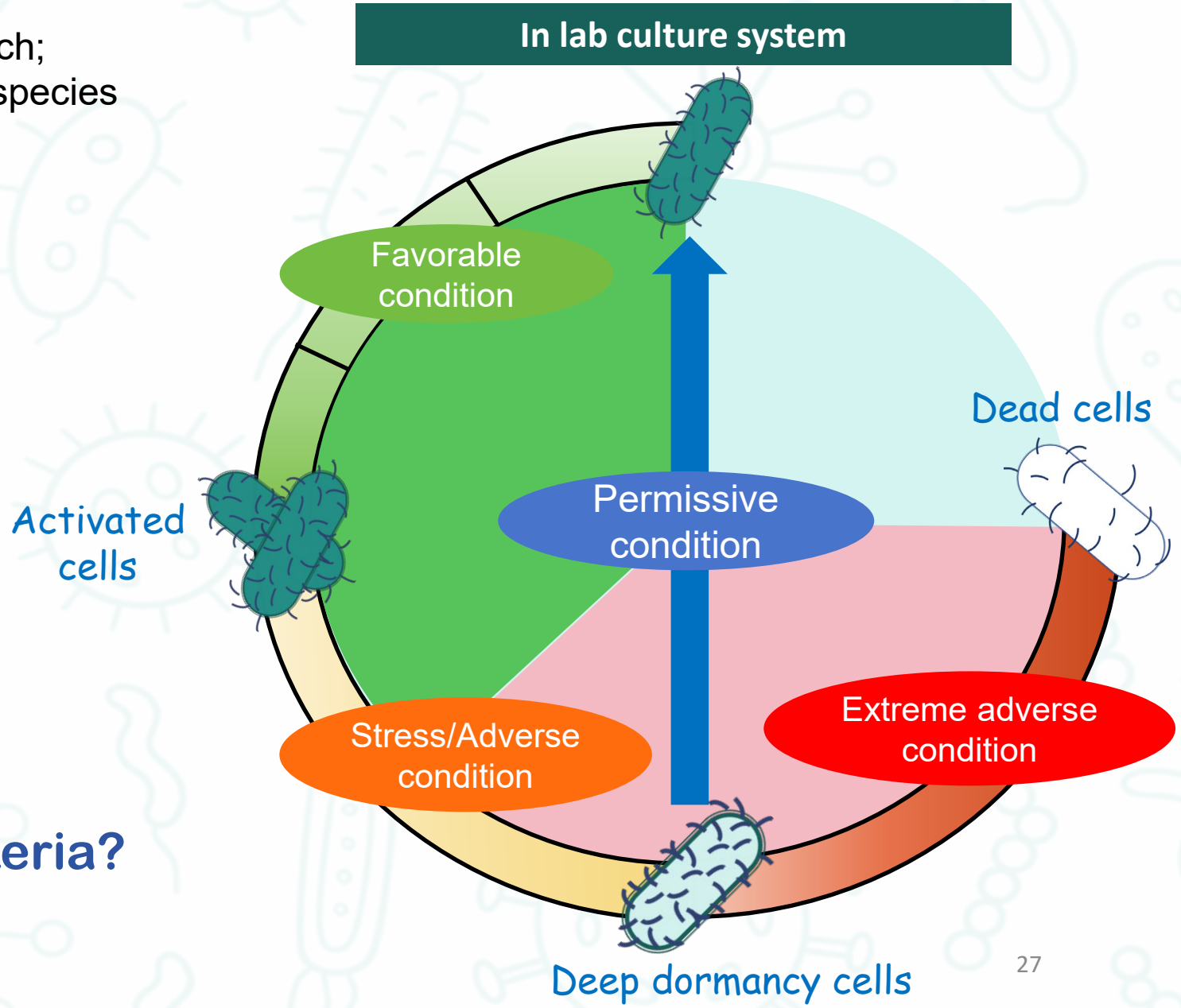
Appendix: Background

- Microbial diversity on Earth is impressively rich; **>99%** of the potentially 10^{11}–10^{12} microbial species remain undiscovered to date;

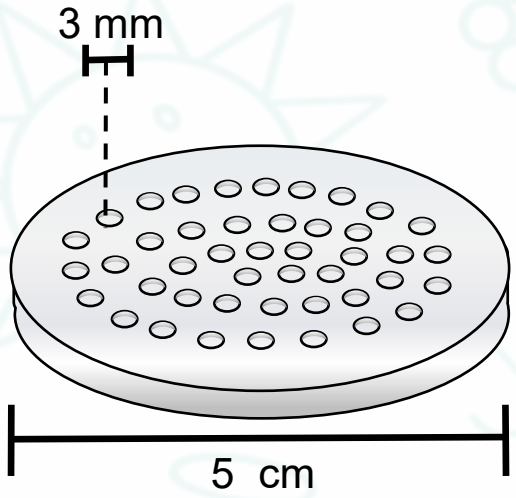
- The deep dormancy state is an adaptive strategy that serves long-term bacterial subsistence under adverse conditions:

- **nutrient starvation**
- extreme temperatures
- increased salinity
- pH changes
- osmotic stress
- heavy metals...

Ideal environment →Culture bacteria?

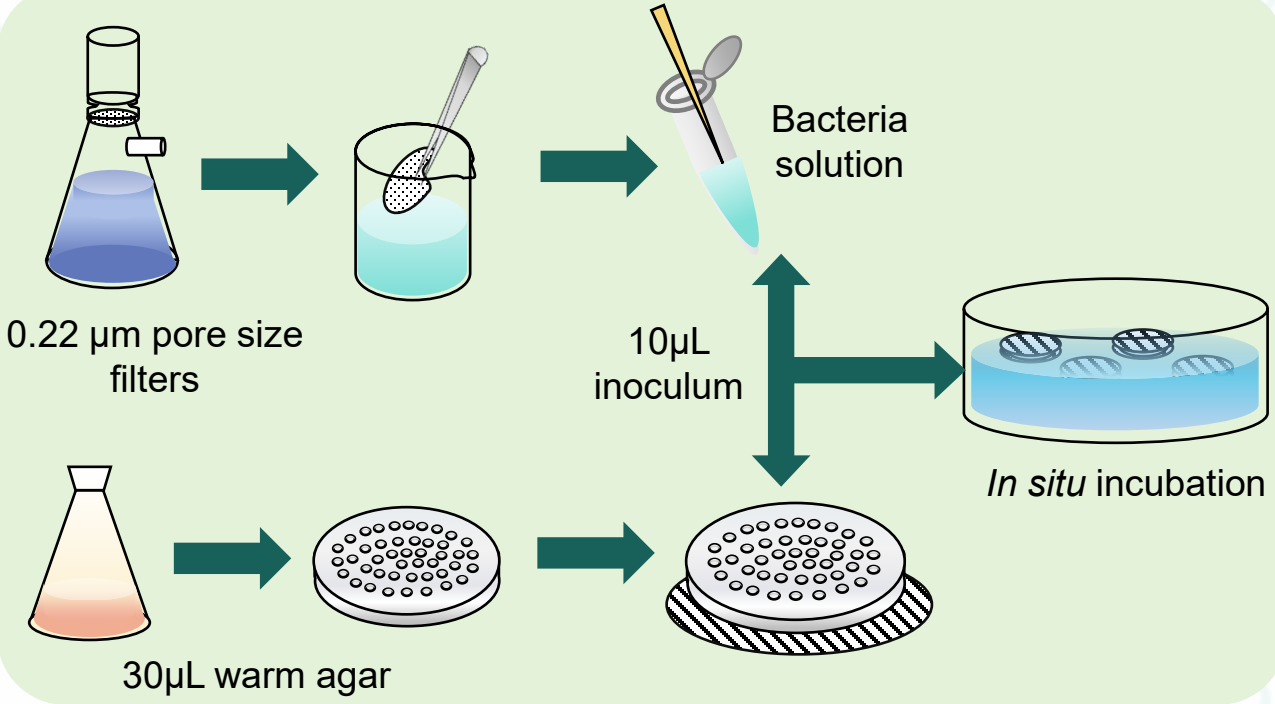


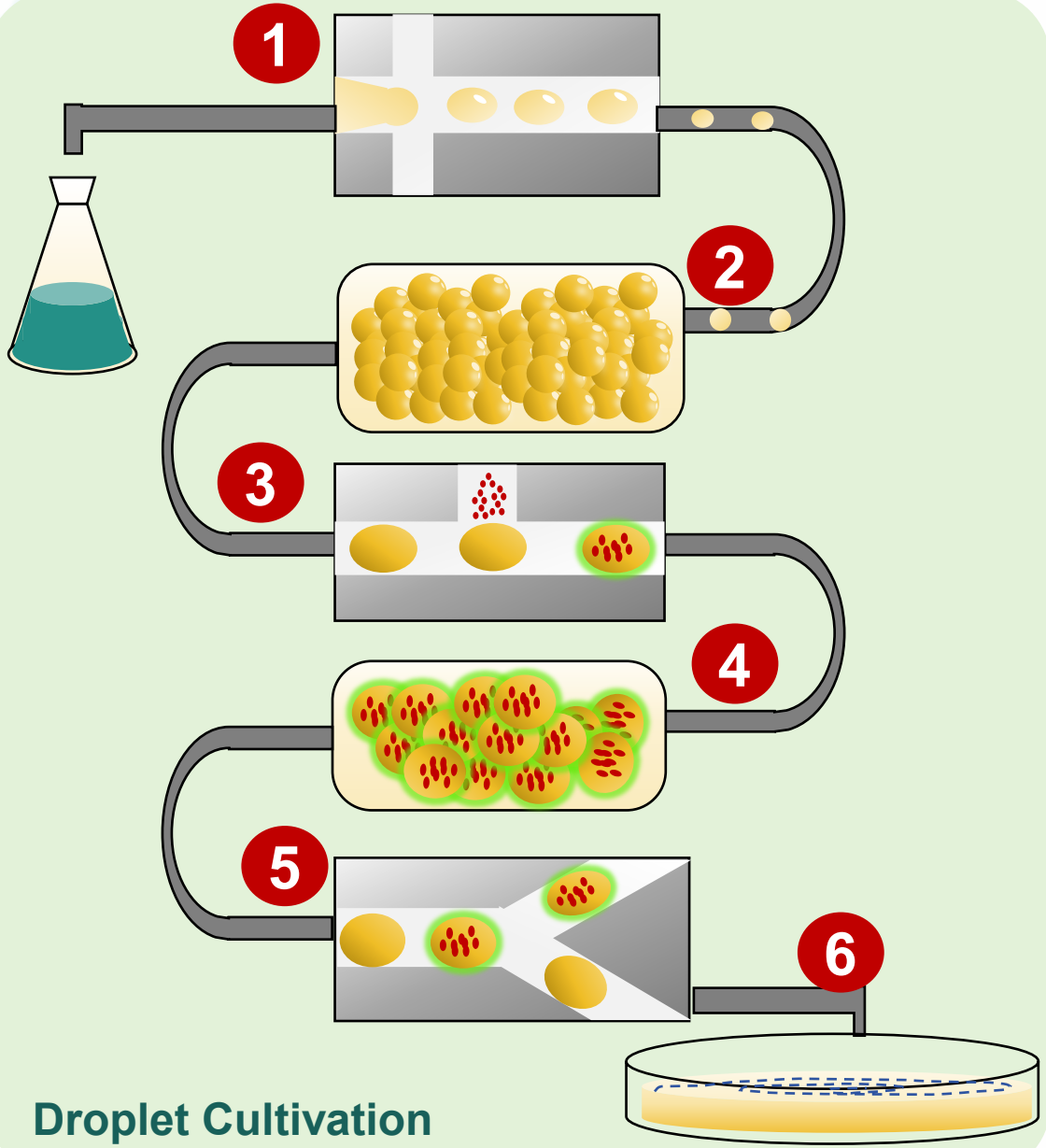
Appendix: Other *in situ* cultivation techniques



Culture Chip

- A polypropylene plastic material sandwiched between two 0.03 µm-pore-size polycarbonate membranes





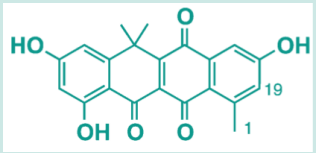
Droplet Cultivation

(A Lodhi et al., Archives of Microbio, 2023; Mahler L et al., Elife 2021)

Appendix: *In situ* cultivation techniques development

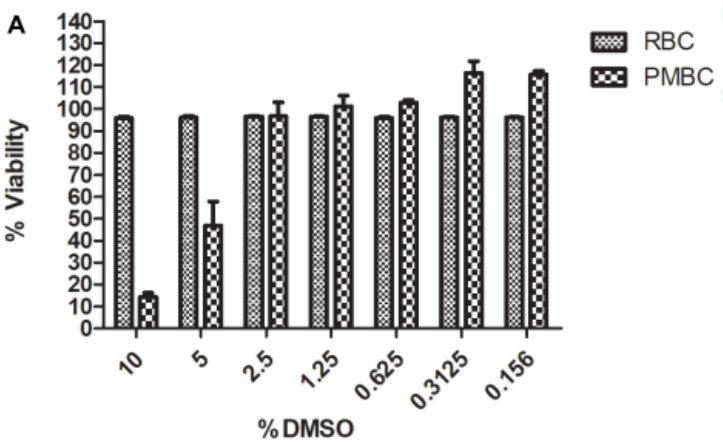
		Petri Dish	Diffusion Chamber	Isolation Chip	Culture Chip	Droplet-microfluidic Platform
Year		1898	2002	2009	2022	2020
Culture events	Pre-treatment	-	-	Enumeration, dilution	-	(Co-)encapsulation, Pico injection
	Medium	Nutrient agar/broth	Soil, aquatic sediment, etc.	Soil, seawater, etc.	Lake water	Bulk
	Resuscitation	+\-	+	+	+	+
	Enrichment	+\-	+	+	+	+
	Isolation	+\-	-	+	-	+
	Antimicrobial screening	+\-	-	-	-	+
Further		Subculture (Isolation/Antimicrobial screening)				Fermentation

Appendix: Antibiotics discovered in unculturable bacteria

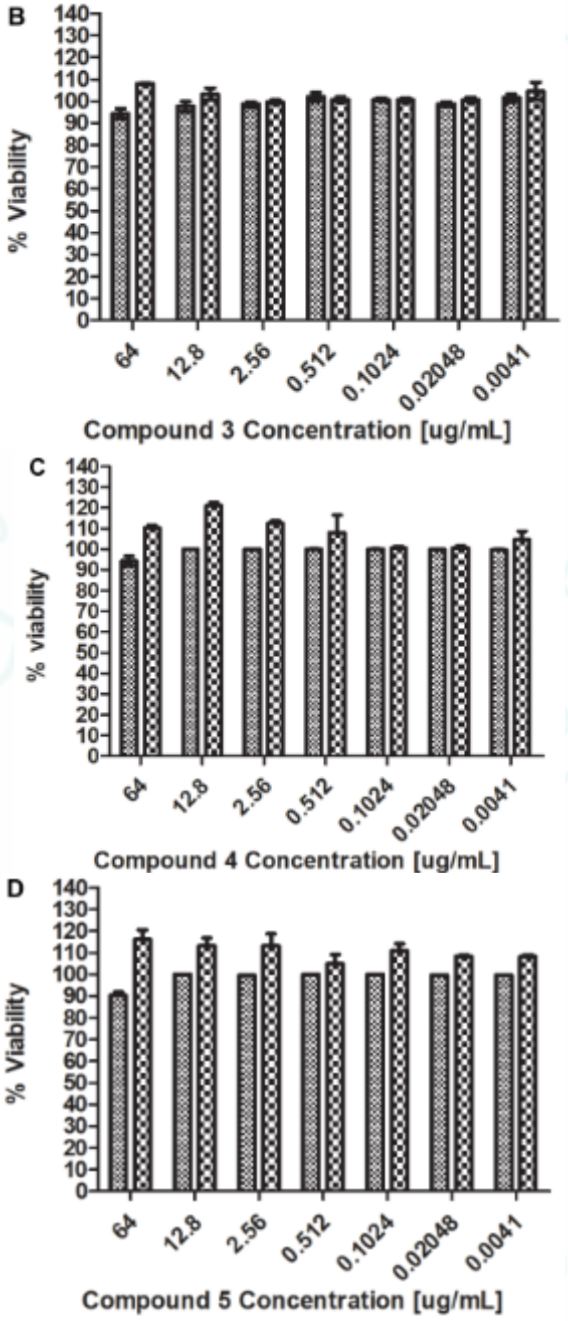
Antibiotics	Isolate strains	Target strains	Characteristics	Mechanisms
Lassomysin	*Actinomycetes*	*Mycobacteria*, including drug-resistant forms of *Mycobacterium tuberculosis*	Ribosomal encoding cyclic peptide	Binding to a highly acidic region of the ClpC1 ATPase complex
Streptomycobactin	*Streptomyces sp.*	*M. tuberculosis*	2259 Da, 20 amino-acid semi-cyclic peptide	Remains to be elucidated
Kitamycobactin	*Kitasatospora sp.*	*M. tuberculosis*	1735 Da, lasso peptide	An analog of lassomycin
Amycobactin	*Amycolatopsis sp.*	*M. tuberculosis*	762 Da, featuring a ketal moiety within a macrolactone backbone	Inhibiting protein secretion via the SecY translocon
Tetarimycin A	*Streptomyces albus*	Methicillin-resistant *Staphylococcus aureus*		Remains to be elucidated

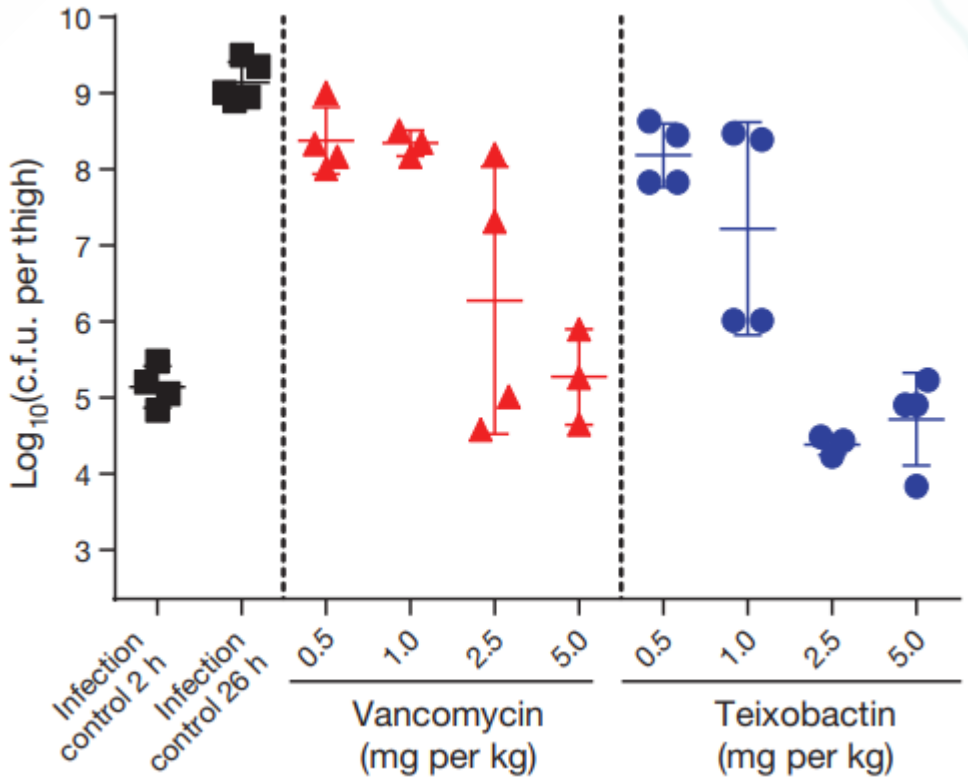
Appendix: No cytotoxicity and high therapeutic potential by Teixobactin

Haemolysis and cytotoxicity effects were evaluated by exposing RBCs and PBMCs to varying concentrations of the Teixobactin derivatives.



Teixobactin had no toxicity against mammalian NIH/3T3 and HepG2 cells at 100 μg/mL.





Single dose (i.v., 2 h post-infection, 4 mice per group) treatment with Teixobactin and vancomycin in neutropenic mouse thigh infection model using MRSA

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