Antimicrobial Production Screening

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Agenda

1. Introduction
2. Scientific principles and context
   - Antimicrobial production triggers
   - Mechanism of Action
3. Procedures and interpretation
   - Agar-based methods
   - Metagenomics
   - High-throughput methods
   - Isolation chip
4. Case study: Soil in Nepal
5. Conclusion

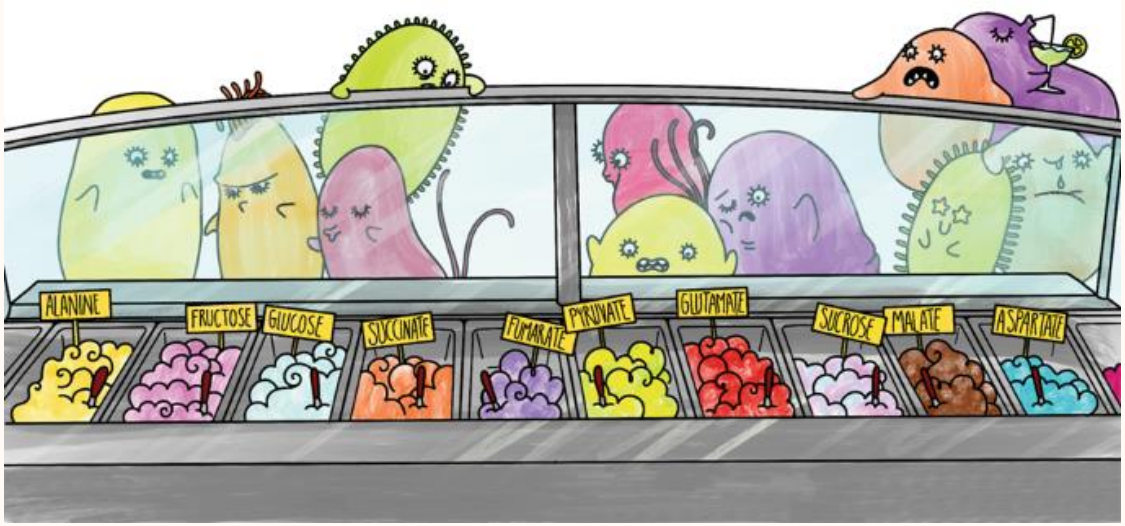
Introduction

Antimicrobial : any substances that impairs or kill other microbe. Microbes produce them to compete for space and nutrients,

Antimicrobial Production Screening:

➔ How can we identify which microorganisms are producing antimicrobial compounds?

➔ What are those compounds?



(Schlechter, R. O. *et al*. (2023). *ISME J* **17**, 1445–1454)

Introduction

In human health:

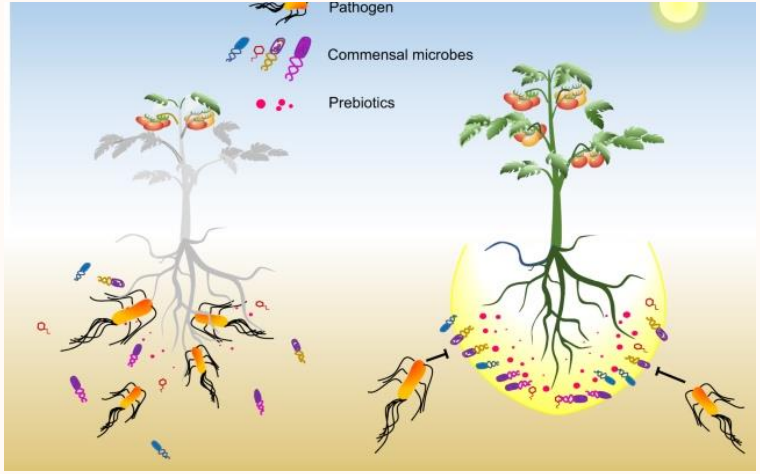
- Long-standing interest in antimicrobial properties
  - Ayurvedic medicine (~2500-600 BCE) use of plant preparations to prevent spoilage and infection.
  - New standardized lab assays led to the golden age of antibiotics (1940-70s)
  - Today, rapid screening is critical as resistance outpaces drug discovery.

(EURACTIV, 2022)

(Agappe, 2021)

In ecology:

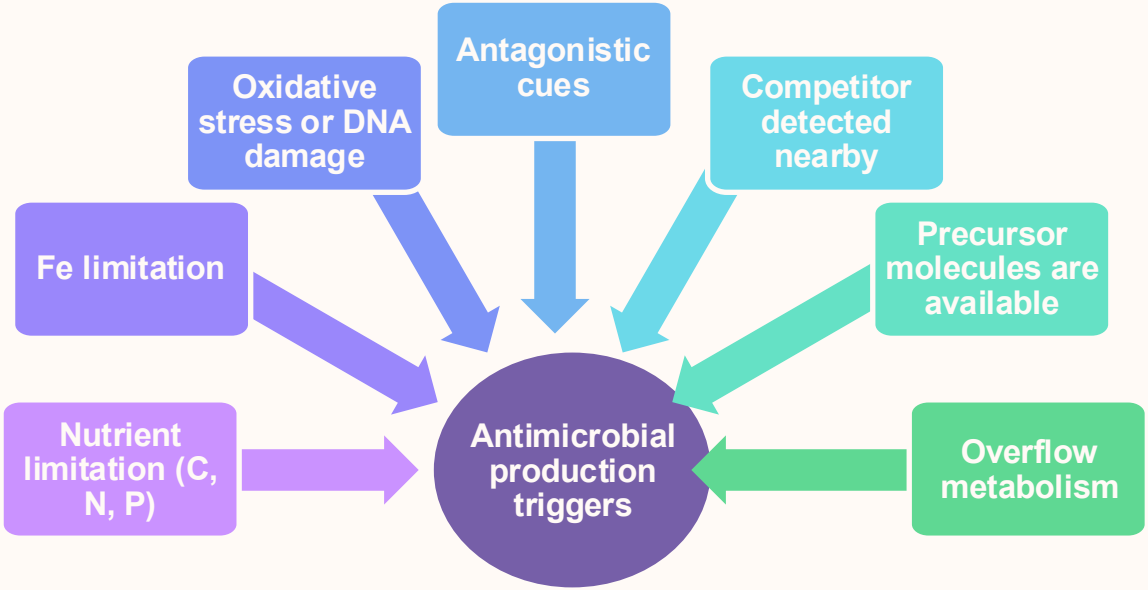
- These compounds structure biofilms, soil communities, and rhizosphere interactions.
- Sub-inhibitory levels act as signals that coordinate behaviour or modulate competition amongst communities.



(Wen *et al.* (2023). *Nature Communications* **14**, Article 4497.)

What triggers antimicrobial production?

Microbes do not produce antimicrobials constantly; they produce them **under specific ecological and physiological conditions**. Some triggers are:

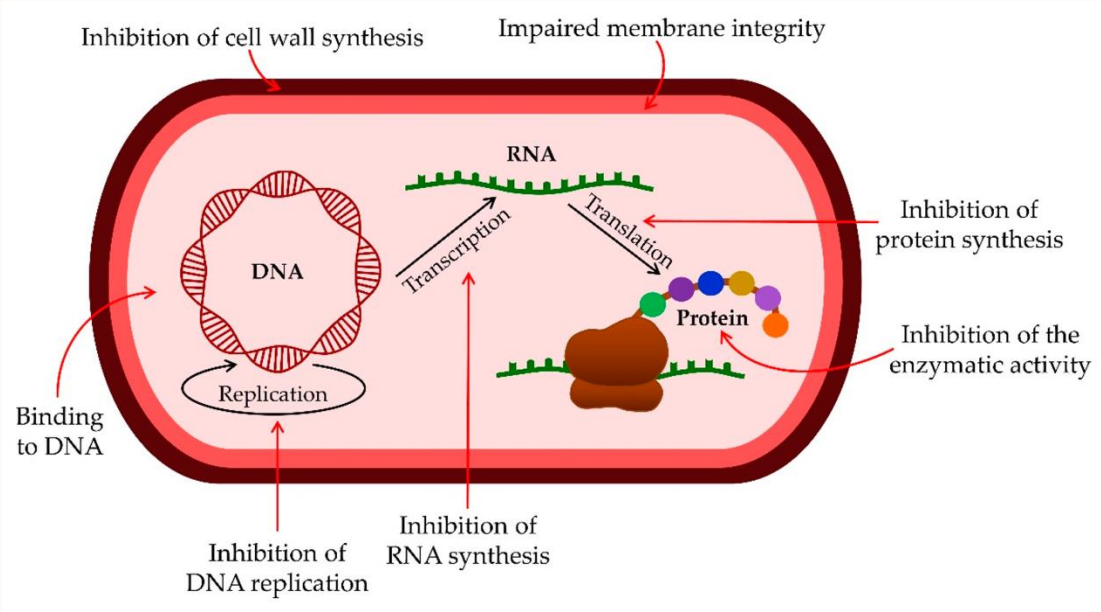


Antimicrobial pathways may remain silent in vitro if lab conditions don't replicate the ecological triggers needed.

How do antimicrobials work ?

There exist many different mechanisms, and their ecological impact depends on context:

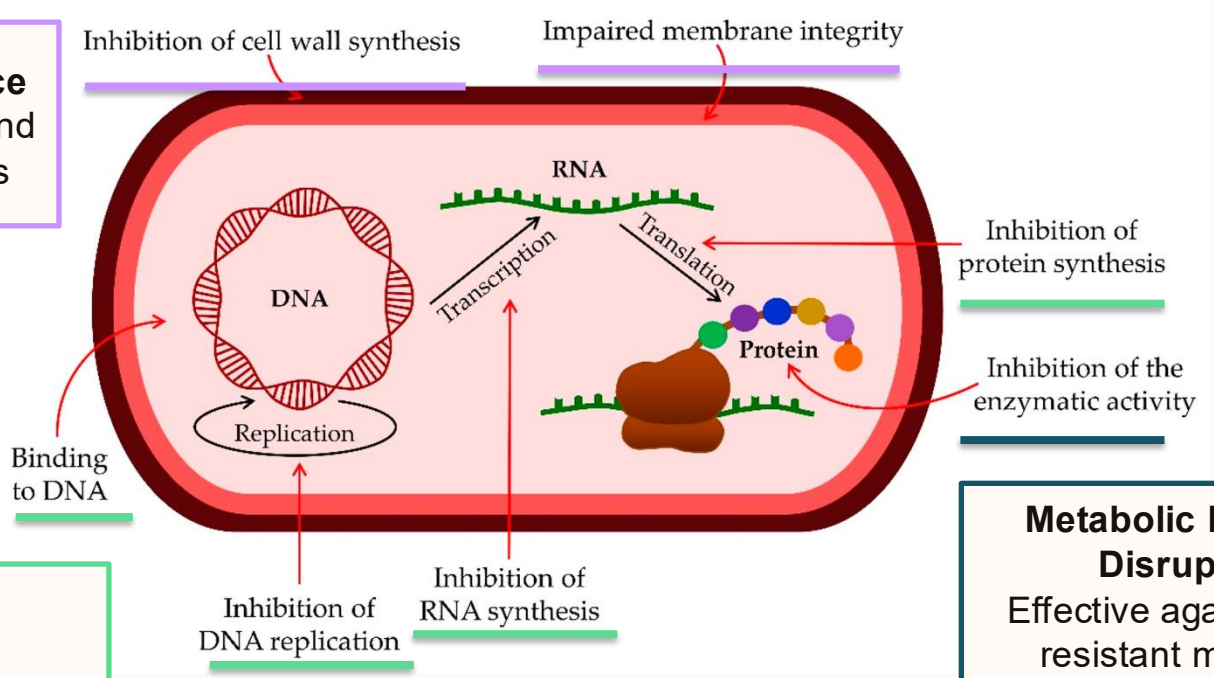
Concentration, spatial structure, nutrient availability → determine if compounds act as a **weapon, signal or no effect**



(Talapko *et al.*, *Antibiotics* (2022))

How do antimicrobials work ?

Structural interference
Rapidly clears space and opens local resources



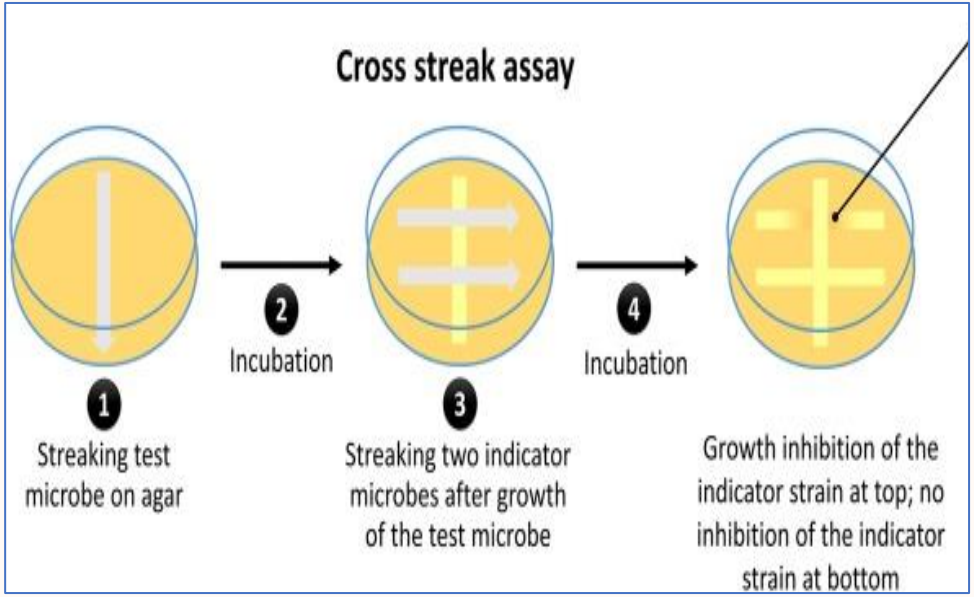
(Talapko *et al.*, *Antibiotics* (2022))

Informational interference
Effective against fast-growing microbes

Metabolic Pathway Disruption
Effective against more resistant microbes

Procedures

Agar based method : cross streak



Eur J Microbiol Immunol (Bp). 2024 Apr 22;14(2):97–115. doi: 10.1556/1886.2024.00035

Principle: Test microbe streaked first; indicator streaked perpendicular. Clear zones = inhibition.

Advantages: Simple, fast, low-cost, multiple indicators per plate.
Limitations: Qualitative, diffusion-dependent, subjective.

Liquid screening

Co-culture

- A test microbe and an indicator grown together in a medium that supports both their growth
- Regular sampling and plating on a medium that only supports the indicator
- Counting of the colonies
- Growth of the indicator over time is compared with a negative control to determine if there is an antimicrobial activity

- Better represents the real interactions than the agar method as there is no problem of diffusion
- But it is possible the inhibition happens for other things than antibiotic production (competition, direct cell to cell interaction)

Cell free supernatant

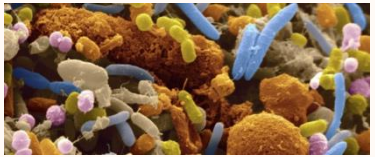
- The test microbe is cultivated alone
- It is then filtered to remove the microorganisms
- We then grow the indicator in the same broth to see if there is inhibition

- Can be used to confirm that the antimicrobial activity is caused by a compound produced by the tested organism

Metagenomics

- The DNA of the entire community is extracted
- Primers are chosen to select relevant section, then these get replicated with PCR
- The DNA chains are then sequenced
- Then we can do a bioinformatics analysis to compare them to the databases, differences with all the may indicate that the gene is involved in the production of a new antibiotic
- Example on this article [11] they screened the NRPS genes (nonribosomal peptide synthetases), as these are enzymes that are involved in the production of some antibiotics

community



DNA extraction

PCR + Sequencing

Analysis + potential discovery of new intibiotics

Hight throughput methods

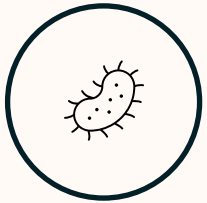
- These are processes that can test for a lot of different organisms in a very short time thanks to highly parallelised operations
- Ex : on this paper : [13]
  they reached > 100000 tests/d
- They were able to do this by isolating single cells and growing them in tiny bioreactors in the form of microdroplets
- They used it to make a mass spectrometry analysis to look for the presence of a known antibiotic and also to inject it with reporter cells to measure the antimicrobial activity of each population

Thousands of droplets containing 1 cell each before the cultivations

Isolation chip

- Single-cell isolation in diffusion chambers.
- Grown in natural environment → allows growth of previously unculturable microbes.
- Opens the possibility for lots of new antibiotics to be discovered
- Example: discovery of teixobactin from *Eleftheria terrae*.

The chamber prevents other cells to enter but not the nutrients and chemicals from the environment



Case study:

Isolation, Characterization, and Screening of Antimicrobial-Producing Actinomycetes from Soil Samples

Anupama Sapkota, Aishwarya Thapa, Anupa Budhathoki, Muskan Sainju, Prativa Shrestha, Sagar Aryal

- Eleven soil samples from different altitude range of Nepal (organically cultivated fields, rhizospheric area, and river banks)
- Microbial group studied: **Actinomycetes**

https://www.nepaltripplanners.com/package/delights-of-nepal (2025)

Actinomycetes

- Aerobic, Gram-positive, spore-forming bacteria.
- Belong to the order *Actinomycetales*
- Exhibit filamentous growth with substrate and aerial mycelium, giving them a fungus-like appearance.
- Produce Geosmin, the compound responsible for the characteristic “earthy smell” of healthy soil.
- Produce a wide range of secondary metabolites of various medical values like antibiotics, antifungal, antiprotozoal, antiviral, anticholesterol, antihelminth, anticancer and immunosuppressant.

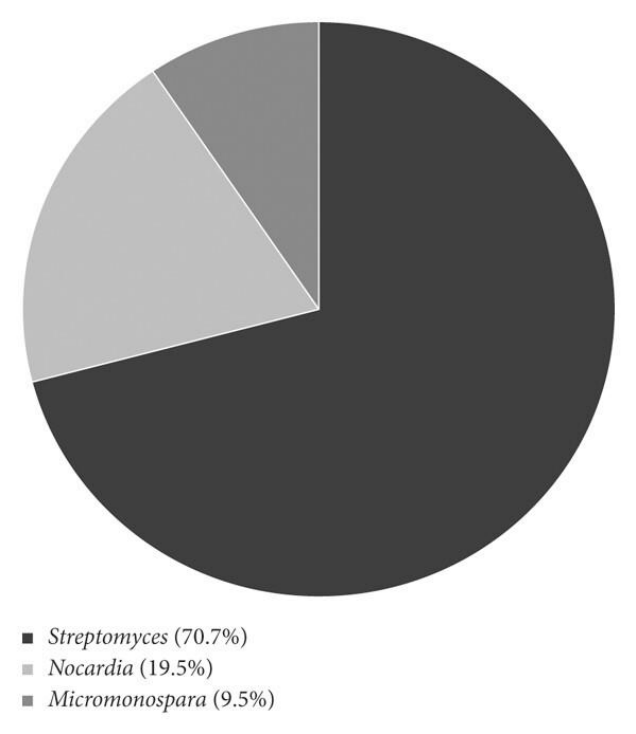
The bacterium *Streptomyces griseus* is an example of an actinomycete.
Source:https://www.britannica.com/science/actinomycete

Goal of the study: Isolate, identify, and screen the potential antimicrobial-producing actinomycetes from soil samples.

Methods & results

- Soil sample collection
- Isolation of Actinomycetes
- Identification
- Macroscopic characterization
- Microscopic observation
- Biochemical Tests

→ 41 actinomycete isolates recovered from 11 soil samples.



Primary screening: perpendicular streaking method

Indicator bacteria:

- *Staphylococcus aureus* (Gram +)
- *E. coli* (Gram -)
- *Pseudomonas aeruginosa* (Gram -)
- *Klebsiella pneumoniae* (Gram +)

→ Out of 41 pure isolates, 19 isolates (~46%) showed antimicrobial activity against the test organisms

→ All active isolates inhibited *Staphylococcus aureus.*

→ Only a few inhibited *E. coli* or *Klebsiella pneumoniae.*

→ None inhibited *Pseudomonas aeruginosa.*

→ 12.19% of total isolates was found to be active against both Gram-positive and Gram-negative test organisms

Secondary screening: agar well diffusion method

- **Step 1:** Production of Crude Extract: Isolates are fermented, incubated and centrifuged to obtain a concentrated mixture of antibiotics and metabolites produced by the Actinomycete.

- **Step 2:** Agar Well Diffusion Method:
  - *Negative control:* Ethyl acetate
  - *Positive control:* antibiotic discs (oxacillin, nitrofurantoin, and ciprofloxacin)

→ Only **13 isolates** showed inhibition from crude extracts, demonstrating that:
→ Some isolates show activity on agar but do not produce stable antimicrobials in liquid culture.
→ This difference is common due to media composition, fermentation conditions, or loss/inactivation of compounds in broth.

Conclusions

- Antimicrobial screening remains essential for uncovering microbial interactions and novel bioactive compounds, especially in the context of increased antibiotic resistance .
- It offers powerful functional and ecological insights with clear biotechnological potential.
- Its impact is greatest when paired with genomic and chemical analyses for deeper resolution.
- Our case study highlights the value of dual-screening approaches to capture a broader spectrum of activities.

Thank you!

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