Influence of composition and physical properties of the surrounding medium on cell-to-cell communication in *Streptococcus salivarius*

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Antimicrobial resistance has become a major global health threat, highlighting the need for alternative strategies to control pathogenic bacteria. One promising approac the use of commensal bacteria that naturally secrete antimicrobial peptides to inhibit competitors.

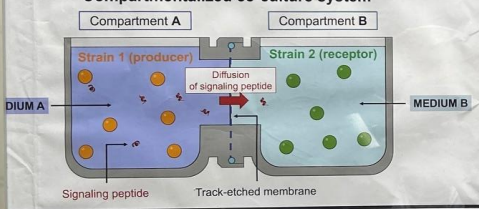
Streptococcus salivarius, the production of such peptides is tightly regulated by **cell-to-cell communication**. This signaling process between cells involves a cas ents: (i) a **producer strain** (**1**) releases a **signaling peptide**, (ii) a **receptor strain** (**2**) senses the signal and activates transcriptional pathways, leading to (iii) the **se antimicrobial molecules** acting on target **pathogens**.

While the genetic and molecular basis of this communication system has been extensively studied, the **influence of the surrounding medium** on signal propagation r orly understood. Physical and chemical properties of the medium such as nutrient composition and diffusional constraints may strongly affect the timing and effici cterial cross-talk.

Here, we investigate how the **composition and physical state of the environment** modulate intercellular signaling between *S. salivarius* strains, with the goal of ide ategies to better control commensal activity in complex settings and ways to control the temporal dynamics of this activity.

EXPERIMENTAL SET-UP

Compartmentalized co-culture system



Procedure: **producer** inoculated first in A; **receptor** added **1.5 h later** to synchronize exponential growth phases.

Reporter sys luminescence (~ bacteriocin

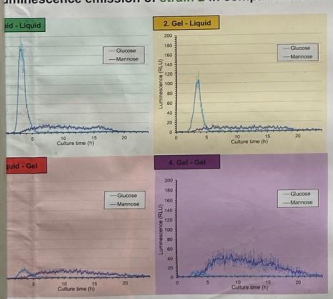
Conditions tested

	Medium A	Medium B
Condition 1	Liquid	Liquid
Condition 2	Gel	Liquid
Condition 3	Liquid	Gel
Condition 4	Gel	Gel

Carbon source = glucose *OR* mannose

RESULTS

Luminescence emission of strain 2 in compartment B

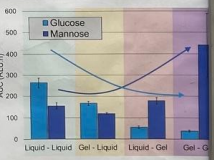


Signal confirmed: luminescence detected in compartment B under all tested conditions → **peptide is produced in A and diffuses across the membrane.**

- **Carbon source effect**:
  - **Glucose** → strong but short response (~4 h).
  - **Mannose** → weaker but long-lasting response (~20 h).
- **Impact of physical environment**:
  - **Liquid–liquid & Gel–liquid** → similar profiles; only a slight decrease of intensity when gel is present in A.
  - **Liquid–gel** → dramatically decreased response with glucose; effect mainly linked to the presence of gel in B.
  - **Gel–gel** → response with glucose almost abolished, but response with mannose **strongly increased** (>2x), suggesting enhanced production/diffusion/activation under these conditions.

→ Carbon source is the dominant factor; gel in **compartment B** strongly limits glucose-induced signaling but unexpectedly boosts mannose-induced response.

Areas under the luminescence cu



- **Area under the curve (AUC)** quantif luminescence over time.
- **With glucose**: AUC decreases from **liquid gel–liquid > liquid–gel > gel–gel**.
- **With mannose**: opposite trend, with AUC i across the same sequence of conditions.

→ The presence of gelled medium, e in **compartment B**, enhances mannose signaling and reduces glucose-induced sig

CONCLUSION

Cell-to-cell communication in *S. salivarius* was successfully reconstituted in a compartmentalized co-culture device: signaling peptide produced in compartment cross the membrane and activates the luminescent reporter (~ bacteriocin secretion process) in compartment B.

Carbon source strongly shapes the cascade response:

- **Glucose** → rapid but short cell activation, highest in liquid–liquid conditions, progressively lost with mechanical properties of the medium increasing.
- **Mannose** → slower but long-lasting cell activation, remarkably **enhanced under gel–gel conditions**.

Physical environment has a net impact on **receptor** response: the presence of gel medium around the receptor (compartment B) drastically reduces glucos signaling but unexpectedly boosts mannose-induced signaling.

Take-home message: both **nutrient composition** and **physical constraints** of the medium modulate bacterial cross-talk. Harnessing these pa could guide the **design of smart commensal systems** with tunable antimicrobial activity and dynamics.