Streptomyces exploration is triggered by fungal interactions and volatile signals

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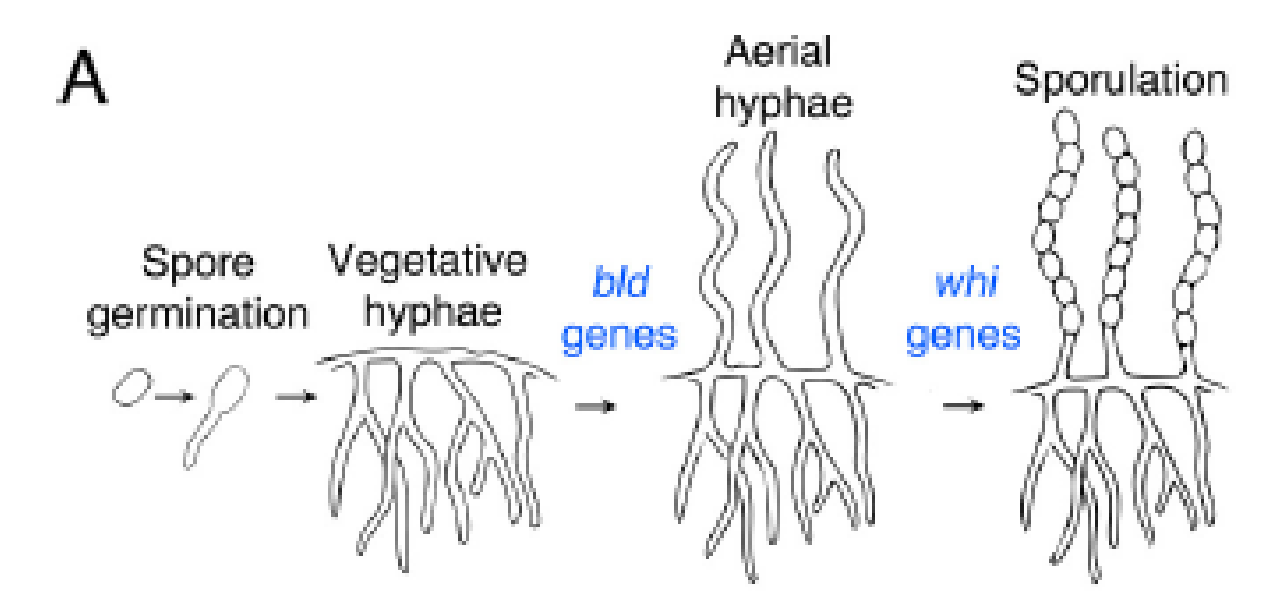
Objective

Understanding microbiol behavior: multi-species cultures unveil new and unexpected microbiol growth strategies.

A novel interaction between *Streptomyces venezuelae* and fungi was identify; that induces a unknown mode of bacterial growth – “exploratory growth”

Life cycle of Streptomyces



Germ tubes emerge from a single spore, and grow by apical tip extension and hyphal branching, In response to unknown signals, non-branching aerial hyphae coated in a **hydrophobic** sheath, escape into the air. Aerial hyphae differentiate into chains of dormant, stress-resistant non-motile spores.

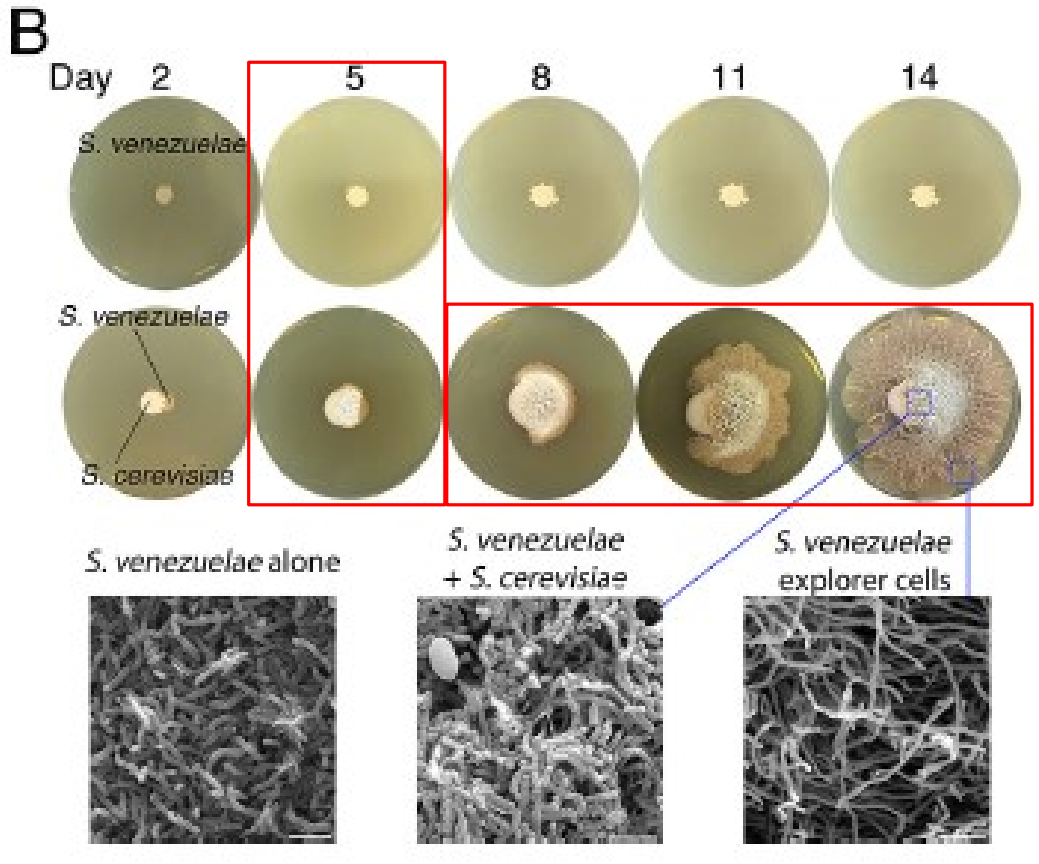
- The **bld gene** products are required for the transition from vegetative growth to aerial hyphae formation.
- The **whi gene** products are required for the differentiation of aerial hyphae into spore chains.

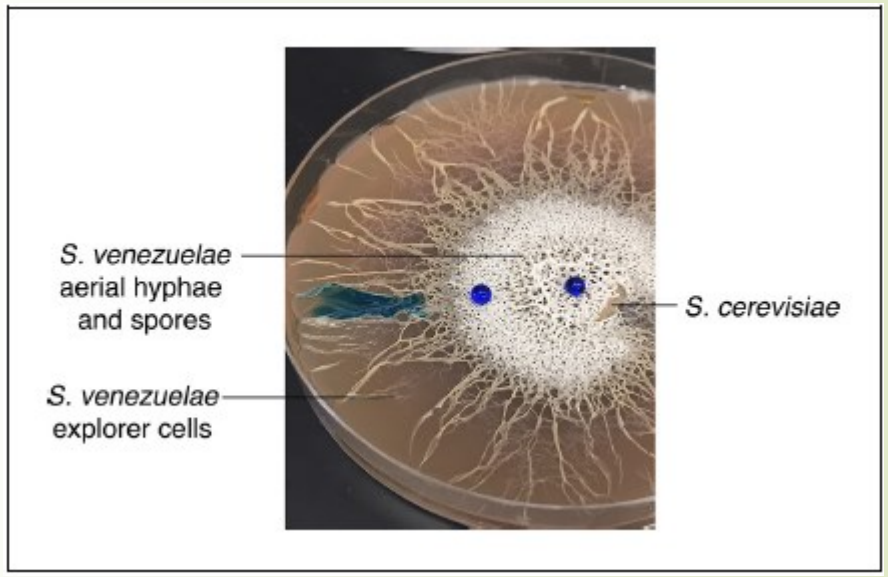
Results

Physical association with yeast stimulates Streptomyces exploratory behaviour

S. venezuelae grown alone

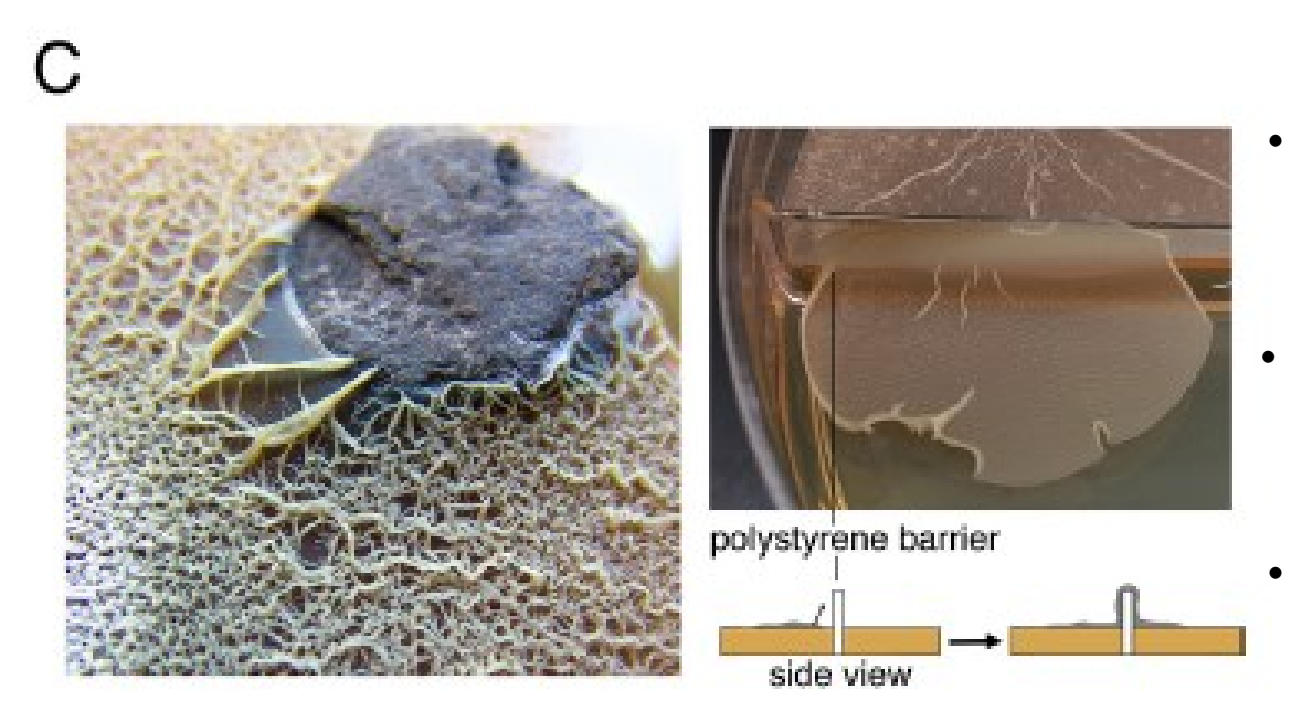
S. Venezuelae beside *S. cerevisiae*





Explorer cells are hydrophilic. S. venezuelae growing on top of S. cerevisiae cells **raise hydrophobic aerial hyphae and spores.**

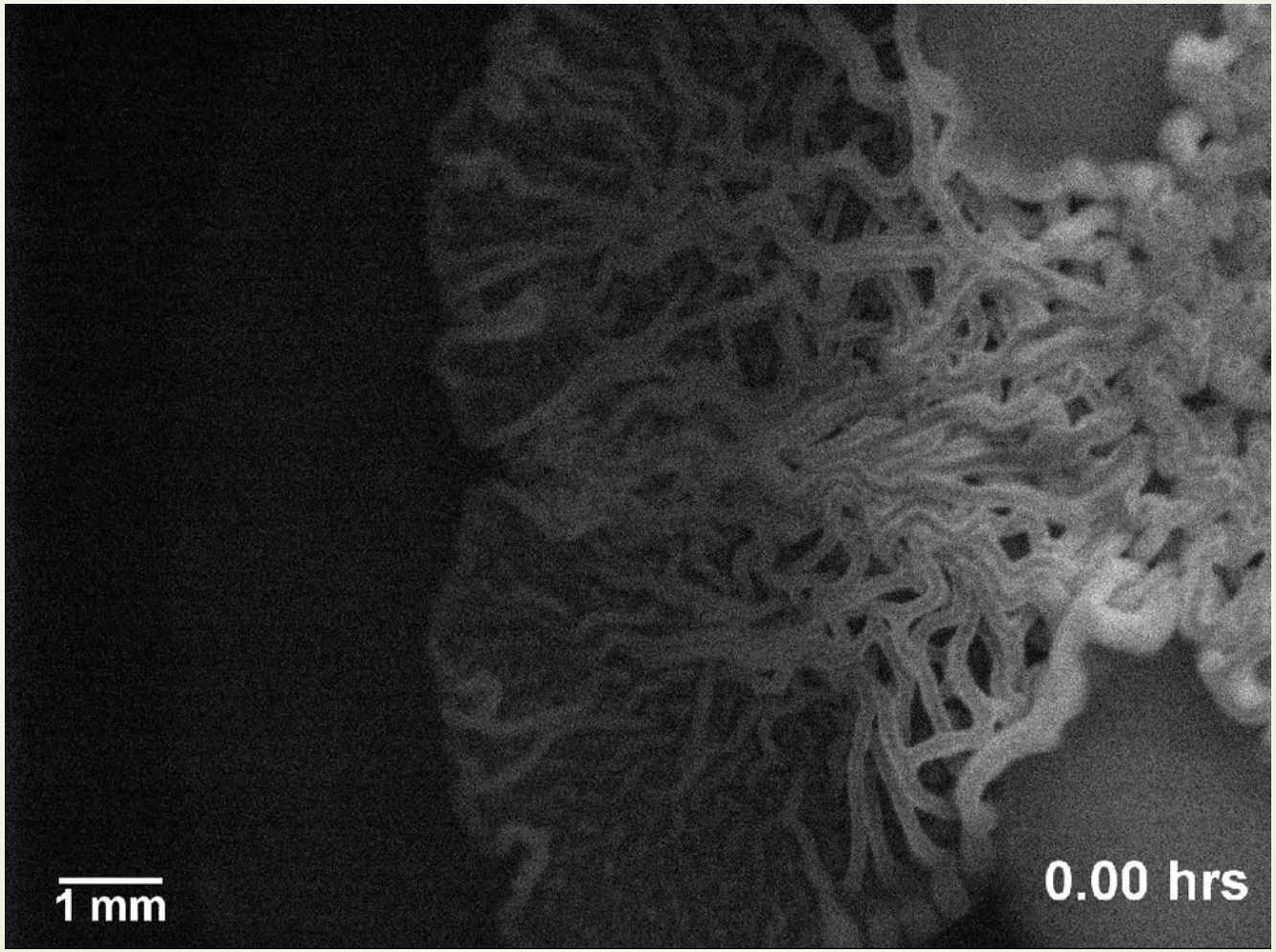
Physical association with yeast stimulates Streptomyces exploratory behaviour



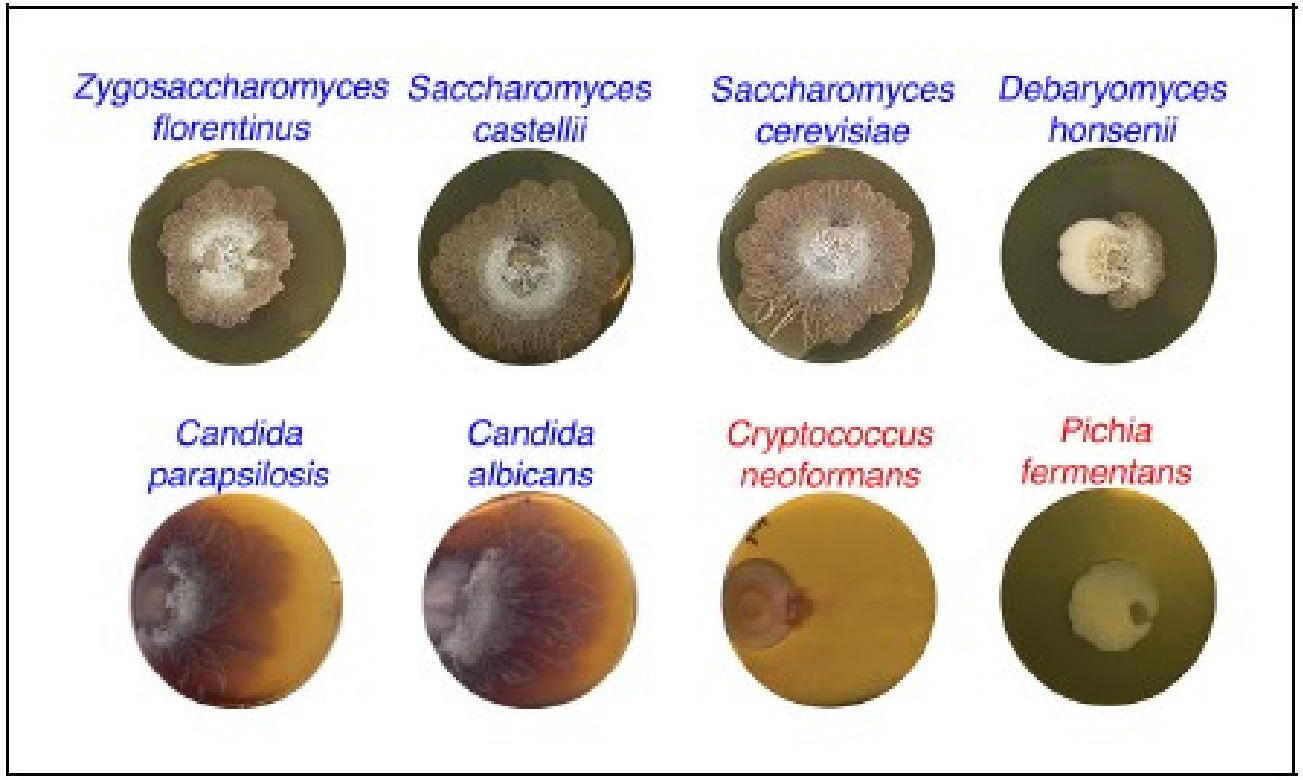
S. venezuelae explorer cells growing up a rock, and over a polystyrene barrier within a divided petri dish

- 'exploratory growth' - ability to effectively transverse both biotic and abiotic surfaces
- *S. venezuelae* growing on *S. cerevisiae* raised aerial hyphae and sporulated.
- Explorer cells revealed failed to branch these filaments were hydrophilic.

Physical association with yeast stimulates Streptomyces exploratory behaviour



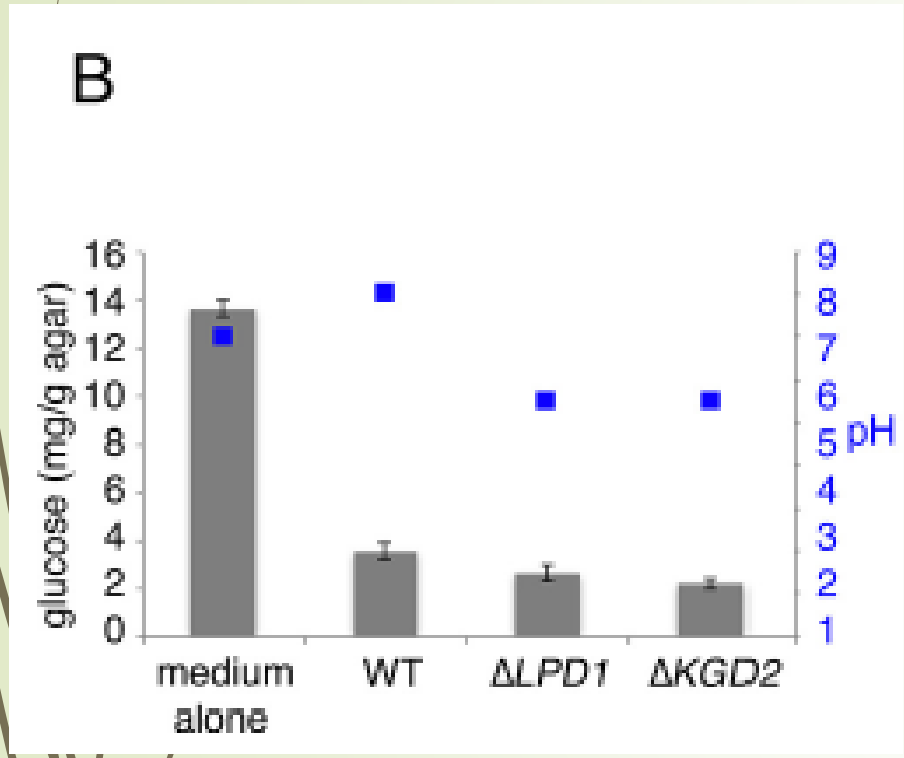
Physical association with yeast stimulates Streptomyces exploratory behaviour



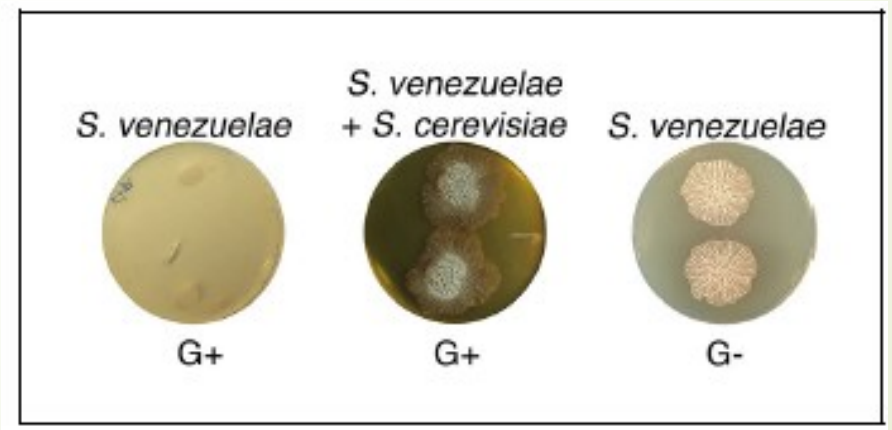
We observed that all species, apart from *C. neoformans* and *P. fermentans*, induced *S. venezuelae* exploratory behaviour. This indicated that a broad range of microbial fungi could trigger exploratory growth.

Exploration is glucose-repressible and pH-dependent

- glucose-repressible



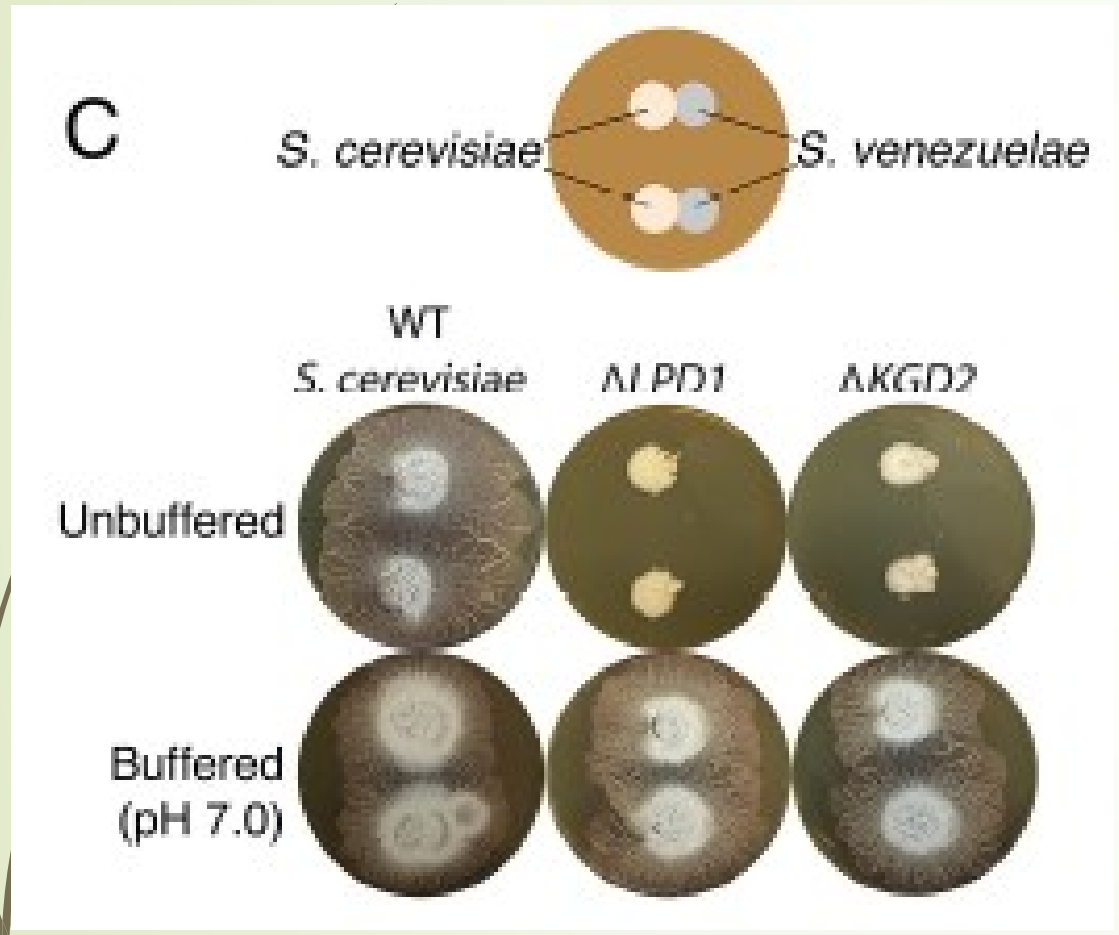
Wild type, D*LPD1* and D*KGD2* *S. cerevisiae* strains consumed similar levels of glucose, suggesting that other factors must be inhibiting *S. venezuelae* exploration when grown adjacent to these TCA cycle mutants.



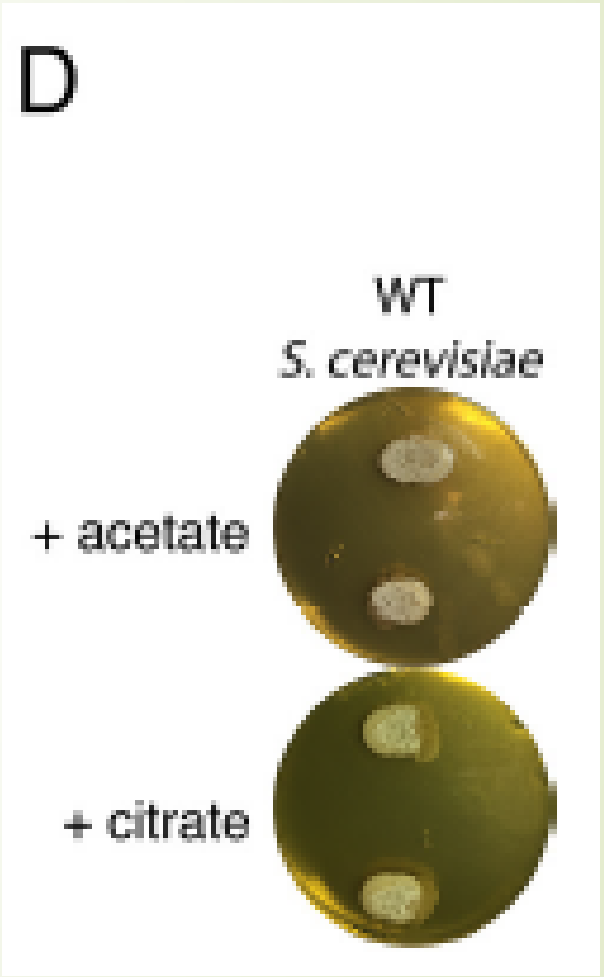
Glucose repressed exploratory growth.

Exploration is glucose-repressible and pH-dependent

- pH-dependent

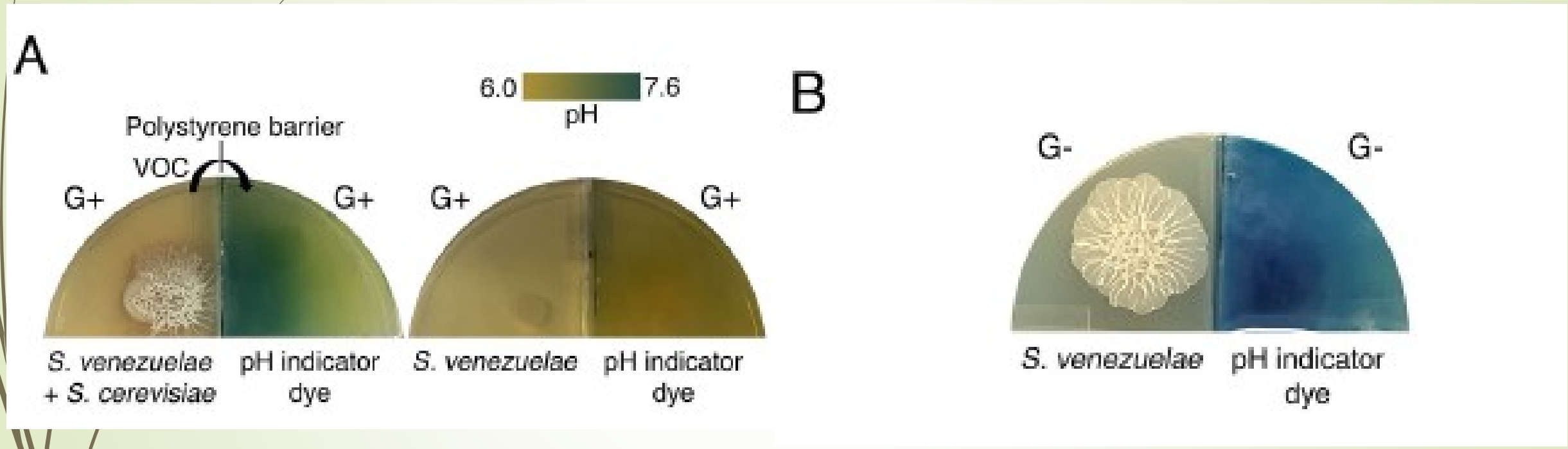


Exploration is pH dependente



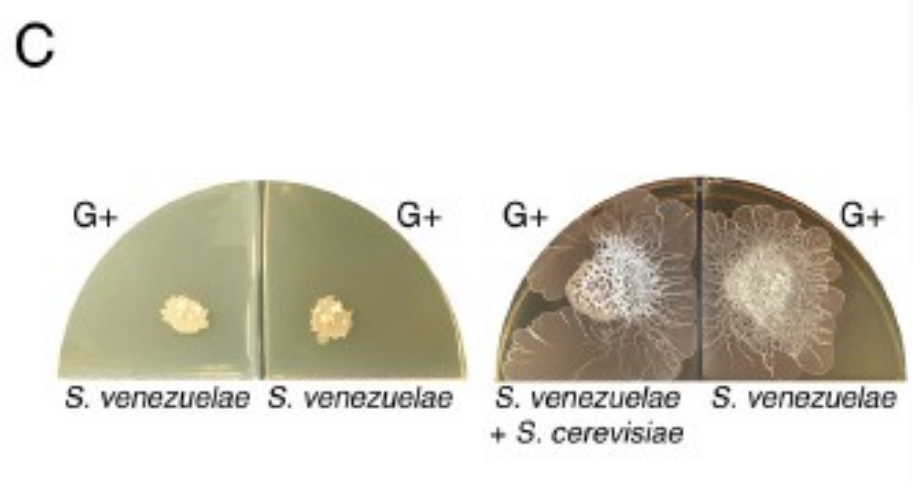
Secreted acids inhibited *S. venezuelae* exploration.

S. venezuelae explorer cells alkalinize the medium using an airborne volatile organic compound

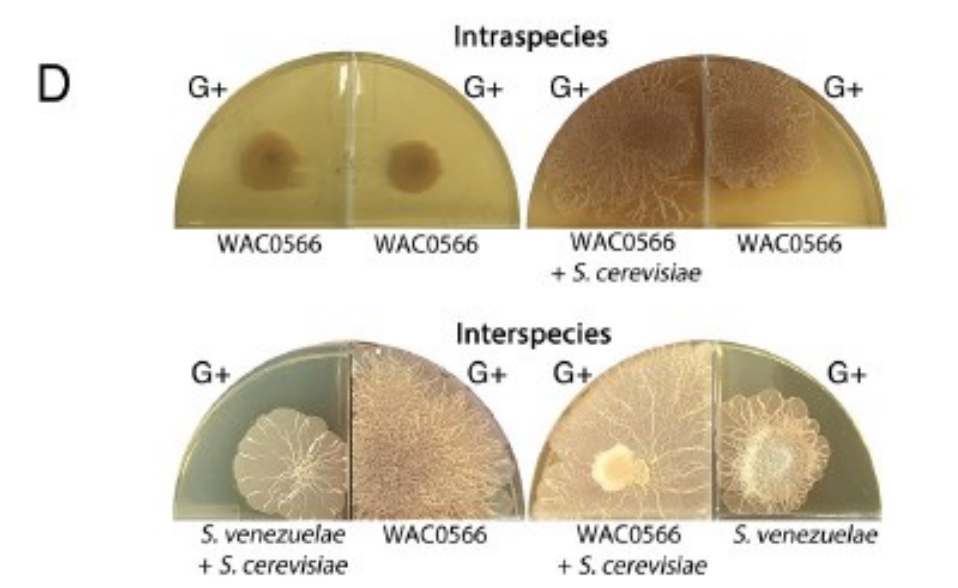


Volatile organic compounds released by *S. venezuelae* raise the medium pH and induce exploratory growth in physically separated Streptomyces.

S. venezuelae exploratory cells use VOCs to induce exploration in other streptomycetes at a distance



These data implied that *S. venezuelae* explorer cells released a VOC that effectively promoted exploratory growth in distantly located *S. venezuelae* cells.



Exploratory growth could be communicated to unrelated streptomycetes.

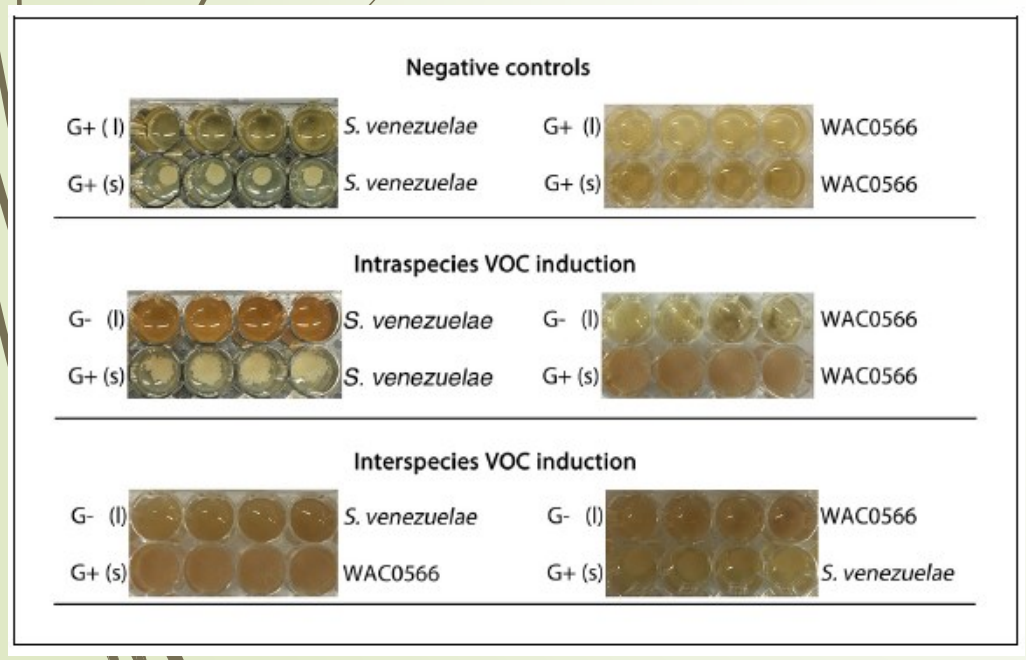
The VOC trimethylamine stimulates Streptomyces exploratory behaviour

S. venezuelae and WAC0566 in G+ and G- liquid culture for three days

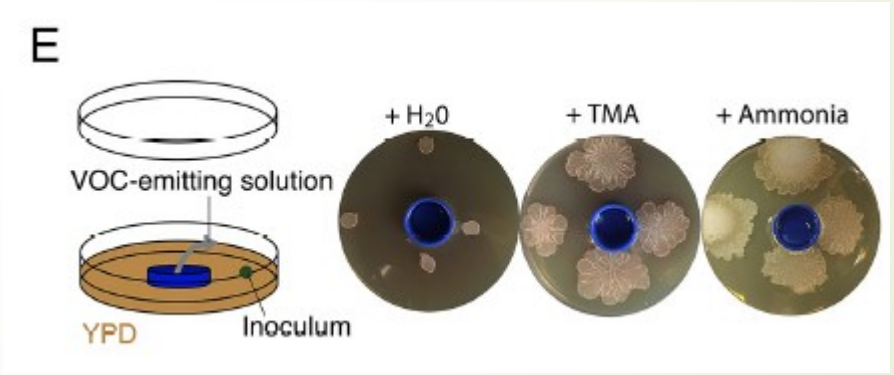
Gas chromatography time-of-flight mass spectrometry

1400 unique compounds were identified.

12 were not detected in the negative controls

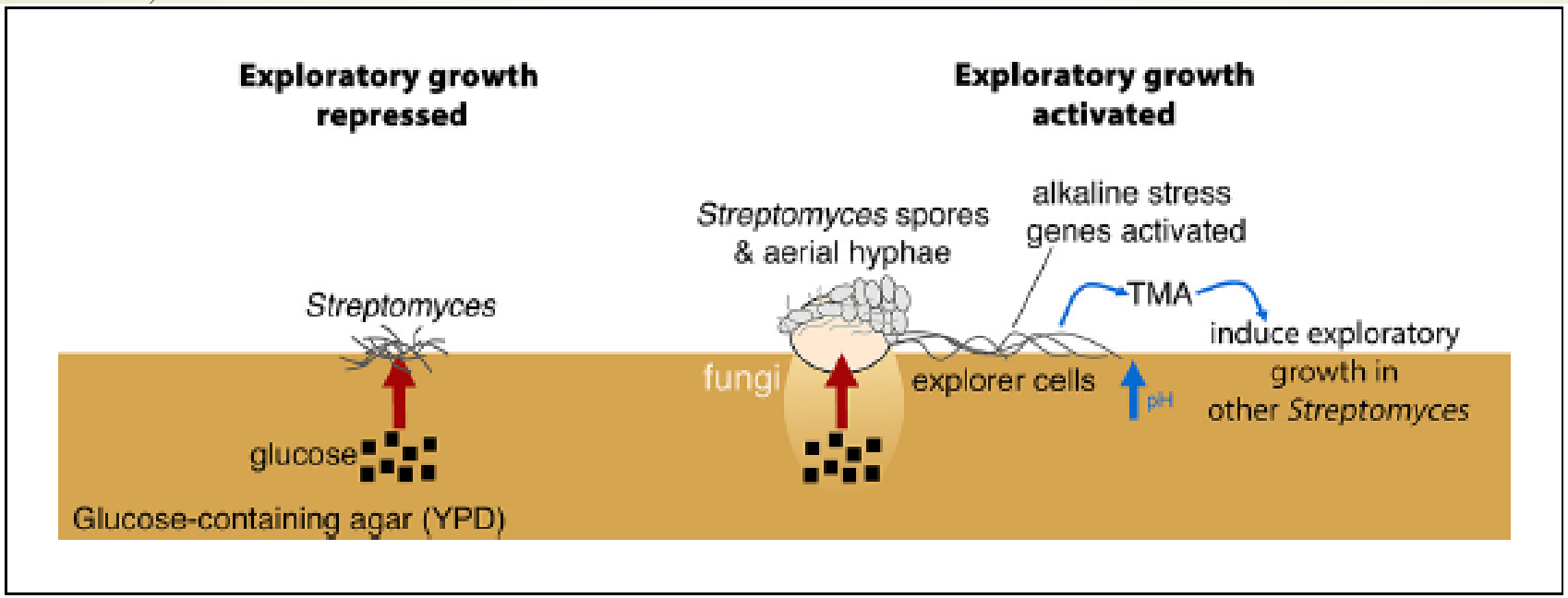


Trimethylamine (TMA) was >10 fold more abundant than the other



TMA production is not well understood.

Ecological implications for exploratory growth within microbial communities



New model for Streptomyces development.

Conclusions

A new developmental behavior for *Streptomyces* that provides them with an alternative means of colonizing new habitats.

In response: to fungal neighbours and nutrient (glucose) depletion, *Streptomyces*

Explorer cells are not limited by inanimate barriers, and can grow over abiotic surfaces.

Explorer cells alter their local environment through the release of the alkaline, volatile compound TMA.

Emitting TMA not only promotes exploratory behaviour by the producing cells, it also functions as an airborne signal that elicits an exploratory response in physically distant streptomycetes, and provides further fitness benefits by inhibiting the growth of other bacteria.

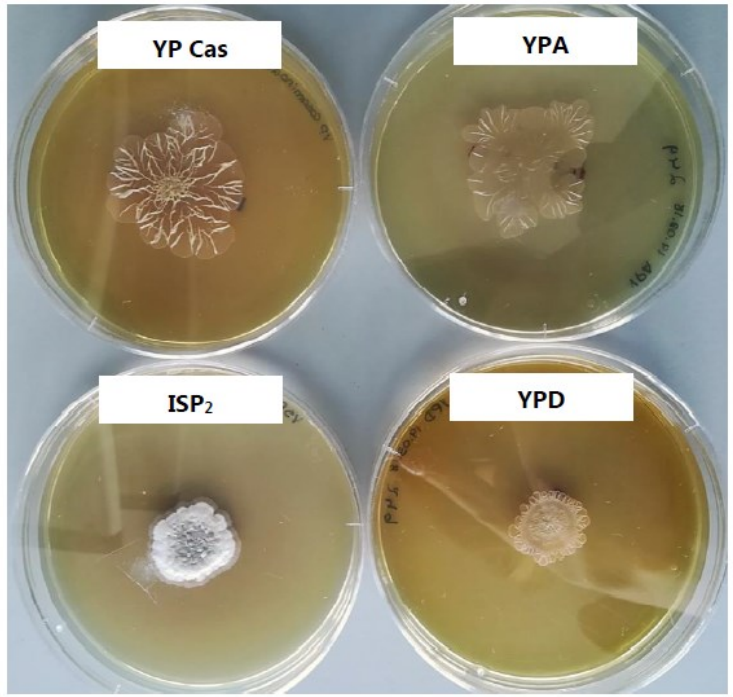


Figura S1. Crescimento e morfologia do *Streptomyces* (Z49) influenciado pelos diferentes meios.

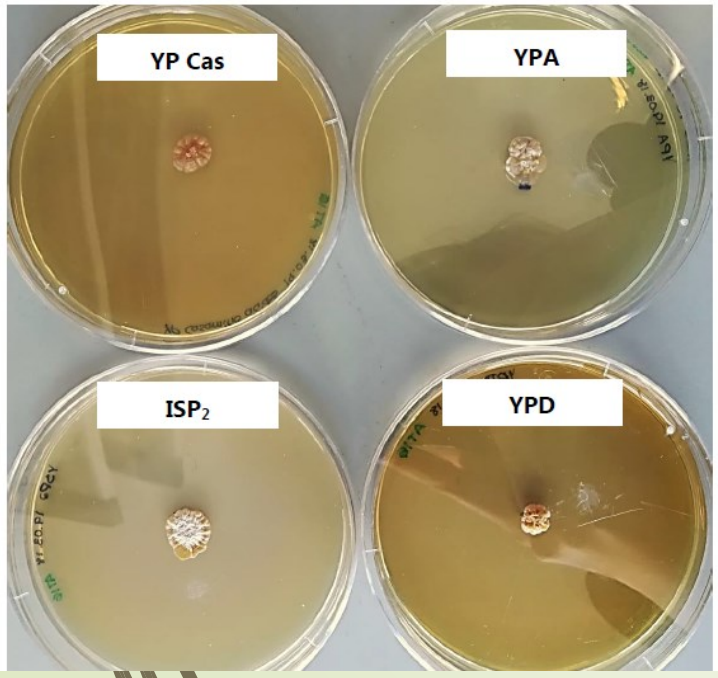


Figura S3. Crescimento e morfologia do *Streptomyces* (AT16) influenciado pelos diferentes meios.

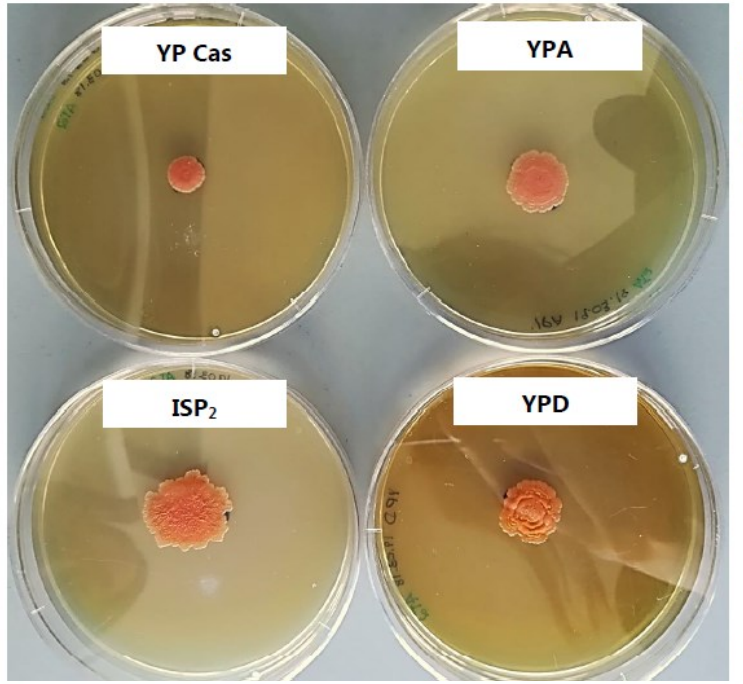


Figura S2. Crescimento e morfologia do *Streptomyces* (AT2) influenciado pelos diferentes meios.

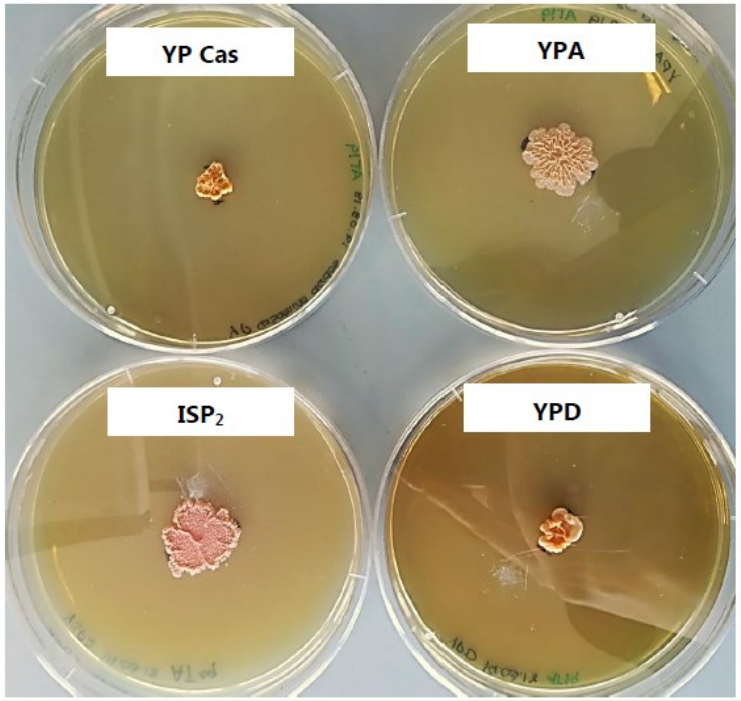
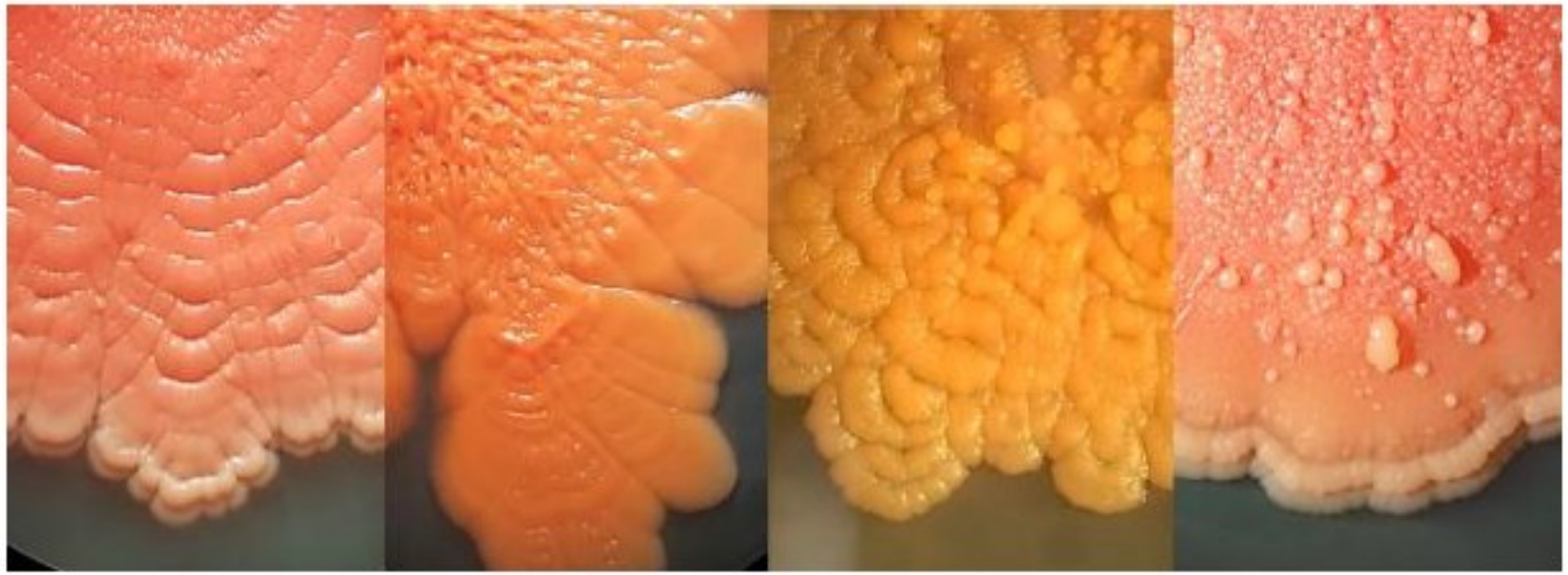


Figura S4. Crescimento e morfologia do *Streptomyces* (AT9) influenciado pelos diferentes meios.



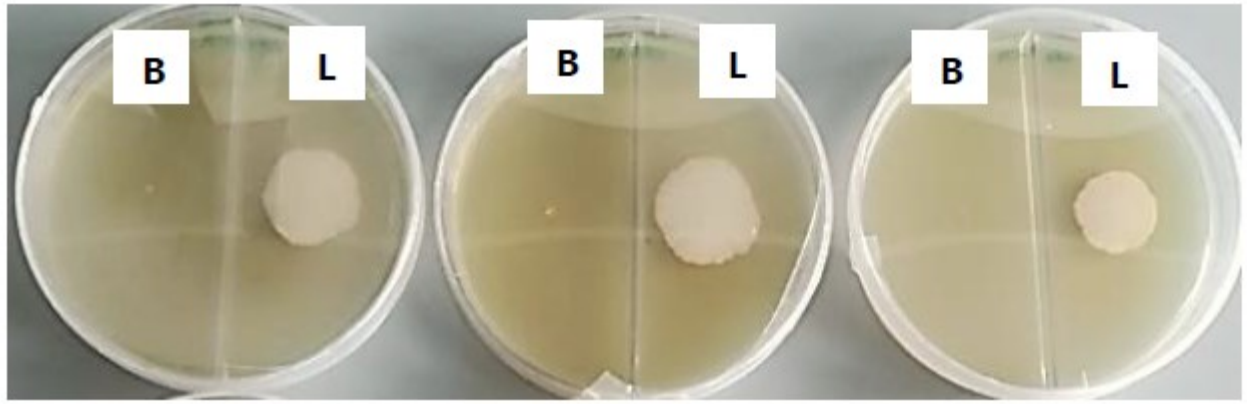


Figura S6. Crescimento influenciado pela interação volátil entre *Streptomyces* AT19 e *S.cerevisiae* em ISP_2.

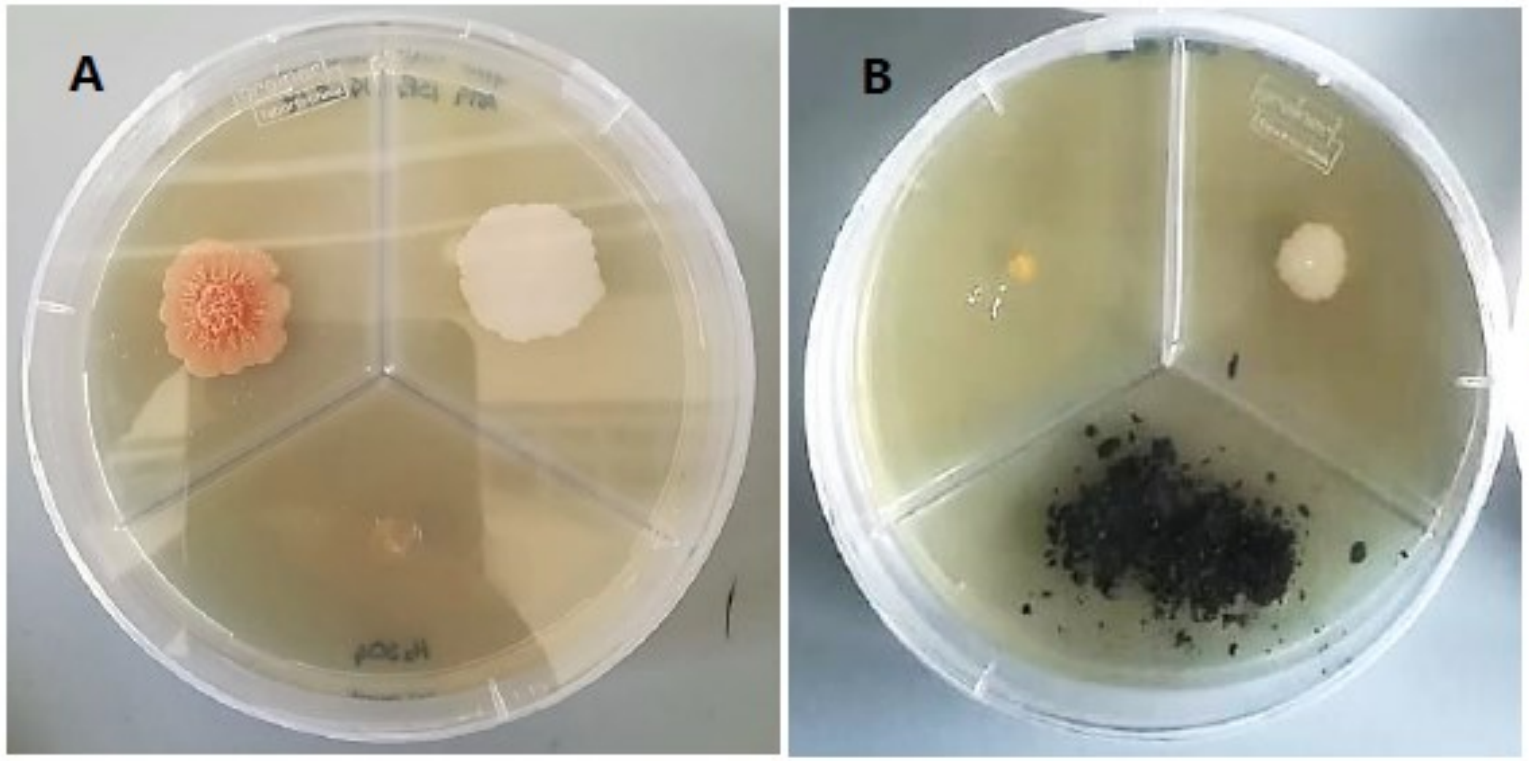


Figura S7. Crescimento influenciado pela interação volátil entre *Streptomyces* AT19 e *S.cerevisiae* em ISP_2 com disco de amónio (A) e carvão ativado (B).

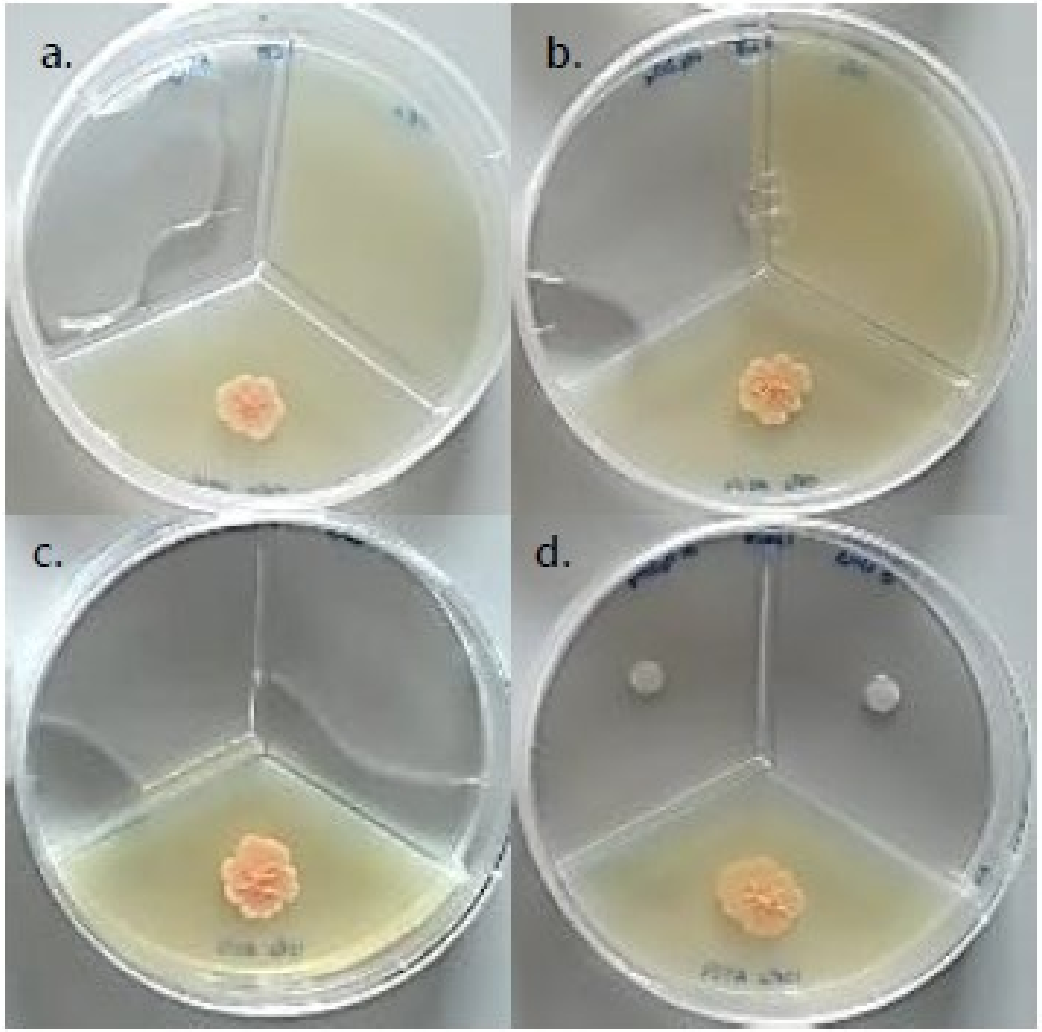


Figura 12. Resultados do crescimento de AT19, influenciado pela amónia em ISP_2. Resultados dos ensaios da Figura 5 e 6: a. NH_4^+ líquido; b. H_2SO_4 liquido; c. H_2SO_4 e NH_4^+ líquidos; d. discos de H_2SO_4 e NH_4^+ .

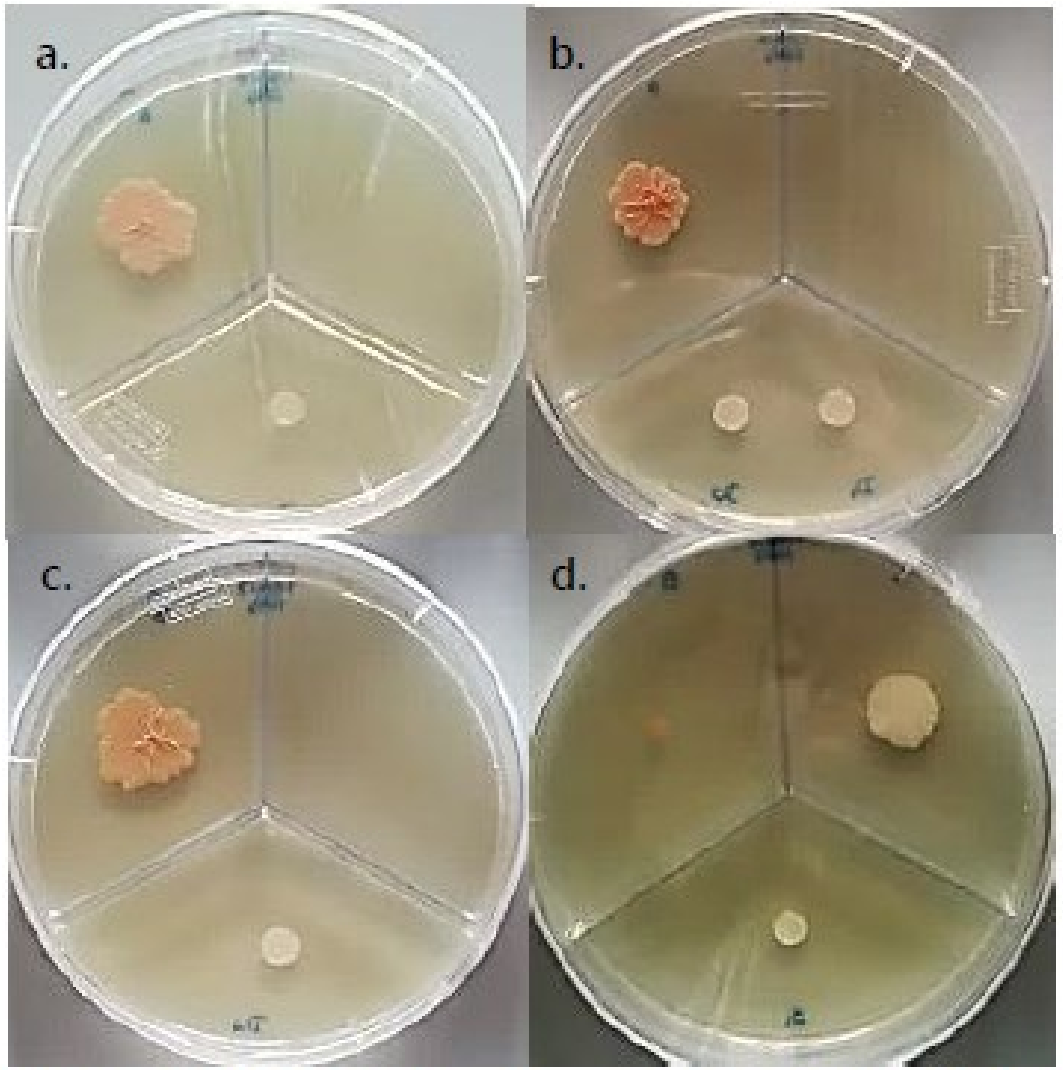


Figura 13. Resultados do crescimento de AT19, influenciado por voláteis em ISP_2. Resultados dos ensaios da Figura 6: a. D1; b. D1 + D2; c. D2; d. S1.

D1 - fonte de amónia; D2 - fonte de óxido nítrico; S1 - *scavenger* H2SO4.

https://www.youtube.com/watch?v=yWOqeyPlVRo

https://www.youtube.com/watch?v=PMmX1iHLs5Y

https://www.youtube.com/watch?v=uukhM3-feQk