Eliciting production of antimicrobial metabolites in *Streptomyces* spp. environmental isolates

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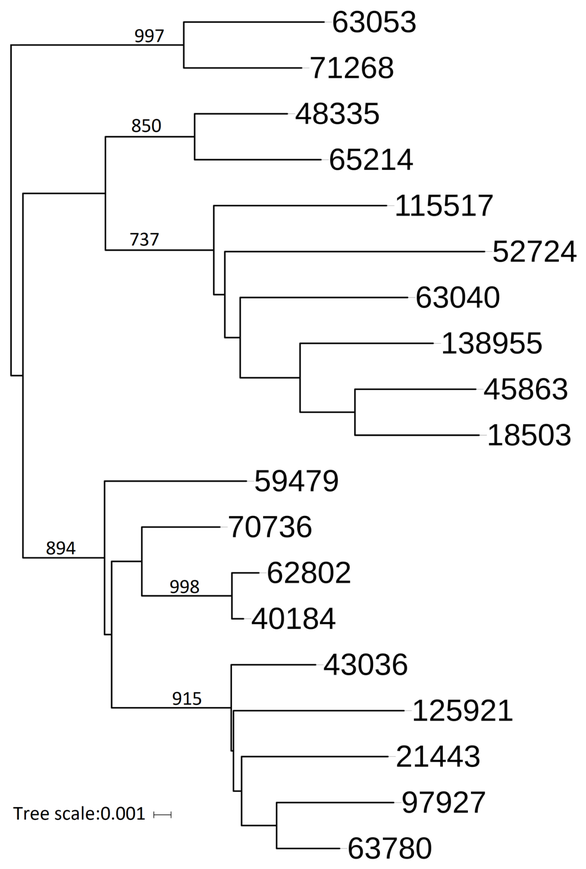
Abstract

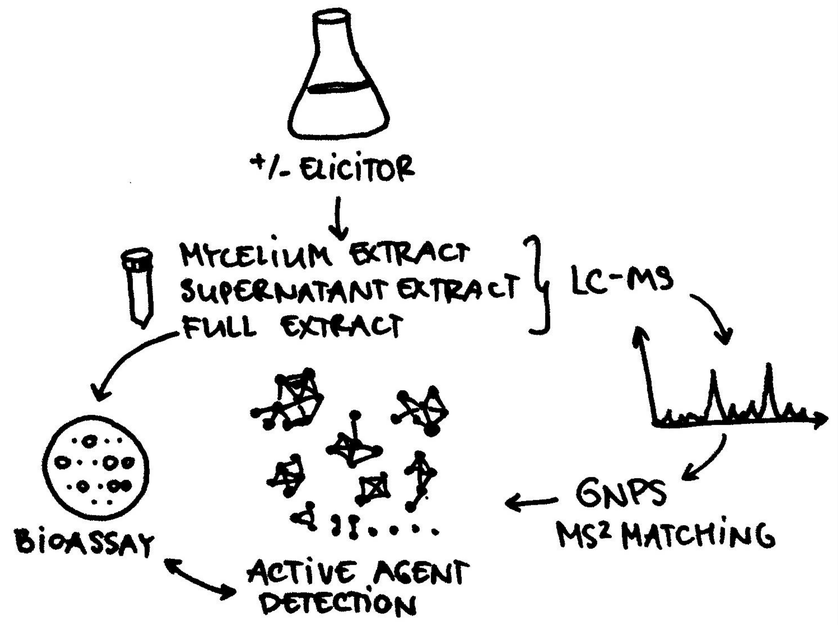
Streptomycetes are well known for their ability to produce bioactive metabolites, used widely in human and animal medicine. Yet, many of the biosynthetic gene clusters they harbor in their genomes remain silent under standard laboratory conditions. We explored the metabolic diversity of 21 randomly picked *Streptomyces* strains from Naicons' collection of 45000 actinomycete environmental isolates. We ventured to enhance the production of specialized metabolites by adding elicitors into the growth medium. As elicitors we used substances that could carry a biological meaning and therefore trigger a reaction that could be seen looking at the metabolic profiles. As a result, we established three strategies for strain prioritization.

Strain Diversity



Neighbor-Joining tree based on 16S phylogeny showing the placement of the investigated strains. Two strains with incomplete sequences were omitted. Bootstrap values (1000 replicates) >70% are shown at nodes. Scale bar: 1 inferred substitution per 1000 nt. *P.rosea* used as the outgroup (not shown). Collection sites of strains used in this study are indicated on the map (left).

Experimental approach

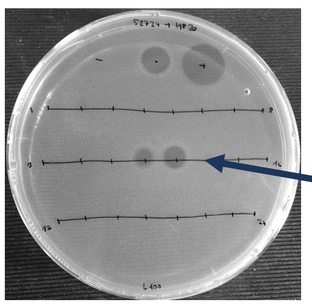


- 21 randomly chosen strains
- 20 cultivation conditions:
  - 12 antibiotics in sub-inhibitory concentrations
  - 2 iron chelating agents (natural and synthetic)
  - 3 lactone-containing entities
  - N-acetylglucosamine
  - HP20 resin
  - control
- 1500 extracts
- Bioassay vs *S. aureus*, *M. luteus* and ΔtolC *E. coli*

Three strategies for strain prioritization

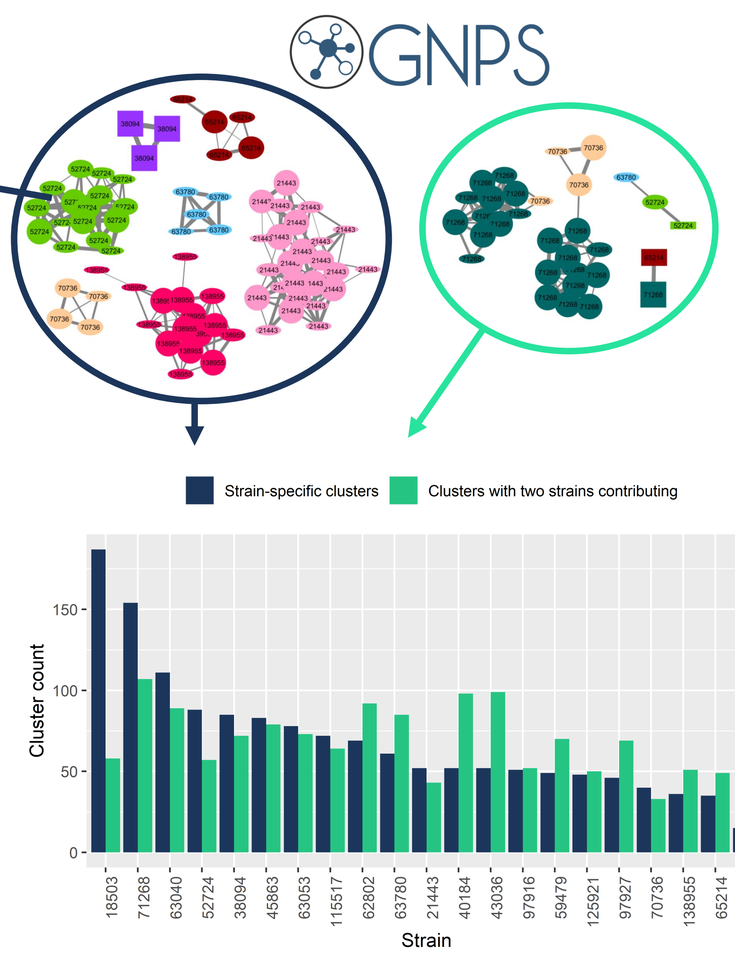
1. Bioactivity + novelty

This method is combining information about extract bioactivity with the novelty of the compound regardless of the presence of elicitors. As the strains are unexplored, they possess the potential of expressing novel metabolites at standard laboratory conditions.



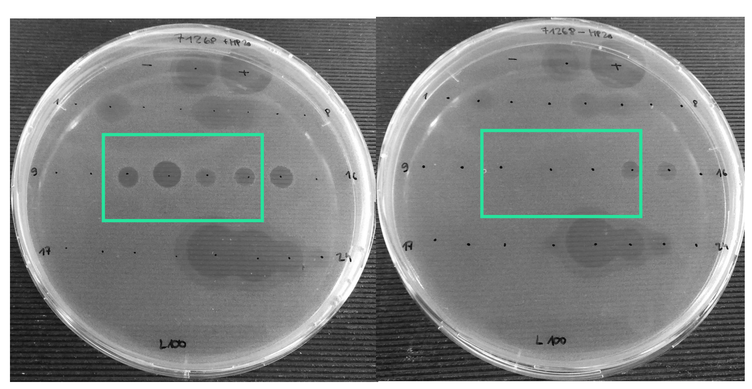
Fractionated extract of strain 52724 harbors a novel *S.aureus*-inhibiting compound regardless of presence of chosen elicitors.

2. Molecular Networking: Rarity



In rarity-based prioritization, the metabolites of interest are not present in the Naicons extract library and they do not have a database match. GNPS[1] serves as a valuable tool for metabolite dereplication. The bioactivity of observed compounds is not known. This untargeted approach is valuable for pinning down potentially new chemistry. Strains that produce the largest number of strain-specific metabolites are prioritized for further work, such as upscaling and metabolite purification.

3. Difference in bioactivity +/- elicitor



Extract fractionations reveal multiple bioactive compounds present in the same extract, containing both known and unknown active agents. Strain 71268 exhibits a clear difference in bioactivity vs *S. aureus* when grown in the presence or absence of adsorbing resin HP20. Preliminary results indicate that even though a number of cases of changed bioactivity were observed in the screening with elicitors, the number of reproducible cases is low.

Conclusions

The key challenge in our research is to pin down the most promising metabolites in the abundance of signals. All three methods have yielded interesting cases to follow up. Upscaling, purification and structure determination of one of the compounds is ongoing. Full genome sequencing of all the strains is in progress to combine the observed metabolic capabilities with corresponding gene clusters.

Acknowledgements

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 765147. Kristiina Vind has received a Research and Training Grant from FEMS. We thank Jos Hagemans for help with R.

References

[1]Mingxun Wang, Jeremy J Carver, Vanessa V Phelan, Laura M Sanchez, Neha Garg, Yao Peng, et al. "Sharing and community curation of mass spectrometry data with Global Natural Products Social Molecular Networking" Nature Biotechnology 34.8 (2016): 828-837. PMID: 27504778