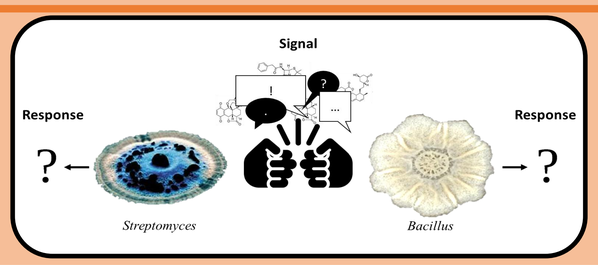
Bacterial Interactions : Influence on Growth and Secondary Metabolism

Déborah Tellatin[1], Augustin Rigolet[2], Pierre Burguet[3], Loïc Martinet[4], Nudžejma Štulanović[1], Loïc Quinton[3], Marc Ongena[2], Sébastien Rigali[1,4]

E-mails: deborah.tellatin@uliege.be ; srigali@uliege.be

Introduction

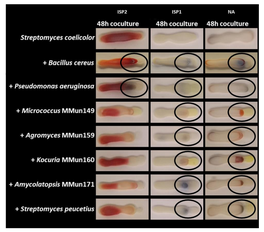
In their natural environment, microbes are living in diverse associations with other organisms and are thus influenced by each other [1]. The microbial cross-talk involves small molecules called “natural products” (NPs), which possess diverse chemical structures and bioactivities [2]. During conventional culture conditions with axenic strains, a large number of these NPs are not expressed, they are defined as cryptic NPs [3]. To trigger the production of these cryptic NPs, the strategy of co-cultivation, which attempt to mimic the ecological situations where those microbes co-exist and interact, shows its importance [1,4,5].



Project purpose

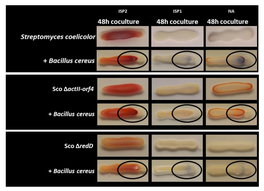
In this project which focuses on bacterial interactions, mainly using *Streptomyces* and *Bacillus* bacterial genera, we are particularly interested in the nature of the stimulus and how it is perceived through the answer and elucidation of the following questions: i) What are the culture conditions that allow the observed response, and, ultimately, what is the signal responsible for these changes, ii) How each bacterium respond to the interaction, in other words, what are the cryptic induced molecules, and finally, in the last case study, iii) Who are these two bacteria that need each other for survival.

Streptomyces coelicolor vs. Bacteria



Observations

S. coelicolor showed a different response to bacterial genera found in the same ecosystem by producing the red-pigmented prodiginines (PdGs) and the blue-pigmented actinorhodin (Act) metabolites. In addition, the response was dependent on the medium used for coculture.

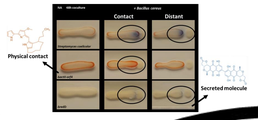


Induced molecules

To confirm our hypothesis of PdGs and Act production during interaction we used mutants of *S. coelicolor* unable to produce these molecules. Both metabolites were induced in interaction. Interestingly, we observed that Act was rather red than blue in ISP2 medium.

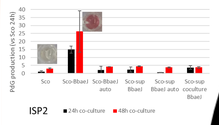
Signal identification

With contact or distant bacterial interactions, we observed that PdGs induction is contact-dependent, while Act is induced by diffusible molecules.

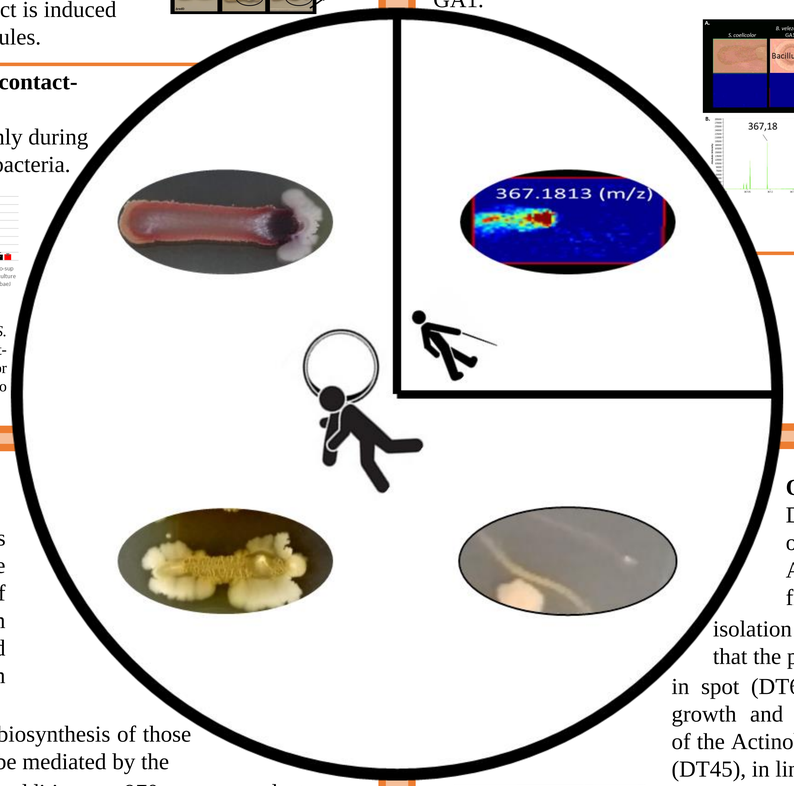


PdGs induction is contact-dependent

PdGs are induced only during coculture with live bacteria.

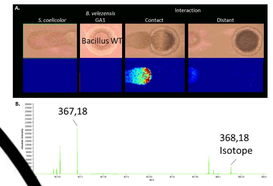


Liquid cocultures of *S. coelicolor* with live or heat-inactivated bacteria or supernatants were performed to support our hypothesis.

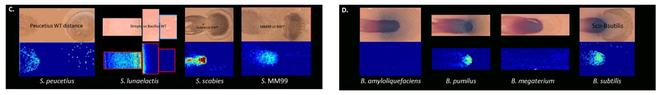


Observations

Analysis with IMS showed the induction of an ion peak at m/z 367.1806, produced in intracellular by *S. coelicolor*, only during interaction with *B. velezensis* GA1.



Streptomyces sp. *vs. Bacillus subtilis*-like



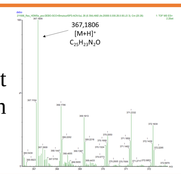
Generality

Surprisingly, the metabolite induction was observed in each *Streptomyces* species tested in coculture with *B. velezensis* GA1 and on different media, indicating its generality in the genus *Streptomyces*.

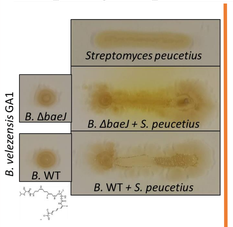
However, the production is mediated only when interacting with *B. velezensis*, *B. pumilus* and *B. subtilis*, indicating the specificity of the inducers to the *B. subtilis* group.

Metabolite identification

The crude acetonitrile extract containing the polar metabolite at m/z 367.1806, was analyzed in LC-MS² with a HILLIC column and we are now working on its structural elucidation.



Streptomyces peucetius vs. Bacillus velezensis GA1



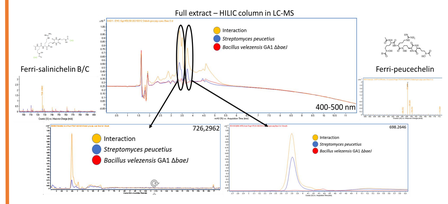
Observations

Yellow diffusible metabolites are induced, only in the absence of bacillaene, during the coculture between *S. peucetius* and *B. velezensis* GA1 on R2YE medium.

Induced molecules

Metabolites that absorb at 400-500 nm are overproduced during the interaction compared to *S. peucetius* cultivated alone.

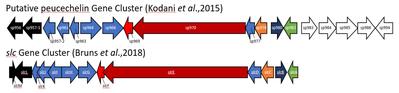
Masses associated with the two main peaks correspond to siderophores peucechelin and salinichelins complexed to iron.



Analyses: The LC-MS profile analyses with an HILIC column were performed with crude ethanol extracts of *S. peucetius* and *B. velezensis* GA1 Δ*baeJ* grown alone or in interaction on R2YE agar plates.

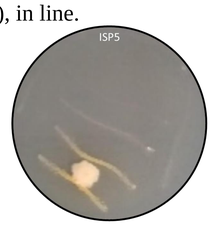
Genome mining

Comparative analysis of BGCs involved in the production of peucechelin from *S. peucetius* and salinichelins from *Salinispora spp.* suggests that the biosynthesis of those molecules might be mediated by the same BGC. In addition, sp970 gene products are siderophore ions at m/z 645.4, 659.4, 673.4, and 687.4 [6], which are actually masses of peucechelin, salinichelin A, salinichelin B/C, and salinichelin D/E, respectively.

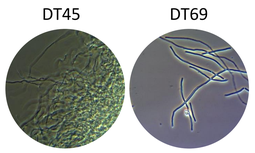


Observations

During the generation of a collection of Actinobacteria isolated from hive elements, the isolation steps on ISP5 showed that the presence of the bacteria in spot (DT69) was necessary for growth and metabolite production of the Actinobacteria-like bacterium (DT45), in line.



Mutually beneficial interaction from hive elements

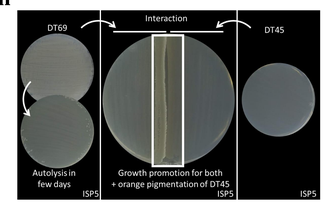


Bacterial identification

Nevertheless, we were able to obtain axenic cultures and genomic DNA of filamentous DT45 and DT69 strains on MHB medium.
Sequencing in progress.

Mutually beneficial interaction

Not only DT45 but also DT69 showed growth disturbances when cultivated alone on ISP5, while their coculture induced mutually beneficial growth and pigmented metabolite production for DT45 strain.



Conclusion

With these four case studies, and even if we are at different steps of elucidation for each interaction, we demonstrate that bacterial coculture is a powerful strategy to trigger the induction of cryptic NPs. In addition, combining bacterial interactions with the OSMAC approach using different culture conditions can also be complementary.

[1] InBioS, Centre for Protein Engineering, Institut de Chimie B6a, University of Liège, Liège, Belgium ;
[2] Terra Teaching and Research Centre, MIPI laboratory, University of Liège, Gembloux, Belgium ;
[3] MolSys Research Unit, Mass Spectrometry Laboratory, University of Liège, Liège, Belgium ;
[4] Hedera22, Liège, Belgium

References:
1. Marmann *et al.*, 2014. *Mar. Drugs.* doi:10.3390/md12021043.
2. Katz and Baltz, 2016. *J. Ind. Microbiol. Biotechnol.* doi:10.1007/s10295-015-1723-5.
3. Ziemert, Alanjary and Weber, 2016. *Nat. Prod. Rep.* doi:10.1039/c6np00025h.
4. Bertrand *et al.*, 2014. *Biotechnol. Adv.*
5. Ueda and Beppu, 2017. *Nat. Publ. Gr.* doi:10.1038/ja.2016.127.
6. Park *et al.*, 2013. *Appl. Microbiol. Biotechnol.* doi:10.1007/s00253-012-4268-9.
7. Kodani *et al.*, 2015. *BioMetals*. doi:10.1007/s10534-015-9866-4.
8. Bruns *et al.*, 2018. *ISME J.* doi:10.1038/ismej.2017.137.