Volatile compounds from Actinobacteria as mediators of microbial interactions

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SUMMARY

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RESÚMEN

Microorganisms live in a complex soil habitat participating in intricate and dynamic interactions. They compete for nutrients or space, therefore, the production of compounds with antibiotic activity results in a convenient trait to have. Molecules with antibiotic activity have a wide range of structures and these include volatile compounds (VCs); the latter have a low molecular weight and low vapour pressure that allows them to evaporate and diffuse through different surfaces like the soil pores, water and through the air. During this PhD work the diversity and functions of volatile compounds were studied. *Streptomyces* are one of the most abundant genus of bacteria in soil, and are the main producers of bioactive compounds, including volatile compounds. This work shows how even very small volatile molecules such as ammonia can act as antibiotics when produced in high amounts, by modifying the surrounding environment. Ammonia is derived from the metabolism of amino acids, in particular from glycine which can be regarded as an abundant molecule in soil as exudate from plants. The production of a bioactive compound would certainly trigger a response by the perceiving bacteria. We observed that *Escherichia coli* responds to the high concentrations of ammonia and other VCs by down-regulating its major outer membrane porins in order to reduce the amount of toxic compounds that enter the cell.

Streptomyces are excellent producers of terpenoid compounds dominating the headspace. Two of these molecules have been known for decades namely geosmin and 2-methylisoborneol, nevertheless their function remains unknown. The volatile character of such molecules could suggest that they participate in “long-distance” interactions. A role in communication has been suggested from plant terpenes; however, only a few examples are known in bacteria where terpenes play a role in interspecies communication.

The high-level production of terpenes under different growth conditions raised the question of their role as intra- and/or interspecies signals. The work in this thesis shows that these molecules are not vital for the growth and development of *Streptomyces*, but instead affect the development when grown under conditions of high osmolarity. The mutants unable to produce terpenes, in particular those lacking geosmin

and 2-methylisoborneol synthase were affected, and the typical mycelial pellet formation was altered, resulting in more open and fragmented mycelia.

VCs have also been suggested as waste products or as a carbon release valve. However, when the headspace of the mutant unable to produce any volatile terpene compound was analysed, almost no VCs were identified. The regulatory mechanism of these molecules is barely known, which makes it more important to continue the study of VCs in order to better understand their biosynthesis, regulatory mechanisms and more importantly, their function. This study sheds some light by showing strong upregulation of sulfide compounds when the major terpenes 2-methylisoborneol, 2-methylenebornane and 2-methyl-2-bornene were not produced anymore. This result suggests a possible link between these completely different and unrelated pathways, which had not been seen before. We hypothesize that the methyltransferase that is encoded by the gene cluster that is responsible for the production of the 2-methylisoborneol, may also participate in the methylation of sulfides, thus giving rise to dimethyl disulfide and dimethyl-trisulfide.

Several volatile compounds have been shown to have antimicrobial activity, and the upregulation of sulfides correlates with the higher antifungal activity shown by the mutant missing the 2-methylisoborneol synthase. The antifungal effect of dimethyl disulfide and dimethyl trisulfide is well known, thus confirming the results obtained in this work. The antimicrobial effect of VCs was also observed against protists known to be the main bacterial predators. VCs released by *Streptomyces* induced the formation of cysts in protists, particularly when a mix of VCs was present in the headspace. Geosmin, 2-methylenebornane and dimethyl disulfide were tested as pure compounds and showed an inhibitory effect in the activity of protists by the higher number of cysts. The effect correlated with in vivo anti-protist activity, and we believe that the effects of molecules produced in a blend could be cumulative. The anti-protist activity of VCs produced by bacteria is a novel concept and there is yet little evidence in the literature; still, it makes sense in the ecological context, considering that bacteria are the

main prey of protists. This idea could also be a very good reason for bacteria to produce VCs like terpenes in high amounts.

In conclusion, the work presented in this thesis shows the potential of VCs as antimicrobials. More effort needs to be put in understanding the role of VCs in microbial interactions and to elucidate the biosynthetic pathway and genetic control of this important class of natural products, which may well find their way towards clinical or agricultural application in the future.