Volatile compounds from Actinobacteria as mediators of microbial interactions

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CHAPTER 1

General introduction and thesis outline

Smell is the most chemically focused of our senses. It is the result of small molecules traveling through the air, passing up through the nasal cavity and reaching the olfactory epithelium where they come into contact with the odour receptors. These molecules are volatile compounds (VCs). It is easy for us to smell them because the compounds have a low vapour pressure and a low molecular weight which means that they can readily evaporate and come into the air we breathe. VCs have diverse origins. They are released from plants, microorganisms, food and even organic and inorganic materials such as compost and plastics. Examples of VCs are the perfume of flowers like the molecule geraniol responsible for the smell of roses. Dimethyl trisulfide is that pungent odour we smell when opening the wrapping of a good Limburger cheese and let's not forget geosmin, the scent that fills the air after the first drops of rain have fallen.

Many VOCs are produced by bacteria, whereby members of the genus *Streptomyces* in the family of Actinobacteria are a major source (Dickschat et al., 2007; Yang et al., 2018). Streptomycetes are soil-dwelling bacteria with a complex mycelial life cycle, that can reproduce via sporulation (Barka *et al*., 2016). Actinobacteria are prolific producers of natural products, including two-thirds of all known antibiotics, the majority of which are produced by *Streptomyces* (Kieser et al., 2000). Some examples of these antibiotics are streptomycin (Distler et al., 1987), vancomycin (Levine, 2006), the more recently discovered daptomycin (Miao, 2005), as well as the β-lactam inhibitor clavulanic acid that, in combination with antibiotics like penicillin, overcomes the antibiotic resistance generated by the secretion of beta-lactamases (Paradkar, 2013). The antibiotic-producing potential of these bacteria includes the 'soluble' secondary metabolites and the smaller volatile compounds, which have not yet been extensively explored. Despite the recent interest in VCs, only a few compounds have been found to have antibiotic activity, namely the sesquiterpenes albaflavenone and pentalenolactone produced by *Streptomyces coelicolor* and *Streptomyces avermitilis* respectively (Zhao et al., 2008; Tetzlaff et al., 2006). The physicochemical properties of volatile compounds make them ideal molecules to participate in microbial communication and interactions. For this reason, the aim of this work is to analyse the potential of volatile compounds as antibiotics as well as to obtain a deeper understanding of the function of these molecules.

Over the last decade, research on microbial volatiles and their biological activity, including potential biotechnological applications has gained attention. **Chapter 2** presents a review of the potential of microbial volatiles as antimicrobials and as modulators of antibiotic resistance. The review highlights the bioactivity of volatiles against pathogenic bacteria and describes the modes of action behind antibiotic volatiles and as modulators of antibiotic resistance.

To identify *Streptomyces* with volatile antibiotic activity, we performed a screening using our actinomycetes collection from the Himalaya and Qinling mountains as well as soil from The Netherlands (Zhu et al., 2014). In **Chapter 3** we show that *Streptomyces* produce an abundance of VCs both organic and inorganic. These VCs show antibiotic activity inhibiting the growth of *B. subtilis* and *E. coli*. The antibiotic effect was strain specific since none of the *Streptomyces* strains that were able to inhibit *E. coli* inhibited the growth of *B. subtilis* and vice versa. Surprisingly, *Streptomyces* can produce high concentrations of volatile ammonia, which has antibiotic activity. This inorganic molecule can be produced by the bacteria at low cost and diffuse over long distances, thereby accumulating in the agar, with an inhibitory effect as the result. The high concentrations of ammonia that accumulated altered the pH of the growth media and influenced the activity of common antibiotics and the behaviour of neighbouring streptomycetes. This shows the importance of VCs in air-borne interactions, not only via a direct effect but also via collateral effects such as pH change. In **Chapter 3** we also analysed the headspace from different *Streptomyces* strains and observed that it is dominated by the terpenes 2-methylisoborneol and its dehydrogenation molecules 2-methylenebornane and 2-methyl-2-bornane.

E. coli is a Gram-negative bacterium commonly found in the intestine of animals, including humans (Gorbach, 1996). Pathogenic strains can cause diarrhea, urinary tract infections, respiratory illness and pneumonia, among others (CDC, 2018). Gram-negative bacteria have an outer membrane that protects the cell by limiting the entrance of toxic substances therefore reducing the efficacy of antibiotics (Zgurskaya et al., 2015). Pathogenic *E. coli* strains can also rapidly develop resistance to antibiotics threatening the life of infected people (Collingnon, 2009). In **Chapter 4** we analyse the response of *E. coli* to ammonia. The target bacteria respond to the high concentrations of ammonia by down-regulating the porin master regulatory two-component system OmpR-EnvZ. The concomitant reduced expression of the outer membrane porins OmpC and OmpF, limits the entrance of ammonia into the cell. In confirmation of the toxic influence of ammonia, *E. coli* also reduces its own ammonia production.

In **Chapter 5** we show that apart from 2-methylisoborneol and its derivatives, the headspace of *Streptomyces griseus* consists mostly of terpenes. 36 out of the 46 VOCs identified belong to the terpene class, including the well-known sesquiterpene geosmin. To study the importance of such molecules, we constructed several mutants lacking one or more genes responsible for the production of the volatile terpene compounds, including a quadruple mutant that was unable to produce any volatile terpenes. In this chapter, the evident and not-so evident phenotypical changes that arose in the different mutants are presented. A first approach towards understanding the biological and ecological role of such compounds in *Streptomyces* is also presented.

In **Chapter 6** we concentrated on the role of VOCs in ecological interactions. The effect of VOCs emitted by *Streptomyces* in its interaction with protists are discussed. When protists were grown in the presence of VOCs emitted by *Streptomyces,* an inhibition of the activity of protists was observed. The inhibitory effect was confirmed when the pure compounds dimethyl disulfide and 2-methylenebornane were used. Bacterial VOCs can be detected by protists and possibly used as food source as previously suggested (Schulz-Bohm et al., 2017). Here we observed a similar behaviour, indicating that some protists can use VOCs emitted by *Streptomyces* as nutrients. At the same time, VOCs released by *Streptomyces* acted as a defence mechanism against protist predators. The overall findings obtained in this work are integrated in the general discussion of **Chapter 7** with a summary of the most important observations and future perspectives.