

Review

Streptomyces Exploration: Competition, Volatile Communication and New Bacterial Behaviours

Stephanie E. Jones¹ and Marie A. Elliot^{1,*}

***Streptomyces* bacteria are prolific producers of specialized metabolites, and have a well studied, complex life cycle. Recent work has revealed a new type of *Streptomyces* growth termed ‘exploration’ – so named for the ability of explorer cells to rapidly traverse solid surfaces. *Streptomyces* exploration is stimulated by fungal interactions, and is associated with the production of an alkaline volatile organic compound (VOC) capable of inducing exploration by other streptomycetes. Here, we examine *Streptomyces* exploration from the perspectives of interkingdom interactions, pH-induced morphological switches, and VOC-mediated communication. The phenotypic diversity that can be revealed through microbial interactions and VOC exposure is providing us with insight into novel modes of microbial development, and an opportunity to exploit VOCs to stimulate desired microbial behaviours.**

The Classic *Streptomyces* Life Cycle

Streptomyces bacteria are abundant in the soil, an environment they share with millions of other microorganisms. *Streptomyces* are renowned for conferring soil with a rich, earthy odor, courtesy of a volatile compound they produce known as geosmin [1]. In addition to geosmin, *Streptomyces* species produce a multitude of specialized metabolites having functions ranging from nutrient acquisition, (e.g., siderophores for iron uptake) [2], through to growth inhibition of competing organisms (e.g., antibiotics and antifungal compounds) [3].

Specialized metabolite production on this scale is unique to the streptomycetes, and it has been proposed that these bacteria require a diverse metabolic repertoire to support their unusual life cycle. *Streptomyces* species are sporulating bacteria, and their life cycle initiates with spore germination (Figure 1). The emerging germ tubes are extended by polar growth at the hyphal tips. Branches emerge at occasional intervals, and this iterative cycle of hyphal tip extension and branching ultimately leads to the formation of a dense network of vegetative cells known as the vegetative mycelium. Polar growth is directed by members of the ‘polarisome’, a dynamic complex that includes DivIVA, which recruits the peptidoglycan biosynthetic machinery to hyphal tips and future branch sites [4–6], the *Streptomyces*-specific cytoskeletal protein Scy, which augments DivIVA positioning and activity [7], and the intermediate filament FIIP, which imparts rigidity to the hyphal tips [8,9].

Streptomyces vegetative cells do not undergo typical cell division, and instead form occasional cross-walls within the vegetative hyphae. These crosswalls presumably serve as diffusion barriers for cellular macromolecules. Chromosomes are replicated within the vegetative

Trends

The classical *Streptomyces* life cycle involves three growth stages: vegetative hyphae, aerial hyphae, and spores. A novel form of *Streptomyces* development termed ‘exploratory growth’ has now been identified. Explorer cells are nonbranching vegetative hyphae that can rapidly move across surfaces.

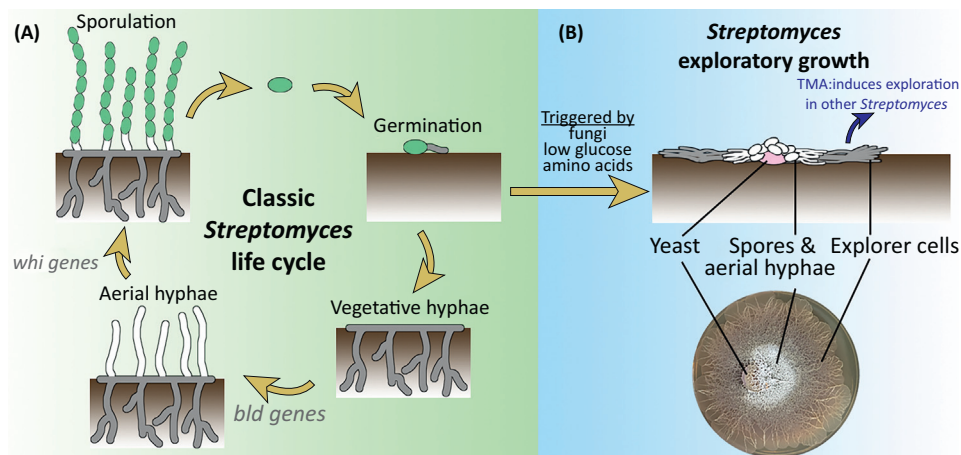
Streptomyces do not typically initiate exploration when grown alone in laboratory environments. Exploration was first observed when *Streptomyces venezuelae* was cultured with yeast. Investigating bacterial development in the context of multispecies interactions therefore has the potential to reveal novel microbial behaviours.

Streptomyces exploration is promoted by the volatile compound trimethylamine.

Different growth conditions, such as alkaline environments, can dramatically alter microbial development.

¹Department of Biology and M.G. DeGroot Institute for Infectious Disease Research, McMaster University, 1280 Main Street West, Hamilton, ON, Canada, L8S 4K1

*Correspondence: melliot@mcmaster.ca (M.A. Elliot).



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Figure 1. The Classic *Streptomyces* Life Cycle and *Streptomyces* Exploratory Growth. (A) The classic *Streptomyces* life cycle begins with spore germination. Germ tubes emerge from a single spore and grow by hyphal tip extension, forming a dense network of branching vegetative hyphae. In response to unknown signals, nonbranching aerial hyphae rise into the air. These aerial hyphae are coated in a hydrophobic sheath that permits their escape from the aqueous vegetative mycelium. Finally, aerial hyphae differentiate into chains of dormant exospores. The resulting spores are stress-resistant, and can be dispersed into the environment. The transition from vegetative growth to aerial hypha formation requires the *bld* gene products, and the transition from aerial hyphae to spore chains requires the *whi* gene products. (B) *Streptomyces* can deviate from the classic vegetative hyphae–aerial hyphae–sporulation life cycle. In response to combinations of the presence of fungi, low glucose and amino acids, *Streptomyces* can initiate exploratory growth. When *Streptomyces* are grown beside yeast, they first form aerial hyphae and spores on and around the yeast colony. Once yeast has consumed the local glucose supply from the medium, *Streptomyces* begin to produce the volatile trimethylamine (TMA). TMA release leads to a localized increase in pH and promotes the initiation of exploration in the TMA producer, and also nearby exploration-competent streptomycetes. Explorer cells grow as nonbranching vegetative hyphae, and are capable of rapidly traversing surfaces. Exploratory growth does not require *bld* or *whi* developmental regulators critical for the classic *Streptomyces* life cycle.

hyphae, and are segregated along the growing filaments [10,11] and into emerging branches; however, visible chromosome compaction is not apparent.

The reproductive phase of the *Streptomyces* life cycle involves the raising of nonbranching aerial hyphal filaments from the vegetative mycelium (Figure 1). These aerial hyphae are coated with a hydrophobic protein layer (comprising the chaplins, and under some growth conditions, SapB) that permits their extension away from the aqueous environment of the vegetative cells [12–15], and protects them from desiccation in the aerial environment. The aerial hyphae are ultimately transformed into chains of dormant exospores through a synchronous round of cell division and chromosome segregation [16] (Figure 1). The resulting spores are resistant to a range of environmental insults, and can be readily dispersed in the environment.

These different growth stages (vegetative growth, aerial hypha formation and sporulation) are subject to distinct genetic control. Aerial hypha formation requires the activity of the *bld* genes (Figure 1), whose products are primarily regulators that direct the production of aerial hypha-specific proteins and, in some cases activate specialized metabolism [17–19]. Subsequently, the conversion of aerial hyphae into spore chains requires the activity of the *whi* gene products (Figure 1); these regulatory proteins promote cell division, chromosome segregation, and spore maturation [19–21].

A filamentous life cycle appears to be particularly effective within the soil environment, having evolved independently in both the streptomycetes and the filamentous fungi. However, it

Glossary

Surfactant: surface-active agent, used to reduce friction during sliding motility (amongst other functions).

Trimethylamine (TMA): a volatile, alkaline molecule that can promote exploration by *Streptomyces* bacteria, and alter antibiotic resistance in other bacteria.

Volatile organic compound (VOC): used by many organisms (including bacteria and fungi) as communication/competition tools.

means that in the absence of sporulation – an energetically expensive process that cannot be reversed once initiated – *Streptomyces* lacks the ability to readily colonize new environments. Instead, the vegetative mycelial network is thought to permit maximal nutrient access within a localized area. This limited mobility may have led to a selective growth advantage for those *Streptomyces* species equipped with a chemical arsenal capable of inhibiting the growth of nearby bacteria and fungi, as this would allow them to protect both their nutrient pool, and themselves, from predation or competition by other microbes [22].

A New Mode of *Streptomyces* Development

Courtesy of technical advances in both genomics and cell biology, significant progress has been made in understanding the mechanisms underpinning the different developmental transitions during the *Streptomyces* life cycle. To date, however, these investigations have focussed on the study of single-species cultures. As the soil is replete with microorganisms, the growth of any individual soil microbe in the laboratory may not effectively recapitulate or capture the full developmental potential of these organisms in the environment.

It was recently discovered that the coculture of many *Streptomyces* species (19 out of 200 species tested) with diverse yeasts leads to a novel developmental behaviour that had not been seen previously for *Streptomyces* cultured alone. This new behaviour, termed ‘exploratory growth’, is a glucose-repressible phenomenon that initiates once the glucose in the immediate vicinity of the *Streptomyces* and yeast colonies has been depleted [23]. Indeed, removing glucose from the medium can obviate the need for yeast in stimulating *Streptomyces* exploration [23]. Exploration involves the rapid outgrowth of seemingly vegetative hyphae. The rate at which colony expansion occurs within the exploratory model system *Streptomyces venezuelae* has been estimated to be $\sim 90 \mu\text{m/h}$ [23]; this is an order of magnitude faster than has been observed for individual *S. venezuelae* vegetative hyphal filaments [24,25]. A closer examination of exploring hyphae has revealed that while these cells are hydrophilic like vegetative hyphae, they do not seem to branch. The absence of branching is a characteristic associated with aerial hyphae, but in this vegetative context, may contribute to the enhanced outward spread of the exploring colonies.

Exploratory growth does not require any of the classical *bld* or *whi* developmental regulators [23]; these findings, coupled with the distinct physical characteristics of explorer hyphae (hydrophilic, nonbranching), suggest that exploration represents a novel mode of *Streptomyces* growth and development.

In addition to glucose-repression, exploration also requires alkaline conditions. *S. venezuelae* produces **trimethylamine** (TMA; see Glossary), a **volatile organic compound** (VOC) that can raise the pH of the adjacent environment, promoting exploration of nearby *Streptomyces* cultures [23]. Notably, the TMA-mediated response is not confined to *S. venezuelae*: all exploration-competent streptomycetes tested to date appear to emit a VOC that promotes the exploration of *S. venezuelae* [23].

Dispersal to New Environments

Exploratory growth provides *Streptomyces* with the ability to colonize new environments without committing to the terminal sporulation process. In addition to effectively colonizing their immediate vicinity, explorer cells can readily traverse nutrient-deficient abiotic surfaces, including plastic dividers, glass coverslips, and rocks [23]. Within a heterogeneous soil environment, this has the potential to confer a profound fitness advantage with respect to accessing nutrients in more disparate locations.

Exploration by *Streptomyces* is analogous to motility in many other bacterial systems. Like exploration, swarming motility (powered by flagella over semi-solid surfaces) is a group

behaviour where cells typically move together as rafts [26], while twitching motility (driven by the repeated extension, attachment, and retraction of type IV pili) is capable of propelling bacteria over abiotic surfaces [27]. Unlike flagellar- and pili-mediated motility, however, exploration is not accompanied by the assembly of any motility organelles. Gliding motility, most extensively studied in *Myxococcus xanthus*, employs a membrane-anchored motor complex to propel cells forward [28,29]; *S. venezuelae* lacks the genes required for motor formation and assembly, suggesting that, if exploration occurs via a gliding motility-type mechanism, it must do so using an entirely different motor system. Instead, exploration is most consistent with sliding motility, a growth-dependent spreading that is facilitated by secreted **surfactants** [30,31]. The lack of branching observed for explorer cells may help to promote rapid colony spreading, and this process may be further enhanced by the secretion of a surfactant that alters the surface properties of the substrate over which exploration is occurring.

Many bacteria move by sliding, including the pathogens *Salmonella* [32], *Legionella* [33], *Vibrio* [34], *Mycobacterium* [35], and *Pseudomonas* [36], as well as soil-dwelling microbes like *Bacillus* [37] and *Sinorhizobium* [38]. There does not, however, seem to be any one conserved mechanism underlying sliding motility: while sliding is not yet well understood, the genetic cues that trigger sliding appear to be genus-specific, as do the associated surfactants. For example, in the mycobacteria, sliding requires the secretion of glycopeptidolipid surfactants, and in mutants unable to produce these, sliding – and biofilm formation – are abolished [39]. In contrast, in *Bacillus*, sliding is promoted by the secretion of the surfactant antibiotic surfactin [37], and is governed by the master sporulation regulator SpoOA [40]. While an exploration-associated surfactant has yet to be identified, the sporulation regulators (*whi* gene products) do not affect exploration in *S. venezuelae* [23]. When grown in conjunction with yeast, however, wild-type *Streptomyces* cells will sporulate prior to initiating exploration. This provides an interesting ‘bet-hedging’ strategy which ensures that there exists a dormant/resistant genetic repository in the event of unproductive or failed exploration (Figure 1).

Conservation of Interkingdom Interactions

Broad-spectrum microbial competition strategies include chemical warfare and localized nutrient depletion, and these can induce a range of responses in the competing organism. In the case of *Streptomyces*, fungal consumption of glucose activates *Streptomyces* exploratory growth. Interestingly, fungi can also change the developmental trajectory of nonexploring streptomycetes through modulating carbon source availability [41]. These interactions are reminiscent of one reported previously for *Saccharomyces cerevisiae* and *Pseudomonas putida*, where glucose depletion by the yeast served to activate exopolysaccharide production by *P. putida* [42]. In the soil environment, localized nutrient depletion by one organism is expected to be a common phenomenon, and as such, responses like those of *Streptomyces* and *Pseudomonas*, involving altered genetic or developmental programs in response to altered nutritional conditions, would be important survival mechanisms.

The *Streptomyces*–yeast interaction is reasonably conserved: at least six types of yeast can induce *Streptomyces* exploration, and ~10% of *Streptomyces* species tested can explore [23]. Such generalized responses seem to be the norm for interkingdom interactions. One of the best studied examples involves the human pathogen *Candida albicans*. *Candida* uses the quorum sensing molecule farnesol to inhibit its own hyphal growth and promote biofilm development [43]. At the same time, farnesol also suppresses the growth of a range of bacteria, including *Staphylococcus aureus* [44] and *Acinetobacter baumannii* [45,46]. Another well-characterized example involves *Pseudomonas* species, and their production of a class of specialized metabolites known as phenazines. Phenazines are important for *Pseudomonas* persistence in the environment, but they also have profound effects on the morphology and virulence of

various fungal species [47–49]. Given that most microbes live within multispecies communities, it seems likely that competition in these communities has selected for the evolution of broad-spectrum fungal and bacterial responses.

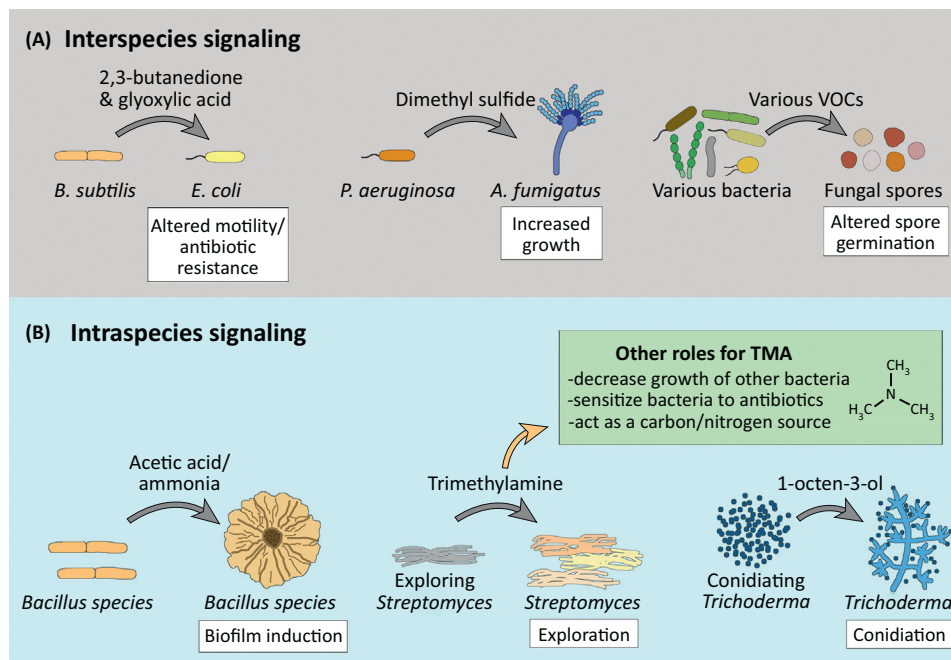
pH and Microbial Morphological Switching

While *Streptomyces* exploration can be induced through yeast association, initiation of exploration also requires alkaline conditions. A high-pH environment can be established either through a self-induced rise in extracellular pH, or by exogenous alkaline compounds. An analogous phenomenon has been observed in fungi, where alkaline growth conditions are known to influence developmental switches. In the human pathogenic fungi *C. albicans* [50] and *Aspergillus fumigatus* [51], a rise in pH promotes the switch from budding yeast to filamentous hyphal growth. This developmental switch is critical for the progressive invasion into host tissues, and fungal mutants unable to undergo this transition are significantly attenuated in virulence [52,53]. The fungal response to existing alkali environments appears to be conserved: the transcription factors Rim101 and PacC govern the primary fungal pH-sensing signal transduction pathways, and they alter the expression of genes involved in cell wall synthesis, filamentation, and hyphal morphogenesis [54]. As has been observed for *Streptomyces*, *C. albicans* can also raise the pH of acidic medium in response to glucose starvation [55]. In *Streptomyces* this is through the production of TMA, while in *C. albicans*, it is mediated by ammonia production, generated through the catabolism of amino acids. The resulting self-induced hyphal switch appears to occur independently of Rim101/PacC, suggesting that the response to a self-induced rise in pH proceeds via a different pathway than that activated by existing alkaline conditions [54].

The conservation of high pH-mediated morphological switching by both *Streptomyces* and fungi raises the question of why a rise in pH triggers such profound morphological changes. For most microbes, alkaline environments are not optimal for growth. Nutrient uptake driven by proton gradients is hindered, and the solubility of iron and other essential elements is reduced [56]. Thus, hyphal/exploratory growth must somehow provide a means of maximizing growth and surface colonization under these high-pH conditions. Given that alkaline-induced switching appears beneficial for survival and is observed for many fungi and streptomycetes, this phenomenon may well be employed by other microorganisms occupying the immense phylogenetic space between fungi and *Streptomyces* bacteria. Supporting this proposal is the observation that *Bacillus* sliding motility is a pH-sensitive phenomenon and is inhibited during growth under low-pH conditions.

VOCs: The Airborne Language of Microorganisms

In both *Streptomyces* and fungi, the ability to modulate environmental conditions (e.g., raise pH) is achieved through the use of VOCs. There is a growing appreciation for the diverse roles played by VOCs in microbial communities (e.g., [57–60]). While the biological functions of microbial VOCs are not yet fully understood, it is clear that these compounds can act as infochemicals, affecting the behaviour, gene expression and group behaviour of responding microorganisms (Figure 2). Most studies to date have explored the ecological roles of VOCs in communication and competition between nonrelated species. Within the bacteria, recent work has revealed that VOCs emitted by *Bacillus subtilis* can induce widespread changes in gene expression, motility and biofilm formation in *Escherichia coli* [61]. Bacterial VOCs can also serve as interkingdom communication signals. For example, dimethyl sulfide emitted by *Pseudomonas aeruginosa* can stimulate the growth of the fungus *A. fumigatus* [62], while other bacterial VOCs can modulate fungal spore germination and mycelial growth [63,64]. There are fewer known cases of VOCs mediating communication between related species. In fungi, *Trichoderma* uses VOCs to coordinate conidiation of separated colonies [65], while in bacteria, *Bacillus* species form biofilms in response to volatile acetic acid (*B. subtilis*) [66] and ammonia



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Figure 2. Examples of Signaling Roles Played by VOC Infochemicals. (A) Interspecies signaling: VOC infochemicals have diverse roles, and can mediate communication between different bacteria. Left: 2,3-butanedione and glyoxylic acid emitted by *Bacillus subtilis* induce changes in the motility and antibiotic-resistance profile of *Escherichia coli*. VOCs can also mediate communication between bacteria and fungi. Middle: dimethyl sulfide emitted by *Pseudomonas aeruginosa* can stimulate the growth of the fungus *Aspergillus fumigatus*. Right: various bacterial VOCs can modulate the germination of fungal spores. (B) Intraspecies signaling: VOC infochemicals have diverse roles in communication between related species. Left: Acetic acid emitted by *B. subtilis* or ammonia emitted by *Bacillus licheniformis* induces biofilm formation in various *Bacillus* species. Middle: *Streptomyces* explorer cells release the alkaline volatile organic compound (VOC) trimethylamine (TMA). This VOC induces exploration in other physically separated *Streptomyces* cells, irrespective of whether it is the same or a different species. TMA emitted by explorer cells can also decrease the growth of other soil bacteria. Based on studies in other bacteria, TMA can sensitize bacteria to antibiotics, and act as a carbon/nitrogen source. Right: conidiating *Trichoderma* fungi use 1-octen-3-ol and related analogs to induce the onset of conidiation in other *Trichoderma*.

(*Bacillus licheniformis*) [67]. *Streptomyces* exploration is coordinated by the alkaline TMA – a VOC produced by *Streptomyces* via an unknown pathway. This TMA-mediated induction of exploration appears to result from a rise in pH of the medium rather than a TMA-specific signal, as other alkaline VOCs (e.g., ammonia) can also induce exploration [23]. Unexpectedly, a high-pH environment was unable to promote exploration, suggesting that there may be an adaptation phase that precedes exploration, during which *Streptomyces* species activate their alkaline stress response. Remarkably, TMA production by exploring *Streptomyces* can induce exploration in other, physically separated *Streptomyces* species [23], suggesting that the pH-modulatory effects of TMA are broadly recognized as exploration permissive signals within the streptomycetes (Figure 2).

In the soil, VOC infochemicals are released into spaces occupied by polymicrobial communities. Once released, these signals become communal public goods, and they can be exploited by nonproducing ('cheater') neighbours [68,69]. In the case of TMA, this provides other soil microbes with the opportunity to either take advantage of the pH-altering effects of TMA, or catabolize this molecule as a carbon or nitrogen source. Significant efforts have been dedicated

to understanding the evolutionary basis for secreting public goods, given the cost associated with production and the benefits associated with cheating [68,70,71]. One theory that has been put forth involves kin selection, whereby the secreted molecules provide the greatest benefit to those species closely related to the producer [72,73]. TMA produced by *Streptomyces* exploring cells could therefore confer a competitive advantage to related streptomycetes by inducing their exploration, and thus enhance their surface colonization and nutrient access capabilities. VOC-mediated communication between closely related species may be a common mechanism to maximize survival of microbial relatives in densely populated polymicrobial communities.

Streptomyces species produce a vast array of specialized metabolites, and in addition to their growth-inhibitory properties, it has been hypothesized that these compounds might also function as signaling molecules acting within or between species [74,75]. Soils are not homogenous environments, and instead comprise a mixture of solid particles, and air- or water-filled pores. Specialized metabolites would be most effectively employed as signalling molecules – or as antibiotics – in short-range interactions. However, these compounds would be unlikely to modulate the behaviour or activity of organisms on the other side of a pore. In contrast, VOCs are low-molecular-weight compounds with relatively high vapour pressures, and consequently could diffuse rapidly through air-filled gaps. Thus, conditions that induce exploration in one streptomycete – high amino acid density, low glucose, or the presence of yeast – could be communicated to distant streptomycetes across air pockets using VOCs, promoting exploration by these related species. The *Streptomyces* arsenal of communication tools may therefore include both specialized metabolites for use in local communication and competition, and VOCs for use as long-distance signals.

TMA as a Competitive Tool

In addition to their roles as signalling molecules, VOCs offer a competitive advantage by directly acting as antibacterial or antifungal agents, or by modulating the antibiotic resistance of other microorganisms (Figure 2). Beyond its role in signalling exploration within the streptomycetes, TMA also confers a competitive benefit by inhibiting the growth of other bacteria. Both exploring *Streptomyces* and aqueous TMA solutions can suppress the growth of the soil bacteria *B. subtilis* and *Micrococcus luteus* [23]. This growth inhibition could be due to the pH-raising effects of TMA, as other soil microbes do not typically thrive in such alkaline environments. It will be interesting to see whether other VOCs with pH-altering capabilities have similar growth-inhibitory effects.

As a complement to its antibacterial effects, TMA can also influence the antibiotic-resistance profiles of Gram-positive and Gram-negative bacteria [76] (Figure 2). The pH-raising effects of TMA alter bacterial transmembrane pH and proton motive forces. This in turn leads to enhanced antibiotic uptake, and a corresponding increase in sensitivity to aminoglycoside antibiotics and chloramphenicol [76]. *Streptomyces* species are prolific antibiotic producers, and included within their production repertoire are both aminoglycosides [3] and chloramphenicol [77], with the latter being produced by *S. venezuelae*. In the soil, TMA may therefore work synergistically with streptomycete-produced antibiotics, sensitizing other bacteria to these compounds. The antibiotic modulatory effects of TMA are not unique. Indeed, there is mounting evidence suggesting that VOCs may have widespread roles in modulating bacterial antibiotic resistance. For instance, ammonia can alter the resistance profiles of physically separated microorganisms through altered membrane permeability [78], while VOCs emitted by *Burkholderia ambifaria* can increase the resistance of *Escherichia coli* to several antibiotics through unknown mechanisms [79]. It will be of interest to explore the synergistic potential of combining VOCs with more traditional antibiotics to treat bacterial infections.

The VOC Potential of *Streptomyces*

Although *Streptomyces* are well known for their production of geosmin, the VOC with a distinct earthy aroma, the VOC repertoire of *Streptomyces* extends far beyond this compound. Schöller *et al.* [80] screened 26 *Streptomyces* species and identified 120 distinct VOCs belonging to various chemical classes. More recently, Cordovez *et al.* [81] identified 536 VOCs from 12 *Streptomyces* species isolated from disease-suppressive soil, and Wang *et al.* [82] identified several VOCs produced by *Streptomyces albolavus* with direct antifungal properties. In recent work by Jones *et al.* [23], *Streptomyces* exploratory and static cultures collectively yielded ~1400 unique VOCs. Each of these compounds has the potential to modulate the behaviour of its streptomycete producer, affect the growth of any nearby microbes in the soil, and/or contribute to cooperation or competition between microbes. An intriguing role for VOCs in the streptomycetes that has yet to be explored is the potential for stimulating *Streptomyces* specialized metabolite production. Most *Streptomyces* biosynthetic gene clusters are not expressed under laboratory (single species) culturing conditions, and recent studies have shown that VOCs can induce antibiotic production in other bacteria. For example, the volatile 2,3-butanediol emitted by *Enterobacter aerogenes* stimulates antibiotic production by *P. aeruginosa* [83]. It is conceivable that, in the soil, *Streptomyces* specialized metabolite production could be stimulated by high-jacking VOCs from non-streptomycetes, or by responding to VOCs produced by neighbouring streptomycete species. Whether it is possible to employ VOCs as a strategy to stimulate new antibiotic production by *Streptomyces* in the laboratory remains to be determined.

Concluding Remarks and Future Perspectives

The traditional *Streptomyces* life cycle involves a progression from single spores to branching vegetative hyphae, and from vegetative hyphae to raised aerial hyphae. The life cycle culminates with the metamorphosis of the aerial hyphae into chains of spores that can be individually dispersed, allowing the cycle to begin anew. We now know that *Streptomyces* can deviate from this classically defined life cycle by initiating exploratory growth. Exploration appears to be initiated in response to low glucose levels, and this in turn induces the production and release of the alkaline VOC TMA by *Streptomyces*. TMA in turn raises the pH of the surrounding environment, and this signals the onset of exploration by nonbranching vegetative-like hyphae – irrespective of glucose levels.

Explorer cells can rapidly transverse biotic and abiotic surfaces, presumably facilitating access to nutrient-rich environments. Exploring cultures are further capable of communicating this mode of growth to distant streptomycetes using an airborne VOC. Exploration is an unexpected mode of microbial development, and suggests that even the best studied model microorganisms may be capable of growing in yet undiscovered ways. Exploratory behaviour is prompted by interactions with fungi, and it seems likely that multispecies analyses will be necessary to obtain a full appreciation for the developmental repertoire of bacteria as a whole. *Streptomyces* exploratory growth can be communicated to distant species by the VOC TMA. VOCs are increasingly being found to have important roles in modulating bacterial growth, enhancing microbial competition, altering antibiotic-resistance profiles, and stimulating antibiotic production. Microbial VOCs represent a largely untapped class of metabolites, and further work will be required to fully appreciate the ecological roles of these compounds, alongside their clinical and agricultural potential.

Streptomyces exploratory behaviour is an exciting new mode of microbial development, and many questions remain open regarding this unexpected behaviour (see Outstanding Questions). The mechanism underlying exploration is not yet understood, and within this, the question of how the colony foregoes the branching associated with classical vegetative hyphal growth is of particular interest. Furthermore, it has yet to be established whether exploration is a

Outstanding Questions

What are the mechanisms underlying *Streptomyces* exploration? Is exploration a form of sliding motility, or is it a completely new form of bacterial movement? Is there a surfactant involved? How do *Streptomyces* cells sense the pH changes that appear to be required for initiating exploration?

Is exploration directional, and if so, what factors (genetic and environmental) influence this chemotaxis-like behaviour?

How pervasive is *Streptomyces* exploration, and what other conditions/interactions can promote this?

How is TMA produced? What function do the other 1400+ volatile compounds produced by *Streptomyces* species serve? How do these compounds contribute to the cooperation and competition of *Streptomyces* with their relatives, and other soil microbes?

What is it about high pH that triggers exploration in *Streptomyces* and hyphal growth in fungi?

Can VOCs be used to activate cryptic specialized metabolic clusters, and stimulate new antibiotic production in streptomycetes?

form of sliding motility, or whether it represents a new mechanism of traversing surfaces. Further investigating these questions will enhance our understanding of the now expanded life cycle of the *Streptomyces* genus, and could lay the foundation for developing new techniques to stimulate *Streptomyces* specialized metabolite production, and exploit the emerging volatile repertoire of these organisms.

Acknowledgments

S.E.J. is supported by a Vanier Scholarship, and this work has been supported by the Canadian Institutes of Health Research (MOP-137004CIHR) and the Natural Sciences and Engineering Research Council of Canada (NSERC) Discovery Grants and Discovery Accelerator Supplements programs.

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