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Review

# Phenotypic heterogeneity in *Streptomyces* colonies

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*Streptomyces* are a large genus of multicellular bacteria best known for their prolific production of bioactive natural products. In addition, they play key roles in the mineralisation of insoluble resources, such as chitin and cellulose. Because of their multicellular mode of growth, colonies of interconnected hyphae extend over a large area that may experience different conditions in different parts of the colony. Here, we argue that within-colony phenotypic heterogeneity can allow colonies to simultaneously respond to divergent inputs from resources or competitors that are spatially and temporally dynamic. We discuss causal drivers of heterogeneity, including competitors, precursor availability, metabolic diversity and division of labour, that facilitate divergent phenotypes within *Streptomyces* colonies. We discuss the adaptive causes and consequences of within-colony heterogeneity, highlight current knowledge (gaps) and outline key questions for future studies.

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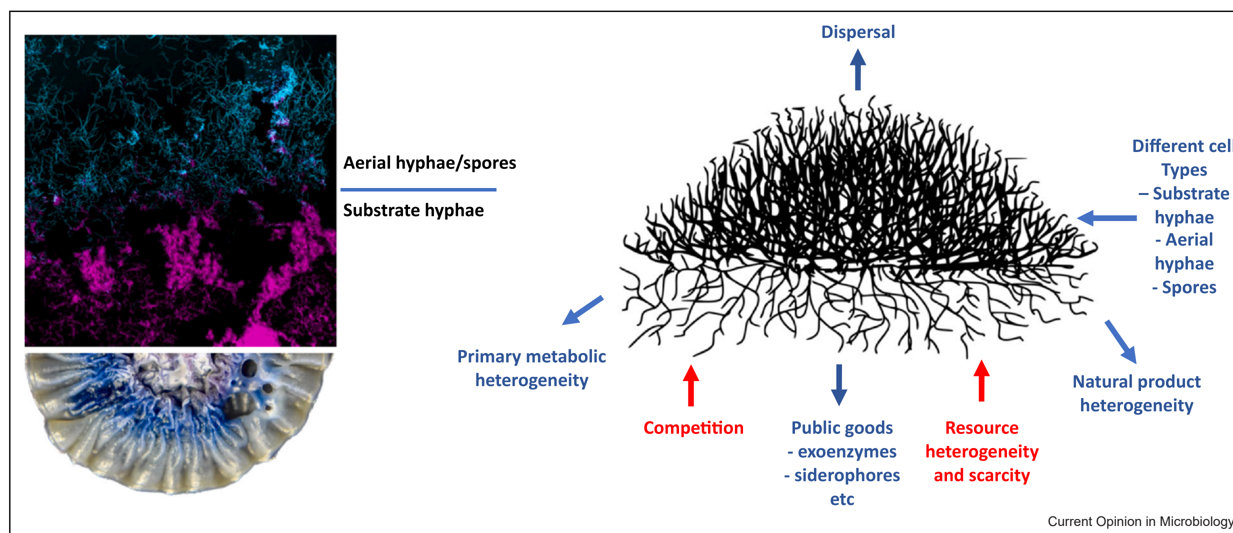
## Introduction

*Streptomyces* are among the most important and best-studied bacterial genera. As saprophytes growing in soil and aquatic sediments, they play a crucial role in the mineralisation of recalcitrant polymers, such as chitin, cellulose and starch, that result from the death of plants, arthropods and fungi [1]. In addition, they are the primary source of natural products used in medicine and agriculture. Some two-thirds of antibiotics owe their origin, directly or indirectly, to streptomycetes, and predictions based on bioinformatic analyses of *Streptomyces* genomes suggest that they remain a vast resource for drug discovery [2]. Finally, *Streptomyces* are multicellular (Figure 1), with a life cycle that is highly convergent with filamentous fungi [3]. These features, on their own, are not unique. However, our aim in this perspective is to argue that the combination of these features has unique consequences for the manner in which *Streptomyces* persist and thrive in spatially and temporally heterogeneous environments.

The *Streptomyces* life cycle begins when a spore lands, or is transported, to a habitat suitable for germination and growth. Thereafter, the colony grows via apical expansion of filamentous hyphae, giving rise to a large interconnected mycelial network reminiscent of branching filamentous fungi. Eventually, upon resource (or other) stress, part of the colony irreversibly differentiates to produce aerial hyphae that hold aloft a new generation of unichromosomal spores [4]. Some species have also been found to produce a rapidly expanding 'explorer' phenotype in response to environmental cues [5]. The transition to sporulation typically coincides with the lysis of parts of the vegetative hyphae and with secondary metabolite production [4].

Although it has received only limited attention, phenotypic heterogeneity (plasticity) can potentially arise at each stage of this multicellular life cycle. By heterogeneity, we refer specifically to functional differentiation that emerges in spatially separated regions of the colony. Phenotypic heterogeneity, where genetically identical cells express different phenotypes in the same environment, has been extensively studied in unicellular bacteria and can be caused by many factors, from stochastic differences due to unequal partitioning of transcripts or proteins, feedback loops that generate bistable subpopulations or differences in cellular vigour that correlate with the expression of 'auxiliary traits' [6–8]. *Streptomyces* can, in principle, share these mechanisms, but the underlying processes would arise within the framework of a multicellular colony rather than among independent cells. This implies that heterogeneity could be evident at different spatial scales: within cells, between adjacent compartments of the multicellular

**Figure 1**




*Streptomyces* colony from above and a section through a colony illustrating the spatial and temporal effects and outputs of growth. Top left: mesoscopic section through a *Streptomyces* colony showing substrate hyphae (magenta) and aerial mycelium and spore chains above the substrate (cyan; courtesy of Dr Liam Rooney, University of Strathclyde). Bottom left: *Streptomyces coelicolor* colony image from above showing the distribution of cell types and heterogeneity in metabolite production. From the centre of the colony, the powdery white/grey aerial mycelium can be seen, followed by an area of the colony producing the blue-pigmented natural product actinorhodin, and finally, the outer region is actively growing vegetative hyphae (photograph: Paul A Hoskisson). Right hand side image: schematic representation of a *Streptomyces* colony (courtesy of Phylopic.org CC0 1.0) indicating the factors that drive heterogeneity in colonies (red) and the resulting cellular outputs and responses of the heterogeneity (blue).

filament and between different filaments of a single larger colony. In this sense, *Streptomyces* colonies are less similar to unicellular bacteria than they are to a single multicellular body with a temporal developmental programme. Spores and young colonies are ontogenetically distinct from older colonies, and one side of the colony is potentially distinct from the other. The integration of responses at these different temporal and spatial scales could potentially enable colonies to optimise behaviours to simultaneously accommodate dynamic needs and requirements.

## Why should *Streptomyces* colonies become heterogeneous?

Soil is a highly diverse environment in terms of resource composition, concentration and distribution [9,10]. Equally, it holds enormous biodiversity that shifts readily upon resource amendment, depletion or other disturbance [11,12]. Competition for resources and space is ubiquitous. As saprophytes, *Streptomyces* grow on resources that are often insoluble and heterogeneously distributed [1]. Their genomes are enriched for genes encoding metabolic pathways to degrade these complex polysaccharides and the uptake systems to transport their simpler degradation products [1]. As noted above, they also encode numerous biosynthetic gene clusters (BGCs) directing the synthesis of secondary metabolites, including antibiotics, siderophores, volatile compounds, pigments and others [1,2], with a variety of specialised functions, and whose costs and benefits depend on the timing and location of their expression.

Patchy environmental resources imply that colonies can experience different resources in different regions of their mycelia. Additionally, because degradation and interference competition are external via exoenzyme or antibiotic secretion, *Streptomyces* metabolism can act as a type of 'public good' that can be used by the entire colony but also exploited by other competitors. Together, these can drive nonuniform availability of the precursors necessary to produce antibiotics and other secondary metabolites as well as spatially segregated interactions with competitors. In addition, heterogeneity can emerge due to division of labour and the intrinsic promiscuity of biosynthetic pathways that can result in multiple metabolites from a single pathway. Our aim in this review is to discuss current knowledge of these sources of heterogeneity and to highlight future areas for research (Figure 1).

## Spatial segregation due to subcellular physical heterogeneity

Physical heterogeneity within bacterial cells creates opportunities for functional differentiation. The first indications of subcellular partitioning were observed for specialised chemoreceptors located at specific cellular locations [13], followed by the identification of cytoskeletal elements [14,15], and the partitioning of

chromosomes and extrachromosomal elements [16,17]. More recently, bacterial organelles, such as the magnetosome [18], the anammoxosome [19] and the cyanobacterial carboxysome [20], were found that segregate specialised metabolic activities into discrete intracellular regions. These discoveries challenged the conventional view of the bacterial cytoplasm as an unstructured milieu. Phylogenetic analyses of the proteins that form these cytoplasmic protein microcompartments appear to be widespread among bacteria but are thus far unknown in *Streptomyces* [21]. Within *Streptomyces*, there are tantalising glimpses into the subcellular heterogeneity of transcriptional activity during development in specific cellular compartment, such as SigN-dependent transcription in apical stem compartments [22] and tissue-specific upregulation of cell division gene expression (*ftsZ*) in aerial hyphae [23], suggesting that there is much to learn if technical challenges can be overcome in this area.

The origin of intracellular functional heterogeneity is not restricted to organelle-like structures. During anaerobic growth in *Escherichia coli*, dynamic localisation of nitrate reductase uses transmembrane proton gradients as a cue for localisation [24]. Polar distribution appears to increase ATP generation, perhaps through closer association with respiratory complexes. These data further supported work that demonstrates the presence of the so-called metabolic channelling [25] — the idea that spatially localising sequential reactions in a metabolic pathway increases pathway efficiency. Building on work that showed clustering and scaffolding of metabolic genes, Castellana et al. [25] quantitatively demonstrated that enzyme clustering in a pathway enhances metabolic efficiency and improves regulation. Evidence of metabolic localisation in Actinomycetota is scarce, although a recent study suggests similar mechanisms may occur [26]. For example, some species express the hybrid pyruvate/2-oxoglutarate dehydrogenase complex (PDH–ODH), rather than the two separate multienzyme complexes found in other eubacteria. The actinobacterial PDH–ODH enzyme complex comprised four subunits (AceE, AceF, Lpd and OdhA) that are localised at the cell poles in *Corynebacterium glutamicum*. Remarkably, the phosphorylated OdhI protein that binds to OdhA to inhibit activity was also located at the poles, while unphosphorylated OdhI is distributed throughout the cytoplasm. The enzymes, glutamate dehydrogenase and isocitrate dehydrogenase that are metabolically linked to Odh activity were also distributed evenly throughout the cystoplasm. These data suggest that central metabolism is, at least in part, spatially organised in Actinomycetota, but it remains to be seen if this is more widely observed in the multicellular species of this phylum.

The dynamic localisation of the Twin-arginine translocation secretion and potentially other systems that facilitate the secretion of hydrolytic enzymes to the tips of apically growing hyphae in *Streptomyces* [27,28] also suggest that active foraging for nutrients through apical extension can be spatially partitioned in *Streptomyces*. The secretion of hydrolytic enzymes and uptake/translocation of soluble nutrients is well established in filamentous fungal hyphae with the same growth habits as *Streptomyces* [29–32]. While the translocation of nutrients in *Streptomyces* hyphae has not been shown unequivocally, this model of phenotypic heterogeneity would suggest translocation was also a feature of hyphal Actinomycetota. If so, it would also suggest that localisation of primary metabolism may be associated with extending apical tips. This is an area that has received little attention in *Streptomyces*, but spatial organisation has been found in terms of DNA replication [33] and transcriptional activity [34] in actively growing hyphae. They have also been shown to modulate their transcriptionally active foci in response to nutrient stress, such as nitrogen starvation (stringent response; [34]). The dynamic localisation of transcriptional activity would also suggest that apically extending tips are sensing and responding to the availability of metabolites, liberated through the localised secretion of hydrolytic enzymes. This would support the idea that primary metabolism can be spatially partitioned either as shown for *C. glutamicum* or in the form of the metabolic channelling.

The results from primary metabolism suggest the possibility that natural product biosynthesis is also partitioned and localised. This is well established in fungal specialised metabolites via biosynthesis and shuttling of precursors [35] but is less well understood, although not unknown, in *Streptomyces*, for example, the localisation of pigmented natural products, such as the prodigiosins [28]. While the localisation of the large multimodular megasynthases of polyketide and nonribosomal peptide biosynthesis is implicit, given the iterative, assembly line nature of their production [36]. There is some evidence of specialised metabolic channelling [37] where multienzyme complexes help to protect substrates and facilitate metabolic channelling. This could be important, given the capability of many *Streptomyces* species to produce numerous natural products from BGCs encoded in their genomes, with metabolic channelling limiting crosstalk/mistargeting of biosynthetic enzymes. This may be less obvious for other natural products where discrete, single-protein enzymology is responsible for biosynthesis and where protein–protein interactions are short lived or weak and may be triggered by transient intermediate metabolites. Yet, it is likely that localisation of biosynthetic enzymes would be beneficial, given the propensity for genes in BGCs to be promiscuous in their substrate preference (see below). Localisation of biosynthesis would also facilitate the provision of key primary metabolites that are required as building blocks, with abundant examples of duplicated primary

metabolic enzymes being found in BGCs [38]. While evidence for colocalisation of biosynthetic enzymes is limited, the biosynthesis of the lantibiotic, nisin, was shown to be highly localised at the old cell poles in *Lactococcus lactis* [39]. This suggests that production is closely associated with the ABC transporter that was responsible for the secretion of the mature lanthipeptide; however, visualisation of these complexes was only possible with the use of inactive nisin exporters [35]. More recently, using *Bacillus subtilis* as a heterologous host, biosynthetic proteins responsible for nisin biosynthesis (the NisBTC biosynthetic complex) were shown to colocalise at the membrane [40]. This level of localisation is yet to be shown for *Streptomyces*, but the spatially restricted induction of development [41], secretion and production of natural products [28,42,43] that has been observed in some strains suggests that this is a major gap in our knowledge of phenotypic heterogeneity in *Streptomyces* and is likely a fruitful area of investigation.

## How metabolic diversity and precursor supply can drive functional heterogeneity

The canonical view that one gene leads to one protein with one function is now known to be incomplete [44]. For instance, moonlighting proteins, where one gene can have multiple functions with different temporal and spatial resolutions, are pervasive [45]. An analogous process may be true at the metabolic level, with enzyme promiscuity adding further diversity upon which evolution can operate. In secondary metabolism, metabolites are actually produced as clouds of related molecular species even when encoded by a single BGC, and secondary metabolites can have multiple biological or ecological functions. The intertwined relationship of these observations is developed below in the context of *Streptomyces* heterogeneity.

It is well known that growth conditions alter the metabolite profile or biosynthetic potential of *Streptomyces* [46]. This chemical diversity has been partially explained by the large number of BGC encoding pathways in the genomes of these microorganisms [47]. In addition, the wealth of knowledge about *Streptomyces* biosynthetic enzymes has revealed that enzyme promiscuity, the ability to accept and convert many substrates into an equally large number of products, is common. The latter is prominent in mega synthetases directing the synthesis of polyketides and peptides, but this feature is present in any *bona fide* natural product biosynthetic enzyme [48,49]. These features suggest the possibility that chemical diversity within *Streptomyces* colonies has been vastly underestimated. Two general questions derive from this: How large, and spatially distributed, is the chemical diversity encoded by known BGCs in colonies of single strains in different environmental conditions? What is the correspondence between chemical or metabolic diversity and genotypic diversity of BGCs across *Streptomyces* strains of the same population?

Although little work has been done to answer these questions, heterogeneity of the *Streptomyces* siderophores desferrioxamine (dFO) may be illustrative. In this system, dFOs exist in multiple forms (linear, cyclic), which can include different chemical moieties (aliphatic, aromatic), known to be produced in response to iron limitation but also by unknown factors [50–53]. Although the biological roles of these different dFO forms are not fully understood, it is known that some dFO variants (e.g. linear vs cyclic) have different morphological and developmental effects on the colony [54–56]. The synthesis of these metabolites, in turn, is regulated by complex pleiotropic networks [57,58] and responds to the presence of other coexisting siderophores and trace elements [59,60]. Interestingly, dFOs have been directly implicated in the recently identified explorer cell type [5,61], a *Streptomyces* developmental innovation leading to rapid colony expansion. dFOs can also play different roles as both siderophores and antibiotics [62] by means of different, yet related, biomolecular activities. This latter scenario is reminiscent of the siderophores produced by *Pseudomonas aeruginosa* [63] and also of the coumarins and phenazines in plant–*Pseudomonas* interactions [64], which highlights both multiple metabolites *and* multiple functions, as siderophores and redox-active metabolites.

Metabolic heterogeneity finds an additional layer of complexity during the interaction between primary and secondary metabolism, for instance, between fatty acid and polyketide biosynthesis [65]. This includes two well-documented observations: (1) natural products precursor-supply biosynthetic enzymes are frequently contained within BGCs; and (2) central metabolic enzymes are subject to different metabolic flux constraints as they serve both central and natural products metabolic pathways. For instance, extra gene copies of most glycolytic enzymes have been found in *Streptomyces* species [66]. This observation was expanded in detail after experimental characterisation of the two constitutively expressed *S. coelicolor* pyruvate kinases, Pyk1 and Pyk2. These paralogous enzymes have the same enzyme activity, yet they have different impacts on the production of known natural products [67]. Given that this observation was attributed to a subtle metabolic regulatory mechanism related to cofactor specificity and allosteric enzyme regulation, it is not difficult to imagine how this could drive metabolic heterogeneity. For instance, the complete removal of BGCs triggers the overproduction of other unrelated secondary metabolites, including both known and previously unnoticed metabolites, as in the discovery of the polyketide alkaloid and yellow-

pigmented coelimycin in *S. coelicolor* [68]. These observations are typically explained as a reconfiguration of the metabolic fluxes in the context of the interaction between primary and secondary metabolism. Similar effects can be anticipated to occur naturally.

Despite advances in *Streptomyces* synthetic and systems biology [69], predicting metabolic outcomes is challenging even in straightforward situations. Consider the overexpression of amino acid biosynthetic pathways aimed at increasing precursor supply, such as the *proABC* genes, needed to synthesise proline used during prodiginines biosynthesis in *S. coelicolor* [70]. In this case, and probably others, this is complicated by at least two factors. First, the product of *proC*, pyrroline-5-carboxylate reductase, was found to be promiscuous in *Streptomyces* and generates crosstalk with the biosynthesis of branched-chain amino acids via the reductase activity of *ilvC* [71]. Second, extra copies of the 'same' precursor-supply or central metabolic genes can also be found within BGCs, presumably to regulate precursor supply for the synthesis of the cognate natural product. Given the physical localisation of the precursor-supply enzyme genes within the BGC, it is reasonable to assume that these enzymes colocalise, forming a metabolic continuum from precursor supply to natural product biosynthesis. However, it is more often the case that the extra central metabolic precursor-supply genes are not sufficient to reconstitute a complete pathway leading to the starting precursor or intermediary. Many examples of this scenario exist in *Streptomyces*, including the branched-chain amino acid gene *ilvC* in the valanamycin BGC of *S. viridifaciens* [72] and the aromatic amino acid *trp* and *aro* biosynthetic genes in the BGC of the calcium-dependent antibiotic (CDA) produced by *S. coelicolor*. Despite the latter being a model lipopeptide produced by a model strain, the outcome of the interaction between precursor synthesis and production of the different CDA lipopeptides [73–75] cannot be predicted. All together, these metabolic interactions highlight crosstalk between primary and secondary metabolism, which could drive colony heterogeneity based on different metabolic outcomes.

## Adaptive benefits of division of labour

In addition to the mechanistic and structural causes of heterogeneity raised above, an additional factor that can underlie within-population heterogeneity at the level of entire colonies is division of labour, where different cells adopt distinct, often mutually incompatible, phenotypes. Division of labour is increasingly studied in unicellular microbes as a means of understanding cooperative dynamics and differentiation [76,77]. However, *Streptomyces* are quite different from unicellular bacteria because their multicellular growth allows them to behave more like a single integrated body than a collection of independent cells [3]. Moreover, their strict clonality reduces the risks of 'cheaters' that profit at the expense of the rest of the colony. These features contribute to their ability to differentiate into multiple specialised cell types [78].

As we have reviewed elsewhere, *Streptomyces* colonies are the bacterial exemplar for a temporal division of labour between vegetative hyphae and spores [3,79]. In addition, metabolic division of labour has recently been detected in *Streptomyces coelicolor* whereby some cells become specialised to produce antibiotics, while others specialise on growth or sporulation [80–82]. These tasks are essential for colony competitiveness and reproduction, respectively, yet one task strongly reduces the ability to perform the other. A division of labour therefore partially offsets this trade-off and allows colonies to maximise the output of each.

Given the importance of sporulation and antibiotic production, it is perhaps not surprising that there are at least two mechanisms to regulate this division of labour. One route involves an irreversible type of differentiation that results from large deletions at the ends of the *Streptomyces* linear chromosomes [80,81]. By mechanisms that remain obscure, these large deletions result in a subpopulation of cells in *S. coelicolor* that show higher expression and increased secretion of antibiotics, especially actinorhodin, coelimycin and CDA [81,83] that, via diffusion, can benefit the whole colony. Because different deletions give rise to diverse outcomes with respect to antibiotic production [81,83], this process may also behave as a type of bet-hedging to pre-emptively respond to unknown competitors. Although chromosome instability appears to be broadly conserved across the genus, it remains to be seen if this process always results in a division of labour. A second route to division of labour for antibiotic production involves the transient phenotypic shifts that arise during colony development [82]. Using reporters for the developmental regulator *bldN* and the antibiotic undecylprodigiosin regulatory protein *redZ*, Zaccharia et al. observed temporal waves of expression that radiated from the centre towards the edge of the developing colony. Intriguingly, they found that these spatial patterns were abolished when colonies were grown on surfaces that removed (or homogenised) diffusing gradients beneath the colonies, indicating the critical importance of positional cues. As yet, it remains unknown how general either mechanism is for division of labour and whether they extend to other species or other 'public goods', for example, exoenzyme secretion or siderophores.

## Competitive drivers of heterogeneity

One of the most important realisations from the *Streptomyces* genomics era is the fact that their genomes

encode many more pathways for secondary metabolites than are known to be expressed in laboratory culture [84]. This has led to a comprehensive effort to identify conditions to elicit these silent BGCs with the hope that some encode new and valuable products. Cocultures, whereby *Streptomyces* strains are grown together with other strains, have proven a valuable, if idiosyncratic, tool in this effort [85,86]. The idea behind cocultures is intuitive: natural environments are diverse, and so BGC may require cues from the 'real world' to be induced. It also builds on the evolutionary framework of 'competition sensing' [87], which argues that metabolically expensive antibiotics should be kept in abeyance until they are elicited by recognisable threats or damage from competitors. Although this framework has been refined in various ways, it has generally been well supported with streptomycetes grown adjacent to other streptomycetes [88–90], bacterial species [91] or fungi [92,93]. A significant fraction of interactions among essentially random strains leads to altered or increased antibiotic production. For example, Uede et al. [94] found that nearly all the *Streptomyces* strains collected from a single soil sample in Japan could increase or broaden antibiotic activity of at least one other strain from the sample. More recently, Abrudan et al. [90] and Westhoff et al. [88] found similar results: nearly all strains, whether isolated from the same soil sample or not, induced at least one other strain, while reciprocally, virtually all strains were induced by at least one other. It is notable that these results are based on the ability of one strain to inhibit another and may therefore underestimate metabolic changes that might be detected with metabolomic profiling.

Although it is clear that interstrain interactions mediated by diffusible compounds are ubiquitous, the mechanisms and spatial localisation of these interactions are less consistent. Broadly, the probability that one strain modifies antibiotic production in another scales with genetic relatedness, and more specifically by whether or not they share related BGCs [88]. In other studies, antibiotic induction, as well as developmental changes, are caused by subinhibitory concentrations of antibiotics (with or without coculture) [95] mycolic acid [96] and possibly by quorum-dependent signals for antibiotic production [88]. The diversity of inducing cues suggests that multiple mechanisms have evolved to detect and respond to competition-mediated stressors in nature.

Antibiotic induction due to resource heterogeneity or scarcity is less well studied, but there is mounting evidence that competition for iron can play a decisive role in BGC induction and development, and, moreover, that changes in BGC expression can be spatially localised within *Streptomyces* colonies [41]. Using MALDI-based imaging mass spectrometry (MS), Traxler et al. [41] showed that siderophore secretion by a neighbouring actinomycete caused increased production of pigmented antibiotics and siderophores in *S. coelicolor*. Strikingly, this response was highly spatially localised at the interface between colonies. It was also highly temporally variable; at least 12 distinct analogues of acyl-dFO were expressed in series as the colonies grew. Similar responses have been seen in *Streptomyces* interactions with *Myxococcus* [97], suggesting that competition for iron may be a general cause of within-colony temporal and spatial heterogeneity.

These examples highlight the strong likelihood that resource and competitive stress driven by external factors can cause *Streptomyces* colonies to adopt spatially and temporally variable phenotypes. It is important to note, however, the limitation that these experiments were conducted in well-defined and otherwise homogeneous laboratory environments. The scale of environmental heterogeneity or diffusion of, for example, siderophores or antibiotics in nature remains largely unknown.

## Conclusion

Although there is compelling evidence for within-colony heterogeneity at different spatial scales, much of what we have discussed is based on results from other systems and conjecture. More importantly, there is limited evidence that these sources of phenotypic variation are adaptive or have evolved in order to generate within-colony heterogeneity. We highlight several questions that we hope will motivate further work in this area: (1) what is the spatial scale of within-colony heterogeneity and is this the same for exoenzymes, antibiotics and siderophores?; (2) how diverse are the products of a single BGC, what fraction of these products are functionally distinct, and is diversity the product of natural selection or of enzymatic imprecision?; (3) which public goods are coordinated by division of labour and are these regulated by genome deletions or by other mechanisms known to drive heterogeneity in other bacteria?; (4) how common, and over what temporal and spatial scales, are metabolites translocated within the mycelium?; (5) how are cues from resources and competitors integrated to drive spatially regulated adaptive responses?; (6) how do these processes unfold in the complex biotic and abiotic environments of natural soils? Some of these questions can be addressed with current methodologies, but many represent biological and technical challenges. Among others, there is a considerable need to develop fluorescent metabolic probes to track uptake and transport of metabolites, higher resolution methods to investigate mRNA localisation and translational activity with spatial and temporal resolution in colonies, and increased sensitivity of desorption electrospray ionisation (DESI) and Matrix Assisted Laser Desorption/Ionization (MALDI) imaging MS to understand the distribution of natural product production and variation in response to a range

of conditions beyond competitive scenarios. Finally, growing these organisms in conditions that better reflect their natural growth environment and habits may reveal the nature, context and adaptive benefits of heterogeneity. Some progress has been made in this area on the development of transparent soil systems for imaging [98,99], but the application of DESI-MS in these systems is yet to be attempted.

## Data Availability

No data were used for the research described in the article.

## Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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