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Unlocking the trove of metabolic treasures: activating silent biosynthetic gene clusters in bacteria and fungi

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Bacteria and fungi are prolific producers of secondary metabolites, yet they house a multitude of silent biosynthetic gene clusters that are poorly expressed and whose products are unknown or 'cryptic'. Stimulating the expression of these clusters and accessing their associated molecules is a major priority, as they are expected to have a veritable cornucopia of bioactivites. Here, we highlight three strategies that have been the focus of recent developments. Co-culture and elicitor screening, genetic regulator investigation and exploitation, and pathway refactoring and heterologous cluster expression, are collectively being employed to activate the expression of cryptic biosynthetic gene clusters (BGCs), and stimulate the production of novel metabolites having diverse activities.

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Secondary metabolites are not essential for microbial viability, and instead are presumed to confer a competitive fitness advantage to the producing organism under specific conditions. Many bacteria and fungi have been deemed 'gifted' producers of secondary metabolites, courtesy of their ability to synthesize a broad repertoire of these compounds, which include molecules having medical and agricultural application (e.g. antibiotics, antifungals, antitumor, immunosuppressants, etc.). Among bacteria, the actinobacteria, and particularly the streptomycetes, are renowned for their metabolic capabilities [1], although groups like the *Burkholderia* [2], myxobacteria [3] and cyanobacteria [4] are also prolific producers of secondary metabolites. Within the fungi, secondary metabolism predominates among the filamentous *Ascomycetes* and *Basidiomycetes* [5].

The arrival of the genomic era led to the realization that the secondary metabolic production potential of these microbes was in fact far more extensive than anyone had appreciated. Indeed, the vast majority of secondary metabolite biosynthetic gene clusters (BGCs) are poorly expressed in lab environments, and consequently, their corresponding products are largely unknown, or 'cryptic'. Stimulating the synthesis of novel silent and otherwise cryptic metabolites has been a major priority for both academia and industry since the discovery of this vast, untapped metabolic repository.

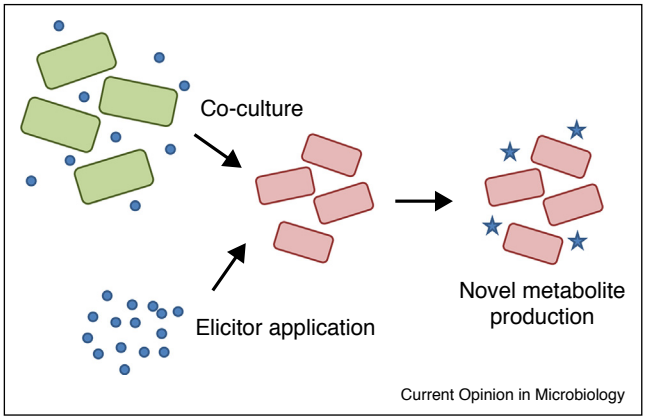
Many creative strategies have been developed to promote silent BGC expression and identify their corresponding metabolites. Recent advances have been concentrated in three areas: (i) ecological or chemical genetic strategies that rely on metabolic exchange or small molecule application to stimulate secondary metabolism; (ii) genetic approaches that leverage our understanding of the regulatory networks governing secondary metabolism; and (iii) synthetic strategies that involve manipulating BGCs in their native background, or capturing clusters and overexpressing them in heterologous hosts.

Exploiting small molecules and nutrients

Microbes use secondary metabolites as tools for communication [6], and as chemical weapons during competition for resources [7]. In their natural environments, sensing the presence of other microbes – and thus competitors for nutrients – is predicted to be one of the cues that stimulate the production of otherwise poorly expressed secondary metabolites. This idea underlies both the co-culture approach to stimulating silent metabolite production, and strategies that mimic such interactions through chemical elicitor application or nutrient manipulation (Figure 1).

Successfully exploiting the effect of chemical elicitors can be achieved using different high-throughput strategies (e.g. Ref. [8]). A recent work involved subjecting *Streptomyces albus* to a wide array of small molecule effectors [9•]. Two compounds – etoposide and ivermectin – led to the production and identification of 14 distinct metabolites having varying bioactivities (*e.g.* antifungal and anti-cancer properties) [9•]. Reciprocal approaches have involved applying a single validated elicitor to a library of bacterial isolates, and screening these for new activities or new metabolites (e.g. Ref. [10•]). Coupling such elicitor screens with imaging mass spectrometry has the potential to provide sensitive detection of newly stimulated metabolites in a wide range of microbes [11].

Figure 1



Activating new secondary metabolism by simulating community interactions. Microbes use metabolites for communication and competition purposes. Co-culturing different microbes together (top left, producing circle metabolites), or simulating co-culture through the application of chemical elicitors (bottom left; blue circles), can effectively stimulate a target bacterium/fungi to produce otherwise silent or cryptic metabolites in response (blue stars).

Chemical elicitors come in many forms. Heavy metals can effectively stimulate secondary metabolism [12], and this characteristic was recently exploited to yield a new (otherwise silent) angucycline-type antibiotic, that was produced in response to nickel supplementation by a marine streptomycetes [13]. Many chemical elicitors are themselves bioactive natural products. For example, ivermectin, recently found to activate new metabolites in *S. albus* [9•], is an anti-parasitic compound derived from the *Streptomyces avermitilis* product avermectin [14]. Components and derivatives of bacterial cell walls (e.g. *N*-acetylglucosamine, mycolic acids), can promote novel metabolite production when applied to cultures directly [15] or as a result of interaction during co-culture experiments [16]. This was observed recently with the co-incubation of *S. coelicolor* M145 with *Amycolatopsis* sp. AA4, where accumulation of galactose (or application of *N*-acetylglucosamine) activated the production of the new antibiotic amycomicin [17•].

In fungi, many metabolic elicitors promote epigenetic modification. Histone deacetylase inhibitors and DNA methylation inhibitors are particularly potent activators of otherwise repressed BGCs (reviewed in Ref. [18]). Notably, fungal secondary metabolism can also be stimulated by bacterial-triggered epigenetic changes, as was reported for *Aspergillus nidulans* in response to physical interaction with the bacterium *Streptomyces rapamycinius* [19]. Co-culturing these two microbes leads to increased acetylation of histone H3 in *A. nidulans*, altered expression of a globally acting transcription regulator, and ultimate activation of a formerly cryptic biosynthetic cluster (for orsellinic acid) in *A. nidulans* [20••].

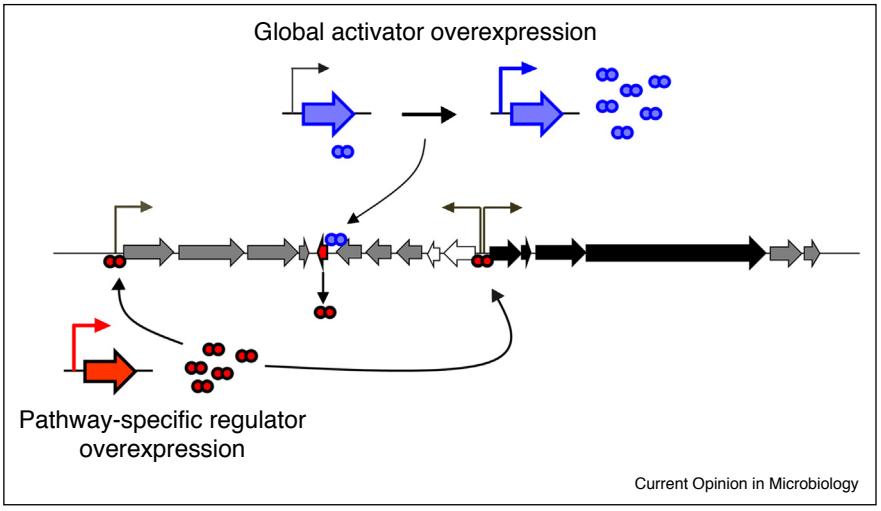
Applying histone deacetylase inhibitors (e.g. sodium butyrate) to *Streptomyces* cultures can also stimulate antibiotic production [21]. Interestingly, this phenomenon extends to *Streptomyces* relatives as well, where sodium butyrate enhanced production levels of the novel antifungal polyene selvamicin in *Pseudonocardia* [22]. While bacteria do not have histones, and consequently these compounds cannot be acting in the same way as in fungi, it will be interesting to see whether disrupting the native chromosome architecture in bacteria also serves to activate the production of new secondary metabolites.

Manipulating the genetic control of secondary metabolic clusters

Secondary metabolism in bacteria and fungi is tightly controlled, and secondary metabolic BGCs are subject to multi-level transcriptional regulation. Many of these BGCs encode dedicated cluster-situated regulators, and these directly control cluster expression [23,24]. Cluster-independent regulators are also pervasive, and these typically govern the expression of multiple BGCs [5,25,26] (Figure 2).

Overexpressing transcriptional activators, or deleting transcriptional repressors, has been a broadly successful approach to stimulating the production of secondary metabolites in bacteria and fungi (e.g. Refs. [27,28••,29]). Notably, however, the regulatory hierarchies governing BGC expression are complex and have not been fully fleshed out for any system. Consequently, global regulator manipulation frequently represents an unbiased approach to silent/cryptic cluster activation, similar to the chemical elicitor or co-culture-based strategies, in that it is not easy to predict which BGCs will be expressed and what molecules will be produced. In bacteria, global regulators typically target cluster-situated regulators, and further control those clusters lacking a dedicated regulator (e.g. Refs. [28••,30,31]). New regulators are continually being identified, and a recent addition to the global metabolic regulator repertoire in the actinobacteria is the conserved MtrAB two component regulatory system. In *Streptomyces venezuelae*, MtrAB govern chloramphenicol biosynthesis [32], while in *Streptomyces coelicolor*, this system directly controls the production of multiple antibiotics [33]. In *Burkholderia*, a conserved LysR regulator dubbed ScmR was recently shown to repress the expression of multiple cluster-situated regulators and their corresponding clusters; its deletion led to the activation of uncharacterized BGCs [28••]. BGC activation has recently been achieved using 'transcription factor decoys', whereby the regulatory regions associated with cluster-situated regulators are cloned onto multi-copy plasmids. These sequences can sequester negative global regulators and enable cluster expression, and detection and characterization of its associated metabolite [34].

Figure 2



Genetic strategies to activating the expression of silent BGCs. The overexpression of global metabolic regulators (encoded outside of the BGC and usually targeting the cluster-situated regulatory genes; top) and cluster-situated regulators (bottom) has proven to be a powerful means of activating the transcription of uncharacterized BGCs.

Within the fungi, secondary metabolism is broadly controlled by the velvet family of regulators, and these in turn associate with the ubiquitous LaeA methyltransferase, which itself influences secondary metabolism and cryptic BGCs in many fungi [35]. As in bacteria, identifying new global regulators is an active area of research, with recent discoveries including novel activators [e.g. Refs. 15,28••] and repressors [e.g. Ref. 29] of secondary metabolism.

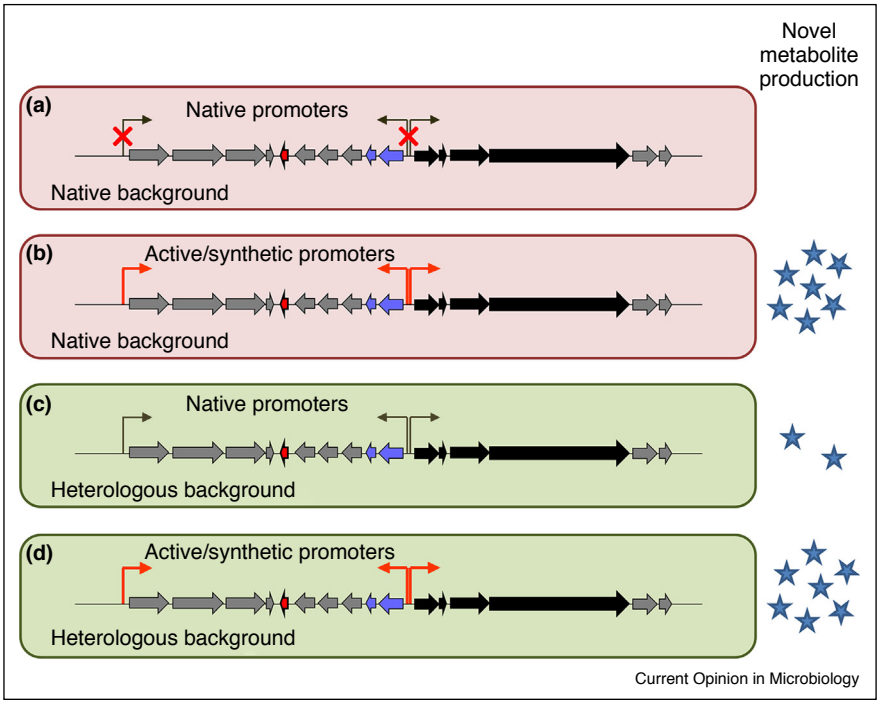
In contrast to the global regulators, exploiting the activity of cluster-situated regulators represents a more targeted approach to stimulating the expression of BGCs of interest. In fungi, ~50% of secondary metabolic BGCs encode cluster-situated regulators [5]. These regulators are typically members of the fungal-specific Zn_2Cys_6-family of transcription factors, although other family types, both fungal-specific and eukaryotic-specific, have also been identified [26,36,37]. In bacteria, cluster-situated regulators typically fall into a handful of transcription factor classes (e.g. Ref. [23]); however, novel regulatory classes are still being uncovered. In *Streptomyces lincolnensis*, the newly discovered cluster-situated activator of the lincomycin A biosynthetic cluster, LmbU, shares little sequence or predicted structural similarity with other transcription factors, and appears to represent a new regulator family [38••,39]. Importantly, LmbU homologues are associated with secondary metabolic clusters throughout the actinobacteria.

There is still much to be learned about the control of BGCs. As the wealth of sequence and genetic/genomic information continue to emerge, the discovery of new regulators and regulatory classes will provide new avenues and opportunities to promote silent or cryptic BGC expression.

BGC refactoring and heterologous expression

Stimulating new secondary metabolites by manipulating genetic regulators, while highly effective, requires some knowledge of the regulatory cascades governing metabolite synthesis. An alternative approach to activating these clusters involves replacing 'inactive' native promoters and associated regulatory elements, with highly active natural (e.g. Ref. [40]) or synthetic (e.g. Refs. [41,42]) variants (Figure 3). One of the first applications of promoter swapping led to the successful activation of multiple classes of formerly cryptic BGCs in diverse *Streptomyces* species, including type I, II, and III polyketide synthases (PKS), non-ribosomal peptide synthases (NRPS), hybrid PKS-NRPS, and phosphonate clusters [43••]. This sort of genetic manipulation is now being achieved using CRISPR/Cas9-mediated homology-directed repair. A range of CRISPR-based systems are now being unveiled, including systems with a catalytically inactive (dead) Cas9 enzyme (dCas9) for use in CRISPR-mediated repression of target genes (CRISPR-interference or CRISPRi) [44,45]. CRISPRi has been successfully used to manipulate antibiotic production in the model species *Streptomyces coelicolor* [44]. More recently, an alternative enzyme system has been developed around the Cpf1 nuclease, which should expand the genome editing potential in the streptomycetes [46•]. Historically, in non-model BGC-containing bacteria there have been fewer tools available for cluster activation; however, this is gradually changing. For example, in *Burkholderia*, a recently discovered recombination system is now

Figure 3



Novel metabolite production as a result of pathway refactoring and/or heterologous expression. The promoters of a transcriptionally silent BGC **(a)** can be swapped out for more active variants **(b)**, and this can lead to expression of the cluster and production of the associated metabolite (blue stars). The cluster can also be cloned and introduced into a heterologous host **(c)**, and sometimes this altered environment can be enough to alleviate the transcriptional repression/stimulate expression of the BGC. In this heterologous system, the cluster can be subjected to promoter alteration or other genetic editing **(d)** in an effort to further enhance metabolite yield.

enabling promoter refactoring and accessing of otherwise cryptic metabolites [47••]. CRISPR-based genome editing technologies are being developed for different fungi [48], but they have yet to gain wide-spread use in stimulating silent BGC expression.

Refactoring or modifying silent BGCs in their native hosts represents the ideal situation; however, not all microbial hosts can be manipulated genetically, at least using the currently available genetic tools. Clusters of interest in these systems can, however, be cloned/captured and introduced into heterologous hosts for manipulation and overexpression, and strategies for doing this have been reviewed recently [49].

To date, a universal host for heterologous cluster expression has proven elusive: fungal clusters are typically expressed best in fungal hosts, while bacterial clusters are thought to be most effectively induced in related hosts, although recent findings are starting to challenge this view [50•]. Extensive efforts have been dedicated to developing *Escherichia coli* as a heterologous system; however, it lacks key metabolic enzymes needed to generate sufficient precursors, and while progress is being made, it is not yet a viable option for many metabolites [51]. A number of *Streptomyces* 'chassis strains' have been developed, including genome minimized strains of *S. avermitilis* [52] and *S. albus* [53], and a metabolically simplified strain of *S. coelicolor* [54]. These have enabled the activation of a wide range of cryptic natural products, including lavendiol (from *S. lavendulae*) [55], venemycin (from *S. venezuelae*) [56] and fralnimycin (from *Frankia* spp.) [53].

In recent years, several cyanobacterial strains have been developed for the purposes of high-level metabolite production. *Anabaena* 7120 has been successfully employed for the production of the *Moorea producens* product lyngbyatoxin A [57], while a *Synechococcus*-based platform has been developed to produce polyketide synthase-derived products [58]. Similarly, multiple *Aspergillus* species have been developed for heterologous BGC expression, and include *Aspergillus oryzae* [59], *Aspergillus niger* [60] and *A. nidulans* [61]. *Saccharomyces cerevisiae* is also emerging as the heterologous host of choice for many fungal-derived biosynthetic clusters. A recent large scale developmental effort by Harvey *et al.* [62••] yielded a *S. cerevisiae* strain with improved growth kinetics and genetic stability, and a suite of autoinducible promoters with delayed induction characteristics and a range of expression levels. Impressively, when

these promoters were used to refactor a library of uncharacterized fungal BGCs, and these were then introduced into the improved *S. cerevisiae* strain, >40 new metabolites were identified [62••].

Considering the explosion of heterologous host strains that are being developed, it is worth noting that the same biosynthetic cluster, when introduced into different hosts, does not yield equivalent metabolic product levels [63], suggesting that achieving optimal induction and yields for any cluster of interest remains a highly empirical process.

Conclusions

The onset of the genomic era has revealed many hidden treasures, with the sheer abundance of metabolic potential hidden within the vast numbers of silent and cryptic clusters being a major discovery. These clusters are rapidly beginning to lose their cryptic designation, as ecological (simulating microbial competition), genetic (pathway and global regulators) and synthetic (promoter refactoring, cluster capture, and heterologous expression) strategies are collectively allowing us to make significant inroads into accessing this rich metabolic reservoir. Combining strategies, and leveraging new technologies, will undoubtedly allow us to more comprehensively mine this increasingly precious metabolic resource.

Conflict of interest statement

Nothing declared.

Acknowledgements

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References and recommended reading

Papers of particular interest, published within the period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Hopwood DA: ***Streptomyces***. *Nature and Medicine: The Antibiotic Makers*. Oxford University Press; 2007.
2. Esmaeel Q, Pupin M, Jacques P, Leclère V: **Nonribosomal peptides and polyketides of *Burkholderia*: new compounds potentially implicated in biocontrol and pharmaceuticals**. *Environ Sci Pollut Res* 2018, **25**:29794-29807.
3. Weissman KJ, Müller R: **Myxobacterial secondary metabolites: bioactivities and modes-of-action**. *Nat Prod Rep* 2010, **27**:1276-1295.
4. Burja AM, Banaigs B, Abou-Mansour E, Burgess JG, Wright PC: **Marine cyanobacteria - a prolific source of natural products**. *Tetrahedron* 2001, **57**:9347-9377.
5. Keller NP: **Fungal secondary metabolism: regulation, function and drug discovery**. *Nat Rev Microbiol* 2019, **17**:167-189.
6. Romero D, Traxler MF, López D, Kolter R: **Antibiotics as signal molecules**. *Chem Rev* 2011, **111**:5492-5505.
7. Davies J: **Specialized microbial metabolites: functions and origins**. *J Antibiot* 2013, **66**:361-364.
8. Seyedsayamdost MR: **High-throughput platform for the discovery of elicitors of silent bacterial gene clusters**. *Proc Natl Acad Sci U S A* 2014, **111**:7266-7271.
9. • Xu F, Nazari B, Moon K, Bushin LB, Seyedsayamdost MR: **Discovery of a cryptic antifungal compound from *Streptomyces albus* J1074 using high-throughput elicitor screens**. *J Am Chem Soc* 2017, **139**:9203-9212.

Describes an interesting high-throughput screening approach using chemical elicitors targeting a single microbe. This approach permits comprehensive metabolic profiling of an organism of interest.

10. • Craney A, Ozimok C, Pimentel-Elardo SM, Capretta A, Nodwell JR: **Chemical perturbation of secondary metabolism demonstrates important links to primary metabolism**. *Chem Biol* 2012, **19**:1020-1027.

Investigation that laid the groundwork for high-throughput screening using chemical elicitors as a tool for activating cryptic metabolism in the streptomycetes.

11. Xu F, Wu Y, Zhang C, Davis KM, Moon K, Bushin LB, Seyedsayamdost MR: **A genetics-free method for high-throughput discovery of cryptic microbial metabolites**. *Nat Chem Biol* 2019, **15**:161-168.
12. Weinberg ED: **Roles of trace metals in transcriptional control of microbial secondary metabolism**. *Biol Met* 1990, **2**:191-196.
13. Akhter N, Liu Y, Auckloo BN, Shi Y, Wang K, Chen J, Wu X, Wu B: **Stress-driven discovery of new angucycline-type antibiotics from a marine *Streptomyces pratensis* NA-ZhouS1**. *Mar Drugs* 2018, **16** pii: E331.
14. Campbell WC, Fisher MH, Stapley EO, Albers-Schönberg G, Jacob TA: **Ivermectin: a potent new antiparasitic agent**. *Science* 1983, **221**:823-828.
15. Rigali S, Titgemeyer F, Barends S, Mulder S, Thomae AW, Hopwood DA, van Wezel GP: **Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces***. *EMBO Rep* 2008, **9**:670-675.
16. Onaka H, Mori Y, Igarashi Y, Furumai T: **Mycolic acid-containing bacteria induce natural-product biosynthesis in *Streptomyces* species**. *Appl Environ Microbiol* 2011, **77**:400-406.
17. • Pishchany G, Mevers E, Ndousse-Fetter S, Horvath DJ, Paludo CR, Silva-Junior EA, Koren S, Skaar EP, Clardy J, Kolter R: **Amycomicin is a potent and specific antibiotic discovered with a targeted interaction screen**. *Proc Natl Acad Sci U S A* 2018, **115**:10124-10129.

A community-based co-culture screen that ultimately led to the identification of a pair-wise interaction that promoted cryptic antibiotic production by *Amycolatopsis*.

18. Gacek A, Strauss J: **The chromatin code of fungal secondary metabolite gene clusters**. *Appl Microbiol Biotechnol* 2012, **95**:1389-1404.
19. Nutzmann H-W, Reyes-Dominguez Y, Scherlach K, Schroeckh V, Horn F, Gacek A, Schumann J, Hertweck C, Strauss J, Brakhage AA: **Bacteria-induced natural product formation in the fungus *Aspergillus nidulans* requires Saga/Ada-mediated histone acetylation**. *Proc Natl Acad Sci U S A* 2011, **108**:14282-14287.
20. •• Fischer J, Müller SY, Netzker T, Jäger N, Gacek-Matthews A, Scherlach K, Stroe MC, García-Altares M, Pezzini F, Schoeler H *et al.*: **Chromatin mapping identifies BasR, a key regulator of bacteria-triggered production of fungal secondary metabolites**. *eLife* 2018, **7** pii: e4096.

Following up on the Nutzmann *et al.* [19] investigation, this work revealed the mechanistic basis underlying the cryptic metabolite activation stemming from *Aspergillus-Streptomyces* co-culture, courtesy of the activity of a methylation-dependent global transcription regulator.

21. Moore JM, Bradshaw E, Seipke RF, Hutchings MI, McArthur M: **Use and discovery of chemical elicitors that stimulate biosynthetic gene clusters in *Streptomyces* bacteria**. *Methods Enzymol* 2012, **517**:367-385.
22. Van Arnam EB, Ruzzini AC, Sit CS, Horn H, Pinto-Tomás AA, Currie CR, Clardy J: **Selvamicin, an atypical antifungal polyene from two alternative genomic contexts**. *Proc Natl Acad Sci U S A* 2016, **113**:12940-12945.

23. Bibb MJ: **Regulation of secondary metabolism in streptomycetes**. *Curr Opin Microbiol* 2005, **8**:208-215.

24. Brakhage AA: **Regulation of fungal secondary metabolism**. *Nat Rev Microbiol* 2013, **11**:21-32.

25. Martín JF, Liras P: **Engineering of regulatory cascades and networks controlling antibiotic biosynthesis in *Streptomyces***. *Curr Opin Microbiol* 2010, **13**:263-273.

26. Macheleidt J, Mattern DJ, Fischer J, Netzker T, Weber J, Schroeckh V, Valiante V, Brakhage AA: **Regulation and role of fungal secondary metabolites**. *Annu Rev Genet* 2016, **50**: 371-392.

27. Daniel-Ivad M, Hameed N, Tan S, Dhanjal R, Socko D, Pak P, Gverzdys T, Elliot MA, Nodwell JR: **An engineered allele of *afsQ1* facilitates the discovery and investigation of cryptic natural products**. *ACS Chem Biol* 2017, **12**:628-634.

28. •• Mao D, Bushin LB, Moon K, Wu Y, Seyedsayamdost MR: **Discovery of *scmR* as a global regulator of secondary metabolism and virulence in *Burkholderia thailandensis* E264**. *Proc Natl Acad Sci U S A* 2017, **114**:E2920-E2928.

Unlike in the actinobacteria, few global secondary metabolic regulators in *Burkholderia* have been identified. This work reports on the characterization of the global repressor ScmR, which targets multiple secondary BGCs, as well as virulence-associated genes.

29. Bok JW, Hoffmeister D, Maggio-Hall LA, Murillo R, Glasner JD, Keller NP: **Genomic mining for *Aspergillus* natural products**. *Chem Biol* 2006, **13**:31-37.

30. Gao C, Hindra, Mulder D, Yin C, Elliot MA: **Crp is a global regulator of antibiotic production in *Streptomyces***. *mBio* 2012, **3** pii: e00407-12.

31. McKenzie NL, Nodwell JR: **Phosphorylated AbsA2 negatively regulates antibiotic production in *Streptomyces coelicolor* through interactions with pathway-specific regulatory gene promoters**. *J Bacteriol* 2007, **189**:5284-5292.

32. Som NF, Heine D, Holmes NA, Munnoch JT, Chandra G, Seipke RF, Hoskisson PA, Wilkinson B, Hutchings MI: **The conserved actinobacterial two-component system MtrAB coordinates chloramphenicol production with sporulation in *Streptomyces venezuelae* NRRL B-65442**. *Front Microbiol* 2017, **8**:1145.

33. Som N, Heine D, Holmes N, Knowles F, Chandra G, Seipke R, Hoskisson P, Wilkinson B, Hutchings M: **The MtrAB two component system controls antibiotic production in *Streptomyces coelicolor* A3(2)**. *Microbiology* 2017, **3**:1-5.

34. Wang B, Guo F, Dong S-H, Zhao H: **Activation of silent biosynthetic gene clusters using transcription factor decoys**. *Nat Chem Biol* 2019, **15**:111-114.

35. Bayram Ö, Braus GH: **Coordination of secondary metabolism and development in fungi: The velvet family of regulatory proteins**. *FEMS Microbiol Rev* 2012, **36**:1-24.

36. Pfannenstiel BT, Zhao X, Wortman J, Wiemann P, Throckmorton K, Spraker JE, Soukup AA, Luo X, Lindner DL, Lim FY *et al.*: **Revitalization of a forward genetic screen identifies three new regulators of fungal secondary metabolism in the genus *Aspergillus***. *mBio* 2017, **8**:e01246-17.

37. Oakley CE, Ahuja M, Sun WW, Entwistle R, Akashi T, Yaegashi J, Guo CJ, Cerqueira GC, Russo Wortman J, Wang CCC *et al.*: **Discovery of McrA, a master regulator of *Aspergillus* secondary metabolism**. *Mol Microbiol* 2017, **103**:347-365.

38. •• Hou B, Lin Y, Wu H, Guo M, Petkovic H, Tao L, Zhu X, Ye J, Zhang H: **The novel transcriptional regulator LmbU promotes lincomycin biosynthesis through regulating expression of its target genes in *Streptomyces lincolnensis***. *J Bacteriol* 2018, **200** pii: e00447.

This work reveals the discovery of a new family of cluster-situated regulator governing antibiotic production in *S. lincolnensis*. Importantly, homologous regulators are found in BGCs throughout the streptomycetes.

39. Ju K-S, Zhang X, Elliot MA: **New kid on the block: LmbU expands the repertoire of specialized metabolic regulators in *Streptomyces***. *J Bacteriol* 2018, **200** pii: e00559-17.

40. Luo Y, Zhang L, Barton KW, Zhao H: **Systematic identification of a panel of strong constitutive promoters from *Streptomyces albus***. *ACS Synth Biol* 2015, **4**:1001-1010.

41. Bai C, Zhang Y, Zhao X, Hu Y, Xiang S, Miao J, Lou C, Zhang L: **Exploiting a precise design of universal synthetic modular regulatory elements to unlock the microbial natural products in *Streptomyces***. *Proc Natl Acad Sci U S A* 2015, **112**:12181-12186.

42. Horbal L, Siegl T, Luzhetskyy A: **A set of synthetic versatile genetic control elements for the efficient expression of genes in Actinobacteria**. *Sci Rep* 2018, **8**:491.

43. •• Zhang MM, Wong FT, Wang Y, Luo S, Lim YH, Heng E, Yeo WL, Cobb RE, Enghiad B, Ang EL *et al.*: **CRISPR-Cas9 strategy for activation of silent *Streptomyces* biosynthetic gene clusters**. *Nat Chem Biol* 2017, **13**:607-609.

This study paved the way for using CRISPR/Cas9 for the purposes of promoter refactoring, where it was used successfully to activate the expression of BGCs of multiple classes.

44. Tong Y, Charusanti P, Zhang L, Weber T, Lee SY: **CRISPR-Cas9 based engineering of actinomycetal genomes**. *ACS Synth Biol* 2015, **4**:1020-1029.

45. Zhao Y, Li L, Zheng G, Jiang W, Deng Z, Wang Z, Lu Y: **CRISPR/dCas9-mediated multiplex gene repression in *Streptomyces***. *Biotechnol J* 2018, **13**:e1800121.

46. • Li L, Wei K, Zheng G, Liu X, Chen S, Jiang W, Lu Y: **CRISPR-Cpf1-assisted multiplex genome editing and transcriptional repression in *Streptomyces***. *Appl Environ Microbiol* 2018, **84** pii: 300827-18.

Multiple CRISPR/Cas9 systems have been developed for use in *Streptomyces* sp. This work introduces a new nuclease, which has the potential to expand the gene editing potential in these bacteria.

47. •• Wang X, Zhou H, Chen H, Jing X, Zheng W, Li R, Sun T, Liu J, Fu J, Huo L *et al.*: **Discovery of recombinases enables genome mining of cryptic biosynthetic gene clusters in *Burkholderiales* species**. *Proc Natl Acad Sci U S A* 2018, **115**:E4255-E4263.

This work describes an important tool for use in exploiting the metabolic potential of the *Burkholderia* bacteria.

48. Hillman ET, Readnour LR, Solomon KV: **Exploiting the natural product potential of fungi with integrated -omics and synthetic biology approaches**. *Curr Opin Syst Biol* 2017, **5**:50-56.

49. Xu M, Wright GD: **Heterologous expression-facilitated natural products' discovery in actinomycetes**. *J Ind Microbiol Biotechnol* 2019, **46**:415-431.

50. • Zhang JJ, Tang X, Zhang M, Nguyen D, Moore BS: **Broad-host-range expression reveals native and host regulatory elements that influence heterologous antibiotic production in Gram-negative bacteria**. *mBio* 2017, **5**:e01291-17.

Most studies to date have suggested that the heterologous expression of BGCs is best achieved in related hosts. This work convincingly shows that this is not always the case.

51. Ahmadi MK, Pfeifer BA: **Recent progress in therapeutic natural product biosynthesis using *Escherichia coli***. *Curr Opin Biotechnol* 2016, **42**:7-12.

52. Komatsu M, Komatsu K, Koiwai H, Yamada Y, Kozone I, Izumikawa M, Hashimoto J, Takagi M, Omura S, Shin-Ya K *et al.*: **Engineered *Streptomyces avermitilis* host for heterologous expression of biosynthetic gene cluster for secondary metabolites**. *ACS Synth Biol* 2013, **2**:384-396.

53. Myronovskyi M, Rosenkränzer B, Nadmid S, Pujic P, Normand P, Luzhetskyy A: **Generation of a cluster-free *Streptomyces albus* chassis strains for improved heterologous expression of secondary metabolite clusters**. *Metab Eng* 2018, **49**:316-324.

54. Gomez-Escribano JP, Bibb MJ: **Engineering *Streptomyces coelicolor* for heterologous expression of secondary metabolite gene clusters**. *Microb Biotechnol* 2011, **4**:207-215.

55. Pait IGU, Kitani S, Roslan FW, Ulanova D, Arai M, Ikeda H, Nihira T: **Discovery of a new diol-containing polyketide by heterologous expression of a silent biosynthetic gene cluster from *Streptomyces lavendulae* FRI-5**. *J Ind Microbiol Biotechnol* 2018, **45**:77-87.

56. Thanapipatsiri A, Gomez-Escribano JP, Song L, Bibb MJ, Al-Bassam M, Chandra G, Thamchaipenet A, Challis GL, Bibb MJ:

Discovery of unusual biaryl polyketides by activation of a silent *Streptomyces venezuelae* biosynthetic gene cluster. *ChemBioChem* 2016, **17**:2189-2198.

57. Videau P, Wells KN, Singh AJ, Gerwick WH, Philmus B: **Assessment of *Anabaena* sp. strain PCC 7120 as a heterologous expression host for cyanobacterial natural products: production of lyngbyatoxin A**. *ACS Synth Biol* 2016, **5**:978-988.

58. Roulet J, Taton A, Golden JW, Arabolaza A, Burkart MD, Gramajo H: **Development of a cyanobacterial heterologous polyketide production platform**. *Metab Eng* 2018, **49**:94-104.

59. Sakai K, Kinoshita H, Nihira T: **Heterologous expression system in *Aspergillus oryzae* for fungal biosynthetic gene clusters of secondary metabolites**. *Appl Microbiol Biotechnol* 2012, **93**:2011-2022.

60. Gressler M, Hortschansky P, Geib E, Brock M: **A new high-performance heterologous fungal expression system based on regulatory elements from the *Aspergillus terreus* terrein gene cluster**. *Front Microbiol* 2015, **6**:184.

61. van Dijk JWA, Wang CCC: **Heterologous expression of fungal secondary metabolite pathways in the *Aspergillus nidulans* host system**. *Methods Enzymol* 2016:127-142.

62. •• Harvey CJB, Tang M, Schlecht U, Horecka J, Fischer CR, Lin HC, Li J, Naughton B, Cherry J, Miranda M *et al.*: **HEx: a heterologous expression platform for the discovery of fungal natural products**. *Sci Adv* 2018, **4**:eaar5459.
Representing a herculean body of work, this manuscript describes the optimizing of both expression systems and a *Saccharomyces cerevisiae* heterologous host, and employed these in producing 40+ cryptic secondary metabolites from a wide range of classes and organisms.

63. Beites T, Mendes MV: **Chassis optimization as a cornerstone for the application of synthetic biology based strategies in microbial secondary metabolism**. *Front Microbiol* 2015, **6**:906.