

## REVIEW

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# Streptomyces as a versatile host platform for heterologous production of microbial natural products

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Focus on 2004 to 2024

The rediscovery of natural products (NPs) as a critical source of new therapeutics has been greatly advanced by the development of heterologous expression platforms for biosynthetic gene clusters (BGCs). Among these, *Streptomyces* species have emerged as the most widely used and versatile chassis for expressing complex BGCs from diverse microbial origins. In this review, we provide a comprehensive analysis of over 450 peer-reviewed studies published between 2004 and 2024 that describe the heterologous expression of BGCs in *Streptomyces* hosts. We present a data-driven overview of expression trends across time, BGC types, donor species, and host strain preferences, offering the first quantitative perspective on how this field has evolved over two decades. Our review discusses the key factors influencing successful BGC expression in *Streptomyces*, including genomic integration strategies, regulatory elements, codon optimization, and precursor supply. We also examine the impact of synthetic biology tools, genome engineering, and host strain tailoring in overcoming common expression barriers. Special emphasis is placed on the role of heterologous expression in accessing silent or cryptic BGCs, elucidating biosynthetic pathways, and generating new-to-nature analogues through combinatorial biosynthesis. By integrating technological advances with practical case studies, we highlight how *Streptomyces*-based heterologous expression is enabling not only the efficient production of known compounds but also the discovery of structurally novel and biologically potent metabolites. This review aims to serve as a resource for researchers in natural products, synthetic biology, and drug discovery who seek to harness the full potential of microbial biosynthetic diversity.

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## 1. Introduction

Natural products represent a uniquely rich source of bioactive compounds, characterized by their high specificity for biological targets and structural complexity, offering a broader chemical space than most synthetic molecules.<sup>1,2</sup> These features make them exceptionally valuable for pharmaceutical, agricultural, and biomedical innovation. In light of growing antibiotic resistance and persistent unmet medical needs, the search for new and effective bioactive compounds remains a critical global challenge.<sup>3–6</sup>

Historically, actinomycetes have been prolific producers of natural products, yielding numerous clinical and commercial successes.<sup>7</sup> However, conventional approaches such as traditional bioactivity-guided screening and chemical profiling are now yielding diminishing returns, with the rediscovery of known molecules becoming a common and costly bottleneck.<sup>8,9</sup> This is largely because highly expressed, well-conserved BGCs tend to dominate in standard cultivation conditions.

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Advances in genome sequencing and mining have revealed a vast, untapped reservoir of cryptic and silent BGCs within actinobacterial genomes—many of which encode unknown secondary metabolites.<sup>10</sup> Unfortunately, these clusters are often not expressed under laboratory conditions or produce metabolites at levels too low for successful isolation and analysis. Unlocking this hidden biosynthetic potential requires a new paradigm: a robust heterologous expression platform capable of activating and producing these compounds in scalable quantities.<sup>11</sup>

One of the most promising strategies involves the systematic cloning, refactoring, and expression of BGCs in optimized microbial hosts (chassis strains).<sup>12</sup> This approach not only facilitates access to cryptic metabolites, but also enables: (1) discovery of entirely new bioactive compounds; (2) consistent production of known natural products previously limited by supply constraints; (3) biosynthetic tailoring and derivatization of valuable scaffolds; and (4) elucidation of complex biosynthetic pathways.

To implement such a platform effectively, three essential components must be in place: (1) a curated library of BGCs prepared for expression; (2) modular genetic elements for regulating expression; and (3) a panel of microbial hosts capable of reliably expressing diverse BGCs.<sup>13–17</sup>

Among potential hosts, *Streptomyces* strains stand out as the most suitable chassis for heterologous BGC expression.<sup>18,19</sup> This is due to several intrinsic advantages:

**Genomic compatibility:** *Streptomyces* share high GC content and codon usage bias with many natural BGC donors, reducing the need for extensive gene refactoring and codon optimization.

**Proven metabolic capacity:** these organisms naturally produce complex polyketides and non-ribosomal peptides and possess the necessary enzymatic machinery to support large and modular biosynthetic pathways.

**Advanced regulatory systems:** *Streptomyces* have evolved highly sophisticated regulatory networks that govern the expression of secondary metabolite BGCs. These include pathway-specific regulators, sigma factors, and global transcriptional regulators that can be co-opted or engineered to enhance BGC expression. This regulatory compatibility allows for efficient transcription and translation of heterologous BGCs, especially those from related actinobacterial sources, often without the need for extensive promoter replacement or rewiring.

**Tolerant physiology:** these bacteria can tolerate the accumulation of potentially cytotoxic secondary metabolites, making them ideal for producing bioactive compounds that inhibit growth in simpler hosts.

**Scalability:** fermentation processes for *Streptomyces* are well established, enabling smooth transition from lab-scale production to industrial biomanufacturing.

In contrast, standard model host microorganisms such as *Saccharomyces cerevisiae*, *Pseudomonas putida*, *Escherichia coli* and *Bacillus* offer fast growth and ease of manipulation but struggle with expression of large, GC-rich gene clusters, often lacking essential co-factors, resistance mechanisms, or tailoring enzymes.<sup>20–23</sup> Technological advancements in DNA capture and assembly have further accelerated the development of heterologous platforms. Conventional large-insert libraries using bacterial artificial chromosomes (BACs) provide access to entire BGCs, though constructing these libraries from high-GC actinomycete genomes remains labor-intensive. Faster alternatives like cosmid libraries offer technical ease but often miss large or complex gene clusters, especially those encoding modular PKS (polyketide synthase) or NRPS (non-ribosomal peptide synthetase) systems. To address this, recent innovations such as transformation-associated recombination (TAR), Cas9-assisted targeting of chromosome segments (CATCH), and linear-linear homologous recombination (LLHR) now allow direct, high-fidelity capture of entire BGCs from native chromosomes, streamlining the path to expression and characterization.<sup>24–28</sup>

Efficient heterologous expression of BGCs in actinomycetes relies not only on the compatibility of the host strain but also on the availability of well-characterized genetic control elements that can drive and fine-tune gene expression. Over the past

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decade, a large and expanding toolbox of such regulatory parts has been developed specifically for *Streptomyces* and other actinomycetes, enabling predictable and robust expression of native and heterologous genes.

A wide variety of constitutive and inducible promoters are now available, many of which have been engineered or selected for strength, tunability, and compatibility with GC-rich actinomycete genomes. These include strong constitutive promoters like ermEp and kasOp as well as synthetic variants with defined activity profiles.<sup>13,29,30</sup> In parallel, inducible systems responsive to tetracycline, thiostrepton, cumate, and other small molecules allow temporal and conditional control over gene expression—an important feature when expressing potentially toxic biosynthetic enzymes or pathways.<sup>31–36</sup>

Ribosome binding sites (RBSs) have also been systematically characterized in *Streptomyces*, with libraries available that allow modulation of translation efficiency across a wide dynamic range. Modular RBSs can be paired with synthetic or native promoters to fine-tune expression of individual genes within a cluster or pathway.<sup>14</sup>

In addition, a collection of well-defined transcriptional terminators is available to ensure transcriptional fidelity and prevent unwanted read-through between genes, which can be particularly important when expressing large multi-cistronic BGCs.<sup>14</sup>

The modularity of these regulatory parts facilitates the construction of synthetic operons and the refactoring of entire gene clusters for optimized expression in heterologous hosts. Combined with tools for CRISPR interference, recombineering, and plug-and-play DNA assembly (e.g., Golden Gate, Gibson), these elements form the backbone of advanced synthetic biology platforms in actinomycetes.

Together, these tools not only enable high-level expression of BGCs but also provide precise control over gene dosage, timing, and stoichiometry—critical parameters for successful reconstitution of complex biosynthetic pathways and for the discovery and production of novel natural products.<sup>37–39</sup>

We have analysed over 450 scientific articles published between 2004 and 2024 that report on the heterologous expression of BGCs across a variety of *Streptomyces* hosts and research objectives. These studies cover applications ranging from the activation of cryptic pathways and structure

elucidation to the production of valuable natural products at scale (Fig. 1 and Table S1).

The data show a clear upward trajectory in publication activity over the years, reflecting growing interest and progress in this field. In the early years (2004–2006), the number of publications was relatively modest, likely due to technical limitations in genome sequencing, cloning, and host engineering. From 2007 to 2012, there was a steady increase, driven by early genome mining efforts and the development of advanced genetic tools for *Streptomyces* and other actinomycetes. The period 2013–2018 saw a sharp rise in publications, coinciding with the expansion of synthetic biology platforms, improved BGC capture methods (e.g., TAR, CATCH), and increased awareness of the metabolic potential hidden in microbial genomes. The number of articles peaked between 2016 and 2021, with nearly 90 articles published in each 3-year interval. This period reflects a mature phase in the field, where heterologous expression became an established strategy in natural product research. From 2022 to 2024, we observe a slight decline, though publication numbers remain high. Whether this is a lasting trend remains to be seen in the next few years.

## 2. *Streptomyces* hosts for heterologous expression

The success of heterologous expression of BGCs depends critically on the choice of the microbial host. While advances in synthetic biology and DNA assembly have made it increasingly feasible to clone and refactor large gene clusters, efficient expression and production of the desired natural products remain highly host-dependent. Host selection is influenced by a combination of biological, technical, and practical factors. These include genetic compatibility with high-GC BGCs, the ability to supply biosynthetic precursors and cofactors, tolerance to toxic or bioactive metabolites, and the presence of a clean metabolic background to simplify product detection. Additionally, factors such as host availability, ease of genetic manipulation, community familiarity, and the existence of established protocols play key roles in host preference.

We have analysed several hosts used by the community over the years for heterologous expression of BGCs and observed clear trends in their adoption and performance (Fig. 2).

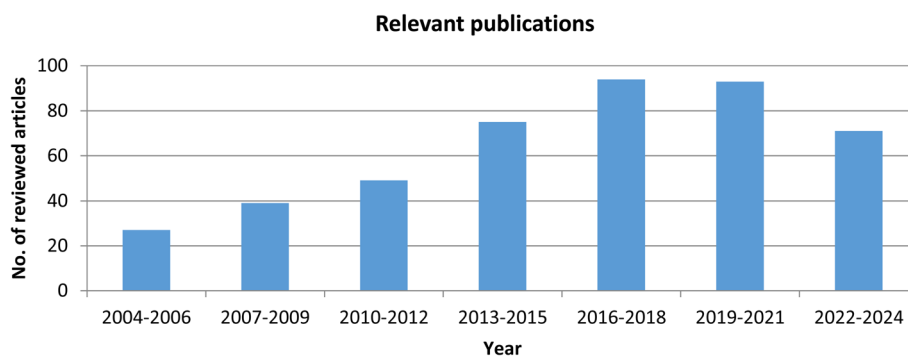


Fig. 1 Number of relevant publications that we reviewed for this article (referenced in Table S1).



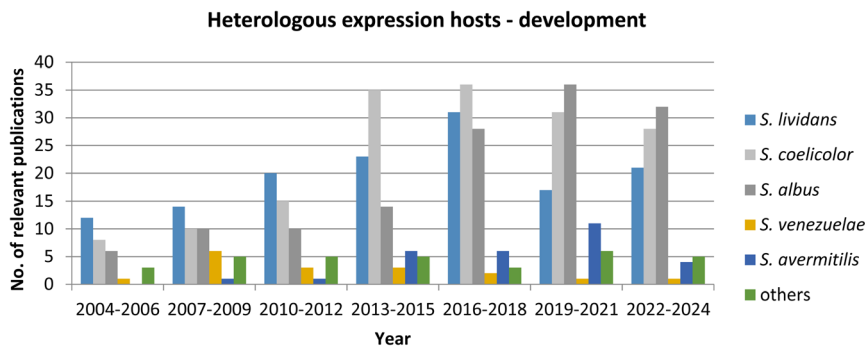


Fig. 2 Prevalence of *Streptomyces* key heterologous host strains over the years.

The data show a significant shift toward *Streptomyces albus*, which has steadily gained popularity and has become the most widely used host in recent years. Its rise is attributed to its fast growth, a clean metabolic background, broad BGC compatibility, and strong genetic tractability, making it ideal for detecting and producing diverse natural products.<sup>40</sup>

In contrast, *Streptomyces lividans*, once one of the most frequently used hosts, has seen some decline in use. Despite its historical significance, it has gradually been replaced by more efficient and versatile strains. *Streptomyces coelicolor* remains a stable, often used host. Its status as a well-characterized model organism makes it a reliable choice. *Streptomyces avermitilis* shows a slight increase in application but remains largely limited to Japanese research groups. While it possesses favorable features such as a reduced native metabolite profile, its broader use may be hindered by limited availability and fewer community-developed tools. *Streptomyces venezuelae*, despite initial interest due to its rapid growth, liquid sporulation, and compatibility with modern genetic tools, has not established itself as a mainstream host. Its low tolerance toward bioactive heterologous products likely explains its limited success, as growth inhibition or instability often occur during expression of certain BGCs. Despite their potential, newly engineered strains, such as *S. chattanoogensis*, have not yet gained widespread adoption within the community. Overall, host selection remains influenced by a combination of practical experience, genetic accessibility, metabolic compatibility, and physiological robustness. These factors continue to shape the evolution of preferred chassis strains in the pursuit of unlocking novel natural products and the overproduction of known ones.

As a next, we have analysed BGC types used for heterologous expression in each host across the period 2004–2024 and identified some trends in both cluster type preferences and host-specific compatibilities (Fig. 3).

The most frequently expressed clusters are polyketide synthase (PKS) types, reflecting their prominence in natural product biosynthesis and their pharmaceutical importance. PKS clusters dominate across all hosts, underlining their central role in heterologous expression efforts. In the three most commonly used hosts—*Streptomyces coelicolor*, *S. lividans*, and *S. albus*—the number of successful expressions for PKS and NRPS clusters is roughly equal, suggesting that these strains

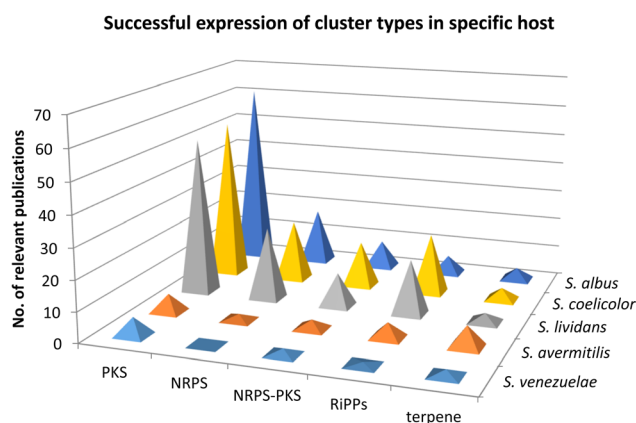


Fig. 3 Preferred heterologous host strains for the expression of specific gene cluster types.

have remained reliable platforms for both BGC classes. When it comes to RiPPs (ribosomally synthesized and post-translationally modified peptides), *S. coelicolor* and *S. lividans* are the most widely used hosts, followed by *S. albus* and *S. avermitilis*. Interestingly, terpenoid BGCs have been expressed predominantly in *S. avermitilis*. This specialization may be due a strong research focus in Japan, where this strain has seen broader development and application.<sup>41–45</sup>

Finally, we have analysed the origin of expressed BGCs spanning the years 2004 to 2024, and the results reveal clear shifts in the sources of BGCs chosen for heterologous expression over time (Fig. 4). In the early years (2004–2006), the vast majority of BGCs expressed were derived from cultured *Streptomyces* strains, representing nearly 100% of all reported cases. This dominance reflects the historical reliance on well-characterized, easily accessible actinomycetes with known genetic tools and predictable behavior. However, starting from around 2010–2012, there is a gradual diversification in BGC origins. The share of non-*Streptomyces* actinomycetes steadily increased, reaching 23% in the most recent intervals. This shift indicates growing interest in rare actinomycetes, which often harbor novel and chemically distinct secondary metabolites not found in canonical *Streptomyces* species. Simultaneously, BGCs from distant bacterial taxa (non-actinomycete sources such as *Sorangium*, *Pseudomonas*, *Myxococcus*, or *Lysobacter*) have also





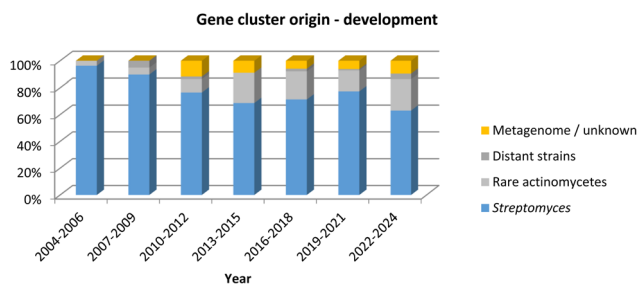


Fig. 4 Origin of the expressed gene clusters: relative share of *Streptomyces* and more distant donor species.

appeared, albeit in small numbers.<sup>46–49</sup> These cases remain limited due to challenges in expression compatibility and the need for pathway refactoring but reflect the broader scope of genome mining efforts. Importantly, the number of BGCs originating from metagenome or unknown sources has shown a modest yet steady increase. These clusters, often retrieved from uncultured organisms or environmental DNA, now represent 10% of recent studies (2022–2024). Their rise is driven by advances in metagenomic sequencing, clone-based capture techniques, and interest in accessing the “microbial dark matter” for novel compound discovery.<sup>50–53</sup> While *Streptomyces* remains the most dominant source of heterologously expressed BGCs, the last decade has seen a notable shift toward diversification.

### 3. Heterologous expression for drug discovery

Heterologous expression has become a powerful and widely adopted strategy for the discovery of natural products. By transferring BGCs from their native organisms into genetically tractable and well-characterized microbial hosts, researchers can unlock the production of bioactive compounds that are otherwise silent or produced in very low amounts under standard laboratory conditions.<sup>12</sup> This approach enables access to novel chemical scaffolds encoded by cryptic or unexpressed BGCs, facilitates the structural and functional characterization of natural products, and allows for the scalable production of valuable metabolites. It also circumvents challenges associated with culturing rare or slow-growing microbes, including unculturable environmental strains accessed through metagenomics.

An analysis of published work from 2004 to 2024 (Fig. 5) illustrates the distribution of heterologous hosts that have successfully yielded novel metabolites. It is worth noting that most of the novel metabolite discoveries occurred after 2010, in parallel with advancements in genome mining, synthetic biology, and pathway engineering. Earlier work in heterologous expression primarily focused on functional validation of BGCs, characterization of known compounds, and reconstitution of metabolite biosynthesis, rather than the identification of new chemical entities.

#### 3.1. *Streptomyces albus*

Surprisingly, *S. albus* J1074 and its engineered variants account for the largest share (34%) of all newly discovered compounds.

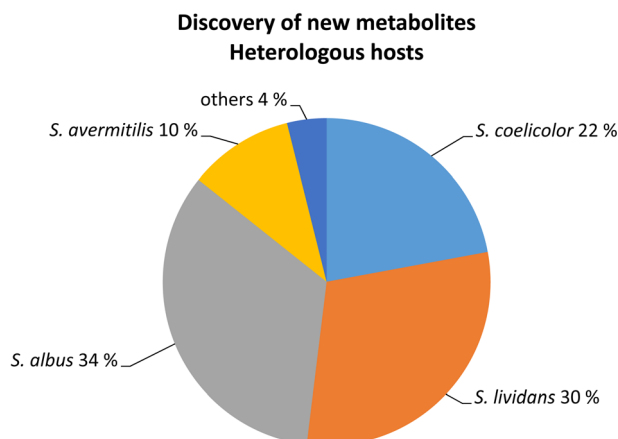


Fig. 5 Heterologous host strains that yielded novel metabolites.

*S. albus* J1074, which is a derivative of the *S. albus* G strain, became a widely used host in natural product research. It was originally selected for its lack of a functional *SalI* restriction-modification system, making it particularly amenable to genetic transformation and an ideal platform for cloning and expressing genes from other *Streptomyces* species.<sup>54</sup> Over time, a comprehensive and highly efficient genetic toolbox has been developed for this strain, including both replicative and integrative plasmid systems, straightforward methods for conjugation and protoplast transformation, and a rich set of well-characterized regulatory elements such as promoters, terminators, and reporter genes. Among heterologous hosts, *S. albus* J1074 is also notable for its rapid growth, completing its full developmental cycle in just four days on solid media—a clear advantage in time-sensitive expression studies. Genomically, it features a compact 6.8 Mb linear chromosome, the smallest among commonly used *Streptomyces* expression strains, which contributes to its streamlined metabolic behavior.<sup>55</sup> Despite its reduced genome, *S. albus* J1074 retains 25 native secondary metabolite BGCs encoding a wide array of compounds, including alteramides, candicidins, mansouramycins, and paulomycins. Thanks to its genetic accessibility, rapid growth, and metabolic versatility, *S. albus* J1074 has emerged as one of the most successful and reliable hosts for heterologous expression of secondary metabolite pathways.

In addition to novel compounds derived from favorable BGCs such as polyketide synthases (PKS) and non-ribosomal peptide synthetases (NRPS), *Streptomyces albus* J1074 has established itself as a fully functional host for underrepresented cluster types, recently demonstrating its extensive capabilities in metabolite production utilizing various precursors. Over the past decade, cyclodipeptide synthase (CDPS) clusters have garnered significant attention due to the diverse biological activities associated with their metabolites, including antibacterial, antiviral, and anti-inflammatory properties.<sup>56</sup> In this context, a cryptic CDPS gene cluster from *Streptomyces chrestomyceticus* was identified and heterologously expressed in *S. albus* J1074, leading to the isolation of a novel, highly modified cyclodipeptide, purincyclamide, from the ex-conjugant culture.



**Table 1** Summarizes new natural products that have been identified or verified through the heterologous expression of BGCs in *S. albus* and its engineered derivatives

Compound name	BGC type	Compound type	Native strain	Ref.
Maramycin	[Complex]	Isoquinolinequinone terpenoid	<i>S. mirabilis</i>	58
Atralabdans	Terpene	Diterpenoid (labdan)	<i>S. atratus</i>	72
Microechmycins	[Complex]	Benzoxazole	<i>Micromonospora</i> sp. SCSIO 07395	71
Lipothrenins	Complex FAS	Lipo-amino acid	<i>S. aureus</i>	67
Acidonemycins	PKS type II	Aromatic polyketide (angucycline)	<i>S. indonesiensis</i>	73
Miramides	NRPS-PKS	Depsipeptide	<i>S. mirabilis</i>	68
Marinolactam	PKS type I	Macrolactam	<i>Micromonospora</i> sp. 181	70
Cacaoidin	RiPPs	Lanthipeptide	<i>S. cacaoi</i> CA-170360	74
Bonsecamin	NRPS-amino acid ligase	Cyclic pentapeptide	<i>S. albus</i> ssp. <i>chlorinus</i>	66
Shuangdaolides	<i>trans</i> -AT PKS type I	Macrolide	<i>S. sp.</i> B59	75
Metathramycin	PKS type II	Aromatic polyketide (aureolic acid)	[Metagenome]	51
Epemicins	PKS type I	Macrolide	<i>Kutzneria</i> sp. CA-103260	63
Dudomycins	NRPS	Depsipeptide	<i>S. albus</i> subsp. <i>chlorinus</i>	64
Loseolamycins	PKS type III	Aromatic polyketide (alkylresorcinol)	<i>Micromonospora endolithica</i>	65
Bosamycins	NRPS	Linear peptide	<i>S. sp.</i> 120454	76
Benzanthric acid	[Unusual]	Anthranilate	<i>S. albus</i> subsp. <i>chlorinus</i>	59
Huimycin	[Unusual]	Pyrrlopyrimidine	<i>Kutzneria albida</i>	69
Purincyclamide	CDPS	Cyclodipeptide	<i>S. chrestomyceticus</i>	57
Scleric acid	NRPS	benzoyl-amino acid	<i>S. sclerotialis</i>	77
9401-LP1, 9810-LP	RiPPs	Lasso peptide	[Several <i>Streptomyces</i> ]	78
Frlnimycin	[Unusual]	Indole alkaloid	<i>Frankia alni</i>	16
Metatricycloene	PKS type II	Tricyclic polyene	[Metagenome]	52
Lazarimides	[Complex]	Indolotryptoline	[Metagenome]	79
Calixanthomycin, arenimycins	PKS type II	Aromatic polyketide	[Metagenome]	80
Borregomycin	Indolocarbazole	Indolotryptoline/indolocarbazole	[Metagenome]	53
KB-3346-5, [compound 2]	PKS type II	Aromatic polyketide	[Metagenome]	81

Unfortunately, the authors did not provide insights into the potential biological activities of this intriguing molecule.<sup>57</sup>

Another noteworthy example of the successful application of *S. albus* J1074 as a versatile heterologous host is the production of the new isoquinolinequinone terpenoid maramycin, derived from a gene cluster of *Streptomyces mirabilis*.<sup>58</sup> A well-documented characteristic of *S. albus* J1074 as a chassis is its ability to produce new metabolites resulting from the interaction between newly introduced gene clusters and host genes.<sup>59–62</sup> This untargeted natural combinatorial biosynthesis does not necessarily yield undesirable shunt or side products; rather, it can lead to valuable compounds, as evidenced by maramycins, which have demonstrated cytotoxic activity against human prostate cancer cell lines, indicating their potential for further development as therapeutic agents.

Furthermore, *S. albus* J1074's capability to produce metabolites from gene clusters of actinomycete genera that are phylogenetically distant from *Streptomyces* is an additional advantage of this chassis strain. For instance, the newly identified macrolides, epemicins, were successfully isolated from the native producer strain *Kutzneria* sp. CA-103260 and subsequently produced following heterologous biosynthetic gene cluster expression in *S. albus* J1074.<sup>63</sup> This opens avenues for in-depth genetic studies and manipulation of their gene cluster. Additionally, the production of the new aureolic acid compound metathramycin from an environmental DNA sample further underscores the versatility and flexibility of *S. albus* J1074 in the discovery of new bacterial metabolites.<sup>51</sup>

In 2018, a significant advancement was made in the development of *Streptomyces albus* as a chassis for heterologous expression. We have reported the development of a cluster-free *S. albus* chassis strain (*S. albus* Del14) specifically engineered to improve the heterologous expression of secondary metabolite BGCs and to facilitate the discovery of novel natural products.<sup>16</sup> This work represents a major advance in chassis design, pushing the boundaries of microbial platforms used in genome mining and synthetic biology for drug discovery. Using a marker-free deletion strategy, 15 endogenous BGCs were sequentially removed from the chromosome of the parental *S. albus* J1074 strain. The resulting strain, *S. albus* Del14, is devoid of most of the native secondary metabolite production and shows a significantly simplified metabolic background, as confirmed by LC-MS profiling. This metabolic clean background dramatically improves the detectability of heterologously produced compounds and reduces false positives during screening.

To further enhance expression capacity, the authors introduced additional *attB* sites for  $\phi C31$  integrase into the genome, enabling the multi-copy integration of BGCs. The resulting strains, *S. albus* B2P1, B3P1 and B4, allow stable incorporation of up to four copies of a gene cluster, leading to significantly increased production yields of diverse natural products.

Importantly, the newly engineered *S. albus* strains demonstrated remarkable capabilities in activating cryptic and previously uncharacterized BGCs. Since its development, the engineered *S. albus* strain Del14 has been systematically employed for the heterologous expression of BGCs from our in-house genomic libraries. The substantial number of novel



metabolites discovered between 2018 and 2023 from various actinomycetes underscores the efficacy of this host strain.<sup>59,64–69</sup> Notably, two significant examples from recent years are the NRPS compounds bonsecamin and dudomycin. The gene clusters responsible for these metabolites were both cryptic and inactive in the native producer strain, *S. albus* subsp. *chlorinus*. Through heterologous expression in *S. albus* Del14, we successfully activated these clusters, leading to the production of the corresponding metabolites. The minimal bonsecamin peak was nearly undetectable but became discernible in our cluster-minimized host due to reduced background interference, facilitating its subsequent characterization.<sup>66</sup> In contrast, the prominent signals of dudomycins simplified metabolic profiling, suggesting that the genome reduction likely enhanced the precursor pools of amino acids and malonyl-CoA essential for dudomycin biosynthesis.<sup>64</sup> Fralnimycin and huimycin further exemplify the capability of *S. albus* Del14 to express gene clusters from rare actinomycetes, specifically *Frankia alni* and *Kutzneria albida*, respectively.<sup>16,69</sup>

The utility of *S. albus* Del14 extends beyond our research group; other scientists have reported promising outcomes using this chassis. For instance, a cryptic type I PKS cluster from *Micromonospora* sp. 181 was successfully captured using the novel CAT-FISHING cloning method. CAT-FISHING (CRISPR/Cas12a-mediated fast direct biosynthetic gene cluster cloning) is a recently developed high-fidelity cloning technique that enables the direct capture of large BGCs from genomic DNA. This method combines CRISPR/Cas12a-guided cleavage with *in vitro* recombination, streamlining the retrieval of complex gene clusters. Heterologous expression in the cluster-free *S. albus* derivative led to the production of marinolactam, a novel bioactive macrolactam.<sup>70</sup> Additionally, the production of new benzoxazole alkaloids, microechmycins, encoded by the *mich* BGC of *Micromonospora* sp. SCSIO 07395, was achieved through heterologous expression in *S. albus* Del14.<sup>71</sup> These examples highlight the expanding chemical diversity accessible through *S. albus* Del14, with more novel compounds already in the pipeline for discovery (Table 1).

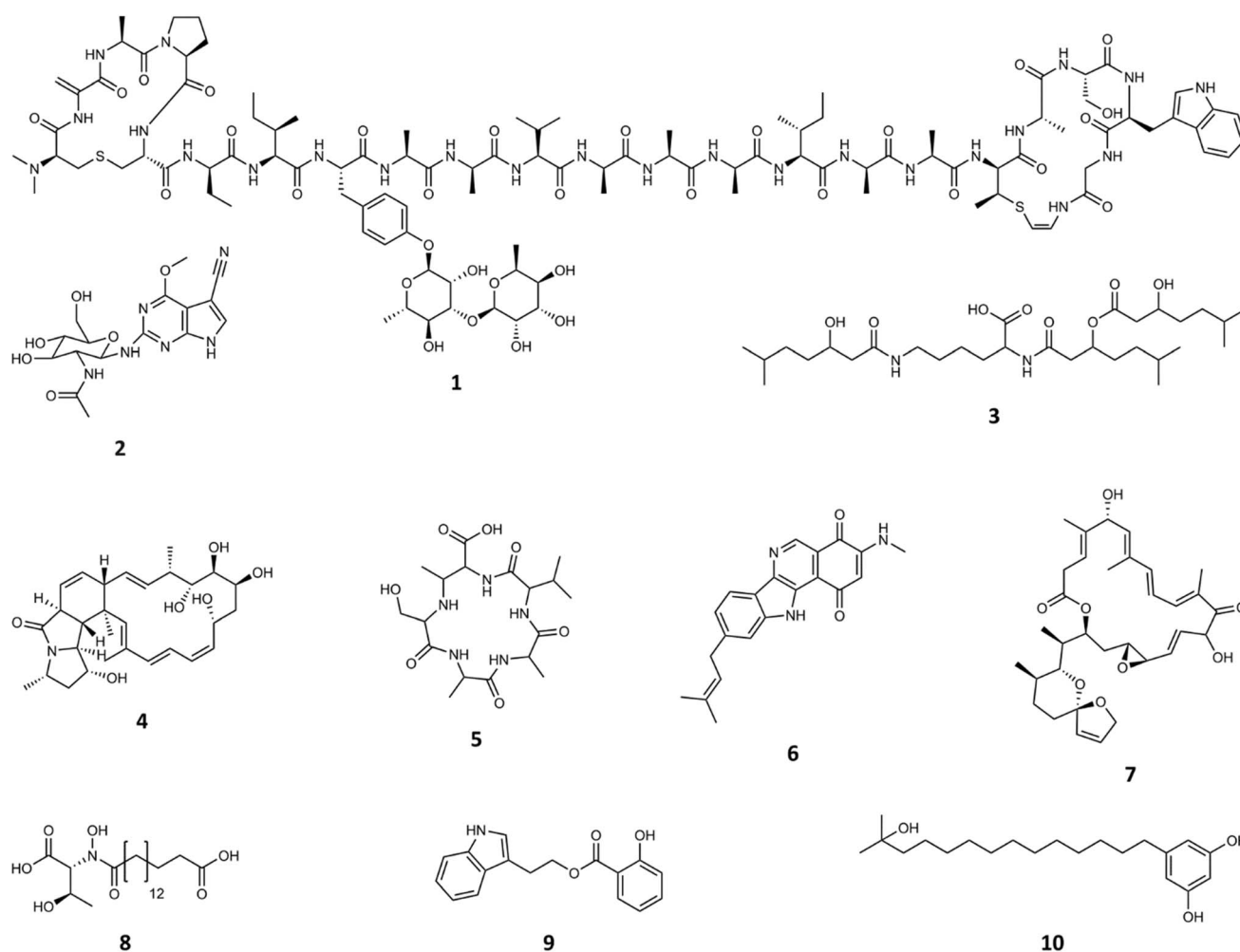


Fig. 6 Natural compounds produced by heterologous expression in *S. albus* and its engineered derivatives: (1) – cacaoidin, (2) – huimycin, (3) – dudomycin A, (4) – marinolactam A, (5) – bonsecamin, (6) – maramycin, (7) – shuangdaolide A, (8) – lipothrenin A, (9) – fralnimycin, (10) – loseolamycin A 1-1.



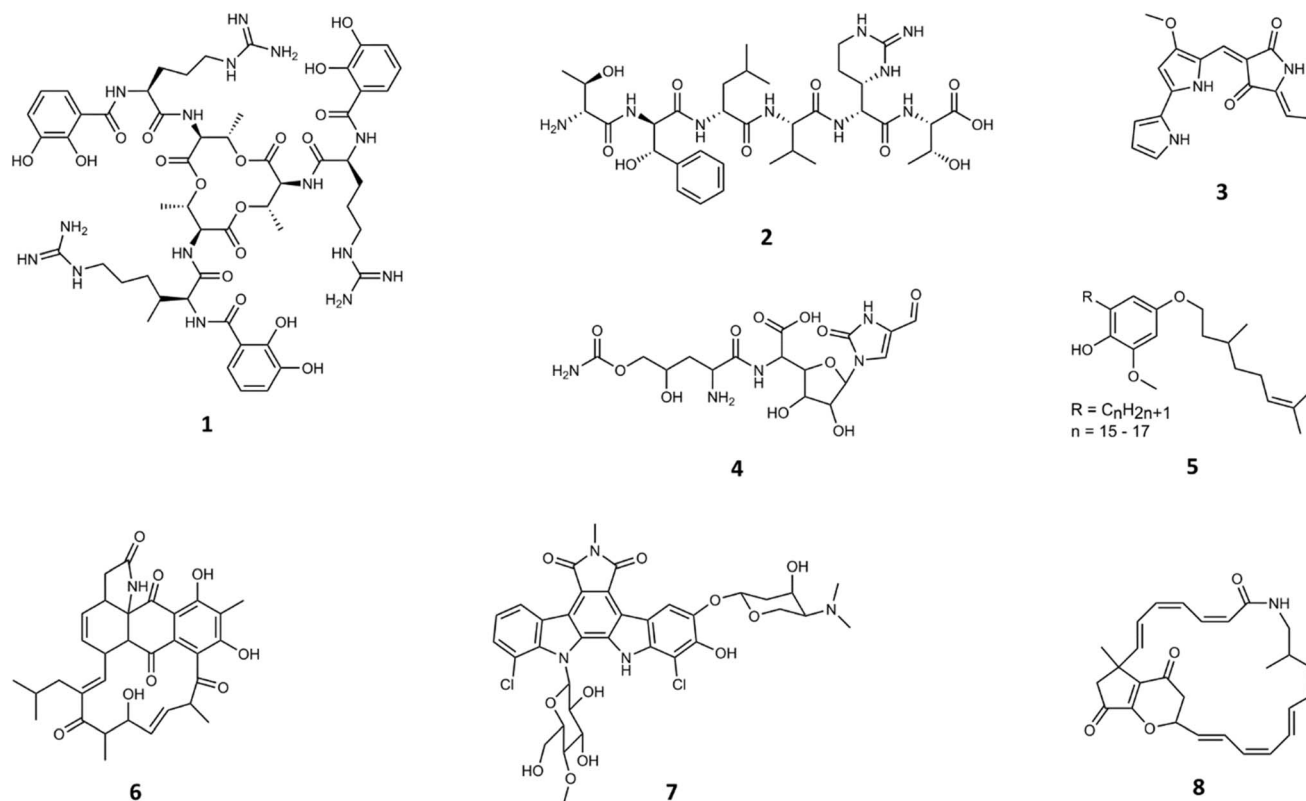


Fig. 7 Natural compounds produced by heterologous expression in *S. lividans* and its engineered derivatives: (1) – griseobactin, (2) – faulknamycin, (3) – hybrubin A1, (4) – polynik A, (5) – alkyl-*O*-dihydrogeranyl-methoxyhydroquinones, (6) – ansaseomycin A, (7) – loonamycin A, (8) – weddellamycin.

In addition to its genetic and biosynthetic advantages, the use of metabolically streamlined *S. albus* chassis strains offers clear environmental benefits. The absence of native secondary metabolites in these clean-background strains reduces the formation of toxic by-products, minimizing the environmental burden associated with downstream waste disposal. Moreover, the simplified metabolic profile significantly eases the purification of target compounds, often requiring fewer chromatographic steps and less use of hazardous organic solvents. This not only lowers processing costs and time but also aligns with the principles of green chemistry by reducing chemical waste and energy input—making *S. albus* not only a powerful tool for discovery, but also a more environmentally responsible platform for natural product discovery and production (Fig. 6).

### 3.2. *Streptomyces lividans*

*S. lividans* follows with 30%, demonstrating its historical importance and reliable performance in heterologous expression workflows, particularly in the earlier phases of BGC exploration. The strain is a close relative of the model organism *S. coelicolor*, but with a slightly smaller genome (~8.3 Mb). Its genetic accessibility, particularly its ability to accept methylated DNA, makes it more amenable to transformation than many other *Streptomyces* strains. Additionally, its low intrinsic protease activity has made *S. lividans* a favored host for the

production of recombinant proteins, a role it has served for decades.<sup>82</sup>

Among its derivatives, *S. lividans* TK24 is the most widely used strain for heterologous expression. This strain carries the RpsL[K88E] mutation, which has been shown to enhance the production of natural products.<sup>83</sup> Numerous successful examples underscore *S. lividans*' effectiveness in producing complex peptide natural products, such as capreomycin, daptomycin, bottromycin, viomycin, and labyrinthopeptins, among others.<sup>35,84–87</sup> Its superior performance in this area is likely linked to its low protease background, which reduces degradation of peptide products. To improve its performance further, several engineered variants of *S. lividans* have been developed. Ziermann and colleagues generated strains K4-114 and K4-155 by deleting the entire actinorhodin (*act*) gene cluster from TK24, aiming to eliminate competition for resources and simplify metabolite detection.<sup>88</sup> Expression of erythromycin precursor biosynthetic genes (6-deoxyerythronolide B, 6-dEB) in these strains resulted in production levels similar to the parental TK24. However, when expressing the mithramycin A pathway, these engineered hosts performed significantly better, with K4-114 producing up to 3 g L<sup>-1</sup>, compared to just 0.86 g L<sup>-1</sup> in the unmodified TK24.<sup>89</sup>

More recently, additional enhancements have been introduced through the stepwise integration of global regulatory genes (*nusG<sub>sc</sub>* and *afsR*), deletion of negative regulator *wblA*, and





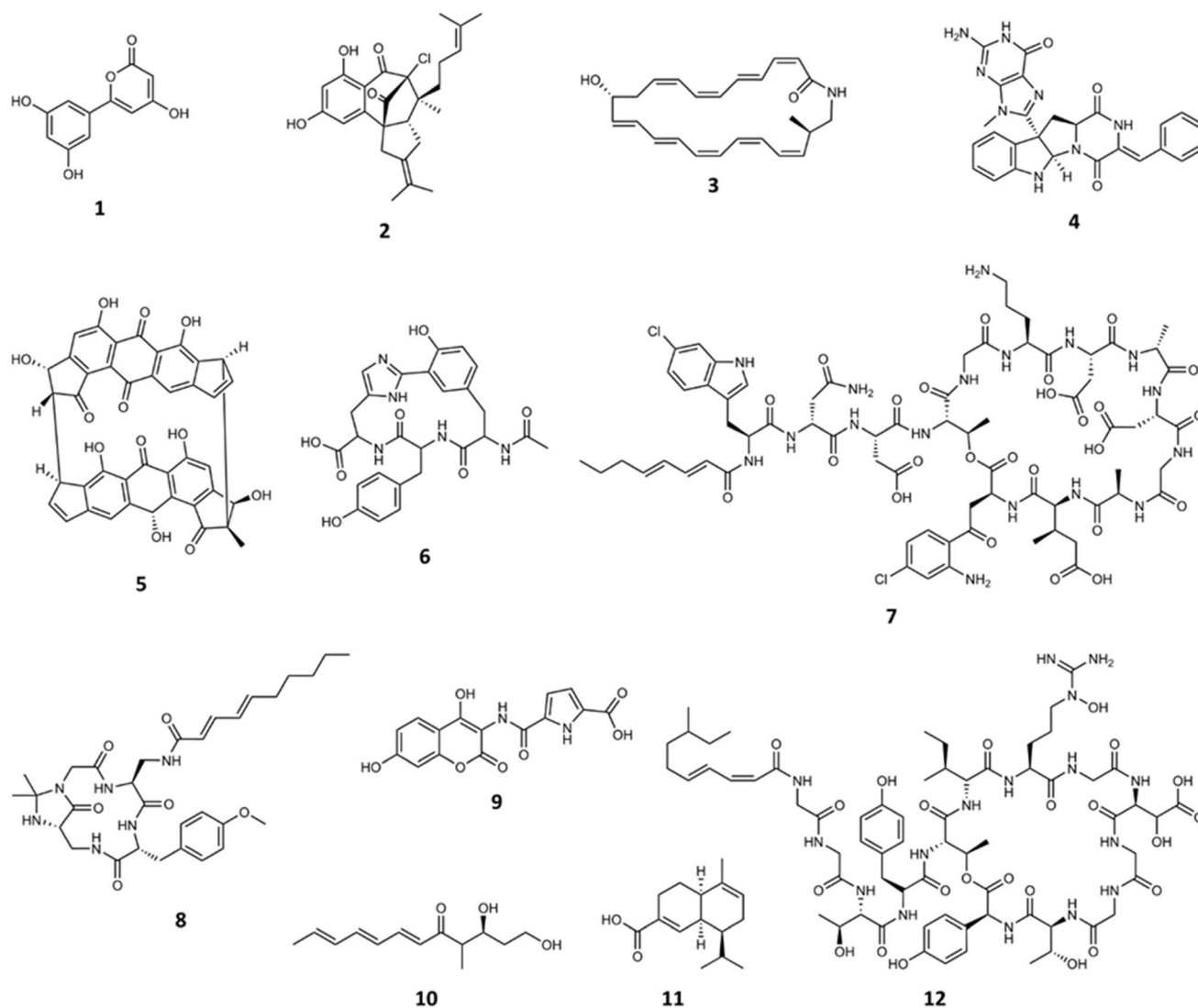


Fig. 8 Natural compounds produced by heterologous expression in *S. coelicolor*, *S. avermitilis* and their engineered derivatives: (1) – venemycin, (2) – merochlorin A, (3) – JBIR-156, (4) – guanitrypmycin A1-1, (5) – bipentarmycin A, (6) – biaryllyte YYH, (7) – taromycin A, (8) – kutzneridine A, (9) – cacibocin A, (10) – lavendiol, (11) – levinoid A, (12) – ambocidin A.

introduction of codon-optimized efflux pump genes (*lmrA* and *mdfA*).<sup>90</sup> These modifications led to significantly improved yields of several natural products, including hybrubins, piericidin, dehydrorabelomycin, and actinomycin D.<sup>91</sup>

In our 2020 study, Ahmed *et al.* presented a significant advancement in the development of *S. lividans* as a heterologous expression host for secondary metabolite BGCs. Recognizing the limitations of native *S. lividans* TK24—such as interference from endogenous metabolite pathways—the authors systematically engineered clean-background chassis strains by deleting up to 11 native BGCs, simplifying downstream metabolite analysis and improving strain fitness in liquid media.<sup>17</sup>

The engineered strains, named *S. lividans*  $\Delta$ YA9,  $\Delta$ YA10, and  $\Delta$ YA11, featured not only reduced metabolic background but also additional  $\phi$ C31 *attB* integration sites, facilitating the multi-copy expression of foreign BGCs. Comparative analyses demonstrated that these strains were superior to the parental

strain in expressing diverse classes of BGCs, including those encoding tunicamycin, deoxycinnamycin, and griseorhodin. One of the study's key contributions was the demonstration of these strains' value in natural product discovery. Using a BAC library derived from *S. albus* subsp. *chlorinus*, the authors expressed 17 BGC-containing clones in *S. lividans*  $\Delta$ YA9 and *S. albus* Del14. This led to the identification of seven new metabolites, including novel pyrrolbenzodiazepine (PBD)-like compounds. Notably, some compounds were detected only in *S. lividans* and not in *S. albus*, highlighting the host-specific expression potential and the need for complementary chassis strains in screening efforts. The study reinforces the idea that no single *Streptomyces* strain is universally optimal for all BGC types. These findings underscore the importance of expanding and diversifying the chassis strain repertoire for more comprehensive genome mining and natural product discovery (Table 2 and Fig. 7).



**Table 2** Summarizes new natural products that have been identified or verified through the heterologous expression of BGCs in *S. lividans* and its engineered derivatives

Compound name	BGC type	Compound type	Native strain	Ref.
Weddellamycin	PKS type I	Polyene macrolactam	<i>S. sp.</i> DSS69	92
Morphosins	RiPPs	Lasso peptide	<i>S. sp.</i> L06	93
Cihanmycins	Complex NRPS	Bicyclic CCNP <sup>a</sup>	<i>Amycolatopsis cihanbeyliensis</i>	94
Lipothrenins	Complex FAS	Lipo-amino acid	<i>S. aureus</i> LU18118	67
JBIR-159	NRPS-PKS type 1	Oxazole-polyene	<i>S. versipellis</i>	95
Stlassin	RiPPs	Lasso peptide	<i>S. sp.</i> PKU-MA01240	96
Loonamycin	Indolocarbazole	Indolocarbazole	<i>Nocardiosis flavescens</i>	97
Faulknmycin	NRPS	Linear peptide	<i>S. griseus</i>	98
Pentangumycin, SEK90	PKS type II	Aromatic polyketide (angucyclinone)	<i>Saccharotrix espanaensis</i>	99
Ansaseomycins	PKS type I	Polyketide (ansamycin)	<i>S. seoulensis</i>	100
Ashimides	NRPS	Cyclopeptide	<i>S. sp.</i> NA03103	101
Snou-LP, 9401-LP1, 9810-LP	RiPPs	Lasso peptide	[Several <i>Streptomyces</i> ]	78
Albusnodin	RiPPs	Lasso peptide	<i>S. albus</i>	102
Polynik	[Hybrid - combinatorial]	Nucleoside	<i>S. ansochromogenes</i>	103
Pactamides	NRPS-PKS	PoTeM <sup>b</sup>	<i>S. pactum</i>	104
Rimosamides	NRPS-PKS	Depsipeptide	<i>S. rimosus</i>	105
Ketomemicins	Dipeptide ligase	Pseudotripeptide	<i>Micromonospora sp.</i> ATCC 39149, <i>S. mobaraensis</i> , <i>Salinispora tropica</i>	106
Hybrubins	Complex NRPS-PKS type I	Bipyrrole tetramic acid	<i>S. variabilis</i>	91
s56-p1	[Unusual]	Dipeptide - hydrazone	<i>S. sp.</i> SoC090715LN-17	107
Erythreapeptins	RiPPs	Lanthipeptide	<i>Saccharopolyspora erythraea</i>	108
alkyl-O-dihydrogeranyl-methoxyhydroquinones	PKS type III	Aromatic polyketide (alkylresorcinol)	<i>Actinoplanes missouriensis</i>	109
Griseobactin	NRPS	Catechol-peptide	<i>S. sp.</i> ATCC 700974	110
[Several phenolic lipids]	PKS type III	Aromatic polyketide (alkylresorcinol)	<i>S. griseus</i>	111

<sup>a</sup> CCNP – cinnamoyl-containing non-ribosomal peptide. <sup>b</sup> PoTeM – polycyclic tetramate macrolactam.

### 3.3. *Streptomyces coelicolor*

*S. coelicolor*, the best-characterized species of the actinomycetes, accounts for 22% of new discoveries. This strain has long served as a foundational model for studying bacterial differentiation and secondary metabolism. Over the past decade, it has also become a prominent heterologous expression host for BGCs sourced from a wide range of actinomycetes, including rare or genetically intractable strains.<sup>112–114</sup> The pioneering work by Gomez-Escribano and Bibb focused on engineering *S. coelicolor* derivatives specifically optimized for heterologous production of natural products. By systematically deleting the four major endogenous secondary metabolite gene clusters—those for actinorhodin, prodiginine, coelimycin, and the calcium-dependent antibiotic (CDA)—the authors created *S. coelicolor* M1146, a clean-background strain.<sup>115</sup> This simplification of the metabolic profile not only minimized native interference but also enhanced the detectability of new products via LC-MS and bioassays. Further enhancements yielded strains M1152 and M1154, incorporating point mutations in *rpoB* and *rpsL*—known regulators of secondary metabolism. Importantly, the engineered *S. coelicolor* strains demonstrated broad compatibility across BGC classes, including polyketides, non-ribosomal peptides, RiPPs, aminocoumarins, and nucleoside antibiotics. Our data indicate that the limitations of the

hosts, as anticipated by the authors—particularly regarding the expression of gene clusters from more distantly related taxa—are not attributable to the strain improvements.

The combination of predictable growth, rich genetic tools, high production yields, and well-understood regulatory architecture makes *S. coelicolor*—particularly the M1152/M1154 chassis—a versatile and powerful platform for the expression and discovery of microbial natural products (Table 3 and Fig. 8).

### 3.4. *Streptomyces avermitilis*

*S. avermitilis* contributed to 10% of the newly identified metabolites. This strain has emerged as a highly promising chassis for the heterologous production of secondary metabolites, particularly due to its robust genetic stability, rapid growth, and industrially optimized primary metabolism. Originally known for the industrial production of the antiparasitic compound avermectin, *S. avermitilis* has been systematically repurposed into a versatile expression host through targeted genome minimization and regulatory engineering.

Komatsu and colleagues constructed a suite of genome-reduced strains (designated SUKA series), such as SUKA2, SUKA5, SUKA17, and SUKA22, by deleting over 1.4 Mb of non-essential genomic regions, including gene clusters for endogenous secondary metabolites (e.g., avermectins, filipins,



**Table 3** Summarizes new natural products that have been identified or verified through the heterologous expression of BGCs in *S. coelicolor* and its engineered derivatives

Compound name	BGC type	Compound type	Native strain	Ref.
Morphosins	RiPPs	Lasso peptide	<i>S. sp. L06</i>	93
Levinoids	Terpene	Sesquiterpenoid	<i>S. levis</i>	116
Kutzneridine	NRPS	Cyclic lipo-tetrapeptide	<i>Kutzneria sp. CA-103260</i>	113
Griseocazines	CDPS	Prenylated cyclodipeptide	<i>S. griseocarneus</i>	117
Biarylittides	RiPPs	Cyclic tripeptide	<i>Planomonospora sp</i>	112
Stlassin	RiPPs	Lasso peptide	<i>S. sp. PKU-MA01240</i>	96
Leepeptin	RiPPs	Lasso peptide	<i>S. leeuwenhoekii</i>	118
Guanitrypmysins	CDPS	Pyrrolindoline	<i>S. monomycini</i>	119
Ansaseomycins	PKS type I	Polyketide (ansamycin)	<i>S. seoulensis</i>	100
Albusnodin	RiPPs	Lasso peptide	<i>S. albus</i>	102
Venemycin	PKS type I-PKS type III	Biaryl polyketide	<i>S. venezuelae</i>	120
Streptocollin	RiPPs	Lanthipeptide	<i>S. collinus</i>	121
Alkyldihydropyrones	PKS type III	Dihydropyran	<i>S. reveromyceticus</i>	122
Taromycin	NRPS	Lipopeptide	<i>Saccharomonospora sp. CNQ-490</i>	123
Cacibiocin	Aminocoumarin	Aminocoumarin	<i>Catenulispora acidiphila</i>	114
Merochlorins	PKS-terpene	Polyketide-meroterpenoid	<i>S. sp. strain CNH-189</i>	124
Erythreapeptins	RiPPs	Lanthipeptide	<i>Saccharopolyspora erythraea</i>	108

**Table 4** Summarizes new natural products that have been identified or verified through the heterologous expression of BGCs in *S. avermitilis* and its engineered derivatives

Compound name	BGC type	Compound type	Native strain	Ref.
Morphosins	RiPPs	Lasso peptide	<i>S. sp. L06</i>	93
Ambocidins	NRPS	Cyclic lipodepsipeptides	<i>S. ambofaciens</i>	133
Bipentarymycins, allenomycins	PKS type II, PKS type I	Aromatic polyketide, allene	<i>S. sp. NRRL F-6131, S. griseofuscus</i>	134
JBIR-156	PKS type I	Polyene macrolactam	<i>S. rochei</i>	135
Neothioviridamide	RiPPs	Thioamide	<i>S. sp. MSB090213SC12</i>	136
Lavendiol	PKS type I	Linear polyketide	<i>S. lavendulae</i>	137
[Several terpenes]	Terpene	Sesquiterpene, diterpene	[Several]	44
[Several terpenes]	Terpene	Sesquiterpene, diterpene	[Several]	45

oligomycins).<sup>41,125,126</sup> These deletions not only cleared the metabolic background, allowing simplified detection and isolation of heterologous products, but also redirected precursor flux and biosynthetic energy toward the expression of exogenous pathways.

The deletion strains demonstrated enhanced heterologous production of diverse natural products. For example, SUKA strains expressing the streptomycin and cephamycin gene clusters produced higher titers than the original native producers (*S. griseus* and *S. clavuligerus*, respectively). Expression of cryptic or poorly expressed clusters—such as the pladienolide BGC—was achieved by supplementing with heterologous regulatory elements (e.g., alternative promoters or regulatory genes like *pldR*).<sup>41</sup>

In total, more than 20 biosynthetic gene clusters from diverse actinomycetes have been successfully expressed in *S. avermitilis* SUKA strains. The chassis supported a wide range of compound classes, including polyketides, non-ribosomal peptides, terpenoids, alkaloids, and even plant-like metabolites.<sup>127–131</sup> Importantly, in several cases, production levels in *S. avermitilis* exceeded those of the original producers, confirming its utility for scalable production and discovery.<sup>126,132</sup>

One of the key advantages of *S. avermitilis* over other *Streptomyces* hosts is its remarkable genetic and phenotypic stability. In comparative studies under stress conditions (e.g., elevated temperature), *S. avermitilis* exhibited significantly lower rates of genetic instability (e.g., bald mutants) than other *Streptomyces* such as *S. coelicolor* or *S. griseus*. This trait, along with its short terminal inverted repeats (TIRs) and lower frequency of transposon activity, underpins its suitability for industrial and long-term biosynthetic applications.<sup>42</sup>

Despite its favorable biosynthetic capacity—particularly for terpenoids—it remains underutilized outside of Japan, where it has seen focused development (Table 4).

The remaining 4% of discoveries were made in other or engineered hosts, indicating that non-canonical strains have yet to make a major impact in the field, likely due to challenges in standardization, compatibility, or accessibility.<sup>49,138</sup>

## 4. Heterologous expression for drug development

A persistent challenge in early-stage drug development is the limited supply of promising natural products for biological evaluation and preclinical testing. Although many natural



products exhibit potent and selective bioactivities, they are often produced in extremely low quantities by their native microbial producers—frequently in the microgram range—and making further development impractical. This is particularly true for actinomycetes, whose genomes encode a wealth of BGCs, many of which remain silent or poorly expressed under standard laboratory conditions. Even when these strains are culturable, optimizing fermentation conditions to support metabolite production can be complex, time-consuming, and not easily scalable. Heterologous expression provides a practical and increasingly effective solution to these problems. By transferring BGCs into genetically tractable and well-characterized host strains, researchers can bypass the regulatory complexity of native producers and activate silent pathways under controlled conditions. Importantly, many of these heterologous hosts have well-established fermentation protocols, enabling more straightforward scale-up and reproducibility. Furthermore, they often possess clean or minimal metabolic backgrounds, which simplifies downstream processing and purification of the compound of interest—another critical advantage in the early phases of drug development. In addition to supply issues, early-stage discovery also depends heavily on the ability to diversify lead compounds. Natural products, while structurally complex and often pharmacologically attractive, are notoriously difficult to modify chemically. Biosynthetic engineering offers powerful strategies to generate analogues, allowing for structure–activity relationship (SAR) studies and lead optimization. However, such modifications are frequently unfeasible in the native producer strains due to genetic intractability or metabolic burden. Heterologous systems, in contrast, provide a flexible and modular platform for pathway engineering, enabling the incorporation of mutations, domain swaps, or tailoring enzymes to expand chemical diversity. By addressing these two major bottlenecks—compound supply and structural diversification—heterologous expression has become an indispensable tool in the early stages of natural product-based drug discovery.

#### 4.1. Natural products yield improvement through heterologous expression

The aforementioned examples of bonsecamin and dudomycin illustrate the efficacy of heterologous expression as a tool for directly accessing compounds from silent gene clusters.<sup>64,66</sup> Furthermore, this technique has the potential to enhance the titers of metabolites that are produced in only minimal quantities by the native producer strain. Utilizing an industrial strain of *Streptomyces cinnamonensis* as a host for the production of the antitumor polyketide tetracenomycin (TCM), the total production rate of TCM was increased tenfold compared to the native producer strain *Streptomyces glaucescens*, resulting in a yield of 5 g L<sup>-1</sup>. However, substantial amounts of TCM accumulated within the bacterial cells, as the host appears to lack an effective excretion mechanism to release the product into the medium.<sup>139</sup> It is important to note that this instance of achieving multigram quantities of product solely through heterologous expression is more of an exception than the norm.

While heterologous expression is a powerful strategy for unlocking and accessing natural products from silent or poorly expressed BGCs, it does not inherently guarantee high or optimal production yields. Transferring a gene cluster into a new host can mitigate regulatory silencing, but this is often insufficient to satisfy the requirements of early-stage drug development, where multigram quantities of pure compounds are necessary for pharmacological evaluation, lead optimization, and preclinical studies. Therefore, to fully exploit the potential of heterologous production, it is frequently essential to (1) metabolically tailor the host strain and/or (2) refactor the BGCs to enhance precursor availability, pathway balance, and compound yield.

In 2010, Gomez-Escribano *et al.* constructed derivatives of *Streptomyces coelicolor* M145 that lacked four endogenous secondary metabolite gene clusters and contained two additional point mutations in pleiotropic regulator genes *rpoB* and *rpsL*. The authors compellingly demonstrated the superiority of the resulting M1152 and M1154 strains as chassis for the production of secondary metabolites, such as chloramphenicol and congoicidine, with heterologous expression leading to production levels that were up to 40 times greater than those of the wild-type strain.<sup>15</sup> Since then, both advanced chassis strains have become well-established, widely used and successful hosts within the scientific community for heterologous expression studies.<sup>118,140–143</sup>

Another proven approach is transcriptional refactoring of the BGC, where native regulatory elements are replaced with well-characterized synthetic promoters. This strategy was successfully applied to the bottromycin gene cluster: by systematically generating a library of cluster variants with randomized synthetic promoters and expressing them in *Streptomyces* heterologous hosts, production of bottromycin was increased by up to 50-fold compared to the native producer.<sup>35</sup> This not only facilitated the generation of previously uncharacterized derivatives but also enabled biosynthetic derivatization that was previously impossible due to limited material.

A similar strategy was employed for pamamycins, a family of macrodiolide polyketides with strong antimicrobial and anticancer properties. In this case, random promoter insertion in front of key operons within the *pam* BGC, followed by expression in a genetically optimized *S. albus* host, led to a significant shift toward higher-molecular-weight and more bioactive derivatives.<sup>144</sup> Notably, new analogues such as pamamycin 663A and homopamamycin 677A were discovered, compounds that would remain undetectable in the native producer due to extremely low yields. Beyond transcriptional engineering, host metabolic rewiring plays a crucial role in improving yield and tailoring the product profile. Pamamycin biosynthesis, for example, depends heavily on the availability of various CoA-activated extender units. By knocking out or modulating specific genes involved in the supply of methylmalonyl-CoA and ethylmalonyl-CoA in *S. albus* J1074, researchers were able to redirect flux toward desirable derivatives and reduce the formation of undesired side products.<sup>145</sup> This strategy led to a more defined production profile and simplified compound





isolation—important steps toward preclinical development. Process engineering approaches can further support these biosynthetic improvements. For instance, cultivation of *S. albus* harboring the *pam* BGC in the presence of talc microparticles resulted in improved morphology, altered precursor availability, and up to a threefold increase in pamamycin production.<sup>146</sup> Transcriptomic analyses revealed a broad upregulation of genes, including those within the *pam* BGC (up to 1024-fold), demonstrating how physical process enhancements can synergize with genetic modifications. In summary, yield improvement in heterologous systems requires more than just transferring a gene cluster into a new host. It depends on a combination of strategies—BGC refactoring, precursor engineering, resistance adaptation, and process optimization—to establish a production platform that can meet the stringent demands of early drug development.

Similar to the strategies successfully applied to bottromycin and pamamycins, spinosad—a complex polyketide insecticide produced by *Saccharopolyspora spinosa*—has also been the subject of extensive heterologous expression efforts. Spinosad's industrial potential is high due to its broad-spectrum insecticidal activity and environmental safety. However, *S. spinosa* is genetically recalcitrant, and its native biosynthetic machinery is difficult to manipulate. To address this, multiple synthetic biology strategies have been implemented in heterologous hosts such as *Streptomyces albus* and *S. coelicolor*, achieving strong yield improvements.<sup>147–151</sup>

As with pamamycins, enhancing precursor supply has proven crucial. In *S. albus* B4, deletion of the transcriptional repressor BkdR, a TetR-family regulator of the *pccAB* operon (involved in propionyl- and acetyl-CoA carboxylation), led to a significant increase in intracellular pools of methylmalonyl-CoA and malonyl-CoA, key building blocks in spinosad biosynthesis. The engineered strain produced 29.4% more spinosad than its parental strain, especially when supplemented with propionate.<sup>151</sup>

Additionally, the fine-tuning of tailoring enzymes was essential to reduce the formation of less active analogues. In a study using *S. albus* J1074, unbalanced expression of the forosamine methyltransferase SpnS led to the accumulation of *N*-monodesmethyl spinosad—an undesired derivative with much lower insecticidal activity. By placing *spnS* under the control of a tunable promoter and co-overexpressing *spnP* (the forosaminyl transferase), researchers achieved a 5.3-fold increase in desired spinosad titer while eliminating ~90% of unwanted derivatives.<sup>150</sup> This highlights how expression balancing within a refactored BGC directly impacts both yield and product purity—echoing similar findings in the pamamycin pathway.

Other yield-enhancement strategies have focused on gene dosage and dynamic precursor control. In *S. coelicolor* M1146, the entire spinosad BGC was amplified using a ZouA-dependent tandem amplification system, resulting in a 224-fold increase in spinosad production. When combined with dynamic regulation of intracellular triacylglycerol (TAG) degradation—which mobilizes carbon toward polyketide precursors—titers reached nearly 2 mg L<sup>-1</sup>, a ~347-fold improvement over the baseline.<sup>149</sup>

Finally, the construction of a 79-kb synthetic multi-operon gene cluster in *S. albus* further demonstrated the potential of BGC refactoring. Dividing 23 spinosad biosynthetic genes into 7 operons under strong constitutive promoters yielded a 328-fold increase in spinosad production compared to the native gene cluster.<sup>148</sup> This synthetic system exemplified how modular pathway architecture and rational promoter assignment can successfully overcome regulatory incompatibility between the native cluster and the heterologous host. Together, these case studies—bottromycin, pamamycin, and spinosad—illustrate a common principle: heterologous expression must be supported by host engineering, transcriptional refactoring, precursor balancing, gene dosage control and bioprocess engineering to achieve better production levels for drug development (Table 5).

#### 4.2. Natural products diversification through heterologous expression

The unparalleled structural complexity of natural products poses significant challenges for their modification and diversification. Unlike synthetic small molecules, natural products often contain densely functionalized, stereochemically rich cores that are difficult to access or alter through traditional medicinal chemistry. Selective derivatization is frequently limited by the lack of functional handles, the need for protecting groups, or lengthy synthetic routes, making the generation of analogues labor-intensive, costly, and often impractical—especially in the early discovery phase when rapid structure-activity relationship studies are crucial.

Diversification within native producers is equally constrained. Many natural product-producing microbes are genetically intractable, exhibit low production yields, or harbor tightly regulated BGCs that are only weakly expressed under laboratory conditions. Even in genetically accessible strains, pathway engineering can trigger metabolic burden, instability, or interference with native regulatory networks, severely limiting the scope for introducing mutations, tailoring modifications, or combinatorial biosynthesis efforts.

To overcome these limitations, heterologous expression systems have emerged as versatile and powerful platforms for natural product diversification. By transferring BGCs into well-characterized and genetically flexible hosts it becomes possible to bypass native regulatory constraints and refactor biosynthetic pathways for controlled expression. These engineered systems provide a clean background for introducing biosynthetic modifications—including gene deletions, domain swaps, tailoring enzyme variations, or hybrid pathway assemblies—that generate new-to-nature compounds with improved or altered properties. Number of strategies such as pathway refactoring, mutasynthesis, precursor-directed biosynthesis, and tailoring enzyme engineering are enabling the efficient generation of diverse analogue libraries for lead optimization and early-stage drug development.

A recent example comes from thioholgamide, a thioamide RiPP (ribosomally synthesized and post-translationally modified peptide) with potent anticancer properties. Traditional



**Table 5** Summarizes metabolite overproduction that has been realized through the heterologous expression of BGCs in various hosts

Compound name	BGC type	Compound type	Heterologous host	Ref.
Spinosad	PKS type I	Macrolide	<i>S. albus</i> B4, <i>S. coelicolor</i> M1146, <i>S. albus</i> J1074	147–151
Mellein	PKS type I	Aromatic polyketide	<i>S. albus</i> B4	152
Staurosporine	Indolocarbazole	Indolocarbazole	<i>S. albus</i> J1074, <i>S. coelicolor</i> M1146	153 and 154
Indigoidine	NRPS	Azachinone	<i>S. lividans</i> TK24	155
Neoaurerethin	PKS type I	Polyketide	<i>S. coelicolor</i> M1152	156
Oviedomycin	PKS type II	Aromatic polyketide	<i>S. coelicolor</i> M1152, <i>S. coelicolor</i> $\Delta$ abrA1/A2	157 and 158
di-AFN A1	NRPS	Cyclohexapeptide	<i>S. coelicolor</i> M1154, <i>S. lividans</i> TK24	159
Neotetrafibricin	PKS type I	Linear polyketide	<i>S. lividans</i> TK21	160
Thaxtomin	NRPS	Cyclic dipeptide	<i>S. coelicolor</i> M1154, <i>S. albus</i> J1074	161–163
Tetracenomyces	PKS type II	Aromatic polyketide	<i>S. coelicolor</i> M1146, <i>S. cinnamonensis</i> sp	139 and 164
Salinomycin	PKS type I	Polyether	<i>S. lividans</i> K4-114, <i>S. albus</i> J1074	165
Moenomycin, nosokomycin	[Unusual]	Phosphoglycolipid	<i>S. albus</i> J1074, <i>S. coelicolor</i> M1152, <i>S. albus</i> J1074 deriv	166–168
Brasilicardin	Terpene	Diterpene	<i>S. griseus</i> sp	169
Totopotensamides	NRPS-PKS	Polyketide-cyclic peptide	<i>S. lividans</i> TK64	170
Chlortetracycline	PKS type II	Aromatic polyketide	<i>S. rimosus</i> sp	171
Chromomycins	PKS type II	Aromatic polyketide	<i>S. lividans</i> K4–114	172
Mithramycin	PKS type II	Aromatic polyketide	<i>S. lividans</i> TK24 deriv	89
Oxytetracycline	PKS type II	Aromatic polyketide	<i>S. venezuelae</i> WVR2006	173
Tautomycetin	PKS type I	Linear polyketide	<i>S. coelicolor</i> M145	174
Goadsporin	RiPPs	Linear azole peptide	<i>S. lividans</i> TK23	175
Bafilomycin, lactacystin, holomycin, pholipomycin, chloramphenicol	[Several]	[Several]	<i>S. avermitilis</i> SUKA 22	42
Tacrolimus	NRPS-PKS type I	Macrolide	<i>S. coelicolor</i> M1146	176
Gougerotin	[Complex]	Peptidyl nucleoside	<i>S. coelicolor</i> M1146	177
Muramycin	Complex NRPS	Peptidyl nucleoside	<i>S. lividans</i> TK24	178
Aloesaponarin II	PKS type II	Aromatic polyketide	<i>S. coelicolor</i> ESK104	179
YM-216391	RiPPs	Cyclopeptide	<i>S. lividans</i> 1326	180
Actinorhodin, chloramphenicol, congoicidine	[Several]	[Several]	<i>S. coelicolor</i> M1146, M1152, M1154	115
Iso-migrastatin	PKS type I	Macrolide	<i>S. albus</i> J1074	181
Caprazamycin	[Complex]	Liponucleoside	<i>S. coelicolor</i> M1154	140
Clorobiocin, coumermycin, novobiocin, novclobiocin	Aminocoumarin	Aminocoumarin	<i>S. coelicolor</i> M512, M1146, M1154	81,125 and 126
Flaviolin	PKS type III	Aromatic polyketide	<i>S. venezuelae</i> YJ028 deriv	182–184

chemical derivatization of this complex, post-translationally modified molecule is virtually impossible. Using a heterologous expression system in a *S. lividans*  $\Delta$ YA8 chassis, our group successfully implemented a codon-randomization strategy in the core peptide gene of the thioholgamide BGC. This system enabled the generation of a focused derivative library with over 85 new variants, many of which retained high production yields and bioactivity. Notably, several derivatives revealed novel post-translational modifications—including thiazoline rings and *S*-methylmethionine—that had not previously been observed in this class, underlining the value of this system not just for diversification but also for biosynthetic discovery.<sup>185</sup> A further impressive case is that of cinnamycin, a lantibiotic with antiviral and anticancer potential. Utilizing a heterologous *S. albus* platform, we introduced site-specific stop codons into the cinnamycin prepeptide and employed the pyrrolsyt-tRNA synthetase/tRNA<sup>Py1</sup> system to incorporate non-canonical amino acids at specific positions. This method led to the production of multiple cinnamycin analogues with ncAAs (noncanonical

amino acids) bearing reactive side chains.<sup>186</sup> These analogues showed varied bioactivity profiles, illustrating how structural tuning at single amino acid positions can modulate function in complex RiPPs.

A particularly elegant demonstration of pathway remodeling through heterologous expression is seen in thaxtomin, a phytotoxin with herbicidal potential. The biosynthetic genes from *S. scabies* were expressed in *S. albus* along with a promiscuous tryptophan synthase from *Salmonella typhimurium*. This system enabled the *in situ* biosynthesis of modified tryptophans, which were incorporated into the thaxtomin scaffold by the native NRPS machinery. As a result, a suite of non-natural thaxtomin analogues was obtained—each with different substituents on the indole ring—demonstrating the power of precursor-directed biosynthesis coupled with heterologous expression.<sup>187</sup>

In their investigations of thiopeptide biosynthesis, the Walsh group introduced mutations into the gene cluster of GE37468 derived from *Streptomyces* ATCC 55365, a genetically unstable and unreliable native producer of the thiopeptide. A



specific gene inactivation targeted the P450 enzyme GetJ, which is responsible for the conversion of Ile8 to mhP8, while a gene replacement focused on an Ile8Ala mutation. The heterologous expression of the modified gene clusters in an *S. lividans* TK24 host resulted in the production of the expected GE37468 analogs, both of which exhibited antibiotic activity against MRSA, albeit at a reduced level compared to the native product.<sup>188</sup>

The clorobiocin gene cluster, classified as aminocoumarin, from *Streptomyces spheroides* has been thoroughly characterized since 2005. Utilizing this knowledge, several attempts were made to create new analogs and hybrid compounds using *S. coelicolor* M512 as the expression system. A deletion mutant of the methyltransferase gene *cloP* yielded a series of derivatives with alterations in the sugar moieties, referred to as novclorobiocin.<sup>189,190</sup> In a mutasynthesis approach, the native amide synthetase gene *cloL* from the clorobiocin pathway was replaced with the corresponding gene from the coumermycin biosynthesis pathway, *coul*. This strategy led to the identification of three novel compounds: ferulobiocin, 3-chlorocoumarobiocin, and 8'-dechloro-3-chlorocoumarobiocin, demonstrating the effectiveness of this approach while also highlighting the unpredictability of the resulting substitutions.<sup>191</sup> All newly synthesized derivatives exhibited antibacterial activity, although at a lower potency than the highly effective native product, clorobiocin.

## 5. Outlook

Heterologous expression in *Streptomyces* hosts has already proven to be a transformative approach for accessing cryptic natural products, improving production yields, and enabling structural diversification. Nevertheless, several important challenges remain that must be addressed to unlock the full biosynthetic potential of microbial genomes. One key future direction lies in improving the success rate of cluster expression, particularly for BGCs derived from rare actinobacteria and expressed across different chassis. While current *Streptomyces* strains perform well for *Streptomyces*-derived clusters, the expression of non-*Streptomyces* BGCs—especially those originating from phylogenetically distant actinobacteria—remains a significant challenge.<sup>99</sup> These clusters most likely face issues related to promoter incompatibility, enzyme folding, missing cofactors, or substrate limitations in current *Streptomyces* hosts. To address this, there is a pressing need to develop new chassis strains from other actinobacterial genera, particularly from rare or understudied lineages.<sup>192</sup> These alternative hosts could provide the native-like intracellular environment required for effective expression of non-*Streptomyces* actinobacterial pathways.

Another critical area is yield enhancement, especially for compounds advancing toward preclinical development or industrial scale-up. While engineered *Streptomyces* strains like *S. albus* Del14, *S. coelicolor* M1152, and *S. lividans* ΔYA9 have demonstrated impressive capabilities, consistent high-titer expression remains the exception rather than the norm. Future solutions will likely rely on systems-level metabolic

engineering, AI-driven pathway design, and dynamic regulatory tools to fine-tune metabolic flux and improve precursor availability.

Finally, minimizing metabolic background and streamlining downstream processing will be essential not only for natural product discovery but also for the sustainable production of lead compounds. Continued advances in genome reduction, synthetic biology toolkits, and chassis standardization will support more predictable and scalable platforms. In parallel, expanding the diversity of heterologous hosts—including from within the broader actinobacterial clade—will be key to fully realizing the promise of genome mining and pathway engineering for next-generation drug discovery.

## 6. Conflicts of interest

The authors declare no conflicts of interest.

## 7. Data availability

All data supporting the findings of this review are available within the article and its SI.

A comprehensive table listing all heterologous expression experiments, including donor organisms, host strains, BGC types, expression outcomes, and corresponding literature references, is provided as SI Table S1. This curated dataset enables reproducibility and facilitates further analysis by researchers in the field. See DOI: <https://doi.org/10.1039/d5np00036j>.

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