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Awakening Silent Antibiotic Arsenals in Streptomyces: CRISPR-Guided Rewiring of Transcriptional Regulators to Combat Antimicrobial Resistance in a One Health Era

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ABSTRACT

Antimicrobial resistance is now among the leading causes of death worldwide, yet the clinical antibacterial pipeline remains critically thin. Genome sequencing has revealed that Streptomyces species carry far more biosynthetic gene clusters than the metabolites they express, indicating a large reservoir of cryptic antibiotics that remains transcriptionally silent under laboratory conditions. This review examines how CRISPR-based genome and transcriptome engineering can be directed at the regulatory layer that keeps these clusters dormant. We summarise the architecture of cluster-situated and global regulators, compare CRISPR-Cas9, base-editing and CRISPR interference or activation strategies for promoter replacement and repressor removal, and consider reported yields, off-target risks and scale-up constraints. Finally, we place cryptic-metabolite discovery within a One Health framework, in which human, veterinary and environmental antibiotic use jointly shape resistance. Coupling regulatory rewiring with stewardship offers a realistic route to replenishing the antibacterial pipeline.

Keywords: Streptomyces; biosynthetic gene clusters; CRISPR interference; transcriptional regulators; antimicrobial resistance; One Health

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Introduction

Antimicrobial resistance (AMR) has shifted from a projected threat to a measured burden. Global modelling attributed approximately 1.27 million deaths directly to bacterial resistance in 2019, with a further 4.95 million deaths associated with resistant infections, and the burden fell most heavily on low- and middle-income regions [1]. Earlier policy analyses warned that unchecked resistance could cost ten million lives annually by 2050, a figure that continues to frame international funding priorities [2]. Against this background, the discovery of structurally novel antibacterial scaffolds has stagnated, and most recent approvals are derivatives of established classes.

Actinomycetes, and the genus Streptomyces in particular, supplied the majority of clinically used antibiotics during the mid-twentieth century golden age, including streptomycin, tetracyclines, macrolides and glycopeptides. Sequencing of Streptomyces coelicolor A3(2) and Streptomyces avermitilis showed that each genome encodes more than twenty secondary metabolite pathways, far exceeding the number of compounds previously isolated from these strains [3,4]. Subsequent comparative genomics confirmed that this discrepancy is general: the biosynthetic potential of the genus is substantially uncharacterised, and the limiting factor is expression rather than genetic capacity [5].

Clusters that are genetically intact yet transcriptionally inactive under laboratory conditions are described as silent or cryptic. Because silence is fundamentally a regulatory phenomenon, tools that act on transcription are conceptually better matched to the problem than the classical approaches of varied media, co-culture or chemical elicitation. CRISPR-derived technologies provide precisely this capability. This review outlines the regulatory logic governing cryptic clusters, evaluates CRISPR-guided strategies for rewiring it, and argues that discovery efforts must be embedded in a One Health response to resistance.

The regulatory basis of cluster silence

Secondary metabolism in Streptomyces is controlled by a hierarchical network that couples morphological differentiation, nutrient status and developmental timing to biosynthetic output [6]. At the lowest level, cluster-situated regulators are encoded within the biosynthetic gene cluster itself. The Streptomyces antibiotic regulatory protein family typically acts as pathway-specific activators, binding operator sequences upstream of structural genes; deletion of such an activator abolishes production, whereas its overexpression can induce it. TetR-family repressors perform the opposite function, occluding promoters until a pathway intermediate or ligand relieves repression.

Superimposed on this are pleiotropic regulators, including the bld and whi gene products that link aerial mycelium formation and sporulation to metabolite synthesis, and nutrient-responsive systems such as PhoP for phosphate limitation and GlnR for nitrogen control. Gamma-butyrolactone signalling molecules, exemplified by A-factor in Streptomyces griseus, act as quorum-type signals that relieve repression at a defined culture density [6]. Chromatin-level and translational constraints add further layers: many clusters lie in the variable chromosomal arms, and pathways of foreign genomic-island origin may carry promoters or codon usage poorly matched to the host transcriptional machinery.

The practical consequence is that silence usually arises from one of three tractable defects: absence of an activating signal, dominance of a repressor, or a weak native promoter. Each is addressable by targeted genetic intervention, and each defines a distinct CRISPR strategy.

CRISPR platforms for actinomycete engineering

Genetic manipulation of Streptomyces was historically constrained by high genomic GC content, low transformation efficiency and slow growth, so that a single marker-free deletion could require several months. Adaptation of CRISPR-Cas9 to actinomycetes transformed this position. Independent systems reported in 2015 achieved efficient single and multiplex deletions, including removal of entire biosynthetic gene clusters, within a single round of conjugation [7,8]. Codon-optimised cas9 expressed from a thiostrepton- or tetracycline-inducible promoter, paired with a single-guide RNA cassette and homology arms on a temperature-sensitive or conjugative vector, has since become the standard configuration.

Three functional modes are now routinely deployed. First, nuclease-active Cas9 with a homology-directed repair template supports precise edits: deletion of a repressor gene, in-frame replacement of a native promoter with a strong constitutive element such as kasOp*, ermEp* or a synthetic variant, or insertion of a reporter for expression monitoring. Promoter knock-in has proved especially productive, since a single replacement upstream of a cluster operon can convert an undetectable pathway into a measurable one [9]. Second, catalytically dead Cas9 fused to activator or repressor domains, or used alone as a steric block, enables CRISPR interference and CRISPR activation. These approaches leave the genome unaltered, permit reversible and titratable control, and allow rapid interrogation of candidate regulators before committing to a permanent edit [10]. Third, base and prime editing derivatives introduce point changes without double-strand breaks, which is advantageous in strains where break repair is inefficient or where clean start codon and operator mutations are preferable to gene loss.

Multiplexing extends all three modes. Arrays of guide RNAs allow simultaneous removal of competing high-flux pathways, deletion of a repressor and insertion of a strong promoter in one construct, so that carbon flux is redirected toward the target metabolite while its transcription is derepressed.

Rewiring regulators to awaken silent clusters

Four intervention designs recur in the literature. Repressor knockout is the most direct: CRISPR-mediated deletion of a cluster-situated TetR-family or MarR-family repressor frequently yields detectable product, and the resulting strain remains genetically stable without antibiotic selection. Activator overexpression, achieved by replacing the promoter of a Streptomyces antibiotic regulatory protein gene with a strong constitutive promoter, exploits the amplification inherent in the regulatory cascade, since one activator may control several operons.

Direct promoter refactoring bypasses the native regulatory layer altogether. Inserting characterised constitutive or inducible promoters upstream of each operon within a cluster places biosynthesis under the control of a defined synthetic circuit; this approach has been applied successfully to clusters with no identifiable pathway-specific regulator [9]. Finally, global regulator modulation tunes the host background. CRISPR interference directed at nutrient-responsive or developmental regulators can shift the strain into a secondary-metabolite-permissive physiological state, although the pleiotropic nature of these factors makes growth defects a common trade-off.

Heterologous expression remains complementary rather than competing. Capturing a cluster and refactoring its promoters in an optimised chassis such as Streptomyces coelicolor M1152 or M1146, from which major endogenous clusters have been deleted, reduces background metabolites and simplifies detection [11]. CRISPR tools facilitate both the construction of such chassis and the subsequent editing of the captured cluster. Analytical resolution has kept pace, with mass spectrometry-based molecular networking enabling rapid comparison of engineered and parental strain metabolomes and dereplication of known compounds [12].

Limitations and translational barriers

Several constraints temper expectations. Off-target cleavage and Cas9 toxicity are reported in high-GC actinomycetes, and inducible or transient expression is generally necessary to preserve viability. Awakening a cluster does not guarantee useful chemistry: many derepressed pathways yield known compounds, analogues of limited novelty, or metabolites with no antibacterial activity, so dereplication must precede any investment in scale-up. Titres obtained after a single regulatory intervention are often at the milligram-per-litre level, insufficient for structure elucidation and bioassay without additional strain and fermentation optimisation. Precursor supply, transporter capacity and self-resistance determinants frequently become limiting once transcription is restored, and post-transcriptional bottlenecks are not addressed by promoter engineering alone.

Economic and regulatory factors compound these technical issues. Antibacterial development suffers from a well-documented market failure, since new agents are reserved rather than widely prescribed, and push-and-pull incentives remain incompletely implemented. Discovery programmes must therefore be judged not only by technical yield but by the realistic probability of clinical translation.

A One Health perspective

Resistance determinants circulate continuously between human, animal and environmental compartments, and the One Health framework recognises that interventions confined to clinical medicine cannot contain this exchange [13]. Agricultural and aquacultural antibiotic use, together with pharmaceutical manufacturing effluent, sustains selective pressure in soils and waterways, which are also the natural habitat of Streptomyces. Environmental resistomes serve simultaneously as reservoirs of mobilisable resistance genes and as sources of the biosynthetic diversity that discovery programmes seek to exploit [14].

Two implications follow. First, discovery and stewardship are not sequential but concurrent obligations, since a novel scaffold introduced without deployment controls will attract resistance along the same pathways as its predecessors. Second, biosynthetic gene clusters commonly co-encode self-resistance determinants, so characterising a cryptic pathway yields advance intelligence on the resistance mechanism likely to emerge clinically, information that can inform surveillance design [15]. Integrating CRISPR-guided discovery with environmental resistome monitoring and coordinated usage policy across sectors therefore strengthens both arms of the response.

Conclusion

Streptomyces genomes contain a substantial unexploited antibiotic reservoir whose principal barrier is regulatory rather than genetic. CRISPR-based deletion, interference, activation and promoter refactoring provide a precise and increasingly routine means of addressing that barrier, and have already converted silent clusters into detectable metabolites. Realising clinical value will require coupling these tools with high-throughput dereplication, strain and fermentation optimisation, and sustained economic incentives. Situated within a One Health strategy that simultaneously restrains selective pressure across human, animal and environmental sectors, regulatory rewiring represents one of the more credible routes to replenishing the antibacterial pipeline.

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