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Application of Metabolic Engineering Strategies in Streptomyces Species for Secondary Metabolite Production: A Systematic Review

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Protocol status: Working

We use this protocol and it's working

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Keywords: Streptomyces, Actinobacteria, metabolic engineering, genetic engineering, pathway engineering, CRISPR, regulatory engineering, heterologous expression, secondary metabolites, antibiotics, natural products, biosynthetic gene clusters, BGCs, streptomyces species for secondary metabolite production, metabolic engineering strategies influence metabolite yield, secondary metabolites in streptomyces species, secondary metabolites from streptomyce, metabolic engineering strategies on the production, metabolic engineering strategy, major advances in metabolic engineering, application of metabolic engineering strategy, metabolic engineering, future research directions in microbial biotechnology, microbial biotechnology, streptomyces species, streptomyce, secondary metabolite production, activation of cryptic biosynthetic gene cluster, pathway optimization, cryptic biosynthetic gene cluster, crucial role in biotechnology, secondary metabolite, biotechnology, including crispr

Abstract

This systematic review protocol aims to synthesize current evidence on the impact of metabolic engineering strategies on the production of secondary metabolites in *Streptomyces* species. Secondary metabolites from *Streptomyces* play a crucial role in biotechnology, particularly in antibiotic, anticancer, and industrial applications. Despite major advances in metabolic engineering, including CRISPR-Cas systems, pathway optimization, and regulatory network modification, the overall effectiveness and outcomes of these interventions remain scattered across the literature. This review will systematically identify, evaluate, and compare relevant studies to determine how metabolic engineering strategies influence metabolite yield, diversity, and activation of cryptic biosynthetic gene clusters. The findings will provide an integrated understanding of current approaches and inform future research directions in microbial biotechnology.

Guidelines

SEARCH STRATEGY

A comprehensive search will be performed in the following electronic databases: PubMed, and Scopus. The search will cover all years from 2015 to the 2025. No language restrictions will be applied at the search stage.

PUBMED SEARCH QUERY : (("Streptomyces/genetics"[Mesh]) AND ("Streptomyces/metabolism"[Mesh])) AND "Metabolic Engineering"[Mesh]

SCOPUS : streptomyces AND secondary metabolites AND metabolic engineering AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (EXACTKEYWORD , "Streptomyces") OR LIMIT-TO (EXACTKEYWORD , "Secondary Metabolites") OR LIMIT-TO (EXACTKEYWORD , "Genetic Engineering")) = 2071 docs

streptomyces AND secondary metabolites AND metabolic engineering = 10930 docs

streptomyces AND secondary metabolites AND metabolic engineering AND PUBYEAR 3e 2014 AND PUBYEAR 3c 2026 = 8866 docs

streptomyces AND secondary metabolites AND metabolic engineering AND PUBYEAR 3e 2014 AND PUBYEAR 3c 2026 AND (LIMIT-TO (DOCTYPE , "ar")) = 4680 docs

streptomyces AND secondary metabolites AND metabolic engineering AND PUBYEAR 3e 2014 AND PUBYEAR 3c 2026 AND (LIMIT-TO (DOCTYPE , "ar")) AND (LIMIT-TO (EXACTKEYWORD , "Metabolic Engineering") OR LIMIT-TO (EXACTKEYWORD , "Secondary Metabolites") OR LIMIT-TO (EXACTKEYWORD , "Genetic Engineering") OR LIMIT-TO (EXACTKEYWORD , "Biosynthesis")) = 1882 docs

SCREENING PROCESS

1. Title 26 Abstract Screening

- Quickly check if the article seems relevant.
- Remove clearly irrelevant records (e.g., animal studies, wrong population, wrong intervention).

2. Full-Text Screening

- Read the full article to confirm it meets all inclusion criteria.
- Use the predefined inclusion/exclusion criteria (no improvisation).

Data extraction

Data will be extracted independently by two reviewers using Rayyan.ai and a standardized data extraction form designed in Microsoft Excel. Extracted information will include: study identifiers (authors, year, country), study design, Streptomyces species, metabolic engineering strategy (gene overexpression, knock-out, CRISPR editing, pathway refactoring, or heterologous expression), characteristics of the targeted gene (if applicable),

characteristics of the studied secondary metabolite, experimental conditions, analytical methods, and outcomes related to secondary metabolite production (yield, fold-change, activation of silent BGCs, new metabolites detected).

Methodological details such as culture conditions (Temperature, pH, Presence of Oxygen), controls, limitations reported by authors, and funding sources will also be collected.

Disagreements during data extraction will be resolved through discussion or by consultation with a third reviewer. When essential data are missing, the original authors will be contacted. If no response is obtained within four weeks, missing elements will be documented as such.**

Quality assessment

Quality assessment will be performed independently by two reviewers. The primary tool used will be ROBINS-I, which is appropriate for evaluating risk of bias in non-randomized experimental and Quasi-experimental interventions such as metabolic engineering modifications (gene knock-outs, overexpression, CRISPR editing, heterologous expression).

In addition, a custom quality checklist adapted from the Joanna Briggs Institute (JBI) will be applied to assess methodological rigor, including description of genetic manipulation techniques, culture conditions, control strains, replication, analytical methods (e.g., LC-MS, NMR), and completeness of reporting.

DATA SYNTHESIS

A narrative synthesis will be conducted to summarize study characteristics, metabolic engineering strategies, and reported outcomes. When at least three studies provide comparable quantitative data, a meta-analysis will be performed. Continuous outcomes measured in the same units (e.g. mg/L of metabolite) will be pooled using Mean Difference (MD); when units or scales differ, Standardized Mean Difference (SMD) will be used. Dichotomous outcomes (e.g. activation of a biosynthetic gene cluster) will be synthesized using Risk Ratios (RR), Odds Ratios (OR), or pooled proportions, depending on data availability.

A random-effects model will be applied by default due to anticipated methodological and biological variability across studies. Statistical heterogeneity will be assessed using the I^2 and t^2 statistics. Where significant heterogeneity is observed ($I^2 \geq 75\%$), results will be explored through subgroup analyses or presented narratively.

Predefined subgroup analyses will investigate potential sources of heterogeneity, including: (i) type of metabolic engineering approach (overexpression, gene knockout, CRISPR-based editing, refactoring, heterologous expression), (ii) host strain (native vs heterologous chassis), (iii) analytical method used for metabolite quantification, (iv) culture conditions, and (v) biosynthetic gene cluster characteristics. Meta-regression will be considered if at least ten studies are available for a given outcome.



Sensitivity analyses will be performed by excluding studies at high risk of bias, comparing fixed- and random-effects models, and conducting leave-one-out analyses. Publication bias will be assessed using funnel plots and Egger's test when ≥ 10 studies are included.

Data will be harmonized by converting units to common measures and, when required, estimating means and standard deviations from medians, interquartile ranges, or other estimates.

Before start

Exclusion Criteria

Population/Participants: Studies not involving *Streptomyces* spp., or focusing on other genera (e.g., *Actinomyces*, *Mycobacterium*).

Intervention/Exposure: Studies not applying metabolic engineering (e.g., fermentation optimization, random mutagenesis, or cultivation condition changes without genetic intervention).

Comparator: Studies lacking a control or reference strain (wild-type or unmodified strain) for comparison.

Outcomes: Studies not reporting measurable changes in secondary metabolite production (e.g., only focusing on growth rate, primary metabolism, or enzyme kinetics without metabolite quantification).

Study design: Reviews, editorials, conference abstracts without data, Computational modeling without experimental validation

Document: Articles with no full-text access, incomplete data, or non-peer-reviewed materials (e.g., theses, patents, preprints not validated).

Language: Languages outside your selection criteria.

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Data will be harmonized by converting units to common measures and, when required, estimating means and standard deviations from medians, interquartile ranges, or other estimates.
- 3 Summary statistics using established methods. Multi-arm studies will be handled according to Cochrane recommendations to avoid double counting.



Statistical analyses will be performed using R (packages meta and metafor) and/or RevMan. Results will be presented using forest plots, summary tables, risk-of-bias assessments, and a PRISMA flow diagram.

Timeline

- 4 The review will be conducted over an estimated period of 1 to 2 months. The planned timeline is as follows:
 - Protocol development and registration in Protocols.io : 3 days
 - Search strategy development and pilot testing: 3 days
 - Database searching and record retrieval: 1 week
 - Title and abstract screening: 3 days
 - Full-text screening: 1 week
 - Data extraction: 5 days
 - Quality assessment (risk of bias): 4 days
 - Data synthesis and meta-analysis: 1 week
 - Manuscript preparation: 1 week
 - Manuscript submission and revisions: 2 weeks