



Genome-scale metabolic models of *Streptomyces clavuligerus*: Retrospectives and perspectives

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The worldwide crisis of bacterial antimicrobial resistance (AMR) has placed a high burden on the pharmaceutical industry. As reviewed by Naghavi *et al.* [1], an estimation of 4.71 million deaths was linked to bacterial AMR in 2021. This led to the development of ground-breaking strategies for enhancing antibiotic production and discovering new antimicrobial compounds in the near future to mitigate AMR-related deaths. Here, the genus *Streptomyces* is highlighted as the leading natural source of clinically relevant antibiotics, responsible for most of the compounds currently used in the market. This biosynthetic capacity stems from their large and GC-rich genomes, which harbor numerous biosynthetic gene clusters for secondary metabolites [2–4]. Among *Streptomyces* species, *Streptomyces clavuligerus* (*S. clavuligerus*) produces the β -lactamase inhibitor clavulanic acid (CA). CA, a compound with modest antibiotic activity but high inhibition capacity of β -lactamase enzymes, possesses high pharmaceutical value [5,6]. The sales of Augmentin® (Amoxicillin trihydrate/potassium clavulanate) in 2024 reached 628 £m [7].

The development of genome-scale metabolic models (GEMs) for *S. clavuligerus* has emerged as a tool to understanding and optimizing its complex metabolic networks [8]. This computational and mathematical platform integrates omics information (genomics, transcriptomics, proteomics, and metabolomics), and biochemical knowledge to provide comprehensive representations of cellular metabolism, enabling the prediction of metabolic behavior, identify engineering targets, and optimize bioprocess conditions (Fig. 1).

1. Retrospectives on *S. clavuligerus* GEMs

The first GEM of *S. clavuligerus* ATCC 27064, designated iMM864, was reconstructed in 2010 by Medema

et al. [9] using the available genome annotation. This model included 1,492 reactions, 971 metabolites, and 864 genes, enabling simulations of growth on various carbon and nitrogen sources and assessment of the essentiality of the 1.8 Mb megaplasmid present in this strain. Later, a study demonstrated that flux balance analysis (FBA) accurately predicted the metabolic fluxes through the precursors of CA, in agreement with gene expression profiles from overproducer strains (*claR/ccaR* overexpression and $\Delta gap1$) [10].

In 2018, iMM864 underwent two independent updates. The first, named iLT1021, expanded the network to 1,494 reactions, 1,360 metabolites, and 1,021 genes [11]. Using data from chemostat cultures, ATP yield was identified as the most appropriate objective function, while growth rate was optimal for batch simulations. In the same year, Ramirez-Malule *et al.* [12] refined iMM864, and the updated model consisted of 1,510 reactions and 1,187 metabolites. This improved GEM was employed to investigate the accumulation of tricarboxylic acid (TCA) cycle intermediates linked to CA biosynthesis under batch and subsequent continuous cultures with steadily declining feed rates. The authors reported a positive correlation between CA production and the accumulation of oxaloacetate, succinate, and acetate. In contrast, a negative correlation with malate levels was found. Furthermore, FBA-based simulations suggested that phosphoenolpyruvate carboxylase activation stimulates CA biosynthesis, whereas activation of the glyoxylate shunt via isocitrate lyase is associated with carbon limitation and reduced CA titers.

A subsequent update in 2019 expanded the network to 1,534 reactions, 1,199 metabolites, and 871 genes. In this GEM, 18 alternative objective functions for evaluating metabolic phenotypes related to CA and cephamycin C production were *in silico* explored [13].

In 2020, an independent reconstruction, iDG1237, was released. This model, topologically and thermodynamically curated, comprised 2,177 reactions, 1,518 metabolites, and 1,237 genes, and was validated with experimental data [14]. It provided insights into the metabolic reprogramming of *S.*

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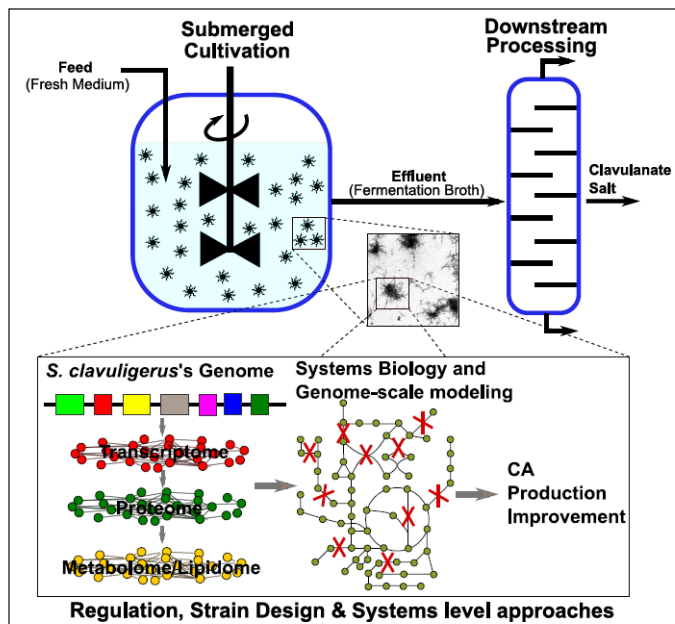


Figure 1. Rational and holistic approach for understanding *S. clavuligerus* physiology to increase CA yield. Taken from López-Agudelo *et al.* [8].

clavuligerus under varying (high and low) shear stress and nutrient availability.

Collectively, these reconstructions demonstrate that GEMs are powerful tools for dissecting the metabolic complexity of *S. clavuligerus*. Their integration with multi-omics datasets offers a promising avenue for uncovering regulatory connections between genes, proteins, and metabolism, ultimately guiding strategies to optimize CA production at the bench, pilot, and industrial scales.

1.1. Current perspectives and applications

Metabolic engineering represents perhaps the most direct application of these GEMs, where computational predictions guide rational strain design strategies. In practice, these models enable to predict the effects of gene knockouts, overexpressions, and pathway modifications on CA production, leading to the development of engineered strains with improved yields and productivity. Even these GEMs can be potentially used to predict the metabolic flux distribution in already reported CA-overproducer strains like *S. clavuligerus* IDG3 [15], and based on that, propose further genetic modification.

In the case of *S. clavuligerus*, a combination of experimental and GEMs helps to explain the associations between TCA cycle intermediates accumulation and CA, and identifies bottlenecks in its biosynthesis [12,16].

Additionally, the use of omics data could help to refine and improve the findings achieved by employing GEMs. Transcriptome analysis in *S. clavuligerus* have already been reported [17,18].

Bioprocess optimization represents another critical application area where GEMs provide valuable insights for CA industrial applications. These models have helped to optimized fermentation conditions by predicting how different media

compositions, feeding strategies, and environmental conditions affect metabolic flux distributions and CA formation (as described by Gómez-Rios *et al.* [19] and Gómez-Gaona *et al.* [20]).

Likewise, GEMs of *S. clavuligerus* has also proven valuable for understanding the complex dynamics of fed-batch fermentation processes, where nutrient availability and environmental conditions change over time [21].

Another equally important phenomenon is the degradation of CA, which needs to be considered to increase its yield in submerged cultures of *S. clavuligerus* [22,23].

Lastly, drug discovery and development applications are useful for identifying potential targets to enhance CA production or to develop new compounds. *Streptomyces clavuligerus* has been reported to produce naringenin, desferroxamines, several N-acyl tunicamycins, among others [24]. Undoubtedly, GEMs could help to increase the yield of such metabolites.

1.2. Future directions and challenges

Streptomyces clavuligerus exhibits a complex morphological development and differentiation patterns that significantly affect CA production [12,25,26]. This phenomenon is not captured in the current GEMs of *S. clavuligerus*. Thus, integration of population dynamics and spatial organization in GEMs development represents one of the major challenges. Future models will need to incorporate the effects, such as cell-cell communication, morphological differentiation, and environmental gradients, on metabolic phenotype behavior.

As described by Zampieri *et al.* [27], machine learning offers many opportunities to boost model predictive accuracy and enables better handling of complex regulatory networks and enzyme kinetics. Since constraint-based modeling approaches are limited by their reliance on stoichiometric relationships and steady-state assumptions, the integration of machine learning approaches in GEMs can help overcome these limitations by incorporating dynamic regulatory information, enzyme kinetics, and environmental responses. Deep learning approaches also show particular promise for predicting gene expression patterns, metabolite concentrations, and flux distributions under different environmental conditions [27].

Synthetic biology applications also represent an increasingly important application domain where GEMs could guide the design of synthetic circuits for producing novel antibiotics or optimizing existing pathways for heterologous expression in the genus *Streptomyces*. As reviewed by Del Carratore *et al.* [4], the recent advances in synthetic biology open a new door for the exploitation of the biotechnological and pharmaceutical potential of this genus.

Regarding the challenges and limitations, the published GEMs of *S. streptomyces* do not capture regulatory mechanisms such as transcriptional, translational, and post-translational control. Thus, regulatory information remains of one of the major challenges. Additionally, the enzyme kinetics and thermodynamic constraints present another significant task. Furthermore, GEMs generally do not to capture the effects of enzyme saturation, allosteric regulation, and metabolite inhibition. In general, developing frameworks that integrate

kinetic information with GEMs remains an active area of research for *S. clavuligerus*.

Additionally, many GEMs were developed and validated using data from specific bench-scale conditions and may not accurately predict behavior under large-scale conditions or in genetically modified strains, which constitutes a limitation. Large-scale processes are characterized by inhomogeneous conditions, with gradients of oxygen, nutrients, and pH, among others, which could limit the model's prediction.

2. CONCLUSION

The development and evolution of GEMs of *S. clavuligerus* represents an active topic in systems biotechnology, demonstrating how computational and mathematical-based approaches can enhance the understanding of complex biological systems and guide practical applications in CA production. The *S. clavuligerus* modeling efforts also provide essential tools for optimizing existing antibiotic production processes and developing new therapeutic strategies, which is currently a hotspot topic. Finally, looking ahead, the integration of multi-scale modeling approaches, machine learning techniques, and synthetic biology applications promises to further advance the understanding and engineering of this important organism.

3. AUTHOR CONTRIBUTIONS

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be an author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

4. CONFLICTS OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

5. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing and editing of the manuscript, and no images were manipulated using AI.

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How to cite this article:

Ramirez-Malule H. Genome-scale metabolic models of *Streptomyces clavuligerus*: Retrospectives and perspectives. *J Appl Pharm Sci*. 2026;16(01):001-004. DOI: 10.7324/JAPS.2025.276494.ed