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Review Article

Unleashing the Power of Artificial Intelligence-Driven Drug Discovery in *Streptomyces*

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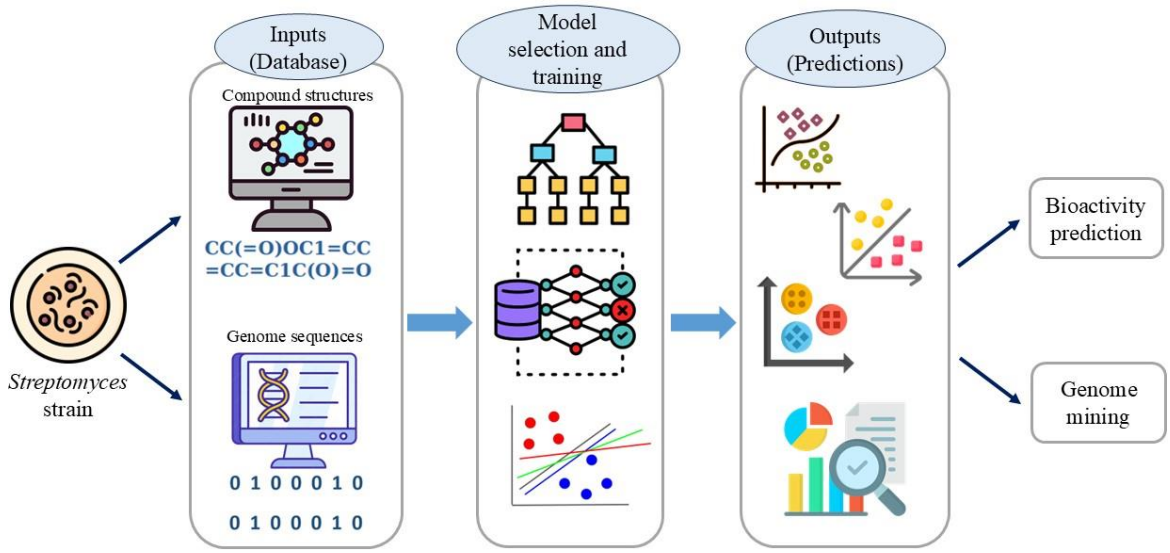
Abstract: The rise of antibiotic resistance has created an urgent need for the discovery of new antibiotic compounds. Streptomycin, the first antibiotic isolated from *Streptomyces* sp., paved the way for discovering other antibiotics for combating bacterial infections. By exploring the genome-based biosynthetic potential of various *Streptomyces* species, a vast array of secondary metabolites with potential therapeutic applications can be identified, contributing a transformative impact on the field of medicine. However, conventional screening approaches on novel natural products (NPs) from *Streptomyces* sp. have entered a bottleneck due to inefficiency. Fortunately, artificial intelligence (AI) and machine learning (ML) models enable rapid exploration and prediction of potential antibiotic compounds, increasing the probability of discovering new antibacterial compounds. AI-driven drug discovery in *Streptomyces* sp. represents a paradigm shift in the future quest for novel pharmaceutical agents. Various ML models have been developed and applied in different practical applications. Overall, the ML model is trained using input data and generates

outcomes based on prediction output. This review discusses the continued potential of *Streptomyces* sp. as a source of novel NPs, along with the application of ML throughout the NP drug discovery pipeline involving genome mining, biological activities prediction, and optimization compound production in *Streptomyces* microbial systems.



Graphical abstract. The role of machine learning in drug discovery from *Streptomyces.*

Keywords: *Streptomyces*; Secondary metabolites; Natural products; Drug discovery; Machine Learning; Artificial Intelligence; SDG 3 Good health and well-being

1. Introduction

Bioprospecting has played an important role in natural product-based drug discovery through the exploration, extraction, and screening of new natural compounds derived from plants, microorganisms, and animals for commercially valuable applications, particularly in harnessing their therapeutic effects in the pharmaceutical industry [1-5]. During the 'Golden Age' of antibiotic discovery (1940 to 1970), this approach was used in identifying 23 classes of antibiotics that are currently in clinical settings to prevent and treat human diseases [6]. Major antibiotic classes include aminoglycosides, cephalosporins, fluoroquinolones, macrolides, tetracyclines, and β-lactam. The introduction of antibiotics has remarkably changed the therapeutic paradigm and saved millions of lives from a wide range of bacterial infections [7]. Most of the new drug classes were identified originating from natural resources such as microbes and screened based on molecular, species, and genetic levels [8]. Whole-cell screen strategies have been implemented extensively on microorganisms for exploring potential biotherapeutic activity within the cellular context [9].

Over two-thirds of the clinically used antibiotics are natural products produced by the genus *Streptomyces* which have been considered as a bio-factory of a diverse range of natural compounds with antagonistic or pharmacological properties [10]. This high biosynthetic potential of *Streptomyces* was explored actively due to its large genome of 8-10 Mbp with high GC contents and large biosynthetic gene clusters (BGCs) that are able to synthesize a large variety of secondary metabolites [11, 12].

Nowadays, widespread antibiotic resistance has raised alarms in healthcare systems across the globe [13, 14]. Antibiotic resistance emerges when bacteria, viruses, fungi and parasites evolve different mechanisms to evade the therapeutic effects of antibiotics [15, 16]. One of the most widespread infections worldwide is Methicillin-resistant *Staphylococcus aureus* (MRSA) infection where *S. aureus* becomes resistant to methicillin and other β-lactam antibiotics in hospital settings, leading to significant morbidity, and mortality [17, 18]. The excessive and inappropriate use of antibiotics in both human medicine and agriculture has significantly accelerated the development of antibiotic resistance, making it one of the top 10 global public health threats as recognized by the World Health Organization (WHO) [15]. Discovering new antibiotics has been an alternative approach to combat this growing serious health threat. However, nature-driven drug discovery research on microbes has taken a back seat and evolved over the years. Traditional drug discovery approaches often face technical difficulties, particularly in screening programs, separation and isolation techniques of natural products produced from the primary or secondary metabolism of bacterial species under laboratory conditions. To address these issues, several technologies, such as combinatorial chemistry, high throughput screening (HTS), computational modeling, and artificial intelligence (AI), have been developed to accelerate the drug discovery process for natural products. [19]. The application of AI and machine learning (ML) incorporated with algorithms marks a revolutionary shift in drug discovery and development. AI refers to the development of machine learning ML models that simulate human-like intelligence and perform tasks adaptively [20]. In natural product discovery, ML techniques have been applied throughout the process involving compound screening, detecting biosynthetic gene clusters (BGCs), drug target interaction, compound optimisation and compound dereplication [21].

Herein, this review highlights the continued potential of *Streptomyces* as a source of novel natural products with the current state-of-the-art technology in antimicrobial natural product drug discovery and the utility of ML approaches in advancing microbial natural product discovery focusing on *Streptomyces sp*. Employing ML approaches in *Streptomyces* natural products discovery is still relatively nascent and needs to be explored more.

2. *Streptomyces* as a Source of Valuable Compounds

Presently, two-thirds of commercial and therapeutical antibiotics are derived from actinomycetes, nature's topmost antibiotic producers, and almost exclusively from *Streptomyces* sp. [22, 23]. Several previous studies have extensively reported the morphology, taxonomy, and genetics of *Streptomyces* as well as various metabolic pathways and enzymatic functions [24-26]. *Streptomyces* is a Gram-positive bacteria and the largest genus in the *Actinobacteria* phylum living in a wide range of environments, such as harsh, underexplored

habitats, terrestrial, marine regions and mangroves [22, 27]. It undergoes a complex life cycle that includes vegetative growth, sporulation, and antibiotic production. Besides, *Streptomyces* undergo multiple levels of morphological differentiation in response to the growing environments [28]. When a typical *Streptomyces* spore encounters favourable conditions, it forms filamentous branching structures of vegetative mycelium, further differentiates into the reproductive aerial mycelium and eventually leads to the formation of sporulation septa under nutrient depletion or stressed environment [29]. The mature spore is often associated with the production of secondary metabolites, including antibiotics as a result of nutrient limitation. Unlike other bacteria, *Streptomyces* have a large genome size of 8-10 Mb with an exceptionally high G+C content of > 70% and multiple biosynthetic gene clusters (BGCs) [30].

Remarkably, *Streptomyces* account for over 70% of commercially useful antibiotics, major types of antibiotics such as aminoglycosides, anthracyclines, glycopeptides, β-lactams, macrolides, ansamycins, nucleosides, peptides, polyenes, polyesters, and tetracyclines (Table 1). The diverse range of natural products with high structural diversity exhibits broad-spectrum activity against both Gram-positive and Gram-negative bacteria, antiviral, antifungal, cytotoxic, antitumor, anti-protozoal, anti-hypertensive, immunosuppressive, insecticide, and antioxidative properties [31-33]. Bioactive compounds with different biological activities isolated from different *Streptomyces* are represented in Table 2. In 1943, the first aminoglycoside antibiotic, streptomycin was discovered by Albert Schatz and Selman Waksman [34]. This antibiotic was isolated from *Streptomyces griseus* and contributed significantly to the treatment of various bacterial infections, including tuberculosis, plague, tularemia, and brucellosis. In recognition of his achievements in the discovery, Selman Waksman was awarded the Nobel Prize for Medicine in 1952 [35]. Besides, erythromycin is an antibiotic produced by *Streptomyces erythreus* that is used to treat a variety of bacterial infections, particularly respiratory tract and skin infections [36]. *Streptomyces* have profoundly revolutionized medicine with the discovery of antibiotics leading to the antibiotic era and have significantly reduced live mortality. Exploration of bioactive compounds from *Streptomyces* remains a valuable ally in the search for new solutions to overcome antibiotic resistance [37]. The unique and diverse range of bioactive compounds synthesised by *Streptomycetes* have high versatility and broad-spectrum antagonistic activity against both Gram-positive and Gram-negative bacteria [38-40].

3. Secondary Metabolite Biosynthetic Gene Clusters (smBGCs) in *Streptomyces*

Secondary metabolite production in *Streptomyces* was triggered during the stationary phase in response to environmental stress or lack of nutrients. Secondary metabolites are natural products that, different from primary metabolites involve normal growth, development, and reproduction. In contrast, secondary metabolites are primarily involved in the defence system [61]. In *Streptomyces*, the biosynthesis of secondary metabolites is mainly regulated by nonribosomal polyketide synthetase (NRPS) pathways and polyketide synthetase (PKS) [62]. Polyketides are secondary metabolites or natural products produced by *Streptomyces* and synthesized through sequential reactions catalyzed by a set of enzyme

complexes known as polyketide synthases (PKSs) [63]. *Streptomyces*-derived bioactive natural products are produced by means of complex 'secondary metabolic' pathways encoded by the secondary metabolite biosynthetic gene clusters (smBGCs) [64]. smBGCs is a grouping of genes in genome-sequenced bacteria that encode the enzymes and proteins involved in the pathways of precursor biosynthesis, assembly, modification, transport, and regulation of a particular secondary metabolite. Different modular structures within the gene clusters are responsible for distinct steps in the secondary metabolite biosynthetic pathway [65]. Each smBGC contains a set of core biosynthetic genes, accessory genes, and regulatory elements that work synergistically to synthesize secondary metabolites. The expression of these gene clusters is tightly controlled by molecular mechanisms in complex regulatory networks in response to environmental stresses found in the bacteria's native habitats [66]. Advances in sequencing technology have demonstrated that a typical *Streptomyces* genome encodes around 25–50 BGCs. However, approximately 90% of them are cryptic or silent under laboratory fermentation conditions, limiting the synthesis of secondary metabolites [67]. Therefore, to maximize secondary metabolite production by discovering the unexplored biosynthetic potential of *Streptomyces*, methods to activate silent BGCs are crucial to current natural product-derived drug discovery research.

Table 1. Lists of classes of antibiotics and their examples from *Streptomyces*.

Class	Antibiotics	References
Aminoglycosides	gentamicin streptomycin tobramycin neomycin kanamycin	[41]
Anthracyclines	doxorubicin	[42]
β-lactams	monobactams, cephalosporin carbapenems	[43]
Macrolides	clarithromycin erythromycin azithromycin	[44]
Ansamycins	rifamycin	[45]

Table 2. Bioactive compounds isolated from *Streptomyces* species with biological activities.

Bioactive molecules	Bioactivities	Species	References
Bleomycin	Anticancer	*S. verticillus*	[46]
Chloramphenicol	Antibiotic	*S. venezuelae*	[47]
Clavulanic acid	β-lactamase inhibitor	*S. clavuligerus*	[48]
Clindamycin	Antibiotic	*S. lincolnensis*	[49]
Daptomycin	Antibiotic	*S. roseosporus*	[50]
Daunomycin	Antitumor	*S. peucetius*	[51]
Erythromycin	Antibiotic	*S. erythraeus*	[36]
FK506 (Tacrolimus)	Immunosuppressant	*S. tsukubaensis*	[52]
Ivermectin	Antiparasitic	*S. avermitilis*	[53]

Kanamycin	Antibiotic	*S. kanamyceticus*	[54]
Lincomycin	Antibiotic	*S. lincolnensis*	[55]
Nystatin	Antifungal	*S. noursei*	[56]
Streptomycin	Antibiotic	*S. griseus*	[57]
Tetracycline	Antibiotic	*S. aureofaciens and S. rimosus*	[58, 59]
Vancomycin	Antibiotic	*S. orientalis*	[60]

4. Traditional approaches for drug discovery in *Streptomyces*

In 1941, Selman Waksman known as the Father of Antibiotics discovered streptomycin isolated from *Streptomyces griseus* through *in vitro* screening tests against pathogenic bacteria, including tuberculosis-causing mycobacteria Woodruff [34]. During the golden era of antibiotic discovery in the 1940s to 1960s, his systematic screening approach known as the Waksman Platform was widely adopted and implemented by researchers to identify antimicrobial agents produced by actinomycetes, particularly *Streptomyces* [68]. Traditionally, the identification and discovery of secondary metabolites and their associated biosynthetic gene clusters in *Streptomyces* were achieved through a combination of classical microbiological and molecular biology techniques [69]. To get the first insight into the biological activities of soil-borne *Streptomyces sp*. against pathogens, plenty of *Streptomyces* species were isolated from various environments and screened for the production of secondary metabolites through phenotypic screening approaches based on physical observations such as pigment production or inhibition zones and biochemical assays [70]. The isolation of secondary metabolites by *Streptomyces* is first performed by fermentation under various culture conditions, fractional extraction, and a series of purification steps involving chromatography techniques such as column chromatography, thin-layer chromatography (TLC), or high-performance liquid chromatography (HPLC). To identify and characterize the natural products, the purified compounds are then subjected to structural elucidation using spectroscopic methods such as nuclear magnetic resonance (NMR) and mass spectrometry [71]. Bioassay-guided fractionation is also used to investigate the bioactivity of fractions of a crude extract and isolate the active fractions indicating the specific secondary metabolites [72, 73]. However, these methods are time-consuming and laborious, and a large fraction of secondary metabolites are not expressed actively and cannot be identified under laboratory culture conditions.

In the early 2000s, classical molecular technologies such as genome mining and bioinformatic analysis revealed enormous numbers of BGCs in the *Streptomyces* genomes [74]. Unlike the traditional bioactivity-guided isolation of NPs, the genome sequencing and bioinformatic analysis of sequenced *Streptomyces* genomes lead to the exploration and prediction of the cryptic or silent smBGCs for novel NPs, which are silent under standard laboratory conditions and are potential targets to be activated [75]. To identify smBGCs within the *Streptomyces* genome sequences, several bioinformatics tools have been developed such as antiSMASH [76], PRISM [77], NP.searcher [78], ClustScan [79], BLAST [80] and BAGEL [81] integrated with databases such antiSMASH database, Minimum Information about a Biosynthetic Gene cluster (MIBiG) [82], the biosynthetic gene cluster families database (BIG-

FAM) [83] or the Integrated Microbial Genomes Atlas of Biosynthetic gene Clusters (IMG-ABC) [84]. The urgent need to discover novel secondary metabolites has resulted in the development of genomic engineering to activate these cryptic or silent gene clusters [85]. Silent smBGCs can be activated through heterologous expression and *in situ* activation. Heterologous expression was established by cloning the target silent gene cluster and heterologous expressed in a heterologous host. Besides, the manipulation of smBGCs for unlocking silent or cryptic gene clusters can be achieved by introducing constitutive promoters, regulating the transcription factors (TFs), and modifying ribosomes through targeted mutagenesis involving knock-in or knock-out techniques [67, 86]. In the past studies reported by Ochi [85], the CRISPR-Cas9 system was applied to promote the effective activation of silent BGCs in five *Streptomyces* species [85]. In addition, overexpression of activator gene under promoter such as *bldA* successfully triggered the expression of cryptic biosynthetic gene clusters for the production of the antibiotics actinorhodin, undecylprodigiosin, and methylenomycin [87]. Although all these techniques have shown efficacy, there is a high investment and low return rate in silent BGC activation. Nonetheless, the application of these methods in studies on NPs discovery provides better insights into the hidden potential of microorganisms (Figure 1). By leveraging techniques such as genome mining, predictive analysis, and targeted genetic manipulation, new bioactive compounds or biosynthetic pathways of NPs can be identified and elucidated from metabolomics which involve metabolite profiling.

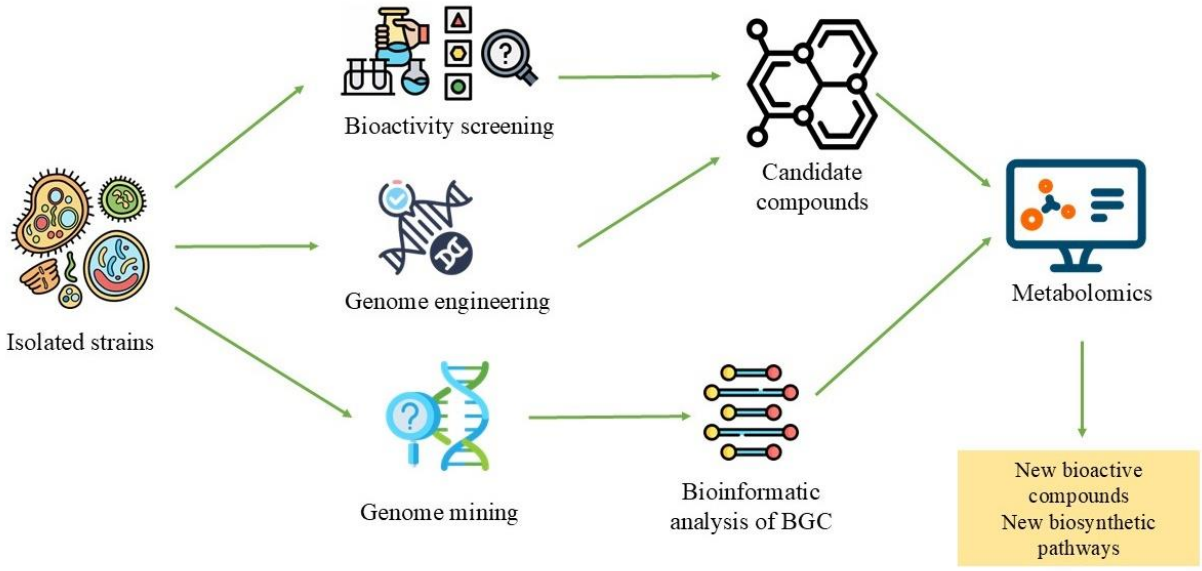


Figure 1. Summary workflows of natural product discovery in *Streptomyces.* The icons in the figure are generated by Freepik from https://www.flaticon.com/.

5. Application of artificial intelligence in natural product-derived drug discovery research

In 2020, group researchers at MIT's Jameel Clinic discovered the first powerful antibiotic using AI named Halicin and it has made a ground-breaking drug discovery [88]. Halicin was initially used for diabetes treatment and was accidentally rediscovered to exhibit broad-spectrum antibacterial activity, particularly against multidrug-resistant pathogens like *Clostridiodes difficile*, *Acinetobacter baumannii*, and *Mycobacterium tuberculosis* [89]. This discovery demonstrated the potential of AI to repurpose existing drugs for new therapeutic applications. The researchers employed an ML model and trained using a deep learning algorithm with a diverse dataset of about 2,500 FDA-approved drugs and natural products. The ML model was trained to recognize patterns of different compounds associated with various chemical structures and further correlate with their antibacterial properties [90]. The algorithm was designed to predict the effectiveness of potential antibiotics. Remarkably, there is a study that highlights the efficacy of Halicin in both *in vitro* and *in vivo* settings, with particular success in murine models infected with *Clostridioides difficile* and pan-resistant *Acinetobacter baumannii* [89]. While the research was in its early stages, the discovery of Halicin highlighted the potential of AI in accelerating the identification of novel antibiotic compounds. It raised hopes to combat the rising threat of antibiotic resistance. AI-based drug discovery also showed another breakthrough in discovering a new antibacterial molecule, namely abaucin that targets *Acinetobacter baumannii*, which causes blood, urinary tract, and lung infections [65, 91]. The convergence of AI and drug discovery has catalyzed a paradigm shift in the pharmaceutical industry to speed up the discovery of new antimicrobial drugs. Exploring new NPs with biological activity has been a cornerstone of drug discovery research. However, conventional drug discovery in *Streptomyces* sp. namely, the Waksman Platform and other approaches are usually characterized by time-consuming processes, high costs and low success rates. Therefore, AI and machine learning (ML) approaches have been utilized to bypass the limitations and challenges of traditional approaches hence accelerating the drug discovery process in a better efficient, more cost-effective and time-effective way. These advanced technologies enable researchers to analyze large number of datasets, predict molecular properties, and identify potential drug candidates with greater precision and speed. As a result, AI and ML have emerged as invaluable tools in the quest for novel therapeutics from natural sources.

Machine learning (ML) is a subset of AI, which mimics the human cognitive processes to interpret information. It is a mathematical model that learns from data, understands the patterns and makes predictions or decisions [92]. ML techniques can be classified into supervised, unsupervised and semi-supervised learning. In supervised learning, a given dataset with known class labels is used to train the algorithm for the classification or regression tasks. For unsupervised learning algorithms, the model learns from unlabelled data without any explicit guidance or predefined outputs. It is used for the three main tasks including clustering, association and dimensionality reduction. Semi-supervised learning is a hybrid model that includes both supervised and unsupervised learning [93]. Supervised learning algorithms, such as Random Forest (RF) [94], Support Vector

Machines (SVM) [95], Naive Bayes (NB) [96], decision tree (DT) [97] and linear regression are the most commonly used supervised algorithms for NP discovery. Unsupervised learning algorithms like Hierarchical Clustering [98] and Chemical Space Mapping [99] also facilitate NP discovery research. The choice of ML algorithm depends on several factors, such as the size and quality of the data, the type of machine learning task, and the interpretability of outputs. In general, constructing a machine learning model consists of multiple steps involving data collection and preprocessing, model selection and training, evaluation and validation of the model's performance and eventually ends with model deployment and continuous monitoring (Figure 2). Initially, the workflow of machine learning starts with data collection from various sources and data preprocessing to remove duplicates, handling missing values, and normalizing or scaling features. Once the data is pre-processed, a model architecture such as decision trees and neural networks with optimal hyperparameters such as learning rate, regularization strength, and batch size is selected and trained on the data [100]. The model learns patterns and relationships from the input features to make predictions. After the model is trained, the model's performance metrics, such as precision, accuracy, or F1 score are evaluated and validated. After evaluating and validating the model, it can be deployed for real-world application or production. To evaluate the model's performance consistency across different data partitions, cross-validation is utilized by randomly dividing the original datasets into multiple training and testing sets, typically referred to as "folds." K-fold cross-validation is employed to compare different models, evaluate efficient models with hyperparameters, and subsequently obtain greater model performance with high reliability and robustness [101]. This approach enhances the generalization ability of a learning algorithm on unseen data so that it can be used to make predictions on new data, resulting in more accurate and dependable results in practical applications. In natural product-derived drug discovery, ML tools could play a vital role in processes involving detecting BGCs, drug target identification, lead compound prioritization and optimization as well as compound screening and drug design [102].

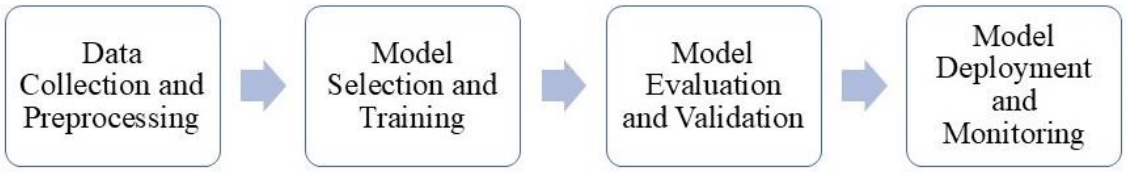


Figure 2. Machine learning workflow.

5.1. Genome mining

Genome mining refers to the analysis of genetic information within the genome of sequenced microorganisms to identify or characterise bioactive compounds such as natural products (NPs) or secondary metabolites [103]. Especially in the case of *Streptomyces*, a genus known for its prolific production of bioactive compounds, genome mining is indispensable for identifying novel drug candidates. Utilising NGS platforms like MiSeq (short-read sequencing) or PacBio (long-read sequencing), numerous studies have sequenced the whole genome of *Streptomyces* isolated from various environments [104-106]. These advanced

approaches have significantly contributed to drug discovery research and expanded our understanding of their biosynthetic capabilities. To enhance the efficiency of discovering NPs and characterization of their bioactivity, various approaches such as computational tools, bioinformatics techniques, and experimental validation methods have been employed to establish the gene–metabolite link by identifying biosynthetic genes that encode enzymes involved in the biosynthesis of bioactive metabolites [65, 107].

The biosynthetic machinery of *Streptomyces* is a highly regulated system, where the biosynthetic gene cluster (BGCs) responsible for the production of various NPs such as polyketides, nonribosomally synthesized peptides (NRPs), ribosomally synthesized and posttranslationally modified peptides (RiPPs), along with alkaloids, and terpene. A novel pentacyclic polyketide named formicamycin has been discovered and identified by using antiSMASH, which employs profile-hidden Markov models (pHMMs) to identify the BGC [108]. In another study, the RiPPER genome mining tool enabled the isolation of novel thioamidated RiPPs [109]. RiPPs differ from other natural products like polyketides and NRPs, which are typically synthesized by multi-modular enzyme complexes. Unlike other classes of NP, RiPPs exhibit unique biosynthetic pathways that lack universal signature biosynthetic genes across all RiPP families, making it challenging to develop universal bioinformatics tools or predictive models for RiPP genome mining [110].

To fully reveal the biosynthetic potential of *Streptomyces*, genome mining tools that incorporate machine learning approaches could be used to detect different classes of NPs. To aid in the identification of BGCs for all major NP classes, DeepBGC, Deep-BGCpred and BIGCARP were introduced by Hannigan et al. [111], Yang et al. [112], and Rios-Martinez et al. [113], respectively. Each genome mining tools implement a deep learning approach such as neural networks combined with vector representations of protein family (Pfam) to predict BGC boundaries and annotate BGC function associated with the biosynthesis of secondary metabolites. It also adopted word embedding techniques such as Word2vec, a natural language processing (NLP) algorithm to analyse literature, patents and databases to extract information about gene functions, gene-drug associations, natural products, and their biosynthesis pathways facilitating the understanding of drug mechanisms and identification of potential therapeutic targets [114]. This has streamlined the target selection process, accelerating the process of identifying novel targets for drug development. Furthermore, NRPSpredictor2 and SANDPUMA (Specificity of Adenylation Domain Prediction Using Multiple Algorithms) were developed to predict NRPS adenylation domain specificity [115, 116]. Both of the tools employ SVM algorithms or other machine learning algorithms to identify NRPS BGCs. Deep learning models such as DeepRiPP, Data-driven Exploratory Class-independent RiPP TrackER (decRiPPter) and NeuRiPP have been developed to tackle these challenges for mining RiPP BGCs [117-119]. A recent study reported that a new class of lanthipeptides (termed “class V”) and 42 new RiPP family candidates were identified with the help of decRiPPter on genome mining of 1295 *Streptomyces* genomes. Unlike traditional methods, decRiPPter does not rely on prior knowledge of core enzymatic machinery or specific modifications. It employs an SVM classifier trained on 175 known RiPP precursors regardless of RiPP subclasses. This approach incorporates pan-genomic analysis to identify

putative precursor genes located within specialized genomic regions. These genomic regions contain multiple enzyme-coding genes and are part of the accessory genome of a genus [120]. By employing decRiPPter, researchers have the potential to unlock a treasure trove of previously undiscovered natural products of RiPP, paving the way for groundbreaking advancements in drug discovery.

Over the years, other ML-assisted genome mining tools such as the hidden Markov model-based method ClusterFinder [121], GECCO17 [122], RiPPMiner-Genome [123], Pytorch [124] and SanntiS [125] have been developed and utilized to detect and annotate potential BGCs that encoded putative bioactive compounds different classes of NPs. Integrating AI technologies with classical approaches might hold tremendous potential for accelerating the discovery process of discovering novel drugs from the vast genomic reservoir of *Streptomyces* sp. AI systems help to integrate various data types such as genomics, transcriptomics, proteomics, metabolomics, structural data, and bioactivity data. This integration enables the discovery of the complex relationships between features and supports the development of finely tuned hypothesis [126].

5.2. Biological activities prediction

The predictive power of AI extends beyond gene cluster identification. The incorporation of automation and AI-powered systems into in silico screening of natural products has also revolutionized the initial phase of drug discovery. ML plays a crucial role in advancing molecular property prediction (MPP) and chemical reaction prediction (CRP) [127] contributing to the discovery of lead compounds. This enables the rapid screening of compound libraries against specific drug targets to identify potential bioactive compounds with therapeutic activity in *Streptomyces* strains. The virtual screening of natural products (NPs) derived from *Streptomyces* sp. utilises datasets from the StreptomeDB 2.0 database which includes about 2,877 NPs originating from *Streptomyces* [128]. In ML technology, molecular featurization is implemented to digitize chemical structures of novel molecules from natural products into a machine-readable format [127]. Molecules have been featured through various techniques such as molecular representations, descriptors, fingerprints and latent vectors derived from molecular embedding [129]. During the molecular featurization process, the chemical structures of the molecules are represented as SMILES (Simplified Molecular Input Line Entry System) [130] or international chemical identifier (InChES) [131] annotations, images, strings or molecular graphs to serve as input information and datasets for machine learning models including various neural network, SVM, multilayer perceptron (MLP) and random forest (RF) [132], subsequently generate outputs to predict the biological activities of molecules such as antibacterial, antifungal, antiviral, antitumor, or immunomodulatory activity (Figure 3).

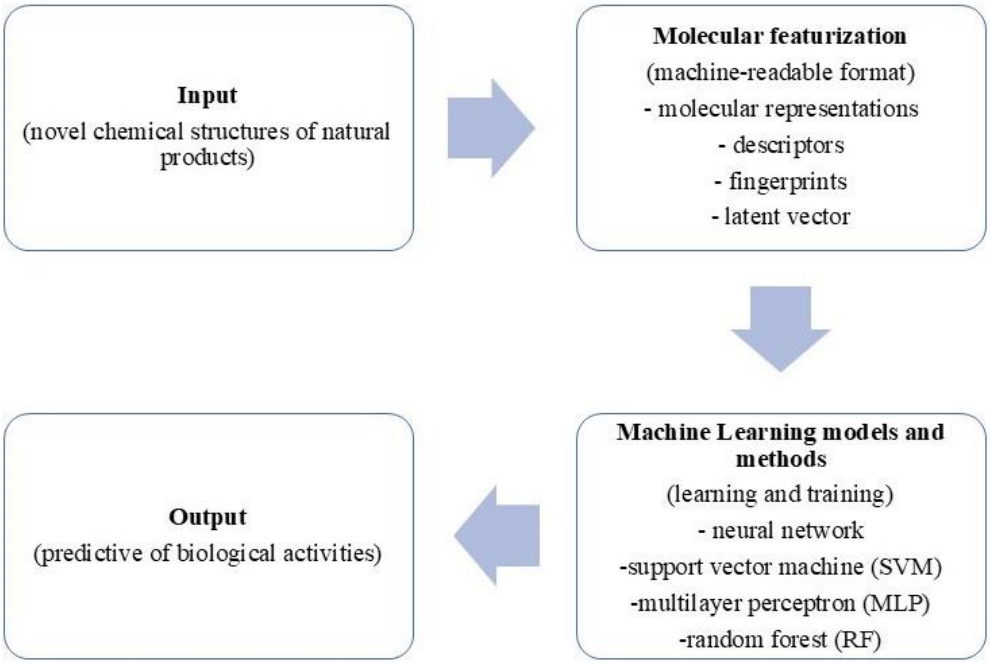


Figure 3. General overview of ML approaches in natural products drug discovery.

In the context of structural elucidation, computational prediction of spectroscopic data, mainly NMR, gas/liquid chromatography (GC/LC) or mass spectrometry (MS) could be integrated with improved performance of deep learning models for more accurate prediction of chemical structures of natural products and theoretical NMR correlation data [133]. Deep learning models employ deep neural networks such as Artificial Neural Network (ANN), Convolutional Neural Network (CNN) and Graph Neural Network (GNN) can be applied in ADMET (Adsorption, Distribution, Metabolism, Excretion, and Toxicity) modelling and Quantitative Structure-Activity relationship (QSAR) modelling involving various aspects of drug discovery including drug target identification, drug target interaction prediction, pharmacokinetics and toxicity of compound [134-137]. This approach could computationally predict the biological activity of *Streptomyces*-derived compounds to target proteins based on chemical structures and select targeted compounds with favourable pharmacokinetic properties and reduced toxicity risks [138]. The interpretation of results could help identify potential compounds, prioritize targeted compounds for testing, and reduce the experimental workload in screening [139]. Moreover, AI algorithms such as Random Forest and SVM speed up multi-omics data processing [140] and hence dereplicate known compounds in natural extracts efficiently [141]. This speeds up the process of identifying novel compounds by eliminating redundancy and focusing on unique chemical entities.

5.3. Optimisation of bioactive compound production

The natural fermentation process has been employed for drug discovery in microbes where cultivated microbes synthesise naturally occurring bioactive compounds through fermentation technology. Some major challenges of microbial fermentation are low-cost efficiency of raw material inventory, low quality of biomass concentration and low yield of fermentation products. The drug discovery process could be enhanced by improving fermentation strategies for the production of secondary metabolites in *Streptomyces* sp. [142]. Therefore, fermentation optimization in terms of incubation time, medium compositions, and environmental factors such as temperature and pH are crucial steps to optimize the conditions for growing *Streptomyces* cultures maximize the valuable secondary metabolite yields, and determine and minimize the input variables. Conventionally, non-statistical techniques such as One-Factor-at-Time (OFAT) and statistical methods such as response surface methodology (RSM) have been practised widely for medium optimization [143, 144]. OFAT is a traditional experimental design method where one independent variable is varied at a time while all other variables remain unchanged. In the context of fermentation optimization, OFAT involves altering one fermentation parameter while keeping all other parameters constant and observing the resulting changes in fermentation yield. This traditional approach could be time-consuming and may overlook synergistic effects that impact overall outcomes due to the difficulty in estimating interactions between multiple variables from the experiments. RSM is a mathematical approach that employs a few experimental designs such as Box-Behnken design (BBD) or Central Composite Design (CCD) to model the relationship between multiple explanatory variables and one or more response variables. The experimental data is then analysed using statistical analysis techniques including multiple regression analysis and analysis of variance (ANOVA) to fit mathematical models such as higher-order polynomial equations, to predict the response surface and identify the optimal combination of fermentation conditions that maximizes the production of desired metabolites or bioactive compounds. Even though RSM is extensively used, some associated limitations are the assumption of linearity and high dependence on the experimental design that might yield biased or unreliable results. Fermentation is a complex system where the output results can be influenced by multiple variables [145, 146]. Compared to conventional methods, AI-driven approaches can analyse complex interactions between variables and adaptively refine solutions over time. ML-based predictive models namely ANNs and statistical models specifically RSM can be coupled with four evolutionary algorithms (EAs) such as GA, DE, simulated annealing algorithm, and particle swarm optimization to optimize the fermentation parameters [147-150]. This coupling of machine learning model and EAs signifies powerful, hybrid optimisation techniques leveraging the strengths of both paradigms, where machine learning models provide predictions while EAs fine-tune and optimize parameters more precisely [151].

Introducing AI or ML-based approaches in genome mining, compound screening, identification of metabolites or bioactive compounds and optimisation of metabolite expression have contributed significant impacts in *Streptomyces*-related drug discovery. Research studies involving the application of AI algorithms or models related to

Streptomyces-related drug discovery are presented in Table 3. Despite the transformative potential, AI systems might have key challenges such as limited data quality, availability, heterogeneity, dataset size, and data privacy [152]. During predictive model construction, unbalanced active to inactive compound datasets with limited coverage of inactive compounds might influence the accuracy of prediction results. Additionally, AI models might lack explainability, interpretability, reproducibility and validation on diverse datasets due to their underlying complexity. Besides, overfitting might occur when the ML model fits the training data too precisely, resulting in poor generalization of new test data [153]. Biases might be present in training data including systemic biases, selection bias, and automation bias, which could potentially lead to biased predictions and ethical considerations [154]. Hence, the accuracy of the prediction may be uncertain when biased data are utilized. Other significant challenges in developing and implementing AI in drug discovery are the need for significant computational power, expertise, and financial investment.

Table 3. Research studies implementations of AI algorithms or models in *Streptomyces sp*.

Aim of the study	AI algorithms or models	*Streptomyces* strains	Findings	Ref.
Genome mining	decRiPPter (Data-driven Exploratory Class-independent RiPP TrackER) combines a Support Vector Machine (SVM)	*Streptomyces pristinaespiralis* ATCC 25468	• Identify 42 novel Ribosomally synthesized and post-translationally modified peptides (RiPP) families • Discover novel family of lanthipeptides within the RiPP biosynthetic gene cluster	[120]
	DeepT2, DeepBGC and antiSMASH model with four machine learning algorithms (random forest, XGBoost, SVM, and MLP)	37 selected *Streptomyces* isolates	DeepT2 outperforms both DeepBGC and antiSMASH in the prediction of type II polyketides (T2PK) using only KS_{β} sequences	[155]
	genetic algorithm (GA)	*Streptomyces coelicolor*	• Identify 11 secondary metabolite gene clusters of the antibiotic-producing eubacterium *Streptomyces coelicolor* • identified gene regulator based on transcriptomic and expression data.	[156]
	SVM-based model	*Streptomyces coelicolor*	Predict and verify the operon structure using different binary classifiers.	[157]
	NRPSpredictor2	*Streptomyces lincolnensis*	Characterized a new nonribosomal peptide namely cysteoamide, 1 and identified its NRPs biosynthetic gene cluster	[158]
Strain identification	artificial neural network (ANN)	three putatively novel *Streptomyces* species	Identify members of the three target streptomycete taxa.	[159]

Optimisation of compound production	MSHub/GNPS (Global Natural products Social Molecular Networking)	*Streptomyces Volatilomes* (37 selected isolates)	• Detect and annotate more volatile organic compounds (VOCs) than using the conventional method. • Remove the volatilome variability between media and isolates.	[160]
	ANN	*Streptomyces flavolimosus*	• Predict the optimal conditions that maximize the biosynthesis of AuNPs using the cell-free supernatant of *Streptomyces flavolimosus* at high efficacy compared to mathematical models, central composite design. • Demonstrate antitumor properties in-vitro (MCF-7 human breast cancer and Hela carcinoma cell lines) and in vivo against Ehrlich ascites carcinoma	[161]
	Response Surface Methodology-Genetic Algorithm (RSM-GA)	*Streptomyces rimosus* MTCC 10792	This combination approach optimizes the medium components for extracellular cholesterol oxidase (COD) production in *Streptomyces* (3.6 folds higher compared to un-optimized medium)	[162]
	ANN coupled with GA and Nelder-Mead downhill simplex (NMDS)	*Streptomyces sindenensis* MTCC 8122	ANN-NMDS optimization was found to be more efficacious compared to the ANN-GA optimization maximum antibiotic production where 197 microgram/ml was obtained in ANN-NMDS optimization; 176 microgram/ml was obtained in ANN-GA optimization	[163]
	ANN/GA	*Streptomyces triostinicus*	ANN/GA prediction model shows better optimal performance in actinomycin V yield of 36.7% higher than RSM model.	[164]
	ANN/GA	*Streptomyces* sp. NICM 5500	ANN/GA prediction model shows better optimal performance with 60% higher COD concentration than RSM model.	[165]
	GA	*Streptomyces hygroscopicus*	GA predicts optimal cultivation parameters which contribute maximum antifungal activity	[166]

ANN	*Streptomyces microflavus* strain NEAE-83	ANN prediction model shows better accuracy than CCD model and aligns closely with the validation experimental in optimization of the chitosan nanoparticles biosynthesis	[167]
ANN	*Streptomyces noursei*	ANN prediction model shows better accuracy than RSM model and aligns closely with the validation experimental in fermentation optimisation	[168]
ANN	*mutant Streptomyces durhamensis GC23*	ANN optimization shows higher predictive efficiency than RSM and aligns closely with the validation experimental for cellulase production.	[169]

6. Conclusion

The global spread of multidrug-resistant bacterial pathogens has become a major threat to the healthcare system and hence driven the urgent need to discover new sources of natural products or antibiotics. In the past few decades, *Streptomyces* sp. has been the largest bio-factory for the production of secondary metabolites with a wide range of biological activities. However, *Streptomyces* sp. possesses large smBGCs in which numerous cryptic BGCs are silenced under laboratory culture conditions with only a limited number of secondary metabolites that have been expressed actively, rendering a largely untapped source of drugs. Drug discovery was traditionally performed through random screening and this traditional compound screening might be challenging. Besides, optimisation of cultivation parameters for bacteria is crucial for enhanced production of secondary metabolites, which is significant for the drug discovery process. Indeed, AI tools are increasingly used to enhance drug discovery efficacy by offsetting the limitations of ineffective traditional approaches in drug discovery. Therefore, applying AI integrated with multidisciplinary techniques is critical to unlocking hidden pathways and discovering new natural products in *Streptomyces* sp.

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