Faculty of Medical and Health Sciences, University of Poonch Rawalakot
Journal of Pharma and Biomedics
ISSN: 3007-1984(online), 3007-1976 (Print)
https://www.jpbsci.com/index.php/jpbs

Connecting the Dots in Secondary Metabolite Detection for Next-Generation Therapeutics: Combating Antibiotic Resistance and Cytotoxic Challenges

Mazia Amber[1]*, Mehral Aslam[2], Erum Naz[3], Javeria Suhail[4], Sareen Fatima[5], Jawad Amin[6]**

[1] School of Biological Sciences, Queen's University Belfast, United Kingdom.
[2] Department of Nutrition and Health Promotion, University of Home Economics Lahore, Pakistan.
[3] Department of Medical Technology, Malir University of Science and Technology, Pakistan.
[4] Department of Biological Life Sciences, Forman Christian College Lahore, Pakistan.
[5] Department of Microbiology, Bolan University of Medical and Health Sciences, Quetta, Pakistan
[6] Department of Horticulture, University of Sargodha, Sargodha, Pakistan.

Received: May 12, 2025; *Revised: June 08, 2025;* *Accepted: June 30, 2025*

A B S T R A C T

Secondary metabolites (SMs) are the bioactive compounds produced mainly by microorganisms and fungi, comprising with ecological and physiological roles. They have been recognized as a primary clinically important source for antibiotics, anticancer agents, and immunosuppressants. However, with the increasing emergence of antibiotic resistance the situation has been intensified, and there is a need for novel natural products with antimicrobial and cytotoxic potential globally. This review depicts the need of microbial and fungal secondary metabolites, with the strong emphases on their biosynthesis, regulation, and therapeutic applications. Particular attention is given to fungal metabolites such as beauvericin, gliotoxin, and chaetoglobosins, which exhibit potent cytotoxic activity against tumor cells. Furthermore, the review also draws the picture of endophytic fungi contributing as an unexplored reservoir of new metabolites, with advances in genome mining, and CRISPR-Cas-based activation of silent gene clusters to promote the discovery of bioactive compounds. The antimicrobial and cytotoxic functions together of several secondary metabolites underline their potential as next-generation therapeutics. Understanding their biogenesis, mechanism of action, and resistance modulation can significantly contribute to combating infectious and neoplastic diseases in the post-antibiotic era.

Keywords: Secondary metabolites; Antibiotics; Cytotoxicity; Endophytic fungi; CRISPR-Cas9.

Corresponding Author: *Mazia Amber; Mehral Aslam*
Email: maziaamber_65@hotmail.com; mehral.92@gmail.com

INTRODUCTION

Overview of Secondary Metabolites

The compounds with varied and most probably sophisticated chemical structures , produced by different microbial strains and some plant species are termed as "Secondary metabolites" (SM) (Anisova LN *et al* .,1984). They are a low-molecular-weight organic compounds which are not involved in the growth or reproduction of microbes directly, but are essential for their survival and adaptation towards the competitive environments. They are biosynthesized through complex enzymatic pathways, often encoded by biosynthetic gene clusters (BGCs) located on chromosomal or plasmid DNA (Martín et al., 2013). These compounds further exhibit a remarkable diversity in structure and function ranging from antibiotics, immunosuppressants, and antitumor agents to enzyme inhibitors and metal chelators (Demain & Fang, 2000). This review highlights the strength and usage of secondary metabolites against antibiotic resistance, it also aids regulating and promoting appropriate use of antibiotics.

Industrial and Pharmaceutical Significance

Secondary metabolites hold immense industrial and pharmaceutical significance due to their structural diversity, complex biosynthesis, and wide range of biological activities (Ramirez-Rendon et al., 2022). These bioactive compounds, are primarily produced by actinomycetes; the *Streptomyces* genus and fungi, which contribute significantly to global health and the economy. Furthermore these secondary metabolites when produced comprises of enormous ecological and biological purposes (Jakubiec-Krzesniak et al., 2018; Demain & Fang, 2000). Interestingly, although antibiotics are not essential for sporulation, several secondary metabolites, including antibiotics themselves, can regulate spore formation and germination. Therefore these compounds are not only important for the survival and adaptation of microorganisms but they also impart a strong role in the growth, development and communication of microbes (Čihák et al., 2017).

The secondary metabolites are widely used and consumed in modern biotechnology and medicine era, where thanks to them they have been a pivotal source for pain releive, cancer treatment, anti-inflammatory and insecticidal agent (Seca and Pinto, 2018). These molecules have profoundly influenced human health, nutrition, and socioeconomic development. Structurally, microbial secondary metabolites exhibit remarkable diversity and are regulated by multiple factors unlike th eprimary metabolites (Figure 1) such as nutrient availability, growth rate, feedback control, enzyme induction or inactivation, and genetic regulation through clustered biosynthetic genes—primarily located on chromosomal DNA, though occasionally on plasmids (Fouillaud and Dufossé, 2022). Unlike primary metabolism, which is well characterized, secondary metabolism remains partially understood, offering opportunities for in-depth studies of enzymatic pathways, metabolic regulation, and cellular differentiation (Bocso and Butnariu, 2022).

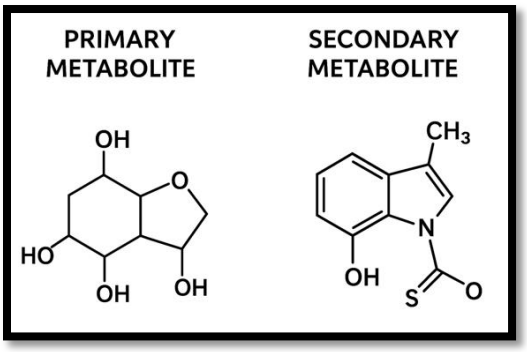

Figure 1. Structural represeantaion of Metabolies; Primary and secondary.

Over the past few years there has been an rapid increase seen in the discovery and production of secondary metabolites, which have further been revolutionized by advances in metabolic engineering, synthetic biology, and genome editing. Classic examples underscore their continuing impact: penicillin from *Penicillium chrysogenum* remains a cornerstone antibiotic (de Frias et al., 2018) cyclosporin A from *Tolypocladium inflatum* is indispensable in organ transplantation due to its immunosuppressive effects (Anke & Thines, 2007) and so on. Modern tools like CRISPR-Cas9 are now enabling activation of silent biosynthetic gene clusters, leading to the discovery of novel compounds with antimicrobial and anticancer potential (Zhang et al., 2017). These technological advancements, together with the increasing understanding of fungal and bacterial genomics, have expanded the potential of secondary metabolites as an inexhaustible source for new pharmaceuticals and industrial biomolecules (Andryukov et al., 2019).

Fungal Secondary Metabolites with Cytotoxic Activity

The secondary metabolites are also produced from fungi, Figure 2 (Salvatore et al., 2025). They make an extremely important input in disease management in humans and other animals. Fungi have been engaged in industrial processing of more than 10 of the 20 most profitable products used in human medicine till date (Ślusarczyk et al., 2021). Two anti-cholesterol statins, the antibiotic penicillin and the immunosuppressant cyclosporin A are among the top 10. Fungi are extremely useful organisms in biotechnology. Fungi construct unique complex molecules using established metabolic pathways (Wisecaver et al., 2014). Different taxa produce sets of related molecules, each with slightly different final products. Metabolites formed along the metabolic pathway may also be biologically active (Anke T & Thines E 2007).

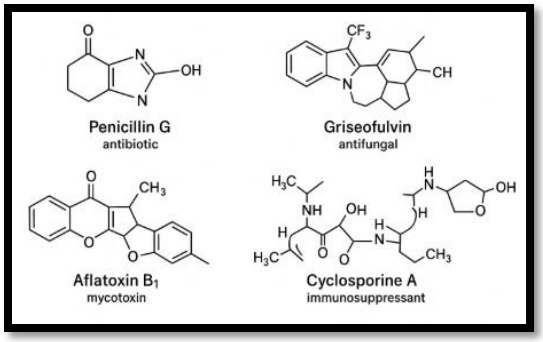

Figure 2. Secondary Metabolies from Fungi.

In addition, the final compounds are often released into the environment. Manipulation of the genome, and environmental conditions during formation of compounds, enable the optimisation of product formation. On the negative side, single isolates of fungi in manufacture may lose their capacity to form or release the target molecules. Indeed, the target compound may only be expressed under specific conditions, or at a specific point in the life cycle of the fungus (Rafeeq et al., 2023). It is amazing that so many biologically active compounds have been discovered and taken to the point where they are medically important. Indeed, attempts to 'discover' new and exciting molecules remain the core activity of many research groups Few antibiotics produced from fungi include penicillin (secondary metabolite) cyclosporineA (primary metabolite), where penicillin is used to treat infection against bacterium and cyclosporine A is a powerful an immunosupressent used widely before and after bone marrow and organ transplants in humans (Anke T & Thines E 2007).

"Beauvericin" (Figure 3) is a famous mycotoxin produced by many fungal species like *Beaveria bassiana* and *Fusarium* spp (Logrieco et al., 1998) This highly toxic secondary metabolite is a cyclic hexadepsipeptide compound that belongs to the enniatin "antibiotic family", and contains three D-hydroxyisovaleryl and three N-methylphenylalanyl residues in an alternating sequence. It has structural resemblance to the enniatins, which are also produced by a number of *Fusarium* species, but above all it differs in the nature of the N-methylamino acid. (Hamill R.L et al., 1969).

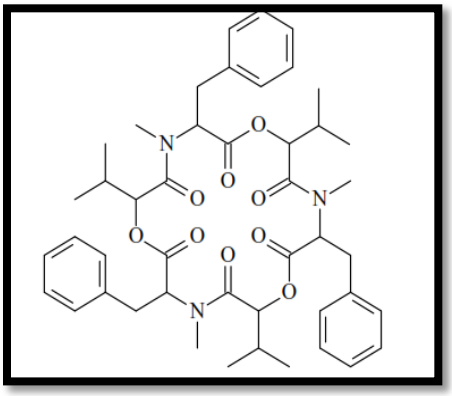

Figure 3. Beauvericin structure.

Antibiotic Resistance and Novel Metabolites

One of the most life-threatening global health challenges of the 21st century is the antibiotic resistance, which poses serious life threats to human helath agriculture, and the environment. A comprehensive snapshot taken in the United States in 2013 revealed the alarming scale of the issue, emphasizing the urgent need for global action to curb the spread of resistant infections (Lessa et al., 2012). The Centers for Disease Control and Prevention (CDC) estimated that more than two million Americans acquire antibiotic-resistant infections annually, resulting in approximately 23,000 deaths (CDC, 2010–2012). Particularly concerning is the rising resistance not only among bacteria but also in fungal species such as *Candida*,

which increasingly causes life-threatening infections in hospitalized patients (Hall et al., 2012). Although antibiotics have long been considered one of the greatest medical breakthroughs of the 20th century, their widespread misuse and overuse in healthcare, agriculture, and animal husbandry have fueled the evolution of resistant strains (Mahalel, 2012). Microorganisms possess remarkable genetic adaptability, enabling them to acquire and disseminate resistance genes through horizontal gene transfer, thereby developing multiple resistance mechanisms against nearly every antibiotic introduced for therapeutic use (Alekshun & Levy, 2007).

Antibiotics, primarily microbial secondary metabolites, remain vital tools in controlling infectious diseases, though only a small fraction of the thousands discovered have achieved clinical success due to limitations such as toxicity, inefficacy, or high production costs. Many of these bioactive compounds are produced naturally by fungi and bacteria, making microbial fermentation a preferred method over chemical synthesis. Since the 1940s, fungi have been particularly valuable sources of antibiotics, with purification and structural identification of bioactive compounds achieved through advanced techniques such as silica gel chromatography and GC-MS analysis. Despite these advances, the alarming rise in antibiotic resistance across multiple pathogenic species underscores the need for continuous exploration of novel antimicrobial sources (Saeed et al., 2007). Researchers emphasize the importance of discovering naturally occurring microbial and fungal metabolites with potent antibacterial and antifungal properties (Chaudhry et al., 2007). Among these, endophytic fungi—symbiotic organisms residing asymptomatically within plant tissues such as pine and yew—are gaining prominence as reservoirs of chemically diverse and biologically active secondary metabolites with potential therapeutic applications (Katoch, 2014).

Secondary metabolites, although traditionally associated with antibiotics, encompass a broader class of bioactive molecules that hold significant industrial and pharmaceutical relevance (Bainton et al., 1992). Unlike primary metabolites, these compounds are produced during the idiophase, typically when key nutrients such as carbon, nitrogen, or phosphate become limited, triggering specialized metabolic pathways (Ruiz-Villafán et al., 2022). In this context, fungal and microbial metabolites are increasingly recognized as promising sources for novel antibiotics capable of overcoming resistance. Many secondary metabolites target unique or multiple cellular pathways, minimizing the emergence of resistant strains (Gorlenko et al., 2020). For example, teixobactin, a recently discovered compound from *Eleftheria terrae*, has shown exceptional efficacy against Gram-positive bacteria, including MRSA and *Mycobacterium tuberculosis*, without detectable resistance (Piddock, 2015). These developments underscore the urgent need for continued exploration of microbial and fungal biodiversity, coupled with advances in genomics and metabolomics, to discover next-generation antibiotics that can outpace the global threat of antimicrobial resistance.

Cytotoxicity and Antimicrobial Screening

Cytotoxicity refers to the ability of certain substances or agents that cause severe damage to cells and very seldom are also involve in cell death. Cytotoxic agents may be chemical compounds or biological toxins (Istifli et al., 2019). Cytotoxicity mechanisms are broadly categorized into antibody-dependent cell-mediated cytotoxicity (ADCC)—where immune effector cells recognize and lyse target cells coated with antibodies—and cell-mediated cytotoxicity, which involves direct killing by cytotoxic T lymphocytes or natural killer (NK) cells (Janeway et al., 2001). When cells are exposed to cytotoxic compounds, they can be the reason behind any kind of cellular injury to cell death. In cases of lethal exposure, they may undergo necrosis, or follow regulated death pathways such as apoptosis or simply goes to autophagy phase for maintaining cellular homeostasis (Galluzzi et al., 2018). Understanding these mechanisms is crucial for distinguishing between therapeutic cytotoxicity (e.g., against cancer cells) and undesirable toxicity toward normal cells.

One of the most crucial steps in natural product research or drug discovery or pharmacological testing is the evaluation of cytotoxicity. It serves as an initial screening measure to identify potentially toxic or therapeutically useful compounds. A variety of in vitro assays are routinely employed for this purpose, such as the MTT assay (which measures mitochondrial activity as an indicator of cell viability), LDH release assay (which quantifies lactate dehydrogenase released from damaged cells), and Trypan Blue exclusion test (which distinguishes viable from nonviable cells) (Riss et al., 2016). Other advanced assays include the resazurin (Alamar Blue), neutral red uptake, and flow cytometric apoptosis detection assays, which provide more detailed insights into cytotoxic mechanisms (Fotakis & Timbrell, 2006). Compounds showing broad cytotoxicity may be discarded from further testing, whereas those exhibiting selective cytotoxicity—for instance, killing cancer cells while sparing healthy ones—are considered

valuable leads in anticancer drug development. Controlled cytotoxicity is particularly relevant for secondary metabolites, where the goal is to exploit their bioactivity against pathological targets while minimizing off-target effects.

Interestingly, several microbial secondary metabolites and antibiotics display both cytotoxic and antimicrobial properties, representing potential dual-function therapeutic candidates. Some antibiotics, initially developed for antimicrobial use, have been found to exert cytotoxic effects on cancer cells through mechanisms such as inhibition of mitochondrial biogenesis, DNA replication, or protein synthesis. For example, doxycycline, a tetracycline antibiotic, selectively targets mitochondrial ribosomes in cancer stem cells, thereby impairing their energy metabolism and self-renewal capacity, suggesting promising applications in oncology (Lamb et al., 2015). Similarly, anthracyclines such as doxorubicin, originally derived from *Streptomyces peucetius*, exhibit potent anticancer activity by intercalating into DNA and generating free radicals (Weiss, 1992). The dual nature of such compounds underscores the overlapping biochemical targets shared between microbial pathogens and cancer cells. Consequently, integrating cytotoxicity and antimicrobial screening not only aids in identifying safe therapeutic windows but also opens new avenues for developing multifunctional agents derived from natural products, especially microbial and fungal secondary metabolites, which continue to be an invaluable source for drug discovery (Genilloud, 2017).

Biotechnological Approaches for Metabolite Discovery

Recent advances in biotechnology have transformed the landscape of secondary metabolite discovery, enabling the identification of novel bioactive compounds that were previously undetectable through conventional screening methods. Modern biotechnological strategies integrate genomics, metagenomics, transcriptomics, proteomics, metabolomics, and synthetic biology to explore the biosynthetic potential of microorganisms in unprecedented depth. Traditional culture-based methods often fail to access the majority of microbial metabolites, as over 99% of microorganisms in nature are unculturable under standard laboratory conditions (Stewart, 2012). To overcome this limitation, metagenomics—the direct extraction and sequencing of environmental DNA—has emerged as a powerful approach for identifying biosynthetic gene clusters (BGCs) from complex microbial communities without the need for culturing (Handelsman, 2004; Charlop-Powers et al., 2015). Through metagenomic libraries and next-generation sequencing, previously "silent" or "cryptic" metabolites from soil, marine, and extreme environments can be uncovered, expanding the chemical diversity available for pharmaceutical and industrial exploitation.

Genome mining represents another cornerstone of modern metabolite discovery. Computational tools such as antiSMASH (antibiotics & Secondary Metabolite Analysis Shell), PRISM (Prediction Informatics for Secondary Metabolomes), and SMURF (Secondary Metabolite Unknown Region Finder) allow systematic identification and annotation of BGCs from microbial genomes (Medema et al., 2011; Skinnider et al., 2015; Khaldi et al., 2010). These platforms enable prediction of metabolite structures, biosynthetic enzyme classes, and potential bioactivities, greatly accelerating the discovery process. Comparative genomic analyses further reveal the evolutionary relationships between BGCs and guide pathway engineering for novel compound production. Integration of multi-omics data (e.g., combining transcriptomics and metabolomics) provides dynamic insights into metabolite biosynthesis under varying environmental conditions (Ziemert et al., 2016). Moreover, CRISPR-Cas9-based genome editing has emerged as a versatile tool for activating silent gene clusters or deleting competing pathways, thereby enhancing secondary metabolite yields and diversity (Zhang et al., 2022; Hussin et al., 2022).

In addition to in silico and genetic strategies, heterologous expression systems and co-culture techniques have proven instrumental in unlocking cryptic metabolic potential. Heterologous expression involves transferring a BGC from its native producer into a more genetically tractable host, such as *Streptomyces coelicolor*, *Escherichia coli*, or *Aspergillus nidulans*, allowing efficient production and characterization of novel metabolites (Yamanaka et al., 2014; Luo et al., 2016). Such systems facilitate controlled expression, combinatorial biosynthesis, and pathway optimization, enabling the generation of non-natural analogs with improved pharmacological properties. Similarly, co-culture or mixed-culture approaches, where two or more microorganisms are grown together, have been shown to induce interspecies chemical signaling that activates otherwise silent biosynthetic pathways (Bertrand et al., 2014; Netzker et al., 2018). For instance, co-cultivation of *Aspergillus nidulans* with *Streptomyces rapamycinicus* led to the discovery of orsellinic acid derivatives through histone modification–mediated pathway activation (Schroeckh et al., 2009). Complementing these approaches, metabolomics coupled with mass spectrometry (MS) and nuclear magnetic resonance (NMR) allows high-throughput detection, dereplication, and structural elucidation of newly

produced compounds (Wolfender et al., 2015).

Overall, the integration of computational genome mining, synthetic biology, metagenomic sequencing, co-culture activation, and advanced analytical platforms has revolutionized natural product discovery. These biotechnological approaches not only accelerate the identification of novel metabolites but also enable rational manipulation of biosynthetic pathways for improved yield and activity. The synergy between omics technologies, genome editing, and bioinformatics continues to expand the accessible chemical space, offering promising avenues for the discovery of next-generation antibiotics, anticancer agents, and other valuable therapeutic molecules.

CONCLUSION AND FUTURE PERSPECTIVES

Microbial and fungal secondary metabolites remain one of the most prolific and versatile sources of bioactive compounds, offering immense potential for the development of next-generation therapeutics. Their diverse biological activities—as antimicrobials, cytotoxins, immunomodulators etc underscore their biotechnological and pharmaceutical relevance. Despite the long history of natural product discovery, a significant portion of microbial and endophytic biodiversity remains unexplored, suggesting that many potent and structurally novel metabolites are yet to be revealed. The integration of multidisciplinary biotechnological approaches is therefore crucial to unlocking this hidden metabolic potential. Future research should emphasize comprehensive exploration of uncultured and extremophilic microorganisms, as well as plant-associated endophytes, which are promising sources of novel bioactive secondary metabolites. Advanced genome mining, metagenomics, and metabolomics approaches combined with synthetic biology and systems biology can significantly accelerate the discovery and characterization of these compounds. The adoption of genome editing tools such as CRISPR-Cas systems has revolutionized the activation of silent or cryptic biosynthetic gene clusters (BGCs), enabling the production of previously inaccessible metabolites. Parallelly, heterologous expression systems, co-culture techniques, and adaptive laboratory evolution can be harnessed to enhance metabolite yields and diversify chemical scaffolds.

The future of metabolite discovery also lies in eco-friendly and sustainable bioprocessing, emphasizing green extraction techniques, renewable substrates, and bioreactor-based fermentation optimization to reduce environmental impact while improving yield and purity. Integration of artificial intelligence (AI), machine learning (ML), and computational modeling into natural product research promises to transform the drug discovery pipeline by enabling structure–activity relationship (SAR) prediction, virtual screening, and biosynthetic pathway reconstruction. Furthermore, by combining the AI-assisted dereplication with the metabolomic profiling the novel compounds can be identified and discovered. Addressing the mounting challenge of antibiotic resistance remains a global priority. In this regard, microbial and fungal metabolites especially those with novel mechanisms of action offer tremendous promise as leads for developing next-generation antimicrobials. Overall collaborative efforts across microbiology, genomics, pharmacology, and computational sciences are essential to fully realize the potential of microbial and fungal secondary metabolites. Harnessing the synergy between biotechnology, synthetic biology, and artificial intelligence will ultimately redefine natural product discovery and shape the future of sustainable and precision therapeutics.

REFERENCES

Alekshun, M.N. & Levy, S.B. (2007) ‘Molecular mechanisms of antibacterial multidrug resistance’, *Cell*, 128(6), pp. 1037–1050.

Anisova, L.N., Blinova, I.N., Efremenkova, O.V., Koz’min, Y.P., Onoprienko, V.V., Smirnova, G.M. & Khokhlov, A.S. (1984) ‘Development regulators in *Streptomyces coelicolor* A3(2)’, *Izv Akad Nauk SSSR Ser Biol*, 1, pp. 98–108.

Anke, T. & Thines, E. (2007) ‘Fungal metabolites as lead structures for agriculture’, in Robson, G.D., van West, P. & Gadd, G.M. (eds.) *Exploitation of Fungi*. Cambridge, UK: Cambridge University Press.

Andryukov, B., Mikhailov, V. & Besednova, N. (2019) ‘The biotechnological potential of secondary metabolites from marine bacteria’, *Journal of Marine Science and Engineering*, 7(6), p. 176.

Bainton, N.J., Bycroft, B.W., Chhabra, S.R., et al. (1992) ‘A general role for the lux autoinducer in bacterial cell signalling: control of antibiotic biosynthesis in *Erwinia*’, *Gene*, 116, pp. 87–91.

Bertrand, S., Bohni, N., Schnee, S., et al. (2014) ‘Metabolite induction via microorganism co-culture: a potential way to enhance chemical diversity for drug discovery’, *Biotechnology Advances*, 32(6), pp. 1180–1204.

Bocso, N.S. & Butnariu, M. (2022) ‘The biological role of primary and secondary plant metabolites’, *Journal*

of Nutrition and Food Processing, 5(3), pp. 1–7.

Centers for Disease Control and Prevention (CDC) (2010) *Antimicrobial Susceptibilities Among Group B Streptococcus Isolates, Active Bacterial Core Surveillance (ABCs)*. Available at: http://www.cdc.gov/abcs/reports-findings/survreports/gbs10-suscept.html (Accessed: 23 July 2013).

Centers for Disease Control and Prevention (CDC) (2011) *Active Bacterial Core Surveillance (ABCs) Report, Emerging Infections Program Network, Group B Streptococcus*. Available at: http://www.cdc.gov/abcs/reports-findings/survreports/gbs11.pdf (Accessed: 23 July 2013).

Centers for Disease Control and Prevention (CDC) (2012) *Active Bacterial Core Surveillance Methodology*. Available at: http://www.cdc.gov/abcs/index.html (Accessed: 23 May 2013).

Centers for Disease Control and Prevention (CDC) (2019) *Antibiotic Resistance Threats in the United States, 2019*. Atlanta, GA: CDC.

Čihák, M., Kameník, Z., Šmídová, K., et al. (2017) 'Secondary metabolites produced during the germination of *Streptomyces coelicolor*', *Frontiers in Microbiology*, 8, p. 2495.

Charlop-Powers, Z., Owen, J.G., Reddy, B.V., Ternei, M.A. & Brady, S.F. (2015) 'Chemical-biogeographic survey of secondary metabolism in soil', *Proceedings of the National Academy of Sciences of the USA*, 112(14), pp. 4257–4262.

Chaudhry, N.M.A., Saeed, S. & Tariq, P. (2007) 'Antibacterial activity of *Streptomyces* species isolated from soil', *Pakistan Journal of Pharmaceutical Sciences*, 20(1), pp. 45–50.

de Frias, U.A., Pereira, G.K.B., Guazzaroni, M.E. & Silva-Rocha, R. (2018) 'Boosting secondary metabolite production and discovery through the engineering of novel microbial biosensors', *BioMed Research International*, 2018, p. 7021826.

Demain, A.L. & Fang, A. (2000) 'The natural functions of secondary metabolites', *Advances in Biochemical Engineering/Biotechnology*, 69, pp. 1–39.

Fouillaud, M. & Dufossé, L. (2022) 'Microbial secondary metabolism and biotechnology', *Microorganisms*, 10(1), p. 123.

Fotakis, G. & Timbrell, J.A. (2006) 'In vitro cytotoxicity assays: comparison of LDH, neutral red, MTT, and protein assay in hepatoma cell lines following exposure to cadmium chloride', *Toxicology Letters*, 160(2), pp. 171–177.

Galluzzi, L., Vitale, I., Aaronson, S.A., et al. (2018) 'Molecular mechanisms of cell death: Recommendations of the Nomenclature Committee on Cell Death 2018', *Cell Death and Differentiation*, 25(3), pp. 486–541.

Genilloud, O. (2017) 'Actinomycetes: still a source of novel antibiotics', *Natural Product Reports*, 34(10), pp. 1203–1232.

Gorlenko, C.L., Kiselev, H.Y., Budanova, E.V., Zamyatnin, A.A. Jr. & Ikryannikova, L.N. (2020) 'Plant secondary metabolites in the battle of drugs and drug-resistant bacteria: new heroes or worse clones of antibiotics?', *Antibiotics (Basel)*, 9(4), p. 170.

Hall, A.J., Curns, A.T., McDonald, L.C., Parashar, U.D. & Lopman, B.A. (2012) 'The roles of *Clostridium difficile* and norovirus among gastroenteritis-associated deaths in the United States, 1999–2007', *Clinical Infectious Diseases*, 55(2), pp. 216–223.

Hamill, R.L., Higgens, G.E., Boaz, H.E. & Gorman, M. (1969) 'The structure of beauvericin, a new desipeptide antibiotic toxic to *Artemia salina*', *Tetrahedron Letters*, 49, pp. 4255–4258.

Handelsman, J. (2004) 'Metagenomics: application of genomics to uncultured microorganisms', *Microbiology and Molecular Biology Reviews*, 68(4), pp. 669–685.

Hussin, S.H., Xinlong L., Chunjia L., Mohamed D., Ghulam H. J., Raees A., Muhammad I., & Muhammad A. I. (2022). An updated overview on insights into sugarcane genome editing via CRISPR/Cas9 for sustainable production. *Sustainability*, 14, pp. 12285.

Istifli, E.S., Hüsunet, M.T. & Ila, H.B. (2019) 'Cell division, cytotoxicity, and the assays used in the detection of cytotoxicity', in *Cytotoxicity—Definition, Identification, and Cytotoxic Compounds*. London, UK: IntechOpen.

Jakubiec-Krzesniak, K., Rajnisz-Mateusiak, A., Guspiel, A., Ziemska, J. & Solecka, J. (2018) 'Secondary metabolites of actinomycetes and their antibacterial, antifungal and antiviral properties', *Polish Journal of Microbiology*, 67(3), pp. 259–268.

Janeway, C.A. Jr., Travers, P., Walport, M., et al. (2001) *Immunobiology: The Immune System in Health and Disease*. 5th ed. New York, NY: Garland Science.

Katoch, M., Sharma, S., Sahgal, M., Srivastava, M.M. &

Kumar, S. (2014) 'Secondary metabolites from endophytic fungi and their biological activities', *BMC Complementary and Alternative Medicine*, 14, p. 52.

Khaldi, N., Seifuddin, F.T., Turner, G., et al. (2010) 'SMURF: genomic mapping of fungal secondary metabolite clusters', *Fungal Genetics and Biology*, 47(9), pp. 736–741.

Lamb, R., Harrison, H., Hulit, J., et al. (2015) 'Antibiotics that target mitochondria effectively eradicate cancer stem cells', *Oncotarget*, 6(7), pp. 4569–4584.

Lessa, F.C., Mu, Y., Cohen, J., et al. (2012) *Abstract presented at: IDWeek 2012; Annual Meeting of the Infectious Diseases Society of America, Society for Healthcare Epidemiology, Pediatric Infectious Diseases Society, and HIV Medical Association*; October 2012; San Diego, CA.

Logrieco, A., Moretti, A., Castella, G., et al. (1998) 'Beauvericin production by *Fusarium* species', *Applied and Environmental Microbiology*, 64(8), pp. 3084–3088.

Luo, Y., Enghiad, B. & Zhao, H. (2016) 'Activation and characterization of a cryptic polycyclic tetramate macrolactam gene cluster', *Nature Communications*, 7, p. 11091.

Mahalel, U.A. (2012) 'Antibacterial sensitivity for some chemically diverse steroidal glycosides in vitro', *Journal of Agricultural and Social Sciences*, 8, pp. 24–28.

Martín, J.F., Liras, P. & Castro, J.M. (2013) 'Secondary metabolism in *Streptomyces*: regulation, diversity, and industrial importance', *Microbial Biotechnology*, 6(5), pp. 540–554.

Medema, M.H., Blin, K., Cimermancic, P., et al. (2011) 'antiSMASH: rapid identification, annotation, and analysis of secondary metabolite biosynthesis gene clusters', *Nucleic Acids Research*, 39(Web Server issue), pp. W339–W346.

Netzker, T., Fischer, J., Weber, J., et al. (2018) 'Microbial communication leading to the activation of silent fungal secondary metabolite gene clusters', *Frontiers in Microbiology*, 9, p. 1138.

Piddock, L.J. (2015) 'Teixobactin, the first of a new class of antibiotics discovered by iChip technology?', *Journal of Antimicrobial Chemotherapy*, 70(10), pp. 2679–2680.

Rafeeq, H., Afsheen, N., Rafique, S., et al. (2023) 'Genetically engineered microorganisms for environmental remediation', *Chemosphere*, 310, p. 136751.

Ramirez-Rendon, D., Passari, A.K., Ruiz-Villafán, B., Rodriguez-Sanoja, R., Sanchez, S. & Demain, A.L. (2022) 'Impact of novel microbial secondary metabolites on the pharma industry', *Applied Microbiology and Biotechnology*, 106(5), pp. 1855–1878.

Riss, T.L., Moravec, R.A., Niles, A.L., et al. (2016) *Assay Guidance Manual: Cytotoxicity Assays*. Bethesda, MD: Eli Lilly & NIH.

Ruiz-Villafán, B., Cruz-Bautista, R., Manzo-Ruiz, M., Passari, A.K., Villarreal-Gómez, K., Rodríguez-Sanoja, R. & Sanchez, S. (2022) 'Carbon catabolite regulation of secondary metabolite formation, an old but not well-established regulatory system', *Microbial Biotechnology*, 15(4), pp. 1058–1072.

Saeed, S., Naim, A. & Tariq, P. (2007) 'A study on prevalence of multidrug-resistant Gram-negative bacteria', *International Journal of Biology and Biotechnology*, 4, pp. 71–74.

Salvatore, M.M., Masi, M. & Andolfi, A. (2025) 'The current status on secondary metabolites produced by fungi of the genus *Diplodia* associated with plants', *Phytochemical Reviews*, 24(2), pp. 1565–1589.

Seca, A.M. & Pinto, D.C. (2018) 'Plant secondary metabolites as anticancer agents: successes in clinical trials and therapeutic application', *International Journal of Molecular Sciences*, 19(1), p. 263.

Schroeckh, V., Scherlach, K., Nützmann, H.W., et al. (2009) 'Intimate bacterial–fungal interaction triggers biosynthesis of archetypal polyketides in *Aspergillus nidulans*', *Proceedings of the National Academy of Sciences of the USA*, 106(34), pp. 14558–14563.

Ślusarczyk, J., Adamska, E. & Czerwik-Marcinkowska, J. (2021) 'Fungi and algae as sources of medicinal and other biologically active compounds: a review', *Nutrients*, 13(9), p. 3178.

Skinnider, M.A., Johnston, C.W., Gunabalasingam, M., et al. (2015) 'PRISM 3: expanding the prediction informatics for secondary metabolomes toolkit', *Nucleic Acids Research*, 43(W1), pp. W441–W447.

Weiss, R.B. (1992) 'The anthracyclines: will we ever find a better doxorubicin?', *Seminars in Oncology*, 19(6), pp. 670–686.

Wisecaver, J.H., Slot, J.C. & Rokas, A. (2014) 'The evolution of fungal metabolic pathways', *PLoS Genetics*, 10(12), e1004816.

Wolfender, J.L., Marti, G., Thomas, A. & Bertrand, S. (2015) 'Current approaches and challenges for the metabolite profiling of complex natural extracts', *Journal of Chromatography A*, 1382, pp. 136–164.

Yamanaka, K., Reynolds, K.A., Kersten, R.D., et al. (2014) 'Direct cloning and refactoring of a silent gene cluster for cryptic compound discovery in *Streptomyces*', *Proceedings of the National Academy of Sciences of the USA*, 111(5), pp. 1957–1962.

Zhang, J.J., Tang, X. & Moore, B.S. (2022) 'Genetic platforms for heterologous expression of microbial natural products', *Natural Product Reports*, 39(3), pp. 509–528.

Zhang, M.M., Wong, F.T., Wang, Y., Luo, S., Lim, Y.H., Heng, E., et al. (2022) 'CRISPR–Cas9 strategies for activating silent biosynthetic gene clusters', *Nature Chemical Biology*, 18(4), pp. 314–322.

Ziemert, N., Alanjary, M. & Weber, T. (2016) 'The evolution of genome mining in microbes—a review', *Natural Product Reports*, 33(8), pp. 988–1005.