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Literature Review Paper

Multiplex PCR for Screening PKS-I and NRPS Biosynthetic Genes in Streptomyces: A Methodological Review

Ayu Septia Fatriza*
Biotechnology Study Program, Graduate School, Universitas Andalas, Padang, West Sumatra, Indonesia

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*Corresponding Author: **Ayu Septia Fatriza**, Biotechnology Study Program, Graduate School, Universitas Andalas, Padang, West Sumatra, Indonesia
Email: ayuseptiafatriza1991@gmail.com

Abstract: Streptomyces is an important source of microbial natural products, and many of its biosynthetic pathways are associated with polyketide synthase (PKS) and nonribosomal peptide synthetase (NRPS) gene clusters. PCR-based screening can be used to prioritize isolates for subsequent antimicrobial and chemical investigation, but the evidence for true multiplex detection of these targets has often been mixed with studies that used separate PCR reactions. This review focuses specifically on the methodological use of multiplex PCR for screening PKS-I and NRPS biosynthetic genes in Streptomyces and related actinomycetes. A structured literature search was conducted in PubMed and Google Scholar using combinations of the terms Streptomyces, multiplex PCR, PKS, NRPS, biosynthetic gene cluster, degenerate primer, and mangrove actinomycetes. Studies were classified according to whether multiple targets were amplified in the same reaction or detected by separate PCR assays. The literature shows that simultaneous amplification of PKS-I and NRPS targets is feasible, but the number of studies directly demonstrating this approach remains limited. Degenerate primers broaden target coverage but increase the risk of nonspecific amplification, making primer compatibility, annealing temperature, template quality, magnesium concentration, and amplicon separation critical. Positive PCR results indicate biosynthetic potential rather than expression or metabolite production. Therefore, multiplex PCR is best used as a first-stage screening method followed by sequencing and, when genomic data are available, genome mining and metabolite-based validation.

Keywords: Biosynthetic gene cluster; Degenerate primer; Multiplex PCR; NRPS; PKS; Streptomyces.

Introduction

Antimicrobial resistance continues to reduce the effectiveness of established antibiotics and has increased interest in the discovery of structurally diverse natural products. Microorganisms remain an important source of these compounds, with Streptomyces recognized as one of the most productive bacterial genera for secondary metabolites. Recent reviews have documented substantial numbers of newly reported Streptomyces species and natural products, indicating that the genus remains relevant to natural-product discovery despite its long history of investigation (Barka et al., 2016; Donald et al., 2022; Alam et al., 2022).

The biosynthetic capacity of Streptomyces is closely related to the presence of biosynthetic gene clusters (BGCs). Polyketide synthases (PKSs) and nonribosomal peptide synthetases (NRPSs) are among the major biosynthetic systems responsible for assembling structurally complex natural products. Type I PKS systems contain conserved ketosynthase and acyl-transferase-associated regions, whereas NRPS systems contain conserved adenylation domains involved in substrate activation and selection. These conserved regions have therefore been used as molecular targets for culture-based screening of actinomycetes (Ayuso-Sacido & Genilloud, 2005; Noureen et al., 2020).

PCR-based screening is attractive when a large collection of isolates must be prioritized before more expensive sequencing, fermentation, and chemical analyses. Degenerate primers are particularly useful because target sequences vary among taxa, but their broad sequence recognition also increases the possibility of nonspecific

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products. The problem becomes more pronounced when several primer pairs are combined in a single reaction. In multiplex PCR, primer competition, differences in annealing temperatures, unequal amplification efficiencies, and overlapping product sizes can affect the final pattern observed on an agarose gel (Henegariu et al., 1997; Ghashghaei et al., 2023).

A further problem is the frequent use of the term multiplex PCR for studies that actually performed separate PCR reactions. This distinction matters because simultaneous amplification demonstrates a different level of methodological integration from single-target screening. Ayuso-Sacido and Genilloud (2005), for example, explicitly demonstrated multiplex amplification of NRPS and PKS-I fragments, whereas Noureen et al. (2020) used separate PCR-based screening of several biosynthetic genes. Recent work has also applied multiplex PCR to PKS-I and NRPS-I detection in actinobacterial isolates (González-Nava et al., 2024).

The present review therefore narrows the discussion to the methodological basis and practical limitations of multiplex PCR for PKS-I and NRPS screening. Evidence from singleplex or degenerate PCR is included only when it helps explain primer selection, expected amplicon size, optimization, or interpretation. Particular attention is given to studies involving mangrove-associated actinomycetes because these environments have yielded isolates with antimicrobial activity and detectable biosynthetic genes (Sivalingam et al., 2019; Abidin et al., 2018).

Materials and Methods

This article was prepared as a focused narrative review with a structured search strategy. Literature was searched in PubMed and Google Scholar during August 2026 using combinations of the following terms: “Streptomyces” AND (“multiplex PCR” OR “multiplex polymerase chain reaction”) AND (“PKS” OR “polyketide synthase”) AND (“NRPS” OR “nonribosomal peptide synthetase”); additional searches combined “degenerate primer”, “biosynthetic gene cluster”, and “mangrove actinomycetes”. Reference lists of key primary studies were also examined to identify methodological papers that were not retrieved by the initial keyword combinations.

Studies were considered eligible when they (i) investigated Streptomyces or closely related actinomycetes, (ii) reported PCR detection of PKS and/or NRPS biosynthetic genes or domains, and (iii) provided sufficient methodological information to assess the target, primer set, amplification format, or validation procedure. Studies were classified as true multiplex when two or more target amplicons were generated within the same reaction mixture. Studies using separate PCR reactions were retained as singleplex/degenerate-PCR evidence and were not counted as direct evidence of multiplex performance.

Data were extracted qualitatively for organism or isolate source, target biosynthetic system, primer pair, target domain, expected amplicon size, amplification format, important reaction parameters, and validation method. Particular attention was given to whether the reported PCR product was sequenced and whether the authors linked gene detection to antimicrobial or metabolite data. Because this was a focused narrative review rather than a systematic review with quantitative synthesis, no meta-analysis was performed. Exact database hit counts were not used as an outcome because the search interfaces and duplicate records differed between databases; instead, the review emphasizes transparent search terms and explicit classification of the evidence.

Results and Discussion

Streptomyces and biosynthetic gene screening

Streptomyces has an unusually broad natural-product repertoire. Genomic studies have shown that individual strains may contain numerous BGCs, while many clusters are poorly expressed or silent under routine laboratory conditions. This distinction is important for PCR screening: detection of a biosynthetic gene provides evidence that a biosynthetic system may be present, but it does not demonstrate that the corresponding pathway is expressed or that a metabolite is produced under the tested culture conditions (Barka et al., 2016; Alam et al., 2022; Mao et al., 2021).

PCR screening remains useful because it can reduce the number of isolates taken forward for detailed characterization. Noureen et al. (2020) screened 40 Streptomyces isolates from the Cholistan Desert and detected nrps in 33 isolates and pks-I in 17 isolates. The authors combined PCR screening with sequencing,

bioinformatics, antimicrobial testing, and chemical profiling. This design illustrates the appropriate role of PCR: it is a prioritization step rather than a substitute for downstream characterization.

Similar caution is required for mangrove isolates. Sivalingam et al. (2019) reported an antibacterial Streptomyces sp. from Pitchavaram mangrove sediment and described a distinct phylogenetic pattern in the NRPS adenylation domain. The result supports biosynthetic potential, but it does not by itself establish production of a previously unknown compound. Abidin et al. (2018) also investigated PKS-I and NRPS genes in mangrove actinomycetes from Pahang, Malaysia. These studies support the value of mangrove-derived actinomycetes as a screening source while illustrating the need to separate gene detection from functional confirmation.

Envidence for true multiplex PCR

The strongest methodological evidence for simultaneous PKS-I and NRPS detection comes from Ayuso-Sacido and Genilloud (2005). The study developed degenerate primers A3F/A7R for NRPS adenylation domains and K1F/M6R for PKS-I sequences. The expected products were approximately 700–800 bp for NRPS and 1200–1400 bp for PKS-I. The authors first evaluated the primer pairs individually and then demonstrated multiplex amplification in the same reaction, with both expected bands detected. This study should be treated as the primary methodological reference when discussing simultaneous PKS-I/NRPS amplification.

Table 1. Summary of key studies on PCR-based detection of PKS-I and NRPS biosynthetic genes in actinomycetes

Study	Target(s)	Format	Amplicon	Key methodological point
Ayuso-Sacido & Genilloud (2005)	NRPS A-domain + PKS-I	True multiplex	NR~700–800 bp; PKS-I ~1200–1400 bp	Demonstrated simultaneous amplification of both targets in one reaction.
Abidin et al. (2018)	NRPS + PKS-I	Multiplex	NRPS ~700–800 bp; PKS-I ~1200–1400 bp	Applied multiplex screening to mangrove actinomycetes from Pahang, Malaysia.
González-Nava et al. (2024)	NRPS-I + PKS-I	Multiplex	PKS-I 1200–1400 bp; NRPS-I target reported in protocol	Used multiplex PCR for PKS-I and NRPS-I; PKS-II and NRPS-II were amplified separately.
Noureen et al. (2020)	pks-I, nrps and other BGC genes	Separate PCR assays	PKS-I ~1200–1500 bp; NRPS target reported	Useful evidence for gene-ba screening, but not direct evidence simultaneous multiplex amplification.
Ghashghaei et al. (2023)	PKS-I, PKS-II, NRPS	Separate optimized PCR assays	Target-specific	Showed that annealing temperature and primer concentration strongly influence degenerate-PCR outcomes.

Primer selection and technical considerations

Primer design is central to the performance of multiplex PCR because each primer pair must amplify its intended target without substantially interfering with the other pairs. For PKS-I, K1F/M6R targets conserved regions associated with ketosynthase and acyl-transferase functions and generally produces a fragment of approximately 1200–1400 bp. For NRPS, A3F/A7R targets a conserved region within the adenylation domain and generally produces a fragment of approximately 700–800 bp. PKS-II is commonly targeted with KSα/KSβ primers, producing a fragment of about 600 bp, but evidence for combining PKS-II with PKS-I and NRPS in the same reaction is less consistent and should not be presented as established practice without direct methodological evidence.

Degenerate bases are useful for broadening target coverage but increase the effective complexity of the primer mixture. Ghashghaei et al. (2023) showed that changes in annealing temperature and primer concentration could substantially alter detection frequencies for PKS-I, PKS-II, and NRPS in actinomycetes. Their results are important for multiplex design because a primer pair that performs well in a singleplex reaction may become inefficient when competing with another primer pair.

Table 2. Summary of key studies on PCR-based detection of PKS-I and NRPS biosynthetic genes in actinomycetes

Target	Primer pair	Sequence (5′–3′)	Approx. product	Reference
PKS-I	K1F/M6R	K61F: TSAAGTCSAACATCGGBCA; M6R: CGCAGGTTSCSGTACCAGTA	1200–1400 bp	Ayuso-Sacido & Genilloud (2005)
NRPS	A3F/A7R	A3F: GCSTACSYSATSTACACSTCSG G; A7R: SASGTCVCCSGTSCGGTAS	700–800 bp	Ayuso-Sacido & Genilloud (2005)
PKS-II	KSα/KSβ	KSα: TSGCSTGCTTGGAYGCSATC; KSβ: TGGAANCCGCCGAABCCTCT	~600 bp	Metsä-Ketelä et al. (1999)

Table 3. Key factors affecting the performance of multiplex PCR for PKS-I and NRPS screening

Factor	Expected effect in multiplex PCR	Practical implication
Annealing temperature	Changes specificity and relative amplification efficiency.	Use gradient or stepwise optimization before combining targets.
Primer concentration	An abundant primer pair may dominate the reaction.	Adjust primer concentrations rather than assuming equal concentrations are optimal.
Mg^{2+} concentration	Excess Mg^{2+} can increase nonspecific amplification; insufficient Mg^{2+} reduces polymerase activity.	Optimize within the range recommended for the master mix.
Template quality	Inhibitors or degraded DNA can cause weak or failed amplification.	Use intact genomic DNA and assess purity before multiplex PCR.
Amplicon size	Closely sized products are harder to distinguish on agarose gels.	Prefer sufficiently separated product sizes or use higher-resolution electrophoresis.
Primer interactions	Primer-dimers and cross-reactivity can reduce target yield.	Check primer compatibility in silico and experimentally.
GC-rich template	High GC content can reduce denaturation and polymerase processivity.	Consider a validated GC enhancer only when supported by optimization.
Controls	Lack of controls makes negative or weak bands difficult to interpret.	Include no-template control and, when available, positive controls for each target.

Interpretation of positive and negative PCR Result

A positive PCR band should be described as detection of a target gene or biosynthetic domain, not as proof of antibiotic production. The distinction is particularly important for Streptomyces because many BGCs are silent or poorly expressed under standard laboratory conditions. Genome-mining studies and research on silent-cluster activation show that genetic presence and transcriptional or metabolic activity are separate biological levels (Mao et al., 2021; Liu et al., 2022).

A negative result also requires caution. Degenerate primers may fail when sequence divergence occurs at primer-binding sites, even though a homologous biosynthetic gene is present. A weak or absent band can therefore reflect primer mismatch, suboptimal annealing, template quality, or competition within the multiplex reaction. Confirmation by a separate singleplex PCR using the relevant primer pair is useful when a negative result has biological importance.

Sequencing of representative amplicons provides a stronger basis for assigning the amplified fragment to PKS or NRPS. When whole-genome data are available, genome mining provides a broader assessment because it can identify complete BGC architecture rather than only a short conserved region. Current versions of antiSMASH also provide expanded analyses of BGC types and biosynthetic domains, making genome-based confirmation more informative than PCR alone (Blin et al., 2025).

Multiplex PCR in mangrove-derived actinomycetes

Mangrove sediments are relevant sources for actinomycete bioprospecting because isolates from these environments have yielded antimicrobial activity and biosynthetic genes. However, the environmental setting should not be used as evidence that a strain necessarily contains novel BGCs. Such a conclusion requires direct genetic or genomic comparison. The study by Sivalingam et al. (2019) is a useful example: the NRPS adenylation domain of a mangrove-derived Streptomyces isolate showed a distinct phylogenetic pattern, but the authors framed this as potential for novel antibacterial compounds rather than direct chemical proof.

For a mangrove screening program, multiplex PCR is therefore most useful as a triage method. Isolates that produce clear target bands can be prioritized for amplicon sequencing, antimicrobial assays, fermentation, and chemical profiling. This sequence of steps is more defensible than interpreting a PCR-positive isolate as an antibiotic producer. The same principle applies when isolates are obtained from other underexplored habitats.

Limitations and future directions

The main limitation of the available literature is the small number of studies that clearly document simultaneous amplification of PKS-I and NRPS in a single reaction. Many publications report PCR screening of several biosynthetic genes but use separate reactions. Treating these studies as equivalent would overstate the evidence for multiplex PCR.

A second limitation is methodological heterogeneity. Primer sequences, DNA concentrations, polymerases, Mg^{2+} concentrations, annealing temperatures, cycle numbers, and electrophoresis conditions vary considerably. As a result, a protocol optimized in one actinomycete collection cannot automatically be transferred to another collection. This is especially relevant for degenerate primers, where small changes in annealing temperature can alter the observed detection rate (Ghashghaei et al., 2023).

Future studies should report the complete primer sequences, target domains, degeneracy, expected amplicon sizes, reaction composition, cycling conditions, controls, and validation strategy. A useful workflow is to establish each primer pair in singleplex PCR, confirm expected products, and only then combine compatible pairs into multiplex reactions. Positive multiplex products should be sequenced, while isolates of particular interest should be examined by genome mining and metabolite-based approaches. antiSMASH 8.0 provides an updated computational framework for identifying and characterizing BGCs from genome sequences, including improved analysis of PKS and NRPS domains (Blin et al., 2025).

Conclusion

Multiplex PCR is a practical strategy for early screening of PKS-I and NRPS biosynthetic genes in Streptomyces, but the direct evidence for simultaneous amplification is narrower than the broader literature on PCR-based biosynthetic-gene screening. The clearest methodological evidence comes from studies that explicitly combined the NRPS A-domain and PKS-I primer pairs in a single reaction. Degenerate primers increase target coverage but also increase the need for careful optimization of annealing temperature, primer concentration, Mg^{2+} concentration, DNA quality, and primer compatibility.

Most importantly, PCR detection should be interpreted as evidence of biosynthetic potential rather than evidence of gene expression or metabolite production. Sequencing of representative amplicons and, where possible, genome mining and metabolite-based validation are necessary to strengthen the biological interpretation. For mangrove-derived Streptomyces, multiplex PCR can serve as an efficient first-stage prioritization tool, provided that claims about novelty and antimicrobial production are supported by independent downstream evidence.

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References

Abidin, Z. A. Z., Chowdhury, A. J. K., Abdul Malek, N., & Zainuddin, Z. (2018). Diversity, antimicrobial capabilities, and biosynthetic potential of mangrove actinomycetes from coastal waters in Pahang, Malaysia. *Journal of Coastal Research,* 82, 174–179.

https://doi.org/10.2112/SI82-025.1

Alam, K., Mazumder, A., Sikdar, S., Zhao, Y.-M., Hao, J., Song, C., & Li, W. (2022). Streptomyces: The biofactory of secondary metabolites. *Frontiers in Microbiology*, 13, 968053. https://doi.org/10.3389/fmicb.2022.968053

Ayuso-Sacido, A., & Genilloud, O. (2005). New PCR primers for the screening of NRPS and PKS-I systems in actinomycetes: Detection and distribution of these biosynthetic gene sequences in major taxonomic groups. *Microbial Ecology*, 49(1), 10–24. https://doi.org/10.1007/s00248-004-0249-6

Barka, E. A., Vatsa, P., Sanchez, L., Gaveau-Vaillant, N., Jacquard, C., Klenk, H.-P., Clément, C., Ouhdouch, Y., & van Wezel, G. P. (2016). Taxonomy, physiology, and natural products of Actinobacteria. Microbiology and Molecular Biology Reviews, 80(1), 1–43. https://doi.org/10.1128/MMBR.00019-15

Blin, K., Shaw, S., Augustijn, H. E., Reitz, Z. L., Biermann, F., Alanjary, M., Fetter, A., Terlouw, B. R., Metcalf, W. W., Helfrich, E. J. N., van Wezel, G. P., Medema, M. H., & Weber, T. (2023). antiSMASH 7.0: New and improved predictions for detection, regulation, chemical structures and visualisation. Nucleic Acids Research, 51(W1), W46–W50. https://doi.org/10.1093/nar/gkad344

Blin, K., Shaw, S., Vader, L., Szenei, J., Reitz, Z. L., Augustijn, H. E., & Weber, T. (2025). antiSMASH 8.0: Extended gene cluster detection capabilities and analyses of chemistry, enzymology, and regulation. *Nucleic Acids Research*, 53(W1), W32–W38. https://doi.org/10.1093/nar/gkaf334

Donald, L., Pipite, A., Subramani, R., Owen, J., Keyzers, R. A., & Taufa, T. (2022). Streptomyces: Still the biggest producer of new natural secondary metabolites, a current perspective. *Microbiology Research,* 13(3), 418–465. https://doi.org/10.3390/microbiolres13030031

Epstein, S. C., Charkoudian, L. K., & Medema, M. H. (2018). A standardized workflow for submitting data to the Minimum Information about a Biosynthetic Gene cluster (MIBiG) repository. *Standards in Genomic Sciences,* 13, Article 16. https://doi.org/10.1186/s40793-018-0323-1

Genilloud, O. (2019). Natural products discovery and potential for new antibiotics. *Current Opinion in Microbiology,* 51, 81–87. https://doi.org/10.1016/j.mib.2019.08.001

Ghashghaei, S., Etemadifar, Z., Tavassoli, M., & Mofid, M. R. (2023). Optimization of degenerate PCR conditions for reducing error rates in detection of PKS and NRPS gene groups in actinomycetes. *Avicenna Journal of Medical Biotechnology*, 15(1), 28–37. https://doi.org/10.18502/ajmb.v15i1.11422

Gong, B., Chen, S., Lan, W., Huang, Y., & Zhu, X. (2018). Antibacterial and antitumor potential of actinomycetes isolated from mangrove soil in the Maowei Sea of the southern coast of China. *Iranian Journal of Pharmaceutical Research,* 17(4), 1335–1344. https://pubmed.ncbi.nlm.nih.gov/30568692/

Henegariu, O., Heerema, N. A., Dlouhy, S. R., Vance, G. H., & Vogt, P. H. (1997). Multiplex PCR: Critical parameters and step-by-step protocol. *BioTechniques*, 23(3), 504–511. https://doi.org/10.2144/97233rr01

Huitu, Z., Lin, Z., Ping, L., & Chenglin, J. (2009). PCR screening of 3-amino-5-hydroxybenzoic acid synthase gene leads to identification of ansamycins and AHBA-related antibiotic producers in actinomycetes. Journal of Applied Microbiology, 106(3), 755–763. https://doi.org/10.1111/j.1365-2672.2008.04011.x

Jiang, L., Li, M., Wang, X., Zhang, Y., & Liu, H. (2025). Integrated genome mining and molecular networking uncover the biosynthetic potential of a novel mangrove-derived strain Streptomyces sp. B1866. *BMC Genomics*, 26(1), Article 762. https://doi.org/10.1186/s12864-025-11945-8

Knight, G. M., Davies, N. G., Colijn, C., & Costelloe, C. (2018). Addressing the unknowns of antimicrobial resistance: Quantifying and mapping the drivers of burden. *Clinical Infectious Diseases*, 66(4), 612–616. https://doi.org/10.1093/cid/cix855

Lee, L.-H., Zainal, N., Azman, A.-S., Eng, S.-K., Goh, B.-H., Yin, W.-F., Mutalib, N.-S. A., & Chan, K.-G. (2014). Diversity and antimicrobial activities of actinobacteria isolated from tropical mangrove sediments in Malaysia. *The Scientific World Journal,* 2014, Article 698178. https://doi.org/10.1155/2014/698178

Li, Z.-Y., Wang, Y.-X., Wang, L.-L., Shi, T.-Q., Sun, X.-M., & Huang, H. (2019). Activation of anthrachamycin biosynthesis in Streptomyces chattanoogensis L10 by site-directed mutagenesis of rpoB. *Journal of Zhejiang University Science B,* 20(12), 983–994. https://doi.org/10.1631/jzus.B1900280

Mao, X.-M., Zeng, J., & Jiang, J. (2021). Recent advances in silent gene cluster activation in Streptomyces. *Frontiers in Bioengineering and Biotechnology,* 9, Article 632230. https://doi.org/10.3389/fbioe.2021.632230

Medema, M. H., Kottmann, R., Yilmaz, P., Cummings, M., Biggins, J. B., Blin, K., de Bruijn, I., Chooi, Y. H., Claesen, J., Coates, R. C., Cruz-Morales, P., Duddela, S., Düsterhus, S., Edwards, D. J., Fewer, D. P., Garg, N., Geiger, C., Gomez-Escribano, J. P., Greule, A., ... Glöckner, F. O. (2015). Minimum information about a biosynthetic gene cluster. *Nature Chemical Biology*, 11(9), 625–631. https://doi.org/10.1038/nchembio.1890

Metsä-Ketelä, M., Ylihonko, K., & Mäntsälä, P. (2004). Partial activation of a silent angucycline-type gene cluster from a rubromycin beta producing Streptomyces sp. PGA64. *Journal of Antibiotics,* 57(8), 502–510. https://doi.org/10.7164/antibiotics.57.502

Noureen, N., Cheema, M. T., Anwar, S., Hasnain, S., & Sajid, I. (2020). PCR-based screening approach: A rapid method to detect the biosynthetic potential of antimicrobials in actinobacterial strains. *Poblish Journal of Microbiology*, 69(2), 139–149. https://doi.org/10.33073/pjm-2020-016

Reitz, Z. L. (2024). Predicting metallophore structure and function through genome mining. *Methods in Enzymology*, 703, 1–27. https://doi.org/10.1016/bs.mie.2024.05.014

Sivalingam, P., Muthuselvam, M., Pote, J., & Prabakar, K. (2019). Phylogenetic insight of nonribosomal peptide synthetases (NRPS) adenylate domain in antibacterial-potential Streptomyces BDUSMP 02 isolated from Pitchavaram mangrove. Bioinformation, 15(6), 412–418. https://doi.org/10.6026/97320630015412

Walsh, C. T., & Fischbach, M. A. (2010). Natural products version 2.0: Connecting genes to molecules. *Journal of the American Chemical Society,* 132(8), 2469–2493. https://doi.org/10.1021/ja909118a

Wang, H.-X., Chen, Y.-Y., Ge, L., Fang, T.-T., Meng, J., Liu, Z., & Tan, R.-X. (2013). PCR screening reveals considerable unexploited biosynthetic potential of ansamycins and a mysterious family of AHBA-containing natural products in actinomycetes. *Journal of Applied Microbiology,* 115(1), 77–85. https://doi.org/10.1111/jam.12215

Wood, S. A., Kirby, B. M., Goodwin, C. M., Le Roes, M., & Meyers, P. R. (2007). PCR screening reveals unexpected antibiotic biosynthetic potential in Amycolatopsis sp. strain UM16. *Journal of Applied Microbiology,* 102(1), 245–253. https://doi.org/10.1111/j.1365-2672.2006.03059.x