



Original Research

Azaphilone pigments from *Penicillium maximae*

Filipe Augusto Tironi Alves¹, Alice Ferreira-Silva¹, Ivanildo Evódio Marriel², Gabriel Franco dos Santos¹, Jacqueline Aparecida Takahashi^{1, *}

¹Department of Chemistry, Universidade Federal de Minas Gerais (UFMG), Av. Antônio Carlos, CEP 31270-901 Belo Horizonte, MG, Brazil; ²Empresa Brasileira de Pesquisa Agropecuária, Centro Nacional de Pesquisas de Milho e Sorgo, EMBRAPA/CNPMS, Rod. MG 424, km 45, 35701-970, Sete Lagoas, MG, Brazil. *Email: jacqueline@ufmg.br

Abstract

Azaphilones are an important class of secondary metabolites derived from the polyketide pathway. These metabolites have an intense yellow-orange-red pallet of colour and have been studied as promising natural pigments for use in the food industry. On a screening with 30 fungi isolated from soil and cultivated in different conditions, a fungal species furnished an orange extract containing a very major metabolite, as determined by HPLC analysis. The species was identified *Penicillium maximae* by molecular biology and morphological studies, and it was selected to determine the major secondary metabolites responsible by the colour present in the extract. The dichloromethane extract prepared from *P. maximae* was column chromatographed leading to the isolation of sclerotiorin (1, 16.36 % yield), eupenicilazaphilone B (2, 0.77 % yield) and sclerotioramin (3, 1.05 % yield). Sclerotiorin yield is encouraging for industrial production. These azaphilones of orange, yellow and red colour respectively, are the major metabolites produced by *P. maximae* on a culture medium containing peptone as a differential. High concentration of sucrose and the presence of iron ions in the culture medium did not improve the metabolites yield. *P. maximae* is, therefore, a good source of pigments for potential food, pharmaceutical and dyeing applications.

Keywords: Food industry, Microbial metabolites, Natural dye, Screening, Soil fungi

Citation: Alves, F. A. T., Ferreira-Silva, A., Marriel, I. E., dos Santos, G. F., & Takahashi, J. A. (2025). Azaphilone pigments from *Penicillium maximae*. *Mycological Spectrum* 1(1):18-27.

Received: 2 February 2025 | **Accepted:** 8 May 2025 | **Published:** 18 May 2025

Handling Editor: Dr. Shilpa A. Verekar | **Reviewers:** Prof. Laurent Dufossé, Dr. Sunil Kumar Deshmukh

Copyright: ©2025 Alves, Ferreira-Silva, Marriel, dos Santos, Takahashi. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution, or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution, or reproduction is permitted which does not comply with these terms.

1. Introduction

Fungi produce small molecules with diverse chemical structures to mediate processes such as intra and inter-species communication and self-defence towards biotic and abiotic adversities



present in their natural habitats (Deshmukh et al., 2024; Oliveira et al., 2022). Research in this area has gained strength over the last decades with the decrease in the cost of genome sequence analysis and the development of novel approaches to mine fungal metabolites for pharmaceutical and food and other industrial applications (Kenshole et al., 2021). The advancement of omics techniques has also sped up the development of research with fungal metabolites (Saini et al., 2024; Takahashi et al., 2024).

One of the most common fungal genera is *Penicillium*, which can be found in different ecosystems, having an important role in pharmaceutical area as well as in the environmental balance as decomposers and bioremediation agents (Martins et al., 2016; Saxena et al., 2024; Wang et al., 2010). *Penicillium* species have been described as outstanding producers of metabolites from diversified biosynthetic pathways (Bazioli et al., 2017) and with several medicinal applications such as antiviral, anticancer, immunosuppressant, and neuroprotective activities (Toghueo & Boyom, 2020). Among 354 species of *Penicillium*, *P. maximae* section *Sclerotiora* can be considered an under-reported taxon (Houbraken et al., 2020). This species is reported to grow well in Czapek Yeast Autolysate (CYA) Agar at 30 °C, Malt Extract Agar (MEA) and Yeast Extract Sucrose (YES) Agar and moderately in Dichloran 18% glycerol (DG18) agar, being distinguishable from closely related species *P. sclerotiorum* by the absence of sclerotia (Visagie et al., 2013). *P. maximae* has already been isolated from marine environment in Korea (Park et al., 2019) and as an endophyte capable of producing azaphilone alkaloids (Koyanagi et al., 2021).

Azaphilones are fungi-exclusive secondary metabolites derived from the polyketide pathway with a pyrone-quinone structure and a chiral quaternary carbon atom (Pimenta et al., 2021). The intense yellow-orange-red pallet of colour of azaphilones makes them promising pigments for food and textile applications to replace synthetic dyes (Morales-Oyervides et al., 2020; Venil et al., 2020). In addition to their application as pigments, azaphilones possess antimicrobial, antifungal, antiviral, antioxidant, cytotoxic/antitumor, nematocide and anti-inflammatory activities, which are likely a consequence of their reactions with amino groups present in amino and nucleic acids (Chen et al., 2020). The bioactivity of these pigments adds an important functional appeal to their incorporation in foods, beyond the dyeing property.

This work describes the screening of terrestrial fungal strains capable of producing coloured pigments and the isolation and identification of three azaphilones from *P. maximae*, an understudied species of *Penicillium*.

2. Materials and methods

Thin layer chromatography (TLC) plates were obtained from Silicycle (Canada); silica gel for column chromatography (230-400 mesh) was purchased from Merck (Germany). Analytical grade solvents, chemicals and culture media were purchased from VETEC and KASVI (Brazil), and HIMEDIA (India). Deuterated solvents were acquired from Sigma Aldrich (USA) and Cambridge Isotope Laboratories (USA).

2.1 Isolation and screening of fungal species

Soil samples (1 g) were suspended in aqueous NaCl (0.85 m/v, 4.5 mL) and serially diluted to the final concentration of 10^{-3} gL⁻¹. Aliquots of 100 µL were added to Petri dishes containing bacteriological agar (15.0 gL⁻¹), peptone (50.0 gL⁻¹), yeast extract (50.0 gL⁻¹), and rose bengal (0.033 gL⁻¹). After 24 to 72 h, individual colonies were transferred successively to freshly sterilized agar until recovery of 30 pure cultures that were stored in PDA at -80 °C. A screening



was carried out with 30 fungal species, that were cultivated in 100 mL of three different culture media, as following described. Culture medium 1: Yeast extract (12.0 gL^{-1}), Sucrose (150.0 gL^{-1}), $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (0.5 gL^{-1}), and Glucose (20.0 gL^{-1}). Culture medium 2: Glucose (20.0 gL^{-1}), Bacterial peptone (5.0 gL^{-1}), Yeast extract (3.0 gL^{-1}), and KH_2PO_4 (5.0 gL^{-1}). Culture medium 3: Glucose (20.0 gL^{-1}), Bacterial peptone (5.0 gL^{-1}), Yeast extract (3.0 gL^{-1}), KH_2PO_4 (5.0 gL^{-1}), Fe^{3+} (2.5 gL^{-1}), and Fe^{2+} (2.5 gL^{-1}).

The culture media were distributed to 500 mL Erlenmeyer flasks; the pH was adjusted to 5.12 and the flasks were autoclaved for 15 minutes at 121°C . After cooling, the 30 fungal species were individually inoculated to the media in a laminar flow hood, previously sterilized with 70 % ethyl alcohol. Resting growth was awaited, and three flasks were discarded during the process, as the respective fungi suffered contamination during cultivation. After 14 days, 30 mL of ethyl acetate was added to each Erlenmeyer flask, followed by filtration at reduced pressure to separate the mycelium from the liquid phase containing ethyl acetate and broth. The mycelium was then collected, placed in an Erlenmeyer flask, and left immersed in ethyl acetate for further 24 hours. The extraction procedure was carried out in a separation funnel individually for each of 27 fungi. Vigorous stirring was carried out to remove all secondary metabolites from the broth, collecting the organic phase. The process was repeated twice with 30 mL of extracting solvent and the organic phases were combined. The solvent in which the respective mycelium was immersed was separated from the mycelia and added to the organic phase. Finally, the organic phase was taken to rotary evaporation to remove the solvent and obtain the extracts. The mycelia were autoclaved for 15 minutes at 121°C and subsequently discarded. A species presenting white mycelia orange-coloured reverse which presented an outstanding HPLC profile was selected for identification and large-scale study.

2.2 Identification of *P. maximae*

Fungal mycelium was transferred to microtubes containing buffer (Tris-HCl 0.05 M, EDTA 0.005 M pH 8.0, NaCl 0.1 M and sodium dodecyl sulphate 1 %) (400 μL). Stainless steel beads were added to improve the extraction. The material was homogenized for 5 min in a Bullet Blender24 and then incubated (60°C ; 30 min). After that, cetyltrimethylammonium bromide buffer (200 μL) and β -mercaptoethanol (4 %; 4 μL) were added and the microtubes were stirred and incubated (65°C ; 30 min). Then, chloroform:isoamyl alcohol (24:1, 570 μL) were added and the microtubes were gently shaken followed by centrifugation at 13,200 rpm (15 min). The aqueous phase was then transferred to new microtubes containing a solution of sodium acetate (3 M, 10 %). After mixing, solutions were centrifuged (13,200 rpm, 10 min) and the supernatant was transferred to microtubes and followed by precipitation with isopropanol. The tubes were centrifuged (13,200 rpm, 10 min). The supernatant was removed, and the DNA pellet was washed twice in 70 % cold ethanol, dried down, and resuspended in 50 μL of Tris-EDTA (Tris-HCl 0.01 M and EDTA 0.001 M). Pure genomic DNA was assayed in NanoDrop ND 1000 (NanoDrop Technologies) and stored at 20°C until amplification of the internal transcribed spacer (ITS) region. After DNA extraction, the ITS region was amplified with the universal primers ITS1 and ITS4 (Abrão et al., 2014; White et al., 1990). Successfully amplified PCR products were purified by precipitation with ethanol/ethylenediaminetetraacetic acid. The sequencing reactions and quality control of individual sequences were performed (Applied Biosystems). Molecular fungal identification was performed as described in Ferreira-Silva et al. (2021). The consensus sequence was submitted to GenBank and assigned an accession number: MZ683178. Phylogenetic analysis was performed in MEGA version X (Kumar et al., 2018). The ITS sequences were aligned using MUSCLE. The alignments were prepared including sequences of all relevant ex-type strains,



from sequences obtained from GenBank to ensure an accurate identification. The Bayesian information criterion was used to identify the most appropriate model of evolution. The molecular identification was in accordance with macroscopic examination and optical microscopy (Visagie et al., 2013).

2.3 Fermentation, extraction, and isolation of secondary metabolites

P. maxima was grown (10×1 L) using (g L^{-1}) glucose (20.0), peptone (5.0), yeast extract (3.0), KH_2PO_4 (5.0) at $\text{pH } 5.65 \pm 0.07$, at room temperature (25 ± 4 °C). To maximize pigments recovery, the growth was allowed for 35 days. After this period, the mycelia were filtered under vacuum and the broth was exhaustively extracted with dichloromethane (3×300 mL $\text{CH}_2\text{Cl}_2/\text{L}$ broth), which promoted higher yield of extract than EtOAc. The solvent was removed to furnish the broth extract. The mycelia were soaked overnight with CH_2Cl_2 , the organic fraction was recovered by filtration and the solvent was removed, furnishing the mycelia extract. Broth and mycelia extracts were combined (8.56 g total) and subjected to silica gel column chromatography using hexane, ethyl acetate and methanol gradient. Fractions were combined according to their TLC profiles into nine groups (F1–F9). Group F2 (1.40 g) was constituted by a pure metabolite (1). Groups F7 (0.23 g), and F8 (0.53 g) were further chromatographed to furnish 2 (66.0 mg) and 3 (90 mg), respectively.

2.4 Nuclear Magnetic Resonance (NMR) Analysis

NMR spectra were determined on a Bruker Avance DRX 400 MHz Spectrometer with tetramethylsilane (TMS) as an internal standard. Samples (10 mg) were solubilized in 0.7 mL of chloroform-*d* (1, 3) or methanol-*d*₄ (2). Chemical shifts (δ) are given in parts per million (ppm) relative to the internal standard TMS. Coupling constants (*J*) are given in hertz (Hz).

3. Results and discussion

Thirty fungal species isolated from soil were cultivated in three different culture media. Culture medium 1 was distinguished by its high sucrose content (150 g L^{-1}) as an additional carbon source. Sucrose is associated with the production of azaphilones (Pimenta et al., 2021). Culture medium 2, in turn, contained peptone, as an additional nitrogen source in its composition. Peptone is an ingredient classically present in culture media aimed at producing pigments, also favoring fungal growth and biomass production (Gomes, 2024; Lucas et al., 2007). Culture medium 3 contained the same ingredients as culture medium 2, with the addition of iron ions. The presence of metals in the culture medium causes abiotic stress, which interferes with metabolic pathways, intensifying or reducing metabolic production (Takahashi et al., 2013). The profiles of the extracts, analyzed by HPLC, showed, in general, low metabolic production, however one species stood out for the production of colored metabolites, which was suppressed in the presence of iron (Fig. 1).

This species was identified and selected for large scale cultivation. The identification was based on the analysis of the ITS region. The nucleotide sequence showed 100 % alignment and 99 % identity with *P. maxima* NRRL 2060 (CBS 134565) type species deposited in GenBank [NR_121343]. The phylogenetic analysis confirmed the distinct clustering with bootstrap support (99 %) of the isolate studied with NRRL 2060 strain (Fig. 2). The identification was confirmed morphologically by the absence of sclerotia, lack of sporulation on CYA, and lack of growth in CYA at 37 °C. *P. maxima* presented white and orange mycelia with orange reverse pigmentation on PDA, fading to orange in the center (Visagie et al., 2013) (Fig. 2).

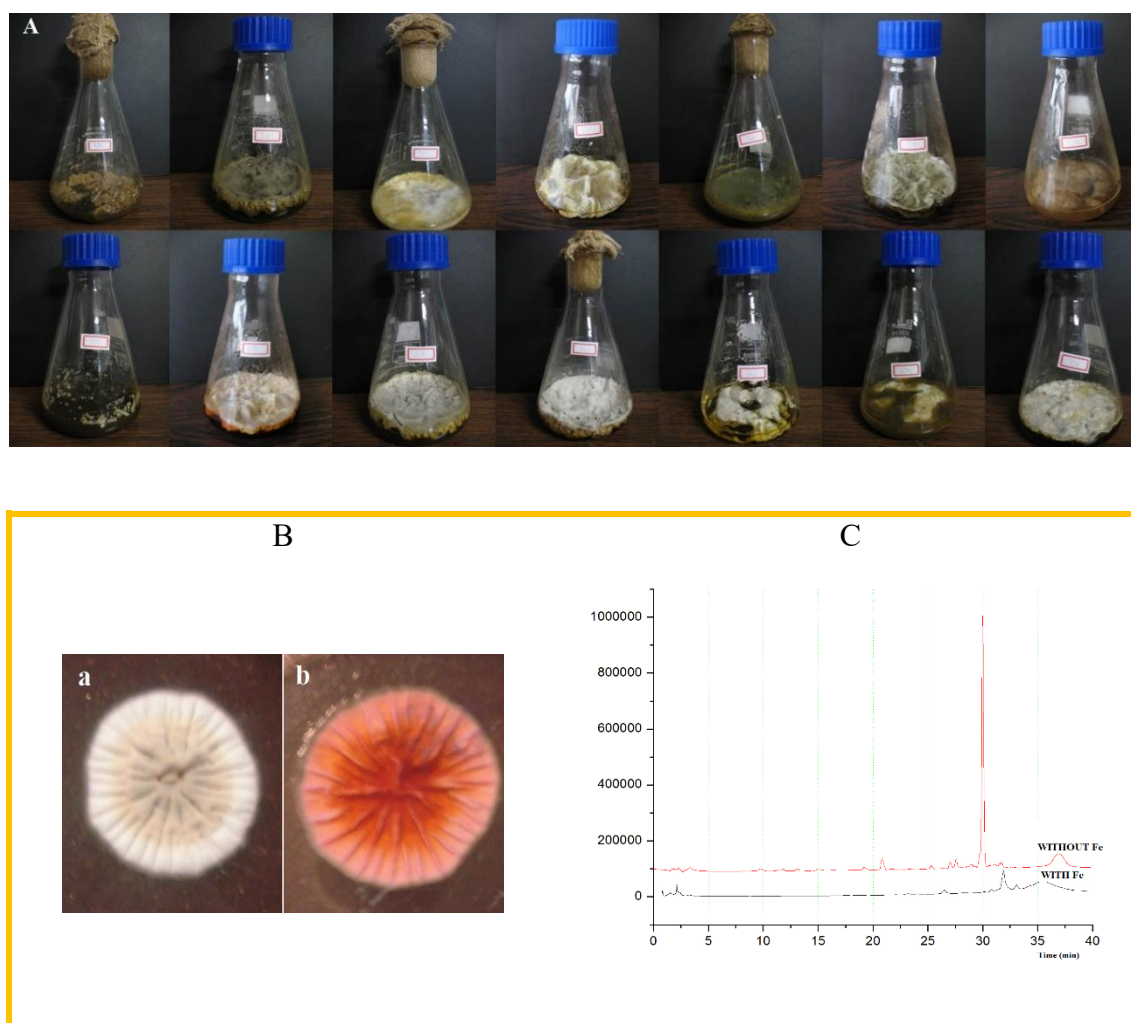


Fig. 1 - A. Overview of the screening step. B. Appearance of *P. maximae* on the surface (a) and back (b) of the Petri dish. C. Chromatogram showing the presence of a major metabolite produced by *P. maximae* grown without iron supplementation.

For the large-scale cultivation, *P. maximae* was grown in liquid media (10 L) generating a dark-orange brownish broth. Three metabolites were isolated from *P. maximae* extract and identified by NMR spectrometry. Compound **1** was isolated as an amorphous solid that furnished an orange solution in chloroform. The ^1H NMR spectrum of **1**, identified as sclerotiorin (Lucas et al., 2007), showed characteristic methyl and methylene groups (0.86–2.18 ppm), conjugated olefinic hydrogen atoms (δ_{H} 5.71–7.94 ppm), and signals attributed to H9 and H10 (δ_{H} 6.08 and 7.08 ppm respectively, $J=15.6$ Hz). The ^{13}C NMR spectrum presented 21 signals, including three carbonyl groups at δ_{C} 186.0 (C6), 191.8 (C8) and 170.1 ppm (C19) and one carbinolic carbon at δ_{C} 84.6 ppm (C7). Sclerotiorin is a natural pigment previously isolated from *P. sclerotiorum*, a closely related species. Antibacterial properties and potential application of sclerotiorin as dye in the food industry have been reported (Gomes and Takahashi, 2016; Lucas et al., 2007). Sclerotiorin (**1**, 16.37 % yield): orange solid; ^{13}C NMR (chloroform-*d*): 152.7 (C1), 158.1 (C3), 106.4 (C4), 138.7 (C4a), 110.8 (C5), 186.0 (C6), 84.6 (C7), 191.8 (C8), 114.5 (C8a), 115.7 (C9), 142.9 (C10), 132.0 (C11), 148.9 (C12), 35.1 (C13), 30.1 (C14), 12.0 (C15), 20.2 (C16), 12.4 (C17), 22.5 (C18), 170.1 (C19), 20.1 (C20).

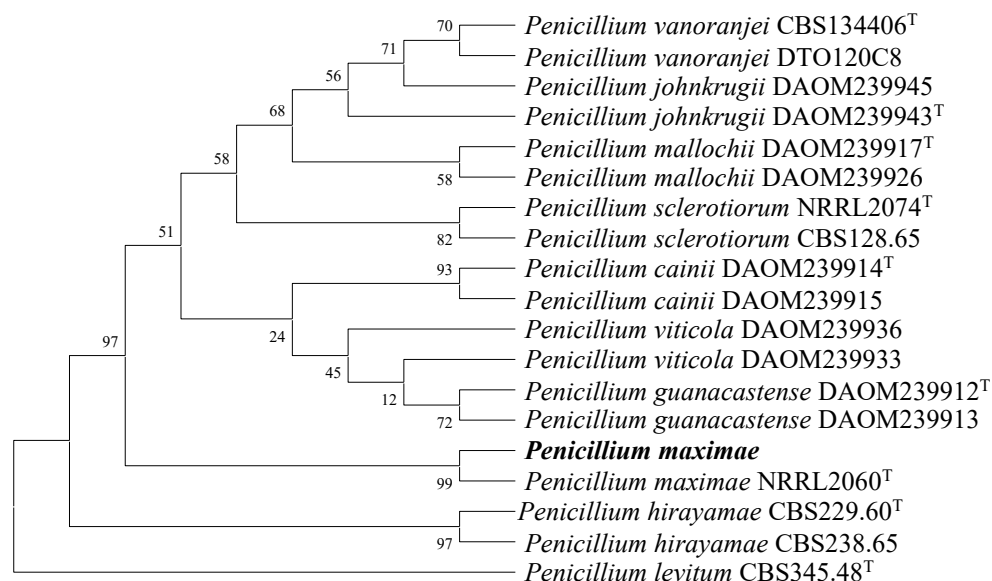


Fig. 2 - Maximum-likelihood tree based on ITS sequences showing the placement of the soil isolate *P. maximae* (in bold, GenBank accession MZ683178) relative to the type strain and closely related species in *Penicillium* section *Sclerotiora*. The tree was rooted with *P. levitum* CBS345.48. Numbers at branches are bootstrap values of 1,000 replications. The final dataset contained 543 aligned positions. The tree was constructed using MEGA software with Tamura 3-parameter model. A discrete Gamma distribution was used to model evolutionary rate differences among sites [5 categories (+G, parameter = 0.0500)].

Compound 2, isolated as a yellow amorphous solid, was identified as eupenicilazaphilone B (**2**) (Gu et al., 2018), according to the signals of methyl and methylene groups (δ_{H} 0.86–1.71 ppm) and olefinic hydrogen atoms at δ_{H} 6.10 (H4), 6.27 and 6.77 ppm (H9 and H10, $J = 15.6$ Hz). The ^{13}C NMR spectrum (19 signals) comprised signals of carbonyl (δ_{C} 191.52 ppm, C6), and carbinolic carbon atoms at δ_{C} 76.8 (C7), 74.5 (C8), 76.6 (C11) and 80.9 ppm (C12). Eupenicilazaphilone B (**2**, 0.77 % yield): yellow amorphous solid; ^{13}C NMR (methanol- d_4): 70.1 (C1), 164.1 (C3), 102.0 (C4), 148.1 (C4a), 120.0 (C5), 191.5 (C6), 76.8 (C7), 74.5 (C8), 38.4 (C8a), 122.6 (C9), 145.2 (C10), 76.6 (C11), 80.9 (C12), 36.4 (C13), 30.0 (C14), 12.2 (C15), 14.3 (C16), 26.4 (C17), 20.1 (C18).

Compound 3 was isolated as an amorphous solid with intense red coloration and showed strong structural similarity with compound 1, according to the NMR data related to the signals assigned to hydrogen atoms from methyl and methylene groups (δ_{H} 0.85 to 2.47 ppm), to olefine hydrogen atoms (δ_{H} 5.74 to 8.07 ppm), and to H9 and H10 at δ_{H} 6.24 and 7.20 ppm, respectively ($J = 16.08$ Hz). A signal at δ_{H} 12.07 ppm indicated the presence of a secondary amine in the chemicals structure of compound 3. The ^{13}C NMR spectrum (21 signals) revealed the presence of three carbonyl groups [δ_{C} 183.6 (C6), 193.4 (C8) and 170.7 ppm (C19)]. A signal at δ_{C} 85.2 ppm, corresponding to a carbinolic carbon was assigned to C7. The data obtained were compared to those in the literature and the compound was identified as sclerotioramin (**3**) (Wang et al., 2010). Sclerotioramin (**3**, 1.05 % yield): red amorphous solid; ^{13}C NMR (chloroform- d): 138.8 (C1), 147.0 (C3), 110.8 (C4), 148.0 (C4a), 101.1 (C5), 183.6

(C6), 85.2 (C7), 193.4 (C8), 114.7 (C8a), 116.6 (C9), 143.5 (C10), 132.2 (C11), 149.0 (C12), 35.2 (C13), 30.2 (C14), 12.1 (C15), 20.3 (C16), 12.5 (C17), 23.8 (C18), 170.7 (C19), 20.6 (C20).

Sclerotioramin is an antibacterial and antifungal agent previously isolated from *P. citreonigrum* (Ferreira-Silva et al., 2021; Wang et al., 2010). Sclerotioramin (3) contains a nitrogen atom in the heterocyclic ring, instead of oxygen, present in sclerotiorin (1). This replacement is common in azaphilones due to their affinity for amine groups present in proteins, amino acids and nucleic acids (Svilar et al., 2012). Chemical structures of compounds 1–3 are shown in Fig. 3.

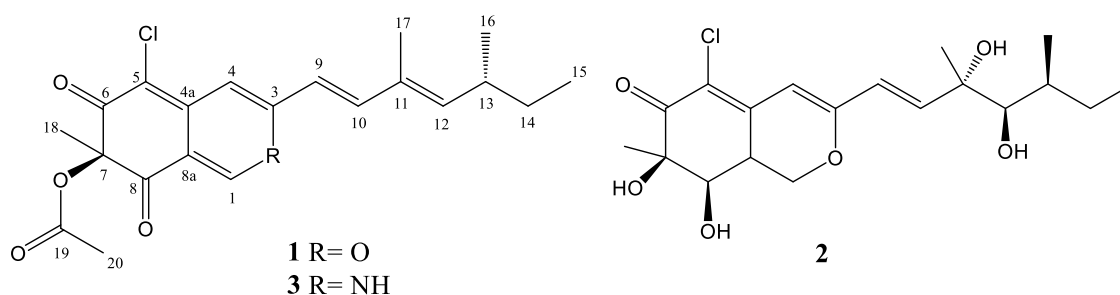


Fig. 3 - Chemical structure of compounds 1–3 from *P. maximae*.

The production of azaphilones by *P. maximae* in a culture medium using glucose, peptone and yeast extract is relevant, as these compounds have broad potential for industrial applications and their production using solid state fermentation was not detected (Rengifo et al., 2023). Sclerotiorin (1) inhibits Hsp90, a therapeutic target for cancer treatment, while the analogue with nitrogen (3) was inactive (Kabbaj et al., 2015), reflecting the role of the heteroatom in the biological activity. Azaphilones with nitrogen in the heterocycle are red while oxygen leads to yellow/orange pigments due to the changes in the chromophore provoked by the heteroatom (Yuliana et al., 2017). Azaphilones were also reported as a promising chemotherapeutic agent to avoid proliferation of cancer-cells (Matsumoto et al., 2023), as well as a natural pigment for several applications in food industry (Dufossé, 2025). *P. maximae* has also been studied due to the high protein content of its biomass, which can be a sustainable source of alternative proteins for human consumption (Moura et al., 2022; Takahashi et al., 2020).

4. Conclusion

P. maximae proved to be a good source of azaphilone dyes, mainly sclerotiorin, produced with a yield of 16.36 % yield. This yield is relevant for large-scale production, given that the initial yield of secondary metabolites by fungi is not always encouraging enough to envisage industrial applications. On the other hand, the use of azaphilones as industrial dyes still needs to be authorized by regulatory bodies, which may occur in the near future, due to the significant increase in cases of allergies, cancer and other diseases directly related to the consume of foods containing some artificial dyes.

Funding Sources

We acknowledge Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG, grant PPM-00255-18), and Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq, grants 158891/2014-7 and 311509/2022-3) for financial support.



Declaration of Competing Interest

The authors declare no conflict of interest.

References

- Abrão, F. O., Duarte, E. R., Freitas, C. E. S., Vieira, E. A., Geraseev, L. C., da Silva-Hughes, A. F., ... Rodrigues, M. (2014). Characterization of fungi from ruminal fluid of beef cattle with different ages and raised in tropical lignified pastures. *Current Microbiology*, 69(5), 649–659. <https://doi.org/10.1007/s00284-014-0633-5>
- Bazioli, J. M., Amaral, L. D. S., Fill, T., & Rodrigues-Filho, E. (2017). Insights into *Penicillium brasilianum* secondary metabolism and its biotechnological potential. *Molecules*, 22(6), 858. <https://doi.org/10.3390/molecules22060858>
- Chen, C., Tao, H., Chen, W., Yang, B., Zhou, X., Luo, X., & Liu, Y. (2020). Recent advances in the chemistry and biology of azaphilones. *RSC Advances*, 10, 10197–10220. <https://doi.org/10.1039/D0RA00894J>
- Deshmukh, S. K., Takahashi, J. A., & Saxena, S. (2024). *Fungi Bioactive Metabolites*. Springer Nature.
- Dufossé, L. (2025). Biotechnological approaches in the production of fungal pigments. In *Fungal Biotechnology* (pp. 449–466). Academic Press.
- Ferreira-Silva, A., Hughes, F. M., Rosa, C. A., & Rosa, L. H. (2021). Higher turnover of endophytic fungal assemblages in the tissues of globose cactus *Melocactus ernestii* from Brazilian semi-arid biome. *Symbiosis*, 85, 79–91. <https://doi.org/10.1007/s13199-021-00795-z>
- Gomes, D. C. (2024). Fungal pigments: Applications and their medicinal potential. In *Fungi Bioactive Metabolites: Integration of Pharmaceutical Applications* (pp. 651–681). Springer Nature Singapore.
- Gomes, D. C., & Takahashi, J. A. (2016). Sequential fungal fermentation-biotransformation process to produce a red pigment from sclerotiorin. *Food Chemistry*, 210, 355–361. <https://doi.org/10.1016/j.foodchem.2016.04.057>
- Gu, B.-B., Tang, J., Jiao, W.-H., Li, L., Sun, F., Wang, S., Yang, F., & Lin, H. W. (2018). Azaphilone and isocoumarin derivatives from the sponge-derived fungus *Eupenicillium* sp. 6A-9. *Tetrahedron Letters*, 59(31), 3345–3348. <https://doi.org/10.1016/j.tetlet.2018.06.057>
- Houbraken, J., Kocsubé, S., Visagie, C. M., Yilmaz, N., Wang, X. C., Meijer, M., ... & Frisvad, J. C. (2020). Classification of *Aspergillus*, *Penicillium*, *Talaromyces* and related genera (Eurotiales): An overview of families, genera, subgenera, sections, series and species. *Studies in Mycology*, 95, 165–169. <https://doi.org/10.1016/j.simyco.2020.05.002>
- Kabbaj, F. Z., Lu, S., Faouzi, M. E. A., Meddah, B., Proksch, P., Cherrah, Y., Altenbach, H.-J., Aly, A. H., Chadli, A., & Debbab, A. (2015). Bioactive metabolites from *Chaetomium aureum*: Structure elucidation and inhibition of the Hsp90 machine chaperoning activity. *Bioorganic & Medicinal Chemistry*, 23(1), 126–131. <https://doi.org/10.1016/j.bmc.2014.11.021>
- Kenshole, E., Herisse, M., Michael, M., & Pidot, S. J. (2021). Natural product discovery through microbial genome mining. *Current Opinion in Chemical Biology*, 60, 47–54. <https://doi.org/10.1016/j.cbpa.2020.07.010>
- Koyanagi, Y., Hitora, Y., Kawahara, T., & Tsukamoto, S. (2021). Peniphilones A and B: Azaphilone alkaloids from the endophytic fungus *Penicillium maximae*. *Heterocycles*, 102(2), 325–332. Retrieved from https://jglobal.jst.go.jp/en/detail?JGLOBAL_ID=202102218984001969



- Kumar, S., Stecher, G., Li, M., Knyaz, C., & Tamura, K. (2018). MEGA X: Molecular evolutionary genetics analysis across computing platforms. *Molecular Biology and Evolution*, 35(6), 1547–1549. <https://doi.org/10.1093/molbev/msy096>
- Lucas, E. M., Castro, M. C., & Takahashi, J. A. (2007). Antimicrobial properties of sclerotiorin, isochromophilone VI and pencolide, metabolites from a Brazilian cerrado isolate of *Penicillium sclerotiorum* Van Beyma. *Brazilian Journal of Microbiology*, 38(4), 785–789. <https://doi.org/10.1590/S1517-83822007000400036>
- Martins, L. R., Lyra, F. H., Rugani, M. M., & Takahashi, J. A. (2016). Bioremediation of metallic ions by eight *Penicillium* species. *Journal of Environmental Engineering*, 142(2), C4015007. [https://doi.org/10.1061/\(ASCE\)EE.1943-7870.0000998](https://doi.org/10.1061/(ASCE)EE.1943-7870.0000998)
- Matsumoto, T., Ohnishi, E., Kitagawa, T., Okayama, M., Saito, Y., Yoshikawa, H., ... & Watanabe, T. (2023). Azaphilones produced by *Penicillium maximae* with their cell death-inducing activity on Adriamycin-treated cancer cell. *Genes and Environment*, 45(1), 5. <https://doi.org/10.1186/s41021-023-00261-w>
- Morales-Oyervides, L., Ruiz-Sánchez, J., Oliveira, J. C., Sousa-Gallagher, M. J., Méndez-Zavala, A., Giuffrida, D., Dufossé, L., ... & Montañez, J. (2020). Biotechnological approaches for the production of natural colorants by *Talaromyces/Penicillium*: A review. *Biotechnology Advances*, 107601. <https://doi.org/10.1016/j.biotechadv.2020.107601>
- Moura, M. A., Alves, V. S., & Takahashi, J. A. (2022). Nutritional quality, techno-functional characteristics, and safety of biomass powder and protein isolate produced from *Penicillium maximae*. *Foods*, 11(23), 3621. <https://doi.org/10.3390/foods11223621>
- Oliveira, G., Barreto, D. L. C., Silva, M., Augusti, R., Marriel, I., Lana, U., & Takahashi, J. A. (2022). Biotic stress caused by in vitro co-inoculation enhances the expression of acetylcholinesterase inhibitors by fungi. *Natural Product Research*, 36(23), 4266–4270. <https://doi.org/10.1080/14786419.2021.1975701>
- Park, M. S., Chung, D., Baek, K., & Lim, Y. W. (2019). Three unrecorded species belonging to *Penicillium* section *Sclerotiora* from marine environments in Korea. *Mycobiology*, 47(2), 165–172. <https://doi.org/10.1080/12298093.2019.1601330>
- Pimenta, L., Gomes, D. C., Cardoso, P. G., & Takahashi, J. A. (2021). Recent findings in azaphilone pigments. *Journal of Fungi*, 7(7), 541. <https://doi.org/10.3390/jof7070541>
- Rengifo, L. R., Rosas Méndez, L., Ludeña, Y., Sirvas, S., Samolski, I., & Villena, G. K. (2022). Comparison of pigment production by filamentous fungal strains under submerged (SmF) and surface adhesion fermentation (SAF). *Journal of Fungi*, 9(1), 48. <https://doi.org/10.3390/jof9010048>
- Saini, R., Verma, A., Tiwari, H., Mishra, S., & Gautam, V. (2024). Omics-based approaches in studying fungal endophytes and their associated secondary metabolites. In *Endophytic Fungi: The Hidden Sustainable Jewels for the Pharmaceutical and Agricultural Industries* (pp. 209–227). Cham: Springer International Publishing.
- Saxena, S., Dufossé, L., Deshmukh, S. K., Chhipa, H., & Gupta, M. K. (2024). Endophytic fungi: A treasure trove of antifungal metabolites. *Microorganisms*, 12(9), 1903. <https://doi.org/10.3390/microorganisms12091903>
- Svilar, L., Stankov-Jovanovic, D., Dossmann, H., & Tabet, J. C. (2012). High-resolution mass spectrometry and hydrogen/deuterium exchange study of mitorubrin azaphilones and nitrogenized analogues. *Journal of Mass Spectrometry*, 47(8), 969–977. <https://doi.org/10.1002/jms.3044>
- Takahashi, J. A., Barbosa, B., Martins, B. D. A., Guirlanda, C. P., & Moura, M. A. F. (2020). Use of the versatility of fungal metabolism to meet modern demands for healthy aging,



- functional foods, and sustainability. *Journal of Fungi*, 6(4), 223. <https://doi.org/10.3390/jof6040223>
- Takahashi, J. A., Marques, J. G. S., Ferreira, M. R., Santos, T., & Rosário, G. O. (2024). Recent advances in pharmaceutically important compounds from endophytic fungi. In *Fungi Bioactive Metabolites: Integration of Pharmaceutical Applications* (pp. 3–28).
- Takahashi, J. A., Teles, A. P. C., de Almeida Pinto Bracarense, A., & Gomes, D. C. (2013). Classical and epigenetic approaches to metabolite diversification in filamentous fungi. *Phytochemistry Reviews*, 12(4), 773–789. <https://doi.org/10.1007/s11101-013-9305-5>
- Toghueo, R. M. K., & Boyom, F. F. (2020). Endophytic *Penicillium* species and their agricultural, biotechnological, and pharmaceutical applications. *3 Biotech*, 10(1), 1–35. <https://doi.org/10.1007/s13205-020-2081-1>
- Venil, C. K., Velmurugan, D., Dufossé, L., Devi, P. R., & Ravi, A. (2020). Fungal pigments: Potential coloring compounds for wide ranging applications in textile dyeing. *Journal of Fungi*, 6(2), 68. <https://doi.org/10.3390/jof6020068>
- Visagie, C. M., Houbraken, J., Rodrigues, C., Pereira, C. S., Dijksterhuis, J., Seifert, K. A., ... & Samson, R. A. (2013). Five new *Penicillium* species in section *Sclerotiora*: A tribute to the Dutch Royal family. *Persoonia: Molecular Phylogeny and Evolution of Fungi*, 31, 42–62. <https://doi.org/10.3767/003158513X667410>
- Wang, X., Sena Filho, J. G., Hoover, A. R., King, J. B., Ellis, T. K., Powell, D. R., & Cichewicz, R. H. (2010). Chemical epigenetics alters the secondary metabolite composition of guttate excreted by an Atlantic-forest-soil-derived *Penicillium citreonigrum*. *Journal of Natural Products*, 73(5), 942. <https://doi.org/10.1021/np100142h>
- White, T. J., Bruns, T., Lee, S. J. W. T., & Taylor, J. (1990). Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In *PCR protocols: A guide to methods and applications* (Vol. 18, pp. 315–322).
- Yuliana, A., Singgih, M., Julianti, E., & Blanc, J. (2017). Derivates of azaphilone *Monascus* pigments. *Biocatalysis and Agricultural Biotechnology*, 9, 183–194. <https://doi.org/10.1016/j.bcab.2016.12.014>