Fungal Biology 126 (2022) 385–394

Contents lists available at ScienceDirect

Fungal Biology

journal homepage: www.elsevier.com/locate/funbio

Chemical modulation of the metabolism of an endophytic fungal strain of *Cophinforma mamane* using epigenetic modifiers and amino-acids

R. Pacheco-Tapia [a, b, *], P. Vásquez-Ocmín [a], S. Duthen [a], S. Ortíz [a, 1], P. Jargeat [c], C. Amasifuen [d], M. Haddad [a, **], M. Vansteelandt [a]

[a] UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, France
[b] Laboratorios de Investigación y Desarrollo, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru
[c] Laboratoire Evolution et Diversité Biologique UMR 5174, Université de Toulouse, CNRS, IRD, France
[d] Instituto Nacional de Innovación Agraria, Dirección de Recursos Genéticos y Biotecnología, Avenida La Molina 1981, La Molina, Lima, 15024, Peru

ARTICLE INFO

Article history:
Received 21 September 2021
Received in revised form
3 February 2022
Accepted 25 February 2022
Available online 2 March 2022

Corresponding Editor: Dr J Slot

Keywords:
Endophytic fungi
Cophinforma mamane
Epigenetic modifiers
Metabolomics
Diketopiperazines

ABSTRACT

Endophytic fungi are capable of producing a great diversity of bioactive metabolites. However, the presence of silent and lowly expressed genes represents a main challenge for the discovery of novel secondary metabolites with different potential uses. Epigenetic modifiers have shown to perturb the production of fungal metabolites through the induction of silent biosynthetic pathways leading to an enhanced chemical diversity. Moreover, the addition of bioprecursors to the culture medium has been described as a useful strategy to induce specific biosynthetic pathways. The aim of this study was to assess the effects of different chemical modulators on the metabolic profiles of an endophytic fungal strain of *Cophinforma mamane* (Botryosphaeriaceae), known to produce 3 thiodiketopiperazine (TDKP) alkaloids (botryosulfuranols A-C), previously isolated and characterized by our team. Four epigenetic modifiers, 5-azacytidine (AZA), sodium butyrate (SB), nicotinamide (NIC), homoserine lactone (HSL) as well as 2 amino acids, L-phenylalanine and L-tryptophan, as bioprecursors of TDKPs, were used. The metabolic profiles were analysed by UHPLC-HRMS/MS under an untargeted metabolomics approach. Our results show that the addition of the two amino acids in *C. mamane* culture and the treatment with AZA significantly reduced the production of the TDKPs botryosulfuranols A, B and C. Interestingly, the treatment with HSL significantly induced the production of different classes of diketopiperazines (DKPs). The treatment with AZA resulted as the most effective epigenetic modifier for the alteration of the secondary metabolite profile of *C. mamane* by promoting the expression of cryptic genes.

1. Introduction

Microorganisms such as bacteria and fungi, have produced most of the known antibiotics due to the large chemical diversity and biological activities of their metabolites (Boufridi and Quinn 2018; Abdel-Razek et al., 2020). Among them, endophytic fungi are considered to be a goldmine of secondary metabolites (Strobel and Daisy 2003), represented by poorly explored and characterized microorganisms capable of producing compounds with potential for pharmaceutical and commercial uses (Strobel 2018; Deshmukh et al., 2018; White et al., 2019). However, the conditions for gene expression in fungi are not fully understood and the presence of silent or lowly-expressed genes represent one of the main challenges for the isolation of novel secondary metabolites (Brakhage and Schroeckh 2011). Biosynthetic gene clusters in fungi are often found in the distal telomeric regions of the chromosome (Shwab et al., 2007; Williams et al., 2008), which are transcriptionally controlled by epigenetic regulation, specifically histone acetylation and methylation (Brakhage 2013; Pfannenstiel and Keller 2019). When fungal DNA is organized into histone proteins and is densely

* Corresponding author. UMR 152 Pharma Dev, Université de Toulouse, IRD, UPS, France.
** Corresponding author.
E-mail addresses: rominapachecotapia@gmail.com (R. Pacheco-Tapia), mohamed.haddad@ird.fr (M. Haddad).
[1] Present address: Therapeutic Innovation Laboratory UMR CNRS 7200, Faculty of Pharmacy, Université de Strasbourg, Strasbourg, France.

https://doi.org/10.1016/j.funbio.2022.02.005
1878-6146/

packed into heterochromatin, it becomes transcriptionally silent (Okada and Seyedsayamdost 2017). In this regard, highly acetylated histones are generally loosely packed into euchromatin and therefore genes are thought to be transcriptionally active (Cichewicz 2010) while methylation of histones often results in gene silencing (Suzuki and Bird 2008).

Recently, a very well-reviewed strategy to induce cryptic genes has consisted in the treatment of fungal endophytes with epigenetic modifiers such as histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi) (Toghueo et al., 2020; Gupta et al., 2020). For instance, the addition of 5-azacytidine, a DNMTi, enhanced the production of the alkaloid camptothecin in *Botryosphaeria rhodina* (Botryosphaeriaceae), a finding that could give insights of the signaling mechanisms between the host plant and its endophytic fungus (Vasanthakumari et al., 2015). Similarly, metabolite production in *Diaporthe* sp. (Diaporthaceae) was enhanced when it was subjected to epigenetic treatment with the HDACi suberohydroxamic acid and valproic acid (Jasim et al., 2019). The addition of the HDACi nicotinamide to the culture of the endophytic fungus *Graphiopsis chlorocephala* (Cladosporiaceae), led to a notable enrichment in secondary metabolite production and also allowed the isolation of new benzophenones and cephalanones (Asai et al., 2013). A clear advantage of using epigenetic-based methods is that they do not require genetic manipulation and they can be applied to any fungal strain, potentially allowing the annotation of new secondary metabolites and the enhancement of the synthesis of low-yield metabolites.

Based on these promising results, we decided to use epigenetic modifiers to induce cryptic genes of an endophytic fungal strain of *Cophinforma mamane* (D.E. Gardner) A. J. L. Phillips & A. Alves (Botryosphaeriaceae) isolated from the leaves of *Bixa orellana* (Bixaceae) (Barakat et al., 2019). The first attempt to induce the metabolite production of *C. mamane* using epigenetic modifiers allowed the detection of 8 metabolites produced *de novo* (Triastuti et al., 2019). Additionally, to date, 10 compounds have been isolated from *C. mamane*, including 3 new TDKPs alkaloids, botryosulfuranols A, B and C isolated by our team, exhibiting cytotoxic activity against different cancer cell lines (Barakat et al., 2019). These compounds are derived from two phenylalanine residues and present an unprecedented skeleton with two spirocyclic centers which are currently under biological investigation to go further in elucidation of their mechanism of action. Moreover, as described in Welch and Williams (Welch and Williams 2014), TDKPs are biogenetically derived from at least one aromatic amino acid (phenylalanine, tyrosine and/or tryptophan), therefore the addition of these type of bioprecursors into the culture media might induce their biosynthetic pathway. The amino-acid directed strategy was shown to induce cryptic biosynthetic pathways, allowing the isolation of many novel fungal bioactive compounds, specifically alkaloids (Huang et al., 2017; Yuan et al., 2019; Qiu et al., 2020; Guo et al., 2020). Other molecules previously isolated from *C. mamane* include a new dihydrobenzofuran derivative, botryomaman and a known molecule, primin (Pongcharoen et al., 2007).

In the present study, the effects on secondary metabolism of *C. mamane* through the addition of four different epigenetic modifiers to the culture medium were investigated in 4 independent experiments. Two HDACi, sodium butyrate (SB) and nicotinamide (NIC), one DNMTi, 5-azacytidine (AZA) and one bacterial *quorum-sensing* (QS) molecule, N-butyryl-DL-homoserine lactone (HSL), were used for this purpose. In a separate experiment, culture medium was supplemented with 2 amino acids, L-phenylalanine and L-tryptophan, precursors of nitrogenous compounds such as DKPs, to evaluate their influence on the metabolism of this strain, especially on the production of the three known phenylalanine-derived TDKPs previously isolated. The presented work has two main objectives: to assess the effects of chemical elicitors on the secondary metabolites profile of *C. mamane* by promoting the expression of cryptic genes, and to obtain a better yield of botryosulfuranols A, B and C for further biological investigations in future research.

2. Materials and methods

2.1. Chemicals and solvents

The culture media used for the experiments are the following: Malt extract broth or agar (MEB/MEA) containing malt extract 20 g, dextrose 20 g, peptone 1 g, copper sulfate pentahydrate 0.005 g, zinc sulfate heptahydrate 0.01 g in 1 L of distilled water and Potato dextrose broth or agar (PDB/PDA) (DifcoTM) containing 4 g of potato extract and 20 g of glucose for 1 L of distilled water.

Epigenetic modifiers, nicotinamide (NIC), sodium butyrate (SB), butyryl-DL-homoserine (HSL) and 5-azacytidine (AZA) and amino acids, L-phenylalanine (L-phe) and L-tryptophan (L-trp) were purchased from Sigma–Aldrich (St. Louis, MO, USA).

For the extraction of the culture and UHPLC-HRMS analyses the following solvents were used: formic acid (FA, Sigma), methanol HPLC grade (Fisher Chemical), ethyl acetate HPLC grade (Fisher Chemical), acetonitrile HPLC grade (Fisher Chemical) and dichloromethane HPLC grade (Fisher Chemical).

2.2. Fungal material

The strain E224 of *Cophinforma mamane* (D.E. Gardner) A. J. L. Phillips & A. Alves was isolated from the fresh leaves of *Bixa orellana* L. collected in November 2013 by C. Amasifuen and M. Haddad, from the National Reserve of Allpahuayo Mishana in the Amazonian forest of Loreto, Peru (GPS coordinates: 3°58'2.3" S, 73°25'3.9" W). The isolation, identification and storage processes followed have been previously described (Triastuti et al., 2019; Barakat et al., 2019). Briefly, the sterilized tissues of the leaves were placed on a Petri dish containing MEA and chloramphenicol (100 mg/L). The ITS sequence is available in the GenBank database under the accession number MG457709.1. The cryotubes are preserved at −80 °C in 30% glycerol in the fungal collection of the UMR 152 laboratory.

2.3. Fermentation conditions

C. mamane was grown on PDA over 7 days prior to inoculation of the seed culture containing 50 mL of PDB, incubated at 27 °C. After 3 days, 250 mL Erlenmeyer flasks containing 50 mL of PDB were inoculated with 2 mL of the seed culture and treated with the corresponding epigenetic modulators (SB, NIC, AZA and HSL) dissolved in DMSO. NIC and SB (El-Hawary et al., 2018) were added at 1 μM AZA (Zutz et al., 2014; Qadri et al., 2017) was added at 25 μM and HSL (Rateb et al., 2013) was added at 100 μM as final concentrations. Fermentations were carried out over 2 weeks at 27 °C under static conditions. All conditions were done in triplicate. DMSO was added to control cultures without epigenetic modifiers. Control media with and without epigenetic modifiers were also included in triplicate.

For the experiment with amino acids, *C. mamane* was cultured in MEB supplemented with 2 mg/L of L-trp and L-phe. Fermentation was carried out in 250 mL Erlenmeyer flasks containing 50 mL of supplemented MEB (4 replicates) over 2 weeks at 27 °C under constant agitation. Control media (MEB and supplemented MEB) were included in triplicate.

2.4. Extraction of culture

Whole culture broths (including mycelium) were extracted with 50 mL of ethyl acetate for 60 min using sonication. Mycelia were removed from the culture supernatant through filtration with glass cotton. Organic phases were then dehydrated on magnesium sulfate and filtered with filter paper. Finally, they were evaporated to dryness under reduced pressure (KNF rotary evaporator RC 600) at 40 °C.

2.5. UHPLC-HRMS profiling

Methanolic solutions at 2 mg/mL of all extracts were prepared for Ultra-High Performance Liquid Chromatography - MS analysis. Quality control (QC) samples were prepared by pooling an aliquot of all extracts for each experiment (epigenetic modifiers and amino acids). Analyses were performed on a UHPLC Ultimate 3000 system (Dionex) coupled with an LTQ Orbitrap XL mass spectrometer (Thermo Fisher Scientific, Hemel Hempstead, UK). Samples were separated on a C18 Acquity column (100 × 2.1 mm i.d, 1.7 μm, Waters, MA, USA) equipped with a guard column. The mobile phase A was ultrapure water acidified with 0.1% FA and mobile phase B was acetonitrile acidified with 0.1% FA. The solvent gradient was: 0 min, 95% A; 10 min, 95% B; 12.5 min, 95% B. The flow rate was 0.45 mL/min, the column temperature was set to 40 °C, the autosampler temperature was set to 15 °C and injection volume fixed to 5 μL for extracts. Mass detection was performed using an electrospray source (ESI) in positive ionization (PI) mode at 15,000 resolving power [full width at half maximum (FWHM) at 400 *m/z*]. The mass scanning range was *m/z* 100–2000 for all samples. Ionization spray voltage was set to 3.5 kV and the capillary temperature was set to 300 °C. Each full MS scan was followed by data-dependent acquisition of MS/MS spectra for the three most intense ions using stepped collision-induced dissociation (CID) at 35 arbitrary energy units.

2.6. Data processing

LC-MS data from each experiment (epigenetic modifiers and amino acids) were separately processed with MS-DIAL version 4.7 (Tsugawa et al., 2015) for mass signal extraction between 100 and 1500 Da from 0.5 to 12.5 min. MS1 and MS2 tolerances were set to 0.01 and 0.025 Da, respectively, in centroid mode for each dataset. Peaks were aligned on a QC reference file with a retention time (RT) tolerance of 0.05 min and a mass tolerance of 0.025 Da. Minimum peak height was set to 70% below the observed total ion chromatogram (TIC) baseline for blank samples. MS-DIAL data was cleaned with MS-CleanR workflow using default parameters (Fraisier-Vannier et al., 2020): all filters with a minimum blank ratio set to 0.8, a maximum relative standard deviation (RSD) set to 30 and a relative mass defect (RMD) ranging from 50 to 3000. The maximum mass difference for feature relationships detection was set to 0.005 Da and maximum RT difference was set to 0.025 min. The Pearson correlation links were considered with correlation $\geq$0.8 and statistically significant $\alpha = 0.05$. Two peaks were kept in each cluster: the most intense and the most connected to other ions. The data was normalized, first, by the Total ion chromatogram (TIC) using MS-DIAL and then re-normalized based on total peak area using MS-CleanR workflow. The kept features were annotated with MS-FINDER version 3.5 (Tsugawa et al., 2016). The MS1 and MS2 tolerances were set to 0.005 and 0.05 Da, respectively. Formula finder were processed with C, H, O, N, P and S atoms. Annotation was done with the use of internal generic databases from MS-FINDER: LipidMaps, YMDB, ChEBI, NPA, NANPDB, COCONUT, KNApsacK and UNPD. Only annotations with a score greater than 5 were retained throughout the analysis while unannotated features are referred to as "unknown". Data were finally exported as.msp file for MSMS spectral information and.csv files for metadata information (peak area, retention times, annotation results, etc.).

The MSP and metadata files generated after the MS-CleanR workflow were imported into MetGem (version 1.3.4) (Olivon et al., 2018). The data were filtered by removing all MS/MS peaks within ±17 Da of the precursor *m/z*. MS/MS spectra were window filtered by using the top 6 peaks in the ±50 Da window throughout the spectrum. MS/MS fragment ion tolerance was set to 0.2 Da. A mass spectral similarity network was created where edges were filtered to have a cosine score above 0.7 and at least six matched peaks. Further edges between two nodes were kept in the similarity network only if each of the nodes appeared on each other's top 10 most similar nodes. The resulting network was then imported into Cytoscape (version 3.8.2, Institute for Systems Biology, Seattle,WA, USA) to improve visualization (Shannon 2003). Nodes were colored according to their culture conditions and the node sizes indicate their normalized total peak area.

2.7. Statistical analysis

Statistical analysis was done by uploading the CSV file to the Metaboanalyst platform version 5.0 (https://www.metaboanalyst.ca/) (Chong et al., 2019). The data were pre-processed, first by normalization (by median) of the samples, then the variables were weighted by auto scale (mean-centered and divided by the square root of the standard deviation of each variable). A Principal Component Analysis (PCA) then a Partial Least Squares Discriminant Analysis (PLS-DA) were carried out. Finally, the *m/z* and the retention time of the top 150 features associated with their normalized peak area were plotted on a heat map (hierarchical clustering) using ANOVA.

3. Results and discussion

3.1. General observations

The general overview of the chromatograms of the experiment carried out with the epigenetic modifiers show five main peaks detected in all the conditions, including the control (untreated *C. mamane*) corresponding to the three TDKPs, botryosulfuranol A, botryosulfuranol B, botryosulfuranol C and two cyclic pentapeptides (Fig. 1). The abundances of the 5 major peaks seem different from one treatment to another. For instance, *m/z* 566.4279 at 9.37 min is the highest peak for AZA, whereas *m/z* 433.0522 at 7.50 min is the highest for SB. Therefore, the epigenetic modifiers do affect the proportions of these metabolites. Moreover, despite the absence of other major peaks observed in the chromatograms, many other minor metabolites may be being produced by *C. mamane*. Besides the 3 botryosulfuranols and the 2 cyclic pentapeptides detected, our strain of *C. mamane* could potentially produce other cyclopeptides and DKPs considering that endophytic fungi have shown a great potential to produce these class of metabolites under standard culture conditions with interesting biological activities (Wang et al., 2017a,b).

A metabolomic profile of the 20 extracts (treatment with 4 epigenetic modifiers and 1 untreated *C. mamane* culture in triplicate, QC and blank samples) obtained in the epigenetic modifiers experiment and analyzed by UHPLC-(+)ESI-HRMS MS afforded 294 features (*m/z*-RT pairs), which were considered for the metabolomics and statistical analysis. Twenty percent of these features were putatively annotated, corresponding to metabolites belonging to different chemical classes such as diketopiperazines, cyclic peptides, fatty acyls, phosphoethanolamine, ubiquinones (Supplementary data).

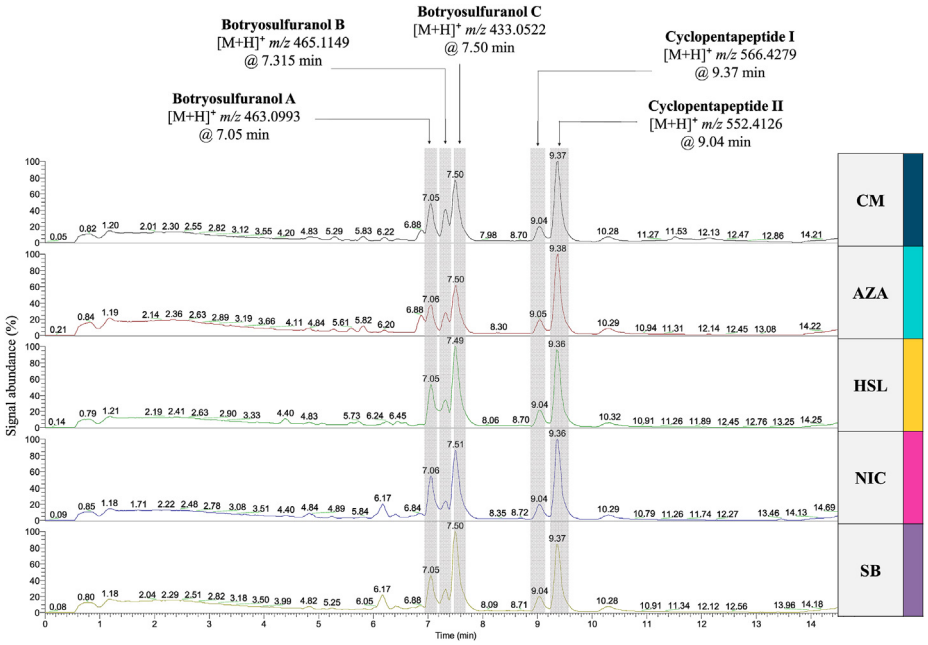

Fig. 1. UHPLC-(+)ESI-HRMS (total ion chromatogram) from crude extracts of *C. mamane* (CM) treated with 5-azacytidine (AZA), homoserine lactone (HSL), nicotinamide (NIC) and sodium butyrate (SB).

3.2. Main discriminating metabolites between different treatment groups

As a first step, a PCA was applied as an unsupervised overview of the general LC-MS profile (data not shown). This analysis clustered all independent biological replicates from the same conditions. Then, a supervised PLS-DA analysis was carried out on these same data which indicated the different responses of *C. mamane* (CM) when treated with the HDACi (NIC and SB), the DNMTi (AZA) and the QS molecule (HSL) in comparison to the untreated culture of *C. mamane* (Fig. 2a). Separate clusters appear on the PLS-DA score plot (65.3% of total variability). The component 1 (X-axis) explained 56.2% of the variability, allowing the discrimination of the AZA cluster. While in the component 2 (9.1%), we can observe two clusters: NIC/SB and CM/HSL, however a slight differentiation can be observed between CM and HSL.

Fig. 2b presents the top 15 metabolites that most contributed to the differences observed in the PLS-DA score plot, regarding component 1. Among them, a metabolite was annotated as terrycoline, a fatty acyl that was previously isolated from an endophytic fungal strain of *Neurospora terricola* (Sordariaceae) isolated from a lichen (Zhang et al., 2009). Another metabolite was annotated as cytidine diphosphate ethanolamine (CDP-ethanolamine), a phospholipid that has previously shown to be critical for mycelial growth and whose biosynthesis is involved in the mycotoxin production and virulence in *Fusarium graminearum* (Nectriaceae) (Wang et al., 2019). The annotated DKP diphenylalazine B has been previously isolated from *Epicoccum nigrum* (Didymellaceae) which colonized *Cordyceps sinensis* (Cordycipitaceae) (Guo et al., 2009) while botryosulfuranol C is one of the TDKPs previously isolated from the strain of *C. mamane* (Barakat et al., 2019) used in this study. The aforementioned metabolites are detailed in the supplementary data.

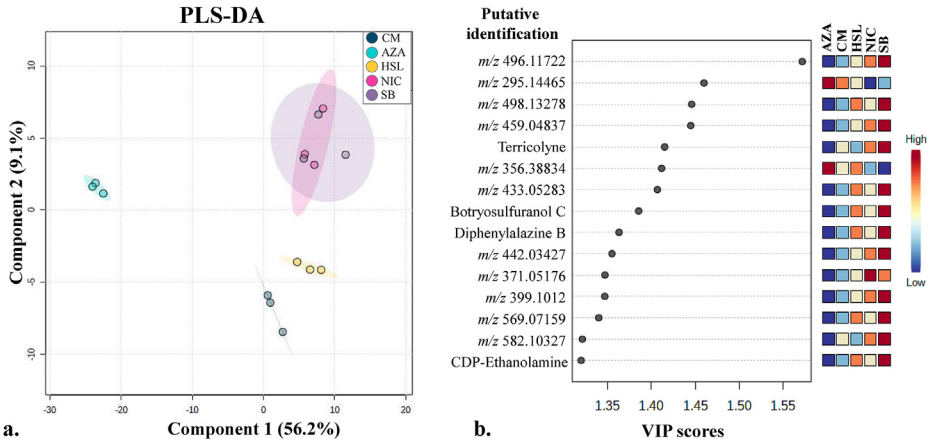

Fig. 2. a) PLS-DA corresponding to the extracts of *C. mamane* (CM) and treated with nicotinamide (NIC), sodium butyrate (SB), 5-azacytidine (AZA) and homoserine lactone (HSL), based on features (*m/z* at Rt to its normalized peak area) detected in LC-MS chromatograms; (b) PLS-DA-VIP projection for the top 15 features corresponding to each group (AZA, CM, HSL, NIC, SB).

3.3. Dynamics of metabolite production

Out of the 294 features, 221 showed significant changes under the treatment with each epigenetic modifier (p-value < 0.05) using the analysis of variance (one way ANOVA) and Fisher test (F-test). The top 150 features with significant changes were plotted in the hierarchical clustering analysis (heat map) where five main clusters of metabolite behaviors are detected (Fig. 3): a cluster corresponding to the up-regulation with AZA treatment, a cluster corresponding to the up-regulation with NIC and SB treatment, two clusters corresponding to the up-regulation with HSL treatment and the last one corresponding to the down-regulation with the 4 epigenetic modifiers treatment (for detailed view, see Supplementary data).). These results are consistent with PLS-DA analysis observed above.

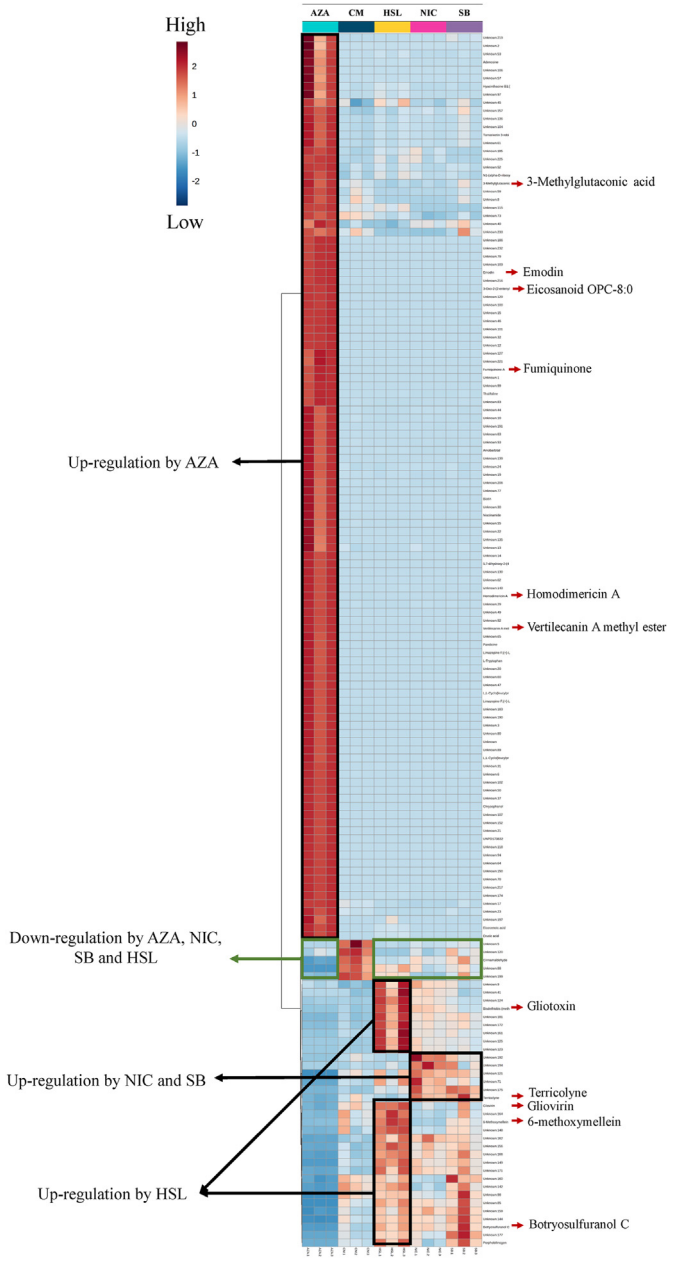

Fig. 3. Hierarchical clustering of the main features observed in *C. mamane* in the positive mode after the treatment with the epigenetic modifiers.

AZA significantly induced the production of 113 metabolites among the top 150, initially lowly produced by *C. mamane*, highlighting the great capacity of this epigenetic modifier to alter the gene expression through the induction of DNA demethylation in this endophytic fungus. An analysis of the differential expression of endophytic fungi under the treatment with 7 different HDACi and DNMTi (including AZA, NIC and SB) published in 2016 showed that AZA was the epigenetic modifier that induced most of the changes in the secondary metabolite profile of the ascomycete *Dothiora* sp. (Dothioraceae) (González-Menéndez et al., 2016), which is consistent with our results. Metabolites annotated as 3-methylglutaconic acid, an eicosanoid, emodin, fumiquinone A, homodimericin A and vertilecanin A methyl ester were also induced under the treatment with AZA as observed in Fig. 3.

Interestingly, the metabolites identified as DKPs gliotoxin, gliovirin and botryosulfuranol C were up-regulated under the treatment with HSL, indicating that the recognition of a bacterial QS molecule by *C. mamane* triggered a signal that induced the production of these type of metabolites. Similarly, the metabolite annotated as isocoumarin 6-methoxymellein, first isolated from the fungus *Preussia bipartis* (Sporormiaceae) was also up-regulated by HSL while different isocoumarin derivatives have been previously isolated from Botryosphaeriaceae family members, including *Botryosphaeria mamane*, *B. obtusa* and *B. rhodina* (Reveglia et al., 2020). Moreover, the fatty acyl terricolyne was up-regulated by NIC and SB but not by AZA or HSL (Fig. 3), suggesting that NIC and SB might trigger similar effects as they are both HDACi.

3.4. Differential production of metabolites

Regarding the influence of the epigenetic modifiers more in details as shown in the Volcano plot representations (Fig. 4), a total of 113, 46, 39 and 18 metabolites were up-regulated when *C. mamane* culture was treated with AZA (Figure 4a, HSL (Fig. 4b), NIC (Fig. 4c) and SB (Fig. 4d), respectively. Notably, as observed above in the heat map, AZA was the epigenetic modifier that induced and down-regulated the highest number of metabolites. On the contrary, SB is the one that alters the metabolism the least, inducing and down-regulating the smallest number of metabolites.

Among the metabolites with the highest significant changes (p-value < 0.05 and fold-change ratio >2) considering their up-regulation under the presence of epigenetic modifiers (see Supplementary data) based on the volcano plot analysis, the one putatively identified as vertilecanin A methyl ester was significantly up-regulated by the addition of AZA but also by NIC. This phenopicolinic acid derivative was previously isolated from the entomopathogenic fungus *Verticillium lecanii* (Soman et al., 2001), a species that is currently classified within the genus *Lecanicillium* (Nicoletti and Becchimanzi 2020). The metabolite annotated as fumiquinone A, previously isolated from *Aspergillus fumigatus* (Hayashi et al., 2007) was significantly up-regulated by AZA. Another ubiquinone, fumiquinone B, was isolated from the endophytic fungus *Neopestalotiopsis* sp. (Grigoletto et al., 2019). AZA, NIC and HSL significantly up-regulated the metabolite annotated as hydroxyanthrequinone emodin, a compound known to be produced by different fungal species as a pigmented metabolite (Wells et al., 1975). Emodin has also been described in different endophytic fungal species including *Aspergillus versicolor* (Hawas et al., 2012), *Alternaria* sp., *Epicoccum nigrum* (Vigneshwari et al., 2019) and an endophytic strain of *Penicillium citrinum* isolated from a medicinal plant (Luo et al., 2019). The metabolite annotated as homodimericin, significantly up-regulated by AZA and SB, has been described as a complex fungal polyketide isolated from *Trichoderma harzianum*, whose biosynthesis occurs in a similar manner to an

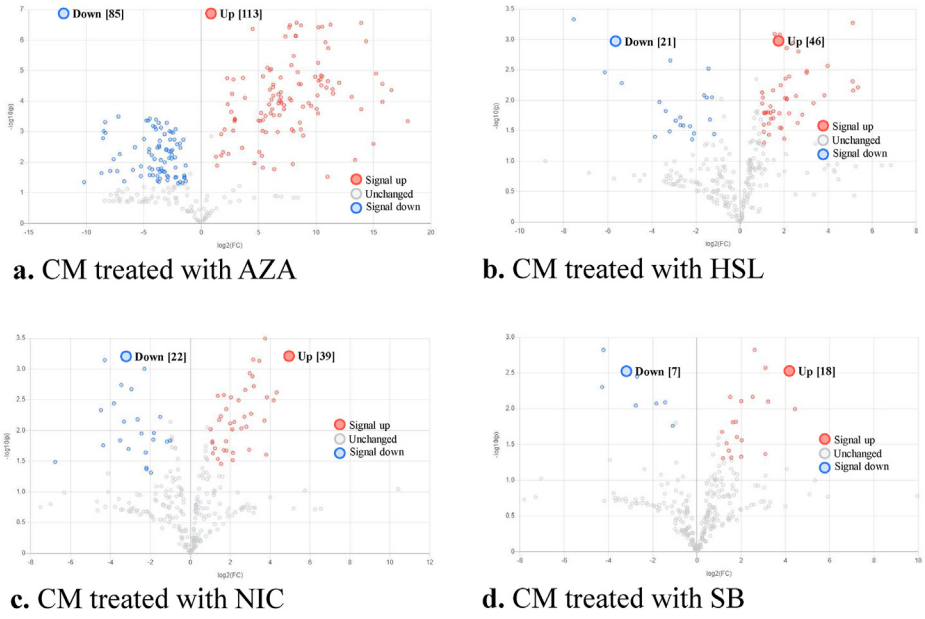

Fig. 4. Volcano plot representations of *C. mamane* response to the treatment with epigenetic modifiers (a) azacytidine (AZA), (b) homoserine lactone (HSL), (c) nicotinamide (NIC) and (d) sodium butyrate (SB). Each dot represents a feature (*m*/*z* at Rt), the red and blue colors indicate a significant change (p-value < 0.05 and fold-change ratio >2) of the features under treatment in up-regulating (red) or down-regulating (blue) their production in comparison with the untreated culture of CM, while the numbers in brackets indicate the number of significant features observed. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

epicolactone produced by an endophytic fungal strain of *Epicoccum* sp. ((Mevers et al., 2016; Long et al., 2018).

Although both NIC and SB inhibit the deacetylation of histones (HDACi), they differently induce the production of compounds in *C. mamane*, probably because each of these HDACi interacts in different manner with different histone deacetylases. As described in the literature, SB inhibits HDAC, classes I and II, while NIC inhibits HDAC class III (Harrison et al., 2019). In agreement with these observations, El-Hawary et al. (2018) observed that when the marine-derived fungus *Penicillium brevicompactum* is treated with NIC and SB, different compounds were induced, obtaining more phenolic metabolites with NIC than with SB (El-Hawary et al., 2018). While the majority of compounds produced by *C. mamane* were up-regulated with AZA treatment, many other compounds were down-regulated. Similar results were reported in the literature. As an example, when the endophytic fungus *Diaporthe* sp. is treated with AZA, Deepika et al. (2020) demonstrated a significant increase in most metabolites, specifically in colchicine production, while a negative regulation in the production of other metabolites in the presence of demethylating agents was also observed (Deepika et al., 2020).

Focusing on the three novel DKPs previously isolated from *C. mamane* (Barakat et al., 2019), botryosulfuranols A, B and C were significantly down-regulated by AZA (Fig. 5 a-c), while only botryosulfuranol C showed a significant up-regulation by HSL(Fig. 5c). The HDACi SB had no influence on their production, contrary to the results of a previous study, where the 3 botryosulfuranols were found to be induced when *C. mamane* culture was treated with the HDACi sodium valproate (Triastuti et al., 2019). This could have been explained by an interaction of the HDACi with different histone deacetylases, however, sodium valproate and SB belong to the same class of HDACi (Shukla and Tekwani 2020) and they were expected to present the same effect on *C. mamane* metabolism.

Homoserine lactones are well-known molecules involved in QS in bacterial communities and their role in gram-negative bacteria has been already reported, indicating their modulatory activity in regulatory proteins for gene expression (Fuqua and Greenberg 2002). The roles of DKPs in QS have also been reported during bacterial interactions (Bofinger et al., 2017), suggesting a similar role of botryosulfuranols in *C. mamane* when treated with HSL. Moreover, mechanisms of QS are also observed in yeast and filamentous fungi through the production of tyrosol, farnesol (Mondal and Majumdar 2019) and mycotoxins (Venkatesh and Keller 2019). As observed with the up-regulation of botryosulfuranol C by HSL, QS are molecules known to be capable of inducing the production of cyclic DKPs, such as emestrin A and emestrin B, produced by *Aspergillus fumigatus* in presence of HSL. In spite of their lack of *in vitro* activity against bacteria, it was hypothesized that these

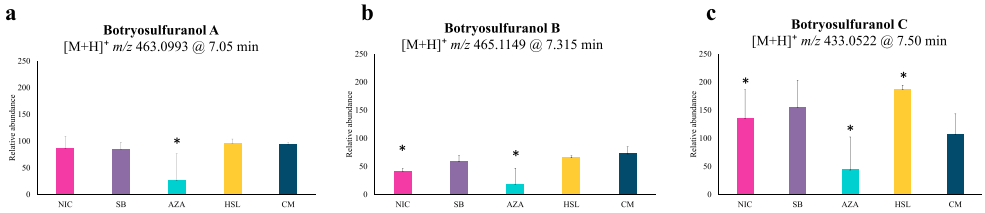

Fig. 5. Normalized peak area of the thiodiketopiperazines (a) botryosulfuranol A, (b) botryosulfuranol B and (c) botryosulfuranol C detected under the treatment with the epigenetic modifiers nicotinamide (NIC), sodium butyrate (SB), 5-azacytidine (AZA) and homoserine lactone (HSL) in comparison to the untreated culture of *C. mamane* (CM). Significant changes with p-value < 0.05 (*) are indicated.

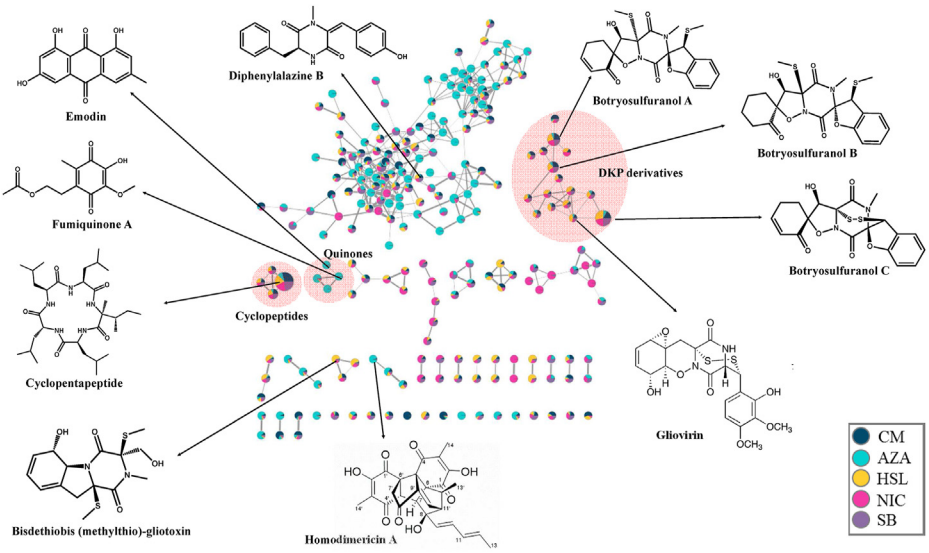

Fig. 6. Molecular networking corresponding to the extracts of *C. mamane* treated with 4 epigenetic modifiers based on features (*m/z* at Rt to its normalized peak area) detected in LC-MS chromatograms.

DKPs could act as QS inhibitors to modulate or attenuate bacterial populations (Rateb et al., 2013).

In our study, the putatively identified DKP diphenylalazine B was also significantly up-regulated by HSL. A similar compound, diphenylalazine C, has been isolated from the fungus *Schizophyllum commune* (Chunyu et al., 2017), recently described as an endophytic fungus isolated from the medicinal plant *Alchornea glandulosa* (Euphorbiaceae), being a source of many other DKPs (Rabal Biasetto et al., 2019). Furthermore, the metabolite annotated as DKP bisdethiobis (methylthio)-gliotoxin was significantly up-regulated by HSL and NIC. In a previous work, this metabolite was isolated from an endophytic fungal strain of *Aspergillus fumigatus* isolated from a Chinese medicinal plant (Zhang et al., 2018).

3.5. Clustering of DKPs and putatively identified metabolites via mass spectral similarity network

To better visualize chemical relationships based on acquired MS–MS data, and to focus on derivatives of annotated metabolites, molecular network analysis was performed. Our analysis displayed a molecular network that contained 294 nodes and 26 spectral families with 109 connected nodes while the rest of the nodes showed no link to others (self-loop). The size of the nodes represent the total average of the detected peak areas for each compound in all the conditions while the colors depend on their detection under the treatment with each epigenetic modifier (Fig. 6).

The metabolite annotated as gliovirin, an epidithiodiketopiperazine obtained from the marine-derived fungus *Trichoderma* sp. isolated from a red alga (Yamazaki et al., 2015), was up-regulated by SB and appeared within the cluster containing the 3 DKPs, botryosulfuranols A, B and C. Additionally, in the same cluster of DKP derivatives, a feature with *m/z* 463.0991 at 6.272 min was found to possess the same molecular formula as botryosulfuranol A, linked with a cosine value of 0.89 (data not shown), possibly indicating an isomer of this metabolite. The putatively identified DKP bisdethiobis (methylthio)-gliotoxin was found in a different cluster linked to two unidentified metabolites that may correspond to two other DKPs (Fig. 6). Moreover, the molecular network highlighted the presence of another cyclopentapeptide linked to the two main cyclopentapeptides observed above in the LC-MS chromatograms (Fig. 1). In the cluster of quinones, we observed two metabolites putatively identified as fumiquinone A and emodin linked to two other metabolites: one annotated as chrysophanol, previously isolated from marine fungus *Aspergillus* sp. (Qian et al., 2011) and a metabolite with *m/z* 285.0763 at 5.56 min ($C_{16}H_{12}O_5$) for which no putative annotation was obtained. The metabolite annotated as homodimericin A was also linked to two unidentified metabolites with *m/z* 505.1832 at 10.04 min ($C_{24}H_{28}N_2O_{10}$) and *m/z* 519.1989 at 10.01 min ($C_{30}H_{30}O_8$).

3.6. Addition of amino acids for DKPs induction

Regarding the experiment with amino acids, we hypothesized that there would be an increase in the production of botryosulfuranol A, B and other alkaloids after the addition of the bioprecursors L-phe and L-trp to the culture medium. Some studies have shown that the addition of different amino acids in the culture of fungi leads to the induction of different metabolites, including

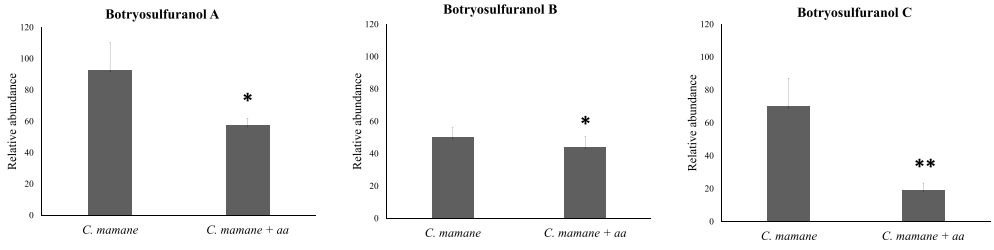

Fig. 7. Normalized peak area of botryosulfuranols A, B and C detected under the addition of amino acids (C.mamane + aa) in comparison to the untreated culture of *C. mamane*. Significant changes with p-value < 0.05 (*) and significant fold-change ratio >2 (**) are indicated.

new compounds such as bioactive alkaloids, in, for example, the fungus *Pseudallescheria boydii* (Microascaceae) (Huang et al., 2017) and in the marine fungus *Aspergillus* sp. (Qiu et al., 2020). However, contrary to what was expected, botryosulfuranols A, B and C showed a significant down-regulation under the addition of L-phe and L-trp (p-value < 0.05) in comparison to the untreated culture of *C. mamane* (Fig. 7). Different situations might explain the observed decrease in the production of botryosulfuranols. The addition of an excess amount of these amino acids might have triggered the activation of negative-feedback mechanisms, as it was also suggested in another study that evaluated the effects of L-trp supplementation in *Fusarium* sp. (Guo et al., 2020). Moreover, botryosulfuranols may be metabolized. For example, the DKP gliotoxin produced by *Aspergillus fumigatus* is metabolized through the activation of defense mechanisms that involves the opening of the di-sulfur bridge (Scharf et al., 2014).

Furthermore, different biosynthetic pathways might have been prioritized for the incorporation of these aromatic amino acids such as those of other alkaloids and non-ribosomal peptides (Keller, 2019). In this regard, other metabolites might have been induced but were neither detected nor identified as DKPs. Indeed, the molecular network showed that the DKPs we could annotate did not necessarily belong to the same cluster, making annotation difficult. Different concentrations of other amino acids as well as different culture media and incubation times will be contemplated for future experiments.

4. Conclusions

Our results show that treatment with HDACi and DNMTi effectively activated biosynthetic pathways due to the induction of lowly produced metabolites when *C. mamane* was grown without any treatment. This indicates that methylation and deacetylation are mechanisms that regulate expression in this endophytic fungus, specifically the up-regulation obtained with the treatment with the DNMTi AZA. Meanwhile, deacetylation or methylation of histones were necessary for those compounds that were down-regulated in presence of the epigenetic modifiers. The induction of different classes of DKPs by the treatment with the QS molecule HSL represents a promising result for future experiments involving a different induction approach such as the co-culture of this endophytic fungus with bacteria. This also suggests a possible role of TDKP botryosulfuranols as QS molecules in *C. mamane* as this potential role is suggested for other DKPs in other fungi and bacteria.

Possible analogues or derivatives of putatively identified metabolites were indeed uncovered thanks to the molecular networking which can be improved by a better adapted LC-MS methodology for a larger diversity of metabolites, including the DKPs.

In an unexpected way, the addition of amino acids did not exert an induction in thiodiketopiperazines production. However, other alkaloids or nonribosomal peptides might be induced with different concentrations of other amino acids. This work consisted in a preliminary step to modulate the culture of *C. mamane* in order to find better conditions to induce the production of botryosulfuranols A, B and C and new metabolites. In view of these results, further experiments will consist in performing large-scale cultures of *C. mamane* with AZA, in order to isolate and to structurally characterize compounds induced by these epigenetic modifiers.

Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgements

This work was supported by the Institut de Recherche pour le Développement of France (IRD) through the Allocations de Recherche pour une Thèse au Sud (ARTS) grant awarded to R. P. The authors also thank the logistic support of Pharma-Dev laboratory at Paul Sabatier University in Toulouse, France and LMI-LaVi laboratory (UPCH-IRD) at Cayetano Heredia University in Lima, Peru.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.funbio.2022.02.005.

References

Abdel-Razek, A.S., El-Naggar, M.E., Allam, A., Morsy, O.M., Othman, S.I., 2020. Microbial natural products in drug discovery. Processes 8, 1–19. https://doi.org/10.3390/PR8040470.

Asai, T., Otsuki, S., Sakurai, H., Yamashita, K., Ozeki, T., Oshima, Y., 2013. Benzophenones from an endophytic fungus, graphiopsis chlorocephala, from Paeonia lactiflora cultivated in the presence of an NAD+-dependent HDAC inhibitor. Org. Lett. 15, 2058–2061. https://doi.org/10.1021/ol400781b.

Barakat, F., Vansteelandt, M., Triastuti, A., Jargeat, P., Jacquemin, D., Graton, J., Mejia, K., Cabanillas, B., Vendier, L., Stigliani, J.-L.L., Haddad, M., Fabre, N., 2019. Thiodiketopiperazines with two spirocyclic centers extracted from Botryosphaeria mamane, an endophytic fungus isolated from Bixa orellana L. Phytochemistry 158, 142–148. https://doi.org/10.1016/j.phytochem.2018.11.007.

Bofinger, M.R., de Sousa, L.S., Jen, Fontes, Marsaioli, A.J., 2017. Diketopiperazines as cross-communication quorum - sensing signals between cronobacter sakazakii and Bacillus cereus. ACS Omega 2, 1003–1008. https://doi.org/10.1021/acsomega.6b00513.

Boufridi, A., Quinn, R.J., 2018. Harnessing the properties of natural products. Annu. Rev. Pharmacol. Toxicol. 58, 451–470. https://doi.org/10.1146/annurev-pharmtox-010716-105029.

Brakhage, A.A., 2013. Regulation of fungal secondary metabolism. Nat. Rev. Microbiol. 11, 21–32. https://doi.org/10.1038/nrmicro2916.

Brakhage, A.A., Schroeckh, V., 2011. Fungal secondary metabolites – strategies to activate silent gene clusters. Fungal Genet. Biol. 48, 15–22. https://doi.org/10.1016/j.fgb.2010.04.004.

Chong, J., Wishart, D.S., Xia, J., 2019. Using MetaboAnalyst 4.0 for comprehensive and integrative metabolomics data analysis. Curr. Protoc. Bioinf. 68. https://doi.org/10.1002/cpbi.86.

Chunyu, W.-X., Ding, Z.-G., Zhao, J.-Y., Wang, Y.-X., Han, X.-L., Li, M.-G., Wen, M.-L., 2017. Two new diketopiperazines from the tin mine tailings-derived fungus Schizophyllum commune YIM DT 10058. Nat. Prod. Res. 31, 1566–1572. https://doi.org/10.1080/14786419.2016.1274894.

Cichewicz, R.H., 2010. Epigenome manipulation as a pathway to new natural product scaffolds and their congeners. Nat. Prod. Rep. 27, 11–22. https://doi.org/10.1039/B920860G.

Deepika, V.B., Vohra, M., Mishra, S., Dorai, K., Rai, P., Satyamoorthy, K., Murali, T.S., 2020. DNA demethylation overcomes attenuation of colchicine biosynthesis in an endophytic fungus Diaporthe. J. Biotechnol. 323, 33–41. https://doi.org/10.1016/j.jbiotec.2020.07.019.

Deshmukh, S., Gupta, M., Prakash, V., Saxena, S., 2018. Endophytic fungi: a source of potential antifungal compounds. J. Fungi 4, 77. https://doi.org/10.3390/jof4030077.

El-Hawary, S., Sayed, A., Mohammed, R., Hassan, H., Zaki, M., Rateb, M., Mohammed, T., Amin, E., Abdelmohsen, U., 2018. Epigenetic modifiers induce bioactive phenolic metabolites in the marine-derived fungus Penicillium brevicompactum. Mar. Drugs 16, 253. https://doi.org/10.3390/md16080253.

Fraisier-Vannier, O., Chervin, J., Cabanac, G., Puech, V., Fournier, S., Durand, V., Amiel, A., André, O., Benamar, O.A., Dumas, B., Tsugawa, H., Marti, G., 2020. MS-CleanR: a feature-filtering workflow for untargeted LC–MS based metabolomics. Anal. Chem. 92, 9971–9981. https://doi.org/10.1021/acs.analchem.0c01594.

Fuqua, C., Greenberg, E.P., 2002. Listening in on bacteria: acyl-homoserine lactone signalling. Nat. Rev. Mol. Cell Biol. 3, 685–695. https://doi.org/10.1038/nrm907.

González-Menéndez, V., Pérez-Bonilla, M., Pérez-Victoria, I., Martín, J., Muñoz, F., Reyes, F., Tormo, J., Genilloud, O., 2016. Multicomponent analysis of the differential induction of secondary metabolite profiles in fungal endophytes. Molecules 21, 234. https://doi.org/10.3390/molecules21020234.

Grigoletto, D.F., Correia, A.M.L., Abraham, W.-R., Rodrigues, A., Assis, M.A., Ferreira, A.G., Massaroli, M., Lira, SP de, 2019. Secondary metabolites produced by endophytic fungi: novel antifungal activity of fumiquinone B. Acta Sci. Biol Sci. 41, e48785. https://doi.org/10.4025/actascibiolsci.v41i1.48785.

Guo, Y.-W., Liu, X.-J., Yuan, J., Li, H.-J., Mahmud, T., Hong, M.-J., Yu, J.-C., Lan, W.-J., 2020. L -tryptophan induces a marine-derived Fusarium sp. to produce indole alkaloids with activity against the zika virus. J. Nat. Prod. 83, 3372–3380. https://doi.org/10.1021/acs.jnatprod.0c00717.

Guo, H., Sun, B., Gao, H., Chen, X., Liu, S., Yao, X., Liu, X., Che, Y., 2009. Diketopiperazines from the Cordyceps -colonizing fungus Epicoccum nigrum. J. Nat. Prod. 72, 2115–2119. https://doi.org/10.1021/np900654a.

Gupta, S., Kulkarni, M.G., White, J.F., Van Staden, J., 2020. Epigenetic-based developments in the field of plant endophytic fungi. South Afr. J. Bot. 134, 394–400. https://doi.org/10.1016/j.sajb.2020.07.019.

Harrison, I.F., Powell, N.M., Dexter, D.T., 2019. The histone deacetylase inhibitor nicotinamide exacerbates neurodegeneration in the lactacystin rat model of Parkinson's disease. J. Neurochem. 148, 136–156. https://doi.org/10.1111/jnc.14599.

Hawas, U.W., El-Beih, A.A., El-Halawany, A.M., 2012. Bioactive anthraquinones from endophytic fungus Aspergillus versicolor isolated from red sea algae. Arch Pharm. Res. (Seoul) 35, 1749–1756. https://doi.org/10.1007/s12272-012-1006-x.

Hayashi, A., Fujioka, S., Nukina, M., Kawano, T., Shimada, A., Kimura, Y., 2007. Fumiquinones A and B, nematicidal quinones produced by Aspergillus fumigatus. Biosci. Biotechnol. Biochem. 71, 1697–1702. https://doi.org/10.1271/bbb.70110.

Huang, L.-H., Xu, M.-Y., Li, H.-J., Li, J.-Q., Chen, Y.-X., Ma, W.-Z., Li, Y.-P., Xu, J., Yang, D.-P., Lan, W.-J., 2017. Amino acid-directed strategy for inducing the marine-derived fungus scedosporium apiospermum F41–1 to maximize alkaloid diversity. Org. Lett. 19, 4888–4891. https://doi.org/10.1021/acs.orglett.7b02238.

Jasim, B., Sahadevan, N., Chithra, S., Mathew, J., Radhakrishnan, E.K., 2019. Epigenetic modifier based enhancement of piperine production in endophytic Diaporthe sp. PF20. Proc. Natl. Acad. Sci. India B Biol. Sci. 89, 671–677. https://doi.org/10.1007/s40011-018-0982-0.

Keller, N.P., 2019. Fungal secondary metabolism: regulation, function and drug discovery. Rev. Microbiol. 17, 167–180. https://doi.org/10.1038/s41579-018-0121-1.

Long, X., Huang, Y., Long, Y., Deng, J., 2018. Biomimetic total synthesis of homodimericin A. Organ. Chem. Front. 5, 1152–1154. https://doi.org/10.1039/C7QO01161J.

Luo, H., Qing, Z., Deng, Y., Deng, Z., Tang, X., Feng, B., Lin, W., 2019. Two polyketides produced by endophytic Penicillium citrinum DBR-9 from medicinal plant stephania kwangsiensis and their antifungal activity against plant pathogenic fungi. Nat. Prod. Commun. 14, 1934578X1984679. https://doi.org/10.1177/1934578X19846795.

Mevers, E., Saurí, J., Liu, Y., Moser, A., Ramadhar, T.R., Varlan, M., Williamson, R.T., Martin, G.E., Clardy, J., 2016. Homodimericin A: a complex hexacyclic fungal metabolite. J. Am. Chem. Soc. 138, 12324–12327. https://doi.org/10.1021/jacs.6b07588.

Mondal, S., Majumdar, S., 2019. The bull effect of endophytic fungi: an approach with quorum sensing. In: Advances in Endophytic Fungal Research, pp. 171–181.

Nicoletti, R., Becchimanzi, A., 2020. Endophytism of Lecanicillium and Akanthomyces. Agriculture 10, 205. https://doi.org/10.3390/agriculture10060205.

Okada, B.K., Seyedsayamdost, M.R., 2017. Antibiotic dialogues: induction of silent biosynthetic gene clusters by exogenous small molecules. In: Shen, A. (Ed.), FEMS (Fed. Eur. Microbiol. Soc.) Microbiol. Rev. 41, 19–33. https://doi.org/10.1093/femsre/fuw035.

Olivon, F., Elie, N., Grelier, G., Roussi, F., Litaudon, M., Touboul, D., 2018. MetGem software for the generation of molecular networks based on the t-SNE algorithm. Anal. Chem. 90, 13900–13908. https://doi.org/10.1021/acs.analchem.8b03099.

Pfannenstiel, B.T., Keller, N.P., 2019. On top of biosynthetic gene clusters: how epigenetic machinery influences secondary metabolism in fungi. Biotechnol. Adv. 37, 107345. https://doi.org/10.1016/j.biotechadv.2019.02.001.

Pongcharoen, W., Rukachaisirikul, V., Phongpaichit, S., Sakayaroj, J., 2007. A new dihydrobenzofuran derivative from the endophytic fungus Botryosphaeria mamane PSU-M76. Chem. Pharm. Bull. 55, 1404–1405. https://doi.org/10.1248/cpb.55.1404.

Qadri, M., Nalli, Y., Jain, S.K., Chaubey, A., Ali, A., Strobel, G.A., Vishwakarma, R.A., Riyaz-Ul-Hassan, S., 2017. An insight into the secondary metabolism of muscodor yucatanensis: small-molecule epigenetic modifiers induce expression of secondary metabolism-related genes and production of new metabolites in the endophyte. Microb. Ecol. 73, 954–965. https://doi.org/10.1007/s00248-016-0901-y.

Qian, Z.-J., Zhang, C., Li, Y.-X., Je, J.-Y., Kim, S.-K., Jung, W.-K., 2011. Protective effects of emodin and chrysophanol isolated from marine fungus Aspergillus sp. on ethanol-induced toxicity in HepG2/CYP2E1 cells. Evid. Based Complement. Alternat. Med. 2011 1–7. https://doi.org/10.1155/2011/452621.

Qiu, Y., Guo, Q., Ran, Y.-Q., Lan, W.-J., Lam, C.-K., Feng, G.-K., Deng, R., Zhu, X.-F., Li, H.-J., Chen, L.-P., 2020. Cytotoxic alkaloids from the marine shellfish-associated fungus Aspergillus sp. XBB-4 induced by an amino acid-directed strategy. RSC Adv. 10, 4243–4250. https://doi.org/10.1039/C9RA10306F.

Rabal Biasetto, C., Somensi, A., Sales Figueiro, F., Beraldo de Moraes, L.A., Silva, G.H., Marx Young, M.C., Da Silva Bolzani, V., Araújo, A.R., 2019. Diketopiperazines and arylethylamides produced by Schizophyllum commune, an endophytic fungus in Alchornea glandulosa. Eclética Química Journal 44, 36–42. https://doi.org/10.26850/1678-4618eqj.v44.3.2019.p36-42.

Rateb, M.E., Hallyburton, I., Houssen, W.E., Bull, A.T., Goodfellow, M., Santhanam, R., Jaspars, M., Ebel, R., 2013. Induction of diverse secondary metabolites in Aspergillus fumigatus by microbial co-culture. RSC Adv. 3, 14444. https://doi.org/10.1039/c3ra42378f.

Reveglia, P., Masi, M., Evidente, A., 2020. Melleins—intriguing natural compounds. Biomolecules 10, 772. https://doi.org/10.3390/biom10050772.

Scharf, D.H., Habel, A., Heinekamp, T., Brakhage, A.A., Hertweck, C., 2014. Opposed effects of enzymatic gliotoxin N - And S -methylations. J. Am. Chem. Soc. 136, 11674–11679. https://doi.org/10.1021/ja5033106.

Shannon, P., 2003. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 13, 2498–2504. https://doi.org/10.1101/gr.1239303.

Shukla, S., Tekwani, B.L., 2020. Histone deacetylases inhibitors in neurodegenerative diseases, neuroprotection and neuronal differentiation. Front. Pharmacol. 11. https://doi.org/10.3389/fphar.2020.00537.

Shwab, E.K., Bok, J.W., Tribus, M., Galehr, J., Graessle, S., Keller, N.P., 2007. Histone deacetylase activity regulates chemical diversity in Aspergillus. Eukaryot. Cell 6, 1656–1664. https://doi.org/10.1128/EC.00186-07.

Soman, A.G., Gloer, J.B., Angawi, R.F., Wicklow, D.T., Dowd, P.F., 2001. Vertilecanins: new phenopicolinic acid analogues from Verticillium l ecanii. J. Nat. Prod. 64, 189–192. https://doi.org/10.1021/np000094q.

Strobel, G., 2018. The emergence of endophytic microbes and their biological promise. J. Fungi 4. https://doi.org/10.3390/jof4020057.

Strobel, G., Daisy, B., 2003. Bioprospecting for microbial endophytes and their natural products. Microbiol. Mol. Biol. Rev. 67, 491–502. https://doi.org/10.1128/MMBR.67.4.491-502.2003.

Suzuki, M.M., Bird, A., 2008. DNA methylation landscapes: provocative insights from epigenomics. Nat. Rev. Genet. 9, 465–476. https://doi.org/10.1038/nrg2341.

Toghueo, R.M.K., Sahal, D., Boyom, F.F., 2020. Recent advances in inducing endophytic fungal specialized metabolites using small molecule elicitors including epigenetic modifiers. Phytochemistry 174, 112338. https://doi.org/10.1016/j.phytochem.2020.112338.

Triastuti, A., Vansteelandt, M., Barakat, F., Trinel, M., Jargeat, P., Fabre, N., Amasifuen Guerra, C.A., Mejia, K., Valentin, A., Haddad, M., 2019. How histone deacetylase inhibitors alter the secondary metabolites of Botryosphaeria mamane, an endophytic fungus isolated from Bixa orellana. Chem. Biodivers. 16. https://doi.org/10.1002/cbdv.201800485.

Tsugawa, H., Cajka, T., Kind, T., Ma, Y., Higgins, B., Ikeda, K., Kanazawa, M., VanderGheynst, J., Fiehn, O., Arita, M., 2015. MS-DIAL: data-independent MS/MS deconvolution for comprehensive metabolome analysis. Nat. Methods 12, 523–526. https://doi.org/10.1038/nmeth.3393.

Vasanthakumari, M.M., Jadhav, S.S., Sachin, N., Vinod, G., Shweta, S., Manjunatha, B.L., Kumara, P.M., Ravikanth, G., Nataraja, K.N., Uma Shaanker, R., 2015. Restoration of camptothecine production in attenuated endophytic fungus on re-inoculation into host plant and treatment with DNA methyltransferase inhibitor. World J. Microbiol. Biotechnol. 31, 1629–1639. https://doi.org/10.1007/s11274-015-1916-0.

Venkatesh, N., Keller, N.P., 2019. Mycotoxins in conversation with bacteria and fungi. Front. Microbiol. 10, 1–10. https://doi.org/10.3389/fmicb.2019.00403.

Vigneshwari, A., Rakk, D., Németh, A., Kocsubé, S., Kiss, N., Csupor, D., Papp, T., Škrbić, B., Vágvölgyi, C., Szekeres, A., 2019. In: Gupta, V. (Ed.), Host Metabolite Producing Endophytic Fungi Isolated from Hypericum perforatum, PLOS ONE, 14, e0217060. https://doi.org/10.1371/journal.pone.0217060.

Wang, X., Li, Y., Zhang, X., Lai, D., Zhou, L., 2017a. Structural diversity and biological activities of the cyclodipeptides from fungi. Molecules 22, 2026. https://doi.org/10.3390/molecules22122026.

Wang, X., Lin, M., Xu, D., Lai, D., Zhou, L., 2017b. Structural diversity and biological activities of fungal cyclic peptides, excluding cyclodipeptides. Molecules 22, 2069. https://doi.org/10.3390/molecules22122069.

Wang, J., Wang, H., Zhang, C., Wu, T., Ma, Z., Chen, Y., 2019. Phospholipid homeostasis plays an important role in fungal development, fungicide resistance and virulence in Fusarium graminearum. Phytopathol. Res. 1, 16. https://doi.org/10.1186/s42483-019-0023-9.

Welch, T.R., Williams, R.M., 2014. Epidithiodioxopiperazines. occurrence, synthesis and biogenesis. Nat. Prod. Rep. 31, 1376–1404. https://doi.org/10.1039/c3np70097f.

Wells, J.M., Cole, R.J., Kirksey, J.W., 1975. Emodin, a toxic metabolite of Aspergillus wentii isolated from weevil-damaged chestnuts. Appl. Microbiol. 30, 26–28. https://doi.org/10.1128/am.30.1.26-28.1975.

White, J.F., Kingsley, K.L., Zhang, Q., Verma, R., Obi, N., Dvinskikh, S., Elmore, M.T., Verma, S.K., Gond, S.K., Kowalski, K.P., 2019. Review: endophytic microbes and their potential applications in crop management. Pest Manag. Sci. 75, 2558–2565. https://doi.org/10.1002/ps.5527.

Williams, R.B., Henrikson, J.C., Hoover, A.R., Lee, A.E., Cichewicz, R.H., 2008. Epigenetic remodeling of the fungal secondary metabolome. Org. Biomol. Chem. 6, 1895. https://doi.org/10.1039/b804701d.

Yamazaki, H., Rotinsulu, H., Narita, R., Takahashi, R., Namikoshi, M., 2015. Induced production of halogenated epidithiodiketopiperazines by a marine-derived

Trichoderma cf. brevicompactum with sodium halides. J. Nat. Prod. 78, 2319–2321. https://doi.org/10.1021/acs.jnatprod.5b00669.
Yuan, M.-X., Qiu, Y., Ran, Y.-Q., Feng, G.-K., Deng, R., Zhu, X.-F., Lan, W.-J., Li, H.-J., 2019. Exploration of indole alkaloids from marine fungus Pseudallescheria boydii F44-1 using an amino acid-directed strategy. Mar. Drugs 17, 77. https://doi.org/10.3390/md17020077.
Zhang, F., Liu, S., Lu, X., Guo, L., Zhang, H., Che, Y., 2009. Allenyl and alkynyl phenyl ethers from the endolichenic fungus Neurospora terricola. J. Nat. Prod. 72, 1782–1785. https://doi.org/10.1021/np900512k.
Zhang, H., Ruan, C., Bai, X., Chen, J., Wang, H., 2018. Heterocyclic alkaloids as antimicrobial agents of Aspergillus fumigatus D endophytic on edgeworthia chrysantha. Chem. Nat. Compd. 54, 411–414. https://doi.org/10.1007/s10600-018-2365-4.
Zutz, C., Bandian, D., Neumayer, B., Speringer, F., Gorfer, M., Wagner, M., Strauss, J., Rychli, K., 2014. Fungi treated with small chemicals exhibit increased antimicrobial activity against facultative bacterial and yeast pathogens. BioMed. Res. Int. 2014 1–13. https://doi.org/10.1155/2014/540292.