Genome sequences of *Rhizopogon roseolus*, *Mariannaea elegans*, *Myrothecium verrucaria*, and *Sphaerostilbella broomeana* and the identification of biosynthetic gene clusters for fungal peptide natural products

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Abstract

In recent years, a variety of fungal cyclic peptides with interesting bioactivities have been discovered. For many of these peptides, the biosynthetic pathways are unknown and their elucidation often holds surprises. The cyclic and backbone N-methylated omphalotins from *Omphalotus olearius* were recently shown to constitute a novel class (borosins) of ribosomally synthesized and posttranslationally modified peptides, members of which are produced by many fungi, including species of the genus Rhizopogon. Other recently discovered fungal peptide macrocycles include the mariannamides from *Mariannaea elegans* and the backbone N-methylated verrucamides and broomeanamides from *Myrothecium verrucaria* and *Sphaerostilbella broomeana*, respectively. Here, we present draft genome sequences of four fungal species *Rhizopogon roseolus*, *Mariannaea elegans*, *Myrothecium verrucaria*, and *Sphaerostilbella broomeana*. We screened these genomes for precursor proteins or gene clusters involved in the mariannamide, verrucamide, and broomeamide biosynthesis including a general screen for borosin-producing precursor proteins. While our genomic screen for potential ribosomally synthesized and posttranslationally modified peptide precursors of mariannamides, verrucamides, broomeanamides, and borosins remained unsuccessful, antiSMASH predicted nonribosomal peptide synthase gene clusters that may be responsible for the biosynthesis of mariannamides, verrucamides, and broomeanamides. In *M. verrucaria*, our antiSMASH search led to a putative NRPS gene cluster with a predicted peptide product of 20 amino acids, including multiple nonproteinogenic isoamines. This cluster likely encodes a member of the peptaibols, an antimicrobial class of peptides previously isolated primarily from the Genus *Trichoderma*. The nonribosomal peptide synthase gene clusters discovered in our screenings are promising candidates for future research.

Keywords: ribosomally synthesized and posttranslationally modified peptide; nonribosomal peptide; peptaibols; verrucamides; broomeanamides; *Rhizopogon roseolus*; *Mariannaea elegans*; *Myrothecium verrucaria*; *Sphaerostilbella broomeana*

Introduction

Borosins, a class of backbone N-methylated ribosomally synthesized and posttranslationally modified peptides (RiPPs), were defined in 2017 following the discovery of the biosynthesis pathway of the founding member omphalotin A (*Ramm et al. 2017; Van Der Velden et al. 2017*). This nematotoxic peptide macrocycle and its variants are produced by the fungus *Omphalotus olearius* via the self-modifying precursor protein OphMA. OphMA contains an N-terminal aN-methyltransferase domain that methylates the precursor’s C-terminal core peptide, followed by cleavage, cyclization and release of omphalotin (*Van Der Velden et al. 2017*). Backbone N-methylations were previously found exclusively in nonribosomal peptides and were even considered a hallmark of this type of peptides. Therefore, it was a surprise to find them in RiPPs (*Vogt and Künzler 2019*). Genome mining led to the discovery of many other potential OphMA-like peptide precursors in fungi, including *Dendrothele bispore* and *Lentinula edodes* (*Quijano et al. 2019*). The genomes of these fungi contain biosynthetic gene clusters with similar composition and organization as the omphalotin cluster. In addition, the encoded OphMA homologs contain core peptide with high sequence similarity to omphalotin A. Analysis of fungal tissue samples confirmed the production of the corresponding peptides, termed dendrotheelins and leentinulins (*Matabaro et al. 2021*). Recent publications demonstrated the presence of borosin clusters with trans-acting aN-methyltransferases in bacteria (*Cho et al. 2022; Imani et al. 2022*). Based on these findings, we were interested in investigating previously discovered, backbone N-methylated cyclic peptides that were hypothesized to be of nonribosomal origin, to represent novel members of the borosin class of RiPPs.

Recently discovered cyclic, backbone N-methylated peptides include verrucamides A-D, tetradecapeptides that are produced...
by the ascomycete *Myrothecium verrucaria* and contain two D-configured amino acids (Zou et al. 2011), and the octapeptides broomeanamides A–C from the mycoparasitic ascomycete *Sphaerostilbella broomeana* where all eight amino acids are L-configured (Fig. 1) (Ekanayake et al. 2021). Another class of cyclic peptides are the octapeptides mariannamides A and B isolated from the filamentous ascomycete *Mariannaea elegans* that are also composed of all L-amino acids amongst three proline residues but do not contain any backbone N-methylations (Fig. 1) (Ishiuchi et al. 2020). Both verrucamides and mariannamides were shown to possess antibacterial properties (Zou et al. 2011; Ishiuchi et al. 2020). The mode of synthesis of all three peptide classes is unknown, the structural similarity of the verrucamides and broomeanamides to the cyclic, backbone N-methylated borosins indicated that they may be RiPPs, although the presence of D-amino acids in the verrucamides rather suggested a nonribosomal origin. Only one fungal RiPP class with a residue in D-configuration has been identified so far (phallotoxins, Hallen et al. 2007).

Here, we report the genome sequences of *M. verrucaria*, *M. elegans*, *Rhizopogon roseolus*, and *S. broomeana*. We mined the genomes of *M. verrucaria*, *M. elegans*, and *S. broomeana* for potential RiPP precursor proteins of the verrucamides, mariannamides, and broomeanamides, respectively. In addition, we performed an antiSMASH search to screen for nonribosomal peptide (NRP) biosynthetic gene clusters that might encode genes for verrucamide, mariannamide, and broomeanamide synthesis. We sequenced the genome of the agaricomycete *R. roseolus*, as the genomes of two species of the genus Rhizopogon were shown in BLAST searches to encode multiple OphMA homologs each (Quijano et al. 2019). Finally, we performed screens to find new OphMA homologs in *R. roseolus*, *M. verrucaria*, *M. elegans*, and *S. broomeana*.

### Materials and methods

#### Strains and cultivation

The sequenced strains of *M. verrucaria*, *M. elegans*, and *S. broomeana* are the authentic producers of the verrucamides, mariannamides, and broomeanamides as seen in Zou et al. (2011), Ishiuchi et al. (2020), and Ekanayake et al. (2021). *Myrothecium verrucaria* DSM 2087 was received by the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures, Germany, *M. elegans* NBRC102301 was ordered from the Biological Resource Center, National Institute of Technology and Evaluation (NITE), Japan, heterokaryotic *R. roseolus* Mykothek Nr 97.03 (CBS 149159) was received from Martina Peter from the Eidgenössische Forschungsanstalt für Wald, Schnee und Landschaft (WSL), Switzerland. *Sphaerostilbella broomeana* TFC201724 is deposited at the Tartu Fungal Culture Collection (TFC) of the University of Tartu, Estonia, and was received by Kadri Poldmaa from the University of Tartu. *Myrothecium verrucaria* was cultivated on Corn Meal Agar (CMA) at 30°C, *M. elegans*...
NBRC102301 on Potato Dextrose Agar (PDA) at room temperature, R. roseolus on Yeast Malt Agar (YMG) at room temperature, and S. broomeana on PDA at room temperature.

Sample preparation and sequencing
The fungi were cultivated on cellophane-covered agar plates before their mycelia were harvested. Myrothecium verrucaria, M. elegans, and R. roseolus mycelia were harvested after 14, 9, and 40 days, respectively, and lysed by grinding with a mortar and pestle in the presence of liquid nitrogen. S. broomeana mycelium was harvested after 7 days, mixed in an Eppendorf tube with 0.5 mm glass beads, frozen in liquid nitrogen and then lysed by vigorous shaking in a Fastprep machine for 2 times 45 s at level 6. Genomic DNA was extracted using the QIAGEN DNeasy plant Mini kit, DNA concentration measured using a Qubit dsDNA kit and DNA quality confirmed by running a fraction of the DNA on an agarose gel. The DNA was sent to Novogene, United Kingdom, for shotgun sequencing on an Illumina Novaseq, producing paired-end 150 bp reads, aiming for approximately 100x coverage.

Quality control
BBDuk (v38.87, Joint Genome Institute) was first used in right-trimming mode with a kmer length of 23 down to 11 and a hammering distance of 1 to filter out sequencing adapters. A second pass with a kmer length of 31 and a hammering distance of 1 was used to filter out PhiX sequences. A third and final pass performed quality trimming on both read ends with a Phred score cutoff of 14 and an average quality score cutoff of 20, with reads under 45 bp or containing Ns subsequently rejected.

Assembly
The paired-end and singleton reads of each read set were assembled using SPAdes (v3.14.0) (Nurk et al. 2013) in isolate mode, but otherwise default parameters.

Gene calling
GlimmerHMM (v3.0.4) (Majoros et al. 2004) was trained on exon sequences taken from phylogenetically close reference genomes (Mariannaea sp. strain FML_226 v1.0 for M. elegans; Myrothecium inunadatum CBS 120646 v1.0 for M. verrucaria; Rhizopogon vulgaris FC72 v1.0 for R. roseolus; Trichoderma reesei QM6a NW_006711148.1 for S. broomeana) and subsequently used to call genes in the genome assemblies. All reference genomes were obtained from JGI (Nordberg et al. 2014) in August 2020 (M. elegans, M. verrucaria, and R. roseolus) and October 2021 (S. broomeana). Average nucleotide identity (ANI) between assemblies and reference genomes were performed with FastANI (Jain et al. 2018) that implements a similar calculation to Goris et al. (2007).

Quality assessment
Completeness of the genome assemblies was assessed using BUSCO (v5.0.0) (Simão et al. 2015) in genome mode with the –auto-lineage-euk parameter to automatically assess the likely lineage of each strain (M. elegans, M. verrucaria, S. broomeana: hypocreales; R. roseolus: boletales). To test for bacterial contamination, the tool moOTUs (v3.0.0) (Milanese et al. 2019) was run on the reads for each sample. M. elegans had 2 inserts that could not be assigned to a specific moOTU, M. verrucaria had 1 insert corresponding to “Phylobacterium species incertae sedis”, R. roseolus and S. broomeana returned no hits. These very low hit counts indicate that it is very unlikely for there to be any contamination by bacteria in the samples.

Taxonomic analysis
The ssu_find function of CheckM (v1.0.13) (Parks et al. 2015) was used to extract 16S and 18S rRNA gene sequences from the assemblies. 18S sequences of 1,726, 1,725, and 1,726 bp were found for M. elegans, M. verrucaria, and S. broomeana, respectively, but no such sequence was found for R. roseolus, likely because its assembly was highly fragmented. The sequences were aligned with the SILVA taxonomy database (v138) (Quast et al. 2013) using the provided software SINA (v1.6.1) (Pruesse et al. 2012). The internal transcribed spacer (ITS) region of each strain was extracted from its assembly using ITSx (Bengtsson-Palme et al. 2013) and its Fungi profile set. The sequences were then analyzed with the UNITE database (Nilsson et al. 2019; Köjõalg et al. 2020).

Genome mining
To search for all possible arrangements of cyclic peptides of interest, a custom Python script generated a fasta file containing all possible variants of linearized peptide sequences for the verrucamides, mariannamides, and broomeamamides. For broomeamide A, for example, these sequences would be VPFAVLIL, PFAVLILV, FAFLILVP, and so on. First, the predicted protein sequences were searched for all peptides with blastp, then the assemblies were searched for all peptides with blastx, both part of the BLAST+ suite (v2.11.0) (Camacho et al. 2009). As a positive control for the functionality of our mining method, we screened the genome of O. olearius using the peptide sequence of the cyclic RIP peptide omphalotin (Ramm et al. 2017; Van Der Velden et al. 2017). We found the omphalotin precursor protein OphMA, thus confirming that our method works. The 300 residue long N-terminal methytransferase domain of the protein OphMA from O. olearius (Quijano et al. 2019) was searched for in the predicted protein sequences with blastp. Further, all assemblies were analyzed with the fungal version of antiSMASH (v5.1.0) (Blin et al. 2019), which ignores contigs of less than 1 kbp in length by default, to look for biosynthetic gene clusters.

Results and discussion
Genome assembly and completeness
The genomes of the fungi M. verrucaria, M. elegans, R. roseolus, and S. broomeana were sequenced using Illumina PE150 and assembled using the reference genomes M. inunadatum CBS 120646 v1.0, Mariannaea sp. strain FML_226 v1.0, Rhizopogon vulgaris FC72 v1.0 and T. reesei QM6a. The M. verrucaria assembly had the highest quality of the 4 assemblies with the lowest contig count of 3,197 and the highest N50 value of 1,666,851, while M. elegans, R. roseolus, and S. broomeana had contig counts of 3,718, 23,000, and 3,882, respectively, and N50 values of 288,335, 61,999, and 295,130 (Table 1). As an alternative assessment of genome assembly and annotation completeness, the open-source software Benchmarking Universal Single-Copy Orthologs (BUSCO) was used (Simão et al. 2015). Myrothecium verrucaria was 98.0% complete as a Euakaryote, while M. elegans, R. roseolus, and S. broomeana were 99.2%, 95.7%, and 98.0% complete, respectively. Completeness for the order Hypocreales (for M. verrucaria, M. elegans, and S. broomeana) and Boletales (for R. roseolus) was 96.6%, 97.4%, 97.5%, and 95.1%, respectively. Potential 16S rRNA sequences were extracted from the assembly to confirm the absence of bacterial contamination.

Myrothecium verrucaria, M. elegans, and R. roseolus had an average nucleotide identity (ANI) of 78.8%, 79.9%, and 90.2% with their reference fungi M. inunadatum, Mariannaea sp. strain FML_226 and Rhizopogon vulgaris (Table 1). No ANI value could be
Table 1. Summary of the assembled genomes of the 4 newly sequenced fungal species M. verrucaria, M. elegans, R. roseolus, and S. broomeana.

| Genome        | M. verrucaria | M. elegans | R. roseolus | S. broomeana |
|---------------|---------------|------------|-------------|--------------|
| All scaffolds |               |            |             |              |
| Count         | 3,197         | 3,718      | 23,000      | 3,882        |
| Length        | 46,297,313    | 52,632,238 | 37,675,430  | 36,266,877   |
| NS0           | 1,066,851     | 288,335    | 61,999      | 295,130      |
| N90           | 2,846,945     | 1,147,592  | 169,220     | 800,567      |
| Max           | 4,080,732     | 2,068,507  | 547,319     | 1,217,981    |
| Scaffolds > 1 kbp |          |            |             |              |
| Count         | 454           | 1,098      | 1,543       | 1,121        |
| Length        | 45,599,860    | 51,857,879 | 33,959,226  | 35,139,140   |
| NS0           | 1,066,851     | 294,018    | 70,807      | 311,573      |
| N90           | 2,846,945     | 1,147,592  | 173,881     | 800,567      |
| Max           | 4,080,732     | 2,068,507  | 547,319     | 1,217,981    |
| BUSCO         |               |            |             |              |
| Completeness  | 98.0% (Eukaryotes) | 99.2% (Eukaryotes) | 95.7% (Eukaryotes) | 98.0% (Eukaryotes) |
| Single copy   | 95.5%         | 96.5%      | 93.8%       | 97.3%        |
| Duplicated    | 1.1%          | 0.9%       | 1.3%        | 0.2%         |
| Fragmented    | 0.4%          | 0.3%       | 0.6%        | 0.2%         |
| Missing       | 3.0%          | 2.3%       | 4.3%        | 2.3%         |
| Number of searched genes | 4,494 | 4,494 | 4,878 | 4,494 |

Average nucleotide identity (ANI) 78.8% (M. inundatum) 79.9% (M. marianiae sp.) 90.2% (Rhizopogon vulgaris) <70% (T. reesei)

Separate values are given for all scaffolds and scaffolds with a size of 1 kbp or more. Given parameters are the scaffold count, scaffold length, NS0 and N90 values, and maximum scaffold length. The NS0 and N90 values describe assembly contiguity by giving the minimal contig size that, together with all larger contigs, covers 50% or 90% of the total genome, respectively. BUSCO values describe the assembly completeness compared to Eukaryotes or the orders Hypocreales or Boletales. Average nucleotide identity describes nucleotide similarity to the reference genomes.

Screening for RiPP precursors and NRP biosynthetic gene clusters

All 4 genomes were screened for potential RiPP precursor proteins. M. verrucaria for verrucamide precursors, M. elegans for marianamide precursors, S. broomeana for broomeanamide precursors, and all 4 genomes, including R. roseolus, for OphMA homologs. Screens were performed using all circular permutations of verrucamide, marianamide, and broomeanamide sequences. In addition, all genomes were screened for the N-terminal methyltransferase domain of OphMA. These searches yielded no hits, indicating that the cyclic backbone N-methylated verrucamides and broomeamides and the cyclic marianamides are not genetically encoded and therefore may indeed be NRPs, and that R. roseolus, unlike many of its relatives from the genus Rhizopogon, does not contain any OphMA homologs.

Following the unsuccessful search for RiPP precursors of verrucamides, marianamides, and broomeamides, an additional search was performed using the fungal version of the “antibiotics and secondary metabolite analysis shell” antiSMASH (Blin et al. 2019) with the goal of finding NRP biosynthetic gene clusters that might direct the biosynthesis of the isolated peptide natural products. antiSMASH currently uses an ensemble prediction method integrating several algorithms to predict the substrate specificity of adenylation domains (Blin et al. 2017). In M. verrucaria, 1 NRP cluster was predicted to produce a verrucamide-like peptide with the correct length and several N-methylated residues, whereas in M. elegans, 1 cluster was predicted to produce a peptide of the same length as the marianamides, containing several leucines and at least 1 proline (Table 2). In S. broomeana, cluster NRPS 77.1 was predicted to produce a 6-residue-long peptide containing 1 isoleucine, 1 leucine and a total of 4 N-methylations. The broomeamides are longer (8 residues), but contain 4 N-methylated residues, 2 leucines and, in the case of Broomeanamide A, 1 isoleucine (Table 2).

Another NRP biosynthetic gene cluster of M. verrucaria was predicted to encode a 20 residue peptide with 11 residues of the non-proteinogenic amino acid isovaline (Table 3). This peptide is likely a peptail. Peptaibols are a class of antimicrobial NRPs from fungi that are 5–20 residues long, linear, N- and C-terminally modified with amino alcohol groups, and defined by the presence of the non-proteinogenic amino acids α-aminoisobutyric acid (Y) and/or isovaline (X) (de la Fuente-Núñez et al. 2013). The predicted peptide from M. verrucaria does not contain α-aminoisobutyric acid, but 11 residues of isovaline. There are no known peptaibols with such a high content of isovaline, so it is likely that some of these predicted iso-valines are rather alpha-aminoisobutyric acids or other residues. To date, over 1,000 peptaibols have been characterized in various members of the order Hypocreales, with the vast majority produced by members of the genus Trichoderma (de la Fuente-Núñez et al. 2013). Our antiSMASH search suggested 2 additional isovaline-containing NRPs in M. verrucaria (NRPS 3 and 4, X?X?L?Q?X and NRPS 15.2, XQ?X?Q?) and 1 in S. broomeana (NRPS 31.1, XXX?X?QX??). Peptaibols have been previously reported in Sphaerothelia toxica (Perlatti et al. 2020), but to our knowledge no peptaibols have been described in the Genus Myrothecium.

In conclusion, we present the complete genome sequences of the fungi M. verrucaria, M. elegans, R. roseolus, and S. broomeana. While our screens of the genomes for genes encoding RiPP precursor proteins of verrucamides, marianamides, broomeamides, and borosins did not yield any hits, we discovered 3 candidate NRP biosynthetic gene clusters that may control verrucamide, marianamide, and broomeanamide biosynthesis, as well as multiple clusters predicted to produce peptaibol-like peptides. These gene clusters will be interesting targets for future research, particularly...
with regard to the antibacterial properties of verrucamides, marianamides, and peptaibols (Zou et al. 2011; de la Fuente-Núñez et al. 2013; Ishiuchi et al. 2020).

Data availability

All relevant data was submitted to ENA with the study accession number PRJEB50709 (secondary accession number ERP135330). The accession numbers of samples, raw reads, and unannotated assemblies are available in Supplementary Table 1. The genome assemblies, predicted gene features and sequences, and antiSMASH annotations are archived on Zenodo with the DOI https://doi.org/10.5281/zenodo.7032226. Supplemental material is available at G3 online.

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Conflicts of interest

None declared.
Table 3. Predicted NRP biosynthetic gene cluster in *M. verrucaria* encoding a putative peptide of the peptaibol class, containing the nonproteinogenic amino acid isovaline (X).

|M. verrucaria NRPS 13.3|
|---|

**Hit region**

| NODE 13_length_1066851_cov_42.619387- Region 3 - NRPS. Location: 807,950–915,464 nt |

**Predicted sequence**

| Trichovirin II 6 b (Trichoderma viride) |
| X PX PX PX P X?? XX?? X X?? X ? |

The peptaibol Trichovirin from *T. viride* is cited as an example (Jaworski et al. 1999). The letters X and Z stand for isovaline and α-aminoisobutyric acid, respectively. Question marks indicate non-specified residues.

**Literature cited**

Bengtsson-Palme J, Ryberg M, Hartmann M, Branco S, Wang Z, Godhe A, De Wit P, Sánchez-García M, Ebersberger I, de Sousa F, et al. Improved software detection and extraction of ITS1 and ITS2 from ribosomal ITS sequences of fungi and other eukaryotes for analysis of environmental sequencing data. Methods Ecol Evol. 2013;4(10):919. doi: 10.1111/2041-210X.12073.

Blin K, Shaw S, Steinke K, Villebro R, Ziemert N, Lee SY, Medema MH, Weber T. AntiSMASH 5.0: updates to the secondary metabolite genome mining pipeline. Nucleic Acids Res. 2019;47(W1): W81–W87. doi: 10.1093/nar/gkx310.

Blin K, Wolf T, Chevrete MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, De Los Santos ELC, Kim HU, Nave M, et al. AntiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. Nucleic Acids Res. 2017;45(W1): W26–W41. doi: 10.1093/nar/gkx319.

Camacho C, Coulouris G, Avagyan V, Ma N, Papadopoulos J, Bealer K, Madden TL. BLAST+: architecture and applications. BMC Bioinformatics. 2009;10(1):9. doi: 10.1186/1471–2105-10-421.

Cho H, Lee H, Hong K, Chung H, Song I, Lee JS, Kim S. Bioinformatic expansion of borosins uncovers trans-acting peptide backbone N-Methyltransferases in bacteria. Biochemistry. 2022;61(3): 183–194. doi: 10.1021/acs.biochem.1c00764.

Ekanayake DI, Perlatti B, Swenson DC, Oldmaa K, Bills GF, Gloer JB. Broomeanamides: cyclic octapeptides from an isolate of the fungicidal ascomycete *Sphaerastilbella broomeana* from India. J Nat Prod. 2021;84(7):2028–2034. doi: 10.1021/acs.jnatprod.1c00414.

2nd ed. Amsterdam: Elsevier Inc; 2013. pp. 150–156. doi: 10.1086/B978-0-12-385095-9.00022-1.

Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. DNA-DNA hybridization values and their relationship to whole-genome sequence similarities. Int J Syst Evol Microbiol. 2007;57(Pt 1):81–91. doi: 10.1099/ijjs.0.04483-0.

Hallen HE, Luo H, Scott-Craig JS, Walton JD. Gene family encoding the major toxins of lethal *Amanita* mushrooms. Proc Natl Acad Sci USA. 2007;104(48):19097–19101. doi: 10.1073/pnas.0707340104.

Imani AS, Lee AR, Vishwanathan N, de Waal F, Freeman MF. Diverse protein architectures and α- N-methylation patterns define split borosin RiPP biosynthetic gene clusters. ACS Chem Biol. 2022; 17(4):908–917. doi: 10.1021/acschembio.1c01002.

Ishiiuchi K, Hirose D, Kondo T, Watanabe K, Terasaka K, Makino T. Mariannamides A and B, new cyclic octapeptides isolated from *Mariannaea elegans* NBRC102301. Bioorg Med Chem Lett. 2020; 30(4):126946. doi: 10.1016/j.bmcl.2019.126946.

Jain C, Rodriguez-R LM, Philippy AM, Konstantinidis KT, Aluru S. High throughput ANI analysis of 90K prokaryotic genomes reveals clear species boundaries. Nat Commun. 2018;9(1):1–8. doi: 10.1038/s41467-018–07641-9.

Jaworski A, Kirschbaum J, Brückner H. Structures of trichovirins II, peptaibol antibiotics from the mold *Trichoderma viride* NRRL 5243. J Peptide Sci. 1999;5(8):341–351. doi: 10.1002/(SICI)1099–1387(199908)5:8<341::AID-PSC204>3.0.CO;2-0.

Kölblaue U, Nilsson HR, Schigel D, Pedersoo L, Larsson KH, May TW, Taylor AFS, Jeppesen TS, Frieslev TG, Lindahl BD, et al. The taxon hypothesis paradigm—on the unambiguous detection and communication of taxa. Microorganisms. 2020;8(12):1910–1924. doi: 10.3390/microorganisms8121910.

Majoros WH, Pertea M, Salzberg SL. TigrScan and GlimmerHMM: two open source ab initio eukaryotic gene-finders. Bioinformatics. 2004;20(16):2878–2879. doi: 10.1093/bioinformatics/bth315.

Matabaro E, Kaspar H, Dahlin P, Bader DLV, Murar CE, Staabli F, Field CM, Bode JW, Küntzer M. Identification, heterologous production and bioactivity of lentimulin A and dendrothelin A, two natural variants of backbone N-methylated peptide macrocycle omphalotin A. Sci Rep. 2021;11(1):1–12. doi: 10.1038/s41598-021–83106-2.

Milanese A, Mende DR, Paoli L, Salazar G, Ruchlewych HJ, Cuenca M, Hingamp P, Alves R, Costea PI, Coelho LP, et al. Microbial abundance, activity and population genomic profiling with mOTUs2. Nat Commun. 2019;10(1):1014. doi: 10.1038/s41467-019–08844-4.

Nilsen RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picarić K, Gockner FO, Tedersoo L, et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 2019;47(D1):D259–D264. doi: 10.1093/nar/gky1022.

Nordberg H, Cantor M, Dusheyko S, Hua S, Poliakov A, Shabalov I, Smirnova T, Grigoriev IV, Dubchak I. The genome portal of the department of energy joint genome institute: 2014 updates. Nucleic Acids Res. 2014;42(D1):26–31. doi: 10.1093/nar/gkt1069.

Nilsson RH, Larsson KH, Taylor AFS, Bengtsson-Palme J, Jeppesen TS, Schigel D, Kennedy P, Picarić K, Gockner FO, Tedersoo L, et al. The UNITE database for molecular identification of fungi: handling dark taxa and parallel taxonomic classifications. Nucleic Acids Res. 2019;47(D1):D259–D264. doi: 10.1093/nar/gky1022.

Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prjibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Smirnova T, Grigoriev IV, Dubchak I. The genome portal of the department of energy joint genome institute: 2014 updates. Nucleic Acids Res. 2014;42(D1):26–31. doi: 10.1093/nar/gkt1069.

Park DM, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from chimeric MDA products. J Comput Biol. 2013;20(10):714–737. doi: 10.1089/cmb.2013.0084.
from isolates, single cells, and metagenomes. Genome Res. 2015; 25(7):1043–1055. doi:10.1101/gr.186072.14.

Perlatti B, Nichols CB, Andrew Alspaugh J, Gloer JB, Bills GF. Sphaerostilbellins, new antimicrobial aminolipopeptide peptabiotics from Sphaerostilbella toxica. Biomolecules. 2020;10(10):1371–1315. doi:10.3390/biom10101371.

Pruesse E, Peplies J, Glöckner FO. SINA: accurate high-throughput multiple sequence alignment of ribosomal RNA genes. Bioinformatics. 2012;28(14):1823–1829. doi:10.1093/bioinformatics/bts252.

Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, Peipples J, Glöckner FO. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(D1):590–596. doi:10.1093/nar/gks1219.

Quijano MR, Zach C, Miller FS, Lee AR, Imani AS, Künzler M, Freeman MF. Distinct autocatalytic α-N-methylating precursors expand the borosin RiPP family of peptide natural products. J Am Chem Soc. 2019;141(24):9637–9644. doi:10.1021/jacs.9b03690.

Ramm S, Krawczyk B, Mühlenweg A, Poch A, Mösker E, Süßmuth RD. A self-sacrificing N-methyltransferase is the precursor of the fungal natural product omphalotin. Angew Chem Int Ed Engl. 2017;56(33):9994–9997. doi:10.1002/anie.201703488.

Simão FA, Waterhouse RM, Ioannidis P, Kriventseva EV, Zdobnov EM. BUSCO: assessing genome assembly and annotation completeness with single-copy orthologs. Bioinformatics. 2015;31(19):3210–3212. doi:10.1093/bioinformatics/btv351.

Van Der Velden NS, Kälin N, Helf MJ, Fiel J, Freeman MF, Künzler M. Autocatalytic backbone N-methylation in a family of ribosomal peptide natural products. Nat Chem Biol. 2017;13(8):833–835. doi:10.1038/nchembio.2393.

Vogt E, Künzler M. Discovery of novel fungal RiPP biosynthetic pathways and their application for the development of peptide therapeutics. Appl Microbiol Biotechnol. 2019;103(14):5567–5581. doi:10.1007/s00253-019-09893-x.

Zou X, Niu S, Ren J, Li E, Liu X, Che Y. Verrucamides A-D, antibacterial cyclopeptides from Myrothecium verrucaria. J Nat Prod. 2011;74(5):1111–1116. doi:10.1021/np200050r.

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