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Short communication

Direct MALDI-TOF/TOF analyses of unnatural beauvericins produced by the endophytic fungus *Fusarium oxysporum* SS46

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ABSTRACT

The best time of production of the cyclohexadepsipeptide beauvericin by the endophytic fungus *Fusarium oxysporum* SS46 in Czapek medium was evaluated. The highest level of beauvericin production was found on day 21 of fermentative culture, as assessed by quantitative analysis by high performance liquid chromatography coupled with a photodiode array detector. Precursor-directed biosynthesis experiments were carried out to produce new analogues of beauvericin by feeding *F. oxysporum* with ten analogues of L-phenylalanine. In order to evaluate which precursor analogues were incorporated by the microorganism, the obtained extracts were analyzed using matrix-assisted laser desorption ionization - time-of-flight mass spectrometry (MALDI-TOF/TOF). The precursor-directed biosynthesis studies led to the biosynthesis of novel beauvericin derivatives by replacement of one, two, or all three L-phenylalanine residues in beauvericin with DL-3-fluorophenylalanine, L-3-fluorophenylalanine, L-4-fluorophenylalanine, or L-tyrosine. Beyond these precursor analogues, one unit of L-4-aminophenylalanine, L-4-chlorophenylalanine, DL-4-bromophenylalanine, or L-4-bromophenylalanine was also incorporated by the endophyte *F. oxysporum* SS46. Units of L-4-nitrophenylalanine and L-histidine were not incorporated by the microorganism to produce unnatural beauvericins.

Introduction

For a long time, natural products have played an important role in drug discovery, since they and their derivatives have been sources of several important therapeutic agents (Koehn and Carter, 2005). Natural products and their derivatives continue to play a significant role in drug discovery (Cragg and Newman, 2013). In order to amplify the chances of

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finding new drugs, strategies are needed to obtain new natural products and their derivatives. There are several approaches to diversify the structures of natural products, one of which is precursor-directed biosynthesis (PDB). PDB is the derivatization of a natural product by feeding biosynthetic precursor analogues to the fermentation broth of the producer organisms (Thiericke and Rohr, 1993). This technique is an efficient and easy method in which addition of biosynthetic precursor analogues to the growth medium allows the production of modified metabolites exclusively by microorganisms and their biosynthetic machinery (Thiericke and Rohr, 1993). This procedure has been successfully applied to the industrial production of antibiotics such as penicillin (Halliday and Arnstein, 1956).

The literature presents some interesting examples of PDB such as the production of derivatives of the antibiotic pacidamycin, which acts specifically against *Pseudomonas aeruginosa* and displays a very narrow spectrum of antibacterial activity. Tryptophan analogues (substituted with chloro, bromo, and methyl groups) were incorporated by *Streptomyces coeruleorubidus*, opening possibilities to obtain new pacidamycins (Grüschow et al., 2009). Cyclohexadepsipeptides can also be produced by PDB experiments. A study was conducted using the fungus *Paecilomyces tenuipes* BCC 1614 in which the culture medium was supplemented with four isomers of isoleucine to produce unnatural derivatives; all three positions in beauvericin were equally replaced with the precursors (Nilanonta et al., 2002). The filamentous fungus *Beauveria bassiana* ATCC 7159 was also used to obtain analogues of beauvericin. Appropriate strategies of feeding analogues of D-2-hydroxyisovalerate and L-phenylalanine led to the biosynthesis of novel compounds, some with improved cytotoxicity (Xu et al., 2007).

Endophytic fungi, microorganisms that live within plant tissues without causing diseases, have been recognized as prolific producers of novel and bioactive secondary metabolites (Borges et al., 2009). The endophytic fungus *Fusarium oxysporum* SS46, isolated from the host plant *Smallanthus sonchifolius* (Gallo et al., 2009), is a producer of the cyclohexadepsipeptide beauvericin (Nascimento et al., 2012). Beauvericin has antibiotic, antifungal, insecticidal, antimycobacterial, antiplasmodial, and nematicidal properties, and also inhibits angiogenesis, cell migration, and cancer cell proliferation (Xu et al., 2007; Zhan et al., 2007; Zhang et al., 2007; Xu et al., 2009; Shimada et al., 2010). Recently, beauvericin displayed promising activity against *Leishmania braziliensis* (Nascimento et al., 2012). Thus, this compound has interesting pharmacological properties that suggest its potential as a candidate for drug development (Tedjiotsop Feudjio et al., 2010). Herein we describe the beauvericin production by *F. oxysporum* SS46, as well as the PDB experiments by feeding amino acid precursor analogues into the cultures that led to the production of twelve beauvericin analogues analyzed by matrix-assisted laser desorption ionization - time-of-flight mass spectrometry (MALDI-TOF/TOF): three beauvericin analogues (**2-4**) previously described in the literature (Xu et al., 2007) and nine new beauvericin analogues (**5-13**).

Material and methods

Reagents

Commercial culture media were used for fungal growth: potato dextrose agar (Acumedia), yeast extract (Fluka), malt extract (Acumedia). Other reagents used were: sucrose (Synth), dextrose (Sigma-Aldrich), triptone (HiMedia Laboratories Pvt. Ltd.), hydrochloric acid (J. T. Baker), $NaNO_3$ (Sigma-Aldrich), K_2HPO_4 (Synth), $MgSO_4.7H_2O$ (Synth), KCl (Synth), $FeSO_4.7H_2O$ (Merck), resazurin and cicloheximide (Actidione®). The amino acid precursor analogues used in PDB experiments were: DL-3-fluorophenylalanine (DL-3-FPA), L-3-fluorophenylalanine (L-3-FPA), L-4-fluorophenylalanine (L-4-FPA), L-4-chlorophenylalanine (L-4-ClPA), L-4-bromophenylalanine (L-4-BrPA), DL-4-bromophenylalanine (DL-4-BrPA), L-4-nitrophenylalanine (L-4-NOPA), L-4-aminophenylalanine (L-4-NHPA), L-tyrosine (L-Tyr), and L-histidine (L-His), all from Sigma-Aldrich. The solvents used were PA grade (hexane, ethyl acetate, methanol, and ethanol) and HPLC grade (acetonitrile and methanol) from Synth, Mallinckrodt, Merck, and J.T. Baker.

Endophytic microorganism and PDB experiments

In the microbial collection of the laboratory of Professor Mônica T. Pupo, the endophytic fungus *Fusarium oxysporum* SS46 was maintained in sterile water according to the Castellani method (Castellani, 1939). *F. oxysporum* SS46 was inoculated onto potato dextrose agar in Petri dishes and incubated at 30°C for 12 days. After this period, three agar plugs (0.5 cm diameter) were cut and inoculated in 10 ml of seed medium (each in 50 ml Falcon flasks, supplemented with 0.5% tryptone, 1.0% dextrose, 0.3% yeast extract, 1.0% malt extract, pH adjusted to 6.2 ± 0.2 with 1M HCl), and the pre-cultures were maintained at 120 rpm and 30°C for 7 days. Later, 20 ml of pre-culture was transferred to 500 ml flasks, each containing 180 ml of Czapek medium (3% sucrose; 0.2% $NaNO_3$; 0.1% K_2HPO_4; 0.05% $MgSO_4.7H_2O$; 0.05% KCl; 0.001% $FeSO_4.7H_2O$, pH adjusted to 6.2 ± 0.2 with 1M HCl), and the cultures were maintained at 120 rpm and 30°C for variable times ranging between 5 and 29 days. A control experiment, one without addition of the fungus, was conducted to obtain extracts from the culture medium. In order to ensure that the concentrations of the precursor analogues used in PDB experiments would be innocuous to the microorganism, the toxicity of the substrates was first evalutated by growing *F. oxysporum* on microdilution plates containing serial dilutions of the precursor analogues (32 mM; 16 mM; 8 mM; 4 mM; 2 mM; 1 mM; 0.5 mM; 0.25 mM; 0.125 mM; 0.0625 mM; 0.03125 mM; and 0.015625 mM). For the PDB experiments, culture conditions were the same as described above, but after the first 24 hours of fermentation, stock solutions of the precursors, prepared in sterile water and adjusted to pH 9 with 0.1M NaOH, were added to the cultures in variable concentrations (0.0625-8 mM). Cultivation was continued for additional 20 days to reach the best time of beauvericin production. Table 1 shows the analogues used and their concentrations in the cultures at the beginning of fungal fermentation.

Extraction and analyses by HPLC and MALDI-TOF/TOF

On days 5, 9, 13, 17, 21, 25, and 29 of culture, two flasks were extracted yielding duplicate fungal extracts. About 200 ml of ethanol were added to each culture flask and the mixture was stirred. After 24 h, the resulting suspension was filtered through filter paper in a Büchner funnel and extracted with ethyl acetate (3 × 400 ml). The organic phases were combined and concentrated under reduced pressure to obtain crude extracts from the culture medium. The resulting mycelia were extracted with 300 ml of methanol for 48 h to yield the crude mycelial extracts. The production of natural beauvericin was analyzed by high performance liquid chromatography coupled with a photodiode array detector (HPLC-PDA), by comparison with a previously isolated beauvericin standard (Nascimento et al., 2012). HPLC analyses were carried out by a Shimadzu® HPLC with LC-6AD pumps, a SCL-10AVP system controller, a diode array detector UV/VIS SPD-M10AVP and RID-10A, SIL-10AF autosampler, and Class VP software. The mass of beauvericin in the crude extracts was calculated from a standard calibration curve (Peak Area=35238*(Concentration)+214360 and R^2=1). Standard solutions of beauvericin were prepared in acetonitrile (ACN) (60, 180, 300, 420, and 540 µg/ml), and injected in triplicate. The analyses were carried out using a CLC-ODS (C-18) column, isocratic mobile phase ACN:H_2O (85:15 v/v), 1.0 ml min^{-1} flow rate and detection at 225 nm. The detection of natural and unnatural beauvericin from PDB experiments was monitored by a MALDI-TOF/TOF instrument (Bruker Daltonics, Bremen, Germany) using the reflectron and positive modes. For external calibration, a mixture of standard peptides was used, and the beauvericin produced was the internal calibrant. Instrument settings were: pulsed ion extraction 120 ns and laser frequency 1000 Hz. The matrix used was 2,5-dihydroxybenzoic acid at 20 mg/ml (in 30% acetonitrile and 70% water with 0.1% trifluoroacetic acid) concentration.

Results and discussion

In order to follow the production of beauvericin, the fermentative cultures were monitored from day 5 through day 29 of culture to determine the time of highest production of beauvericin. HPLC-PDA analyses showed that the highest level of beauvericin production was found on day 21 (Fig. 1), reaching a concentration of 444.5 mg/l in the crude extracts. The establishment of the best time to obtain higher levels of beauvericin was required for further PDB experiments, which were carried out for 21 days. Through serial dilutions of the precursor analogues, it was possible to determine the concentrations of the substrates that were not toxic to *F. oxysporum*. These concentrations are described in Table 1 and were used to carry out the PDB experiments.

To evaluate which precursor analogues were incorporated by *F. oxysporum*, all extracts obtained in the PDB experiments were directly analyzed by MALDI-TOF/TOF. MALDI is a soft ionization technique widely used to analyze proteins, oligosaccharides, oligonucleotides, and polymers (Karas et al., 1985; 1987). However, MALDI has been recently used to analyze compounds of low molecular weight (< 800 Da), but this is still a great challenge (Cohen and Gusev, 2002). The most important advantages of MALDI are the high sensitivity, an unnecessary clean-up process for some samples, and main suppression effects of ionization that are less intense compared with electrospray ionization (Greis et al., 2006).

Analyses by MALDI-TOF/TOF showed that the supplementation of *F. oxysporum* with L-4-BrPA, DL-4-BrPA, L-4-ClPA, and L-4-NHPA (Figs. S11-S13; S14-S16; S17-S20; and S23-S26 in the Supporting Information) afforded compounds **8-10**, with replacement of one L-phenylalanine residue in beauvericin (Table 1). The incorporation of L-3-FPA, DL-3-FPA, and L-4-FPA by the fungus was very efficient

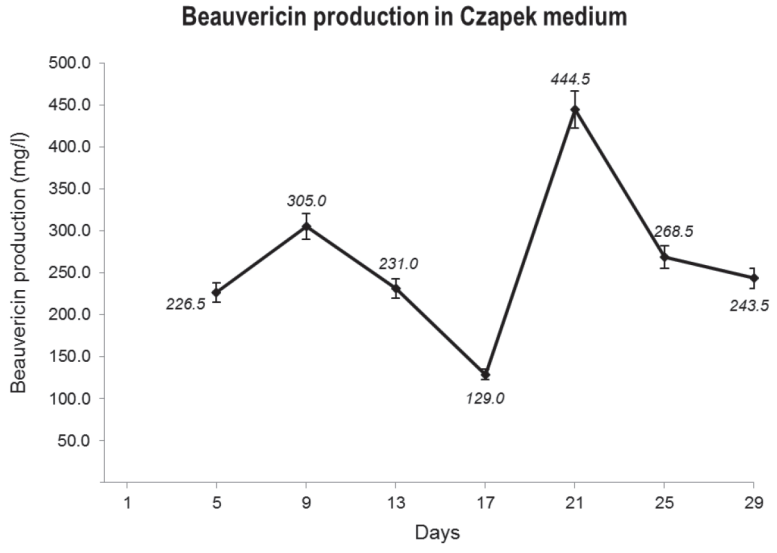


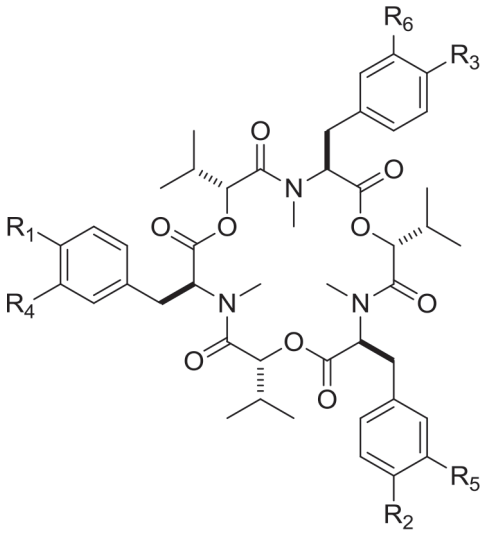
Figure 1 – Yields of beauvericin in crude extracts (days 5-29).

Table 1
Precursor analogues used for PDB experiments and the ions observed at the mass spectra.

Precursors	Conc.[a] (mM)	Ions observed (*m/z*)/error (ppm)	Molecular formula	Products
L-3-FPA-b	1	824.3826 $[M+Na]^+$ / 8.10	$C_{45}H_{56}FN_3O_9$	(1)
		840.3672 $[M+K]^+$ / 4.74		
		842.3726 $[M+Na]^+$ / 8.62	$C_{45}H_{55}F_2N_3O_9$	(3)
		858.3466 $[M+K]^+$ / 8.38		
		860.3636 $[M+Na]^+$ / 7.94	$C_{45}H_{54}F_3N_3O_9$	(4)
		876.3480 $[M+K]^+$ / 4.12		
L-3-FPA-m	1	-	-	
DL-3-FPA-b	1	824.3913 $[M+Na]^+$ / 2.45	$C_{45}H_{56}FN_3O_9$	(2)
		840.3620 $[M+K]^+$ / 1.45		
		842.3783 $[M+Na]^+$ / 1.85	$C_{45}H_{55}F_2N_3O_9$	(3)
		858.3511 $[M+K]^+$ / 3.14		
		860.3657 $[M+Na]^+$ / 5.50	$C_{45}H_{54}F_3N_3O_9$	(4)
		876.3411 $[M+K]^+$ / 3.73		
DL-3-FPA-m	1	-	-	
L-4-FPA-b	0.0625	824.3893 $[M+Na]^+$ / 0.02	$C_{45}H_{56}FN_3O_9$	(5)
		840.3619 $[M+K]^+$ / 1.57		
		842.3743 $[M+Na]^+$ / 6.60	$C_{45}H_{55}F2N_3O_9$	(6)
		858.3486 $[M+K]^+$ / 6.05		
		860.3696 $[M+Na]^+$ / 0.97	$C_{45}H_{54}F_3N_3O_9$	(7)
		876.3449 $[M+K]^+$ / 0.68		
L-4-FPA-m	0.0625	824.3899 $[M+Na]^+$ / 0.75	$C_{45}H_{56}FN_3O_9$	(5)
		842.3748 $[M+Na]^+$ / 6.00	$C_{45}H55F_2N_3O_9$	(6)
		860.3681 $[M+Na]^+$ / 2.71	$C_{45}H_{54}F_3N_3O_9$	(7)
L-4-BrPA-b	0.25	884.3065 $[M+Na]^+$ / 3.57	$C_{45}H_{56}BrN_3O_9$	(8)
		886.3018 $[M+2+Na]^+$ / 2.66		
		900.2779 $[M+K]^+$ / 3.45		
		902.2764 $[M+2+K]^+$ / 4.33		
L-4-BrPA-m	0.25	-	-	
DL-4-BrPA-b	0.25	884.3066 $[M+Na]^+$ / 2.95		(8)
		886.3074 $[M+2+Na]^+$ / 0.13	$C_{45}H_{56}BrN_3O_9$	
		900.2805 $[M+K]^+$ / 2.94		
		902.2797 $[M+2+K]^+$ / 1.91		
DL-4-BrPA-m	0.25	-	-	
L-4-ClPA-b	8	840.3580 $[M+Na]^+$ / 2.06	$C_{45}H_{56}ClN_3O_9$	(9)
		856.3327 $[M+K]^+$ / 1.13		
L-4-ClPA-m	8	840.3576 $[M+Na]^+$ / 2.53	$C_{45}H_{56}ClN_3O_9$	(9)
		856.3391 $[M+K]^+$ / 6.35		
L-4-NOPA-b	8	-	-	
L-4-NOPA-m	8	-	-	
L-4-NHPA-b	8	821.4129 $[M+Na]^+$ / 4.02	$C_{45}H_{58}N_4O_9$	(10)
		837.3875 $[M+K]^+$ / 4.73		
L-4-NHPA-m	8	821.4054 $[M+Na]^+$ / 5.11	$C_{45}H_{58}N_4O_9$	(10)
L-Tyr-b	8	822.3881 $[M+Na]^+$ / 6.71	$C_{45}H_{57}N_3O_{10}$	(11)
		838.3952 $[M+Na]^+$ / 7.99	$C_{45}H_{57}N_3O_{11}$	(12)
		854.3803 $[M+Na]^+$ / 3.68	$C_{45}H_{57}N_3O_{12}$	(13)
L-Tyr-m	8	822.3887 $[M+Na]^+$ / 5.98	$C_{45}H_{57}N_3O_{10}$	(11)
L-His-b	2	-	-	
L-His-m	2	-	-	

[a]Concentrations of precursor analogues at the beginning of fermentations; L-3-FPA (L-3-fluorophenylalanine), DL-3-FPA (DL-3-fluorophenylalanine), L-4-FPA (L-4-fluorophenylalanine), L-4-BrPA (L-4-bromophenylalanine), DL-4-BrPA (DL-4-bromophenylalanine), L-4-ClPA (L-4-chlorophenylalanine), L-4-NOPA (L-4-nitrophenylalanine), L-4-NHPA (L-4-aminophenylalanine), L-Tyr (L-tyrosine), L-His (L-histidine); b-extracts from the liquid broth; m-extracts from the mycelia.

and yielded compounds **2-7**, showing that these precursors were recognized by fungal enzymes replacing one, two, or all three L-phenylalanine residues in beauvericin. The supplementation with L-Tyr also led to replacement of all three L-phenylalanine residues in beauvericin, producing compounds **11-13**.



(1) R_1= R_2= R_3= R_4= R_5= R_6= H
(2) R_1= R_2= R_3= H, R_4= F, R_5= R_6= H
(3) R_1= R_2= R_3= H, R_4= R_5= F, R_6= H
(4) R_1= R_2= R_3= H, R_4= R_5= R_6= F
(5) R_1= F, R_2= R_3= R_4= R_5= R_6= H
(6) R_1= R_2= F, R_3= R_4= R_5= R_6= H
(7) R_1= R_2= R_3= F, R_4= R_5= R_6= H
(8) R_1= Br, R_2= R_3= R_4= R_5= R_6= H
(9) R_1= Cl, R_2= R_3= R_4= R_5= R_6= H
(10) R_1= NH_2, R_2= R_3= R_4= R_5= R_6= H
(11) R_1= OH, R_2= R_3= R_4= R_5= R_6= H
(12) R_1= R_2= OH, R_3= R_4= R_5= R_6= H
(13) R_1= R_2= R_3= OH, R_4= R_5= R_6= H

Beauvericins are biosynthesized in a process of stepwise condensations by nonribosomal peptide synthetases (NRPS) through the continuous use of their modules (Xu et al., 2008). It seems that NRPS of *F. oxysporum* tolerate a variety of substitutions on the benzene ring of phenylalanine, especially fluorine analogues, as molecular recognition is probably due to the hydrogen and fluorine bioisosterism. Moreover, the presence of sodium and potassium adduct ions was notable, since the chemical scaffold of beauvericin is able to form complexes with different metal ions instead of proton interactions (Lopes et al., 2002).

The incorporation of the precursor analogues DL-3-FPA, L-3-FPA, L-4-BrPA, and DL-4-BrPA into beauvericin was found only in the extracts of culture supernatants, while L-4-FPA, L-4-ClPA, L-4-NHPA, and L-Tyr were present in both liquid broth and mycelia extracts. The analogues L-4-nitrophenylalanine (L-4-NOPA) and L-histidine (L-His) were not incorporated by the microorganism, probably due to a defective enzymatic recognition of these precursors by the NRPS. Therefore, our data support that the NRPS modules were able to replace the three L-phenylalanine residues in the beauvericin scaffold with the unnatural precursors L-4FPA, DL-3-FPA, L-3-FPA, and L-Tyr.

In conclusion, we describe beauvericin production by *F. oxysporum* SS46 and PDB experiments, in which amino acid precursor analogues were added to cultures of *F. oxysporum*, generating twelve beauvericin analogs: three known beauvericin analogues (**2-4**) previously produced by the fungus *Beauveria bassiana* (Xu et al., 2007) and nine new unnatural beauvericin analogues (**5-13**), all of which were analyzed by MALDI-TOF/TOF. Therefore, precursor-directed biosynthesis strategy could provide an opportunity to rationally design a broad variety of new molecules and to improve their pharmacological properties with lower toxicity and higher therapeutic effects.

Authors' contributions

MVT (undergraduate student) contributed in running the laboratory work, analyzing the data and drafting the paper. AAL contributed in running the laboratory work and analyzing the data. DBS and NPL were responsible for MALDI-TOF/TOF analyses and contributed in analyzing the data. MTP supervised the laboratory work, analyzed data and contributed to critical reading of the manuscript.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

Borges, W.S., Borges, K.B., Bonato, P.S., Said, S., Pupo, M.T., 2009. Endophytic fungi: natural products, enzymes and biotransformation reactions. Curr. Org. Chem. 13, 1137-1163.

Castellani, A., 1939. Viability of some pathogenic fungi in distilled water. J. Trop. Med. Hyg. 42, 225-226.

Cohen, L.H., Gusev, A.I., 2002. Small molecule analysis by MALDI mass spectrometry. Anal. Bioanal. Chem. 373, 571-586.

Cragg, G.M., Newman, D.J., 2013. Natural products: a continuing source of novel drug leads. Biochim. Biophys. Acta. 1830, 3670-3695.

Gallo, M.B.C., Chagas, F.O., Almeida, M.O., Macedo, C.C., Cavalcanti, B.C., Barros, F.W.A., Moraes, M.O., Costa-Lotufo, L.V., Pessoa, C., Bastos, J.K., Pupo, M.T., 2009. Endophytic fungi found in association with *Smallanthus sonchifolius* (Asteraceae) as resourceful producers of cytotoxic bioactive natural products. J. Basic Microbiol. 49, 142-151.

Greis, K.D., Zhou, S., Burt, T.M., Carr, A.N., Dolan, E., Easwaran, V., Evdokimov, A., Kawamoto, R., Roesgen, J., Davis, G.F., 2006. MALDI-TOF MS as a label-free approach to rapid inhibitor screening. J. Am. Soc. Mass Spectrom. 17, 815-822.

Grüschow, S., Rackham, E.J., Elkins, B., Newill, P.L.A., Hill, L.M., Goss, R.J.M., 2009. New pacidamycin antibiotics through precursor-directed biosynthesis. ChemBioChem. 10, 355-360.

Halliday, W.J., Arnstein, H.R.V., 1956. The biosynthesis of penicillin. 4. The synthesis of benzylpenicillin by washed mycelium of *Penicillium chrysogenum*. Biochem. J. 64, 380-384.

Karas, M., Bachmann, D., Bahr, U., Hillenkamp, F., 1987. Matrix-assisted ultraviolet laser desorption of non-volatile compounds. Int. J. Mass Spectrom. 78, 53-68.

Karas, M., Bachmann, D., Hillenkamp, F., 1985. Influence of the wavelength in high-irradiance ultraviolet laser desorption mass spectrometry of organic molecules. Anal. Chem. 57, 2935-2939.

Koehn, F.E., Carter, G.T., 2005. The evolving role of natural products in drug discovery. Nat. Rev. Drug Discov. 4, 206-220.

Lopes, N.P., Stark, C.B.W., Gates, P.J., Staunton, J., 2002. Fragmentation studies on monensin A by sequential electrospray mass spectrometry. Analyst 127, 503-506.

Nascimento, A.M., Conti, R., Turatti, I.C.C., Cavalcanti, B.C., Costa-Lotufo, L.V., Pessoa, C., Moraes, M.O., Manfrim, V., Toledo, J.S., Cruz, A.K., Pupo, M.T., 2012. Bioactive extracts and chemical constituents of two endophytic strains of *Fusarium oxysporum*. Rev. Bras. Farmacogn. 22, 1276-1281.

Nilanonta, C., Isaka, M., Kittakoop, P., Trakulnaleamsai, S., Tanticharoen, M., Thebtaranonth, Y., 2002. Precursor-directed biosynthesis of beauvericin analogs by the insect pathogenic fungus *Paecilomyces tenuipes* BCC 1614. Tetrahedron 58, 3355-3360.

Shimada, A., Fujioka, S., Koshino, H., Kimura, Y., 2010. Nematicidal activity of beauvericin produced by the fungus *Fusarium bulbicola*. Z. Naturforsch. 65c, 207-210.

Tedjiotsop Feudjio, F., Dornetshuber, R., Lemmens, M., Hoffmann, O., Lemmens-Gruber, R., Berger, W., 2010. Beauvericin and enniatin: emerging toxins and/or remedies? World Mycotoxin J. 3, 415-430.

Thiericke, R., Rohr, J., 1993. Biological variation of microbial metabolites by precursor-directed biosynthesis. Nat. Prod. Rep. 10, 265-289.

Xu, Y., Orozco, R., Wijeratne, E.M.K., Gunatilaka, A.A.L., Stock, S.P., Molnár, I., 2008. Biosynthesis of the cyclooligomer depsipeptide beauvericin, a virulence factor of the entomopathogenic fungus *Beauveria bassiana*. Chem. Biol. 15, 898-907.

Xu, Y., Wijeratne, E.M.K., Espinosa-Artiles, P., Gunatilaka, A.A.L., Molnár, I., 2009. Combinatorial mutasynthesis of scrambled beauvericins, cyclooligomer depsipeptide cell migration inhibitors from *Beauveria bassiana*. ChemBioChem. 10, 345-354.

Xu, Y., Zhan, J., Wijeratne, E.M.K., Burns, A.M., Gunatilaka, A.A.L., Molnár, I., 2007. Cytotoxic and antihaptotactic beauvericin analogues from precursor-directed biosynthesis with the insect pathogen *Beauveria bassiana* ATCC 7159. J. Nat. Prod. 70, 1467-1471.

Zhan, J., Burns, A.M., Liu, M.X., Faeth, S.H., Gunatilaka, A.A.L., 2007. Search for cell motility and angiogenesis inhibitors with potential anticancer activity: beauvericin and other constituents of two endophytic strains of *Fusarium oxysporum*. J. Nat. Prod. 70, 227-232.

Zhang, L., Yan, K., Zhang, Y., Huang, R., Bian, J., Zheng, C., Sun, H., Chen, Z., Sun, N., An, R., Min, F., Zhao, W., Zhuo, Y., You, J., Song, Y., Yu, Z., Liu, Z., Yang, K., Gao, H., Dai, H., Zhang, X., Wang, J., Fu, C., Pei, G., Liu, J., Zhang, S., Goodfellow, M., Jiang, Y., Kuai, J., Zhou, G., Chen, X., 2007. High-throughput synergy screening identifies microbial metabolites as combination agents for the treatment of fungal infections. Proc. Natl. Acad. Sci. USA 104, 4606-4611.