Water-soluble peptides produced in culture by the fungi *Penicillium solitum* IS1-A and *Byssochlamys spectabilis*

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INTRODUCTION

Peptides, modified peptides and peptidic derivatives are typically produced in fungal cultures. Such compounds have attracted much attention in drug discovery and development, because these compounds very often present biological activities, chemical diversity, being of interest in total synthesis, peptide mimetics and suitable pharmacological properties.[1] The investigation of bioactive water-soluble metabolites has been the main focus of our work. [2] Herein we report the isolation of two tetrapeptides and three additional peptide derivatives from aqueous extracts from cultures of two fungal species, *P. solitum* IS1-A and *B. spectabilis*. The absolute configuration of the tetrapeptides IP17 (**1**) and IP16 (**2**) has been established by Marfey's analysis and total synthesis, while the complete configurational assignment of the remaining peptide derivatives **5-7** was possible by Marfey's analysis and electronic circular dichroism analysis.

EXPERIMENTAL SECTION

P. solitum IS1-A and *B. spectabilis* were cultivated and the water-soluble extract was obtained as shown in **Figure 1**. Several chromatographic separations were performed with silica gel derivatized with phenyl-, and and C_{18}, Sephadex G-15 gel permeation chromatography and HPLC purification.

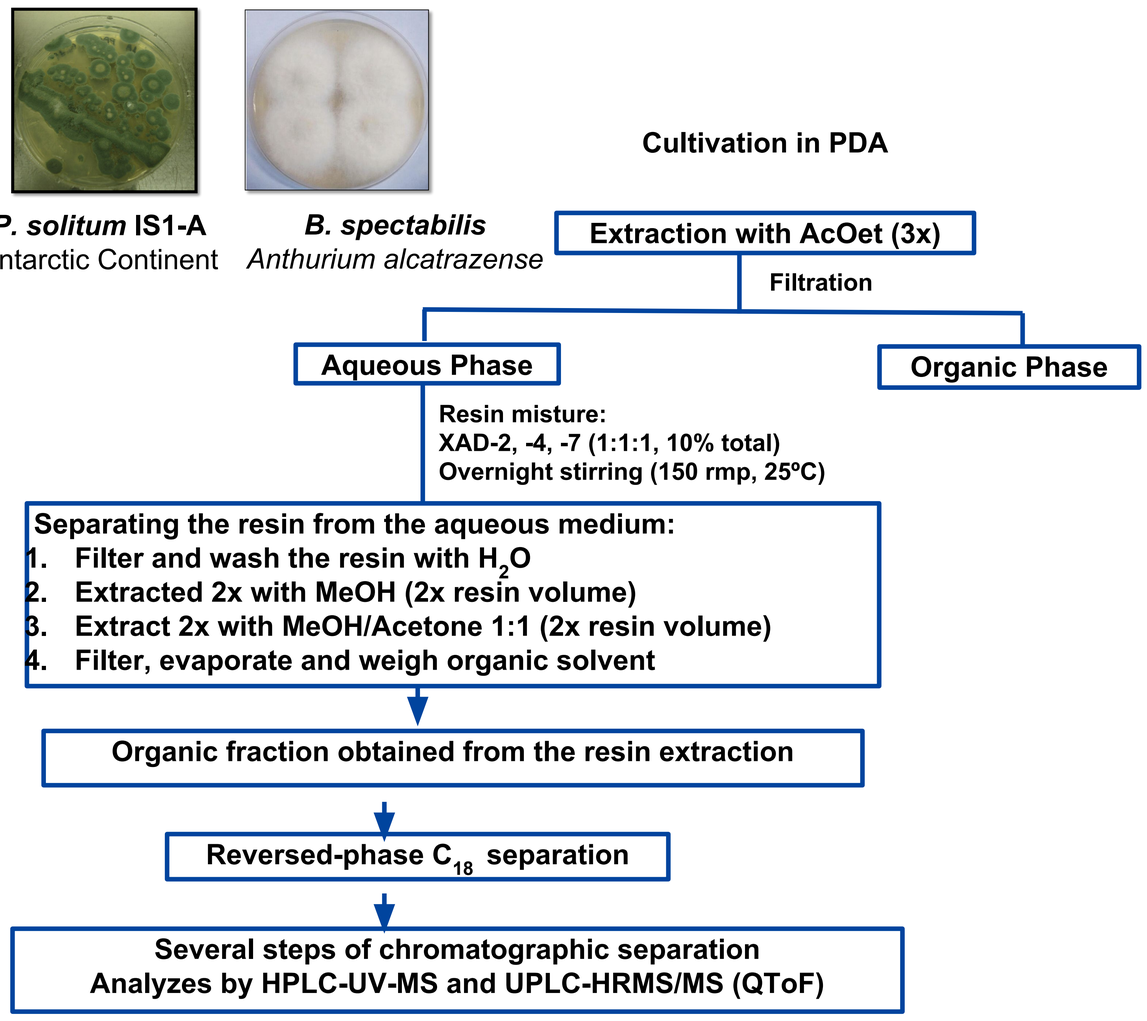

Figure 1. Methodology used for the isolation of hydrophilic metabolites produced by *P. solitum* IS1-A and *B. spectabilis*

RESULTS and DISCUSSION

After several steps of chromatographic purification, the tetrapeptides **IP17 (1)** and **IP16 (2)** were isolated and their structures were determined by NMR spectroscopic data. The absolute stereochemistry was determined by acid hydrolysis and derivatization with Marfey's reagent. Analyses by UPLC-MS revealed the presence of L-Phe and L-Tyr in both peptides. The configuration of the valine residues were not possible to be assigned. The position of the L- and D-valine residues was determined after the total synthesis of the four possible stereoisomers of **(1)** and **(2)**. The anti-fungal activity of natural and synthetic tetrapeptides was conducted against four different phytopathogens *R. solani*, *F. crassistipitatum, F. verticillioides* and *C. gloesporioides*. The antibacterial activity of the tetrapeptides also were investigated against *S. aureus* (ATCC 25923) and *E. coli* (ATCC 25922). However, no biological activity was observed.

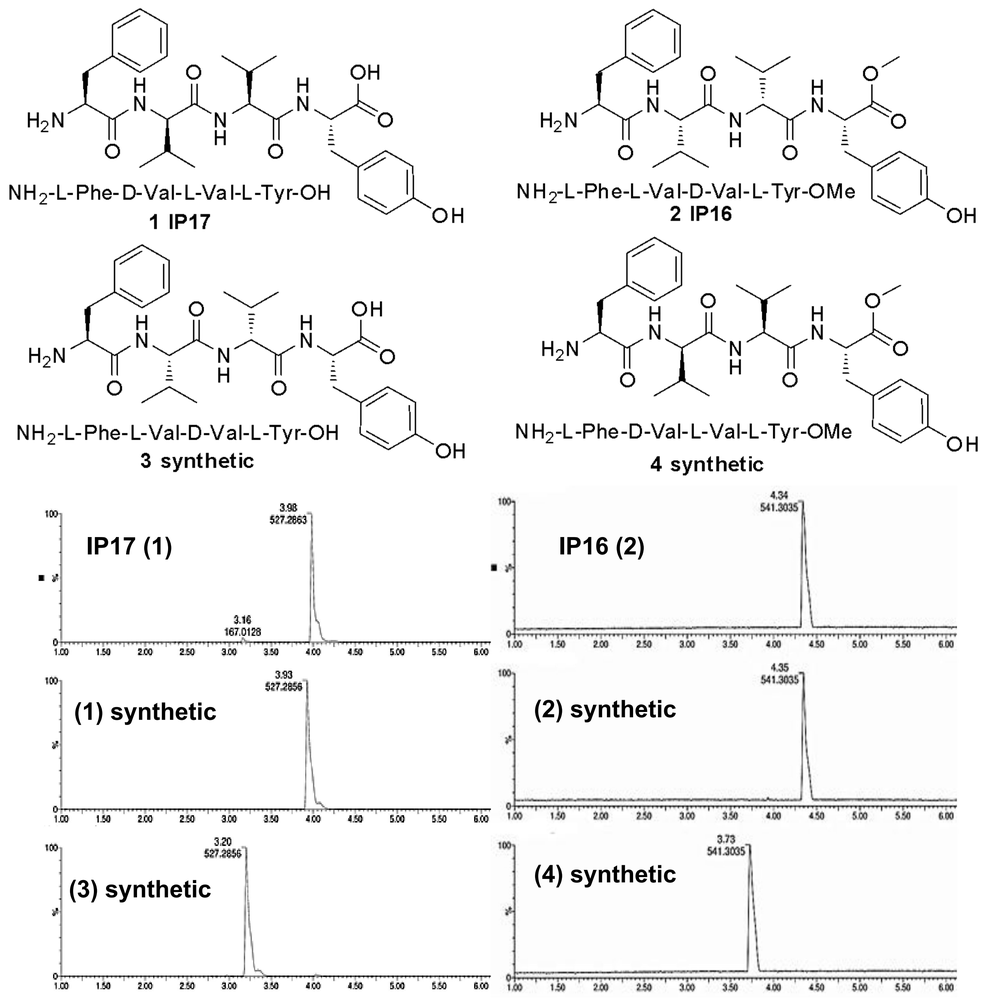

Figure 2. EIC comparison retention time of NP IP17 (1) and IP16 (2) with their respective synthetics stereoisomers

The endophytic strain *B. spectabilis,* isolated from the leaves of *A. alcatrazense*. Produced three peptidic derivatives (**5-7**). The absolute stereochemistry of **5** and **6**, was determined by hydrolysis and Marfey's derivatization. The L-configuration of both tryptophan residues was assigned by electronic circular dichroism analysis. Analyses of ECD of compound **7** is underway. Cytotoxic activity of **5** and **7** was evaluated against OVCAR3 with IC_{50} of 1.12 and 1.07 μM, respectively.

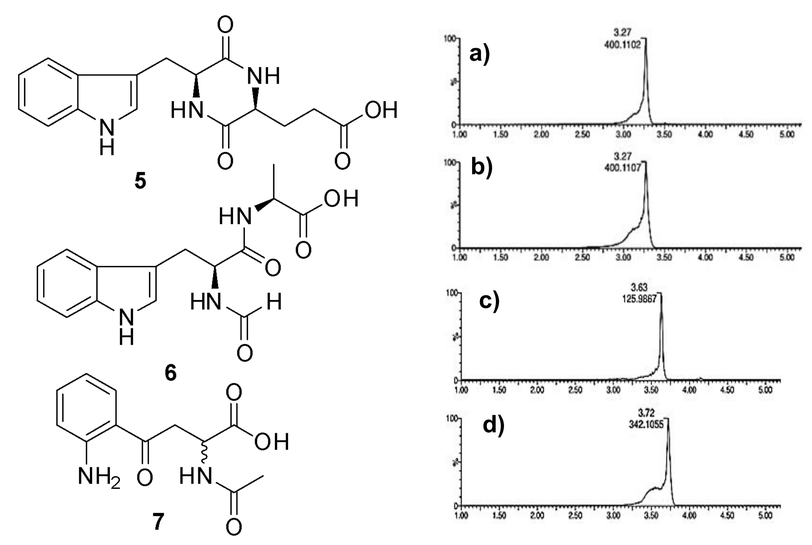

Figure 3. UPLC-MS analysis of L-FDAA derivatives of a) **5**, b) L-glutamic acid and c) **6,** d) L-alanine

CONCLUSION

Investigation of the water-soluble fractions obtained from the growth medium of the fungus *P. solitum* IS1-A and from the endophytic fungus *B. spectabilis* provided four new hydrophilic peptide derivatives (**1**, **2**, **5** and **6**). The absolute configuration was assigned using hydrolysis and derivatization methods with Marfey's reagent and ECD analyses. Both compounds **5** and **7** displayed significant cytotoxic activity against human ovarian cancer cells OVCAR3.

ACKNOWLEDGMENTS

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(2) Rodríguez, J. P. G.; et al. *J. Nat. Prod*. **2020**, *83*, 55-65.