

COMMUNICATION TO THE EDITOR

Verticillin A is likely not produced by *Verticillium* sp.

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Verticillin A is a fungal epipolythiodioxopiperazine (ETP) metabolite with antibiotic properties, which was originally reported to be produced from *Verticillium* sp.¹ It is structurally very similar to Chaetocin from *Chaetomium minutum*, differing only in the position of two hydroxyl groups (Figure 1). Chaetocin is the first described histone methyltransferase inhibitor,² and may therefore influence chromatin remodeling. As many *Verticillium* sp. are known phytopathogens,³ and *Cochliobolus carbonum* has been shown to produce the histone deacetylase inhibitor HC-toxin as a virulence factor,⁴ it is possible that Verticillin A might constitute another fungal means to interfere with the host's chromatin remodeling machinery for enhancing pathogenesis. In fact, two other ETPs, Gliotoxin (from *Aspergillus fumigatus*) and Sirodesmin (from *Leptosphaeria maculans*), have been identified as virulence factors.^{5,6} Thus, a search for *Verticillium* strains producing Verticillin A was initiated to disrupt the genes for toxin production and assess its impact on pathogenicity. Genes involved in ETP biosynthesis are reported to be organized in clusters,⁷ so degenerate primers were designed to amplify genes encoding a aminocyclopropane carboxylic acid synthase, thioredoxin reductase, glutathione S-transferase and methyl transferase from genomic DNA of 39 *Verticillium* strains. No results were obtained from this approach, and also Southern blot analysis of these DNAs with a heterologous thioredoxin reductase probe derived from the corresponding *Leptosphaeria maculans* gene did not result in detection of any signal, except in the positive control. A parallel approach to isolate Verticillin A from the *Verticillium dahliae* strain ST37.01 grown in different media and under different temperatures was also not successful, whereas medium spiked with commercially available Verticillin A (produced from a fungus of the genus *Paecilomyces*; Iris Biotech, Germany) before purification served as

internal positive control in our LC–MS analysis. After the release of the genome sequence of the two *Verticillium* strains *V. dahliae* VdLs17 and *V. albo-altrum* VaMs102 by the Broad Institute, a search for the ETP cluster was performed *in silico*. However, such a gene cluster could not be detected, and hence corroborates the failure to amplify/clone such genes from 39 independent samples described above. This questions the ability of *Verticillium* sp. to produce Verticillin A, because they seem to lack the necessary genes. As the genus *Verticillium* was known to be heterogenous,⁸ it is possible that the fungal strain reported to produce Verticillin A might belong actually to a different genus. Unfortunately we were unable to obtain this original strain to test this hypothesis. Interestingly, Verticillin A has been shown to be produced by *Clonostachys rosea*, formerly known as *Gliocladium roseum*, and this fungus is not only morphologically similar to, but also a mycoparasite of *Verticillium*.^{9,10} Taken

together, it is conceivable that the original *Verticillium* sample was either confused with another fungus or contaminated with *Clonostachys rosea*. Thus, the designation of the isolated ETP as Verticillin A might have been not appropriate.

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Supporting Link: [http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_\(hyaline\)/Gliocladium/](http://www.mycology.adelaide.edu.au/Fungal_Descriptions/Hyphomycetes_(hyaline)/Gliocladium/)

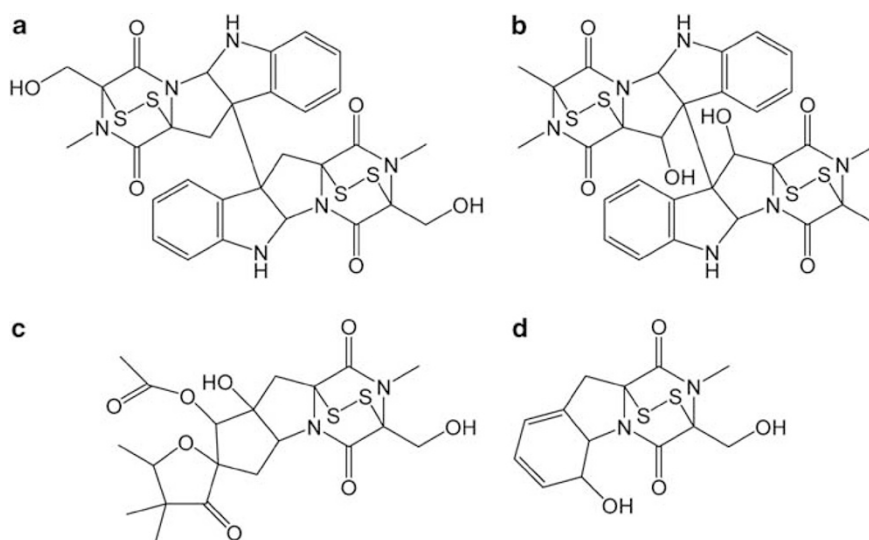


Figure 1 Structural comparison of four epipolythiodioxopiperazine (ETP) secondary metabolites. (a) Chaetocin from *Chaetomium minutum*, (b) Verticillin A from *Gliocladium roseum*, (c) Sirodesmin from *Leptosphaeria maculans* and (d) Gliotoxin from *Aspergillus fumigatus*.

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