



Genome Sequencing of *P. brasilianum* Reveals its Unique Production of Fungic Phenylpropanoids

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The fungus *Penicillium brasilianum* recently had its genome sequenced and the final assembly revealed 252 contigs which underwent gene predictions and annotation. Functional genome analysis revealed 42 putative biosynthetic gene clusters among others, 22 backbone genes, of which 12 were nonribosomal peptide synthetases (NRPSs), 13 polyketide synthases (PKS), 3 terpenes, two NRPS/PKS hybrids, one hybrid terpene/PKS and one NRPS/terpene, indicating the great genetic and enzymatic machinery for secondary metabolites production. Chemical analysis confirmed this great potential of secondary metabolites production with characterization of insecticidal meroterpenes [1], alkaloids [2] and phenylpropanoidic amides, the brasiliamides [3]. The observation of phenylpropanoid biosynthesis putative key genes in *P. brasilianum*'s genome led us to investigate brasiliamides biosynthetic mechanisms. Previous feeding experiments with labelled Phe and LC-MS analysis together with ¹³C NMR indicated the incorporation of two units of Phe on brasiliamide structures. From HRMS analysis of the early days fermentation of *P. brasilianum* we also could confirm the production of all biosynthetic intermediates (cinnamic, p-coumaric, ferulic and sinaptic acids). The first step in the phenylpropanoid pathway was also evaluated through enzymatic bioassays with recombinant PAL obtained from molecular biology studies proving that the enzyme was active towards Phe and the pure recombinant PAL enzyme obtained was characterized by MS and biochemically yielding Km values of 4,8 mM. Finally, knockout studies of *pal* gene were performed through genetic approaches and the mutant Δpal was confirmed by PCR and Southern Blot analysis. The LC-MS analysis of Δpal fermentation indicated decrease in its ability to produce brasiliamides confirming the involvement of *pal* in its production and confirming its phenylpropanoid origin. Interestingly, we found a C4H and 4CL putative genes encoding phenylpropanoid biosynthetic genes very close in *P. brasilianum*'s genome. We evaluated their involvement in brasiliamides biosynthesis through quantitative real time PCR analysis of both WT and Δpal mutant. Analysis indicated great decrease on the expression levels of both genes tested in the Δpal mutant compared to the wild type, indicating that these genes could be involved in brasiliamides production. It is the first time that a phenylpropanoid is genetically proved to be produced by a fungus. Recent studies point out that PAL appears to be involved in defense mechanisms in this fungus, as has been described for plants.

[1] Fill, T. P.; Pereira, G. K.; Santos, R. M. G. & Rodrigues-Filho, E. 2007 "Four additional meroterpenes produced by *Penicillium* sp found in association with *Melia azedarach*: possible biosynthetic intermediates to austin". Z. Naturforsch., 62(b): 1035.

[2] Fill, T. P.; A.; M.; Ferreira, A. G. & Rodrigues-Filho, E. 2013 "Time course production of indole alkaloids by an endophytic strain of *Penicillium brasilianum* cultivated in rice". Nat. Prod. Res., 27(11): 967.

[3] Fill, T. P.; Santos, R. M. G.; Barrison, A.; Rodrigues-Filho, E. & Souza, A. Q. 2009 "Co-production of bisphenylpropanoid amides and meroterpenes by an endophytic *Penicillium brasilianum* found in the root bark of *Melia azedarach*". Z. Naturforsch., 64(c): 355.