Identification of the *Botrytis cinerea* sesquiterpene cyclase involved in botrydial production

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Botrytis cinerea (teleomorph: *Botryotinia fuckeliana*) is the causal agent of grey mould diseases in a broad range of dicotyledonous plants. As a typical necrotroph, its infection strategy includes killing of host cells and feeding on dead tissue; it secretes cell wall degrading enzymes and toxic metabolites, inducing cell death in advance of the invading hyphae. Amongst isolated metabolites, botrydial (**1**), a tricyclic sesquiterpene, and botcinins (before named botcinolides) displayed a high phytotoxic activity. Botrydial (**1**) reproduces the characteristic symptoms of *B. cinerea* infection, and its accumulation *in planta* has been demonstrated during infection process. All these compounds are unspecific phytotoxins which fits well to the broad host range of this pathogen. Although evidence that botrydial is a strain-specific virulence factor, has been reported, no unequivocal proof for an essential role of these toxins in the pathogenicity of *B. cinerea* has been found.

During the screening of calcineurin-dependent (CND) genes of *B. cinerea*, a secondary metabolism gene cluster was identified. Inactivation of one of the clustered genes, *Bcbot1/cnd5* encoding a P450 monooxygenase, demonstrated that it was essential for botrydial synthesis. Here, we present the functional characterisation of the *S1/Cnd15* gene, that is part of the cluster and encodes a putative sesquiterpene cyclase. Study of secondary metabolites produced by the *S1/Cnd15* null mutant, evidenced that the gene *S1/Cnd15* is involved in the first step of botrydial pathway, and correspond to the botryane skeleton cyclase.

Gene inactivation was realised in the B05-10 *ku70* modified strain, that allows a high rate of homologous recombination. The resulting B05-10 *ku70 cnd15⊿* mutant did not show any significant defect in saprophitic growth or virulence. The extracts obtained from fermentation broth of isolates B05-10 *ku70* (reference strain) and mutant strain B05-10 *ku70 cnd15⊿*, were separated by column chromatography and studied by extensive spectroscopic methods, specifically ^{1}H-NMR and ^{13}C-NMR. Botrydial (**1**) and its derivatives **2** and **3** were isolated and its structures characterised from the reference strain, while, from the null mutant, neither botrydial (**1**) nor some botryane derivatives were isolated or detected. Curiously, a high amount of 3-*O*-acetyl botcinic acid (**4**) and botcinin A (**5**) were isolated from the *S1/Cnd15* null mutant.