Characterization of chitinases of *Beauveria bassiana* (Bv062) induced in semisolid-state fermentation

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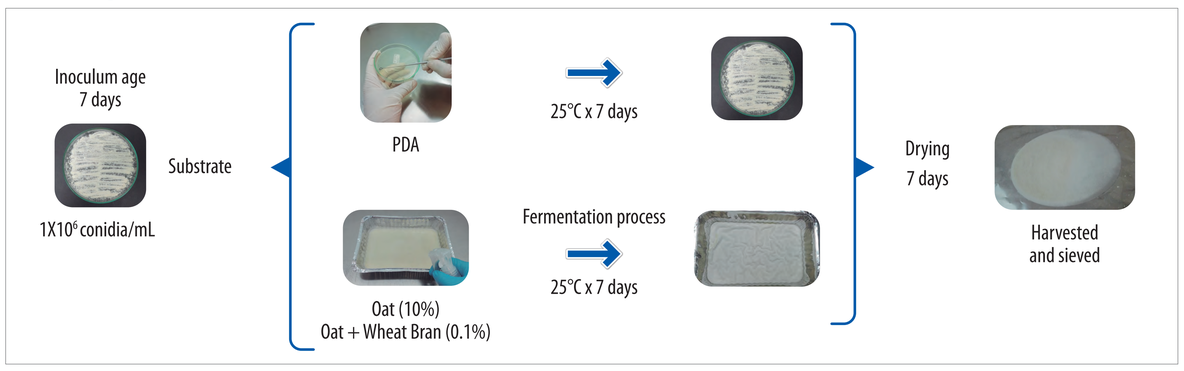
Introduction

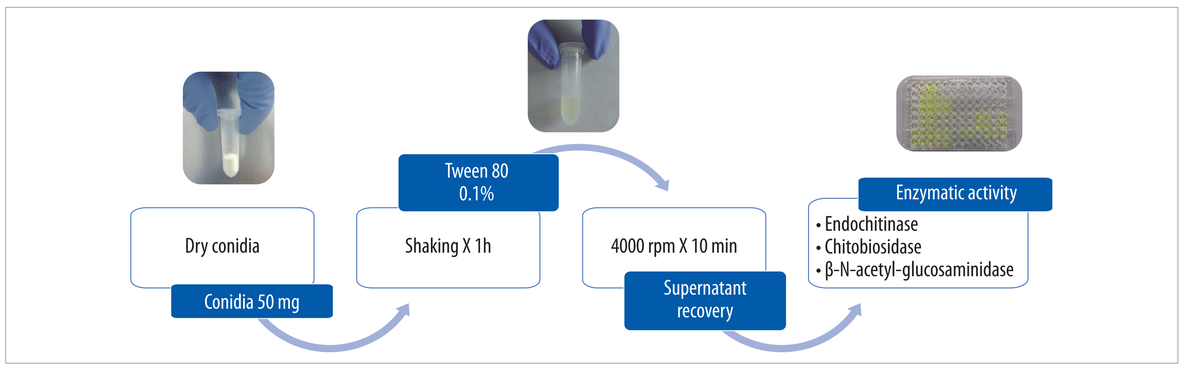
A major consideration in the development of biopesticides based on entomopathogenic fungi has been the virulence and pathogenicity in terms of mean time of mortality and mean lethal concentration against the target insect. Virulence factors as chitinases degrade insect chitin facilitating the hyphal penetration through the cuticle. In this sense, *Beauveria bassiana* chitinases are an important cuticle-degrading group of enzymes implicated in the pathogenesis to insects, with high potential to develop biotechnological tools for in pest control. The aim of this study was to characterize chitinases produced by a Colombian strain of *B. bassiana* (Bv062) isolated from a *Diatraea* sp. larvae naturally infected in a sugar cane crop.

Materials and Methods

Conidia production



Enzymatic activity



Chitinases expression and bioinformatic analysis

In a previous work, the complete genome sequence of Bv062 strain was obtained using the Single Molecule Real-Time (SMRT) Sequencing methodology of Pacific Bioscience company (Eid et al., 2009), where chitinase genes Bb01_00452, Bb01_03008 y Bb01_03026 were identified.

Primer	Sequence (5´- 3´)	Size
Fw_Bb01_03008	ATGGCTCCTTTTCTTCAAACCA	1044 pb
Rev_Bb01_03008	CGCAGTCCCCAAAGTCCCCTTG	
Fw_Bb01_03026	ATGGCAGCATTCAGCCCAG	1269 pb
Rev_Bb01_03026	TTTGCAGGACGACCACCAATCG	

The RNA was purified from conidia produced on PDA agar, oat (10%) and oat with wheat bran (10% and 0.1%, respectively), then analyzed by RT-PCR using primers designed ad hoc in chitinase genes.

The bioinformatic analysis of three chitinases sequences was done using different online software.

Chitinase sequences

Homology	Secretory profile	Characteristics
• P-Blast https://blast.ncbi.nlm.nih.gov • Muscle3.8 https://www.ebi.ac.uk	• SignalP 4.1 server http://www.cbs.dtu.dk • Phobius http://phobius.sbc.su.se/ • Bacello http://gpcr.biocomp.unibo.it	• ProtParam https://web.expasy.org • InterProScan https://www.ebi.ac.uk • ScanProsite https://prosite.expasy.org

Results

A higher sporulation of 2.4 x 10^9 conidia/ g of substrate was obtained when oat supplemented with wheat bran at 0.1% was used as a culture medium compare with 1.5 x 10^9 conidia/ g substrate obtained with oat without additive.

Chitinolytic activity

Conidia showed exoquitinase activity of type b-N acetylglucosamine and quitobiosidase, while the endochitinase activity was very low. The exochitinase activities were increased by the supplemented media (Figure 1).

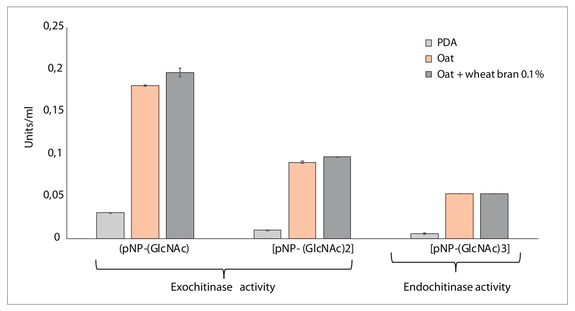


Figure 1. Enzymatic activity of conidia on 4-nitrophenyl-N-acetyl-β-D-glucosaminide (pNP-(GlcNAc), 4-nitrophenyl N,N'-diacetyl β-D–chitobioside [pNP-(GlcNAc)2] and 4-nitrophenyl-β-D-N, N',N''-triacetylchitotriose [pNP-(GlcNAc)3] substrates for determination of NAGase, chitobiosidase and endochitinase activities, respectively.

Bioinformatic analysis

Analysis of chitinase genes of Bv062 shows that Bb01_00452 possesses 8 introns, whereas Bb01_03026 possesses 3 introns (Figure 3). the three studied chitinases belong to family 18 of glycoside hydrolases, where Bb01_00452 and Bb01_03008 are into the subgroup A (related to the development of hyphae and the formation of colonies) and Bb01_03026 is in the subgroup B of fungal chitinases (related with nutritional processes and parasitism) (Li & Greene, 2010; Seidl, 2008; Hartl *et al*., 2012). The three chitinases present a high percentage of identity with those of the strains of *B. bassiana* ARSF2860 and D1-5 (Xiao et al., 2012; Li et al., 2012). Bb01-03008 protein is highly conserved in strains of the same species (99%) and *Metarhizium* (80%). The overexpression of Bbchit increased the virulence of *B. bassiana* on aphids suggesting a possible role in degradation of exogenous chitin (Fang et al., 2005).

Chitinase expression

The RT-PCR showed the expression of chitinases Bb01-03008 and Bb01-03026 with the three production media three media for conidia production (Figure 2). However it is necessary to more deeply study and quantify the enzymatic expression and activity for this strain.

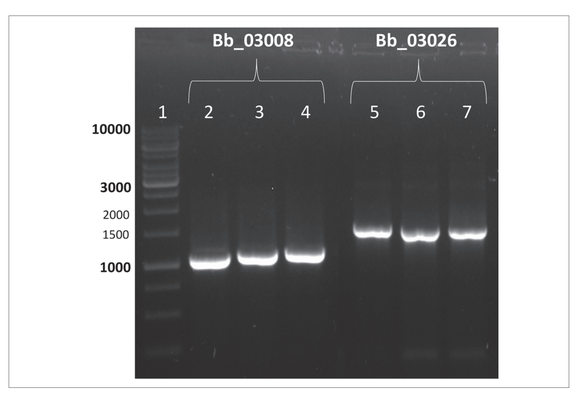


Figure 2. Chitinases expression of conidia produced on PDA agar (lines 2 and 5), oat (lines 3 and 6) and oat supplemented with wheat bran (lines 4 and 7). Line 1: 1Kb DNA Ladder.

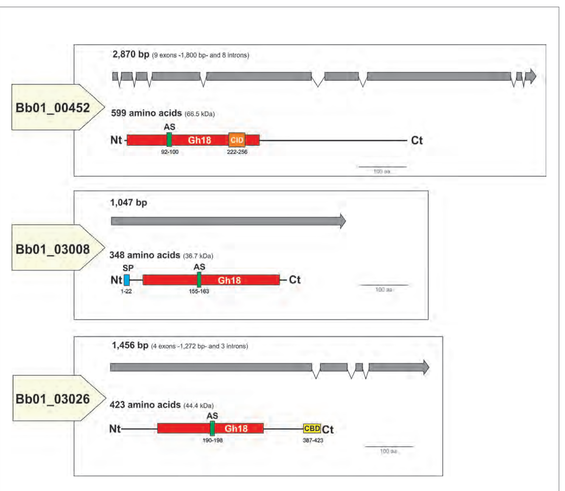


Figure 3. Schematic representation of chitinases genes and corresponding proteins. Signal peptide (S.P), catalytic domain glycoside hydrolase family 18 (GH18), active site (A.S), Chitinase insertion domain (CID), Cellulose binding domain (CBD).

Conclusions

- Composition of culture medium determined chitinase production, which was increased when the fungus grew in a sustrate with higher nitrogen content.
- Chitinases Bb01_03008 and Bb01_3026 are expressed by conidia produced in different media.
- Bioinformatic analysis shows that genes encoding for chitinases Bb01_03008, Bb01_00452 and Bb01_3026 correspond to exo and endochitinases.

References

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