Ga3 subunit Thga3 positively regulates conidiation, mycoparasitism, chitinase activity, and hydrophobicity of *Trichoderma harzianum*

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Abstract

Heterotrimeric G-proteins are key elements of signal transduction pathways, which participate in regulating multiple biological processes in fungi including growth, conidiation, antagonism, and mycoparasitism. Among G protein subunits, Ga3 showed diverse regulatory functions in different fungi. In this study, we cloned a Ga3 subunit coding gene thga3 from T. harzianum Th33 that can antagonize Rhizoctonia solani and some other plant pathogenic fungi. A thga3 deletion strain Δ thga3 was generated using the double-crossover homologous recombination strategy, and R thga3 was generated by transforming thga3-expressing vector into the protoplasts of Δ thga3 by the PEG/CaCl2-mediated method. The biological characteristics of wild-type Th33, Δ thga3 and R thga3 were evaluated. Compared with wild-type Th33, Δ thga3 showed 15%, 94%, and 23% decrease in hyphal growth, conidia yield, and chitinase activity, respectively, and Δ thga3 showed lower antagonistic and mycoparasitism abilities, while there were no significant differences between wild-type Th33 and R thga3. The hyphal surface hydrophobicity of Δ thga3 significantly decreased compared with those of the wild-type Th33 and R thga3. qRT-PCR analysis revealed that transcript abundance of the hydrophobin gene (tha_09745) of Δ thga3 decreased by 80% compared with that of wild-type Th33 and R thga3. The results showed that thga3 positively regulates the growth, conidiation, hydrophobicity, chitinase activities, and mycoparasitism of Th33 towards R. solani. We hence deduced that the expression level of Tha_09745 is correlated to the hyphal hydrophobicity of Th33 and therefore affects the other biological characteristics of Th33. The findings of this report provide a foundation for elucidating the G-protein signal regulatory mechanisms of fungi.

Full Text

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Figures
Figure 1

Construction of thga3 deletion and complemented mutants. (A) Thga3 gene deleting strategy. (B) Thga3 gene complementing vector pKH-KO-Gthga3. (C) PCR and RT-PCR identification of thga3, hyg, and neo genes in Δthga3 and Rthga3. M, 250-bp ladder. PCR templates were genomic DNA and cDNA of wild-type Th33, genomic DNAs of Δthga3 and Rthga3. (D) Southern hybridization analysis. Probe A. Thga3 ORF; Probe B. Hyg gene; Probe C. Neo gene. DNAs of wild-type Th33, Δthga3 and Rthga3 were digested by EcoRI/Xhol.
Figure 2

Growth and conidiation of the T. harzianum wild-type Th33, Δthga3, and Rthga3. (A) Growth of wild-type Th33, Δthga3, and Rthga3 on PDA for 48 h at 25°C. (B) Conidia yield of wild-type Th33, Δthga3, and Rthga3, on PDA for 6 d at 25°C. Error bars represent the SD of three determinations for two independent experiments.

Figure 3
Antagonistic activities of the T. harzianum wild-type Th33, Δthga3, and Rthga3 against R. solani (Rs). (A) Confrontation assay of wild-type Th33, Δthga3, and Rthga3 against Rs. Cultures were grown on PDA plates for 10 d at 25°C. (B) Inhibition ratio of the wild-type Th33, Δthga3, and Rthga3 on the growth of Rs (grown on PDA plates for 6 d at 25°C); error bars represent the SD of five determinations for two independent experiments. (C) Mycoparasitism of wild-type Th33, Δthga3, and Rthga3 against Rs. For wild-type Th33 and Rthga3, the hyphae grew along and coiled the hyphae of Rs; for Δthga3, no coiled growth of the hyphae to the Rs was observed. Bars = 40 μm.

Figure 4

Hydrophobicity and expression of hydrophobin gene Tha_09745 of wild-type T. harzianum Th33, Δthga3, and Rthga3. (A) Hydrophobicity of wild-type T. harzianum Th33, Δthga3, and Rthga3. Fifteen microliters of 0.5% aqueous aniline blue was spotted onto colonies and imaged after 8 h. (B) Relative expression levels of Tha_09745 in wild-type Th33, Δthga3, and Rthga3 grown on PDA plates for 7 d at 25°C (fold-
changes in mRNA expression relative to that of reference gene UCE). Error bars represent the SD of three determinations from two independent experiments.

Figure 5

Chitinase activities of wild-type Th33, Δthga3, and Rthga3 (A) Discoloration of chitinase-inducing medium cultured with wild-type Th33, Δthga3, and Rthga3 at 28°C 5 days later. (B) Diameter of discolored area of chitinase-inducing medium cultured with wild-type Th33, Δthga3, and Rthga3 at 28°C 5 days later. (C) Chitinase activities of the culture filtrates of wild-type Th33, Δthga3, and Rthga3. Error bars represent the SD of three determinations from two independent experiments.

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