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, Gα3 showed diverse regulatory functions in different fungi. In this study, we cloned a Gα3 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.

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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-G*thga3*. (C) PCR and RT-PCR identification of *thga3*, *hyg*, and *neo* genes in $\Delta thga3$ and *Rthga3*. M, 250-bp ladder. PCR templates were genomic DNA and cDNA of wild-type Th33, genomic DNAs of $\Delta thga3$ and *Rthga3*. (D) Southern hybridization analysis. Probe A. *Thga3* ORF; Probe B. *Hyg* gene; ProbeC. *Neo* gene. DNAs of wild-type Th33, $\Delta thga3$ and *Rthga3* were digested by *EcoRI/XhoI*.

Figure 2

Growth and conidiation of the *T. harzianum* wild-type Th33, $\Delta thga3$, and Rthga3. (A) Growth of wild-type Th33, $\Delta thga3$, and Rthga3 on PDA for 48 h at 25°C. (B) Conidia yield of wild-type Th33, $\Delta thga3$, 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, $\Delta thga3$, and *Rthga3* against *R. solani* (Rs). (A) Confrontation assay of wild-type Th33, $\Delta thga3$, and *Rthga3* against Rs. Cultures were grown on PDA plates for 10 d at 25°C. (B) Inhibition ratio of the wild-type Th33, $\Delta thga3$, 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, $\Delta thga3$, and *Rthga3* against Rs. For wild-type Th33 and *Rthga3*, the hyphae grew along and coiled the hyphae of Rs; for $\Delta thga3$, no coiled growth of the hyphae to the Rs was observed. Bars = 40 μ m.

Figure 4

Hydrophobicity and expression of hydrophobin gene *Tha_09745* of wild-type *T. harzianum* Th33, $\Delta thga3$, and *Rthga3*. (A) Hydrophobicity of wild-type *T. harzianum* Th33, $\Delta thga3$, 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, $\Delta thga3$, 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, $\Delta thga3$, and Rthga3 (A) Discoloration of chitinase-inducing medium cultured with wild-type Th33, $\Delta thga3$, and Rthga3 at 28°C 5 days later. (B) Diameter of discolored area of chitinase-inducing medium cultured with wild-type Th33, $\Delta thga3$, and Rthga3 at 28°C 5 days later. (C) Chitinase activities of the culture filtrates of wild-type Th33, $\Delta thga3$, and Rthga3. Error bars represent the SD of three determinations from two independent experiments.

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