

ไคติเนสและการยับยั้งราก่อโรคจากแอคติโนมัยซีทเอนโดไฟต์

Chitinolytic and antifungal activities from endophytic actinomycetes

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บทคัดย่อ

แอคติโนมัยซีทเอนโดไฟต์ 99 สายพันธุ์ ที่แยกได้จากอ้อย สามารถย่อยไคติน (76%) และเส้นใยราได้ (61%) รวมทั้งสามารถยับยั้งการเจริญของรา *Fusarium moniliforme* (71%) และ *Colletotrichum falcatum* (83%) ที่ก่อโรคเหี่ยวเน่าแดงในอ้อย โดยพบสายพันธุ์ที่สามารถยับยั้งราได้มากกว่า 70% และมีหลายสายพันธุ์ที่สามารถยับยั้งการเจริญของราได้ทั้งสองชนิด (71%) เมื่อนำสายพันธุ์ที่สามารถย่อยไคตินในระดับสูง (วงใส ขนาด 0.7–1.2 ซม) 4 สายพันธุ์ มาตรวจหายีน *chi18A* และ *chi19* ด้วยวิธีพีซีอาร์ พบว่าทุกสายพันธุ์มียีนทั้งสองชนิด

ABSTRACT

Ninety-nine strains of endophytic actinomycetes isolated from sugarcane were investigated and found that they could hydrolyze chitin (76%), degrade fungal mycelium (61%) and inhibit *Fusarium moniliforme* (71%) and *Colletotrichum falcatum* (83%), the causative agents of red rot wilt disease in sugarcane. Some strains showed more than 70% inhibition and several strains could inhibit both fungi (71%). When 4 high activity chitinase producing strains (clear zone from 0.7–1.2 cm) were screened for *chi18A* and *chi19* genes by PCR, all of them carried both genes.

คำสำคัญ: ไคติเนส, ยับยั้งรา, แอคติโนมัยซีท, เอนโดไฟต์, อ้อย

Keywords: chitinase, antifungal, actinomycete, endophyte, sugarcane

INTRODUCTION

Endophytes are microorganisms that reside in plants and help plants to enhance growth and protect them from phytopathogens. Endophytic actinomycetes have been reported as promising biocontrol agents because of their property of degradation of fungal cell wall by chitinase (El-Tarabily and Sivasithamparam, 2006). Chitinase is the enzyme that breaks down glycosidic bonds of chitin which is the major component of fungal cell wall. Chitinases are classified into family 18 and 19 of glycosyl hydrolases (Henrissat and Bairoch, 1993). Chitinolytic abilities from actinomycetes have been revealed to play a major role for control plant pathogenic fungi (Joo, 2005).

In this study, chitinase activity was screened from endophytic actinomycetes isolated from sugarcane. Antifungal activity against *Fusarium moniliforme* and *Colletotrichum falcatum*, the causative agents of red rot wilt disease in sugarcane (Jaiswal *et al.*, 1998), was also determined. Genes involved in chitinases were also identified.

MATERIALS AND METHODS

1. Investigation of chitinase activity

Endophytic actinomycetes isolated from sugarcane were obtained from GKU culture collection. They were tested for chitinase activity on colloidal chitin agar (CAA; Nawani *et al.*, 2002) and fungal mycelium fragmented agar (Valois *et al.*, 1996) using *Fusarium moniliforme*. Radiance of clear zone was observed after incubation at 28 °C for 14 days.

2. Antifungal activity of endophytic actinomycetes against pathogenic fungi

Endophytic actinomycetes were cultured on starch casein agar (SCA). *F. moniliforme* and *Colletotrichum falcatum* were inoculated 6 cm away from each of the actinomycete colony. Inhibition zone was observed after incubation at 28 °C for 7 days. Percentage of inhibition was calculated.

3. Identification of chitinase genes

Genomic DNAs of chitinase producing endophytic actinomycetes were prepared according to the protocol described by Kieser *et al.* (2000). Chitinase genes were amplified by PCR using specific primers for *chi18A* (ATT107F, 5'-GACACCTGGGACCAGCCGCTG-3' and ATT107R, 5'-TAGAAGCCGAYGCCGAKSAGCA-3') and *chi19* (ATT092F, 5'-CAGTTCRACCARATGTTCCCG-3') and ATT092R, 5'-CGTTGATSGASC GGATSGTCT-3').

RESULTS AND DISCUSSION

1. Chitinase activity from endophytic actinomycetes

Seventy-five strains from 99 of endophytic actinomycetes showed chitinase activity with various radiance of clear zone from 0.1–1.2 cm on colloidal chitin agar (76%) (Fig. 1a-c) after 14 days of incubation. Amongst these, 11 strains showed high activity level (0.7–1.2 cm). When the strains were tested on mycelium fragmented agar, 61 strains showed clear zone at average radiance about 0.1–1.3 cm (60%) (Fig. 1h-i) with 15 strains high activity level (0.7–1.3 cm).

2. Antifungal activity of endophytic actinomycetes

Seventy strains of 99 endophytic actinomycetes showed antifungal activity against *F. moniliforme* above 5% inhibition (71%) and 6 of the positive strains showed highest activity (more than 70% inhibition). Whereas, 82 strains showed activity against *C. falcatum* above 5% inhibition (83%) and 6 of the positives showed highest activity (more than 70% inhibition). In addition, 70 strains of the endophytes carried antifungal activity against both fungi (71%) (Fig. 1d-g).

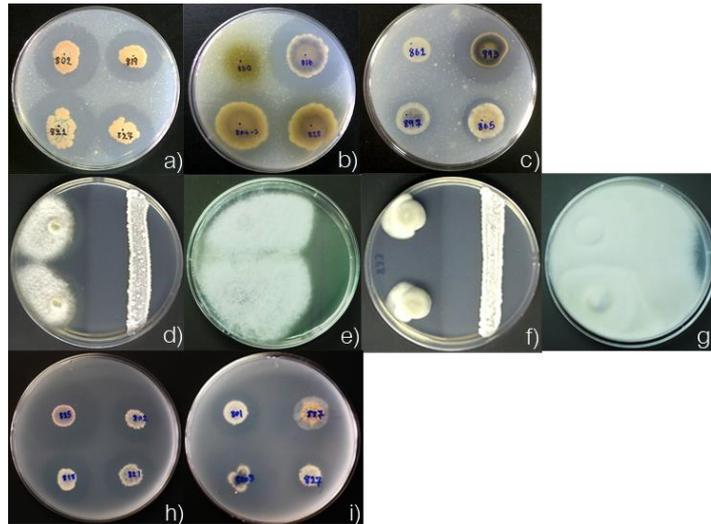


Figure 1 Chitinase activity of endophytic actinomycetes on colloidal chitin agar (a-c). Zones of fungal inhibition against *F. moniliforme* (d), *F. moniliforme* negative control (e), against *C. falcatum* (f) *C. falcatum* negative control (g). Fungal mycelium degradation activity on *F. moniliforme* mycelium fragmented agar (h-i).

3. Chitinase producing endophytic actinomycetes

Endophytic actinomycetes in this study could be divided into three groups. The first group contains strains that carried high chitinase activity. This group could hydrolyzed chitin and degraded fungal mycelium but showed low antifungal activity. The second group contains low chitinase activity but high antifungal activity. The antagonism of this group is likely to be involved with the production of antifungal compounds. The last group contains high chitinase activity and high antifungal activity. The mechanism of this group may implicate in both of the previous mechanisms.

4. Identification of chitinase genes

Genomic DNAs of selected high chitinase producing strains from the first group proposed above namely, *Streptomyces* sp. GKU816, GKU853, GKU893 and a non-streptomycete strain GKU887 were amplified using specific primers for *chi18A* and *chi19* genes. The expected PCR products of 587 bp from *chi18A* and 483 bp for *chi19* were obtained from each strain (Fig. 2). The results were in agreement with previous report that chitinase gene family 18 and 19 were found in *Streptomyces coelicolor* (Kawase et al., 2006).

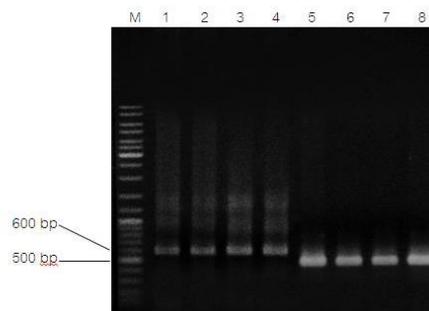


Figure 2 PCR products of *chi18A* and *chi19* genes. Lane M, DNA ladder; Lane 1-4, *chi18A* of GKU816, GKU853, GKU887 and GKU893; lane 5-8, *chi19* of GKU816, GKU853, GKU887 and GKU893.

CONCLUSION

Endophytic actinomycetes isolated from sugarcane revealed a good source for chitinase and antifungal activities against *F. moniliforme* and *C. falcatum*, the causative agents of red rot wilt disease in sugarcane. Further study on application of those strains as biocontrol agents in sugarcane is on the way.

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