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B. Experiment methods

Figures and Tables

Abbreivations

Amp	Ampicillin
APS	Ammonium persulfate
bp	base pair
cDNA	complementary DNA
CpCHI	*Carica papaya* chitinase
DNA	Deoxyribonucleic acid
EDTA	Ethylenediaminetetracetic acid
EtBr	Ethidium bromide
(GlcNAc)	N-acetyl-D-glucosamine
$(GlcNAc)_2$	N-N'-Diacetylchitobiose
$(GlcNAc)_3$	N-N'-N''-Triacetylchitotriose
HCl	Hydrochloric acid
IPTG	Isopropyl thio-β-D-galactoside
kb	Kilobase
kDa	Kilodalton
LB broth	Luria-Bertani broth
NaCl	Sodium chloride
NaOH	Sodium hydroxide
Ni-NTA	Nickel-nitrilotriacetic acid
PAGE	Polyacrylamide gel electrophoresis

PCR	Polymerase chain reaction
PDA	Potato dextrose agar
pI	Isoelectric point
SDS	Sodium dodecyl sulfate
TE buffer	Tris-EDTA buffer
TEMED	N, N, N', N'-tetra-methylethylene diamine
Tris	Tris（hydroxymethyl）aminomethane
UV	Ultraviolet

Abstract

A chitinase cDNA clone (*CpCHI*, 288 bp) was isolated from papaya fruit by substration hybridization. CpCHI encoded a 96-amino-acid protein containing a 28 amino-acid signal peptide in the N-terminal end. The predicted molecular weight of mature protein was 26.2 kDa and its pI value was 6.32. Based on its deduced amino acid sequence homology with other plant chitinases, it was classified as class Ⅳ chitinase.

An active recombinant CpCHI was overexpressed in *Escherichia coli*. The optimal pH, optimal temperature, pH stability and temperature stability of the recombinant CpCHI were pH 6, 30 °C, pH 5.0-9.0 and retained more than 64% activity for 3 week at 30 °C. The hydrolytic product of recombinant CpCHI were $(GlcNAc)_3$, $(GlcNAc)_2$ and (GlcNAc). The recombinant CpCHI showed antifungal activity against *Sclerotium rolfsii*, *Rhizoctonia solani* and *Phytophthora capsici*. Further, various concentration of the recombinant CpCHI was sprayed to the detached leaves of cabbage, bell pepper or tomato, then the agar plugs containing *Sclerotium rolfsii* were placed directly on the center of the leaves. 15 μM recombinant CpCHI was effective in reducing 98% leaf infection by the pathogen. In addition, spore germination of *Alternaria brassicicola* could be completely inhibited by 2 μg mL^{-1} recombinant CpCHI. The recombinant CpCHI did also show 50% antibacterial activity against growth *Escherichia coli* by 2.5 μM recombinant CpCHI.

中文摘要

本實驗利用扣除雜交自木瓜果實選殖到一基因片段，長度為 288 bp，經序列分析發現本片段與幾丁質酶基因序列相似度極高，再根據基因序列設計引子進行聚合酶連鎖反應(Polymerase Chain Reaction, PCR)增幅出全長基因(*CpCHI*)，將此基因構築至表現載體並轉殖到大腸桿菌中表現蛋白質，經蛋白質膠體電泳分析，測得其分子量為 26.2 kDa。另一方面，本基因的胺基酸序列與植物中第四類的幾丁質酶演化程度相近，推測 *CpCHI* 屬於第四類的植物幾丁質酶。

為分析 CpCHI 是否具幾丁質酶活性，將轉殖的大腸桿菌所表現之蛋白質經電泳與活性染色分析，証實經大腸桿菌所表現的 CpCHI 具有幾丁質酶活性。為探討 CpCHI 最適反應條件，以幾丁質為基質進行水解反應，測得其具有廣泛之最適反應 pH 值，pH 5.0-9.0、最適反應溫度為 30 ℃，且於 30 °C 存放三週後仍可維持 64%活性。此外，幾丁質經水解後，測得其水解產物為 N-乙醯幾丁一醣、二醣及三醣。評估 recombinant CpCHI 抗真菌效果方面，於培養基上進行蛋白質與病原真菌對峙試驗，發現 recombinant CpCHI 對 *Sclerotium rolfsii*、*Rhizoctonia solani* 與 *Phytophthora capsici* 具有抗菌活性，進一步將 recombinant CpCHI 分別噴灑於青椒、甘藍、蕃茄葉片上，再接種 *S. rolfsii* 病原真菌菌絲塊，發現 recombinant CpCHI 可抑制 *S. rolfsii* 感染葉片達一週之久。評估 recombinant CpCHI 抑制胞子發芽效果方面，發現 2 μg mL^{-1} recombinant CpCHI 能抑制 98% 的 *Alternaria brassicicola* 胞子發芽。另外在評估 recombinanr CpCHI 抑制細菌生長方面，觀察到 2.5 μM recombinant CpCHI 可有效抑制 50%的大腸桿菌生長。