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Cloning and Analysis of a cDNA Coding a Putative Ribosome-Inactivating Protein from *Cucumis figarei*

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Abstract

A cDNA coding a putative ribosome-inactivating protein (CF-RIP) from *Cucumis figarei* has been cloned and sequenced. The entire cDNA contains an ORF encoding a protein of 286 amino acids which shares conserved residues for ribosome-inactivating proteins (RIPs). CF-RIP shares 54.8-64.5% identity with four RIPs from other cucurbits. RT-PCR analysis indicated that CF-RIP mRNA was expressed in leaf and stem tissues of *C. figarei*.

Many plant species contain ribosome-inactivating proteins (RIPs) (Barbieri and Stirpe, 1982), which are interesting because of their potential usage as therapeutic agents (McGrath *et al.*, 1989; Stirpe *et al.*, 1992). RIPs cleave the *N*-glycosidic bond at adenine 4324 of rat liver 28S rRNA to inactivate the ribosomes, resulting in the inhibition of protein synthesis (Endo *et al.*, 1987; Taylor and Irvin, 1990). RIPs also exhibit antiviral activity against a wide range of plant viruses when applied exogenously to inoculated leaves (Battelli and Stirpe, 1995) and when expressed constitutively in transgenic plants (Lodge *et al.*, 1993; Hong *et al.*, 1996; Wang *et al.*, 1998). We have so far found that *Cucumis figarei*, a wild African cucurbit, is highly resistant to plant virus infection (Saiga *et al.*, 1998). Therefore, we examined the possibility that *C. figarei* contained an RIP homologue in the tissues and obtained a cDNA for it. The putative RIP from *C. figarei* was designated CF-RIP.

To generate a RIP cDNA fragment, two mixed primers were designed for PCR on cDNA sequences for two cucurbit RIPs: α-momorcharin (Ho *et al.*, 1991) from *Momordica charantia* and α-trichosanthin (Chow *et al.*, 1990) from *Trichosanthes kirilowii*. The sense primer, S1 (5'-GCCRTAGAYGTAACNAAYGTNTAYATHATGGG-3'), was derived from the cDNA sequence of 256-287 for α-momorcharin and α-trichosanthin, and the antisense primer, A1 (5'-CCAACTRTTTTCYAAACTTATARTTGC-3'), was derived from the sequence of 619-645 for them. Total RNA was isolated from 0.1 g of *C. figarei* leaves using Total RNA Extraction Kit (Amersham Pharmacia Biotech) and was reverse-transferred to first-strand cDNA with dT(15) primer. RNA isolated from *M. charantia* was used as the control. Using their cDNAs as the template, after preheating (94 ℃, 4 min), 30 cycles of denaturation (94 ℃, 30 sec), annealing (57 ℃, 1 min) and extension (72 ℃ , 30 sec) were carried out to amplify the partial cDNAs by EX Taq polymerase (TAKARA) and GeneAmp® PCR System 9700 (PE Applied Biosystems). The products with an expected size of approximately 390 bp were amplified by PCR and were cloned with TA Cloning Kit (Invitrogen). Their cDNA sequences were then determined using ABI PRISM ® BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (PE Applied Biosystems). The sequence of the fragment for *M. charantia* was identical to nearly half of the cDNA sequence for α-momorcharin and that for *C. figarei* was highly homologous to it.

Then, rapid amplification of cDNA ends (RACE) was used to obtain a full length cDNA for CF-RIP. To execute 5'RACE, three antisense primers were designed for nested PCR based on sequence of the PCR product from *C. figarei*. Firstly, total RNA isolated from *C. figarei* leaves was reverse-transferred to first-strand cDNA with GSP1 (5'-GAAGCTTTTGGTAATTCCCAGAATATGG-3'), and poly dCTP was sequentially extended onto 3' end of its cDNA. Using that as the template, secondly, products were amplified by PCR with dG(15) primer and GSP2 (5'-GGGAGTGTAATACTCTTTGTACCTTG-3'). Using its products as the template, thirdly, product was successfully amplified by PCR with dG (15) primer and GSP3 (5'-GCGTCG-

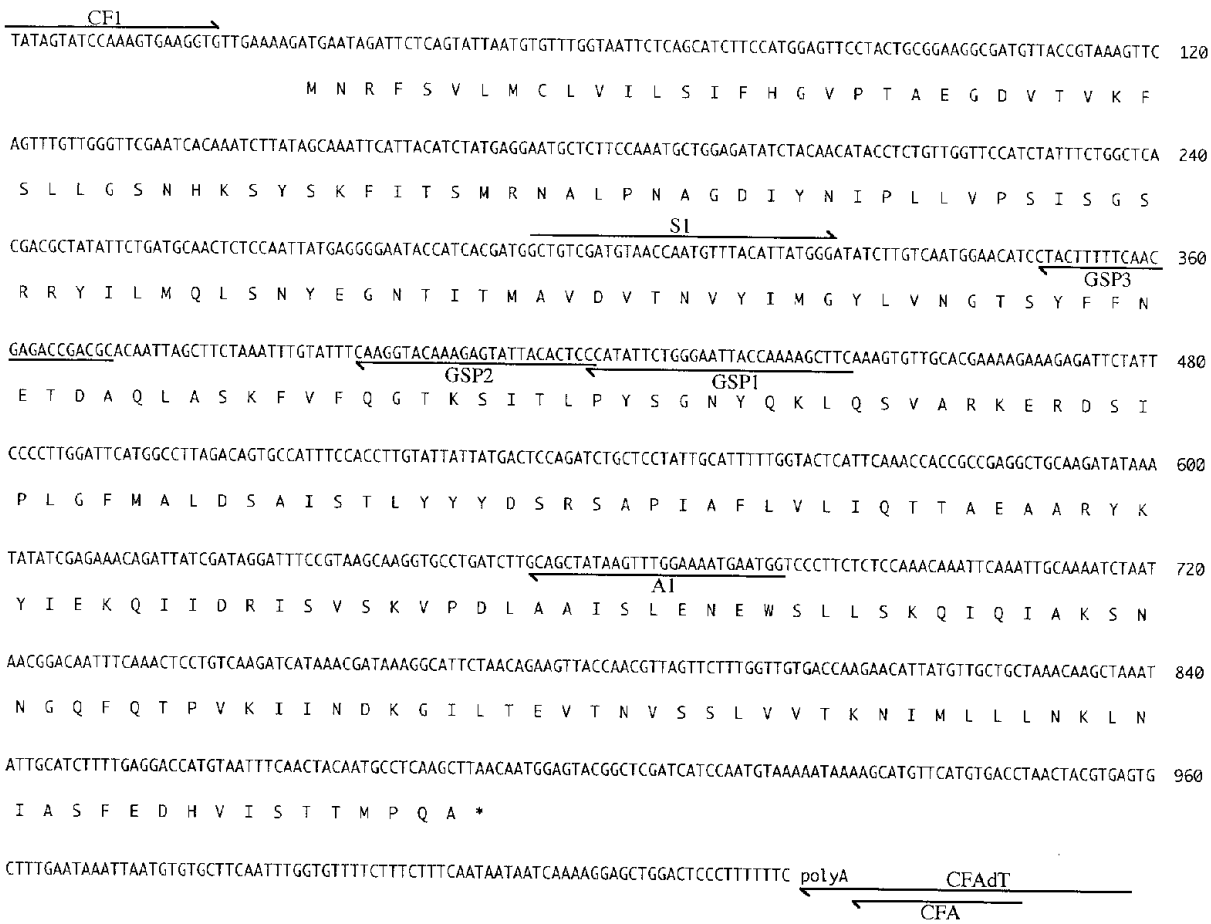

Fig. 1 Nucleotide and deduced amino acid sequence of cDNA for *Cucumis figarei* ribosome-inactivating protein (CF-RIP, accession number AB045560). Sense and antisense primers used for PCR amplification are indicated by arrows.

GTCTCGTTGAAAAAGTAGGATG-3'). The product cloned was sequenced, and the 5' end of the CF-RIP cDNA was determined. To execute 3'RACE, total RNA isolated from *C. figarei* leaves was reverse-transferred to first-strand cDNA with CFAdT (5'-TTCTTGTAGTACTAGATGACACGG-CAGCGACTAGTACTTTTTTTTTTTTT -3') as the anchor primer. Using its cDNA as the template, the full length cDNA was amplified by PCR with CF1 (5'-GTATAGTATCCAAAGTGAAGGTG-3') and with CFA (5'-GACACGGCAGCGACTAG-TAC-3') as the adapter primer. The product was cloned, and the entire sequence for the CF-RIP cDNA was determined.

The CF-RIP cDNA is composed of 1042 nucleotides containing an open reading frame of 286 amino acid residues (**Fig. 1**). The amino acid sequence deduced from cDNA shows high similarity to the four RIPs from cucurbit, α-luffin (Kataoka *et al.*, 1992a) (64.5%), β-luffin (Kataoka *et al.*, 1992b) (62.6%), α-momorcharin (59.4%) and α-trichosanthin (54.8%). Since the deduced protein sequence and the four cucurbit RIPs share many conserved sites (**Fig. 2**), they are considered conspecific proteins. No reported domains with biological activities were detected in the residues.

CF-RIP gene expression in *C. figarei* tissues was verified by reverse transcription-polymerase chain reaction (RT-PCR). Total RNAs isolated from 0.1 g of tissues using Total RNA Extraction Kit (Amersham Pharmacia Biotech) were reverse-transferred to the first-strand cDNAs with CFAdT. Using the cDNAs as the template, CF-RIP gene was amplified by Ampli Taq Gold polymerase (PE Applied Biosystems) with CF1 and CFA, after preheating (94 ℃, 10 min), 45 cycles of denaturation (94 ℃, 30 sec), annealing (58 ℃, 40 sec) and extension (72 ℃, 30 sec). CF-RIP mRNA was detected in leaf and stem tissues of *C. figarei* (**Fig. 3**). Although mechanism of the tissue-specific expression of CF-RIP mRNA remained unclear, it might be related to the protective role of RIPs to pathogens (Battelli and Stirpe, 1995).

From these results we conclude that *C. figarei* produces a putative RIP. CF-RIP may possibly be one of the antiviral factors in *C. figarei* which we have investigated (Saiga *et al.*, 1998). Further studies are needed to recover native CF-RIP from the plant and to analyze its *N*-glycosidase activity (Endo *et al.*, 1987; Stirpe *et al.*, 1992). They will

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CF-RIP          MNRFSVLMCLVILSIFHGVPTAEGDVTVKFSLLGSNHKSYSKFITSMRNALPNAGDIYNI 60
α-luffin        MKRFTVL----ILAI-FVAASTVE-ADVRFSLSGSSSTSYSKFIGDLRKALPSNGTVYNI 54
β-luffin        MNRFTFL-S--LLII-LIAFFTVEGANVSFSLSGADSKSYSKFITALRKALPSKEKVSNI 56
α-momorcharin   MSRFSVL-S--FLILAIFLGGSIVKGDVSFRLSGADPRSYGMFIKDFRNALPFREKVYNI 57
α-trichosanthin MIRFLVL-S--LLILTLFLTTPAVEGDVSFRLSGATSSSYGVFISNLRKALPNERKLYDI 57

CF-RIP          PLLVPSISGSRRYILMQLSNYEGNTITMAVDVTNVYIMGYLVNGTSYFFNETDAQLASKF 120
α-luffin        TLLLSSASGASRYTLMTLSNYDGKAITVAVDVTNVYIMGYLVNSTSYFFNESDAKLASQY 114
β-luffin        PLLLPSASGASRYILMQLSNYDAKAITMAIDVTNVYIMGYLVNSTSYFFNESDAKLASQY 116
α-momorcharin   PLLLPSVSGAGRYLLMHLFNYDGKTITVAVDVTNVYIMGYLADTTSYFFNEPAAELASQY 117
α-trichosanthin PLLRSSLPGSQRYALIHLTNYADETISVAIDVTNVYIMGYRAGDTSYFFNEASATEAAKY 117

CF-RIP          VF-QGTKSITLPYSGNYQKLQSVARKERDSIPLGFMALDSAISTLYYYDSRSAPIAFLVL 179
α-luffin        VFKGST-IVTLPYSGNYEKLQTAAGKIREKIPLGFPALDSAITTLFHYDSTAAAAAFLVI 173
β-luffin        VFKGST-IVTLPYSGNYERLQNAAGKVREKIPLGFRAFDSAITSLFHYDSTAAAGAFLVI 175
α-momorcharin   VFRDARRKITLPYSGNYERLQIAAGKPREKIPIGLPALDSAISTLLHYDSTAAAGALLVL 177
α-trichosanthin VFKDAMRKVTLPYSGNYERLQTAAGKIRENIPLGLPALDSAITTLFYYNANSAASALMVL 177

CF-RIP          IQTTAEAARYKYIEKQIIDRISVSKVPDLAAISLENEWSLLSKQIQIAKSNNGQFQTPVK 239
α-luffin        IQTTAEASRFKYIEGQIIERISKNQVPSLATISLENEWSALSKQIQLAQTNNGTFKTPVV 233
β-luffin        IQTTAEASRFKYIEGQIIERIPKNEVPSPAALSLENEWSALSKQIQLAQTNNGAFRTPVV 235
α-momorcharin   IQTTAEAARFKYIEQQIQERAYRDEVPSLATISLENSWSGLSKQIQLAQGNNGIFRTPIV 237
α-trichosanthin IQSTSEAARYKFIEQQIGKRVDKTFLPSLAIISLENSWSALSKQIQIASTNNGQFETPVV 237

CF-RIP          IINDKGILTEVTNVSSLVVTKNIMLLLNKLNIASFEDHVISTTMPQA------      286
α-luffin        ITDDKGQRVEITNVTSKVVTKNIQLLLN-YKQNVAAFDEDVSAKH--------      277
β-luffin        IIDNKGQRVEIKDVNSKVVTNNIKLLLN--KQNIAAFDDGIPTKH--------      278
α-momorcharin   LVDNKGNRVQITNVTSKVVTSNIQLLLNTRNIAEGDNGDVSTTHGF--SSY--      286
α-trichosanthin LINAQNQRVMITNVDAGVVTSNIALLLNRNNMAAMDD-DVPMTQSFGCGSYAI      289
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Fig. 2 Alignment of the deduced amino acid sequence for CF-RIP, α-luffin (Kataoka *et al.*, 1992a), β-luffin (Kataoka *et al.*, 1992b), α-momorcharin (Ho *et al.*, 1991) and α-trichosanthin (Chow *et al.*, 1990). The alignment was determined using Genetyx-Mac (Software Development). Identical residues are indicated by reversed letters.

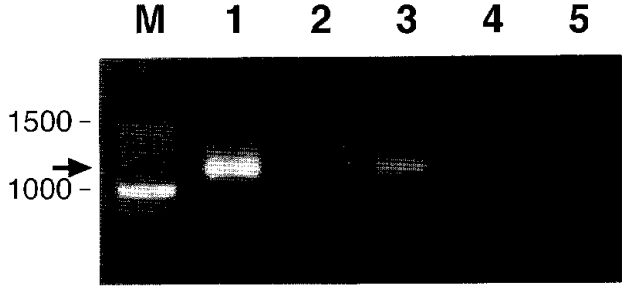

Fig. 3 RT-PCR analysis of CF-RIP mRNA transcript expression in *C. figarei* on agarose gel electrophoresis. Total RNAs from each tissue were subjected to RT-PCR using primers CF1 and CFA. Lane 1, leaf; Lane 2, petiole; Lane 3, stem; Lane 4, root; Lane 5, total RNA (negative control); Lane M, DNA 100 bp ladder; arrow, amplified products with expected size.

contribute better understanding of the antiviral mechanism of RIPs and facilitate development of broad spectrum resistance to disease infection in transgenic plants. Characterization of CF-RIP expressed in *Escherichia coli* is now in progress.

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