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Nucleotide sequence and the action of ribotoxin gene (*sar* gene) of *Penicillium* isolates from Taiwan

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Abstract. Production of ribotoxin from species of *Penicillium* was reported. Among a total of fifty-six strains collected from Taiwan, six strains (*P. resedanum, P. spinulosum, P. daleae, P. digitatum, P. aculeatum,* and *P. chermesinum*) were found to carry ribotoxin gene (*sar* gene), but only four strains (*P. resedanum, P. spinulosum, P. aculeaatum,* and *P. chermesinum*) secreted the ribotoxic protein that cleaves ribosomes. The *sar* gene from the six strains was individually cloned and sequenced. These genes exhibit a typical genomic organization of the fungal-originated ribotoxin gene. The nucleotide and amino acid sequences of these *sar* genes from *Penicillium* are highly conserved and nearly identical. This is the first report that describes the production of ribotoxin by fungal species other than *Aspergillus* spp.

Keywords: *Penicillium*; Ribosome-inactivation; Ribotoxin; rRNA; *sar* gene.

Introduction

Filamentous fungi of *Aspergillus* and *Penicillium* are important in modern medicine and molecular biology, because they produce a wide range of secondary metabolites. Some of the metabolites have antibiotic or toxic properties in plants or animals. *Aspergillus giganteus* carries the *sar* gene that encodes a ribotoxic α-sarcin protein which inhibits protein synthesis of cells. The inhibition results from endonucleolytic cleavage of an RNA domain in 23-28S rRNA (Endo et al., 1983; Wool, 1984). One molecule of α-sarcin could catalyze the cleavage of 5000 molecules of ribosomes. This action has made ribotoxin a potential protein drug in the fight against cancer (Wawrzynczak et al., 1991; Brinkmann and Pastan, 1994; Lin et al., 1994).

Apparently, *Aspergillus* spp. is the only fungal species definitely known to carry the *sar* gene (Lin et al., 1995). Expression of the *sar* gene by several species of *Aspergillus* has been observed (Fernandez-Luna et al., 1985; Lamy and Davies, 1991; Lamy et al., 1991; Huang et al., 1997). The major human pathogenic species of *Aspergillus* is *A. fumigatus*; responsible for a variety of allergenic (Arruda et al., 1990) and invasive diseases (Vanden Bossche et al., 1988). The major cause of aspergillosis is the secretion of a ribotoxic protein by *A. fumigatus* (Lamy et al., 1991). The *sar* gene displays a unique genomic organization, in which an intron is inserted between exons of the signal peptide and the mature ribotoxin gene (Lamy and Davies, 1991; Lamy et al., 1991; Huang et al., 1997). The origin and the need of the *sar* gene in *Aspergillus* have been questioned (Wool, 1984) since the gene is neither an essentially functional nor a house-keeping gene. Furthermore, expression of *sar* gene in cells can stop their growth or cause their death (Miller and Bodley, 1988; Lin et al., 1991; Hwu et al., 2000). This report describes findings of an investigation of possible production of ribotoxin in fungi other than *Aspergillus* spp. Species of *Penicillium* from collections in Taiwan that carried *sar* gene and secreted ribotoxic proteins were identified, and the DNA sequences of the genes were determined and compared.

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Materials and Methods

Fungal Species and Culture Conditions

Species of *Penicillium*, including anamorphs and teleomorphs (Table 1), were obtained from the culture collections of Taiwan (Culture Collection and Research Center, Hsinchiu, Taiwan). All fungi were grown in 500 ml of potato dextrose broth (Difco Lab, Detroit, USA) in one-liter flasks. They were maintained at 30°C, with continuous agitation for 5 days. The mycelium were separated from the culture medium by filtering through Whatman No. 3 filter paper. Total DNA was extracted from the mycelium as follows: mycelium were frozen with liquid nitrogen and ground to a fine powder in a pre-chilled metal Waring blender cup. The powder was first suspended in a high salt containing buffer (1.5 M NaCl, 1.5 M EDTA, 100 mM Tris-HCl, pH 8.1), to which was added a 2% (the final concentration) cetyltrimethylammonium bromide (CTAB), reducing the salt concentration. The reduction

Table 1. A survey on the production of ribotoxins from *Penicellium* spp.

Genus; Subgenus; Species[a]	Immunoreactivity[b]	Western blotting	Ribosomeinactivation	PCR with Primers
Subgenus *ASPERGILLOIDES*				
Penicillium bilaii (CCRC33169)	–			
P. chermesinum (CCRC32572)	+	+	+	+
P. citreonigrum (CCRC32574)	–			
P. glabrum (CCRC32585)	–			
P. lividum (CCRC33164)	–			
P. phoeniceum (CCRC32631)	–			
P. purpurescens (CCRC32570)	–			
P. resedanum (CCRC32037)	+	+	+	+
P. sclerotiorum (CCRC33160)	–			
P. spinulosum (CCRC32565)	+	+/–	+/–	+
P. vinaceum (CCRC33158)	–			
Subgenus *FURCATUM*				
P. canescens (CCRC32050)	+	+	+	+
P. citrinum (CCRC33168)@	–			
P. corylophilum (CCRC33167)	–			
P. daleae (CCRC32392)	+	+/–	+/–	+
P. digitatum (CCRC32571)	+	+	+	+
P. herquei (CCRC32610)	–			
P. janczewskii (CCRC32561)	–			
P. janthinellum (CCRC32644)	–			
P. melinii (CCRC32396)	–			
P. miczynskii (CCRC33163)	–			
P. oxalicum (CCRC4119)	–			
P. paxilli (CCRC33162)	–			
P. rolfsii (CCRC32036)*	–			
P. simplicissimum (CCRC33159)	–			
Subgenus *BIVERTICILLIUM*				
P. aculeatum (CCRC32621)	+	–	–	–
P. erythromellis (CCRC32044)	–			
P. formosanum (CCRC32654)	–			
P. islandicum (CCRC33165)@	–			
P. minioluteum (CCRC32646)	–			
P. pinophilum (CCRC32388)	+	–	–	–
P. purpurogenum (CCRC32601)@	+	–	–	–
P. rugulosum (CCRC33161)	–			
P. variabile (CCRC32651)	–			
P. verruculosum (CCRC32626)	–			
P. vulpinum (CCRC33157)	–			
Subgenus *PENICILLIUM*				
Penicillium aurantiogriseum (CCRC32637)	–			
P. brevicompactum (CCRC32556)	–			
P. chrysogenum (CCRC32635)	–			
P. crustosum (CCRC33166)	–			
P. expansum (CCRC32629)	–			
P. griseofulvum (CCRC32638)	–			
P. hirsutum (CCRC32632)	–			
P. italicum (CCRC32630)	–			
P. olsonii (CCRC32049)	–			
P. ulaiense (CCRC32655)	–			
P. viridicatum (CCRC32022)@	–			
Genus *EUPENICILLIUM*				
Eupenicillium javanicum (CCRC32796)	–			
E. shearii (CCRC32431)	–			
Genus *TALAROMYCES*				
Talaromyces assiutensis (CCRC32401)	–			
T. avellanus (CCRC33170)	–			
T. flavus (CCRC33156)	–			
T. stipitatus (CCRC32411)	–			
T. trachyspermus (CCRC32439)	–			
T. unicus (CCRC32703)	–			
T. wortmanii (CCRC32799)	–			

[a]The number in parentheses is the public depository number from the Culture Collection and Research Center, Hsinchiu, Taiwan.
[b]Tested results of culture medium that reacts to anti-α-sarcin antibody.
Remark: *P. aculeatum* (CCRC32621) fails to produce any products in RCR three times. The PCR product of *P. chermesinum* (CCRC32572) is larger than *α-sar*. The PCR product of is *P. digitatum* (CCRC32571) is smaller than *α-sar*. The protein of *P. canescens* (CCRC32050) produces a smaller sarcin-like protein. *P. rolfsii* (CCRC32036)* reacts positively to anti-tricholin antibody.

in salt concentration resulted in the formation of CTAB/nucleic acid precipitates that were collected by centrifugation (6,000 *g*, 30 min). The pellet was resuspended in CsCl solution A (1 M CsCl, 50 mM Tris-HCl, pH 8, 5 mM EDTA, 50 mM NaCl). The fully resuspended DNA-CsCl solution (3.5-ml) was layered onto a 1.5-ml cushion of CsCl solution B (5.7 M CsCl, 50 mM Tris-HCl, pH 8, 5 mM EDTA, 50 mM NaCl) in an ultracentrifuge tube. After centrifugation at 32,000 *g* for 18 h, DNA fractions were collected from the interfaces and the refractive index was adjusted to 1.399 by adding CsCl solution B for DNA banding. DNA was recovered from equilibrium centrifugation and prepared for cloning.

Preparation of Extra-Cellular Proteins From Fungi and Detection of Immunological Cross Reactivity

The separated filtrate of culture medium was concentrated and dialyzed through a hollow fiber membrane filter (HIP10-20, cut off range of 10,000 daltons, Amicon Inc. MA. USA). The dialyzed filtrate was lyophilized, and its protein concentration was determined by Lowry reaction. The dialyzed filtrate was spotted on nitrocellulose paper (NC paper; Nitro Bind, MSI USA), and the immunological cross reactivity against anti-α-sarcin serum was tested (Lin et al., 1991). Briefly, the spotted NC paper was soaked with 3% (w/v) dried milk powder in phosphate buffer saline (PBS) to block the non-spotted paper area, then incubated with the anti-α-sarcin serum (diluted 1:5000 in PBS) for 1 h, followed by non-radioactive NBT/BCIP chromogen detection (Boehringer Mannheim Co. USA).

Assay of Ribotoxic Ribonuclease Activity

The ribotoxic nuclease activity was examined as follows: 5.7 OD_{260} units of rabbit reticulocyte lysates were treated with the same amounts of dialyzed filtrate in a buffer containing 20 mM Tris-HCl, pH 7.6, and 20 mM EDTA and incubated at 37°C for 15 min. Total RNA was extracted from the reaction mixture by SDS-phenol and analyzed by 1.5% agarose gel electrophoresis in TBE buffer. Ribosomal RNA and α-fragment RNA resultant from ribotoxic action were visualized with ethidium bromide.

Polymerase Chain Reaction Amplification with Genomic DNA

Species that showed positive reactions on immuno dot blotting were selected and their total genomic DNA were prepared for polymerase chain reaction (PCR). Primers used for PCR amplification were derived from the published cDNA sequence of the α-sarcin gene (*sar* gene) (Oka et al., 1990). Two nucleotide sequences, 5'-CCATGGTT GCAATCAAAACCTTGTCCTGG-3' (primer 1), and 5'-GCAAGGAATTCTAATGAGAGCAG-3' (primer 2), were used: primer 1 is a 5' end sequence that matched the amino-terminal sequences of secretory signal peptide; primer 2 is a 3' end sequence, located at the carboxyl-terminal end of the mature α-sarcin and containing a stop codon and non-coding sequence of nucleotides. The PCR amplification was performed according to the following scheme: denaturation at 95°C for 1 min; annealing at 45°C for 1 min; and extension at 72°C for 1 min. After 20 cycles, the amplified products were analyzed by agarose gel electrophoresis, and verified by hybridization with radioactive α-sarcin cDNA (Lin et al., 1995). The sarcin cDNA probe was labeled at the 5' end with [γ^{32}P] ATP using a procedure of T4 polynucleotide kinase (Pharmacia Co.). The hybridization temperature was 50°C.

Results and Discussion

Ribotoxin has been known to be produced only by species of *Aspergillus* (Lin et al., 1995). In this study, six species of *Penicillium* were observed to carry the *sar* gene, and four of them produced ribotoxin that inactivated ribosomes. The initial detection was made by immuno dot

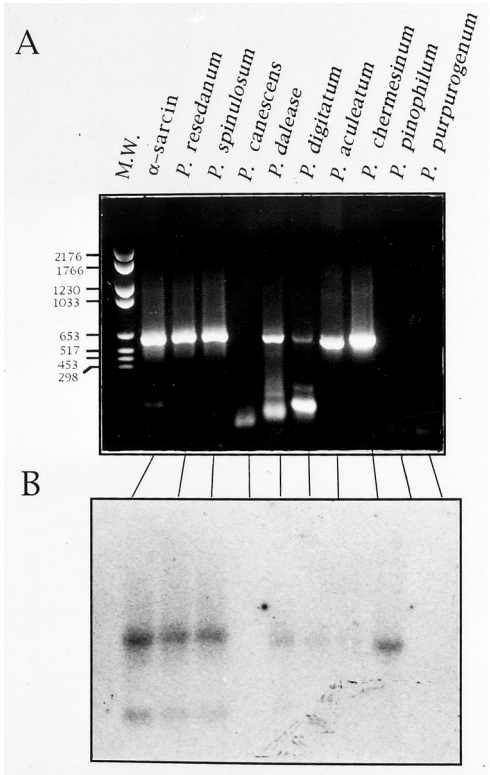

Figure 1. Analyses of polymerase chain reaction (PCR) of *Penicillium* species. The PCR amplification was conducted by using two primers that were described in the text. Total genomic DNA from *P. chermesinum, P. resedanum, P. spinulosum, P. canescens, P. daleae, P. digitatum, P. aculeatum, P. pinophilum, and P. purpurogenum* were subjected to amplification. Panel A gives the PCR products that are analyzed by 1.5% agarose gel electrophoresis in TBE buffer. Panel B contains the results of Southern hybridization with the [^{32}P]labeled α-sarcin cDNA. The molecular weight markers are co-electrophoresed and sizes are indicated; α-sarcin cDNA was an amplified product from the constructed plasmid that contained the α-sarcin gene with intron.

blot screening of fifty-six isolates of *Penicillium* (thirty-six anamorphs and twenty teleomorphs). The immuno dot blot assays (Table 1) indicated that extra-cellular proteins from nine species (*P. chermesinum, P. resedanum, P. spinulosum, P. canescens, P. daleae, P. digitatum, P. aculeatum, P. pinophilum,* and *P. purpurogenum*) reacted with anti-α-sarcin serum. When the Western blot for extra-cellular proteins of these nine species was done, four of them—*P. resedanum, P. spinulosum, P. aculeatum,* and *P. chermesinum*— tested positively to anti-α-sarcin antibody (data not shown).

To resolve the discrepancy between the immuno blots and Western blots, Southern blotting was applied to all fifty-six isolates using radioactive cDNA derived from the *sar* gene of *A. giganteus* as probe (Lin et al., 1995). Instead of finding nine or four positive species, six (*P. chermesinum*, *P. resedanum*, *P. spinulosum*, and *P. aculeatum*, *P. daleae*, and *P. digitatum*) were observed to be hybridized by the *sar*-gene probe (Table 1). These six species, therefore, were genetically cloned and their DNA sequences determined. Prior to cloning the positive genes, the nine species that were positive in immuno-dot blot assay were subjected to PCR amplification. The six species—*P. chermesinum, P. resedanum, P. spinulosum,* and *P. aculeatum, P. daleae,* and *P. digitatum*—generated a comparably-sized PCR fragment that also hybridized with

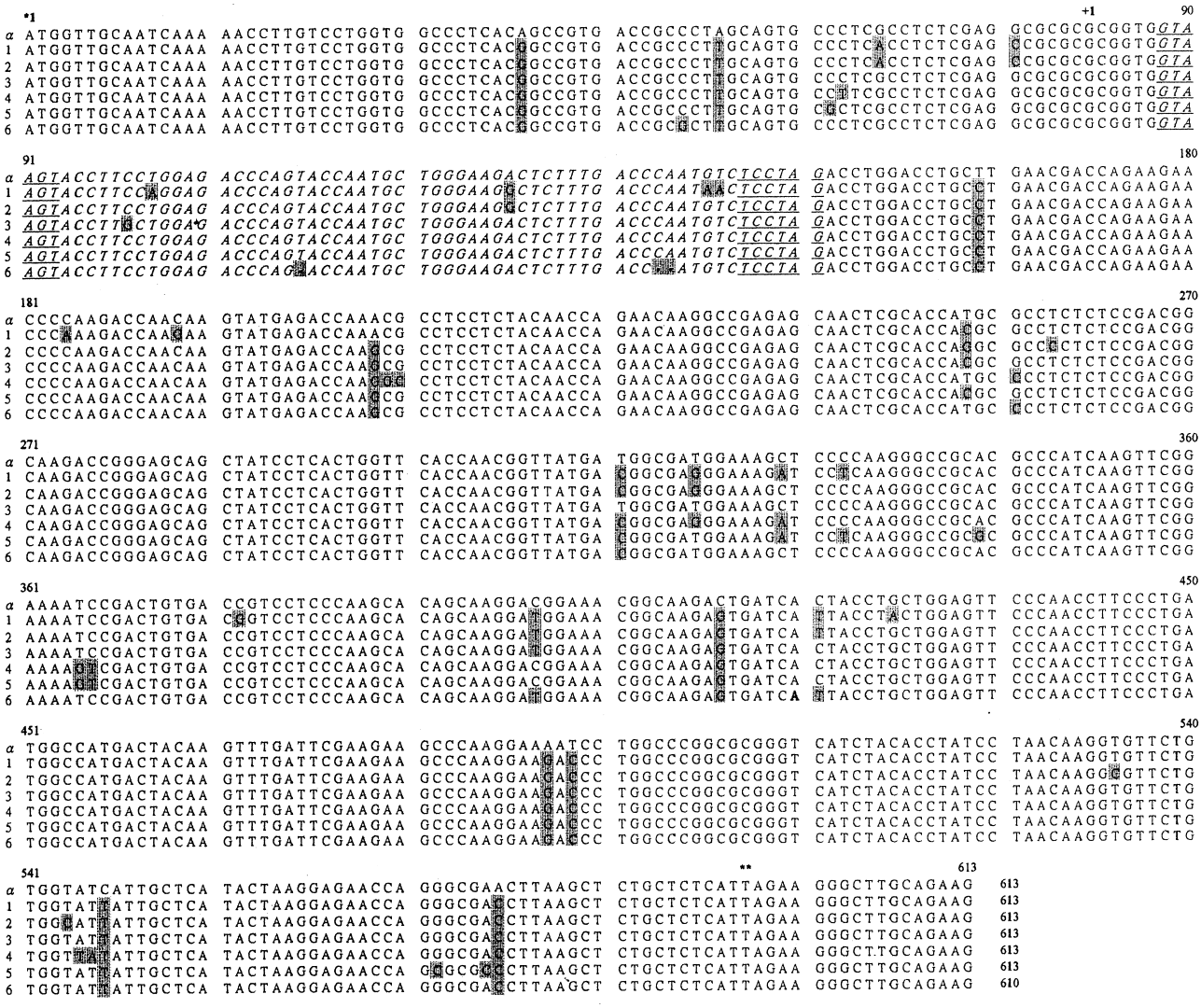

Figure 2. Nucleotide sequences of six ribotoxin genes from *Penecillium* spp. and their comparison to that of α-sarcin of *Aspergillus gigianteus*. The sequences and the genomic organization of ribotoxin gene (*sar* gene) from *P. resedanum* (1), *P. daleae* (2), *P. aculeatum* (3), *P. spinulosum* (4), *P. digitatum* (5), *P. chermesinum* (6), are given, and their GenBank accession Numbers for each species are AF012812, AF012814, AF012816, AF012813, AF012815, and AF012817, respectively. These sequences were compared with the sequence of α-sarcin from *Aspergillus gigianteus* (a). The alignments are given and only the non-identical bases are in shaded boxes. These genes were cloned from their genomic DNA by PCR amplification using two primers (see text) that complement with both ends (underlined). The intron (shown in italics) and the suggested splicing signals (italic and underlined) are putatively based on the sequence of α-sarcin. The first amino acid of signal sequence and the mature protein elucidated are marked with *1 and +1, respectively. The last coding nucleotide is marked with two asterisks.

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                                          +1                                   +30
α  MVAIKNLVLVALTAV  TALAVPSPLEARAVT  WTCLNDQKNPKTNKY  ETKRLLYNQNKAESN
1  MVAIKNLVLVALTAV  TALAVPSPLEPRAVT  WTCLNDQKNPKTKKY  ETKRLLYNQNKAESN
2  MVAIKNLVLVALTAV  TALAVPSPLEPRAVT  WTCLNDQKNPKTNKY  ETKRLLYNQNKAESN
3  MVAIKNLVLVALTAV  TALAVPSPLEARAVT  WTCLNDQKNPKTNKY  ETKRLLYNQNKAESN
4  MVAIKNLVLVALTAV  TALAVPSPLEARAVT  WTCLNDQKNPKTNKY  ETKALLYNQNKAESN
5  MVAIKNLVLVALTAV  TALAVPSPLEARAVT  WTCLNDQKNPKTNKY  ETKRLLYNQNKAESN
6  MVAIKNLVLVALTAV  TALAVRSPLEARAVT  WTCLNDQKNPKTNKY  ETKRLLYNQNKAESN

                                +60                                    +90
α  SHHAPLSDGKTGSSY  PHWFTNGYDGDGKLP  KGRTPIKFGKSDCDR  PPKHSKDGNGKTDHY
1  SHHAPLSDGKTGSSY  PHWFTNGYDGEGKIL  KGRTPIKFGKSDCDG  PPKHSKDGNGKSDHY
2  SHQAPLSDGKTGSSY  PHWFTNGYDGEGKLP  KGRTPIKFGKSDCDR  PPKHSKDGNGKSDHY
3  SHHAPLSDGKTGSSY  PHWFTNGYDGDGKLP  KGRTPIKFGKSDCDR  PPKHSKDGNGKSDHY
4  SHHAPLSDGKTGSSY  PHWFTNGYDGEGKIP  KGRTPIKFGKVDCDR  PPKHSKDGNGKSDHY
5  SHHAPLSDGKTGSSY  PHWFTNGYDGDGKLP  KGRTPIKFGKSDCDR  PPKHSKDGNGKSDHY
6  SHHAPLSDGKTGSSY  PHWFTNGYDGDGKIL  KGRAPIKFGKVDCDR  PPKHSKDGNGKSDHY

                               +120                                   +150
α  LLEFPTFPDGHDYKF  DSKKPKENPGPARVI  YTYPNKVFCGIIAHT  KENQGELKLCSH
1  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKVFCGIIAHT  KENQGDLKLCSH
2  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKAFCGIIAHT  KENQGDLKLCSH
3  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKVFCGIIAHT  KENQGDLKLCSH
4  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKVFCGYIAHT  KENQGDLKLCSH
5  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKVFCGIIAHT  KENQGDLKLCSH
6  LLEFPTFPDGHDYKF  DSKKPKEDPGPARVI  YTYPNKAFCGIIAHT  KENQRALKLCSH
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Figure 3. The comparison of the deduced amino acid sequences of six ribotoxin proteins with that of α-sarcin. Protein sequences of *A. gigianteus* (a), *P. resedanum* (1), *P. daleae* (2), *P. aculeatum* (3), *P. spinulosum* (4), *P. digitatum* (5), and *P. chermesinum* (6) were aligned. The amino acid sequences of six ribotoxin proteins were deduced from their DNA sequence (Figure 2). The differing amino acids are shaded. The signal peptides are underlined. The first amino acid of the mature protein is marked with +1.

the α-sarcin cDNA probe (Figure 1). A lower degree of hybridization was observed in *P. aculeatum, P. daleae,* and *P. digitatum,* even through equal amounts of DNA were applied. *Penicillium canescens* and *P. pinophilum*, and *P. purpurogenum* failed to produce PCR-DNA fragments or to hybridize with α-sarcin cDNA (Figure 1).

The DNA that encoded for the *sar* gene of six positive strains of *Penicillium* were successfully cloned into pGEM (T) plasmid, and sequenced. The sequences of the six DNA were nearly identical (Figure 2), i.e. about 99% identity, indicating that the *sar* gene shared the same genomic organization for the signal peptide exon-intron-ribotoxin exon arrangement (Figure 2). This finding concurs with the previous suggestion for fungal ribotoxin (Lamy et al., 1991; Lin et al., 1995; Huang et al., 1997). The DNA and deduced amino acid sequences were compared with that of the α-sarcin (Oka et al., 1990), and a score of 93% identity was obtained. Ten to seventeen of 595 total nucleotides (plus or minus five nucleotides) were different (Figure 2). The deduced amino acid sequences also showed a 94% similarity to that of α-sarcin (Figure 3). All differences involved a conservative substitution. The Asn^{116} residue of α-sarcin was irreversibly replaced by asparatic acid in all *sar* gene isolates of *Penicillium* spp.

The potential expression of a ribotoxic protein from species carrying the *sar*-gene was further examined by the ribosome-inactivation assay using the rabbit reticulacyte lysate. The assay is sensitive and efficient. It detects the cytotoxic action of ribotoxin, which specifically cleaves

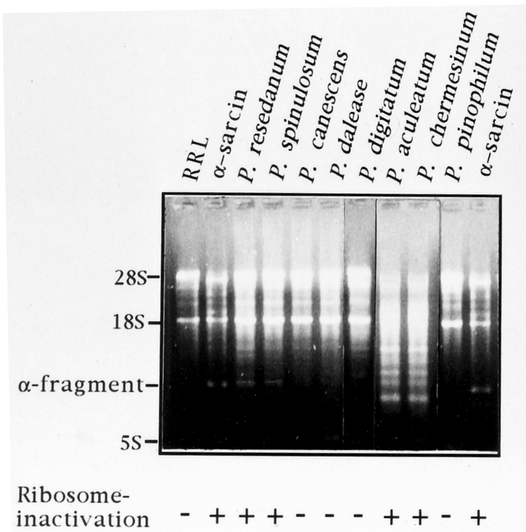

Figure 4. Ribotoxic action of extracellular proteins from *Penicillium* species. Each lane carried 5.7 OD_{260} units of rabbit reticulocyte lysates (RRL) that were treated with prepared extra-cellular protein (2 mg) from species as indicated at top of figure. The reaction occurred at 37°C for 15 min, in a buffer containing 20 mM Tris-HCl, pH 7.6; and 20 mM EDTA. Total RNAs were extracted and analyzed by 1.5% agarose gel electrophoresis in TBE buffer and visualized with ethidium bromide. RRL is rabbit reticulocyte lysate without ribotoxic treatment. α-sarcin is RRL treated with 0.2 mg of α-sarcin and used as the positive control. Positions of α-fragment, 28S, 18S, and 5S rRNA are indicated.

the sarcin domain of the large ribosomal RNA when ribosome is the substrate (Endo and Wool, 1982; Lin and Huang, 1994). The result is an α-fragment rRNA that contains a 469-nucleotide fragment from the 3' end of the large subunit ribosomal RNA (Wool, 1984). The action is distinguishable from that of other ribonucleases on ribosomes since treatment of ribosomes with other ribonucleases, such as RNase A, T2, or U2, caused all species of rRNA to be extensively digested without generating the α-fragment (Endo et al., 1983). When partially purified proteins prepared from species that carried the *sar* gene were tested for the ribosome-inactivation, it was rather surprising that only *P. resedanum, P. spinulosum, P. aculeaatum,* and *P. chermesinum* were able to hydrolyze ribosomes positively (Figure 4). *Penicillium daleae* and *P. digitatum*, also established *sar* gene carriers, were incapable of inactivating ribosomes (Figure 4) even if the amounts of extra cellular proteins were greatly increased (data not shown). The results concur with the findings of the Western blots, but raise the question as to why these species that contain the *sar* gene are unable to generate the gene product. Defects in the secretory mechanism are unlikely, simply because these *sar* genes carried a perfect secretory signal peptide. A defect at the transcriptional level is therefore suspected. Thus, an analysis of the upstream promoter region for these *sar* genes is currently in progress.

The *sar* gene of the filamentous fungi was speculated to originate from a single ancestor of ribonuclease T1. It has also been suggested that it is related to bacterial guanyl-specific ribonuclease (Kao and Davies, 1999). This was based on the close resemblance of the tertiary structure of two ribotoxins from *Aspergillus* (Campos-Olivas et al., 1996; Yang and Moffat, 1996) to that of the guanyl-specific ribonuclease T1 family (Sevcik et al., 1991; Pace et al., 1991). Both protein families share the common structure of a four-stranded β-pleated sheet. The β-pleated sheet structure is indispensable for ribotoxin to digest ribonucleic acids (Kao and Davies, 1999; Hwu et al., 2000).

In this study, species that exhibited ribosome-inactivation, except *P. aculeatum,* belong to the subgenus *Aspergiloides*, which is closely related to *Aspergillus*. The *Penicillium* species that carry *sar* gene, but fail to act in ribosome-inactivation, belong to the subgenus *Furcatum* and are taxonomically distant from *Aspergillus*. The observations of this study might help elucidate the evolutionary relationship between the genera *Aspergillus* and *Penicillium* (Berbee et al., 1995). On the other hand, the finding of near identical genes in *Penicillium* and *Aspergillus* suggests that a horizontal gene transfer might have occurred through the sexual life cycle, although most *Aspergillus*, except for the few that produce teleomorphs, have no sexual cycle. So far, there is no evidence supporting this. Reasons for the carrying of the *sar* gene in *Aspergillus* or in *Penicillium* have been questioned because the *sar* gene is not a constitutive gene for all species of *Aspergillus* (Lin et al., 1995) or *Penicillium* (this study). Moreover, the expression of the *sar* gene is lethal to the carrying host cell itself (Lamy and Davies, 1991; Hwu et al., 2000). The only advantage for fungi that have evolved a sar gene might be enhanced self-defense. Tricholin, a related ribotoxin from *Trichoderma viride* (Lin et al., 1991) that demonstrated an antibiotic effect against the fungi of *Rhizotonia solani* (Lin et al., 1994) in soil ecosystems could support this notion.

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台灣產青黴菌屬核醣毒蛋白基因之鹼基定序及定性

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應用黴菌核醣毒蛋白之抗 α-sarcin 抗體及 α-sarcin 之 cDNA 為探針，以分子生物技術中的西方點漬法（Western blotting）和南方複合法（Southern hybridization），從台灣現有的 54 種青黴菌屬（*Penicillium*）之分離株中篩選及鑑定含核醣毒蛋白之菌種。結果共有六種菌種 *P. resedanum*, *P. spinulosum*, *P. daleae*, *P. digitatum*, *P. aculeatum* 和 *P. chermesinum* 含陽性反應。再經由聚合 鏈鎖反應（Ploymerase chain reaction）複製方法，將這些核醣毒蛋白基因分別篩選出來。經過核酸序列析，發現此六種青黴菌無論是在毒素基因的組合結構或 DNA 序列及蛋白質胺基酸的序列上，帶有的毒素基因皆與麴黴菌屬（*Asperqillus*）的核酸毒素保留著演化上高度共通的特性。經由核醣體水解作用（Ribosome-inactivating protein）之測試，令人驚訝的是在上述之菌種中，僅 *P. resedanum*, *P. spinulosum*, *P. daleae*, *P. digitatum* 可生產及分泌具水解核醣體功能之核醣毒蛋白。推論上，由於此四菌種之基因均含分泌序列 (Signal sequence pepide)，所以可能是在轉錄作用上之機制發生變異致未能一貫分泌出毒蛋白。本報告為初次在青黴菌屬發現核醣毒素的存在，配合核醣毒素基因序列上之變異或保留，對研究核醣毒素之功能與演化具有相當之意義。

關鍵詞：青黴菌；核醣毒蛋白；核醣體水解；核醣毒蛋白基因。