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SHORT COMMUNICATION

Complete nucleotide sequence of *Beauveria bassiana* 5.8s rRNA coding gene and flanking internal transcribed spacers

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The nucleotide sequence of two clones of *Beauveria bassiana* in 5.8s rRNA coding gene and ITS regions were completely sequenced. The overall sequence similarity of these two clones is 96%. The identities of internal transcribed spacer (ITS) regions are 91% (ITSI) and 100% (ITSII), respectively. Both of 5.8s rRNA sequences have 98% homology.

KEY WORDS: 5.8s rDNA, internal transcribed spacer, PCR, sequencing

Beauveria bassiana is an entomopathogenic fungus and is a member of Deuteromycotina which lacks a sexual stage (Paccolla-Meirelles and Azevedo, 1991). Though both heterokaryon incompatibility and parasexuality in *B. bassiana* had been reported in laboratory conditions (Paccolla-Meirelles and Azevedo, 1991), the parasexual cycle has never been demonstrated in nature (St Leger *et al.*, 1992). Thus, mutation was the way responsible for most of the genetic variations in *B. bassiana* population and was maintained from generation to generation by asexual reproduction. Conventional, taxonomic study is primarily based on morphological (Mugnai *et al.*, 1989) and physiological (Mugnai *et al.*, 1989, St Leger *et al.*, 1992) characters. The great heterogeneity demonstrated within this species complex has permitted few conclusions to be drawn about population structures and taxonomy (Mugnai *et al.*, 1989). Both morphological and biological characters (Gerbi, 1985) may reflect the environmental conditions and may thus lead to controversial species reassignments (Curtis *et al.*, 1994).

Darwinian evolution requires selection operating on the phenotype to favor changes in the genotype which are to be maintained and perpetuated (Gerbi, 1985). The ribosomal DNA (rDNA) repeated units contain highly conserved DNA sequence as well as more variable DNA sequence regions and have been used to detect genetic variation in population (White *et al.*, 1990). The products of rDNA are RNAs, this is one case where evolutionary selection may act at the RNA level (Gerbi, 1985). In this report, the 5.8s rRNA gene and the noncoding adjacent regions were amplified by PCR using the ITS1 and ITS4 primers (White *et al.*, 1990) and were sequenced in both orientation, with ITS1, ITS2, ITS3 and ITS4 primers (White *et al.*, 1990). Both ITS1 and ITS4 primers were sequenced completely. We have sequenced a total of tweleve isolates of *B. bassiana* from different geographic areas of the world. The ITS regions and 5.8s rDNA sequences of *B. bassiana* isolate LE73 (from Taiwan) and ARS151 (from France) are shown in Figure 1. The overall similarity of ITS regions and 5.8s rDNA of these two isolates is 98%. The length of ITS1 region is 167 bp (LE73) and 162 bp (ARS151), respectively. The length of ITS2 region is 191 bp in both isolates. The identity of ITS regions is 91% (ITS1 region) and 100% (ITS2 region). The sequence length of both isolates of 5.8s rDNA of *B. bassiana* is 156 bp and has 98% identity. We found that 5.8s rDNA of *B. bassiana* is

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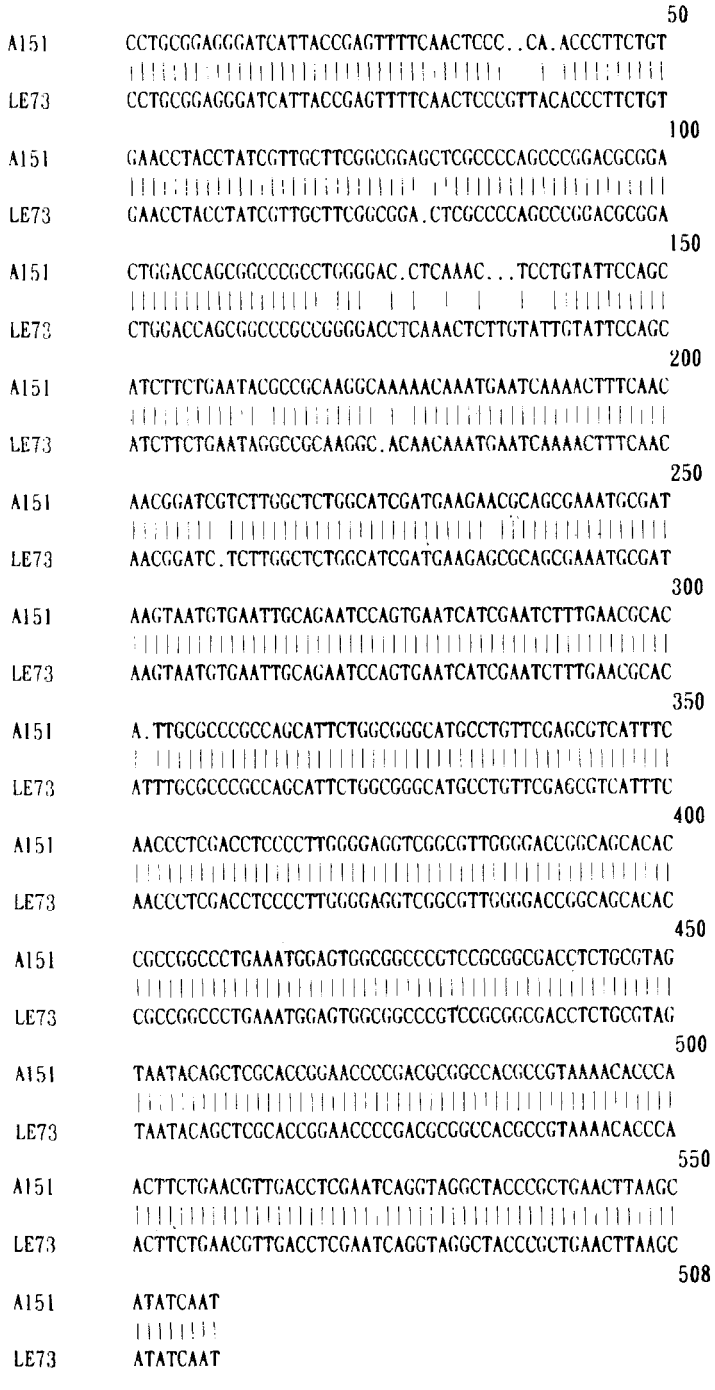

Figure 1 The complete sequence of two clones of *B. bassiana* (A151, LE73) ITS regions and 5.8s rRNA gene. "." indicates deletion of the base. Positions 19–187 and 346–537 are ITSI and ITSII, respectively. Bases 188–305 are 5.8s rDNA gene. Fungal universal primers ITS1 (5′-TCCGTAGGTGAACCTGCGG-3′) and ITS4 (5′-TCCTCCGCTTATTGATATGC-3′) have been used to amplify ITS regions and 5.8s rDNA from fungal genomic DNA (200ng) using Polymerase Chain Reaction (PCR). The contained 10mM Tris-Cl, pH 8.3, 50mM KCl, 1.5mM $MgCl_2$, 0.1% Triton X-100, 100nM each of dATP, dCTP, dGTP and dTTP, 0.2uM primers, and 1 unit of Super Taq DNA polymerase (HT). Amplification was performed with hot start at , 94°C, 3 mim, and then 40 cycles of 1 min at 94°C, 1 min at 50°C, 2 min at 72°C. Amplified products were sequenced directly on both strands using the dideoxynucleotide chain termination method with primers ITS1, ITS2 (5′-GCT-GCGTTCT-TCATCGATGC-3′), ITS3 (5′-GCATCGATG AAGAACGCAGC-3′) and ITS4. ^{35}S-dATP was incorporated during sequencing reaction. The sequencing products were separated by 6% polyacrylamide-urea gel electrophoresis and visualized by autoradiography (Kodak X-OMAT film).

closely related to *Penicillium purpruogenum* (L14506) (92% homology), and *Neurospora crassa* (M13906) (94% homology). The results are not unexpected, since 5.8s rDNA is convinced to be highly conserved (Gerbi, 1985; White *et al.*, 1990).

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