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论文

蝙蝠蛾拟青霉与冬虫夏草关系的分子系统学研究

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中国医学科学院、北京协和医学院 药物研究所 卫生部天然药物生物合成重点实验室, 北京 100050 摘要:

以5月和6月两个不同时段采集的分别产于青海省化隆县和四川省康定县的冬虫夏草(Cordyceps sinensis)新鲜子实体为材料,采用分子生物学方法,以rDNA-ITS序列为分子标记,对蝙蝠蛾拟青霉(Paecilomyces hepiali)与冬虫夏草之间的关系进行了探讨。以真菌通用引物ITS1/ITS4分别对蝙蝠蛾拟青霉及冬虫夏草子座和僵虫基因组DNA进行PCR扩增,获得的片段克隆到pMD18-T Vector上进行测序,结果表明,随机挑取的46个克隆与某些已在GenBank中注册的中国被毛孢(Hirsutella sinensis)或冬虫夏草的rDNA-ITS序列的一致性均在99%以上,但与蝙蝠蛾拟青霉的rDNA-ITS序列的一致性约为72%。根据蝙蝠蛾拟青霉的rDNA-ITS序列设计了两对特异引物,分别以不同产地及不同生长时期共4个批次的冬虫夏草子座和僵虫基因组DNA为模板,进行PCR及巢式PCR扩增,均得到了相应片段,该片段与蝙蝠蛾拟青霉的rDNA-ITS序列具有100%的一致性。在GenBank中注册号为AB067740的另一个标明为冬虫夏草的rDNA-ITS序列与注册号为AJ309353的中国被毛孢的rDNA-ITS序列一致性仅为87.3%,但根据AB067740序列设计特异引物,也从冬虫夏草基因组DNA中扩增到其相应序列。研究结果表明,在天然冬虫夏草中除了中国被毛孢之外,还普遍存在着蝙蝠蛾拟青霉等内寄生菌。

关键词: 蝙蝠蛾拟青霉 冬虫夏草 中国被毛孢 rDNA-ITS区 系统发育分析

Molecular phylogenetic analysis of Paecilomyces hepiali and Cordyceps sinensis

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Abstract:

Phylogenetic relationship between Paecilomyces hepiali and Cordyceps sinensis was studied by analyzing the sequence of rDNA-ITS. The samples of C.sinensis were collected from Hualong County in Qinghai Province and Kangding County in Sichuan Province in May and June, respectively. The rDNA-ITS fragments were obtained by PCR amplification with the template genomic DNA of the fresh stroma or caterpillar body of the collected samples and the cultured mycelium of P. hepiali, with the universal fungal primers ITS1/ITS4. The amplified fragments were cloned into pMD18-T Vector and sequenced. Phylogenetic analysis was performed with these sequences and those from GenBank. The result showed that all of the 46 clones randomly chosen from the amplification of C. sinensis shared identical or almost identical rDNA-ITS regions and had over 99% identity with some rDNA-ITS sequences of Hirsutella sinensis and C.sinensis registered in GenBank, but all of them had only about 72% identity with that of P.hepiali. Two pairs of specific primers were designed based on the rDNA-ITS sequence of P.hepiali, then PCR and Nest-PCR were performed with the template genomic DNA of the stroma or caterpillar body of C.sinensis samples mentioned above. The apparent bands amplified by Nest-PCR were obtained from all of the samples, and the sequences showed 100% identity with the rDNA-ITS sequence of P. hepiali. In addition, another pair of specific primers were designed based on the rDNA-ITS sequence registered in GenBank as the marker of C. sinensis (accession no. AB067740) but the latter only shared 87.3% identity with that of *H.sinensis* (accession no. AJ309353). This pair of primers was used to amplify the *C.sinensis* samples by PCR, and the amplified sequence showed 100% identity with that of AB067740. The result indicated that H.sinensis is the main body of C.sinensis, while some other endoparasitic fungi such as P.hepiali commonly exist in the natural C.sinensis.

Keywords: *Cordyceps sinensis Hirsutella sinensis* rDNA-ITS sequence phylogenetic analysis *Paecilomyces hepiali*

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