

大豆疫霉和苜蓿疫霉rDNA ITS序列比较研究*

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摘要: 采用真菌核糖体基因转录间隔区(ITS)用引物, PCR分别扩增了Phytophthora sojae的5个菌株(Pg1、Pg2、Pg3、CN1和S317)和1个P. medicaginis(菌株44390)的ITS1与ITS2, 并对PCR产物进行了序列测定。根据Bioedit软件中的neighbour-joining methods分析法将上述序列和Genbank中已登录的P. sojae、P. medicaginis、P. megasperma和P. trifolii等4个形态学种10个登录菌株的ITS1与ITS2碱基序列进行聚类分析。结果是聚类组与形态学种有一定差别, 4个种12个菌株分成5个聚类组: 1. 本研究所测的5个P. sojae菌株和Genbank中的2株P. sojae (AF266769和AF228089)成一组, 各菌株的ITS1和ITS2遗传距离分别是0~0.0043和0.0093; 2. 菌株44390和Genbank中2株P. medicaginis(AF266799与L41379)聚为一组, 3个菌株的ITS1、ITS2彼此之间的遗传距离为0.0098~0.0263; 3. Genbank中的AF266800(P. trifolii)和L41380(P. megasperma)为一组, 2菌株的ITS1和ITS2距离分别是0.0049和0.0070; 4. Genbank中的3个P. megasperma菌株(AF266794、L41381和L41382)成为1个聚类组, 3个菌株的ITS1之间遗传距离为0.0000~0.1444, ITS2之间的遗传距离为0.0482~0.1104; 5. 余下的Genbank中的1株(L41385)成为一个独立的聚类组, 其ITS1和ITS2与其他15个菌株的ITS1和ITS2之间的遗传距离为0.2763~0.4430。上述结果表明, 分别属于同一形态学种且可聚为一组的不同个体之间的ITS碱基序列遗传相似性最高, 但是也具有一定的多样性; 形态学上属于不同种的个体的ITS可以聚为一组。上述结果提示L41385可能不属于P. sojae, L41380可能属于P. trifolii, P. megasperma仍是一个复合种。同时提示ITS DNA碱基序列可以区分形态学种。

关键词: Phytophthora megasperma, P. trifolii, 形态学种, 聚类组

中图分类号: 文献标识码: A

大豆疫霉(Phytophthora megasperma)是Drechsler(1931)根据从Althaea rosea上分离的菌株建立的, 它可引起大豆、苹果、草莓、玫瑰、苜蓿及三叶草等多种植物的根腐病, 其中由它引起的大豆疫病是一种毁灭性病害, 是我国A1类进境植物检疫对象, 也是内检对象。该种的形态学鉴别特征是, 孢子囊无乳突、内层出, 同宗配合, 雄器典型侧生。尽管其种内绝大部分菌株都具有上述鉴别特征, 但是不同菌株之间在其它形态学如藏卵器直径和生理生化性状如致病性、生长速率、同工酶谱、菌丝中可溶性蛋白质电泳图谱、染色体数目和线粒体DNA的RFLP等方面变异较大(Hansen & Hamm, 1983; Hansen et al, 1986; Forster et al, 1989; Forster & Coffey, 1993), 且同宗配合性状不是绝对的, Barr(1980)自苜蓿上分离到异宗配合的大豆疫霉菌株, 提示它可能是一个种的复合体(Hansen & Maxwell, 1991), 或该病原菌种下存在有不同的分类亚群。然而这些分类亚群的地位及它们彼此间的关系至今仍不十分清楚。Hansen和Maxwell(1991)主要依据藏卵器大小、孢子囊长度、染色体数目、寄主范围、生长速率、对甲霜素的抗性、最适生长温度等性状, 将上述3个专化型分别建立独立的种, 即大豆疫霉(P. sojae)、苜蓿疫霉(P. medicaginis)和三叶草疫霉(P. trifolii), 其他大豆疫霉菌株仍归为大豆疫霉(P. megasperma)。但是Hansen和Maxwell的分类依据在疫霉菌种内菌株间变异较大, 且这些生物学和形态学性状的种在遗传学上不是同一个种, 不能反应各群体之间进化关系。

最近研究证明核糖体DNA(rDNA)转录间隔区(internal transcribed spacer, ITS)对真菌种、属水平分类是一个最稳定的性状, 该区域DNA核苷酸序列差异比较有助于相似种的鉴别(Bruns et al., 1991; Lee & Taylor, 1992)。因而不少学者应用ITS核苷酸序列来研究Phytophthora形态学种, 且可较充分地反应各种之间系统发育(Forster et al., 1995; Crawford et al., 1996; Cooke & Ducan, 1997; Cooke et al., 2000)。

基于上述研究背景, 本研究对5株P. sojae和1株P. medicaginis的ITS进行了PCR扩增和序列测定, 并将这6个菌株的ITS与Genbank中登录的P. sojae、P. megasperma、P. trifolii和P. medicaginis等4个种共10个登录菌株的ITS序列进行聚类分析, 可望利用ITS对这4个形态学种进行区分, 同时通过ITS DNA序列分析阐明它们彼此间的关系及同一种内ITS碱基序列的多态性。

1 材料与方法

1.1 供试菌株、培养条件及基因组DNA提取

供试菌株包括5株P. sojae和1株P. medicaginis及Genbank中已登录的P. sojae、P. medicaginis、P. trifolii和P. megasperma等4个种 10个菌株, 其编号、种名、寄主、来源及Genbank中的登录号见表1。

菌株的培养和DNA提取参照王源超等(2000)的方法。

1.2 核糖体DNA ITS1和ITS2的扩增与序列测定

参照王源超等(2000)的方法, 采用真菌核糖体基因转录间隔区(ITS)通用引物ITS1和ITS4分别扩增ITS和5.8s rDNA (ITS包括18S rDNA和5.8S rDNA之间的转录间隔区ITS1与5.8S rDNA和28S rDNA之间的转录间隔区ITS2)。PCR反应的混合液总体积为50 ml, 包括: 10 ng模板DNA, 1 mM引物, 4种dNTP各100 mM, 5 ml 10×PCR反应缓冲液, 2.5单位Taq酶(Promega), 离心后, 加两滴矿物油, 在PE2400 PCR仪上进行扩增, 反应条件是: 预变性94℃2 min; 循环, 94℃变性1min, 55℃退火30 sec, 72℃延伸1 min, 30个循环; 72℃延伸10 min。反应结束后取10 ml于1.5%琼脂糖凝胶电泳上电泳40 min (100V), 在紫外光下检测PCR产物大小。扩增的ITS1和ITS2的产物克隆到pGEM-T Easy Vector (美国Promega公司)上委托日本的TaKaRa公司进行DNA测序。

表1 供试菌株编号、种名、寄主及来源

Table 1 Number, species, hosts and origins of tested isolates of Phytophthora spp.

菌株编号或登录号	Isolate No. and Accession No. for Genbank database	种名	Species	寄主	Host	来源	Origins
CN1 S317 Pg1 Pg2 Pg3	P. sojae P. sojae P. sojae P. sojae P. sojae	大豆	Glycine max	大豆	Glycine max	大豆	Glycine max
Glycine max	大豆	Glycine max	加拿大	中国农业大学	南京市出入境检验检疫局	南京市出入境检验检疫局	南京市出入境检验检疫局

44390 P. medicaginis 苜蓿Medicago sativa 美国

L41379 L41380 L41381 L41382 L41385 AF266799 AF228089 AF266769 AF266794 AF266800 P. medicaginis P. megasperma P.

megasperma P. megasperma P. sojae P. medicaginis P. sojae P. sojae P. megasperma P. trifolii 苜蓿Medicago sativa 大豆

■热门文章

■最新更新

为一组, 44390、AF266799和L41379为一组, 余下4个菌株各成独立的聚类组。提示L41385可能不是*P. sojae*, L41380可能是*P. trifolii*; 同时还提示大雄疫霉复合种下可能分成6个群体或种。

图2 4个形态学疫霉菌ITS2聚类分析树状图

Fig.2 Phylogenetic trees indicating the relationships between 4 morphospecies *Phytophthora* spp. based on sequences of ITS2.

3 结论与讨论

本研究对5个*P. sojae*菌株和1个*P. medicaginis*菌株的ITS进行了克隆、序列测定和比较, 并进一步将上述序列与Genbank中已登录的来自不同寄主上的*P. sojae*、*P. medicaginis*、*P. trifolii*和*P. megasperma*等4个形态学种的ITS核苷酸序列进行比较, 同时在对各菌株之间的遗传距离进行分析基础上构建了聚类分析树状图。结果是, 所测定的5株大豆疫霉ITS序列同源性99%, 表明ITS聚类组与形态学分类具有一定的差别, CN1、AF266769和AF228089成一组, AF266800和L41380为一组, 44390、AF266799和L41379为一组, 余下4个菌株各成独立的聚类组。提示L41385可能不属于*P. sojae*, L41380可能是*P. trifolii*。

自Drechsler(1931)建立*P. megasperma*以来, 该形态学种的分类经过了一系列的变化。形态学和生物学性状分类是经典的分类方法, 但是由于形态和生物学特征变化较大, 如*P. megasperma*不同个体的卵孢子直径(42-52 μ m)呈连续性变化, 种内存在有异宗配合的个体, 寄主专化性不是绝对的。线粒体DNA RFLP曾经作为分类依据对不同寄主上的复合种*P. megasperma*进行聚类分析, 将194个菌株划分为9个群体, 但是线粒体DNA RFLP在种内也存在丰富的多态性。Griffith和Shaw(1998)对92株*P. infestans*的线粒体DNA RFLP研究发现*P. infestans*种内存在多样性。可见上述分类依据都具有一定的缺陷。ITS在不同真菌种内保守性强, 而在种间存在丰富的变异, 因而不少学者应用ITS来研究*Phytophthora* (Lees & Taylor, 1992; Lee et al., 1993; Tooley et al., 1997; Cooke et al., 2000; 王源超等, 2000)、*Pythium* (Chen, 1992)、*Verticillium* (Nazar et al., 1991)、*Fusarium* (O'Donnell, 1992)等真菌的系统发育和检测。Cooke等(2000)利用ITS对疫霉菌系统发育研究时发现, 种内不同个体之间的ITS碱基序列完全相同。王源超等(2000)研究发现恶疫霉和苎麻疫霉的ITS序列非常保守, 分别来自构树和苎麻上的苎麻疫霉菌株的ITS碱基序列完全相同, 恶疫霉的ITS与Genbank中的恶疫霉ITS序列同源性高达100%, 认为ITS可以作为疫霉菌分类的辅助性状。本研究结果表明, 种内不同个体之间的ITS碱基序列具有较高的保守性, 提示可以作为疫霉菌分类的依据。

种内个体之间的ITS碱基序列具有一定的多样性。Cooke认为疫霉菌的ITS碱基序列是非常保守的, 种内不同个体之间不存在有差异(私人通讯), 同时Cooke等(2000)报道*P. sojae*、*P. medicaginis*、*P. trifolii*和*P. megasperma*等4个种菌株间的ITS序列完全相同。但是, 本研究将本实验室的1株*P. sojae*和1株*P. medicaginis*的ITS序列与Genbank中已登录的*P. sojae*、*P. megasperma*、*P. medicaginis*和*P. trifolii*等4个形态学种的10个ITS序列进行比较分析结果是, 种内不同个体之间的ITS碱基序列存在一定的差异。表明至少在某些疫霉菌中种内ITS序列存在有微小的差异。

研究中观察到Crawford等(1996)登录的*P. sojae*的L41385(菌株号: 9933R15)的ITS1和ITS2和其它3个*P. sojae*菌株的ITS在碱基序列长度和同源性上均差异较大, 同源性小于80%; 进一步将该序列与Genbank中其它疫霉菌的ITS序列进行比较, 结果显示该菌株的ITS与Genbank中的*P. macrochlamydospora*(登录号: L41367.1; isolate IMI183280)同源性最高, 即两者 ITS1核苷酸序列完全相同, ITS2同源性为93%(研究中未显示)。提示L41385可能属于*P. macrochlamydospora*。

[REFERENCES]

- Barr DJS, 1980. Heterothallic-like reaction in the large-oospore form of *Phytophthora megasperma*. *Can J Plant Pathol*, 2: 116~118
- Bruns T D, White T J, Taylor J W, 1991. Fungal molecular systematics. *Annu Rev Ecol Syst*, 22: 525~564
- Chen W, 1992. Restriction fragment length polymorphism in enzymatically amplified ribosomal DNAs of three heterothallic *Pythium* species. *Phytophthology*, 82: 1467~1472
- Cooke D E L, Duncan J M, 1997. Phylogenetic analysis of *Phytophthora* species based on the ITS1 and ITS2 sequences of ribosomal DNA. *Mycol Res*, 101: 667~677
- Cooke D E L, Drenth A, Duncan J M, Wagels G, Brasier M, 2000. A molecular phylogeny of *Phytophthora* and related oomycetes. *Fungal Genetics and Biology*, 30: 17~32
- Crawford A R, Bassam B J, Drenth A, Maclean D J, Irwin J A G, 1996. Evolutionary relationships among *Phytophthora* species deduced from rDNA sequence analysis. *Mycol Res*, 100: 437~443
- Drechsler R, 1931. A crown rot of hollyhocks caused by *Phytophthora megasperma* n. sp. *Journal of the Washington Academy of Sciences*, 21: 513~526
- Forster H, Coffey M D, 1993. Molecular taxonomy of *Phytophthora megasperma* based on mitochondrial and nuclear DNA polymorphisms. *Mycol Res*, 97: 1101~1112
- Forster H, Kinscherf T G, Leong S A, Maxwell D P, 1989. Restriction fragment length polymorphisms of the mitochondrial DNA of *Phytophthora megasperma* isolated from soybean, alfalfa, and fruit trees. *Can J Bot*, 67:529~537
- Griffith G W, Shaw D S, 1998. Polymorphisms in *Phytophthora infestans*: Four mitochondrial haplotypes are detected after PCR amplification of DNA from pure cultures or from host lesions. *Applied and Environmental Microbiology*, 64: 4007~4014
- Hansen E M, Hamm P B, 1983. Morphological differentiation of host-specialized groups of *Phytophthora megasperma*. *Phytopathology*, 73: 129~134
- Hansen E M, Brasier C M, Shaw D S, Hamm P B, 1986. The taxonomic biological species groups. *Trans Br Mycol Soc*, 87: 557~573
- Hansen E M, Maxwell D P, 1991. Species of *Phytophthora megasperma* complex. *Mycologia*, 83: 376~381

Lees S B, Taylor J W, 1992. Phylogeny of five fungus-like protactistan Phytophthora species, inferred from the internal transcribed spacers of ribosomal DNA. *Mol Biol Evol*, 9: 636~653

Lees S B, White T J, Taylor J W, 1993. Detection of Phytophthora species by oligonucleotide hybridization to amplified ribosomal DNA spacers. *Phytopathology*, 83: 177~181

Nazar R N, Hu X, Schmidt J, Culham D, Robb J, 1991. Potential use of PCR amplified ribosomal intergenic sequences in the detection and differentiation of Verticillium pathogens. *Physiol Mol Plant Pathol*, 39: 1~11

O'Donnell K, 1992. Ribosomal DNA internal transcribed spacers are highly divergent in the phytopathogenic ascomycete Fusarium sambucinum (Gibberella pulicaris). *Curr Genet*, 22: 213~220

Thompson J D, Higgins D G, Gibson T J, 1994. CLUSTAL W: Improving the sensitivity of multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res*, 22: 4673~4680

Tooley P W, Bunyard B A, Carras M M, Hatziloukas E, 1997. Development of PCR primers from internal transcribed spacer region 2 for detection of Phytophthora species infecting potatoes. *Applied and Environmental Microbiology*, 63: 1467~1475

王源超, 张正光, 郑小波, 2000. 核糖体基因ITS作为苎麻疫霉、恶疫霉分类辅助性状的研究. *菌物系统*, 19: 485~491

SEQUENCE COMPARISON OF ITS OF THE RIBOSOMAL RNA GENE REPEAT OF *Phytophthora sojae* AND *P. medicaginis*

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Abstract: The internal transcribed spacer regions (ITS1 and ITS2) of the ribosomal RNA gene from five isolates of *Phytophthora sojae* (Pg1, Pg2, Pg3, Cn1 and S317) and an isolate, 44390 of *P. medicaginis* were amplified using the polymerase chain reaction and sequenced. Sequences from the above isolates were compared with published sequences of *P. sojae* (Accession No.: AF228089, AF266769 and L41385), *P. medicaginis* (Accession No.: L41379 and AF266799), *P. megasperma* (Accession No.: L41380, L41381, L41382 and AF266794) and *P. trifolii* (Accession No.: AF266800) in Genbank database and phylogenetic trees were produced based on ITS sequence data by "Neighbour-joining" methods of the software Bioedit. The resultant grouping of species differed from groupings established using classical morphological and biological characteristics. One group comprised all *P. sojae* isolates excepted L41385. The isolate tested 44390, AF266799 and L41379 were clustered into the second group *P. medicaginis*. The third group consisted of two isolates of two species from Genbank, AF266800 (*P. trifolii*) and L41380 (*P. megasperma*). The isolate L41381 (*P. megasperma*), L41382 (*P. megasperma*), AF266794 (*P. megasperma*) became the fourth group. L41385 was separated from its morphological species to become the fifth group. The results showed that ITS sequences of different isolate of the same phylogenetic group within one morphological species were polymorphic. The results further indicated that L41385 (the accession No. for Genbank database) could not obviously belong to *P. sojae* and L41380, one isolate of *P. megasperma* might belong to *P. trifolii*. Moreover the species *P. megasperma* may be a *Phytophthora* complex. It suggested that the sequence difference within ITS could provide a characteristic for distinguishing morphological *Phytophthora* species.

Key words: *Phytophthora megasperma*, *P. trifolii*, morphological species, phylogenetic group

表2 4个形态学疫霉菌ITS1 DNA距离

Table2 DNA distance matrix between ITS1 of 4 morphospecies *Phytophthora* spp.

Isolate

No. Pg1 Pg2 Pg3 S317 CN1 AF266769 AF228089 AF266799 AF266800 L41379 44390 AF266794 L41380 L41381 L41382 L41385

Pg1 0.0000

Pg2 0.0000 0.0000
 Pg3 0.0000 0.0000 0.0000
 S317 0.0043 0.0043 0.0043 0.0000
 CN1 0.0000 0.0000 0.0000 0.0043 0.0000
 AF266769 0.0043 0.0043 0.0043 0.0086 0.0043 0.0000
 AF228089 0.0043 0.0043 0.0043 0.0086 0.0043 0.0000
 AF266799 0.2923 0.2923 0.2923 0.2998 0.2923 0.2998 0.2849 0.0000
 AF266800 0.3101 0.3101 0.3101 0.3025 0.3101 0.3177 0.3101 0.0351 0.0000
 L41379 0.2923 0.2923 0.2923 0.2998 0.2923 0.2998 0.2849 0.0000 0.0351 0.0000
 44390 0.2998 0.2998 0.2998 0.3073 0.2998 0.3073 0.2923 0.0098 0.0453 0.0098 0.0000
 AF266794 0.3073 0.3073 0.3073 0.3003 0.3073 0.3143 0.3003 0.2107 0.0351 0.2107 0.2239 0.0000
 L41380 0.3094 0.3094 0.3094 0.3171 0.3094 0.3171 0.3094 0.0350 0.0049 0.0350 0.0453 0.2177 0.0000
 L41381 0.3087 0.3087 0.3087 0.3018 0.3087 0.3156 0.3018 0.2382 0.2460 0.2382 0.2519 0.1444 0.2593 0.0000
 L41382 0.3087 0.3087 0.3087 0.3018 0.3087 0.3156 0.3018 0.2382 0.2460 0.2382 0.2519 0.1444 0.2593 0.0000 0.0000
 L41385 0.3914 0.3914 0.3914 0.3823 0.3914 0.3914 0.3914 0.2763 0.2683 0.2763 0.2763 0.3202 0.2763 0.3468 0.3468 0.0000

表3 4个形态学疫霉菌ITS2 DNA距离

Table2 DNA distance matrix between ITS2 of 4 morphospecies Phytophthora spp.

Isolate

No.	Pg1	Pg2	Pg3	S317	CN1	AF266769	AF228089	AF266799	AF266800	L41379	44390	AF266794	L41380	L41381	L41382	L41385
Pg1	0.0000															
Pg2	0.0023	0.0000														
Pg3	0.0023	0.0000	0.0000													
S317	0.0070	0.0046	0.0046	0.0000												
CN1	0.0093	0.0069	0.0069	0.0069	0.0000											
AF266769	0.0046	0.0023	0.0023	0.0023	0.0046	0.0000										
AF228089	0.0046	0.0023	0.0023	0.0023	0.0046	0.0000	0.0000									
AF266799	0.1914	0.1945	0.1945	0.1976	0.2007	0.1945	0.0000	0.0000								
AF266800	0.1938	0.1941	0.1941	0.1972	0.2003	0.1941	0.1941	0.0164	0.0000							
L41379	0.2148	0.2152	0.2152	0.2184	0.2216	0.2152	0.2152	0.0238	0.0360	0.0000						
44390	0.1976	0.1979	0.1979	0.2010	0.2041	0.1979	0.1979	0.0023	0.0188	0.0263	0.0000					
AF266794	0.1553	0.1521	0.1521	0.1521	0.1492	0.1492	0.1492	0.2654	0.2683	0.2925	0.2693	0.0000				
L41380	0.1972	0.1976	0.1976	0.2007	0.2038	0.1976	0.1976	0.0188	0.0070	0.0335	0.0213	0.2722	0.0000			
L41381	0.1299	0.1269	0.1269	0.1269	0.1241	0.1241	0.1241	0.2308	0.2400	0.2475	0.2345	0.0852	0.2438	0.0000		
L41382	0.1248	0.1218	0.1218	0.1218	0.1190	0.1190	0.1190	0.1888	0.1977	0.2086	0.1923	0.1104	0.2012	0.0482	0.0000	
L41385	0.3214	0.3197	0.3197	0.3234	0.3197	0.3197	0.3197	0.3964	0.3836	0.3995	0.4013	0.4430	0.3906	0.3947	0.3899	0.0000

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