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内蒙古苹果树腐烂病病原菌鉴定

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摘 要:【目的】明确内蒙古地区引起苹果树腐烂病的病原种类。**【方法】**采用形态学结合分子生物学的方法,对内蒙古呼和浩特市周边果园的苹果腐烂病病菌进行分离、鉴定和致病力测定。**【结果】**从感病‘金红’苹果树上分离到2株苹果腐烂病病菌,命名为QH1和QH2。QH1与QH2的致病力与分离自陕西杨凌的参照菌株YL1相同;菌落、分生孢子及子实体的形态特征均符合*Cytospora*属的特征;基于内转录间隔区、核糖体大亚基片段、转录延长因子和β微管蛋白4个基因的序列一致性和系统发育的分析证明,QH1属于*Cytospora schulzer*,QH2属于*Cytospora mali*。**【结论】**内蒙古呼和浩特地区苹果腐烂病病菌为*C. schulzer*和*C. mali*两种致病菌。

关键词:苹果树腐烂病;内蒙古;病原菌;分离;鉴定;致病力测定

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Identification of the pathogenic fungi associated with apple valsa canker in Inner Mongolia, China

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Abstract:【Objective】Apple valsa canker, also known as “apple cancer”, is one of the most important diseases of apple trees. Inner Mongolia Autonomous Region, located at the northeast district of apple growing area in China, is famous for its high-quality fruits owing to the abundant sunshine and larger temperature difference between day and night. The climate in Inner Mongolia is dry and cold, which is conducive for the occurrence of apple valsa canker diseases. Little information, however, has been known about the species of the pathogenic fungi associated with apple canker disease in this area. This study aimed to isolate and identify the pathogenic strains associated with apple canker in Inner Mongolia based on morphological and molecular characteristics.【Methods】Symptomatic branches were collected from ‘Jinhong’ trees in the Qianhe apple orchard around Hohhot, Inner Mongolia, China. Bark pieces at the border of healthy and diseased tissue were surface-disinfected with 1% sodium hypochlorite for 3 min and 75% ethyl alcohol for 30 s, and washed three times in sterile distilled water. The surface-disinfected pieces were placed on Potato Dextrose Agar (PDA) plates containing rifampicin, and incubated at 25℃. The hypha at the colony frontier was repeatedly transferred into the new PDA plates, until the pure colonies were received. Isolated fungi were tested for pathogenicity on sterilized healthy apple branches. Specifically, healthy branches on 2-year-old ‘Jinhong’ trees were cut into 20-cm-long segments, surface-disinfected in 1% sodium hypochlorite for 5 min and 75% ethyl alcohol for 30 s, washed three times with sterile distilled water, dried at the room temperature, and both ends were then

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sealed with wax. Subsequently, the sterilized branch segments were scalded by the head of nails, inoculated with the 6 mm diameter fungal mycelial plugs, and wrapped with wet degreasing cotton and parafilm. Inoculated branches were placed in the plastic tray, covered with parafilm, and incubated at 25 ℃, 16 h light/8 h dark cycle and 70% humidity. The degreasing cotton was removed after 3 days. The strain *Cytospora Mali* YL1, isolated from apple in Yangling, Shaanxi, was used as positive control, and PDA disk was used as negative control. The obtained pathogenic isolates were then identified based on their morphological and molecular characteristics. For the morphological characteristics, isolates were cultured on PDA plates at 25 ℃, and their colonial morphology was observed after 3, 10 and 60 days. The mycelium and conidia on the PDA plates were examined under a microscope, and the conidia and pycnidium on the inoculated branches were observed under the microscope or stereoscope. For the molecular characteristics, isolates were cultured at 25 ℃ for 7 days, their mycelium was collected by the sterilized toothpicks, and DNA was extracted from the obtained isolates. The genes of the internal transcriptional spacer (ITS), the ribosomal large subunit (LSU), the transcription prolongation factor (EF) and the beta-tubulin (BTU2) of the obtained isolates were amplified by the ITS1/ITS4, NL1/NL4, EF1-688F/EF1-1251R, and Bt2a/Bt2b primer pairs. The PCR products were purified using the gel purification kit, and cloned into a PMD19-T vector. The reaction mixture was transformed into *Escherichia coli* DH5α competent cells. Three positive colonies from each transformation were selected and sequenced by Sangon Biotech. Obtained sequences were modified by the Vector NTI Advance11, and submitted to the NCBI. The received sequences were aligned with those published in NCBI by Clustal X software, edited manually by the BioEdit software, and connected as the ITS-LSU-EF-BTU2 sequences. The sequence identities based on ITS-LSU-EF-BTU2 sequences between the isolates and those published in NCBI were analyzed using SDT software with the Clustal W method, and phylogenetic trees based on the multi-locus sequences were built by MEGA 6.0 with the neighbor-joining method.【Results】Two strains, named QH1 and QH2, were isolated from the branches. Sterilized healthy ‘Jinhong’ branches inoculated with the QH1, QH2 or YL1 developed typical symptoms of apple canker disease, i.e., black fruiting bodies (pycnidium) formed, and yellow mucilaginous mass of conidia oozed out from the fruiting bodies under humid conditions. Conidia were hyaline, allantoid, and aseptate. The transverse microtome section of the pycnidium showed labyrinthine chambers. The colonies of QH1 and QH2 grown on PDA were white and villiform initially, and then changed moderately to kelly or brown separately after 10 days. Conidiomata began to form abundantly after 60 days grown on PDA plates. The morphological characteristics of QH1 and QH2 coincided with the *Cytospora* species. The sequence identity based on the ITS-LSU-EF-BTU2 genes of the QH1, QH2 and the reported *Cytospora* species was 76.1%-84.5%. Phylogenetic analysis based on the ITS-LSU-EF-BTU2 genes showed that QH1 clustered with C. *schulzer*, and QH2 clustered with *C. mali*.【Conclusion】Two strains of apple valsa canker associated pathogenic fungi were isolated in Inner Mongolia, China, and they were identified as *C. schulzer* and *C. Mali*. This is the first study on identifying the pathogenic fungi of apple valsa canker in Inner Mongolia, via both morphological characteristics and molecular marker genes.

Key words: Apple valsa canker; Inner Mongolia; Pathogenic fungi; Isolation; Identification; Pathogenicity detection

我国是主要的苹果生产大国，根据气候特点、生产规模、主要品种等因素，将苹果生产区划分为西北黄土高原、黄河故道、环渤海湾、西南冷凉高地和东北冷寒5个苹果产区[1]。苹果树腐烂病（Valsa canker of apple）又称“烂皮病”或“臭皮病”，作为苹果生产过程中较为严重的病害之一，在这些地区均

有分布。曹克强等[2]于2008年对中国10个省份的苹果树进行调查，发现其中52%的果树均感染了苹果腐烂病。该病菌主要寄生死组织，侵染树体木质部和韧皮部[3-4]，危害主干和侧枝，导致树势减弱，影响苹果的产量和品质，严重时则造成毁园，给果农造成严重经济损失。

随着苹果树腐烂病的严重发生，目前已有许多对其病原菌分离、鉴定的研究。樊民周等[5]通过形态学和致病性检测，明确陕西省苹果腐烂病主要致病菌为*Cytospora* sp.。臧睿[6]和Wang等[7]从陕西、山西、甘肃、山东、河南、宁夏、西藏、云南和黑龙江9个省区取样，基于内转录间隔区（Internal Transcribed Spacer，ITS）、转录延长因子（Elongation Factor 1-alpha，EF）和β微管蛋白（β-tubulin，BTU2）3个基因的系统发育分析，证明上述9个省苹果腐烂病菌主要分为V*alsa mali*、*Valsa malicola*、*Valsa persoonii* 和*Valsa pyri* 4个种。刘应敏等[8]首次发现*C. schulzeri*在新疆地区引起苹果树腐烂病。王卫雄[9]确定了甘肃省苹果树腐烂病菌分别为*V. malicola*和*V. mali*。刘钰娇等[10]运用随机扩增多态性DNA标记技术（Random Amplification Polymorphic DNA，RAPD），证明河北省苹果树腐烂病病菌的优势菌株为黄色、浅黄色和灰白色菌株，但没有基于分子生物学方法的种群划分。内蒙古属于东北冷寒苹果产区，以生产‘金红’‘黄太平’等小苹果为主[11]。该地区阳光充足、昼夜温差大，果实品质极佳，然而寒冷、干旱的气候很容易造成树势减弱，苹果树腐烂病发生严重[12-13]。目前对内蒙古地区苹果腐烂病的研究较少，仅见庄霞[14]从内蒙古呼和浩特地区感染腐烂病的‘金红’苹果树上分离得到一株病原菌，但没有对该菌株进行进一步的分子鉴定。

为了明确内蒙古呼和浩特地区苹果树腐烂病的种类，笔者基于形态学和分子生物学研究方法对内蒙古呼和浩特地区的苹果腐烂病病菌进行分离和鉴定，对我国苹果产区苹果腐烂病病菌多样性研究进行补充，并为该病害防控提供重要的参考信息。

1 材料和方法

1.1 材料

1.1.1 *样品* 2018年11月19日在内蒙古呼和浩特市大青山谦和果园采集表现苹果腐烂病症状的‘金红’苹果枝条，置于纸质标本袋中保存，用于病原菌的分离。以一株命名为YL1，在分类上属于*V. mali*，分离自陕西省杨凌区的苹果腐烂病菌作为参照菌株。

1.1.2 *主要培养基和试剂* 马铃薯葡萄糖培养基（Potato Dextrose Agar，PDA）：马铃薯200 g，葡萄糖20 g，琼脂粉20 g，蒸馏水1 000 mL。

普通琼脂糖凝胶DNA回收试剂盒，购自天根生化科技（北京）有限公司；PCR扩增试剂盒、pMD™19-T Vector克隆试剂盒，购自宝日医生物技术（北京）有限公司。

1.1.3 *主要仪器设备* 电热恒温培养箱，HPX-9162MBE，上海博讯实业有限公司；实体解剖镜，LEICA EZ4W，德国莱卡仪器有限公司；显微镜，LEICA ICC50W，德国莱卡仪器有限公司；电泳仪，BG-Power 600K 450W，北京百晶生物技术有限公司；PCR仪，624BR47696，美国伯乐Bio-Rad公司。

1.2 试验方法

1.2.1 *病原菌的分离与纯化* 选取采集到的苹果腐烂病枝条病健交界处组织，依次用1%次氯酸钠浸泡3 min，75%乙醇浸泡30 s，灭菌水漂洗3次，放入加有利福平的PDA平板培养基上（每10 mL PDA培养基加入1 μL 50 mg·mL^{-1}利福平），25 ℃恒温培养3~4 d；挑取菌落边缘菌丝移入新的PDA平板，继续恒温培养，重复以上操作直至得到纯培养物。所得菌种均保存在内蒙古农业大学园艺与植物保护学院果树病害病原生物学及综合防控研究室。

1.2.2 *病原菌的致病性检测* 参照乔国彪等[15]的方法进行病原菌的致病性检测。取健康2 a（年）生‘金红’苹果枝条，去除叶片后将其截成20 cm长，依次用1%的次氯酸钠浸泡5 min，75%乙醇浸泡1 min，无菌水冲洗3次，风干后用石蜡封住枝条两端。用6 mm直径的钉帽烫伤树皮后，接种PDA培养基上培养5 d，直径为6 mm的病原菌菌饼，用喷湿脱脂棉裹住接种处后保鲜膜包扎，每个处理10次重复。以只接种PDA培养基的枝条作为阴性对照，接种YL1病原菌枝条为阳性对照。处理枝条置于塑料盘中，封口膜封口保湿，25 ℃、16 h光照/8 h黑暗、70%湿度条件下培养，在处理后3 d去掉脱脂棉，定时观察记录发病情况。

1.2.3 *病原菌形态观察* 将分离菌株接种于PDA平板上，25 ℃恒温培养10 d，观察菌落、菌丝及孢子

形态，60 d后，观察子实体特征。在实体解剖镜下观察发病枝条病部子实体的外部形态，并在显微镜下观察其横切面形态结构特征。

1.2.4　病原菌分子鉴定　将病原菌接种至铺有无菌玻璃纸的PDA平板上，25 ℃恒温培养7 d后，用灭菌牙签收集菌丝体，参照Lee等[16]和臧睿[6]的方法提取分离到菌株总DNA。

以提取的菌株总DNA为模板，分别采用通用引物ITS1/ITS4、NL1/NL4、EF1-688F/EF1-1251R和Bt2a/Bt2b（表1）扩增病原菌的内转录间隔区（Internal Transcribed Spacer，ITS）、核糖体大亚基片段（Ribosomal Large Subunit，LSU）、转录延长因子（Elongation Factor 1-alpha，EF）和β微管蛋白（β-tubulin，TUB2）。PCR反应体系均为10×PCR buffer 2.5 μL，dNTP 2.0 μL（2.5 mmol·L^{-1}），引物（10 μmol·L^{-1}）各1 μL，Taq酶0.5 μL，DNA 1 μL，灭菌双蒸水补足至25 μL。PCR反应条件依次为：94 ℃ 3 min；94 ℃ 30 s，退火30 s（各引物退火温度见表1），72 ℃ 1 min，35个循环；72 ℃ 10 min。最终的PCR扩增产物经1.2%的琼脂糖电泳检测，用普通琼脂糖凝胶DNA回收试剂盒（Tiangen，北京）回收纯化，连接pMD19-T载体，转化到*E. coli*菌株DH5α感受态细胞，涂布于含Amp的LB平板上，37 ℃培养过夜，挑选单菌落将PCR扩增鉴定阳性的单克隆送至上海生工生物工程股份有限公司进行测序。

表1　用于ITS、LSU、EF和BTU2基因PCR扩增的引物信息

Table 1　Primers used for the amplification of ITS, LSU, EF and BTU2 genes

目标基因 Target	引物名称 Primer	引物序列 Primer sequences (5'-3')	退火温度 Annealing temperature/℃	参考文献 Reference
ITS	ITS1	TCCGTAGGTGAACCTGCGG	51	[17]
	ITS4	TCCTCCGCTTATTGATATGC		
LSU	NL1	GCATATCAATAAGCGGAGGAAAAG	55	[18]
	NL4	GGTCCGTGTTTCAAGACGG		
EF	EF1-688F	CGGTCACTTGATCTACAAGTGC	55	[19]
	EF1-1251R	CCTCGAACTCACCAGTACCG		
TUB2	Bt2a	GGTAACCAAATCGGTGCTGCTTTC	61	[20]
	Bt2b	ACCCTCAGTGTAGTGACCCTTGGC		

1.2.5　序列一致性和系统发育分析　采用Vector NTI Advance 11（Thermo Fisher Scientific，USA）软件对测序结果进行校正，校正后的测序结果在NCBI利用BLASTn进行同源序列检索后提交到GenBank数据库。采用ClustalX软件对表2中的菌株序列进行比对分析，用BioEdit软件进行人工调整，按照ITS-LSU-EF-TUB2的顺序进行首尾相连，采用SDT[21]软件分析序列间核苷酸一致性，选择Clustal W算法进行对比[22]，使用MEGA 6.0软件以比邻法（neighbor-joining，NJ）[23]构建多基因序列的系统发育进化树。

2　结果与分析

2.1　分离菌株致病性检测

从采集枝条上共分离、纯化得到2株病原菌，分别命名为QH1、QH2。对分离得到的病原菌进行致病性检测结果显示，接种了QH1和QH2的'金红'苹果枝条均在接种后10 d开始发病，首先是接种部位出现红褐色、凹陷、水浸状、椭圆形病斑，韧皮部变软并开始腐烂，随后逐渐环绕枝条一周，接种后15 d发病率达到100%，病斑表面树皮皱缩，病健交界明显，20 d后在接种部位周边出现黑色的子实体，随后扩散到整枝，子实体的孔口溢出黄色的卷须状物，为分生孢子角（图1），发病特性完全符合苹果腐烂病菌致病特性。依据柯赫氏法则证明分离到的2株病原菌均为苹果树腐烂病菌株，QH1和QH2的致病力与参照菌株YL1无明显差异。

2.2　病原菌形态特征观察

对照菌株YL1在PDA培养基上培养3 d后菌落颜色呈白色，边缘形状呈辐射状，有明显的白色同心轮纹，在同心轮纹上有白色的气生菌丝，10 d后菌丝较为疏松，菌落背面产生黄褐色色素（图2-A1）；60 d后长出球状黑色产孢体（图2-A2）；菌丝直径约3.5 μm（图2-A3）；分生孢子无色、单孢、香蕉型（图2-A4）；产孢体盘为单孔口（图2-A5），单腔室，包含无数不规则且同心的室（图2-A6）。

菌株QH1在PDA培养基上培养3 d后菌落为圆形、白色，菌丝蓬松雪花状；5~6 d后菌落背面中心变为黄褐色，边缘黄绿色；10 d后菌落整体为黄褐色，

表2 用于核酸一致性分析和系统进化树构建的腐烂病菌株基因参考序列

Table 2 The reference gene sequences of valsa canker strains used for nucleotide sequence identity analysis and phylogeny analysis in this study

种名 Species name	寄主 Host	地点 Location	菌株编号 Strain number	GenBank登录号 GenBank No.			
				ITS	LSU	EF	TUB2
Cytospora sacculus	核桃 *Juglans regia*	甘肃 Gansu	CFCC 89624	KR045645	KR045724	KP310860	KR045686
		甘肃 Gansu	CFCC 89625	KR045646	KR045725	KP310861	KR045687
		陕西 Shaanxi	CFCC 89626	KR045647	KR045726	KU710934	KR045688
		陕西 Shaanxi	CFCC 89627	KR045648	KR045727	KU710935	KR045689
Cytospora schulzeri	苹果 *Malus domestica*	宁夏 Ningxia	CFCC 50040	KR045649	KR045728	KU710936	KR045690
	花红 *Malus asiatica*	青海 Qinghai	CFCC 50042	KR045650	KR045729	KU710937	KR045691
	金红苹果 Jinhong	内蒙古 Inner Mongolia	QH1*	MN483200	MN483202	MN491913	MN491915
Cytospora mali	山楂 *Crataegus* sp.	山西 Shanxi	CFCC 50031	KR045636	KR045716	KU710927	KR045677
	山荆子 *Malus baccata*	青海 Qinghai	CFCC 50044	KR045637	KR045717	KU710928	KR045678
	金红苹果 Jinhong	内蒙古 Inner Mongolia	QH2*	MN483201	MN483203	MN491914	MN491916

注：*. 本研究中鉴定菌株。

Note: *. Strains identified in this study.

A. PDA(CK)；B. YL1；C. QH1；D. QH2.

图1 ‘金红’苹果枝条接种20 d的症状

Fig. 1 Symptoms of ‘Jinhong’ branches inoculated after 20 days

其正面有浅灰色气生菌丝形成的晕圈(图2-B1)，60 d后长出黑色球状产孢体(图2-B2)；菌丝直径约3.5 μm(图2-B2)；分生孢子无色、单孢、腊肠型(图2-B4)；产孢体盘为单孔口(图2-B5)，产孢体细胞壁内褶形成花瓣状同心腔(图2-B6)。根据菌落特征及产孢体形态，初步判断菌株QH1符合壳囊孢属(*Cytospora* sp.)基本特征。

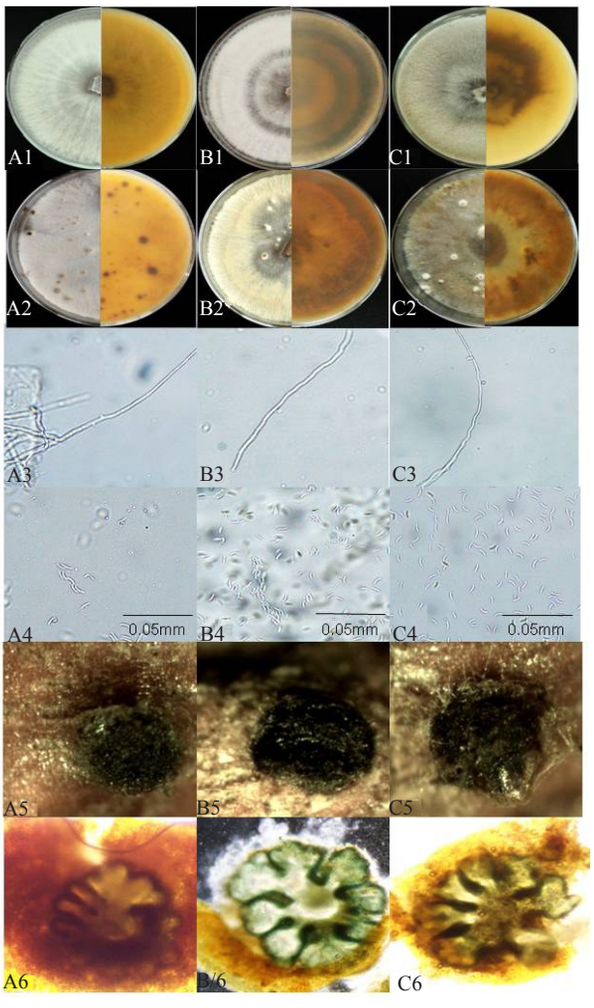
菌株QH2在PDA培养基上培养3 d后菌落呈放射状、白色，菌丝蓬松，5~6 d中央菌丝为浅灰色，边缘为白色，10 d菌落颜色为褐色(图2-C1)；60 d后长出黑色球状产孢体(图2-C2)；菌丝直径约3.3 μm(图2-C3)；分生孢子无色、单孢、香蕉型(图2-C4)；产孢体盘为单孔口(图2-C5)，内有多个腔，每一个腔室都有独立的细胞壁，汇聚于产孢体盘中央形成一个孔口(图2-C6)。根据菌落特征及产孢体形态，初步判断QH2也符合壳囊孢属(*Cytospora* sp.)基本特征。

2.3 病原菌分子鉴定结果

采用通用引物ITS1/ITS4、NL1/NL4、EF1-688F/EF1-1251R和Bt2a/Bt2b，从菌株YL1、QH1、QH2分别扩增到约600、600、630、550 bp的基因片段(图3)，扩增得到的目的条带单一且与预期大小一致，阴性对照无任何条带。

2.4 序列一致性和系统发育分析

应用SDTv.1.2软件分析了菌株QH1和QH2与从GenBank数据库获得的8个不同苹果树腐烂病菌株的ITS-LSU-EF-TUB2基因拼接序列间的核酸(序



A. YL1，B. QH1，C. QH2；1~6 分别为 PDA 培养 10 d 和 60 d 菌落形态，菌丝、分生孢子、产孢体盘和产孢体横切面形态。

A. YL1，B. QH1，C. QH2; 1-6 are the morphology of colony at 10 d, 60 d on PDA medium, mycelium, conidia, conidiomata, and transverse microtome section of the conidiomata.

图2　病原菌形态特征

Fig. 2　Morphological characteristics of pathogenic fungi

列信息详见表2）的一致性。QH1与其他苹果树腐烂病分离菌株之间的一致性为76.7%~84.5%，QH2与其他苹果树腐烂病分离菌株之间的一致性为76.1%~83.5%（图4）。

构建的多基因联合系统发育树结果表明（图5），进化树分为两个大的分支，QH1与分离自宁夏银川市的苹果树（CFCC 50040）和青海省西宁花红沙果（CFCC 50042）的*C. schulzeri*种聚集在一起，形成一个分支。QH2与山西省太原市山楂树（CFCC 50031）和青海省海东市山荆子（CFCC 50044）的*C. mali*聚集在一起。

3　讨　论

由于病原真菌的形态和生理特性多样，有性型经常缺失，对其病原菌的鉴定非常困难[4]。分子生物学的发展，突破了真菌分类学只基于形态特征难于区分的瓶颈。与传统的形态分类法相比，基于单个保守基因的分子分类方法具有简便性、客观准确性、使用方便性、鉴定无限制性等特点，在研究生物进化和物种起源方面具有独特的优势[24]。然而，对大量复合种分析发现，仅仅基于单个基因是很难区分的，如ITS基因在鉴定间座壳属（*Diaporthaceae*）、炭疽菌属（*Colletotrichum*）等一些属的相近种上存在问题[25-26]。Vu等[27]研究认为，丝状真菌在属、科、目和类群水平的分类学阈值分别为94.3%、88.5%、81.2%和80.9%。因此仅依靠ITS单个基因对腐烂病菌从类群到种进行分类有一定的局限性[28-29]。随后LSU、EF和TUB2等保守基因也陆续被用于基于形态学、ITS基因序列不能够区分开的真菌种的分类中，近年来越来越多的研究者采用形态学结合多

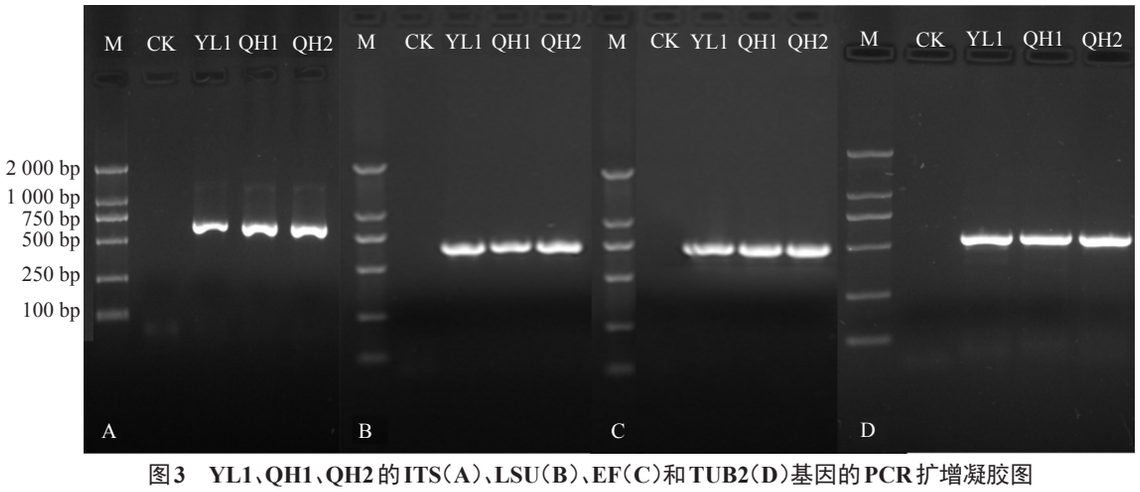


图3　YL1、QH1、QH2的ITS（A）、LSU（B）、EF（C）和TUB2（D）基因的PCR扩增凝胶图

Fig. 3　PCR amplification gel diagram of ITS(A), LSU (B), EF (C) and TUB2(D) genes of YL1, QH1, and QH2

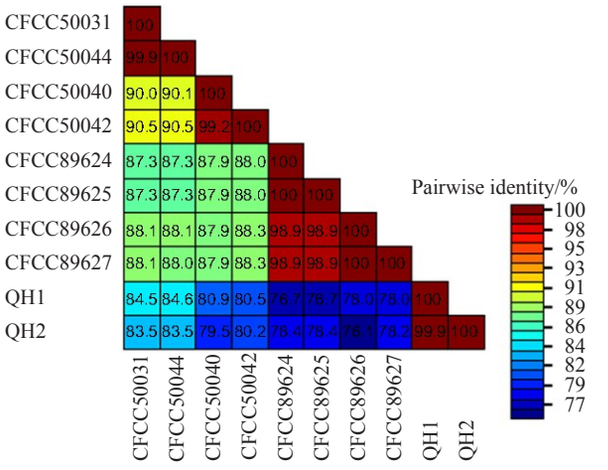


图4 QH1、QH2与其他苹果树腐烂病分离株间基因组核酸一致性矩阵

Fig. 4 Matrixes of nucleotide identity between QH1 and QH2 and other apple tree canker isolates

基因联合分析的方法对真菌进行分类。由此可以看出，形态学结合多基因联合分析对真菌分类更具科学性。

王旭丽[30]发现分离自同一果园内的在分类上属于同一个种的不同菌株在菌落形态特征上也存在多样性，在本研究中QH2与参照菌株YL1虽然都符合*C. mali*的形态描述，但两者在菌落颜色、子实体形态上有较明显的差别。同样QH1符合*C. schulzeri*的形态描述，但在培养形态上，与已报道的分离自新疆的*C. schulzeri*菌株在培养形态上也有差别[8]。结合王旭丽[30]的研究结果可以推测地理环境、培养条件等因素的差异均可以对病原菌的形态特征产生影响。

QH1、QH2与从其他地区分离得到的苹果腐烂

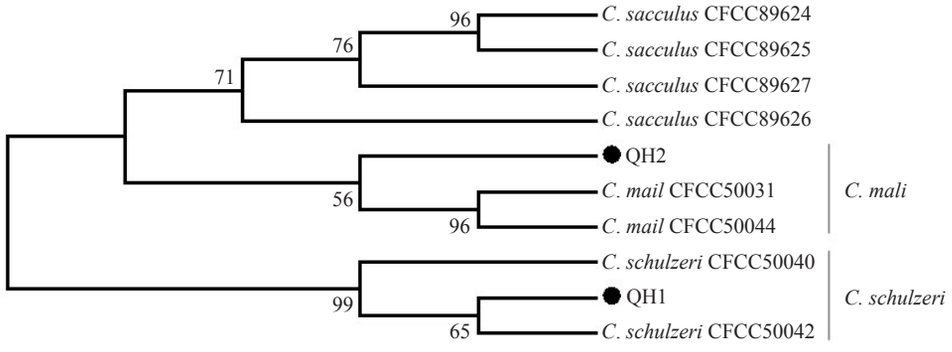


图5 苹果树腐烂病的多基因序列（ITS-LSU-EF-TUB2）系统发育树

Fig. 5 Multi-locus gene phylogram of apple tree canker based on ITS-LSU-EF-TUB2 sequences

病病菌ITS-LSU-EF-TUB2基因拼接序列间的核酸一致性为76%~84%，一致性较低，存在较大核酸水平的变异，这可能与内蒙古地区特殊的地理、环境条件相关。但基于多基因联合分析的系统进化树证明QH1和QH2在分类上仍然分别属于*C. schulzeri*和*C. mali*，均属于中国分布最广的主要致病菌类型[6]，并且具有与YL1相似的致病性，证明QH1和QH2均为致病性菌株。西北黄土高原、黄河故道、环渤海湾、西南冷凉高地等产区苹果均开展过基于形态学结合多基因联合分析的苹果腐烂病病原菌鉴定工作，本研究是内蒙古地区的首次基于形态学结合多基因联合分析对苹果腐烂病病菌进行鉴定的研究，是对上述研究的有力补充。研究中获得的ITS、LSU、EF、TUB2基因丰富了苹果腐烂病病菌的基因库，为苹果腐烂病病菌多样性研究提供了参考序列；分离获得的病原菌为进一步开展内蒙古地区针对苹果腐烂病病菌的药效试验，和病原与寄主互研究提供了参考菌株。对内蒙古其他地区果园苹果腐烂病病菌的分离、鉴定以及是否存在种群上的多样性和优势种的类型，将是下一步工作的重点。

4 结 论

本研究通过生物学结合分子生物学方法首次证明呼市地区苹果腐烂病菌分别属于*C. schulzer*和*C. mali*，两种病原菌均具有强致病性。丰富了我国苹果腐烂病菌的多样性，为开展内蒙古地区腐烂病防治和病原与寄主互作研究奠定基础。

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