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苹果褐腐病拮抗毛壳菌的筛选及鉴定

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摘 要:【**目的**】筛选适合于防治苹果褐腐病(*Monilinia fructigena*)的生防毛壳菌,为苹果采后病害的生物防治提供潜在的菌株材料。【**方法**】选择4株毛壳菌,采用平板对峙法测量抑菌率和抑菌带宽度;通过生防毛壳菌发酵粗提物检测其抑菌活性强弱;通过离体接种苹果果实比较毛壳菌的控病作用。结合形态学特征和核糖体基因内转录间隔区(rDNA-ITS)序列对抑菌活性强的毛壳菌菌株进行鉴定。【**结果**】供试的4株毛壳菌中,菌株24-9在平板对峙试验、发酵粗提物抑菌试验和离体接种试验中均表现出稳定而较强的抑菌活性,通过形态学特征和核糖体基因内转录间隔区(rDNA-ITS)序列构建系统发育树,将菌株24-9鉴定为球毛壳菌 *Chaetomium globosum*。【**结论**】该生防毛壳菌菌株可用于开发苹果采后病害生防制剂,提高植物抗性,丰富我国苹果采后病害的生防菌资源库。

关键词:苹果褐腐病;生防毛壳菌;筛选;鉴定

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Screening and identification of antagonistic *Chaetomium* spp. against *Monilinia fructigena*

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Abstract:【Objective】*Monilinia fructigena* is a common disease in the late growth and storage period of apples. It can cause decay of apple fruit and directly affect its economic value. At present, chemical fungicides are used to prevent and cure them, and long-term use of chemical fungicide pathogens produce drug resistance, while pesticide residues will pollute the environment, break the ecological balance, and also affect human health. *Chaetomium* spp. is a type of fungus with biocontrol potential, and there is no biological control on the brown rot of apples. In order to enrich the biocontrol strains of postharvest diseases of apple trees in China, a series of experiments were carried out to isolate *Chaetomium* spp. from different substrates in Tibet.【Methods】Inhibitory effect of *Chaetomium* spp. against *M. fructigena* was determined by performing an intra-plate antagonistic test. The biocontrol of *Chaetomium* spp. and *M. fructigena* were inoculated on PDA plates, respectively. The biocontrol agent was located 4.5 cm apart from the pathogen. The growth radius of *M. fructigena* and the growth radius of *M. fructigena* fungus were treated respectively at a temperature of 28 ℃ for 10 days, and the inhibitory rate was calculated. The inhibitory rate of the crude extract of *Chaetomium* spp. on *M. fructigena* was determined by using the drilling method. Under aseptic condition, *M. fructigena* was inoculated in the center of the PDA plate, four holes were punched at a 4.5 cm distance from the pathogen using a 6 mm diameter sterile punch respectively, two holes into the 200 mg · L^{-1} crude wool crude extract of dilution, negative control solution or positive control solution of 150 μL, using the mycelial growth rate method, respectively. The antimicrobial activity of the crude ex-

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tracts of *Chaetomium* spp. against *M. fructigena* was determined on the 5th day, the 7th day and the 9th day. The *in vitro* inoculation method was used to determine the control effect of the fermentation liquid of *Chaetomium* spp. on *M. fructigena*. The apples were neat and healthy. The surface was disinfected with 75% alcohol, and sterile nails were used to evenly puncture 3 to 4 mm deep wounds on each apple, after 1 h we added 20 μL biocontrol bacteria fermentation broth, PDB liquid medium, 50% carbendazim solution, 20 μL PDB liquid medium (negative control into the holes to verify the apple fruit's condition without *M. fructigena*). Three hours later, 20 μL of *M. fructigena* spore suspension with a concentration of 1×10^6 per mL was inserted into the three wells by pipetting. The negative control had no *M. fructigena* pathogen spore suspension, and was then dried and wrapped in polyethylene bags wrapped in moisture, stored at 25 ℃, 7 d after measuring the lesion radius. The strains were inoculated on a cornmeal medium (CMA) and cultured in dark at 28 ℃ for 10 days, and the fruiting body was fully matured. The morphology of the ascus, the shape of the appendages, the morphology of the ascus, the morphology of the ascospores, the color, the position and the number of the germination holes were observed with a Leica microscope DM5000, and the morphology of the subcapsules were observed with a Nikon dissecting microscope. Photographs were taken, combined with colony characteristics, ascospores maturation time for measurement, recording and morphological identification. The genome of the strain was amplified by PCR using ITS-1F and ITS4 of the rDNA-ITS internal transcribed spacer (rDNA-ITS). The PCR reaction system was 25 μL: 10 μL PCR buffer 2.5 μL, DNA template 15 ng, 2.5 $\mu L\cdot L^{-1}$ dNTP 2.0 μL, 1 μL each of 10 $\mu mol\cdot L^{-1}$ primer, 0.2 μL of 5 $U\cdot \mu L^{-1}$ *Taq* enzyme, and finally double-distilled water was used to make up to 25 μL. 35 ℃ for 30 s, 35 ℃ for 30 cycles, and 10 min for 72 ℃. The homologous sequence was searched in GenBank by using Blast. The DNA-ITS sequences of 18 related species were cloned using CLUSTALX and constructed phylogenetic trees with MEGA 5.10 under 1 000 replicates (*Melanocarpus thermophile* was selected as outgroup).【Results】The antagonistic effect of *Chaetomium* sp. 24-9 on the growth of *M. fructigena* was the best at the 3rd day, and the inhibition effect was the most obvious at the 7th to 9th days after culture. The inhibition rate to *M. fructigena* was 40.00%, and the inhibition band width to pathogen was 2.0 cm. The results showed that the inhibitory effect of 30 $g\cdot L^{-1}$ 60% carbendazim was 66.67%. The apple pieces treated with the fermentation liquid of *Chaetomium* sp. 24-9 showed good control effects after 4 days of inoculation, and the inhibition rate to *M. fructigena* reached 62.18%. The other three strains of *Chaetomium* spp. in the control of *M. fructigena* showed no significant effects, with the inhibition rates below 30%. The results showed that the growth rate was 7-8 mm, and the aerial mycelium was sparse, with light olive color secretions sporule fruit surface health, spherical to obovate, diameter (232-304) μm×(261-350) μm, with a fixed orifice, ascomycetes began to mature after 7 d, the subcapsular fruit wall in the reflected light was light brown; the fruit wall cell polygonal shape was not corolla, with a separation, the base width of about 3.75 μm; subcapsular clavate, clustered, with stalk, apex acuminate, adaxially glabrous, and adaxially glabrous. Cysts spores were brown, lemon-shaped, smooth on both sides, and with both ends of the protrusions, (7.4-9.8) μm×(9.3-13.5) μm, there is an obvious terminal germination hole. The isolate was identified as *C. globosum* according to its morphological characteristics. DNA was extracted from *C. globosum* 24-9 and amplified with primers ITS-1F and ITS4 to obtain a target fragment of about 600 bp length. The nucleotide sequence of 567 bp was obtained by sequencing. The sequence was submitted to GenBank (accession number: KY132127). The phylogenetic relationship between *C. globosum* and *Chaetomium* sp. 24-9 was confirmed by the homology alignment of the rDNA-ITS sequences of 18 related species.【Conclusion】The results indicated that *Chaetomium* sp. 24-9, which had good antagonistic activity against *M. fructigena*, was identified by laboratory tests. The results indicated that the strain could be

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used as an effective material for apple prevention of postharvest diseases.

Key words: *Monilinia fructigena*; *Chaetomium* spp.; Screening; Identification

苹果是我国北方种植的主要水果，具有耐贮藏、市场供应周期长的特点，成为世界上很多国家的主要消费果品[1]，但我国参与国际市场贸易的苹果仅占水果总量的3%~4%，这与国际平均水平9%~10%还相差甚远，其中病虫害问题和采后产业滞后是我国苹果进入国际市场的约束因子之一[2-3]。

苹果褐腐病是苹果生长后期和贮藏期中的一种常见病害，主要由无性阶段丝孢纲丝孢目丛梗孢属的4种病原菌 *Monilinia fructicola*、*M. fructigena*、*M. iaxa*、*M. polystroma* 所引发。其中，仁果丛梗孢 *M. fructigena* 在我国苹果产区广泛分布[4]，该病原菌可引起苹果果实腐烂，直接影响其经济价值，也是美国、加拿大、澳大利亚等国的检疫对象。目前，主要采用化学杀菌剂，如波尔多液、甲基托布津等对苹果褐腐病进行防治[5]。然而，长期大量使用化学杀菌剂致使病原菌产生抗药性，同时农药的残留还会污染环境、破坏生态平衡，影响人类健康。与化学杀菌剂相比，生物防治是一种高效、无毒、无污染的防治策略，且不易使病原菌产生抗药性，开发和利用生物农药引起了人们的广泛关注[6]。近年来，我国在苹果褐腐病的生物防治方面也开展了一些研究，李海燕等[7]研究发现马兜铃提取物对苹果褐腐病菌菌丝生长的抑制效果能达到49.64%；郭松年等[8]研究表明丁香提取物对苹果褐腐菌有较好的抑制作用；李鹏霞等[9]研究发现丁香精油和丁香酚在苹果采后抑菌防腐和保鲜方面发挥重要作用。

毛壳菌是一种极具生防潜力的真菌，能够产生大量的纤维素酶[10]，可以抑制病原菌生长和发挥生物防治的作用[11-12]。现已发现有些毛壳菌，如球毛壳（*Chaetomium globosum*）、角毛壳（*C. cupreum*）可以用来防治小麦叶锈菌、苹果黑斑病、稻瘟病菌及多种植物种苗猝倒病[13-14]。由此可见，毛壳菌对许多植物病原菌有潜在的生防作用，笔者利用从西藏不同基质上分离到的毛壳菌，通过系列试验，筛选出对苹果褐腐病具有防效的毛壳菌，对丰富我国苹果采后病害的生防菌株具有重要意义。

1　材料和方法

1.1　菌株来源

苹果褐腐病菌（*Monilia fructigena*），毛壳菌24-9、24-28、LZF0006、LZZ0012均来自西藏农牧学院植物病理学实验室，拮抗菌株信息来源见表1。

表1　拮抗毛壳菌菌株来源

Table 1　Antagonistic origin of *Chaetomium* spp. strains

毛壳菌菌株号 Strain number of *Chaetomium* spp.	基物 Substrate	采集地 Acquisition site	海拔 Altitude/m
24-9	树叶 Leaf	林芝排龙 Pailong village, Nyingchi city	2 030
24-28	朽木 Dead wood	林芝排龙 Pailong village, Nyingchi city	2 030
LZZ0012	杨树叶 Poplar leaves	林芝高原生态研究所 Nyingchi Institute of Plateau Ecology	2 982
LZF0006	牛粪 Cow dung	林芝章乡村 Zhangxiang village, Nyingchi city	3 026

1.2　仪器及试剂

1.2.1　*主要仪器*　低温冰箱（海尔）、双人单面水平净化工作台（SF-CJ-20）、扩增仪PTC-200TM PCR、电泳仪DY1002V、自动双重纯水蒸馏器、凝胶成像系统UVIDBT-08、恒温水浴锅、电子天平（JA10003N）、Nikon体式解剖镜，德国徕卡系统显微镜DM5000。

1.2.2　*主要试剂*　异丙醇、无菌水、超纯水、Tris饱和酚、乙醇、氯仿、异戊醇、冰醋酸、十六烷基三甲基溴化铵（CTAB）、氢氧化钠（NaOH）、氯化钠（NaCl）等，二水乙二胺四乙酸二钠（$Na_2EDTA \cdot 2H_2O$）、PCR扩增所用的试剂均购自TaKaRa宝生物工程有限公司。

1.3　皿内拮抗试验

将活化的生防毛壳菌、苹果褐腐病菌用直径为6 mm的无菌打孔器打孔，并分别接种在PDA平板上，生防毛壳菌和苹果褐腐病菌相距4.5 cm，同时接种，以只接种苹果褐腐病菌的处理作为对照，各3次重复。28 ℃恒温培养10 d，分别在培养的第3、5、7和9天测量对峙试验和对照试验的苹果褐腐病菌的

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生长半径，计算生防毛壳菌对苹果褐腐病菌的抑制率，计算公式：抑制率/%=（对照苹果褐腐病菌的生长半径-对峙试验中苹果褐腐病菌的生长半径）/对照苹果褐腐病菌的生长半径×100。

1.4 毛壳菌发酵粗提物的抑菌试验

1.4.1 菌株发酵 一级种子培养基：称取200 g去皮马铃薯，切成小块，在500 mL蒸馏水中煮沸20 min，用纱布过滤后，在滤液中加入20 g葡萄糖、3 g KH_2PO_4、1.5 g $MgSO_4·7H_2O$、1.5 g酵母膏，用蒸馏水补足至1 000 mL，用NaOH将水溶液的pH调至5.8~6.0。

二级培养基：在大米中加入0.3%的蛋白胨，混合均匀，121 °C灭菌30 min。

将生防毛壳菌在无菌条件下接种到已灭菌的一级培养基中，28 ℃、140 $r·min^{-1}$振荡恒温培养3 d，得到一级种子。按10%的接种量将一级种子接种到二级培养基中，摇匀，28 °C恒温培养25 d。

1.4.2 毛壳菌粗提物的提取 室温下用等体积的乙酸乙酯萃取发酵25 d的毛壳菌固态大米培养基3次，收集乙酸乙酯相，用旋转蒸发仪于45 ℃下蒸干，得到淡黄色浸膏，用二甲基亚砜（DMSO）溶解，得到质量浓度为1 $g·L^{-1}$的发酵物浓缩液。

1.4.3 毛壳菌发酵粗提物的抑菌试验 将1 $g·L^{-1}$的毛壳菌粗提物母液用DMSO配置成200 $mg·L^{-1}$的稀释液[14]，将不含生防毛壳菌浓缩液、只含DMSO的稀释液作为阴性对照，以质量浓度为30 $g·L^{-1}$的60%的多菌灵溶液为阳性对照。在无菌条件下，将苹果褐腐病菌菌饼接种在PDA平板中央，在距病原菌4.5 cm处用直径为6 mm的无菌打孔器分别打孔4个，孔中分别两两注入200 $mg·L^{-1}$的毛壳菌发酵粗提物的稀释液、阴性对照液或阳性对照液各150 μL，采用菌落生长速率法，分别在培养的第5、7和9天测定毛壳菌粗提物对苹果褐腐病菌的抑菌活性，计算方法同1.3。

1.5 毛壳菌发酵液对苹果褐腐病的控病作用

参照Bi等[15]的方法，选择外观整齐健康的苹果果实，用75%（φ）酒精进行表面消毒，将每个苹果均匀切成2块，用灭菌铁钉（直径3~4 mm）在每块苹果上均匀刺3~4 mm深的伤口4个，1 h后在其中的2个孔中分别加入20 μL生防菌发酵液、PDB液体培养基，另外2个孔中分别加入30 $g·L^{-1}$的60%的多菌灵溶液、20 μL PDB液体培养基（阴性对照，验证苹果果实本身不带褐腐病菌），3 h后用移液器在其中的3个孔中分别接入每mL $1×10^6$个的苹果褐腐病菌孢子的悬浮液20 μL，阴性对照的孔中不加苹果褐腐病菌孢子悬浮液，晾干后放入聚乙烯袋中密封保湿，25 ℃贮存4 d后测定病斑半径，每个处理10次重复。

抑制率/%=（对照的病斑的半径-处理的病斑半径）/对照的病斑半径×100。

1.6 生防毛壳菌的鉴定

1.6.1 生防毛壳菌的形态学鉴定 将1.3、1.4和1.5试验中筛选出的生防效果好的毛壳菌进行鉴定，形态学鉴定以Arx等[16]的系统为主要基础和依据。将毛壳菌菌株接种到玉米粉培养基（CMA）平板上，28 ℃黑暗培养10 d，定期观察直至子实体完全成熟。在Nikon体式解剖镜下挑取单个子囊果，制成水玻片，用Leica显微镜DM5000观察子囊果形态，附属丝形态，子囊的形态，子囊孢子的形态、颜色、萌发孔位置和数量等特征，并进行拍照，结合菌落特征、子囊果成熟时间进行测量、记录和鉴定。每个菌株测量20个个体。

1.6.2 生防毛壳菌的分子鉴定 生防毛壳菌DNA的提取方法主要参考甘丽萍等[17]的方法，并加以改进。用真菌核糖体基因内转录间隔区（rDNA-ITS）通用引物ITS-1F（5’-CTTGGTCATTTAGAGGAAG-TAA-3’）和ITS4（5’-TCCTCCGCTTATTGATATGC-3’）（上海美吉生物有限公司合成）对菌株的rDNA-ITS区进行PCR扩增，PCR反应体系25 μL：其中10×PCR缓冲液2.5 μL，DNA模板15 ng，2.5 $mmol·L^{-1}$ dNTP 2.0 μL，10 $\mu mol·L^{-1}$引物各1 μL，5 $U·\mu L^{-1}$ *Taq*酶0.2 μL，最后加双蒸水补足至25 μL。反应程序：95 ℃预变性3 min；95 ℃变性30 s，53 ℃退火40 s，72 ℃延伸30 s，扩增35个循环；最后72 ℃延伸10 min。

PCR产物的纯化和序列测定由上海美吉生物有限公司完成，利用Blast将测序结果在GenBank中进行同源序列查找，选择18个相关种的rDNA-ITS序列，使用CLUSTALX对排，用MEGA 5.10在重复1 000次下构建系统进化树（选择*Melanocarpus thermophile*为外群）。

2 结果与分析

2.1 皿内拮抗试验

从表2可以看出，毛壳菌24-9在苹果褐腐病菌

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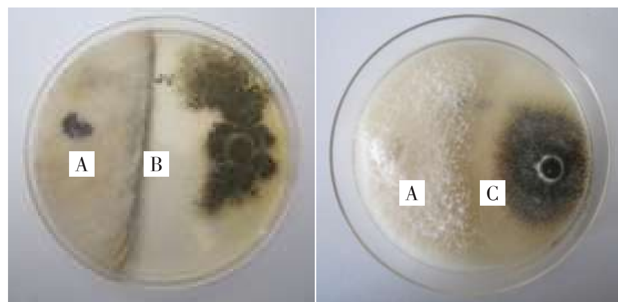
表2　4株毛壳菌对苹果褐腐病菌的抑菌率

Table 2 Inhibition rate of 4 strains of *Chaetomium* spp. on *M. fructigena*

培养时间 Culture time/d	毛壳菌抑制率 Inhibition rate of *Chaetomium* spp./%			
	24-9	24-28	LZZ0012	LZF0006
3	12.00	2.40	4.00	4.00
5	27.03	5.41	27.02	8.11
7	40.00	22.22	37.78	22.22
9	40.00	22.22	37.78	22.22

培养第3天时表现出抑菌效果，而且随着培养时间延长至7~9 d时，抑菌效果最为明显而稳定，对苹果褐腐病菌的抑菌率达40.00%，抑菌效果最好；毛壳菌LZZ0012在苹果褐腐病菌培养第3天时抑菌效果不明显，但是随着培养时间的延长，抑菌效果逐渐稳定，在病原菌培养7~9 d时抑菌率为37.78%；毛壳菌24-28和LZF0006对苹果褐腐病菌的抑菌率，随着培养时间的延长，抑菌作用虽然逐渐增强，但抑菌率最高仅为22.22%。同时，从图1可以看出，毛壳菌24-9与LZZ0012在与苹果褐腐病菌对峙培养时，均形成明显抑菌带，经测量，毛壳菌24-9对病原菌的抑菌带宽度达到2.0 cm，而毛壳菌LZZ0012的抑菌带宽度仅为0.4 cm。



A. 苹果褐腐病菌；B.毛壳菌 24-9；C.毛壳菌 LZZ0012。

A. *M. fructigena*; B. *Chaetomium* sp. 24-9; C. *Chaetomium* sp. LZZ0012.

图1　毛壳菌24-9和LZZ0012对苹果褐腐病的抑菌效果

Fig. 1 Inhibition effects of *Chaetomium* spp. 24-9 and LZZ0012 on *M. fructigena*

2.2　生防毛壳菌发酵粗提物的抑菌效果

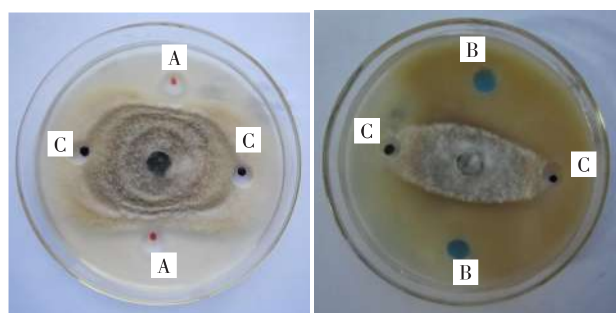
从表3可以看出，毛壳菌24-9的发酵粗提物对苹果褐腐病菌表现出较好的抑菌效果，抑制率达到44.40%，阳性对照的抑菌效果为66.67%；病原菌培养至第7天时，毛壳菌LZF0006抑菌效果逐渐增强，但在病原菌培养至第9天时抑菌效果消失；病原菌培养至第5天时，毛壳菌24-28和LZZ0012对病原菌没有抑菌效果，反而显示促进效果，在病原菌培养第7天时，表现出较弱的抑菌效果，抑制率仅为11.11%，但在病原菌培养第9天时抑菌效果消失。

表3　4株毛壳菌发酵粗提物对苹果褐腐病菌的抑菌率

Table 3 Inhibition rate of crude extract of 4 *Chaetomium* spp. strains on *M. fructigena*

培养时间 Culture time/d	各处理的抑制率 Inhibition rate of different treatments/%				
	24-9	24-28	LZZ0012	LZF0006	阳性对照 Positive control
5	20.00	-8.00	-4.00	20.00	44.00
7	44.40	11.11	11.11	33.33	66.67
9	44.40	0.00	0.00	0.00	66.67

从图2可以看出，毛壳菌24-9的发酵粗提物对苹果褐腐病菌具有明显的抑菌作用，当病原菌生长至注有该毛壳菌发酵粗提物的小孔时，停止生长。



A. 毛壳菌 24-9 发酵粗提物；B. 阳性对照；C. 阴性对照。

A. Crude fermentation extract from *Chaetomium* sp. 24-9; B. Positive control; C. Negative control.

图2　毛壳菌24-9发酵粗提物对苹果褐腐病的抑菌效果

Fig. 2 Antibacterial effect of crude fermentation extract from *Chaetomium* sp. 24-9 on *M. fructigena*

2.3　毛壳菌发酵液对苹果褐腐病的控病效果

从表4可以看出，在控病试验中，通过毛壳菌24-9发酵液预先处理过的苹果块，在离体接种4 d后，表现出较好的控病效果，对苹果褐腐病的抑制率达到62.18%，其他3株毛壳菌在控制苹果褐腐病上均无显著作用，抑制率均在30%以下。从图3也可明显看出，预先接种过毛壳菌24-9发酵液的苹果块，再接种病原菌后，病斑半径明显小于未预先接种毛壳菌发酵液的处理。

表4　接种4 d后4株毛壳菌对苹果褐腐病的控病效果

Table 4 Control effect of 4 days after inoculation of 4 strains of *Chaetomium* spp. on *M. fructigena*

	24-9	24-28	LZZ0012	LZF0006	阳性对照 Positive control
控病率 Control effect/%	62.18	15.63	28.57	6.55	100.00

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A. 毛壳菌 24-9 发酵液；B. 阴性对照；C. 阳性对照。

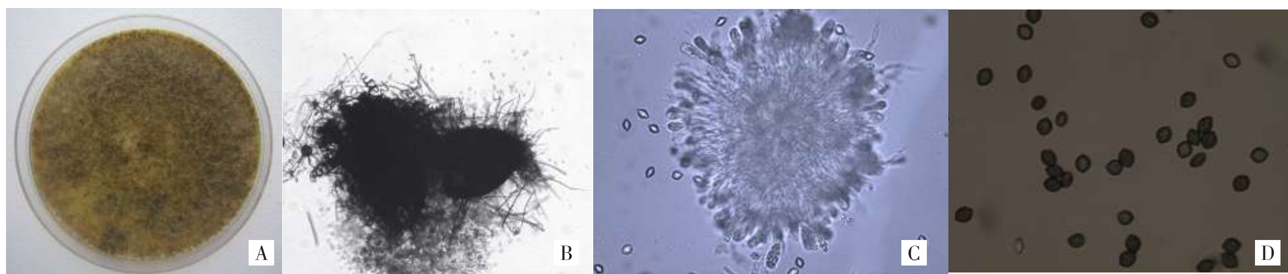
A. Fermentation liquid of *Chaetomium* 24-9; B. Negative control; C. Positive control.

图 3　毛壳菌 24-9 发酵液对苹果褐腐病的控病效果

Fig. 3 Control effect of fermentation liquid of *Chaetomium* 24-9 on *M. fructigena*

2.4　生防毛壳菌的形态学鉴定

采用Arx等[16]的鉴定方法，对具有较好防效的生防毛壳菌24-9进行鉴定（图4），该菌株具有以下特点：菌落浅橄榄绿色，日生长速率7~8 mm，气生菌丝稀疏，有浅橄榄色分泌物溢出；子囊果表生，球形至倒卵形，直径（232~304）μm×（261~350）μm，有固定孔口，子囊果7 d后开始成熟，子囊果果壁在反射光下呈浅褐色；果壁细胞多边形状至不规则形，有棱角，4~5 μm；附属丝多，周生，直或弯曲，波浪状，褐色，具疣状突起，具分隔，基部宽约3.75 μm；子囊棍棒状，簇生，具柄，内含8个子囊孢子，容易消解，44 μm×（27~31）μm×（12.5~17.0）μm；子囊孢子褐色，柠檬形，两侧平滑，两端突起，（7.4~9.8）μm×（9.3~13.5）μm，有1个明显的顶生萌发孔，根据形态学特征将该菌鉴定为球毛壳菌 *Chaetomium globosum*。该毛壳菌来源于土壤分离物，采集于西藏林芝市通麦大桥沿线的树叶，当地海拔2 030 m。



A. 菌落形态；B. 子囊果形态（×100）；C. 子囊形态（×400）；D.子囊孢子形态（×1 000）。

A. Colony morphology; B. Ascomata morphology（×100）; C. Appearance of ascus（× 400）; D. Ascospores morphology（×1 000）.

图 4　毛壳菌 24-9 的形态特征

Fig. 4 Morphological characteristics of *Chaetomium* sp. 24-9

2.5　生防毛壳菌的分子鉴定

对毛壳菌24-9进行DNA的提取，用引物ITS-1F和ITS4进行扩增后得到长度600 bp左右的目的片段，测序获得567 bp核苷酸序列，将测定的序列提交GenBank（登录号：KY132127），通过选择18个相关种的rDNA-ITS序列进行同源序列比对，用MEGA 5.10软件构建系统发育树，从图5可以看出，毛壳菌24-9与 *C. globosum*（KY132140、FN868476、GU563374、KY132166）在99%水平上，相聚为一群，从分子水平上证明了毛壳菌24-9与球毛壳菌 *C. globosum* 的亲缘关系最近。

3　讨　论

在皿内拮抗试验中，苹果褐腐病菌培养至第3天时，4株毛壳菌对其均没有明显抑菌作用，主要因为此时毛壳菌在生长速率上不占优势，当毛壳菌和苹果褐腐病菌培养至7~9 d时，4株毛壳菌生长成熟，开始释放抑菌物质，发挥出对病原菌的抑制作用，但总体来说，毛壳菌24-9在生长速率和抑菌作用上都表现出优势。在毛壳菌发酵粗提物的抑菌试

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括号中为相关菌株在 GenBank 中的登录号,分支处数值表示自举值。尺标表示每个核苷酸位点上的 0.1 替换值。

Numbers in parentheses represent the accession numbers of the related isolates in GenBank, the numbers in each branch points denote the percentages supported by bootstrap. Scale bar represents 0.1 substitutions per nucleotide position.

图5 基于 rDNA-ITS 序列构建的毛壳菌 24-9 的系统发育树

Fig. 5 Phylogenetic tree of *Chaetomium* sp. 24-9 constructed based on rDNA-ITS sequences

验中,毛壳菌 LZZ0012、24-28 和 LZF0006 在病原菌生长至第7天时表现出较弱的抑菌活性,但随着病原菌培养时间的延长,抑菌活性逐渐消失,而毛壳菌24-9在该试验中同样表现出稳定而明显的抑菌效果。在离体控病试验中,4株毛壳菌对苹果褐腐病均表现出不同程度的抗病作用,经毛壳菌24-9发酵液处理过的苹果块虽然发病,但是病斑半径最小,说明该毛壳菌具有抵抗病原物侵入寄主的作用,可用于苹果运输和贮藏期间褐腐病的预防。

我国苹果出口在国际果品市场上占据了一席之地,但是严重的绿色贸易壁垒问题,要求出口苹果必须带有绿色环境标志,其资格审核极为严格[18],成为我国苹果对外贸易的最大障碍,而苹果褐腐病在我国主要苹果产区广泛分布[19],也是加拿大、美国、澳大利亚等国的进境检疫对象,因此研发有效的采后处理技术,减小出口苹果传带苹果褐腐病的风险,可以保障苹果出口贸易健康发展[20],扩大苹果出口规模,提高我国出口苹果在国际市场中的竞争力。目前,我国主要用植物提取物对苹果褐腐病进行生物防治,而植物提取物受植物生长周期、季节等因素的影响,而且在植物中含量有限、提取工艺复杂、研发成本较高,而利用真菌发酵物防治苹果采后病害具有发酵技术容易操作、周期短、研发成本低、不受时间和季节限制的特点,在生产中更易推广和应用。

笔者通过系列试验发现毛壳菌24-9在抑制苹果褐腐病上发挥着比较明显而稳定的抑菌作用,该菌可用来开发生物制剂用于苹果采后病害的预防,也可以提高植物的抗病能力,这与印敬明等[18]的研究结果相一致,他们发现毛壳菌能够诱导植物某些组织抗氧化能力的增强,从而提高植物的抗病能力。本试验筛选出的球毛壳菌24-9,其发酵粗提物也具有明显的抑菌效果,可丰富我国苹果褐腐病的生防菌株材料。

4 结 论

通过室内试验筛选出的对苹果褐腐病具有较好拮抗作用的毛壳菌24-9,经过形态学与分子鉴定确定为球毛壳菌,具有较好的生防潜力,可将其进一步开发用于苹果采后病害的预防。

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