

## 核桃炭疽病原菌鉴定及生物学特性

王清海<sup>1,2</sup>,刘幸红<sup>2</sup>,范昆<sup>3</sup>,段春华<sup>2</sup>,牛贍光<sup>2</sup>,吴小芹<sup>1\*</sup>

1. , , 210037
2. , 250014
3. , 271018

摘要:

TS-09  
rDNA-ITS ACT  $\beta$ -tub2 GPDH TS-09  
*Colletotrichum gloeosporioides* TS-09 PDA 25~30  
28 pH4.0 pH7.0  
60 15 min 62

10 min

关键词:

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## Identification and Biological Characteristics of Pathogen from *Colletotrichum gloeosporioides*

WANG Qing-hai<sup>1,2</sup>, LIU Xing-hong<sup>2</sup>, FAN Kun<sup>3</sup>, DUAN Chun-hua<sup>2</sup>, NIU Shan-guang<sup>2</sup>, WU Xiao-qin<sup>1\*</sup>

1. Co-innovation Center for Sustainable Forestry in Southern China, College of Forestry/Nanjing Forestry University, Nanjing 210037, China
2. Shandong Provincial Academy of Forestry, Ji'nan 250014, China
3. Institute of Pomology, Tai'an 271018, China

**Abstract:** Walnut anthracnose disease is a kind of serious disease of walnut all over the world. In this paper, the identification and the biological characteristics of strain TS-09 isolated from the walnut fruits were studied. The results showed that the strain TS-09 was identified as *Colletotrichum gloeosporioides* based on its morphologic characters, cultural characteristics and four conserved genes(ribosomal DNA-ITS, ACT,  $\beta$ -tub2 and GPDH)sequences analysis and the pathogenicity was powerful. For the growth of strain TS-09, the best medium was PDA. The optimum temperature was 28 and the optimum pH for mycelium growth was 7.0. The condition of weak acid was favorable for spore production (pH 4.0). During continuous illumination, the mycelium growth was inhibited while the condition of continuous darkness was better for mycelium growth. The mycelium had stronger resistance against temperature and the lethal temperature for mycelium was 60 (15 min) or 62 (10 min). Our research expected to offer theory support for prediction and effective control of walnut anthracnose.

**Keywords:** *Colletotrichum gloeosporioides*; identification; biology

*Juglans regia* “ ”  
“21 ”  
FAO 2013  
4.25×10<sup>5</sup> hm<sup>2</sup> 4.0×10<sup>4</sup> Hg/hm<sup>2</sup> 1.70×10<sup>6</sup> t 9.13×10<sup>4</sup>  
hm<sup>2</sup> 9.3×10<sup>4</sup> t  
80% 50%  
[1,2] (*Colletotrichum*  
Corda.) [3]  
*Colletotrichum acutatum*  
[4-6] *Colletotrichum kahawae* [7,8] *Colletotrichum*

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作者简介:

(1978-), , , , , (PAPD) . E-mail:wqhhai@126.com

\*通讯作者: Author for correspondence. E-mail:xqwu@njfu.edu.cn

*gloeosporioides* [9,10] *Colletotrichum graminicola*  
 [11]  
*C. acutatum* *C. gloeosporioides* [6] *C.*  
*gloeosporioides* *Colletotrichum fructicola* [12] 2012

# 1 材料与方法

## 1.1 供试材料

## 1.2 核桃病果菌株分离培养

70% 3  
 28 1~2 d  
 PDA

## 1.3 致病性测定

75% 10<sup>5</sup> mL<sup>-1</sup>  
 3 20 μL  
 5 4 3  
 28 1.2

## 1.4 病原菌鉴定

1.4.1 形态特征观察 TS-09 PDA 28 5 d

1.4.2 TS-09 菌株部分保守基因序列分析及系统发育树构建 DNA CTAB  
 1<sup>[13-16]</sup> 25 μL Taq mix 12.5 μL 1  
 μL ddH<sub>2</sub>O 8.5 μL DNA 2 μL 95 5 min 94 0.5 min  
 59 0.5 min ITS 58 GPDH 56 72 1.5 min 36  
 72 7 min 4  
 PCR NCBI BLAST

GenBank <http://www.ncbi.nlm.nih.gov>

DNAMAN

MEGA5.05 Neighbor-Joining NJ  
 Bootstrap 1000

表 1 引物序列  
 Table1 Sequence of the primers

Gene	Primer	Primer sequences
ACT	ACT-512F	5'-ATGTGCAAGGCCGGTTTCGC-3'
	ACT-783R	5'-TACGAGTCCTTCTGGCCCAT-3'
ITS	ITS1	5'-TCCGTAGGTGAACCTGCGG-3'
	ITS4	5'-TCCTCCGCTTATTGATATGC-3'
GPDH	GDF-1	5'-GCCGTCAACGACCCCTTCATTGA-3'
	GDR1	5'-GGGTGGAGTCGTACTIONTGGAGCATGT-3'
β-tub2	βt2a	5'-GGTAACCAAATCGGTGCTGCTTTC-3'
	βt2b	5'-ACCCTCAGTGTAGTGACCCTTGGC-3'

## 1.5 核桃炭疽菌生物学特性测定

1.5.1 不同培养基对菌丝生长和产孢影响测定

PDA	PSA	PMA	OA	Φ9 mm
(Martin)	(SDA)			(CMA)
		10 d	28	5 d
			[17,18]	4 3

1.5.2 温度对菌丝生长和产孢影响测定

20	25	28	30	35	Φ9 mm	PDA	5	15
						17		10 d

1.5.1

1.5.3 pH 值对菌丝生长和产孢影响测定

pH	PDA	pH	PDA
	59		PDA

Φ9 mm	pH	28	5 d	1.5.1
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1.5.4 光照条件对菌丝生长影响测定

24 h	3	28	12 h	1.5.1
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1.5.5 菌株致死温度测定

60	65	10 min	15 min	10	45	50	55	PDA
	28		2 d		1	1		
	10	15 min			4	3		

1.6 数据处理与分析

Spss20.0                      Duncan

2 结果与分析

2.1 核桃病果菌株分离及致病性

100%                      TS-09                      TS-09

TS-09

2.2 核桃炭疽病菌 TS-09 菌株鉴定

TS-09	PDA				
		10.15±0.21×3.37±0.09 μm	1	ACT	rDNA-ITS
4			TS-09	GPDH	β-tub2
			TS-09		TS-09

*C. gloeosporioides*

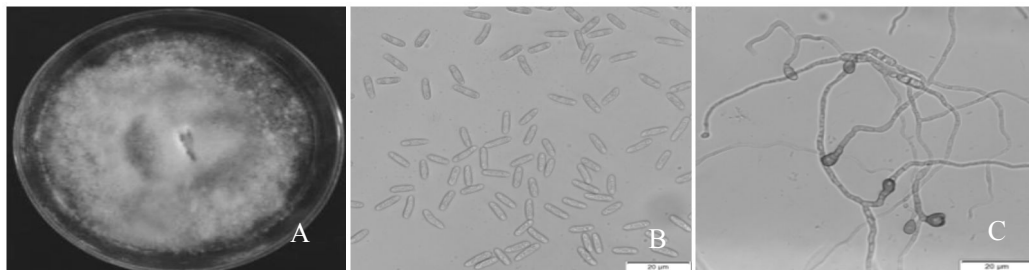


图 1 TS-09 菌株形态特征

A. 菌落培养性状; B. 分生孢子; C. 附着胞

Fig.1 The culture characters of strain TS-09

A. Colony characteristics on PDA for 15d; B. Conidia; C. Appressoria

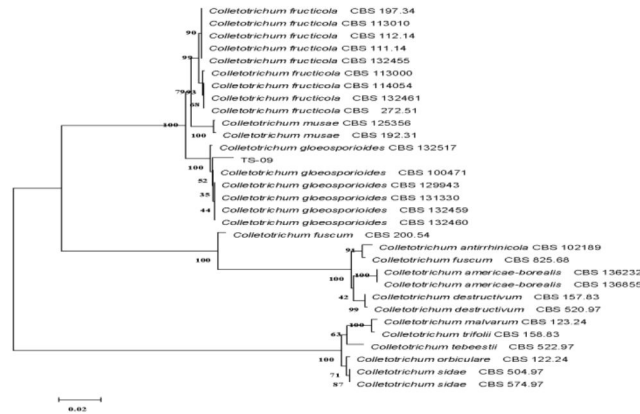


图 2 基于 ACT, rDNA-ITS, GPDH 和  $\beta$ -tub2 序列的 TS-09 菌株系统发育树

Fig.2 Phylogenetic tree of strain TS-09 based on ACT, ITS, GPDH and  $\beta$ -tub2 genes sequences data

2.3 不同培养基对核桃炭疽菌培养性状及菌丝生长的影响

TS-09 3 PDA PMA  
 PSA SDA  
 CMA OA  
 SDA CMA OA Martin

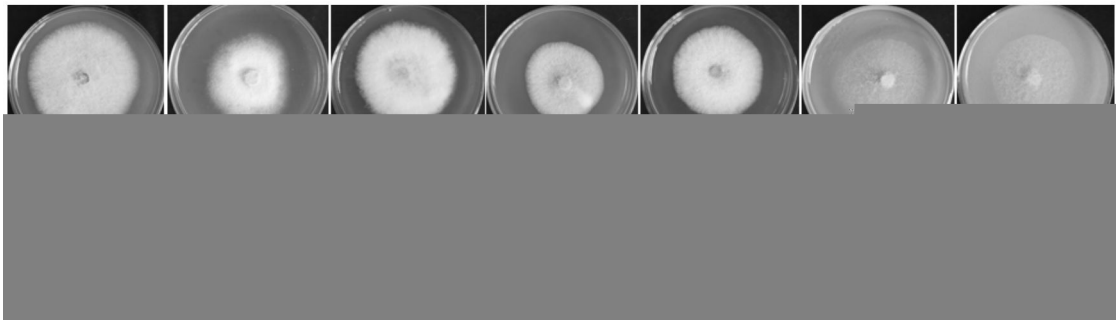


图 3 TS-09 菌株在不同培养基上的形态特征

Fig.3 The morphological characteristics of strain TS-09 on various culture media for 5 d growth

A.PDA, B.PMA, C. PSA, D. Martin, E.SDA, F. OA, G.CMA

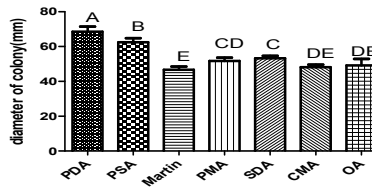


Fig.4 Effects of different medium on colony diameter of strain TS-09.

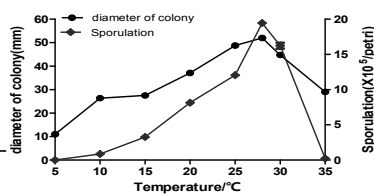


Fig.5 Effect of different temperature on mycelial growth and sporulation of strain TS-09.

4 TS-09 PDA 68.58±1.18 mm  
 P=0.01  
 PSA  
 46.67±0.74 mm  
 Martin

2.4 温度对核桃炭疽菌 TS-09 菌株菌丝生长和产孢的影响

5 TS-09 35  
 5 TS-09 25~30 28  
 7 35 20

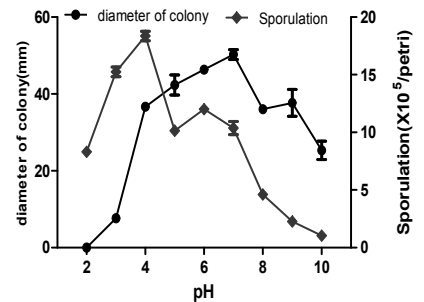


Fig.6 Effect of different pH on mycelial growth and sporulation of strain TS-09.

2.5 pH 对核桃炭疽菌 TS-09 菌株菌丝生长和产孢的影响

TS-09 pH pH3.0~10  
pH7.0 pH4.0 6

2.6 光照条件对 TS-09 菌株菌丝生长的影响

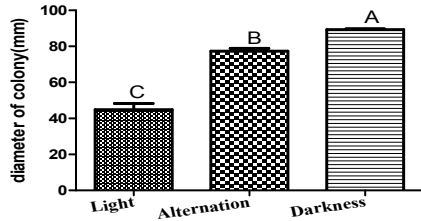


Fig.7 Effects of different light on colony diameter of strain TS-09.

2.7 核桃炭疽菌 TS-09 菌株菌丝致死温度

TS-09 65 10 min 15 min  
P=0.01 TS-09 12 h  
60 10 min 15 min 60 61 62 63  
64 4 TS-09 60 15 min  
62 10 min 2

表 2 菌丝的致死温度  
Table 2 The lethal temperature of mycelium

Time/min	Temperature/								
	45	50	55	60	61	62	63	64	65
10	+	+	+	+	+	-	-	-	-
15	+	+	+	-	-	-	-	-	-

注: + 活的; - 死的。Note: + live; - dead

3 结论与讨论

1790 1831 Corda  
TS-09  
ACT rDNA-ITS GPDH β-tub24  
TS-09 C. gloeosporioides  
TS-09 PDA  
10.15±0.21×3.37±0.09 μm [19]  
Embaby  
C. acutaum C. gloeosporioides [20] C.  
acutatum C. truncatum, C. fructicola C. siamense 4 [21-23]  
TS-09  
TS-09  
28 TS-09 [24]  
24 h

4

7

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