

Research Article



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## 자두 탄저병균의 분리 및 동정

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### Isolation and Characterization of *Colletotrichum* Isolates Causing Anthracnose of Japanese Plum Fruit

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**Abstract**

**BACKGROUND:** Although the filamentous fungal pathogen *Colletotrichum* species causing anthracnose disease on various fruits including peach, apple, persimmon and grape, there is no report on Japanese plum in Korea.

**METHODS AND RESULTS:** In 2016, diseased fruits showing typical anthracnose symptoms of Japanese plum were collected in market and orchards. Diseased tissue was cut off and disinfected subsequently with 70% ethanol for 1 min, and in 1% sodium hypochloride solution for 1 min, followed by three washes with sterile distilled water. The disinfected tissues were placed onto potato dextrose agar (PDA), and incubated at 25°C in the dark for 5 to 7 days. For single-spore isolation, conidia were scraped off the plate using a loop, and suspended with 10 mL sterile distilled water. One hundred microliter of the conidial suspension was spread on PDA plates and incubated at 25°C. Finally, one germinated conidium was transferred onto PDA plates. Morphological and cultural characteries of colonies and spores of isolated *Colletotrichum* were observed after 7 to 10 days incubation on PDA. Molecular identification of

isolates were analyzed by comparing rDNA-ITS gene sequences with NCBI GeneBank.

**CONCLUSION:** Of eleven isolates of *Colletotrichum* isolated from anthracnose diseased Japanese plum fruits, six were identified as *C. acutatum*, and five as *C. gloeosporioides* based on diagnostic characteristics such as colony growth rate, shape and size of conidia, and rDNA-ITS sequences. This is the first report of *Colletotrichum* causing the anthracnose on Japanese plum in Korea.

**Key words:** Anthracnose, *C. gloeosporioides*, *Colletotrichum acutatum*, Japanese plum

**서 론**

(*Prunus salicina* Lindl.)  
 Japanese plum      Chinese plum

			2015
5,920 ha,	67,810 ton	5,803 ha	64,816
ton	2007	가	
	2009		

*Botrytis cinerea*, *Monilinia fructicola*, *Polystigma rubrum*, *Septobasidium tanakae*, *Taphrina pruni*

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, *Podosphaera tridactyla* 가 5 mm PDA  
 (Lee et al., 2012) *Rosellinia necatrix* 25°C 7  
*P. rubrum* 2014 (Nikon Eclipse  
 50i, Japan)  
*Xanthomonas aboricola* pv. *pruni*  
 (Ryu et al., 2012) rDNA - ITS 유전자 염기서열분석에 의한 동정  
 (Choi et al., 2000) 2015 PDA 25°C 7  
 Lee Taylor(1990) genomic  
 가 DNA  
*Colletotrichum* rDNA-ITS White (1990)  
 primer ITS1 5' (TCC GTA GGT GAA CCT GCG  
 G) 3' ITS4 5' (TCC TCC GCT TAT TGA TAT GC) 3'  
 (Jeger and Bailey, 1992; PCR ITS1, 5.8S rRNA gene, ITS2  
 Perfect et al., 1999). 18S 28S rRNA gene 570 bp  
 (Kim et al., 2001; Kim et al., 2002; Lee et al., 2007;  
 Kim and Hong, 2008; Kim et al., 2016; Jeon et al.,  
 2017), BigDye (R)  
 Terminator v3.1 Cycle Sequencing Kits (Applied Biosystems,  
 Thermo Fisher, USA) Primer PCR  
 2016 5 9 DNA Engine Tetrad 2 Peltier  
 Thermal Cycler (BIO-RAD, USA) PCR  
 . PCR dNTP  
 , ABI PRISM 3730XL Analyzer (96 capillary type,  
 Hitachi, Japan) loading  
 NCBI GenBank

**재료 및 방법**

**탄저병균의 분리**

5 mm× 10 PDA  
 5 mm 70% Ethanol 1 (10<sup>5</sup> spore/mL)  
 , 1% NaOCl 3 ( : ) micro pipette (Gilson, P 100, France)  
 filter paper water 20 µL 2 mm  
 agar(1.5%) 25°C  
 potato dextrose agar(PDA, Difco Laboratories) 25°C  
 PDA 6  
 5-7 4 1 2 , 3  
 (10<sup>2</sup> spores/mL) PDA  
 100 µL 25°C 2-3  
 (colony)  
 PDA 25°C  
 4°C  
 cork borer PDA 5 mm  
 7 25°C 가 10

**병원성 검정**

. PDA  
 4  
 (10<sup>5</sup> spore/mL)  
 ( : ) micro pipette (Gilson, P 100, France)  
 20 µL 2 mm  
 25°C  
 PDA 6  
 1 2 , 3

**결과 및 고찰**

**탄저병 발생 및 병징의 특징**

2016 5 9 , ,  
 3 6 가 가 가  
 가 가

**배양적 특성 및 포자형태적인 특징**

PDA 7 가

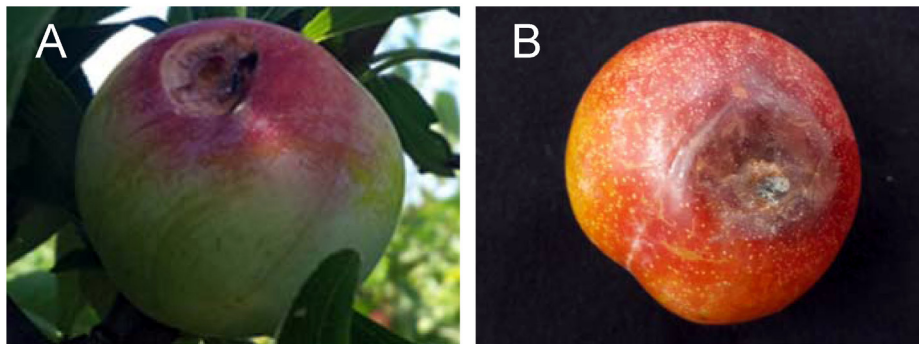


Fig. 1. Anthracnose symptoms on plum fruits collected from orchards (A) and markets (B).



Fig. 2. Anthracnose symptoms on plum fruits artificially inoculated with *Colletotrichum* spp. (A: 71501, B: 92206) isolated from anthracnose of plum fruits.

Table 1. Isolates of *Colletotrichum* spp. isolated from anthracnose of plum fruits

Isolate No.	Region isolated	Cultivar
71501	Gyeongbuk Gunwi	Humoosa
71502	Gyeongbuk Gunwi	Humoosa
91901	Gyeongbuk Yeongcheon	Akihime
92201	Gyeongbuk Gyeongsan	Akihime
92202	Gyeongbuk Gyeongsan	Akihime
92205	Daegu Market	Akihime
92206	Daegu Market	Akihime
92207	Daegu Market	Akihime
92208	Daegu Market	Akihime
92240	Daegu Market	Akihime
M	Daegu Market	Akihime

Table 2. Pathogenicity of *Colletotrichum* spp. isolated from anthracnose of plum fruits

Isolate	Lesion diameter <sup>a)</sup> (mm)
71501	39.3±1.2
71502	38.0±2.0
91901	36.3±1.5
92201	18.7±1.5
92202	19.3±1.2
92205	31.3±1.5
92206	14.7±1.2
92207	18.0±1.0
92208	15.0±1.0
92240	22.3±2.1
M	18.3±1.5

<sup>a)</sup> Lesion diameter was measured six days after artificial inoculation. The diameter represent means ± standard deviations of three replicates.

가 가  
가 ,  
가 (Fig. 1).

3  
6  
가 . 71501, 71502, 91901 92205  
가  
, 9226 가

병원균의 분리 및 배양적 특성  
(Table 1)

(Fig. 2).  
71501, 71502,

**Table 3. Cultural characteristics of the *Colletotrichum* spp. isolated from anthracnose of plum fruits. Cultures of each isolate were inoculated on potato dextrose agar for 7 days at 25**

Isolate No.	Aerial mycelium, color	Reverse color	Mycelium growth (mm)	Mycelium growth (mm/day)
71501	dense cottony, gray to dark gray	white gray	79.9±3.2	11.4±0.5
71502	dense cottony, gray	white gray	78.4±3.5	11.2±0.5
91901	dense cottony, gray	white gray	70.2±3.2	10.0±0.5
92201	sparse, gray, more or less colourless towards edge	dark gray	43.4±2.2	6.2±0.3
92202	sparse, pale gray	light orange	54.7±1.0	7.8±0.1
92205	dense cottony, gray	dark gray	82.6±2.5	11.8±0.4
92206	sparse, dark red purple, colourless edge	red purple	54.2±4.0	7.7±0.6
92207	sparse, gray, more or less colourless towards edge	light yellow to gray	58.9±2.3	8.4±0.3
92208	sparse, gray, more or less colourless towards edge	gray	55.1±1.8	7.9±0.3
92240	sparse, pale orange	pale orange	78.1±2.6	11.2±0.4
M	sparse, dark gray, more or less colourless towards edge	orange	55.3±1.3	7.9±0.2

91901 92205 가 92206 가 (Table 2).

**병원균 동정 및 병원성**

*Colletotrichum*

(Sutton, 1980; Freeman *et al.*, 1998; Kim *et al.*, 2006; Sato *et al.*, 2013).

가 가 (Freeman *et al.*, 1998; Sato *et al.*, 2013) RAPD ribosomal DNA-ITS

(Abang *et al.*, 2002; Martinez-Culebras *et al.*, 2003; Talhinhas *et al.*, 2005; Weir *et al.*, 2012; Sato *et al.*, 2013; Jeon *et al.*, 2017).

*C. gloeosporioides*

가 *C. acutatum*, *C. gloeosporioides*, *C. acutatum*

(Bernstein *et al.*, 1995; Shi *et al.*, 1996; Freeman *et al.*, 1998).

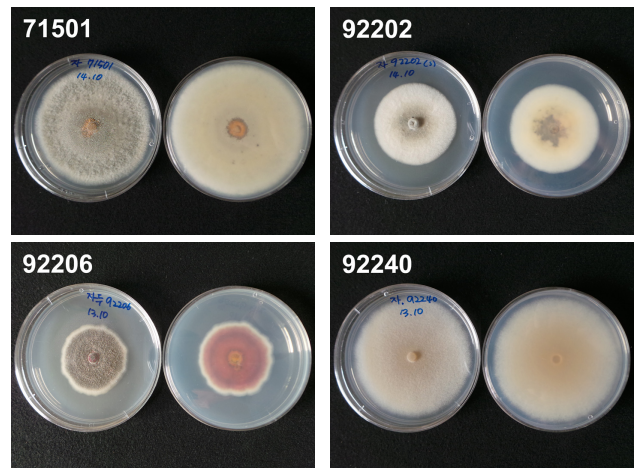
PDA 25°C 7

4 group

(Table 3). Humoosa 71501, 71502

Akihime ( ) 91901, 92205

(Fig. 3).

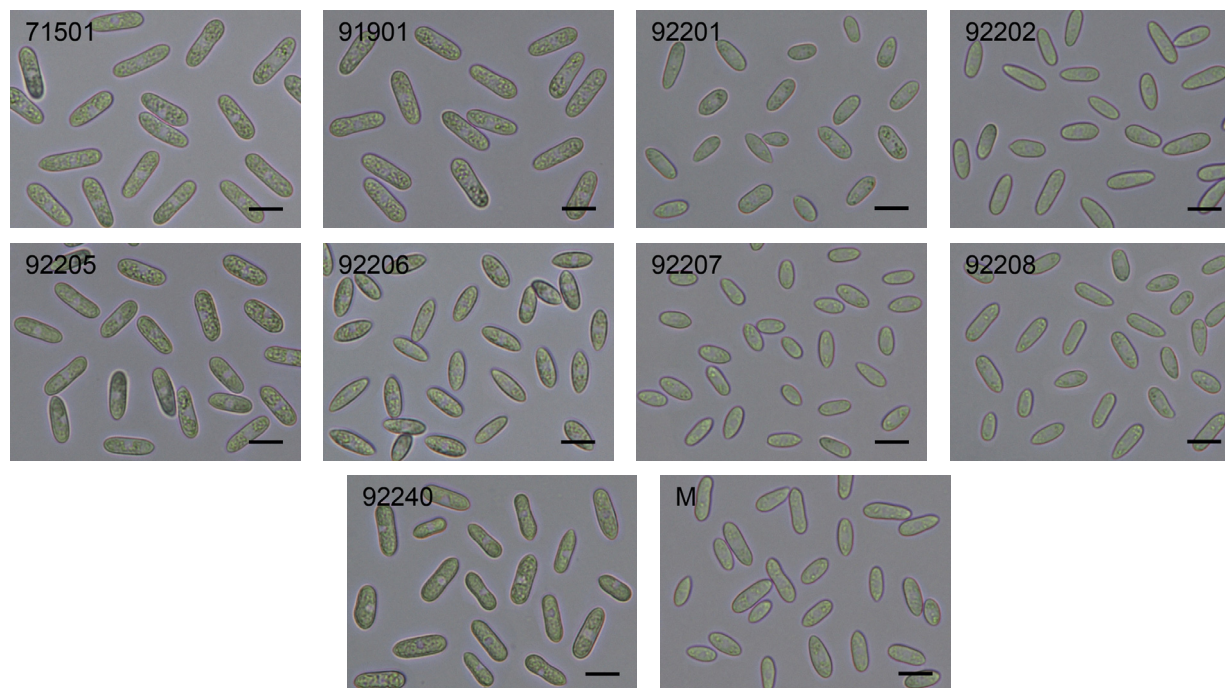


**Fig. 3. Colony morphology of *Colletotrichum* spp. isolated from anthracnose of plum fruits on potato dextrose agar medium (PDA) incubated for 7 days at 25 .**

10-11.8 mm/day 7 80 mm  
 . 92201, 92202, 92207, 92208 M  
 가 6.2~8.4 mm/day 7 43-58 mm  
 (Fig. 3). 2006  
 , 7.7  
 . 92240 가 11.2 mm/day  
 가 71501  
 , colony . 7151,  
 71502, 91901, 92205 92240

**Table 4. Conidial morphology of *Colletotrichum* spp. isolated from anthracnose of plum fruits. Cultures of each isolate were inoculated on potato dextrose agar for 10 days at 25 °C. Fifty conidia of each isolate were measured in length and breadth, and the experiment was conducted twice**

Isolate	Shape	Length (µm)		Breadth (µm)		Length/breadth (L/B) ratio	
		Range	Average	Range	Average	Range	Average
71501	cylindrical, round both end	14.0~18.0	16.37±1.29	4.3~5.6	5.06±0.34	2.45~4.25	3.26±0.43
71502	cylindrical, round both end	14.0~18.8	16.61±1.07	4.6~5.4	4.95±0.27	2.79~3.76	3.37±0.29
91901	cylindrical, round both end	15.1~18.3	16.21±0.81	4.3~5.4	5.09±0.28	2.79~4.19	3.20±0.30
92201	subcylindrical, round both end, sometimes tapered end	8.1~17.2	11.49±1.89	3.8~5.4	4.62±0.51	1.55~3.38	2.51±0.44
92202	subcylindrical, round both end, sometimes tapered end	7.5~15.9	11.99±1.87	3.8~5.1	4.28±0.38	2.13~3.69	2.81±0.41
92205	cylindrical, round both end	11.6~18.3	14.27±1.13	4.0~5.9	4.90±0.43	2.23~4.00	2.94±0.38
92206	straight, fusiform	9.1~15.1	12.13±1.26	3.8~5.4	4.63±0.39	1.70~3.73	2.64±0.39
92207	subcylindrical, round both end, sometimes tapered end	8.1~13.4	9.83±1.42	3.5~5.6	4.39±0.48	1.68~3.00	2.26±0.36
92208	subcylindrical, round both end, sometimes tapered end	7.5~16.1	10.22±2.04	3.5~5.4	4.22±0.45	1.40~3.53	2.44±0.49
92240	cylindrical, round both end, sometimes tapered end	9.9~16.4	13.79±1.84	3.8~5.6	4.85±0.52	2.27~3.57	2.85±0.31
M	subcylindrical, round both end, sometimes tapered end	8.1~14.5	11.15±1.94	3.5~5.4	4.31±0.41	1.76~3.38	2.59±0.42



**Fig. 4. Conidial morphology of *Colletotrichum* spp. isolates from anthracnose of plum fruits. The isolates were grown on potato dextrose agar. Scale bar=10 µm.**

10~11.8 mm/day *C. gloeosporioides* PDA 6.2~8.4 mm/day , 5 45  
 5 55~69 mm (Adaskaveg and mm *C. acutatum* (Kim  
 Hartin, 1997; Kim *et al.*, 2006) *et al.*, 2006)  
 92201, 92202, 92206, 92207, 92208 M Table 4

71501, 71502 91901  
cylinder ,  
가 . 92205  
cylinder 71501  
가 (Fig. 4). 4  
가 C.  
*gloeosporioides* (Kim and Hong, 2008)  
92201, 92202, 92207, 92208 M 71501  
가 , subcylinder  
(Fig. 4).  
*C. acutatum* (fusiform) C.  
*gloeosporioides*  
(Sutton, 1980), 92206  
*C. acutatum* ,  
*C. acutatum* (Kim and Hong,  
2008) . 92240 cylinder  
71501 , 13.8 μm×4.9 μm  
가  
rDNA-ITS NCBI  
GenBank Table 5  
가 7 80 mm  
71501, 71502, 91901, 92205 92240  
colony NCBI  
*C. gloeosporioides* 가  
99-100% . 7 가 43.4-55.3 mm  
가 92201, 92202, 92206, 92207,  
92208 M NCBI *C. acutatum*  
가 99-100%  
*C. acutatum* *C. gloeosporioides*  
(Freeman et  
al., 1998; Hu et al., 2015),  
PDA colony  
*C. acutatum* *C. gloeosporioides*  
*C. acutatum* *C. gloeosporioides*가  
(Kim et al., 2006) (Lee et al.,  
2007)  
In vitro *C. gloeosporioides*  
*C. acutatum*

**요 약**

11  
PDA 25°C 7-10

**Table 5. Blast results with rDNA-ITS sequences *Colletotrichum* spp. isolated from anthracnose of plum fruits. Partial sequence of 18S and 28S ribosomal RNA gene, complete sequence of internal transcribed spacer 1, 5.8S ribosomal RNA gene and internal transcribed spacer 2 were analyzed**

Isolate	Species	NCBI GeneBank Accession No.
71501	<i>C. gloeosporioides</i>	KC493156.1
71502	<i>C. gloeosporioides</i>	HQ645082.1
91901	<i>C. gloeosporioides</i>	HQ645079.1
92201	<i>C. acutatum</i>	KX344998.1
92202	<i>C. acutatum</i>	KX344998.1
92205	<i>C. gloeosporioides</i>	KT282667.1
92206	<i>C. acutatum</i>	KX108948.1
92207	<i>C. acutatum</i>	KX344998.1
92208	<i>C. acutatum</i>	KX344998.1
92240	<i>C. gloeosporioides</i>	KY302642.1
M	<i>C. acutatum</i>	KX344998.1

, colony  
genomic  
DNA rDNA-ITS , PCR  
NCBI GenBank  
6 *Colletotrichum acutatum* , 5  
*C. gloeosporioides*

**Notes**

The author declare no conflict of interest.

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