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Vegetative Incompatibility and Hypovirulence Conversion of *Cryphonectria(Endothia) parasitica*(Murr.)Barr

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Cryphonectria(Endothia) parasitica(Murr.)Barr의 體細胞 不和合性과 低病原性 變換

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ABSTRACT: One hundred and two virulent(V) strains of *Cryphonectria parasitica* were isolated from the cankers of American chestnut (*Castanea dentata*) trees in western Massachusetts, USA. The diversity of vegetative compatibility groups (VCGs) of *C. parasitica* was investigated. One hundred and two strains represented 54 VCGs; 38 VCGs had only one strain each, 6 VCGs had 2 strains each, and 10 most common VCGs had 52 strains. Great diversity in VCGs may due to the increasing numbers of VCGs with time since the pathogen has been in Massachusetts for 80 years. Ten vegetative compatibility representative strains were selected from the 10 most common VCGs and converted to hypovirulent (H) strains through the pairing and hyphal anastomosis of H strains (4 strains with French dsRNA elements and 17 strains with Italian dsRNA elements). All of the 10 representative strains were converted to H strains by at least more than one of the H strains.

KEYWORDS: chestnut blight, vegetative compatibility, hypovirulence conversion, *Cryphonectria* (*Endothia*) parasitica, anastomosis, double stranded RNA

Introduction

Since the determinants of hypovirulence could be potentially useful as biocontrol agents of chestnut blight in northern Italy, much efforts have been done to prove the transmissibility of the factors responsible for hypovirulence. Normal strains became hypovirulent through hyphal anastomosis with hypovirulent(H) strains. Virulent(V) strains that become hypovirulent after fusion with a H strain are said to be converted. If conversion takes place in a canker on a host tree(*Castanea* spp.) infected with chestnut blight, the tree produces callus tissue at the edge of the canker. The use of H strains in eastern North America, however, has not been as successful(Anagnostakis, 1986). Vegetative incompatibility in *C. parasitica* had great concern because of its potential for restricting transmission of hypovirulence and successful spread of H strains(Anagnostakis, 1977). Vegetative incompatibility restricts the transfer of nuclei and cytoplasmic elements during vegetative growth. In order to control chestnut blight effectively in a forest situation, it is necessary to understand this incompatibility. Almost all infectious H strains examined contained dsRNA virus, which vary widely in cultural characteristics, pigmentations, pathogenicity, and so on. Transmission of dsRNA

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virus and the resulting conversion occurs following hyphal anastomosis between H and V strains (Grente & Bertheley-Sauret, 1978; Van Alfen *et al.*, 1975). The converted strain will usually have the same dsRNA banding pattern profile as the donor H strain used in conversion, and will be changed in the morphology and virulence, etc (Anagnostakis, 1981). This study was carried out to investigate vegetative compatibility groups (VCGs) of *C. parasitica* in western Massachusetts and convert V strains from the 10 most common VCGs with H strains to use them for the biological control of chestnut blight.

Materials and Methods

Fungal isolation

Bark samples from chestnut blight infected American chestnut(*C. dentata*) were collected in Pelham, Holyoke, and Shutesbury, Massachusetts, USA. Samples were obtained from the advancing margins of canker by using a 7 mm cork borer dipped in 70% ethanol. *C. parasitica* strains were isolated by placing each sample, bark side up, on 2% water agar. Mycelium grew around each bark sample onto the agar at 25℃. Two or three days later, an agar plug with mycelium was then transferred to potato dextrose agar(PDA) and incubated in the dark at 25℃. Isolated fungal strains were maintained on PDA and transferred weekly. Stock cultures were kept on PDA slants at 4℃.

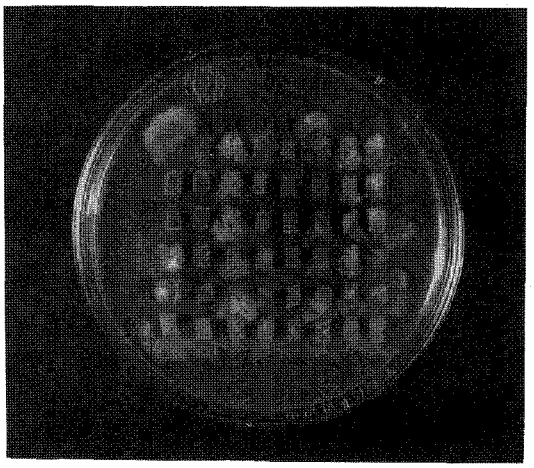

Fig. 1. Vegetative compatibility pairings of *Cryphonectria parasitica* strains. Agar plugs containing actively growing mycelium placed, up side down, 3 mm apart on fresh PDA plates. Plate shows 2 days after inoculation.

Vegetative compatibility determination

Vegetative compatibility groups(VCGs) were determined by cross-pairing the entire 102 V strains of *C. parasitica*. Small plugs(3 mm×4 mm×2 mm) of PDA containing rapidly growing mycelium, were cut from the edge of isolated fungal cultures that were less than 5 days old. Several plugs were placed 3 mm apart on fresh PDA plates, and then incubated in the dark at 25~27℃ for 5~7 days (Fig. 1). Formation of a barrage line between two different fungal strains was indicative of vegetative incompatibility.

Hypovirulent strains

Twenty-two H strains(17 strains containing Italian dsRNA, 4 strains with French dsRNA and 1 strain with north American dsRNA) were provided by S.L. Anagnostakis, Conn. Agric. Exp. Sta., New Haven, CT(Table 1). All H strains were used for the conversion of representative strains from the 10 most common VCGs of 102 V strains.

Hypovirulence conversion

One representative V strain from each of the 10 vegetative compatibility groups were paired with each of 22 H strains of *C. parasitica*. Agar plugs(5 mm in diameter) containing mycelium from rapidly growing fungal cultures were used as inoculum for pairing experiments. One plug of a V strain, and one of a H strain were placed 5 mm apart on fresh PDA plates, and incubated at 25~27℃ for 3~7 days. A 16 hour photoperiod in white fluorescent light was provided during incubation. The V and H strains were grown until mycelial contact between the two strains was apparent. Conversion of the V strain was indicated if cultural sectoring became evident. Also, the growth rate of the sectored V culture usually became noticeably decreased. Mycelium from the margin of the converted V strain, as distant from the H

Table 1. Description and source of hypovirulent strains of *Cryphonectria parasitica* used in hypovirulence conversion

Strain No.	Description and Source				
	ATCC #	Conversion[1]	Origin[2]	Phenotype[3]	VCGs[4]
CP6[I]	22508	CP6[Italian H]	CP6(UV mutant,CT)	met$^-$	8
CP29[I]	38754	CP29[Italian H]	CP29(Guilford,CT)	W	16
CP30[I]		CP30[Italian H]	CP30(Palmerton,PA)	W	24
CP37[I]		CP37[Italian H]	CP37(Kentucky)	W	19
CP42[F]	38751	CP42[French H]	CP42(Mt.Carmel,CT)	W	5
CP46[I]		CP46[Italian H]	CP46(Tuscany, Italy)	W	11
CP49	38759	Original H	Tuscany, Italy	W	12
CP59[F]		CP59[French H]		W	9
CP59[I]		CP59[Italian H]		W	9
CP62[I]		CP62[Italian H]	CP62(SCI of CP49)	W	12
CP74[I]		CP74[Italian H]	CP74(Hamden,CT)	W	18
CP88	38757	Original H	Rockford,MI	O	13
CP110[I]		CP110[Italian H]	CP110(Hamden,CT)	W	5
CP713	52571	EP155[EP113]	EP113(Les Mayons,France)	W	40
CP747	52575	EP155[EP419]	EP419(Mt.amaita, Italy)	W	40
CP748	52572	EP67 [EP420]	EP420(Mt.amaita, Italy)	W	10
CP752	52573	EP42 [EP420]	EP420(Mt.amaita, Italy)	W	5
CP802	52574	EP67 [EP113]	EP113(Mt.amaita, Italy)	F	10
K1		Original H	Natural Bridge, KN	N	
AA3		AAI[French H]	Arnold Arboretum,MA	W	
AA14		AAI[French H]	Arnold Arboretum,MA	W	
AA22		AAI[French H]	Arnold Arboretum,MA	W	

[1]H=Hypovirulent strain, AAI=Arnold Arboretum Isolate, Virulent strain used in conversion [donor Hypovirulent strain]
[2]Origin of virulent or hypovirulent strains. SCI=Single conidium isolate, CT=Connecticut, KN=Kentucky, PA=Pennsylvania, MA=Massachusetts, MI=Michigan
[3]W=White, N=Normal pigment, F=Almost flat, O=Orange, met$^-$=Methionine-requiring.
[4]VCGs=Vegetative Compatibility Groups

strain as possible, was transferred to fresh PDA to confirm that the cultural characteristics had been successfully converted to those similar to the H strains(Anagnostakis, 1979).

Results

Vegetative Compatibility Groups(VCGs)

Some pairings grew together. However, some pairings produced strong barrage line with pycnidia along the line, others produced faint line with no pycnidial formation(Fig. 2). Fifty four vegetative compatibility groups(VCGs) were found. These groups were designated as UM groups. Ten groups had more than 2 strains in each, and the remaining 38 VCGs had only one strain per group. About half of the strains (52 out of 102), comprised the 10 groups designated UM-A through UM-J(Table 2).

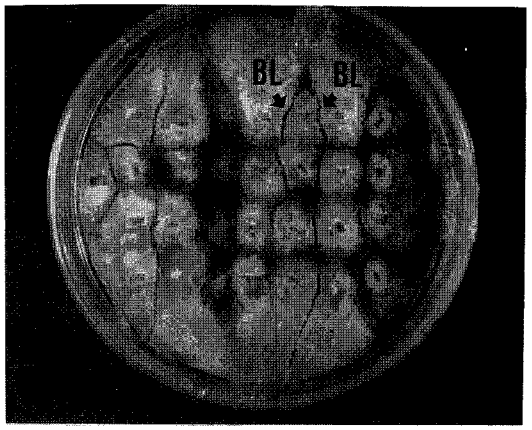

Fig. 2. Formation of barrage lines (BL), which indicates vegetative incompatibility between two paired strains of *Cryphonectria parasitica*. Pycnidia usually formed along this line.

Hypovirulence Conversion

All 10 V strains could be converted to hypovirulence by at least one of the 22 H strains from other locations. Three V strains were converted by H strains in VCG 16, 19, or 24 and six were converted by H strains in VCG 5, 10, or 40. Two of the 4 French H strains, 10 out of the 17 Italian H strains, and the single American H strain, were unable to convert any of the V strains(Table 3). Conversion morphology was usually evident 5~7 days after pairing, but a few pairings showed conversion within 2 days. In almost all cases, the growth rate of the V strains were noticeably faster than that of the H strains. When the conversion occurred, the growth rates of V strains usually noticeably decreased and colony morphology also changed to those similar to the H strains used

Table 2. Vegetative compatibility groupings of virulent strains of *Cryphonectria parasitica*

Location[1]	Pelham	Holyoke	Shutesbury	
VCGs[2]		# of strains		# of strains/VCG
UM-A	3	–	–	3
UM-B	10	–	–	10
UM-C	4	–	–	4
UM-D	3	–	–	3
UM-E	3	–	–	3
UM-F	4	–	–	4
UM-G	3	4	–	7
UM-H	–	8	–	8
UM-I	–	5	1	6
UM-J	–	1	3	4
UM-K	–	2	–	2
UM-L	–	2	–	2
UM-M	–	–	2	2
UM-N	1	–	1	2
UM-O	–	1	1	2
UM-P	–	1	1	2
Single isolate groups[3]	23	11	4	38
TOTALS	54	35	13	102/54 VCGs

[1]Fungi isolated from American chestnut trees (*Castanea dentata*) in Pelham, Holyoke, and Shutesbury, MA.
[2]VCGs=Vegetative Compatibility Groups, determined by cross-pairing strains.
[3]38 VCGs contained only one isolate.

Table 3. Conversion of virulent strains of *Cryphonectria parasitica* to hypovirulence

VCGs[1] Virulent[2]	UM-A PE18	UM-B PE20	UM-C PE23	UM-D PE26	UM-E PE27	UM-F PE51	UM-G HK18	UM-H HK27	UM-I HK34	UM-J SB2
Hypovirulent[3]										
CP42[F][4]	–[5]	–	–	–	–	–	–	–	–	–
CP59[F]	–	–	–	–	–	–	–	–	–	–
CP713	–	–	–	–	–	C[6]	–	–	C	–
CP802	–	–	–	–	–	–	–	–	C	–
CP6[I][7]	–	–	–	–	–	–	–	–	–	–
CP29[I]	C	C	–	–	–	–	–	–	–	–
CP30[I]	C	C	C	–	–	–	–	C	–	–
CP37[I]	–	–	C	–	–	–	–	–	–	–
CP46[I]	–	–	–	–	–	–	–	–	–	–
CP49[I]	–	–	–	–	–	–	–	–	–	–
CP59[I]	–	–	–	–	–	–	–	–	–	–
CP62[I]	–	–	–	–	–	–	–	–	–	–
CP74[I]	–	–	–	–	–	–	–	–	–	–
CP88	–	–	–	–	–	–	–	–	–	–
CP110[I]	–	–	–	–	–	–	–	–	–	–
CP747	–	–	–	C	C	C	–	–	C	–
CP748	–	–	–	C	–	C	C	–	C	C
CP752	–	–	–	C	C	C	C	–	C	C
AA3	–	–	C	–	–	–	–	C	–	–
AA14	–	–	–	–	–	–	–	–	–	–
AA22	–	–	–	–	–	–	–	–	–	–
K1[A][8]	–	–	–	–	–	–	–	–	–	

[1]VCGs=Vegetative Compatibility Groups.
[2]Virulent strains, one representative from each VCGs. PE=Pelham, MA; HK=Holyoke, MA; SB=Shutesbury, MA.
[3]Hypovirulent strains provided by S.L. Anagnostakis, Conn. Agric. Exp. Sta., CT.
[4,7,8]F=French dsRNA; I=Italian dsRNA; A=American dsRNA (Kentucky).
[5]–=Virulent strains unable to be converted to hypovirulence.
[6]C=Converted, virulent strains successfully converted to hypovirulence.

in conversion(Fig. 3). The average conversion capacity of 22 H strains was 12.3% and ranged from 0% to 60%.

Dicussion

Great diversity in vegetative compatibility(v-c) was observed among the V strains. This may be attributed to the normal rate of natural genetic variation of *C. parasitica* over 80 year period of time since chestnut blight was first reported in Massachusetts. Different degrees of vegetative incompatibility were noticed among the V strains, and this result supports the suggestion that some kind of network for v-c types exists that allows some cytoplasmic transmission in spite of a basic

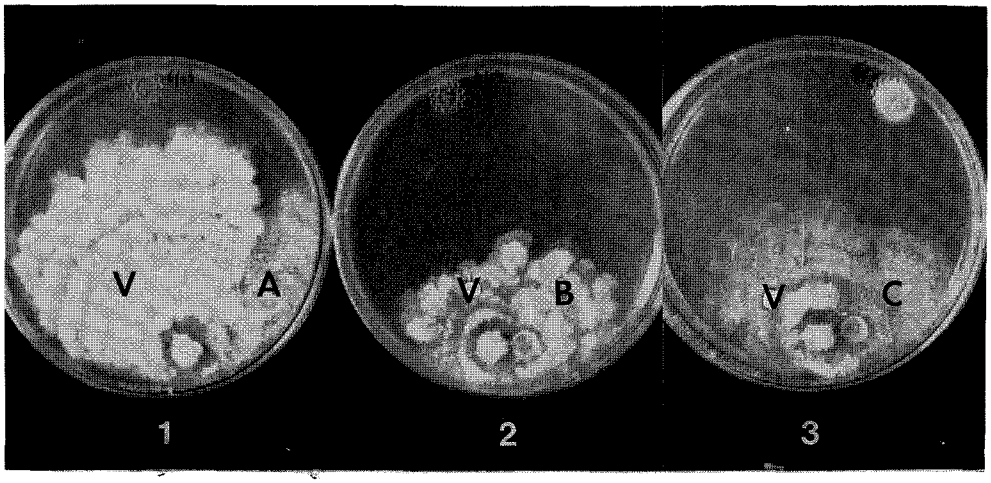

Fig. 3. Conversion and non-conversion of virulent strains of *Cryphonectria parasitica* to hypovirulence. Virulent strain PE51 (V) paired with hypovirulent strains CP42[F] (A), CP752 (B), and CP748 (C). Plate 1 demonstrates the inability of an incompatible strain CP42[F] to convert PE51 to hypovirulence. Plates 2 and 3 show successful conversion of PE51 to hypovirulence.

incompatibility. This may be the result of several levels of vegetative incompatibility, or may be due to strains being different in single or more of v-c loci. It is known that vegetative compatibility of *C. parasitica* is controlled by seven genes, each with a different allele from each parent, and has been differentiated into more than 100 VCGs in the United States(Anagnostakis, 1988).

All of the representative strains from the 10 most common VCGs could be converted by pairing with at least one of the H strains, and one H strain converted several V strains from different VCGs. This means that some vegetatively incompatible pairings allow the transmission of dsRNA virus. Therefore, hypovirulence conversion capacity is more broader than vegetative compatibility. Using two H strains, CP30(v-c 24) and CP752(v-c 5), all representative strains from the 10 most common VCGs of V strains could be converted to hypovirulence. Day *et al.*(1977) showed that the conversion of V strains from 38 VCGs occurred after contact with H strains from only 4 VCGs.

Martin and Van Alfen(1991) measured the movement of dsRNA virus between colonies by using colony *in situ* hybridization techniques. They detected dsRNA in virulent colonies between 1 and 2 days after the colonies touched, and throughout the periphery of the virulent colonies by 2 days after touching. The time required for hypovirulence conversion was various; rapid conversion was detected by the changed morphology of V strain in 2 days after pairing as compared to the 5~7 days for slow conversion.

摘 要

밤나무 胴枯病에 심하게 感染된 미국밤나무의 罹病줄기로부터 病原菌인 *Cryphonectria parasitica*를 102菌株 分離하여 배지상에서 培養하면서 體細胞和合性을 조사한 결과, 102 균주는 54개의 和合性群으로 나누어 졌으며 그중 38개의 화합성군에 단지 한 균주씩, 6개의 화합성군에 각각 2균주씩 포함되었으며 나머지 52개 균주는 10개의 화합성군에 포함되어 다양한 화합성군으로 分化되었음을 보여주었다. 체세포 화합성에 있어서 이러한 多樣한 분화는 병원균이 분리된 지역에서 80년 이상 存在해 오면서 시간이 經過함에 따라 遺傳的으로 많은 분화가 일어나고 이로 인하여 화합성군의 숫자가 增加한 것에 起因하는 것으로 추측된다. 가장 頻度가 높은 10개의 화합성군으로부터 각각 한 균주씩을 대표적인 균주로 選拔하고 이들을 여러 지역에서 분리된 저병원성 균주와 菌絲融合을 시켜면서 低病原性 균주로의 變

換을 試圖하였다. 10개의 대표적인 균주는 모두 最少한 1개 이상의 저병원성 균주에 의해서 저병원성 균주로 변환되었다.

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I thank S.L. Anagnostakis, Connecticut Agricultural Experiment Station, New Haven, CT., for supplying hypovirulent strains of *Cryphonectria parasitica* and T.A. Tattar, Department of Plant Pathology, University of Massachusetts, MA., for providing opportunity to carry out this study.

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