Differentiation of monospore cultures within a isolate of *Fusarium oxysporum vasinfectum*

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[**Abstract**] Restriction fragment length polymorphism (RFLP) was undertaken to assess genetic diversification among 15 monospore cultures within a isolate of *F. oxysporum vasinfectum*. The 15 monospore cultures were categorized into 2 hybridization pattern types. The pathogenicity test on the varieties shown no difference within the 15 monospore cultures. The function of the new DNA fragments in culture No. 1 and No. 6 should be confirmed further.

[**Key words**] *F. oxysporum vasinfectum*; RFLP analysis; Monospore culture

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Cotton wilt is a vascular disease caused by the soilborn pathogen *F. oxysporum vasinfectum*. The disease is widespread and causes substantial crop losses in most of the major cotton producing areas of the world. The pathogen can survive for several years in a dormant state in plant debris or in the soil. It invades the plants through the roots, especially through root wounds caused by nematodes, and subsequently infects the vascular system, resulting in wilt symptoms[1]. This fungus has a wide host rang and displays variation in virulence on distinct host species and genera. Determination of both host specificity and genetici diversity in *F. oxysporum vasinfectum* populations are of great importance in plant breeding for resistance.

Direct analysis of DNA polymorphisms is a more general approach to establish genetic variation in organisms. Restriction fragment length polymorphism (RFLP) analyses of nuclear DNA have been used to estimate the genetic diversity of *F. oxysporum* within and between formae speciales[2] and among nonpathogenic strains. In the present study we tested whether monospore cultures within a isolate displayed DNA diversification by RFLP analysis of nuclear DNA.

1 Materials and methods

1.1 Isolates

CF3 of *F. oxysporum vasinfectum* was isolated from the field in Yangling of Shaanxi province where cotton has been cultivated on a large scale for a long time. The culture was single spored and mycelia were maintained on potato dextrose agar slants for short time storage.

1.2 DNA extraction

The fungal mycelium was cultivated in flasks containing 200 mL of potato-dextrose broth for 4 days at 25 ℃ without shaking. The mycelium was harvested by filtration and lyophilized at −87 ℃ for 48 h. Each lyophilized culture was ground directly in the mortar with liquid nitrogen. The ground culture was transferred to a 2 mL Eppendorf microtube and suspended in 1 mL of extraction buffer (EDTA 50 mmol/L, SDS 0.2%). Total DNA extraction then was performed according to the minipreparation method of Lee and Taylor[3].

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Finally, DNA dissolved in TE buffer (10 mmol/L Tris HCl 1 mmol/L EDTA) to a final concetration of 2 μg/μL and stored at 4 ℃ until use.

1.3 Restriction endonuclease digestion and electrophoresis

For each isolate approximately 10 μg of total genomic DNA was digested with 2 μmol/min of the restriction enzymes for 12 h at 37 ℃. Restriction fragments were separated by electrophoresis in 0.8% agarose gel with 0.2 μL of ethidium bromide (0.5 μg/mL) in TAE buffer at 21 V overnight. Gels were photographed on a UV transilluminator. DNA fragments were blotted onto Nylon membranes using the Vacugene XL apparatus (Pharmacia) according to the manufacturer's instruction.

1.4 Labelling of probes and hybridaization

The hybridization probes used in this study are *impala*[4]. The DNA probes were labeled by a nonradioactive DNA labeling chemiluminescence. Membrane bound DNA fragments were hybridized to denatured probe at 68 ℃ overnight in hybridization buffer. Membranes were washed in washing buffer and probe detection was done with detection kit (Boehringer Mannheim) according to the supplier's instruction.

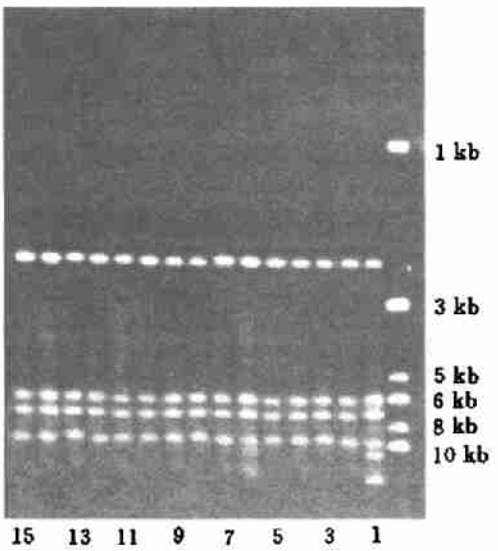

Fig. 1 Examples of Southern blots with probe *impala* of *Eco*R I -digests from 15 monospore cultures

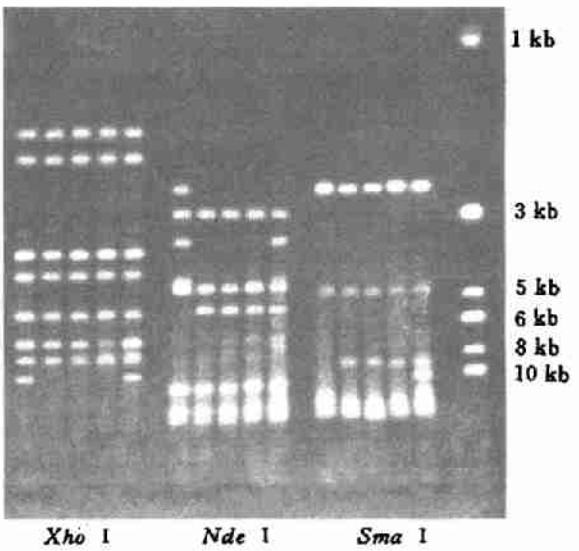

Fig. 2 Examples of Southern blots with probe *impala* of *Xho* I, *Nde* I and *Sma* I -digests from 5 monospore cultures

2 Result and discution

2.1 Pathogenicity tests

The 15 monospore cultures used in this study were randomly selected within isolate Cf3 from cotton field in Yangling. Root-dip inoculation tests confirmed the mild pathogenicity of all 15 monospore cultures on the cotton variety Lumian 1 and the weak pathogenicity on Zhongmian 12. No difference was find among 15 cultures in the pathogenicity on the varieties.

2.2 Hybridization patterns with a random probe

Probe *impala* was hybridized to the *Eco*R I -digested DNA from each of the 15 monospore cultures. The resulting patterns of 13 monospore cultures consisted of 4 bands. There are 6 bands in culture No. 1 and No. 6. The cultures that displayed the same hybridization pattern were assigned the same type. The 15 monospore cultures were categorized into 2 hybridization pattern types. Examples of hybridization patterns are shown in Figure 1.

5 monospore cultures (1, 2, 3, 4, 6) were

selected to digest with *Xho* I, *Nde* I and *Sma* I separately. The hybridization pattern with probe *impala* in culture No. 1 and No. 6 were also the different type with others. Examples of hybridization patterns are shown in Figure 2. The pathogenicity test on the varieties shown no difference within the 15 monospore cultures. The function of the new DNA fragments in culture No. 1 and No. 6 should be confirmed further.

[参考文献]

[1] Hillocks R J. Fusarium wilt[A]. Hillocks R J. Cotton Diseases[C]. London:CAB International,1992. 127—160.

[2] Edel V,Steinberg C,Avelange I. Comparison of three molecular methods for the characterization of Fusarium oxysporum strains[J]. Phytopathology,1995,85:579—585.

[3] Lee S B,Taylor J W. Isolation of DNA from fungal mycelia and single spores[A]. Innis M A,Gelfand D H,Sninsky J J,et al. PCR protocols. A Guide to Methods and Applications[C]. San Diego:Academic Press,1990. 282—287.

[4] Langin T,Capy P,Daboussi M J. The transposable element *impala*,a fungal member of the *Tc1-mariner* superfamily[J]. Mol Gen Genet,1995,246:19—28.

棉花枯萎菌(*Fusarium oxysporum vasinfectum*)单孢菌系间的遗传差异

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[摘　要] 提取棉花枯萎菌 CF3 中的 15 个单孢菌系基因组 DNA,用 RFLP 分析其遗传多样性,可以将 15 个单孢菌系划分为 2 个杂交类型。致病性测定没有差异。对于 1 号和 6 号单孢菌系中新出现的 DNA 片断的功能还需进一步证实。

[关键词] 棉花枯萎菌;RFLP 分析;单孢菌系

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