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Fusarium solani species complex isolates conspecific with *Fusarium solani* f. sp. *cucurbitae* race 2 from naturally infected human and plant tissue and environmental sources are equally virulent on plants, grow at 37°C and are interfertile

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Summary

In a previous taxonomic study based on multilocus sequencing of *Fusarium* from clinical specimens and hospital environments, the most common lineage was *Fusarium solani* species complex group 1 (FSSC 1) which is conspecific with *F. solani* f. sp. *cucurbitae* race 2, a pathogen of cucurbit fruits. The aims of our study were to determine if clinical and environmental isolates of FSSC 1 are plant pathogens and members of the same biological species as cucurbit isolates, and to determine if all isolates can germinate, grow and sporulate at 37°C. Isolates from the different sources did not differ in virulence on zucchini fruits. All FSSC 1 isolates were pathogenic and produced more rot than FSSC isolates from plant hosts other than cucurbits. Both mating types were found among isolates from each of the sources, and all isolates were sexually compatible with cucurbit isolates. All isolates germinated, grew and sporulated at 37°C. This is the first report in which plant pathogenicity has been verified for a collection of human clinical isolates. Our data are consistent with the hypothesis that all FSSC 1 isolates, regardless of source, are a single biological species, equally virulent plant pathogens and tolerant of the human body temperature.

Introduction

In April 2006, the US Centers for Disease Control announced an outbreak of sight-threatening *Fusarium* keratitis, i.e. fungal corneal infections, in contact lens wearers that resulted in 176 confirmed cases in 164 patients in 33 states (Chang *et al*., 2006). Subsequently, three-quarters of the 39 available clinical isolates associated with the *Fusarium* keratitis in the USA were identified via multilocus sequence typing as members of six different species within the *Fusarium solani* species complex (FSSC) (Chang *et al*., 2006). One-third (13/39) of the isolates from patients in the USA were identified as FSSC group 1 (FSSC 1), which was designated as a phylogenetic species by Zhang and colleagues (2006). Based on multilocus sequencing, FSSC 1 is conspecific with *F. solani* f. sp. *cucurbitae* race 2 (Fsc2) (teleomorph, *Nectria haematococca* mating population V), a minor pathogen of cucurbit fruits including pumpkins and squash (O'Donnell, 2000; Zhang *et al*., 2006).

Zhang and colleagues (2006) also used multilocus sequence typing to identify *Fusarium* spp. from a diversity of clinical specimens and selected hospital environments. Sixty-one per cent (350/570) of the isolates were members of the FSSC (O'Donnell *et al*., 2004; Zhang *et al*., 2006). Selected clinical ($n = 278$) and hospital environment ($n = 21$) isolates in the FSSC were compared with FSSC isolates from a variety of non-clinical sources ($n = 172$) including diseased plants and animals, plant debris and soil (Zhang *et al*., 2006). A majority of the clinical FSSC isolates (74.5%) were associated with four major lineages: FSSC groups 1 and 2, which were strongly supported as phylogenetic species based on DNA sequence data, and groups 3 and 4, which were not. The most common lineage of clinical isolates (38/278 = 14%) and hospital environment isolates (10/21 = 48%) was FSSC 1, which is conspecific with isolates of Fsc2 from cucurbit fruits. Thus, in both studies, DNA sequence from three taxonomically informative regions, the ribosomal internal transcribed spacers (ITS), an intron-rich portion of the translation elongation factor 1-α (TEF) gene, and a region of the nuclear large subunit ribosomal RNA gene (LSU), indicates that human clinical isolates in FSSC 1 are indistinguishable from plant pathogenic isolates from fruits in the squash family. This is particularly interesting because pathogens of both humans and plants are extremely rare and generally only cause infections in immunocompromised people. However, in the keratitis study, 93% ($n = 164$) of the patients were not immunocompromised (Chang *et al*., 2006).

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Table 1. Distribution of haplotypes among human clinical, hospital environment, sewage and cucurbit isolates of FSSC 1 conspecific with *F. solani* f. sp. *cucurbitae* race 2 collected previously and during this study.

	Source of isolate						
	Human, cornea[b]	Human, nail or skin[c]	Human, other or unknown[c]	Hospital environment[c]	Sewage[d]	Cucurbit[e]	Total
Haplotype[a]	No. of isolates						
1-a	15	5	4	9	3	6	42
1-b	3	16	7	1	3	4	34
Singletons	0	1	1	0	1	7	10

a. Haplotypes were determined by sequencing portions of the translation elongation factor 1-α (TEF) and internal transcribed spacer (ITS) regions. *Fusarium solani* species complex (FSSC) group 1 (FSSC 1) haplotypes 1-a and 1-b were defined by Chang and colleagues (2006): FSSC 1-a is equivalent to FSSC group 1, haplotype class 1; and FSSC 1-b is equivalent to FSSC group 1, haplotype class 2 as defined by Zhang and colleagues (2006). The two haplotypes differ by a single polymorphism in the ITS. Haplotypes designated as singletons do not have an exact DNA sequence match with any other isolate.
b. Eleven and two of the corneal isolates from haplotypes FSSC 1-a and FSSC 1-b, respectively, were from the study by Chang and colleagues (2006). Other corneal isolates were from Zhang and colleagues (2006).
c. Nail, skin and other human clinical isolates and hospital environment isolates were from Zhang and colleagues (2006).
d. Sewage isolates were collected and sequenced as part of this study.
e. One and two of the cucurbit isolates from haplotypes FSSC 1-a and FSSC 1-b, respectively, and one of the singletons were collected as part of this study. All other cucurbit isolates were from Zhang and colleagues (2006).

Historically, human clinical and environmental isolates of *Fusarium* have only been identified morphologically to 'genus' or to 'species' such as *F. solani*. However, mating studies, for isolates with a sexual stage, and multilocus sequence typing indicate that the anamorph *F. solani* and its teleomorph *N. haematococca* constitute a group of at least 50 related but distinct species (O'Donnell, 2000; Zhang *et al*., 2006). Although researchers now recognize that *F. solani–N. haematococca* is a species complex (O'Donnell, 2000; Suga *et al*., 2000; Summerbell and Schroers, 2002; Aoki *et al*., 2003; Geiser *et al*., 2004; Honraet *et al*., 2005; Zhang *et al*., 2006), many of the historical names in which species have subspecies status are still used: *N. haematococca* mating populations where a teleomorph has been observed (Matuo and Snyder, 1973; VanEtten, 1978; Hawthorne *et al*., 1992) and *forma specialis*, which is sometimes subdivided into race, for those with specialized plant hosts (Snyder and Hansen, 1954; Toussoun and Snyder, 1961). Recently, species within the *F. solani* species complex have been assigned FSSC group numbers that are more phylogenetically meaningful but do not correspond to the earlier classifications. For example, FSSC 1 and FSSC 2 were identified in recent studies of human clinical isolates (Chang *et al*., 2006; Zhang *et al*., 2006); the plant pathogens *F. solani* f. sp. *cucurbitae* race 1 and *F. solani* f. sp. *pisi* are phylogenetic species FSSC 10 and FSSC 11, respectively (K. O'Donnell, pers. comm.). Within the FSSC, FSSC 1, which based on multilocus DNA sequence is conspecific with Fsc2, is the only plant pathogenic species that is consistently associated with human infections. In addition, based on taxonomic studies of medical isolates in the FSSC, FSSC 1 appears to be the most common single species in the FSSC that is involved in human infections (Chang *et al*., 2006; Zhang *et al*., 2006).

Now that a species of *Fusarium* that causes plant disease has been associated with infections in humans, further characterization of the clinical isolates compared with cucurbit isolates is important for determining the relationship between the human and plant pathogens. The aim of our study was to further characterize and compare clinical, environmental and plant FSSC 1 isolates. Our objectives were (i) to collect additional FSSC 1 isolates from plants and environmental sources and to compare their multilocus haplotypes with those of archived FSSC 1 isolates from human, plant and environmental sources, (ii) to determine if clinical and environmental FSSC 1 isolates are *bona fide* plant pathogens and members of the same biological species as FSSC 1 from cucurbits, and (iii) to determine if plant, environmental and clinical isolates can germinate, grow and sporulate at the human body temperature of 37°C.

Results

Isolation and multilocus sequencing of FSSC 1 from cucurbit fruits and sewage

In this study, additional FSSC 1 isolates were collected from post-harvest cucurbit fruits and from sewage wastewater. The cucurbit and sewage isolates of FSSC 1 collected in this study comprises two major haplotypes: one cucurbit and three sewage isolates were haplotype FSSC 1-a, and two cucurbit and three sewage isolates were FSSC 1-b (Table 1). One cucurbit and one sewage isolate each were singletons and did not correspond to any of the haplotypes identified in previous studies.

Table 2. Source, substrate type and geographic origins of *Fusarium solani* species complex group 1 isolates conspecific with *F. solani* f. sp. *cucurbitae* race 2 used in the pathogenicity and mating studies.

Source of isolate[a]	Substrate	*n*	Geographic origin (No. isolates)[c]
Clinical	Skin	9	Brazil (1), Germany (1), CA (4), NY (1), TX (2)
Clinical	Cornea	4	FL (2), MA (1), TX (1)
Clinical	Nail	4	Cuba (1), New Zealand (1), CT (2)
Clinical	Blood	2	CT (1), MA (1)
Clinical	Other[b]	2	TN (1), TX (1)
Hospital environment	Drains	6	TX (1 sink, 4 shower), WA (1 sink)
Hospital environment	Plaster from ceiling	1	OH
Cucurbit	Cucurbit fruit	5	New Zealand (1), CA (4)
Wastewater	Sewage	7	CA

a. Clinical isolates, hospital environment isolates and the cucurbit isolate from New Zealand were obtained from K. O'Donnell (Zhang *et al*., 2006). The California cucurbit and wastewater isolates were collected as part of this study. Identification of isolates was verified by translation elongation factor 1-α and internal transcribed spacer region sequences.
b. Clinical isolates with a substrate listed as other include one isolate from a bronchial alveolar lavage and one human isolate for which the substrate is not known.
c. Isolates from the USA are designated by the two-letter abbreviation for the state in which they were collected.

Multilocus sequencing of the TEF and ITS regions indicated that the *F. solani* f. sp. *cucurbitae* isolated from pumpkins in the field were not FSSC 1 but instead were *F. solani* f. sp. *cucurbitae* race 1 (FSSC 10), a related but distinct species that also is pathogenic on cucurbit fruits.

Pathogenicity bioassay

Zucchini fruits were inoculated with FSSC 1 from cucurbit, human clinical, sewage and hospital environment sources (Table 2, Fig. 1). In addition, fruits were inoculated with FSSC 10 (*F. solani* f. sp. *cucurbitae* race 1) and FSSC isolates from other plant hosts as pathogenic and non-pathogenic controls, respectively. FSSC 1 produced significantly less rot ($P < 0.0001$) than the cucurbit pathogen FSSC 10, indicating that FSSC 1 is a less virulent pathogen than FSSC 10 (Table 3). The FSSC 1 isolates from clinical, sewage and environmental hospital sources were not significantly different in pathogenicity on zucchini fruits compared with cucurbit FSSC 1 isolates ($P = 0.15$). FSSC 1 isolates from all sources produced significantly more rot ($P < 0.0001$) than the FSSC isolates from other plant hosts, indicating that FSSC 1 is a *bona fide* plant pathogen. Mock-inoculated controls did not have any fruit rot (Fig. 1).

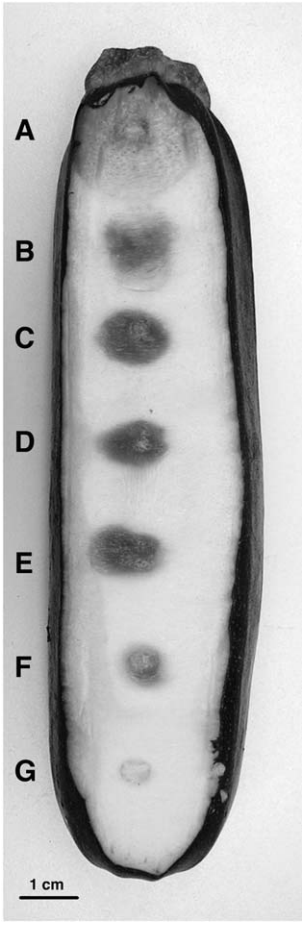

Fig. 1. Rot on a bisected zucchini fruit as a measure of the virulence of *Fusarium solani* f. sp. *cucurbitae* race 1 (FSSC 10), *F. solani* species complex (FSSC) group 1 (FSSC 1) isolates conspecific with *F. solani* f. sp. *cucurbitae* race 2 from various sources, and FSSC from a potato. A = FSSC 10 from a cucurbit; B = FSSC 1 from a cucurbit fruit; C = FSSC 1 from a clinical specimen; D = FSSC 1 from sewage; E = FSSC 1 from a hospital environment; F = FSSC from potato; G = mock-inoculated control.

Matings

All isolates in each of the groups were sexually compatible with cucurbit isolates of the compatible mating type. That is, all produced perithecia with germinable ascospores. Both mating types were present among cucurbit, human clinical, sewage and hospital environment isolates (Table 4).

Isolates obtained from similar geographic areas but isolated from different sources also were from both mating

Table 3. The volume of rot in zucchini fruits inoculated with members of the *Fusarium solani* species complex from either human clinical, hospital environment, wastewater or plant origin.

Source of isolate[a]	Anamorph[b]	Teleomorph[c]	*n*	Log volume of rot, µl ± SE[d]
Cucurbit	FSSC 10	Nh MPI	10	3.27 ± 0.07 a
Cucurbit	FSSC 1	Nh MPV	5	2.52 ± 0.10 b
Clinical (human)	FSSC 1	Nh MPV	21	2.43 ± 0.05 b
Wastewater (sewage)	FSSC 1	Nh MPV	7	2.37 ± 0.07 b
Hospital environment	FSSC 1	Nh MPV	7	2.28 ± 0.07 b
Plant hosts other than cucurbits	FSSC	Nh	10	2.01 ± 0.06 c

a. Isolates are described in Table 2. *Nectria haematococca* mating population I (Nh MPI) was included as a positive control. Hospital environmental isolates, obtained from K. O'Donnell, were isolated from drains and surfaces in hospital rooms. Isolates that were pathogenic on plant hosts other than cucurbits included *F. solani* f. sp. *pisi* (Nh MPVI) (FSSC 11) and *F. solani* species complex (FSSC) isolates from potato and tomato that were identified by classical criteria as *F. solani* f. sp. *eumartii*. Mock-inoculated controls did not have any fruit rot.
b. FSSC 10, *F. solani* f. sp. *cucurbitae* race 1. FSSC 1, *F. solani* species complex isolates conspecific with *F. solani* f. sp. *cucurbitae* race 2.
c. Nh, *N. haematococca*. MP, mating population. Mating populations are designated by roman numerals. For non-cucurbit plant hosts, Nh mating populations are either in MPVI (*F. solani* f. sp. *pisi*) or unknown.
d. The trial was performed three times and data were analysed by ANOVA with trials as blocks. Volume data were log-transformed to satisfy the assumption of homogeneity of variance. An ANOVA of log volume showed a significant difference in volume of rot between groups of isolates ($P < 0.0001$). Values with the same letter are not significantly different by the Tukey–Kramer method ($P \geq 0.05$).

types. For example, in California, both mating types were present in each of the following groups: clinical, sewage and cucurbit isolates. Similarly, both mating types were present among both clinical and environmental hospital isolates from Texas. Besides the cucurbit tester strains, only one cucurbit isolate was hermaphroditic; all other isolates functioned only as males in the crosses.

The *MAT-1* tester NRRL-22141 is a singleton haplotype and the *MAT-2* tester NRRL-45865 is haplotype FSSC 1-a. Among the isolates tested, haplotypes FSSC 1-a and FSSC 1-b were both compatible with haplotype FSSC 1-a (NRRL-45865) and singleton (NRRL-22141) tester strains, confirming that different haplotypes occur within the same biological species.

Germination, growth, sporulation and microconidial morphology at 37°C

FSSC 1 isolates from clinical, hospital environment, cucurbit and sewage sources all germinated and grew at 37°C. However, germination and growth were reduced at 37°C compared with 25°C by 26% and 74% respectively (Table 5). A greater percentage of conidia germinated at 25°C compared with 37°C ($P < 0.0001$). However, germination was not significantly different between isolates from different sources ($P = 0.19$). FSSC 1 grew significantly faster at 25°C versus 37°C ($P < 0.0001$). There were no significant differences in growth between isolates from different sources ($P = 0.11$). As observed for germination and growth, conidial production was not significantly affected by isolate source ($P = 0.58$). However, there was a 63% reduction in conidial production at 37°C compared with 25°C ($P < 0.0001$) (Table 5). Nonetheless, abundant microconidia were produced at 37°C.

Although isolate source did not have a significant effect on length ($P = 0.47$) or width ($P = 0.57$) of microconidia, their morphology was affected by temperature. FSSC 1 microconidia were allantoid to oval at 25°C, but at 37°C, they were oval to subglobose (Fig. 2). That is, at 37°C, microconidia were 9% wider than at 25°C (4.8 versus

Table 4. The mating types and fertility of *Fusarium solani* species complex group 1 isolates conspecific with *F. solani* f. sp. *cucurbitae* race 2 (teleomorph, *Nectria haematococca* mating population V) from human clinical, hospital environment, sewage and cucurbit sources.

	MAT-1,[b] no. isolates			*MAT-2*, No. isolates		
Source of isolate[a]	*n*	Male only[c]	Hermaphrodite	*n*	Male only	Hermaphrodite
Clinical (human)	14	14	0	7	7	0
Hospital environment	4	4	0	3	3	0
Sewage wastewater	3	3	0	4	4	0
Cucurbit fruit	4	2	2	1	0	1

a. Clinical and hospital environment isolates were obtained from K. O'Donnell (Zhang *et al*., 2006).
b. Mating types were first determined by PCR amplification of the *MAT-1*-specific α box and *MAT-2*-specific HMG box (Kerényi *et al*., 2004). Fertility and mating type of all isolates was confirmed by production of perithecia and viable ascospores in crosses with hermaphroditic *N. haematococca* mating population V cucurbit tester strains of known mating types.
c. Isolates were functionally fertile as males or as both males and females (= hermaphrodites) in compatible crosses.

Table 5. Germination, growth and sporulation of human clinical, hospital environment, cucurbit and wastewater *Fusarium solani* species complex group 1 isolates conspecific with *F. solani* f. sp. *cucurbitae* race 2 at 25°C versus 37°C.

Source of isolate[a]	Germination, % ± SE[b]		Colony diameter, mm ± SE[c]		Sporulation, cfu × 10^8 ± SE[d]		Microconidium length, µm ± SE[e]		Microconidium width, µm ± SE	
	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C	25°C	37°C
Clinical	91 ± 1	66 ± 8	36.1 ± 0.9	9.6 ± 0.6	5 ± 2	2 ± 0.5	9.1 ± 0.3	8.6 ± 0.3	4.7 ± 0.2	4.8 ± 0.2
Hospital	89 ± 2	51 ± 8	36.1 ± 0.4	9.3 ± 0.5	5 ± 1	2 ± 0.7	9.1 ± 0.1	8.7 ± 0.6	4.2 ± 0.2	4.8 ± 0.1
Cucurbit	86 ± 2	71 ± 5	36.3 ± 0.6	9.8 ± 0.6	6 ± 1	3 ± 0.5	9.3 ± 0.3	9.3 ± 0.2	4.5 ± 0.3	4.8 ± 0.3
Sewage	86 ± 3	72 ± 7	34.4 ± 0.8	9.0 ± 0.7	8 ± 0	2 ± 0.5	9.3 ± 0.3	8.6 ± 0.3	4.3 ± 0.1	4.8 ± 0.1

a. Four isolates from each source were tested. A two-way ANOVA and *F*-test indicated no significant interaction term between the factors. There were no significant differences in germination, growth, sporulation or microconidium length or width between different sources of isolates ($P > 0.05$). However, germination ($P < 0.0001$), growth ($P < 0.0001$) and sporulation ($P < 0.0001$) were significantly reduced at 37°C compared with 25°C.
b. The percentage of microconidal germination was assessed after 8 h of incubation on V8 juice agar.
c. Colony diameter was measured after 5 days of growth on V8 juice agar.
d. Microconidia were quantified with a haemocytometer after 7 days of growth on V8 juice agar.
e. Microconidia were measured after 7 days of growth on V8 juice agar. Microconidia were significantly wider ($P = 0.006$) at 37°C compared with 25°C.
cfu, colony-forming units.

4.4 µm) ($P = 0.0064$). Microconidial length was not significantly affected by temperature ($P = 0.09$). At both 37°C and 25°C, FSSC 1 cultures from all sources were dominated by microconidia and had relatively scant mycelial production.

Discussion

Zhang and colleagues (2006) used multilocus sequence typing to demonstrate that clinical and hospital environment isolates of FSSC 1 were conspecific with plant isolates of Fsc2 (teleomorph, *N. haematococca* mating population V), a pathogen of cucurbits. Here, we expanded the collection of isolates of FSSC 1 from cucurbits and identified a reservoir of isolates in sewage. In accordance with previous work (O'Donnell, 2000; Zhang *et al*., 2006), we used multilocus sequencing of the TEF and ITS to identify species and haplotypes within the FSSC. We conclude that Fsc2 and FSSC 1 are synonyms because plant isolates and clinical isolates are sexually compatible, all isolates regardless of source are equally virulent on a plant host, all isolates tolerate 37°C, and we failed to find any phenotypic differences associated with isolate source.

Our data also indicate that clinical, sewage and hospital environment FSSC 1 isolates are *bona fide* plant pathogens. Although there are incidental reports of fungal plant pathogens, including *Colletotrichum* spp. (Ritterband *et al*., 1997; Guarro *et al*., 1998), *Phaeoacremonium* spp. (Crous *et al*., 1996; Mostert *et al*., 2005) and *Fusarium* spp. (Honraet *et al*., 2005) causing human disease, this is the first time clinical isolates within a well-defined phylogenetic species have been identified as pathogenic to plants, albeit at a level in which the pathogen could be classified as opportunistically pathogenic on cucurbits. In this study, the fruit rot caused by another cucurbit pathogen, FSSC 10, was almost an order of magnitude greater than the amount of rot produced by FSSC 1 isolates, supporting the idea that FSSC 1 is a facultative pathogen on plants, and a less aggressive pathogen than FSSC 10.

The identification of FSSC 1 as a human pathogen instead of strictly a plant pathogen is relatively recent (Summerbell and Schroers, 2002; Chang *et al*., 2006; Zhang *et al*., 2006) and consequently the epidemiology of the human disease caused by this organism is not well understood. Here, we isolated FSSC 1 from sewage wastewater. Chang and colleagues (2006) concluded that the patients in their study acquired *Fusarium* from their local environments; indeed, some of the most common lineages implicated in the ocular infections, including FSSC 1, were identified in sink and shower drains in other studies (Anaissie *et al*., 2001; O'Donnell *et al*., 2004; Zhang *et al*., 2006). Consequently, keratitis-causing FSSC 1 may have splashed out of sink drains into contact lens-holding cases and then onto contact lenses. Anaissie and colleagues (2001), whose strains were later identified by Zhang and colleagues (2006), speculated that patients infected with members of the FSSC were infected from the hospital water system. However, they did not rule out the possibility that patients' pre-existing infections were the source of FSSC in sink and shower drains. Because Anaissie and colleagues (2001), Chang and colleagues (2006) and Zhang and colleagues (2006) focused on infections caused by *Fusarium* spp. rather than FSSC 1, in Table 1 we show data on all the published FSSC 1 isolates. Interestingly, 83% ($n = 18$) of the FSSC 1 recovered from infected corneas were haplotype FSSC 1-a isolates whereas 73% ($n = 22$) of skin or nail infections were caused by haplotype FSSC 1-b isolates ($\chi^2 = 13.7$,

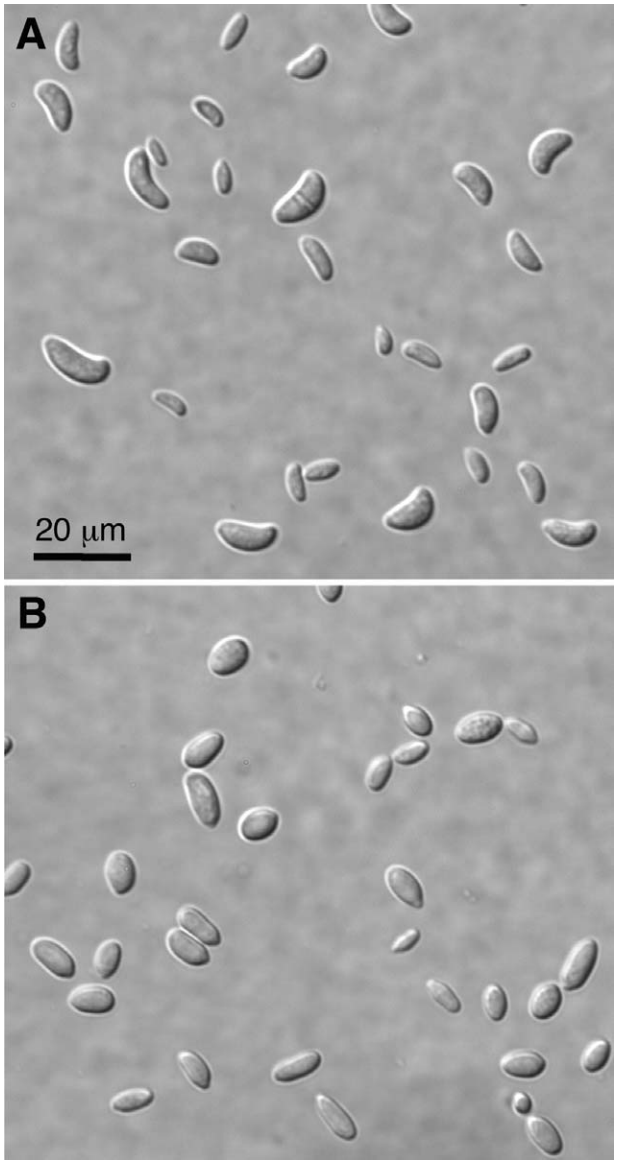

Fig. 2. Microconidial morphology of an isolate of *Fusarium solani* species complex group 1 conspecific with *F. solani* f. sp. *cucurbitae* race 2 produced at 25°C (A) and 37°C (B). NRRL 28545, a human nail isolate, was grown on 30% V8 juice agar for 7 days. Conidia were viewed at 400× with differential interference contrast microscopy.

$P = 0.0003$). Although this may reflect sampling error, it also could indicate that there are different sources of inoculum for infections of human eyes and either human skin or nails. In contrast, 41% ($n = 17$) of the cucurbit infections were caused by isolates that were neither in haplotype FSSC 1-a nor in FSSC 1-b. The sewage and wastewater isolates ($n = 7$) were too few and were from too limited of a geographic area to determine whether or not they are less genotypically diverse than cucurbit FSSC 1; cucurbit FSSC 1 isolates had a diverse geographic origin. If only California isolates are considered, cucurbit isolates are no more likely to be singleton haplotypes versus one of the two major haplotypes than sewage isolates (Fisher's exact test, $P = 0.34$). The same is true even if all cucurbit isolates are compared with California sewage isolates (Fisher's exact test, $P = 0.22$).

The epidemiology of the disease caused by FSSC 1 in plants is also poorly understood; publications on FSSC 1 in plants focus on differences between the disease caused by it and FSSC 10 (Toussoun and Snyder, 1961; Snyder *et al*., 1975; Hawthorne *et al*., 1992; 1994) and not its epidemiology. It is unclear if there is a connection between the life cycle of FSSC 1 in plants and in humans. In contrast to our frequent isolation of FSSC 1 from sewage, our survey data indicate that FSSC 1 is not common in either cucurbit production in California or in soil (data not shown). It is possible that the FSSC 1-infected cucurbit fruits identified here were originally infested via contaminated wastewater used during crop production or post-harvest procedures.

In general, organisms that cause disease in both animal and plant hosts are rare. The bacteria *Pseudomonas aeruginosa*, *Serratia marcescens*, *Burkholderia cepacia* and *Erwinia carotovora* cause both plant and human infections, although usually in circumstances in which the humans are predisposed to illness (Cao *et al*., 2001; Tan, 2002). Only rarely has an individual isolate been shown to cause disease in both animal and plant hosts, i.e. plants exhibiting symptoms after inoculation with isolates from a human source (Rahme *et al*., 1997; Plotnikova *et al*., 2000). In the case of *B. cepacia*, clinical isolates may lack the ability to act as plant pathogens and vice versa (Butler *et al*., 1995; Govan and Deretic, 1996). In contrast, in our study, all FSSC 1 clinical isolates caused rot on zucchini fruits.

In comparison with bacteria, there are even fewer examples of plant pathogenic fungi causing disease in animals, and only incidental reports of plant pathogenic *Fusarium* spp. causing disease in a mammal. Ortoneda and colleagues (2004) induced a systemic infection in immunocompromised mice with the tomato pathogen *Fusarium oxysporum* f. sp. *lycopersici*, but the fungus was not derived from a naturally occurring clinical infection. Indeed, most *F. oxysporum* associated with human infections are in a single apparently clonal lineage (O'Donnell *et al*., 2004) which does not include *F. oxysporum* f. sp. *lycopersici*. Honraet and colleagues (2005) isolated a member of the FSSC from the vocal prothesis of a single immunocompetent patient and based on ITS and LSU sequences, identified an isolate that is conspecific with isolates of *F. solani* f. sp. *radicicola* genotype 2 from ginseng and konjak. However, the phylogenetically more informative TEF region was not sequenced, and they did not demonstrate plant pathogenicity. In our study, the immune status of the patients from whom the clinical

specimens were obtained is unknown. However, the majority of our isolates were from skin, nails and corneal tissue, and fungal infections of these tissues are generally independent of immune status (Nucci and Anaissie, 2002; Dignani and Anaissie, 2004; Nir-Paz *et al*., 2004; Chang *et al*., 2006). Regardless, an immunocompromised individual with a superficial FSSC 1 infection may have a higher risk of developing a systemic infection from FSSC 1, particularly after surgery, than one without FSSC 1. Almost all immunocompromised patients with systemic *Fusarium* infections die (Nucci and Anaissie, 2002).

Comparative genomic analyses have been made between the plant pathogenic bacterium *E. carotovora* ssp. *atroseptica* and related enterobacterial human and animal pathogens in order to elucidate mechanisms of differential pathogenicity and survival in different ecological niches (Toth *et al*., 2006). A microarray analysis of FSSC 1 on plant and animal tissue would provide an interesting comparison of fungal pathogenesis of plants and mammals. The genome sequence of the related species *F. solani* f. sp. *pisi* (teleomorph, *N. haematococca* mating population VI = FSSC 11), a pathogen of pea, has been determined (http://genome.jgi-psf.org/Necha1/Necha1.home.html). Genomic and microarray comparisons of FSSC 10 and FSSC 1, two related but distinct species that both cause disease on cucurbits but with differing levels of virulence, would also provide an interesting comparison.

Clinical and environmental isolates of FSSC 1 are sexually compatible with isolates from cucurbits and therefore are members of the same biological species (teleomorph, *N. haematococca* mating population V) (Matuo and Snyder, 1973; Hawthorne *et al*., 1992). This corroborates the multilocus sequence data that place all of these isolates in the same phylogenetic species (Zhang *et al*., 2006). The mating behaviour of these isolates also is important because it indicates a potential for sexual reproduction in nature and therefore an expected greater genetic diversity than in a strictly clonal organism. Both mating types were isolated in close proximity geographically, so compatible mating types may come in contact with one another. However, with the exception of three cucurbit isolates, two of which were selected as testers, the FSSC 1 isolates in this study functioned only as males. Female fertility is often lost in culture over time (Snyder *et al*., 1975).

Although we did not test for pathogenicity to humans or animals, we demonstrate here that FSSC 1 germinates and grows at 37°C, as required for all human pathogenic fungi (Van Burik and Magee, 2001; Casadevall, 2005). No differences were detected in thermotolerance between the isolates from clinical, hospital environment, plant and sewage sources. In general, fungi, including some *Fusarium* spp., do not tolerate 37°C (Sugiura *et al*., 1999). In fact, FSSC 10 isolates did not grow or survive at this temperature (data not shown). Nonetheless, while FSSC 1 is thermotolerant, its optimal temperature is in the range of 20–25°C. At 25°C, FSSC 1 produced macroconidia and abundant numbers of microconidia, but at 37°C, it produced microconidia almost exclusively. Regardless, even at 37°C, all of the FSSC 1 isolates produced prodigious quantities of microconidia with a 10^6-fold increase in microconidia in 1 week; the production of abundant microconidia may be important in the dissemination of the fungus in a human host. We note that at 37°C, the microconidia of FSSC 1 were 8.8×4.8 μm and resembled yeast cells; indeed some reports of *Fusarium* spp. from clinical specimens state that microconida initially were misidentified as yeast cells (Schell, 1995; Thrall and Cartwright, 2005). If diagnoses of clinical fungal infections are based on microscopic examination of infected tissue, recognition of FSSC 1 microconidia produced at 37°C is important.

To summarize, DNA multilocus sequencing (O'Donnell, 2000; Aoki *et al*., 2003) indicates that classical morphological species such as *F. solani* and *F. oxysporum* are species complexes, and consequently, identification of an isolate to either *F. solani* or *F. oxysporum* provides little information about the ecology and epidemiology of an isolate. Overall, *Fusarium* spp. are primarily either plant pathogens or saprophytes. In humans and other animals, *Fusarium* spp. are opportunistic pathogens that cause disease, either on superficial tissues (e.g. cornea, skin or nails) or traumatized tissues in immunocompetent individuals, or on a wider variety of tissues in immunocompromised individuals. Multilocus DNA sequencing of clinical isolates indicates that only some phylogenetic species within *F. solani* and *F. oxysporum* are involved in human infections and that the only phylogenetic species that has both human clinical and plant pathogen isolates is FSSC 1 (Chang *et al*., 2006; Zhang *et al*., 2006). Here, we demonstrated that human clinical isolates of FSSC 1 can infect host plants, are as virulent on plant hosts as plant isolates and can interbreed with plant isolates. Thus, FSSC 1 provides an apparently rare example of a phylogenetic species within the genus Fusarium that causes both a disease in plants in the cucurbit family and in humans.

Experimental procedures

Collection of isolates

In order to collect additional FSSC 1 plant isolates for use in this study, cucurbit fruits were sampled for FSSC 1 both in the field and post harvest. *F. solani* f. sp. *cucurbitae* race 1 (synonym, FSSC 10) (teleomorph, *N. haematococca* mating population I), a phylogenetically related but distinct species in the FSSC that also infects cucurbit fruits, was isolated concurrently and included in this study. University of California (UC) cooperative extension farm advisors in the cucurbit-

growing regions of California indicated that FSSC 1 had been observed recently only on pumpkins in the Pescadero/Half Moon Bay region. Pumpkin fields in this region were surveyed, and if a suspect *Fusarium* fruit rot lesion was present, fruit flesh from the edge of the lesions was plated on *Fusarium* selective medium (Mehl and Epstein, 2007), which is semi-selective for *Fusarium* spp. Plates were incubated at room temperature under lights for 4–5 days and observed for fungal growth. Cucurbit fruit samples were also collected from grocery stores in Davis, CA and from a produce distributor in San Francisco, CA; symptomatic tissue was cultured as described above. In all cases, isolates tentatively identified as FSSC 1 or FSSC 10 were tested further as described below.

We sampled from sewage wastewater because many *Fusarium* spp. are water-disseminated, members of the FSSC have been identified in drains (Zhang *et al*., 2006), and preliminary surveys indicated that FSSC 1 could be recovered from sewage wastewater (data not shown). Sewage samples were collected from UC Davis campus buildings and wastewater treatment plant (WWTP) and the City of Davis, CA WWTP. Each sample consisted of 10–25 ml of wastewater from which solids were obtained by passing the liquid through a 1.0 μm cellulose nitrate membrane filter (Whatman, Florham Park, NJ). Filters were placed upside-down on Komada's medium (Komada, 1975), and incubated at room temperature under lights for 3–4 days.

Putative isolates of FSSC 1 and FSSC 10 were screened with taxon-specific primers Fsc2-EF1 (5′-GTTGGTGACATA TCTCCC-3′)/Fsc2-EF3 (5′-GAGTGAGAGACATGACGG-3′) and Fsc1-EF1 (5′-GCTAACAATCATCTACAGAC-3′)/Fsc1-EF-2 (5′-GACGGATGAGAGAGCAAC-3′) respectively (Mehl and Epstein, 2007). Identification with specific primers was confirmed by sequencing portions of the intron-rich TEF gene, and the ITS of the ribosomal DNA (O'Donnell, 2000; Zhang *et al*., 2006) of 11 FSSC 1 and three FSSC 10 isolates used in this study (GenBank Accession No. DQ913748, DQ913750, DQ913751, DQ913753, DQ913755–DQ913761, DQ913771–DQ913773, DQ913778, DQ913780, DQ913781, DQ913783, DQ913785–DQ913791, DQ913801–DQ913803). Each isolate was subcultured from a single spore on V8 agar, and stored as conidia at –80°C in 25% glycerol. Isolates collected in this study were deposited at the Agricultural Research Service (NRRL) Culture Collection, National Center for Agricultural Utilization Research, Peoria, IL (NRRL Accession No. 45 862–45 879). Information on all isolates used in this study is in Table S1.

Comparison of multilocus haplotypes

The distribution of multilocus haplotypes among the 11 cucurbit and sewage isolates collected as part of this study were compared with the haplotypes identified in FSSC 1 by Zhang and colleagues (2006) and Chang and colleagues (2006). Zhang and colleagues (2006) and Chang and colleagues (2006) identified two major haplotypes among their FSSC 1 isolates, FSSC 1-a and FSSC 1-b, which are only distinguished by a single polymorphism in the ITS region. Singletons, which did not have an exact sequence match with any other isolate but only differed by a few base pairs, were also identified by Zhang and colleagues (2006). As it was previously determined that all FSSC 1 isolates had identical DNA in the sequenced region of the LSU (Zhang *et al*., 2006), only the TEF and ITS regions were sequenced, as described previously (O'Donnell, 2000; Zhang *et al*., 2006). A 678 bp portion of the TEF and a 342 bp portion of the ITS region from isolates collected in this study were aligned with TEF and ITS sequences representative of previously identified haplotypes using AlignX in VectorNTI (Informax). Isolates with identical ITS and TEF sequences were regarded as the same haplotype.

Isolates used in pathogenicity and mating studies

Multilocus sequence data for clinical and hospital environment isolates were provided by Dr Kerry O'Donnell, USDA-ARS, Peoria, IL (Zhang *et al*., 2006; Table S1). The 21 clinical isolates used in the pathogenicity and mating studies primarily were from skin, cornea and nails of patients in the USA (Table 2). The seven hospital environment isolates primarily were from drains in patients' rooms. Isolates that we received and that were collected before 1990 were not used in the pathogenicity bioassay because fungi often lose pathogenicity in culture over time. In addition, isolates with extremely atypical morphology (e.g. loss of sporulation) on potato dextrose agar and/or V8 juice agar also were excluded from our study.

We also obtained isolates of members of the FSSC that are pathogenic on hosts other than cucurbits. FSSC isolates from solanaceous crops (potato and tomato) were provided by M.K. Romberg, Department of Plant Pathology, UC, Davis, CA. *F. solani* f. sp. *pisi* (synonym, FSSC 11) isolates were provided by H. VanEtten, Department of Plant Pathology, University of Arizona, Tucson, AZ. Isolates that were identified here as FSSC 10 were obtained from the following sources: B.J. Aegerter, Department of Plant Pathology, UC, Davis, CA; E. Vivoda, Harris Moran Seed Company, Davis, CA; and C.A. Wyenandt, Department of Plant Pathology, Ohio State University, Columbus, OH.

All donated isolates were single-spore purified, cultured and stored as indicated above (see Table S1).

Pathogenicity bioassay

Conidia from freezer stocks were used to seed the centre of 2% water agar plates. Plates were wrapped with Parafilm and incubated in the dark at 25°C for 4 days. Plugs of mycelium-covered agar were then used as inoculum in the pathogenicity assay.

Both FSSC 10 and FSSC 1 cause a dry rot of many different species and varieties of cucurbit fruits (Toussoun and Snyder, 1961). Based on a pathogenicity assay used for FSSC 10 (Jones and Epstein, 1990; Hawthorne *et al*., 1994), we developed a quantitative virulence assay on zucchini fruits. Zucchini fruits were purchased from a local grocery store. Fruits of uniform size and shape and with no obvious blemishes or infections were selected. After fruits were gently scrubbed, they were surface-sterilized by swirling briefly in 70% ethanol and then soaked in 0.5% sodium hypochlorite for 20 min. Fruits were then rinsed with distilled and deionized water and air-dried.

Plugs of fruit tissue approximately 5 mm in diameter and 5 mm deep were removed from zucchini fruits with a cork borer. Five plugs were removed from each fruit, with 2.5 cm

between each plug. A 5 mm plug of mycelium-covered agar was inserted into each hole with the mycelium face down. Negative controls were prepared by wounding fruit as described above and inserting sterile agar plugs into a wound. Zucchini fruits were inoculated with isolates in a completely randomized design. Fruits were placed on racks in crispers and incubated in the dark at 25°C for 5 days. The extent of fruit rot from each inoculation was measured by removing the macerated tissue from the infection site and quantifying the volume of water required to fill the cavity. As a plug of tissue was removed before inoculating the fruit, the volume of water required to fill the initial cavity was also determined and subtracted from the final amount.

Matings

FSSC 1 isolates included in the pathogenicity bioassay were tested for their ability to mate with cucurbit tester strains of FSSC 1 (teleomorph, *N. haematococca* mating population V). FSSC 1 is heterothallic, so mating type primers were used to detect the presence of *MAT-1* and *MAT-2* sequences (Kerényi *et al*., 2004) in clinical, hospital environment, cucurbit and wastewater isolates of FSSC 1. Once mating types were determined, isolates were crossed with compatible cucurbit tester strains. The hermaphroditic isolates NRRL-22141 (= PGB 153) (Hawthorne *et al*., 1992) and NRRL-45865 (this study) from cucurbits were used as *MAT-1* and *MAT-2* tester strains respectively. Each FSSC 1 strain was tested as a male and a female with the tester strains.

The mating protocol was based on previously reported protocols for crossing *N. haematococca* (Toussoun and Weinhold, 1967; Snyder *et al*., 1975; VanEtten, 1978; Hawthorne *et al*., 1992). To prepare the 'female' isolates, plates of V8 agar were seeded with a conidial suspension and incubated in the dark at 22°C for 13 days. For microconidial production from the male isolate, V8 agar plates were seeded with conidia, wrapped in Parafilm and incubated in the dark at 27°C for 7 days. Two days before fertilization, the female plates were transferred to continuous light. Male plates were flooded with sterile water to produce a conidial suspension, 0.5 ml of which was spread over the surface of the appropriate plate. Plates thus fertilized were incubated under continuous light at 22°C. After 15 days, plates were checked periodically for perithecial production. To confirm viability, ascospores were cultured on water agar and scored for germination.

Germination, growth and sporulation at 37°C versus 25°C

Sixteen isolates of FSSC 1, four each of clinical, hospital environment, plant and sewage samples, were randomly selected for the germination and growth assays. Microconidia were used in the germination assays because this is the predominant spore type produced by FSSC 1; macroconidia are often absent, particularly at 37°C. The germination assay was modified from the procedure described by Liebman and Epstein (1992). Microconidia were produced by incubating isolates in the dark at 27°C for 6 days. After plates were flooded with an aqueous solution of 0.1 M 2-(4-morpholino)-ethane sulfonic acid, pH 6.1 in 0.05% Tween 20 (MES), conidia were washed twice, and suspensions were adjusted to a final concentration of 1×10^6 conidia ml^{-1}. Twenty-five microlitres of conidial suspension was vacuum-filtered onto a 0.8×0.8 cm polycarbonate membrane (Nucleopore Corporation, Pleasanton, CA) with 0.2 μm pores. Membranes were placed on 30% V8 juice in 2% agar, and each isolate was incubated simultaneously in the dark at 25°C and 37°C. After 8 h, membranes were removed from V8 agar plates, fixed and stained in an aqueous solution of 10% (w/w) lactic acid, 0.05% (w/w) Cotton Blue, and mounted in 80% glycerol. Conidia were examined at 200× with a light microscope. For each isolate, two replicate membranes were examined per temperature. Two hundred conidia were assessed for germination per membrane. A conidium was considered germinated if it produced a germ tube at least one half of the width of its conidium.

For growth assays, isolates were cultured on 2% water agar, and 5 mm plugs from colony margins were used to start cultures on 30% V8 juice in 2% water agar. Plates of each isolate were incubated simultaneously in the dark at 25°C and 37°C. After 5 days, colony diameters were measured. Germination and growth assays were conducted twice.

The 16 isolates used for germination and growth assays also were tested for microconidial production and morphology at 37°C and 25°C. V8 agar plates were seeded with microconidial suspensions and incubated in the dark at both temperatures. After 7 days, plates were flooded with MES to dislodge conidia, and microconidia in the resulting suspensions were quantified using a haemocytometer. Microconidia were mounted on slides, observed at 400× with a light microscope, and digital images were taken from two randomly selected fields of view. For each temperature, length and width were measured for 15 randomly selected microconidia per field of view for each isolate.

Data analysis

In the pathogenicity assay, a total of 60 isolates were classified based on their genotype and source into the following six groups: FSSC 1 clinical; FSSC 1 hospital environment; FSSC 1 sewage; FSSC 1 cucurbit; FSSC 10 cucurbit; and FSSC from other plant hosts. Isolates were used as replicates within groups. After the volume of rot was log-transformed, the data met the assumptions of ANOVA for homogeneity of variance. Results from all three trials were similar. Data were analysed by an ANOVA as a randomized complete block design with the three trials treated as blocks. Means were compared using the Tukey–Kramer method.

For germination, growth, conidial production and conidial morphology, the four isolates within each of the categories (clinical, hospital, cucurbit and sewage) were considered replicates. The effects of source of isolate and temperature were analysed with a two-way ANOVA. Means were compared using the Tukey–Kramer method. All statistical analyses were performed using JMP 5.1 (SAS Institute, Cary, NC).

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Supplementary material

The following supplementary material is available for this article online:

Table S1. Information on all isolates used in this study.

This material is available as part of the online article from http://www.blackwell-synergy.com

