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Fusarium oxysporum as a Multihost Model for the Genetic Dissection of Fungal Virulence in Plants and Mammals

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Fungal pathogens cause disease in plant and animal hosts. The extent to which infection mechanisms are conserved between both classes of hosts is unknown. We present a dual plant-animal infection system based on a single strain of *Fusarium oxysporum*, the causal agent of vascular wilt disease in plants and an emerging opportunistic human pathogen. Injection of microconidia of a well-characterized tomato pathogenic isolate (isolate 4287) into the lateral tail vein of immunodepressed mice resulted in disseminated infection of multiple organs and death of the animals. Knockout mutants in genes encoding a mitogen-activated protein kinase, a pH response transcription factor, or a class V chitin synthase previously shown to be implicated in virulence on tomato plants were tested in the mouse model. The results indicate that some of these virulence factors play functionally distinct roles during the infection of tomato plants and mice. Thus, a single *F. oxysporum* strain can be used to study fungal virulence mechanisms in plant and mammalian pathogenesis.

Fungi are an extremely versatile class of organisms comprised mostly of saprophytes thriving on dead organic material. A relatively small number of fungal species have developed a parasitic lifestyle, associated with the ability to recognize and penetrate a specific host, exploit its nutrient reserves, overcome its innate defense responses, and cause disease. The list of organisms attacked by fungi encompasses evolutionary distinct groups from lower to higher eukaryotes, most prominently plants, insects, and mammals, including humans. To cause disease, fungal pathogens rely on an arsenal of pathogenicity and virulence factors, whose spatially and temporally correct deployment determines the basic pathogenic potential and the extent of infection, respectively. The advent of protocols for targeted gene knockout or random insertional mutagenesis has led to the identification of an increasing number of genes encoding pathogenicity or virulence factors from plant and animal pathogens (12, 34). In spite of these advances, many crucial aspects underlying fungal pathogenesis remain poorly understood.

One of these questions concerns host specificity. Certain fungi cause disease on a single host species, whereas others have extremely broad host ranges. The molecular mechanisms that determine fungal host range specificity are not fully understood. Thus, although a number of virulence determinants are clearly host specific (36), there is also evidence for the existence of universal virulence mechanisms shared by fungal pathogens with highly diverse host ranges (17, 20). Interestingly, common patterns of host defense also are found in evolutionary diverse groups such as plants, insects and mammals (23). Thus, evolutionary ancient mechanisms of fungal virulence and host defense might coexist with highly specific virulence and resistance traits that have arisen during later stages of pathogen-host coevolution.

The availability of the complete genome sequences of fungal plant and human pathogens and their model hosts, as well as the development of high-throughput protocols for gene function analysis, hold a huge potential for advancing our understanding on fungal pathogenesis and host defense (33). However, pathogen host range imposes an important limitation on the comparative analysis of virulence mechanisms in evolutionary distant hosts because it requires gene identification and knockout in different pathogenic organisms. This constraint has been overcome in bacterial systems by developing multihost pathogenesis models, as exemplified by the *Pseudomonas aeruginosa* strain PA14 (29). Application of these models to phylogenetically diverse host species such as *Arabidopsis* spp., *Caenorhabditis elegans*, or mice has led to the identification of both common and contrasting virulence mechanisms in bacterial plant and animal pathogenesis (28, 32). In fungi, no such model system allowing simultaneous testing of virulence factors on plant and mammalian hosts is currently available.

The soil-borne fungus *Fusarium oxysporum* is the causal agent of vascular wilt, a disease that affects a large variety of economically important crops worldwide (1). Besides its well-studied activity as a plant pathogen, *F. oxysporum* is known as a serious emerging pathogen of humans due to the increasing number of severe cases reported and to its broad resistance to the available antifungal drugs (2, 24). *Fusarium* now represents the second most frequent mold causing invasive fungal infections in immunocompromised patients, frequently with lethal outcomes (22, 27, 35). *F. oxysporum*, together with *F. solani* and *F. verticillioides*, are responsible for practically all of the cases of invasive fusariosis in humans (14). Given the dual ability to cause disease both on plants and on humans, we reasoned that *F. oxysporum* could serve as a universal model for studying fungal virulence mechanisms. In the present study, we tested the ability of *F. oxysporum* f.sp. *lycopersici* strain

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4287, a well-characterized model pathogen of tomato plants, to cause disease in a mammalian pathogenesis model. We found that strain 4287 is able to produce systemic infections in immunodepressed mice, resulting in a high death rate. By applying the mouse model to a number of knockout mutants previously shown to exhibit altered virulence on tomato plants, we show that specific virulence factors in a single fungal strain play distinct functional roles in plant and animal pathogenesis.

MATERIALS AND METHODS

Fungal isolates and culture conditions. *F. oxysporum* f.sp. *lycopersici* strain 4287 (race 2) was originally obtained from J. Tello, Universidad de Almería, Almería, Spain, and stored at −80°C with glycerol as a microconidial suspension (11). The pathotype of the isolate on tomato was routinely confirmed by plant infection assays. The generation and characterization of the following mutant strains, all derived from wild-type strain 4287, were described previously: mitogen-activated protein kinase (MAPK) mutant Δ*fmk1* (9), *pacC* loss-of-function mutant $pacC^{+/-}12$ and dominant activating mutant $pacC^{c}9$ (5), and class V chitin synthase mutant D1 and complemented strain C2 (21).

For preparation of inocula, cultures were grown in potato dextrose broth (Difco, Detroit, Mich.) at 28°C and 150 rpm for 4 days, and microconidia were obtained by filtration as described previously (11). The microconidial suspension was centrifuged and the pellet was resuspended in sterile saline. The concentration of conidia was adjusted to 10^8 CFU/ml with a hemocytometer and checked by plating serial dilutions on potato dextrose agar (PDA; Pronadisa, Madrid, Spain) plates that were incubated at 28°C for 3 days. To prepare heat-killed conidia for testing their activity in the murine model, conidial suspensions were incubated in a thermostatic bath at 60°C for 60 min. Complete heat-killing of conidia was confirmed by plating serial dilutions on PDA plates.

Animal infection. OF-1 male mice (Charles River, Criffa S.A., Barcelona, Spain) weighing ca. 30 g were used. Five mice were housed per cage in standard conditions with free access to food and water. Conditions were approved by the Animal Welfare Committee of the Faculty of Medicine of Universitat Rovira i Virgili. Mice were immunosuppressed with a single intraperitoneal 200-mg/kg dose of cyclophosphamide (Laboratorios Funk S.A., Barcelona, Spain) and with an intravenous 150-mg/kg dose of 5-fluorouracil (Fluoro-uracil; Roche S.A., Madrid, Spain) on day 0.

Groups of 10 animals were infected by injecting 0.2 ml of an inoculum of 10^8 conidia/ml into a lateral vein of the tail on day 0. Survival was recorded each day for 13 days. Infection experiments with each individual strain were performed between 3 and 6 times. Survival was estimated by the Kaplan-Meier method and compared among groups by using the log-rank test.

Tissue burden and histopathology. Randomly chosen surviving mice were sacrificed 13 days after inoculation with an overdose of halotane (Fluothane; Zeneca Farma, S.A., Pontevedra, Spain). Livers, spleens, kidneys, lungs, and brains were aseptically removed, and one-half of each organ was weighed and homogenized in 1 ml of sterile saline. Tenfold serial dilutions of this homogenate were made with sterile saline and spread onto PDA. Plates were incubated at 28°C, colonies were counted after 3 days, and the numbers of CFU per gram of organ were calculated. Fungal colony counts were converted to $\log_{10}$ values and compared by using the analysis of variance test. Calculations were performed by using SPSS for Windows, version 10.0.

The remaining halves of the organs were fixed for 10 days in 10% neutral buffered formaldehyde, embedded in paraffin wax, and automatically processed. Sections of the embedded tissues (3 μm in thickness) were stained with hematoxylin-eosin, periodic acid-Schiff, and methenamine silver (Grocott) for light microscopy observations.

RESULTS

***F. oxysporum* f.sp. *lycopersici* causes systemic infection and death in immunodepressed mice.** *F. oxysporum* strains have been reported either as plant or human pathogens. We tested the hypothesis that a single strain of *F. oxysporum* can produce disease both on plant and mammalian hosts. As a candidate we chose strain 4287, a tomato pathogenic isolate belonging to *F. oxysporum* f.sp. *lycopersici* race 2, because the genetic interaction between this pathogen race and its host plant has been well characterized (1, 31). Isolate 4287 has been previously used in numerous molecular studies, resulting in the availability of genomic and cDNA libraries and optimized plant infection assays (10). Studies in our laboratory with targeted gene knockout mutants (5, 9, 21) have led to the identification of several genes involved in the virulence of *F. oxysporum* on tomato plants (Fig. 1).

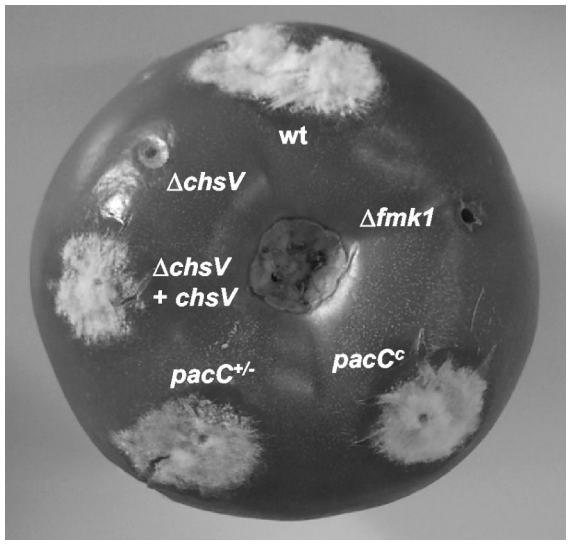

FIG. 1. Invasive growth of *F. oxysporum* strains on tomato fruits. Fruits were inoculated by injecting 5×10^5 microconidia of the following strains: wild-type strain 4287, MAPK mutant Δ*fmk1*, *pacC* loss-of-function mutant $pacC^{+/-}$, dominant-activating mutant $pacC^{c}$, chitin synthase mutant Δ*chsV* complemented with the wild-type *chsV* allele, and chitin synthase mutant Δ*chsV*. The photograph was taken after 5 days of incubation at 100% relative humidity.

In preliminary experiments, we found that strain 4287 grew and sporulated well in submerged culture on porcine blood plasma at 37°C (data not shown). We next tested the capacity of the strain to cause infections in mice. The murine model was chosen because of its high relevance to infectious diseases in humans (3). Inoculation of immunocompetent mice with microconidia of strain 4287 did not produce any detectable symptoms (data not shown). In contrast, inoculation of immunodepressed mice with 2×10^7 microconidia reproducibly resulted in the death of ca. 70% of the animals (Fig. 2A and Table 1). Importantly, injection of heat-killed conidia caused no mortality, suggesting that death was not simply a consequence of physical presence of the microconidia but required living fungal propagules. To study the ability of *F. oxysporum* strain 4287 to produce disseminated infections in different organs, fungal tissue burden was determined in the livers, spleens, kidneys, and lungs of surviving mice sacrificed 13 days after challenge. The presence of fungal propagules was detected in multiple organs, with the highest concentration found in the spleen, followed by kidney, liver, and lung (Fig. 2B).

Role of fungal virulence factors in plant and animal infection. The ability of *F. oxysporum* 4287 to survive and reproduce in immunodepressed mice, colonize multiple organs, and kill the host suggested that this strain contains the basic pathogenicity determinants required to cause disease on mammalian hosts. To further test the usefulness of the *F. oxysporum* model

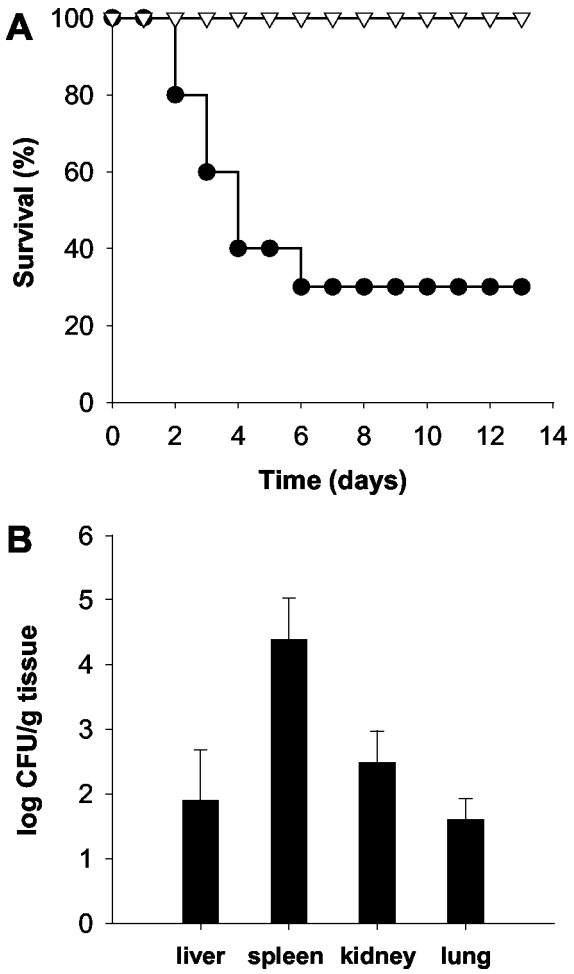

FIG. 2. *F. oxysporum* strain 4287 causes systemic infection and death in immunodepressed mice. (A) Groups of 10 immunodepressed mice were infected with 2×10^7 living (●) or heat-killed (▽) microconidia by lateral tail vein injection. The percent survival was plotted for 13 days. The data shown are from one representative experiment. (B) Four surviving mice were sacrificed on day 13 postinfection, and homogenates from the indicated organs were quantitatively cultured on PDA medium.

for comparing mechanisms of plant and animal pathogenesis, we examined a number of previously obtained knockout mutants in genes involved in virulence on tomato. Among these mutants, Δ*fmk1* strains that lack an MAPK structurally related to the yeast MAPKs Fus3 and Kss1 exhibit an extreme nonpathogenic phenotype on plants (9). Δ*fmk1* mutants not only fail to produce vascular wilt symptoms when inoculated onto tomato roots but also are unable to grow invasively on living tomato fruit tissue (Fig. 1). We found that the Δ*fmk1* mutant was at least as virulent as the wild-type strain in the murine model (Fig. 3 and Table 1), indicating that Fmk1 is dispensable for virulence on immunodepressed mice.

We next examined two different classes of mutants in *pacC*, encoding a zinc finger transcription factor that mediates ambient pH response in fungi (26) and negatively regulates virulence of *F. oxysporum* on tomato (5). *pacC*$^{+/-}$ loss-of-function mutants mimic growth at acidic ambient pH and exhibit increased virulence in a root inoculation assay. In contrast,

TABLE 1. MST of mice infected with the *F. oxysporum* wild-type strain 4287 and different mutants derived therefrom[a]

Strain	MST (days) ± SD[b]	95% Confidence interval	Significance (*P*)
4287 (wild type)	7.9 ± 0.5	6.8–9.0	
Δ*fmk1*	5.6 ± 0.8	4.2–6.9	0.01
pacC$^{+/-}$	12.3 ± 0.2	11.9–12.8	<0.01
pacC^{c}	8.7 ± 0.7	7.4–10.0	0.57
Δ*chsV*	1.7 ± 0.3	1.0–2.3	<0.01
Δ*chsV* + *chsV*	9.2 ± 1.0	7.1–11.3	0.49

[a] Groups of 10 immunodepressed mice were infected with 2×10^7 microconidia by lateral tail vein injection, and survival was recorded daily for 13 days. Data represent the means and standard deviations from at least four separate experiments.

[b] Groups marked with an asterisk were significantly different from the wild type ($P < 0.05$).

merodiploid *pacC*^{c} strains expressing a dominant-activating *pacC* allele mimic growth at alkaline pH and show significantly reduced virulence in the root inoculation assay (5), although invasive growth on tomato fruits is not affected (Fig. 1). Inoculation of immunosuppressed mice with a *pacC*$^{+/-}$ loss-of-function mutant resulted in a significantly higher survival rate and mean survival time (MST) than in mice inoculated with the wild-type strain (Fig. 3; Table 1). In contrast, survival and MST of mice infected with a dominant-activating *pacC*^{c} mutant were not statistically different from those infected with the wild-type strain. Thus, PacC is required for full virulence of *F. oxysporum* on mice but not on tomato plants.

A third class of knockout mutants tested were those lacking a functional copy of the *chsV* gene (21), encoding a class V chitin synthase required for virulence on tomato (Fig. 1). Δ*chsV* mutants have defects in cell wall integrity and show morphological alterations such as abnormally enlarged, lemon-like microconidia. When mice were inoculated with 2×10^7 microconidia of the Δ*chsV* mutant, most of the animals died

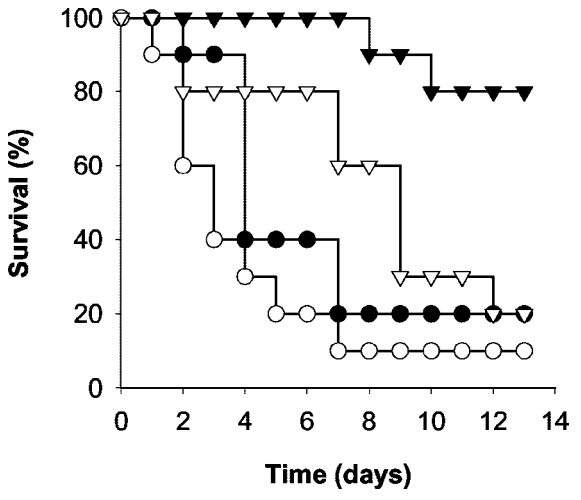

FIG. 3. Virulence of gene knockout mutants of *F. oxysporum* on immunodepressed mice. Groups of 10 immunodepressed mice were infected with 2×10^7 microconidia of the wild-type strain 4287 (●), MAPK mutant Δ*fmk1* (○), loss-of-function mutant *pacC*$^{+/-}$ (▼), or dominant-activating mutant *pacC*^{c} (▽). The percent survival was plotted for 13 days. The data shown are from one representative experiment.

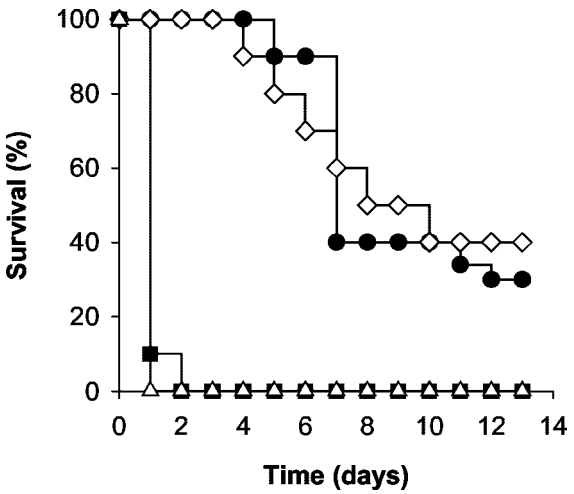

FIG. 4. *F. oxysporum* mutants lacking the chitin synthase ChsV cause "fast killing" on mice. (A) Groups of 10 immunodepressed mice were infected with 2×10^7 microconidia of the wild-type strain 4287 (●), chitin synthase mutant Δ*chsV* (■), heat-killed conidia of the Δ*chsV* mutant (△), or a Δ*chsV* mutant complemented with a wild-type *chsV* allele (◇). The percent survival was plotted for 13 days. The data shown are from one representative experiment.

within 24 h (Fig. 4 and Table 1). We termed this response "fast killing" as opposed to "slow killing" caused by the wild-type strain 4287. A Δ*chsV* mutant that had been transformed with the wild-type *chsV* allele reverted to the slow-killing phenotype, indicating that fast killing was determined by lack of a functional Δ*chsV* allele. Importantly, fast killing was provoked both by living and heat-killed conidia of the *chsV* mutant (Fig. 4) and occurred both in immunocompetent and in immunosuppressed mice (results not shown). Taken together, these data indicate that the slow- and the fast-killing responses have distinct underlying mechanisms.

Postmortem studies in mice that had died 24 h after injection with conidia of the Δ*chsV* mutant revealed an important amount of serum in the upper respiratory tract, suggesting respiratory insufficiency as a possible cause of death. In support of this hypothesis, histolopathological analysis of lung tissue of these animals showed an enlargement of the interstitial walls at the alveolar level. We noted the presence of numerous large (30 by 25 μm), lemon-shaped or irregularly swollen conidia (Fig. 5C to E) which obstructed the blood flow in the interstitial capillaries, leading to congestion, edema, and haemorrhagic foci (Fig. 5E). Some of the conidia had germinated and, ocasionally, branching of the germ tubes was observed. Lung sections of mice infected with heat-killed conidia of the *chsV* strain revealed a highly similar pattern except that these conidia failed to germinate (data not shown). In contrast, the lungs of animals infected either with the wild-type strain or with the complemented strain were considerably less damaged (Fig. 5A) than those of the group infected with the Δ*chsV* mutant. The presence of germinated and ungerminated microconidia of normal shape and size (10 by 3 μm) was detected in multiple organs (Fig. 5A and B). Taken together, these findings indicate that the fast-killing response might be caused by respiratory insufficiency due to early lung damage provoked by the conidia of the *chsV* strain.

DISCUSSION

Within the fungal kingdom, two highly successful groups of pathogens have evolved the ability to infect either plant or animal hosts. Whether these two classes of pathogens use similar mechanisms to cause disease in phylogenetically divergent hosts is currently an open question. Bacterial multihost pathogen models have been highly successful in revealing both conserved and contrasting virulence mechanisms in plant and animal pathogenesis (28, 32). Therefore, the availability of such multihost models for fungal pathogenesis might prove similarly useful. In the present study we have developed a dual plant-animal pathogen system based on a single strain of the vascular wilt fungus *F. oxysporum*, which allows the simultaneous testing of fungal virulence factors in plant and mammal host models.

In search for a fungal multihost pathogen model, *F. oxysporum* was an obvious candidate because of its well-documented ability to infect both plants and immunocompromised humans. Due to the economical and historical importance as a causal agent of vascular wilt, a much larger body of information is currently available on the phytopathogenic aspects of *F. oxysporum* than on its facet as an emerging human pathogen. Plant pathogenic strains of *F. oxysporum* exhibit a complex pattern of host specificity, prompting their classification into formae speciales and physiological races (1). Whereas race-cultivar specificity within a given forma specialis was recently shown to be controlled by a classical gene-for-gene system (16, 31), the genetic basis underlying plant host specificity in *F. oxysporum* remains unclear. The finding that isolates from a particular forma specialis which infect the same host plant have independent evolutionary origins suggests that the ability to infect a given plant species may have arisen convergently (25).

We decided to examine the ability of a genetically well-characterized plant pathogenic strain of *F. oxysporum* to cause disease in a standard animal infection model. An implicit concern with this approach was whether the behavior of a plant pathogenic strain would reflect that of a "true" human pathogen in the murine model. Our results suggest that strain 4287 is not a highly virulent animal pathogen, as shown by the inability to cause disease in immunocompetent mice. However, they also clearly demonstrate that this strain has the basic capacity to infect and to kill immunosuppressed animals in a highly reproducible manner. These findings are consistent with the fact that systemic infections in humans caused by *F. oxysporum* are predominantly reported in immunocompromised individuals (22, 27, 35). The presence of germinating microconidia in different organs suggests that strain 4287 can grow actively on mammalian tissue and may also undergo cycles of conidiation in the host, as reported previously for pathogenic *Fusaria* (19). This notion is further supported by the fact that significant amounts of fungal propagules were detected in multiple organs 13 days after inoculation. Even though quantitative CFU data obtained from filamentous fungal pathogens have to be interpreted with caution since they may vary depending on the spore/mycelium ratio, these results clearly indicate that strain 4287 has a high persistency within the mammalian host.

Once we confirmed the basic ability of *F. oxysporum* strain 4287 to cause disease in a mammalian model, we further tested its usefulness by examining knockout mutants in three func-

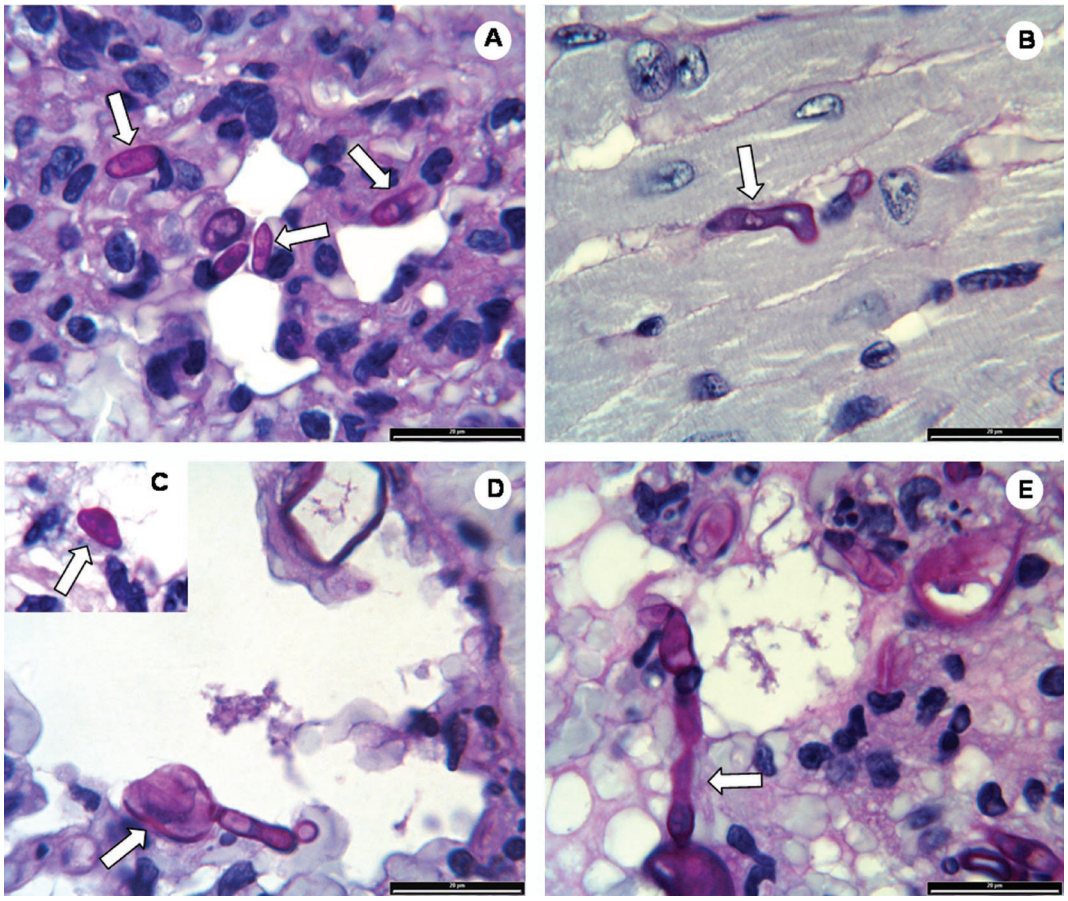

FIG. 5. Histological analysis of mice infected with *F. oxysporum* strains. Sections of embedded tissues from different organs (A and C to E, lung; B, heart) of mice inoculated with microconidia of *F. oxysporum* wild-type strain (A and B) or chitin synthase mutant Δ*chsV* (C to E) and sacrificed 24 h after inoculation were stained with hematoxylin-eosin, periodic acid-Schiff, and methenamine silver and then examined by light microscopy. (A) Characteristic ellipsoidal microconidia of wild-type strain 4287 in the interstitial space; (B) germinated microconidium of wild-type strain 4287; (C) lemon-shaped microconidium of the Δ*chsV* mutant; (D) swollen ungerminated and germinated microconidia of the Δ*chsV* mutant; (E) germinated conidia of the Δ*chsV* mutant growing in the interstitial spaces and provoking an edema. Bar, 20 μm.

tionally distinct classes of genes that were previously found to be involved in virulence on tomato plants. One of them, *fmk1*, encodes an MAPK that functions in a highly conserved signaling module that is essential for virulence in all fungal plant pathogens examined so far (18). Interestingly, we found that Fmk1 is dispensable for the virulence of *F. oxysporum* in the disseminated mouse model. This finding is intriguing, because it was suggested that this highly conserved signaling cascade might play functionally similar roles in fungal plant and animal pathogenesis (18). However, a recent study showed that the orthologous MAPK cascade of the human pathogen *C. neoformans* is also dispensable for virulence in a disseminated mouse model (7). Moreover, the same pathway in *C. albicans* was previously reported to contribute only partly to virulence (6). Although the role of this pathway has not been tested thus far in topical fungal infection models such as the skin or the oral cavity, these results, taken together, indicate that the Fus3/Kss1 MAPK cascade plays functionally distinct roles in plant and animal pathogenesis. Our data further suggest that such divergent functions not only apply to different pathogen species but also reside within a single fungal strain.

Similarly contrasting results were obtained with mutants in the zinc finger transcription factor PacC. In this case, we found that PacC is required for full virulence on mice, although it was previously shown to be dispensable or even negatively affected virulence on tomato plants (5). Interestingly, the *pacC* homologue of *C. albicans*, *RIM101*, is also required for virulence in a hematogenously disseminated murine model (8). A likely explanation for the contrasting role of PacC in animal and plant pathogenesis is the diverging pH of the host niche: slightly alkaline during systemic animal infection versus acidic in plant tissue. Depending on the ambient pH, PacC may function either as a positive or a negative virulence determinant by activating or preventing expression of distinct groups of pathogenicity genes.

A third class of knockout mutants tested were those affected in the class V chitin synthase ChsV. Chitin synthases catalyze the biosynthesis of chitin, a β-1,4-linked polysaccharide made of *N*-acetylglucosamine and an essential component of the fungal cell wall (4). *F. oxysporum* mutants that lack ChsV are unable to infect tomato and hypersensitive to antifungal plant defense compounds such as α-tomatine or H_2O_2 (21). Surprisingly, most of the mice injected with microconidia of the Δ*chsV* strain died within 24 h, a phenomenon that we termed "fast

killing." Postmortem studies and histopathological analysis suggested that in these animals death was caused by respiratory insufficiency as a consequence of severe lung damage. In contrast, death in mice infected by filamentous fungi is usually not provoked by initial lung damage but by more generalized lesions affecting numerous organs (13). We found evidence suggesting that this unusual fast-killing effect could be caused by physical obstruction of interstitial capillaries by the abnormally enlarged Δ*chsV* mutant conidia. The presence of morphological aberrations in fungal class V chitin synthase mutants such as balloon-like swellings and abnormally shaped, lemon-like microconidia, has been reported previously and is probably caused by defects in cell wall integrity, because it can be reversed by addition of an osmoprotectant to the growth medium (15, 21). Two lines of evidence strongly suggest that fast killing is mechanistically unrelated to slow killing caused by the wild-type *F. oxysporum* strain. First, unlike slow killing, fast killing was induced both by living and heat-killed conidia of the Δ*chsV* mutant, suggesting that the sole physical presence of the conidia is sufficient to provoke death. Second, in contrast to slow killing, fast killing occurred both in immunocompetent and in immunosuppressed mice, indicating that this mechanism acts independently of the immunological status of the animals.

F. oxysporum strain 4287 is to our knowledge the first fungal model strain reported to reproducibly infect both plant and mammalian hosts. The availability of such a multihost model strain represents a key step toward improving our understanding of how fungal pathogenesis on evolutionary distant hosts has evolved. The results obtained with the *F. oxysporum* knockout mutants suggest that certain virulence factors essential in plant pathogenesis are dispensable in animal pathogenesis and vice versa. In both cases where such divergent roles were detected between the tomato and the mouse model, the virulence pattern of the *F. oxysporum* mutants in mice mimics that previously reported for two well-established animal model pathogens, *C. albicans* and *C. neoformans*. This finding is important because it addresses the underlying concern whether the virulence pattern of an opportunistic pathogen such as *F. oxysporum* will reflect that of a "true" human pathogen. Our results with the *fmk1* and the *pacC* mutants suggest that *F. oxysporum* strain 4287 indeed behaves like a "true" animal pathogen in the immunosuppressed mouse model. Thus, *F. oxysporum* strain 4287 could be a useful model for the identification of conserved and divergent fungal virulence determinants in plant and animal pathogenesis, similar to the *P. aeruginosa* strain PA14 (30). Importantly, 4287 is also amenable to random insertional mutagenesis (21), and rapid and reliable in vivo virulence assays on tomato fruits are already available for this strain (9). The *F. oxysporum* model may therefore prove highly useful in providing new insights into the molecular basis of host specificity in fungal pathogenesis.

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