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rodletless Mutants of *Aspergillus fumigatus*

NATHALIE THAU,[1] MICHEL MONOD,[2] BRUNO CRESTANI,[3] CORINE ROLLAND,[3] GUY TRONCHIN,[4] JEAN-PAUL LATGÉ,[1] AND SOPHIE PARIS[1]*

Unité de Mycologie, Institut Pasteur,[1] and Unité INSERM 408, Faculté Bichat,[3] Paris, and Laboratorie de Parasitologie Mycologie, Centre Hospitalier Universitaire, Angers,[4] France, and Institut de Microbiologie, Centre Hospitalier Universitaire Vaudois, Lausanne, Switzerland[2]

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Conidia of *Aspergillus fumigatus* adhere in vitro to host proteins and cells via the outer cell wall layer. The *rodA* gene of *A. fumigatus* was cloned by homology with the *rodA* gene of *Aspergillus nidulans*, which is involved in the structure of the rodlets characteristic of the surface layer. The *A. fumigatus* RODA protein sequence has 85% similarity to that of *A. nidulans* RODA; the sequence codes for a hydrophobin, a low-molecular-weight protein moderately hydrophobic and rich in cysteines. The gene was disrupted with the hygromycin B resistance gene. By transformation of protoplasts with the disrupted gene, RodA$^-$ mutants were generated. These mutants are deficient in the ability to disperse their conidia; their conidia lack the rodlet layer and are hydrophilic. The adhesion of the *rodletless* conidia to collagen and bovine serum albumin was lower than that of the wild type; in contrast, there was no difference between RodA$^-$ and RodA$^+$ conidia in adhesion to pneumocytes, fibrinogen, and laminin, suggesting that RODA is not the receptor for these cells and proteins. RodA$^-$ conidia were pathogenic for mice.

The opportunistic fungus *Aspergillus fumigatus* may cause several respiratory diseases: allergic bronchopulmonary aspergillosis, resulting from repeated inhalation of conidia with limited fungal growth; aspergilloma, in which colonization of preexisting pulmonary cavities forms a fungal ball; and invasive aspergillosis, in which *A. fumigatus* invades lung parenchyma and disseminates to other organs (6). The mechanisms by which this fungus persists in the lung and causes diseases in certain individuals are still unclear. The small size of the conidia, their prevalence in the atmosphere, and their ability to grow at 37°C are not sufficient to explain the pathogenic development of *A. fumigatus*. It has been suggested that adhesion of the conidia, the infectious propagules, to host proteins and/or host cells is a primary event during the establishment of infection (1). Previous studies have shown that conidia of *A. fumigatus* in vitro adhere to fibrinogen, laminin, and complement via proteins of the outer cell wall (1, 23, 24).

One approach to understanding the mechanisms by which the conidia adhere to host proteins and cells is through an analysis of proteins that are present at the surface of the conidium. We have focused our attention on the conidial outer cell wall as the source of macromolecules which participate in various aspects of pathogen-host interactions. Electron microscopic and biochemical studies have shown that the outermost cell wall layer of *Aspergillus* conidia is characterized by the presence of interwoven fascicles of clustered proteinaceous microfibrils called rodlets (3, 8). This rodlet layer might play a role in the adhesion of *A. fumigatus* to host proteins and cells. In *Aspergillus nidulans* the *rodA* gene, encoding a small, secreted, moderately hydrophobic polypeptide, is involved in the formation of the rodlet layer. The conidia of RodA$^-$ mutants lack their external rodlet layer and are hydrophilic, while wild-type conidia are hydrophobic (22). Since cell surface hydrophobicity in *Candida albicans* is associated with enhanced virulence, the role of this hydrophobic layer in the pathogenicity of *A. fumigatus* was also investigated.

Using the *rodA* gene of *A. nidulans* as a probe, we cloned and sequenced the *rodA* gene of *A. fumigatus*. Mutants with a defective copy of this gene were then generated by gene disruption in order to test its importance in adherence to host proteins and cells.

MATERIALS AND METHODS

***Aspergillus* strains and culture conditions.** *A. fumigatus* CBS 144-89 is a clinical isolate from a patient with aspergillosis. G10, a spontaneous nitrate reductase mutant (Nia$^-$) of strain CBS 144-89, was chosen as the recipient strain for transformation (17).

The strains were maintained on 2% malt agar slants. To score for the *rodletless* mutation, transformants were grown on agar medium for 7 days at 25°C.

Mycelia for DNA preparation were grown for 18 h at 25°C in Sabouraud medium (2% glucose, 1% Mycopeptone Biokar; Prolabo, Beauvais, France). For transformation experiments, minimal medium with 5 mM ammonium tartrate as a nitrogen source was used (9).

Bacterial strains and plasmids. *Escherichia coli* DH5α was used for plasmid propagation. Plasmid Bluescript KS (Stratagene, La Jolla, Calif.) was used in subcloning procedures. Plasmid pAN7-1 (19) carrying the *E. coli* hygromycin B phosphotransferase gene (*hph*) was kindly provided by P. J. Punt (TNO Medical Biological Laboratory, Rijswijk, The Netherlands). Plasmid pMS14 (22), which contains the *A. nidulans rodA* gene in an 800-bp *Eco*RI-*Nco*I fragment, was kindly provided by W. E. Timberlake (Department of Genetics, University of Georgia, Athens).

Cloning procedures, DNA sequencing, and DNA manipulation. Approximately 50,000 recombinant plaques of the *A. fumigatus* genomic library in λ EMBL3A (14) were immobilized on nylon membranes (Genescreen; DuPont NEN). These filters were probed with the ^{32}P-labelled 800-bp *Eco*RI-*Nco*I fragment of pMS14 (containing the *A. nidulans rodA* gene) in a 5× SSC solution (1× SSC is 150 mM NaCl plus 15 mM Na_3

* Corresponding author. Mailing address: Unité de Mycologie, Institut Pasteur, 25 rue du Dr. Roux, F-75724 Paris Cedex 15, France. Phone: 33 1 45 68 82 25. Fax: 33 1 45 68 84 20.

citrate, pH 7.0) containing 20% formamide, 1% sodium dodecyl sulfate (SDS), and 10% dextran sulfate at 42°C for 20 h. The membranes were exposed to X-ray film after two 30-min washes in 2× SSC–1% SDS at 42°C. Positive plaques were purified, and the DNA was isolated (20). Agarose gel electrophoresis of restricted recombinant bacteriophage and Southern blotting were performed according to standard protocols (20). Plasmids were constructed by using standard techniques (20).

The nucleotide sequence of the *rodA* gene was determined from both strands by the dideoxy nucleotide method of Sanger et al. (21). Double-stranded DNA subcloned into plasmid Bluescript was sequenced with a Sequenase 2.0 sequencing kit (U.S. Biochemicals, Cleveland, Ohio) as recommended by the supplier. DNA sequence analysis was performed by using the University of Wisconsin Genetics Computer Group programs (11). Kyte-Doolittle hydropathy plots were constructed with a window size of seven by using the program DNA Strider, version 1.0 (16). *A. fumigatus* chromosomal DNA was isolated according to the procedure of Girardin et al. (12).

***A. fumigatus* transformation.** The transformation of *A. fumigatus* G10 was done as previously described (17).

Electron microscopy. Conidia replicas were prepared and examined by electron microscopy as described by Cole et al. (8).

Adhesion experiments. For radiolabelling of conidia, strains were cultured on 1 ml of 2% malt agar slants containing 36 μCi of [^{35}S]methionine (Trans ^{35}S-label; ICN, Irvine, Calif.) for 1 week at 37°C.

In order to study the adherence of conidia to lung pneumocytes, alveolar type II cells were isolated from the lungs of adult Sprague-Dawley rats by enzymatic dissociation and purified by differential adherence to plastic as previously described (10). The cells were used for the adherence assay 40 h after isolation. At that time, the cells formed confluent monolayers. The monolayers were washed twice with 10 mM phosphate buffer (pH 7.0)–0.15 M NaCl (PBS), and 5×10^5 radiolabelled spores in 500 μl of Dulbecco's modified Eagle's medium without fetal bovine serum were added to each well. After 6 h at 37°C, the supernatant and the first washing were recovered, and then the monolayer was disrupted by sonication to recover the adherent spores. The radioactivities of the supernatant and of the cells were measured. The percent attachment of fungal spores was expressed as $[A/(A+B)] \times 100$, where A is the number of radiolabelled spores bound to the rat alveolar type II cell monolayer and B is the number of radiolabelled spores free in the medium. Results were expressed as averages from three different experiments performed in triplicate.

Assays of conidium attachment to collagen, fibrinogen, laminin, and bovine serum albumin (BSA) were performed on microtiter plates coated with collagen (Serva), human fibrinogen (Kabi Diagnostica, Stockholm, Sweden), laminin isolated from the Englebreth-Holm-Swarm sarcoma tumor (7), or BSA (fraction V; Serva) as previously described (24). All proteins were applied at 100 μg/ml in PBS and left for 1 h at 37°C and overnight at 4°C, and 10^6 ^{35}S-radiolabelled conidia in PBS were added to each well. After 1 h of incubation at 37°C, the supernatant and the first washing with PBS containing the nonadherent conidia were recovered. Adherent conidia were removed by washing with 10% TFD4 detergent (Franklab, Saint-Quentin-en-Yvelines, France). The radioactivities of the supernatants in PBS and of the washings with TFD4 were measured. The percent attachment of fungal spores bound to protein was expressed as $[A/(A+B)] \times 100$, where A is the number of radiolabelled spores bound to protein and detached with TFD4 and B is the number of radiolabelled spores free in PBS. Results were expressed as the means from three experiments performed in duplicate.

Statistics. Means were compared by using the Mann-Whitney nonparametric U test.

Mouse infection. Wild-type and transformant strains (monospore isolates) were inoculated by inhalation into cohorts of 10 Swiss mice (16 to 18 g) at doses of 10^8, 10^7, 10^6, and 10^5 conidia per mouse as described by Moutaouakil et al. (18). All animals were pretreated with cortisone (two doses of 5 mg of cortisone acetate). Mortality evaluated after 1 week was expressed as the total number of dead mice.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been submitted to the GenBank database under accession number U06121.

RESULTS

Cloning and sequencing of the genomic *A. fumigatus rodA* gene. By using the *rodA* gene of *A. nidulans* to screen a genomic library of *A. fumigatus*, 10 hybridizing clones were identified. Restriction enzyme analysis of purified DNA showed that the 10 clones had common *Nco*I and *Hin*dIII fragments (2.8 and 0.5 kb, respectively) which hybridized with the *A. nidulans rodA* probe (data not shown). In one of these clones, the *A. fumigatus rodA* gene was localized in a 5-kb *Sal*I fragment and was subcloned in plasmid Bluescript to generate the plasmid pAF1. The restriction map of this *Sal*I fragment is illustrated in Fig. 1.

The nucleotide sequence of the *Hin*dIII fragment and flanking region (969 bp) is reported in Fig. 2. In plasmid pAF1 two adjacent *Hin*dIII sites were found in the first intron. The deduced amino acid sequences of the three possible reading frames determined by the nucleotide sequence in Fig. 2 were compared with the amino acid sequence of *A. nidulans* RODA, and this suggested that the *A. fumigatus rodA* gene consisted of three exons (334, 89, and 54 bp) and two introns (49 and 46 bp). The introns of *A. fumigatus rodA* were slightly shorter than the ones of the *A. nidulans rodA* gene (52 and 60 bp). Moreover, their lariat sequences were different from those of *A. nidulans* even if they matched the consensus sequences for fungal introns. The 290 bp of the 5′ upstream region of *rodA* contained a putative TATA box at position −180 bp relative to the ATG and contained a CAAT box located 60 bp upstream from this TATA box. These consensus features are characteristic of fungal promoter sequences (13).

Amino acid sequence analysis. The *rodA* gene was predicted to encode a 159-amino-acid polypeptide. The presence of a highly hydrophobic amino-terminal sequence with a predicted α-helix suggested the existence of a leader sequence required for secretion. The polypeptide had an internal hydrophobic domain with 43% hydrophobic residues (A, F, I, L, M, P, and V), 17% charged amino acid residues (D, E, K, R, and H), no tryptophan residues, and eight cysteine residues. Consequently, the *rodA* sequence predicted a small (133 amino acid residues and a calculated molecular mass of 13.4 kDa), secreted, moderately hydrophobic protein.

Comparison of the polypeptide sequence encoded by *A. fumigatus rodA* with that of the *A. nidulans* protein showed amino acid identity and similarity of 75 and 85%, respectively. Amino acid alignments of the *A. fumigatus* and *A. nidulans* RODA proteins showed a similar spacing and similar environment of the cysteine residues (Fig. 3). The *A. fumigatus* RODA protein had no putative glycosylation site, while the *A. nidulans* protein had one. An alignment of the hydrophobicity plots for

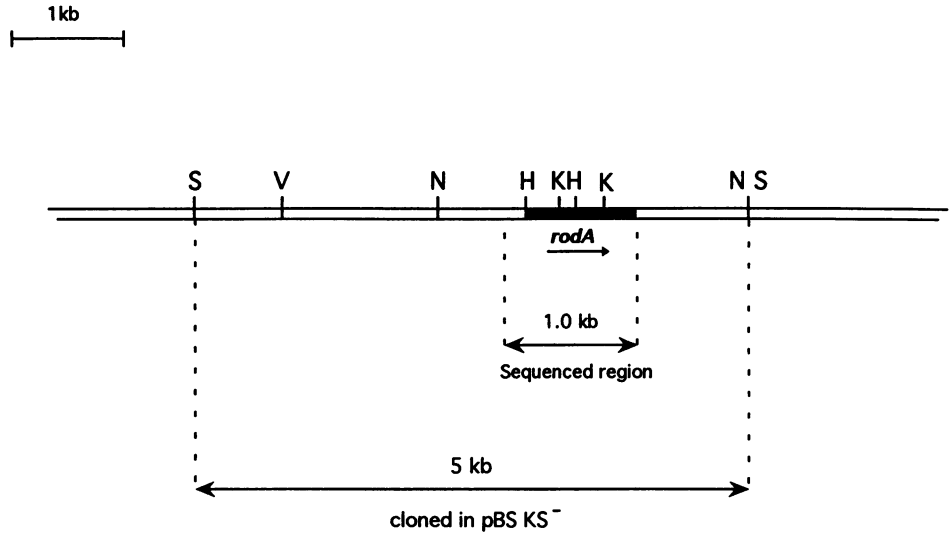

FIG. 1. Restriction map of the *rodA* locus region of *A. fumigatus*. The 5-kb *Sal*I fragment was subcloned in plasmid Bluescript (pBS) KS$^-$ to give pAF1. The black box designates the coding region of the *rodA* gene. Restriction site abbreviations: H, *Hin*dIII; K, *Kpn*I; N, *Nco*I; V, *Eco*RV; S, *Sal*I.

the two genes showed that the *A. fumigatus* and *A. nidulans* RODA proteins had similar characteristics.

Gene disruption of *A. fumigatus rodA*. In order to produce *A. fumigatus* strains with a nonfunctional copy of *rodA* by gene disruption, it was important to determine the number of copies of *rodA* in the genome. Southern hybridization analysis of genomic DNA digested with five enzymes (*Sal*I, *Pst*I, *Kpn*I, *Eco*RI, and *Bgl*II) yielded only one band in each case (7.5, 9, 10, 8.5, and 6.5 kb, respectively [data not shown]). This result was consistent with the presence of a single *rodA* gene.

The strategy outlined in Fig. 4 was used to inactivate the *rodA* gene. A 500-bp *Hin*dIII fragment of pAF1, containing most of the coding region, was replaced by the 3.65-kb *Hin*dIII fragment of pAN7-1H containing the *hph* gene flanked by the

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1    cacgatggattccttgcataccacattccactcagtgattcaagctggatagctaccatgagagaagactcaatcaagggcgggtagaacgagcatcaac   100

101  aggagctcgctgcctccaccggagtctatataaggatctctttacttcatctatggccgttctctttcctctccatcaaccatcactctcgttgacaagc   200

201  ttttccttcgaagtacaatcttctcacctttttccaagcccaaaaattcgctccgagtcttcactccctttttaaagccccaccgtcaca ATG AAG    296
1                                                                                              M   K      2

297  TTC TCT TTG AGC GCT GCT GTC CTC GCT TTC GCC GTC TCC GTG GCC GCC CTT CCT CAG CAC GAT GTT AAC GCT GCT    371
3     F   S   L   S   A   A   V   L   A   F   A   V   S   V   A   A   L   P   Q   H   D   V   N   A   A     27

372  GGC AAT GGT GTT GGC AAC AAG GGC AAC GCC AAC GTC CGC TTC CCC GTT CCT GAC GAC ATC ACC GTG AAG CAG GCA    446
28    G   N   G   V   G   N   K   G   N   A   N   V   R   F   P   V   P   D   D   I   T   V   K   Q   A     52

447  ACC GAG AAG TGT GGT GAC CAG GCT CAG CTC TCT TGC TGC AAC AAG GCC ACC TAC GCC GGT GAT GTC ACC GAC ATT    521
53    T   E   K   C   G   D   Q   A   Q   L   S   C   C   N   K   A   T   Y   A   G   D   V   T   D   I     77

522  GAC GAG GGC ATC CTT GCT GGT ACC CTC AAG AAC CTC ATC GGT GGT GGA TCC GGC ACC GAG GGC CTT GGT CTC TTC    596
78    D   E   G   I   L   A   G   T   L   K   N   L   I   G   G   G   S   G   T   E   G   L   G   L   F    102

597  AAC CAG TGC TCC AAC GTG GAC CTC CAG A gtacgttaagcttcatcaaagactggtcgtccgtactgacatatcttag TC CCC GTC    681
103   N   Q   C   S   N   V   D   L   Q                                                   I   P   V    114

682  ATT GGT ATT CCA ATC CAA GCT CTG GTC AAC CAG AAG TGC AAG CAG AAC ATT GCC TGC TGC CAG AAC TCT  CCT TCC    756
115   I   G   I   P   I   Q   A   L   V   N   Q   K   C   K   Q   N   I   A   C   C   Q   N   S   P   S    139

757  GAT GCC gtaagtgttctctatatatttgtagttctctactaacgtccatcag AGC GGT TCC CTC ATT GGA CTG GGT CTT CCT TGC    841
140   D   A                                                 S   G   S   L   I   G   L   G   L   P   C    152

842  ATT GCC CTT GGT TCT ATC CTG TAA gctactcgtcgcttctgctgttggcgctctcagccagcatttggagattcgatatggcagctgggtag   933
153   I   A   L   G   S   I   L  stop                                                                    159

934  gaacaggtaccggagtctttagataggtactgttaa                                                                969
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FIG. 2. DNA sequence of the *rodA* gene. Putative TATA and CAAT boxes in the 5′ region of the gene are double underlined. Two introns (49 and 46 bp) were identified by comparison with the *rodA* sequence of *A. nidulans* and are shown in lower case; the consensus splice signals are underlined. The putative translation open reading frame is given in one-letter code below the DNA sequence.

A

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RODAFUM      1  MKFSLSAAVLAFAVSVAALPQ-HDVNAAGNGVGNKGNANVRFPVPDDITV  49
RODANID      1      IA   V   A      PA  SQF          S  K    ENV    50

RODAFUM     50  KQATEKCGDQAQLSCCNKATYAGDVTDIDEGILAGTLKNLIGGGSGTEG   98
RODANID     51     SD                   T TV   L S A SG   A   A    99

RODAFUM     99  GLFNQCSNVDLQIPVIGIPIQALVNQKCKQNIACCQNSPSDASGSLIGLG 149
RODANID    100     D   KL VAV-L   -- D                   S D N   V  147

RODAFUM    150  LPCIALGSIL   159 amino acids
RODANID    148     V        157 amino acids
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B

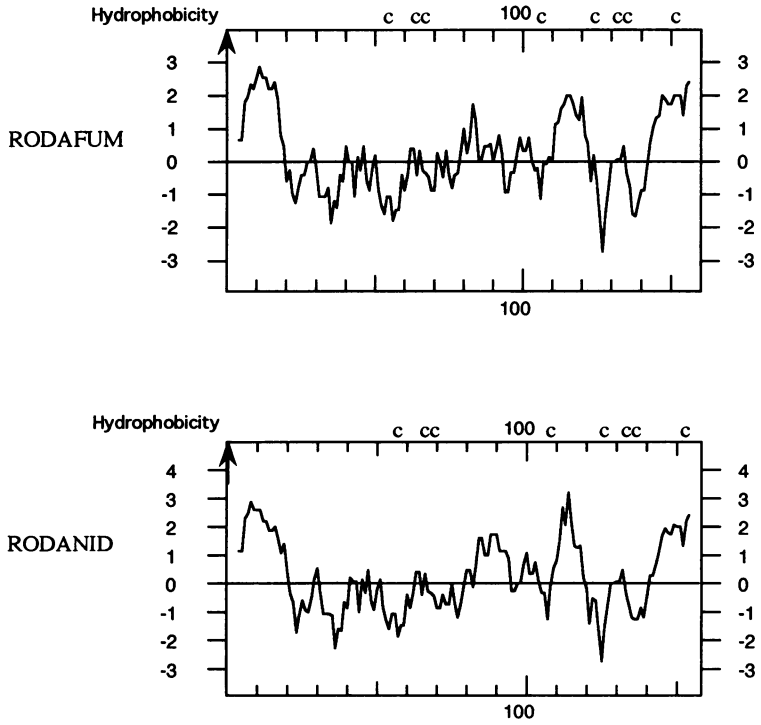


FIG. 3. Comparison of the polypeptide sequences of RODA proteins from *A. fumigatus* (RODAFUM) and *A. nidulans* (RODANID). (A) The predicted RODA sequence was aligned with the RODA sequence of *A. nidulans* on the basis of the conserved cysteine residues (underlined). Only different amino acids are shown. (B) Hydropathy plots were calculated with a window size of seven. Hydrophobic regions are shown above the center line, and hydrophilic regions are shown below the line.

trpC terminator and *gpd* promoter, resulting in pAF3*hph*. This plasmid was cut with *Eco*RV and *Apa*I, and the linear 7.3-kb fragment was used for transformation of *A. fumigatus* G10. Forty-two hygromycin B-resistant transformants were selected and purified. Five *rodletless* mutants, ΔR11, ΔR29, ΔR36, ΔR41, and ΔR47, among the HmB^r transformants were selected on the basis of the phenotype of the colonies on malt extract agar.

Phenotype of RodA⁻ mutants. After 1 week of growth at 25°C, the conidiating mutant colonies looked darker than the wild-type colonies (Fig. 5). A drop of water deposited on top of the RodA⁻ sporulated colony was readily adsorbed, while it remained as an individual drop on the top of the wild-type colony. In addition, the conidia of the mutant transformants were easily wettable but remained aggregated and did not disperse easily in water. To break up clumps of spores, ultrasonic treatment in a water bath (Branson 2200) was required. RodA⁻ conidia were not dispersed in the air as a

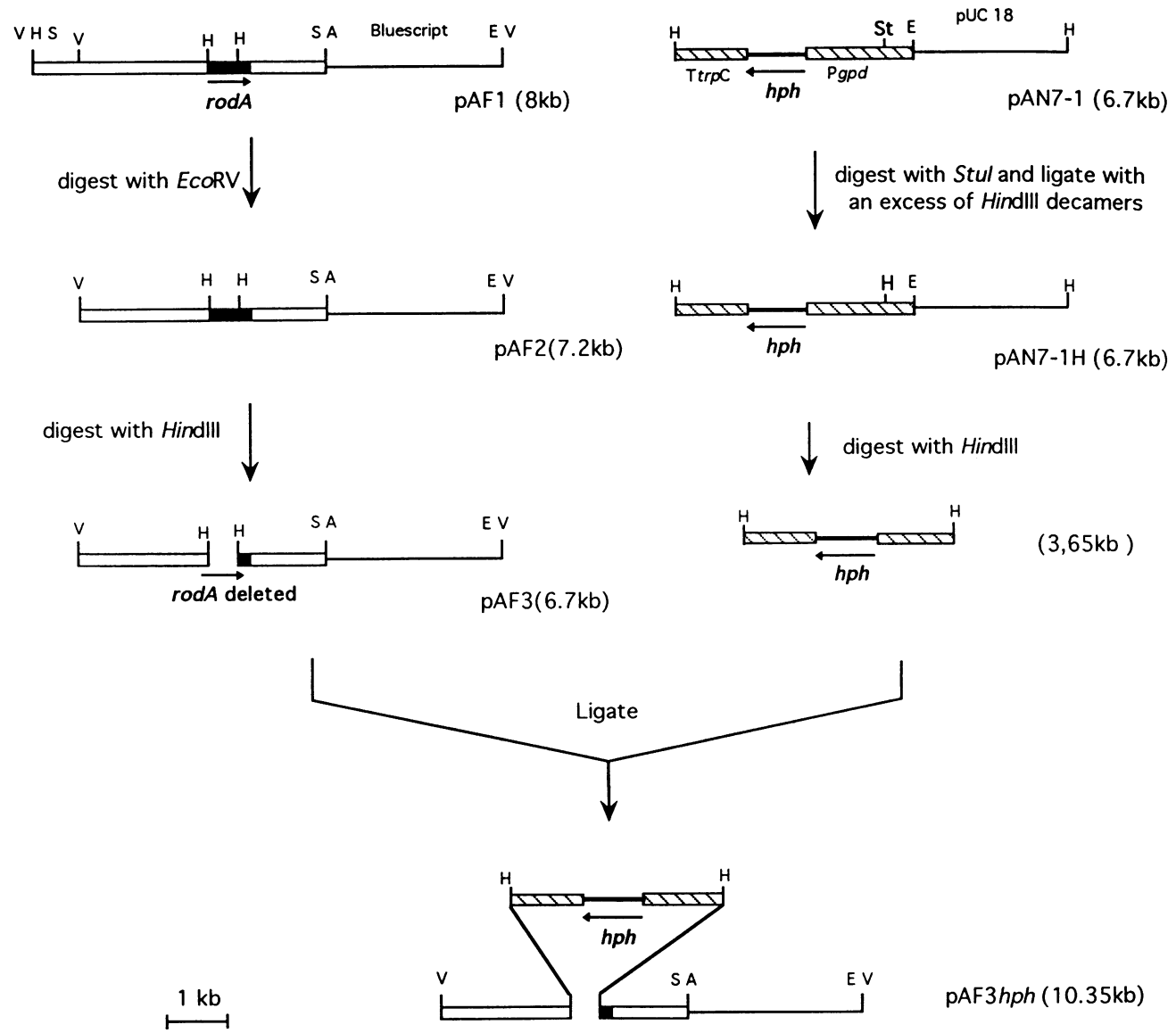


FIG. 4. Construction of *rodA* gene disruption plasmid pAF3*hph*. Boxes represent *Aspergillus* DNA; the black bar is the *A. fumigatus rodA* gene coding region. Lines represent plasmid DNA; the thick one is the *hph* gene, which is flanked by P_{gpd} (the promoter of the *A. nidulans gpd* gene) and T_{trpC} (the terminator of the *A. fumigatus trpC* gene) (hatched boxes). Only restriction sites relevant to the vector construction and to the analysis of the transformants have been included: A, *Apa*I; E, *Eco*RI; V, *Eco*RV; H, *Hin*dIII; S, *Sal*I; and St, *Stu*I.

conidial cloud when agar slants were shaken by hand. Both features are indicative of a lack of outer conidial rodlet fascicles. Electron microscopy was performed on the ΔR11 and ΔR29 mutants. Surface carbon-platinum replicas of RodA$^-$ conidia revealed an amorphous outermost wall layer, while conidia of the wild-type G10 strain showed interdigitated fascicles of parallel bundles of rodlets (Fig. 6).

Southern hybridization analysis. *Sal*I- and *Nco*I-digested genomic DNAs of phenotypic mutants were subjected to Southern analysis with the 2.8-kb *Nco*I fragment of pAF1 containing *rodA* as a probe (Fig. 7). If the desired recombination event occurred, leading to the incorporation of the *rodA*Δ::*hph* construct, then the 5-kb *Sal*I and 2.8-kb *Nco*I fragments would be expected to be replaced by two *Sal*I fragments (5 and 3.0 kb) and two *Nco*I fragments (3.7 and 2.1 kb). Southern analysis verified that three RodA$^-$ mutants (ΔR11, ΔR29, and ΔR47) had the expected enzyme profiles and were *rodA*Δ::*hph* disruptants.

Adhesion to pneumocytes II, collagen, fibrinogen, laminin, and BSA. The percentage of conidia adherent to pneumocytes II was not significantly higher with the RodA$^-$ mutant (54%) than with the wild type (45%) (Fig. 8). There is no difference between RodA$^-$ and RodA$^+$ conidia in adhesion to fibrinogen and laminin. By using fluorescent antilaminin antibody, soluble laminin was also found to bind to both RodA$^+$ and RodA$^-$ conidia (data not shown). In contrast, the adherence of RodA$^-$ conidia to collagen and BSA was lower than that of the wild type (Fig. 9).

Pathogenicity tests. Doses of 10^5, 10^6, 10^7, and 10^8 conidia were given to groups of 10 mice by nasal injection. The

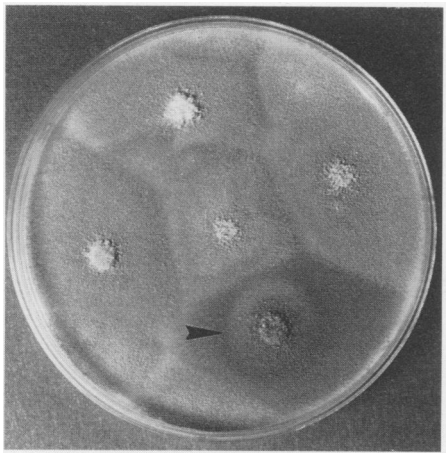


FIG. 5. Petri dish with four colonies of RodA$^+$ and one colony of RodA$^-$ (arrowhead) transformants. Strains were grown on 2% malt agar for 7 days at 25°C.

cumulative mortality 7 days after injection was 10, 30, 60, and 60%, respectively, for the wild-type strain. The mutant strain ΔR29 resulted in similar values, giving 20, 50, 50, and 40% mortality with similar doses of conidia, respectively. All fungi recovered from the lungs of mice inoculated with RodA$^-$ disruptants were HmB^r and NO_3^- (deficient in nitrate reductase) and had the *rodletless* phenotype on plate culture; no revertants were found.

DISCUSSION

We have cloned, sequenced, and disrupted the *rodA* gene of *A. fumigatus*; it codes for a hydrophobin, a class of small (100 to 160 amino acids) hydrophobic proteins with a signal sequence, an internal hydrophobic domain, and eight cysteine residues, including a tripeptide CCN, arranged in a conserved pattern (25). This protein is required for formation of the rodlet layer of conidia. No major differences between the RODA proteins of *A. fumigatus* and *A. nidulans* were observed. The amino acid substitutions are minor, and the hydrophobicities of the two proteins are similar.

A. fumigatus apparently contains only one gene with detectable homology to *rodA*: when a Southern blot of *A. fumigatus* DNA was probed at low stringency with the 500-bp *Hin*dIII fragment, which contains most of the coding region of *rodA* but little flanking DNA (Fig. 2), only those bands previously identified in high-stringency Southern hybridizations appeared (data not shown). Because of the similar characteristics of the RODA proteins in *A. fumigatus* and *A. nidulans*, the differences in the capacity for these two species to be dispersed in the air might not be due to this single protein. Most authors agree that RODA of *A. nidulans* and CCG-2 or EAS of *Neurospora crassa*, hydrophobins involved in rodlet formation, are excreted and are the main component of the rodlet layer (5, 15, 22). Recent experiments have even shown that the purified hydrophobin Sc3 from *Schizophyllum commune* can form rodlets in vitro (25). These results are in agreement with those of previous biochemical studies which showed that the rodlet layer is composed mainly of proteins (4, 8). However, no biochemical analysis of the *Aspergillus* rodlet layer has been done.

The outer cell wall layer of RodA$^-$ and RodA$^+$ strains can now be biochemically analyzed to test whether RODA is the only component of the rodlet. Other hydrophobins might exist,

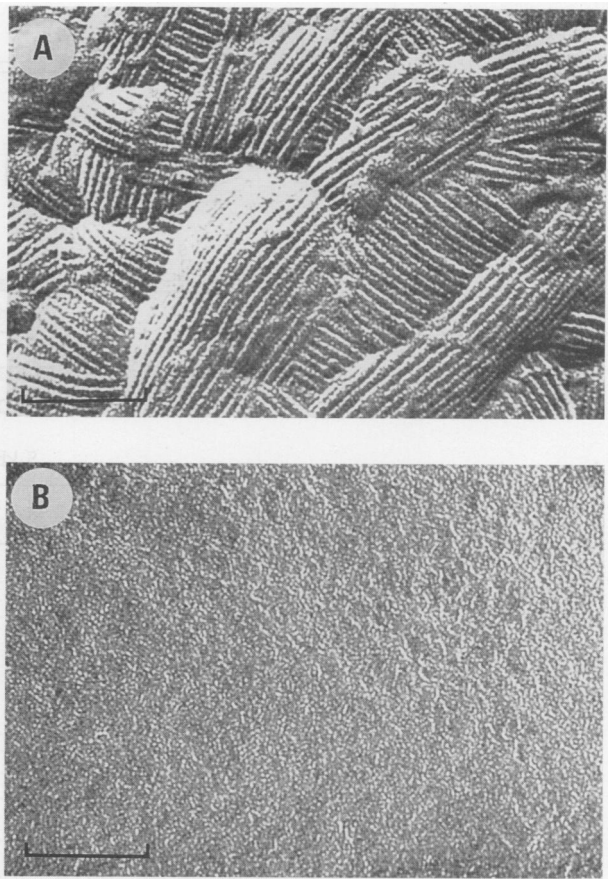
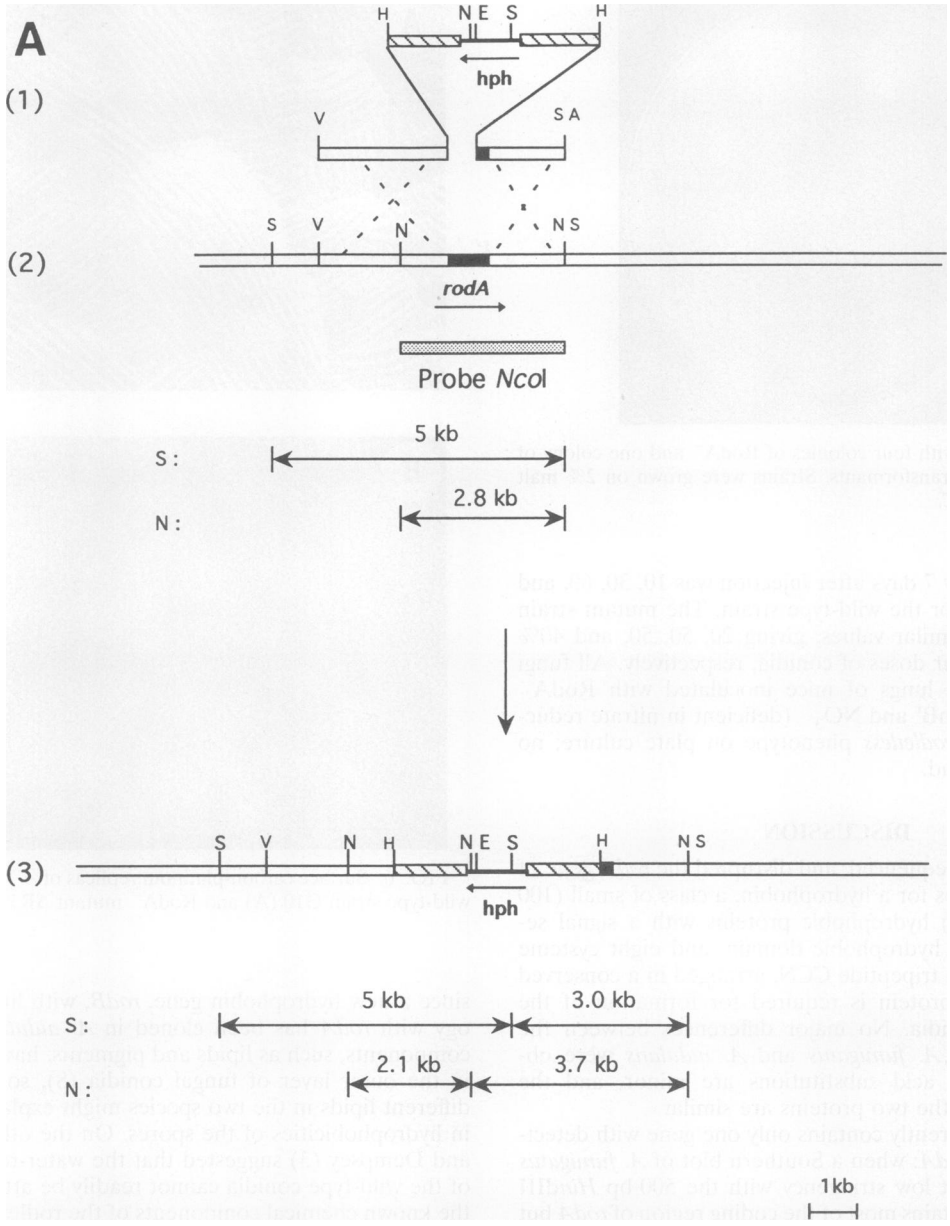


FIG. 6. Surface carbon-platinum replicas of *A. fumigatus* conidia of wild-type strain G10 (A) and RodA$^-$ mutant ΔR11 (B). Bar, 100 nm.

since a new hydrophobin gene, *rodB*, with little DNA homology with *rodA* has been cloned in *A. nidulans* (23a). Other components, such as lipids and pigments, have been identified in the outer layer of fungal conidia (8), so the presence of different lipids in the two species might explain the difference in hydrophobicities of the spores. On the other hand, Beever and Dempsey (3) suggested that the water-repellant property of the wild-type conidia cannot readily be attributed to any of the known chemical components of the rodlet layer but can be reasonably ascribed to the surface conformation.

In *C. albicans*, cell surface hydrophobicity is associated with enhanced virulence (2). In contrast to the results of our studies on *A. fumigatus*, no gene involved in the hydrophobicity of *C. albicans* has been identified and sequenced, and neither hydrophobin nor rodlets have been observed in *C. albicans*. The difference in hydrophobicities in the strains of *C. albicans* might be a phenotypic characteristic linked to other types of molecules. In spite of the absence of rodlets and of their hydrophilicity, RodA$^-$ *A. fumigatus* mutants are able to invade lung tissues, and there was no significant difference in the survival of mice inoculated with RodA$^-$ and RodA$^+$ strains. These results suggest that the mechanisms of infection of *A. fumigatus* are quite different from those of *C. albicans*, in which surface hydrophobicity of the growing yeast cells contributes to their virulence.

Binding of *A. fumigatus* conidia to host proteins and cells seem to involve two different phenomena: a nonspecific binding to BSA and collagen and a more specific binding to



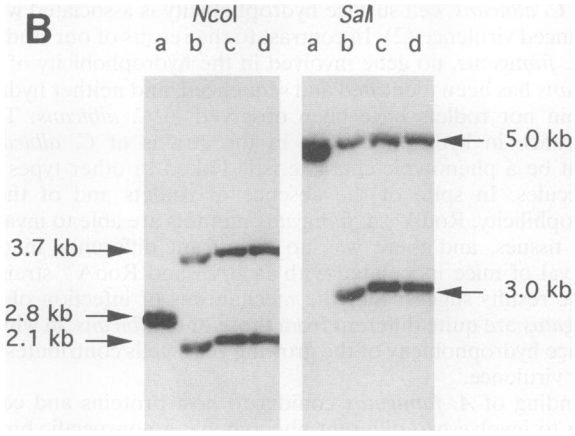


FIG. 7. (A) Disruption of *rodA* in *A. fumigatus*. 1, Map of the *Eco*RV-*Apa*I fragment of plasmid pAF3*hph* containing *rodA* disrupted by the *hph* gene; 2, genomic DNA of the G10 recipient strain; 3, genomic DNA of RodA$^-$ transformants. Open box, *A. fumigatus* DNA; black box, coding region of the *rodA* gene; hatched box, *A. nidulans* DNA; solid bar, *E. coli hph* gene. A, *Apa*I; E, *Eco*RI; V, *Eco*RV; H, *Hin*dIII; N, *Nco*I; S, *Sal*I; St, *Stu*I. (B) Southern hybridization of *Nco*I- and *Sal*I-digested genomic DNAs of wild-type strain G10 (lanes a) and mutants ΔR11, ΔR29, and ΔR47 (lanes b, c, and d, respectively). The filter was probed with the 2.8-kb *Nco*I fragment at 65°C.

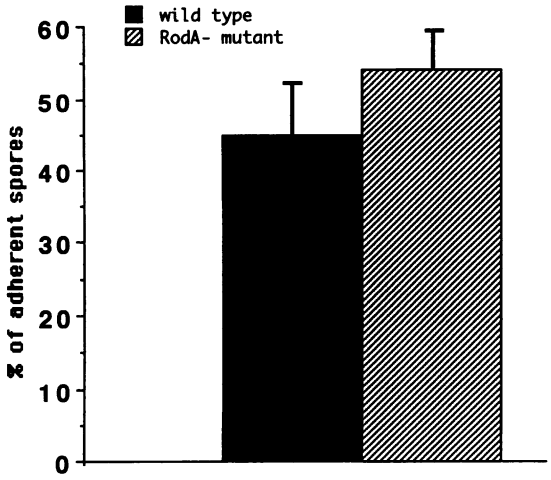


FIG. 8. Adhesion of conidia to pneumocytes II in vitro. The wild-type strain was G10, and the RodA⁻ mutant was ΔR29 (ΔR47 [not shown] gave identical results). Bars indicate the standard deviation from the mean.

fibrinogen, laminin, and pneumocytes. The binding to BSA and collagen decreases in the RodA⁻ mutants (by factors of 5 and 2, respectively). This attachment might be due to nonspecific hydrophobic interactions present mainly with wild-type conidia. The receptors for the specific binding to fibrinogen, laminin, and pneumocytes have not been identified so far. The similar binding of RodA⁻ mutant and wild-type conidia to fibrinogen, laminin, and pneumocytes suggests that RODA is not the receptor specifically involved in the adherence to these proteins and cells. This is consistent with experiments involving binding to pneumocytes, in which no inhibition by maltose, mannose, and lactose was observed (9a). These results are also in agreement with the experiments of Tronchin et al. (24), in which labelling of thin sections of RodA⁺ conidial wall with laminin was distributed essentially at the level of the electron-dense outer layer of the conidial wall but also on underlying lamellar layers of the cell wall (23b).

Although RODA does not seem to be the major surface protein responsible for the adherence of conidia to host cells and proteins, it contributes to the efficiency of dispersion by air

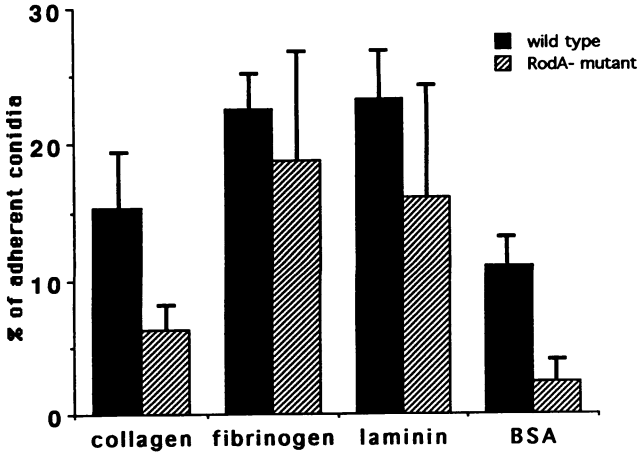


FIG. 9. Adhesion of conidia to collagen, fibrinogen, laminin, and BSA. See the legend to Fig. 8 for details.

of *A. fumigatus* conidia. If present in nature, RodA⁻ mutants will never be dispersed to reach the respiratory tract of a putative patient as occurs with *A. fumigatus* conidia from wild strains. The immunological role of rodlets during infection of host tissue remains unknown, but since hydrophobin and rodlets are quite resistant to various chemical and enzymatic treatments (8, 25), they might play a role in the protection of the conidia from the hydrolytic activity of the phagocytic cells.

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