Generating and Testing Molecular Hypotheses in the Dermatophytes[▽]

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Dermatophytes are the most common cause of fungal infections worldwide, impacting millions of individuals annually. In the United States alone, this translates into an economic impact on the health care system estimated to exceed $400 million a year for treatment alone (8, 46). A recent survey conducted in 16 European countries has shown that more than one-third (35 to 40%) of the 90,000 participants were suffering with a fungal foot disease, mainly caused by dermatophytes (4). In a recent study, 22 to 50% of children in a U.S. day care center exhibited symptoms of dermatophytic scalp infections (2). Despite the prominence of dermatophyte infections and their resulting socioeconomic consequences, the research and medical communities lack a sophisticated understanding of these organisms' biology. Consequently, effective preventatives and therapeutics are lacking. These deficiencies are in large part due to the lack of genetic tools available for the study of these fungi and their host specificities. Several research efforts are now poised to produce genomic and molecular resources that will enable the molecular characterization of dermatophytes. These resources will soon be available for use by the larger research community to address many questions about the biology and pathogenesis of dermatophytes. This review will elaborate on the current status of these resources and their importance to the study of the dermatophytes.

DERMATOPHYTE BIOLOGY AND DISEASE

Disease. Tinea is a fungal infection of the skin, hair, or nails (reviewed in reference 34). The phrase is made specific by the addition of another term referring to a specific body location. Thus, the term "tinea pedis" refers to a fungal infection of the feet. Other tinea infections include tinea capitis (scalp or head), tinea corporis (body or trunk), and tinea unguium (nails, also known as onychomycosis). These terms do not indicate the infecting organism but the type of infection and the body location. Tinea infections are usually localized to the surface and are rarely systemic or disseminated.

Fungal infections can be asymptomatic, they can be acute and severe, associated with inflammation, or they can be chronic (most common) (34). Tinea pedis usually occurs in the toe webs, with considerable scaling, fissuring, maceration, and erythema, accompanied by severe itching or burning. Tinea pedis can also localize to the sole of the foot, extending up the

sides of the foot. The destruction of the skin barrier can result in bacterial superinfection. Nail infections are associated with thickening, discoloration, and pain. Scalp infections can cause irreversible hair loss.

Organisms. All of the dermatophytes are ascomycetous molds, members of the class *Euascomycetes* that also includes *Aspergillus* species and the dimorphic fungi (Fig. 1) (34). The dermatophytes are classified into three genera: *Trichophyton*, *Microsporum*, and *Epidermophton*. As can be seen in Fig. 2, the dermatophytes are a monophyletic clade, although the assigned genus designations within the clade do not strictly segregate (24). At least 31 of the dermatophyte species are known to be human pathogens, although additional unidentified species (including pathogens) are likely to exist.

Species of dermatophytes are characterized based on their environmental niche: geophilic (soil dwelling), zoophilic (animals), and anthropophilic (human specific). The geophilic species (Fig. 2, highlighted in green) are commonly found in soil and only rarely are found in human infections. The zoophilic species (Fig. 2, highlighted in red) colonize animals and can be transmitted occasionally to humans. These species are responsible for about 30% of human dermatophytoses and usually cause acute inflammation upon infection. The anthropophilic species (Fig. 2, highlighted in black) have adjusted to growth in humans and do not appear to have an animal reservoir. They are responsible for the majority (ca. 70%) of human dermatophytoses. These species cause chronic, slowly progressing disease, suggesting that the fungi have adapted to human hosts. Genome sequences from each of these types are currently being prepared and will enable a comparative genomic approach to host specificity.

Many of these species have a sexual cycle. The geophilic species commonly have a viable sexual cycle, the zoophilic species frequently retain the sexual cycle, and the anthropophilic species have, for the most part, lost the ability to complete a sexual cycle. This suggests that most anthropophilic species reproduce clonally and that one or a few clonal lines may be responsible for chronic fungal infections (20, 21, 45). It has also been hypothesized that those clonal lineages are derived from + or − mating types of their sexual relatives (22). Comparative analysis of genome sequence from geophiles, zoophiles, and anthropophiles will identify loci required for mating competence as well as loci required for successful infection of humans. It will be of interest to compare the mating loci of the dermatophytes with the recently described mating loci from related euascomycetes, including *Aspergillus fumigatus*, *Aspergillus oryzae*, and *Aspergillus nidulans* (15) and from *Histoplasma capsulatum*, *Coccidioides immitis*, and *Coccidioides posadasii* (13). The mating loci of these fungi all include

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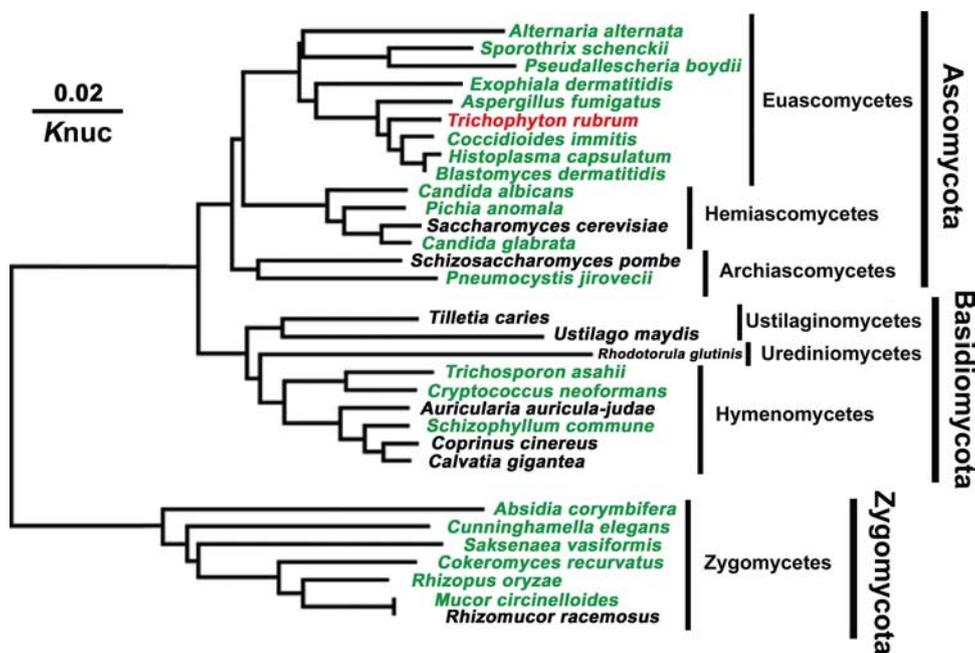
FIG. 1. Phylogeny of a dermatophyte (*T. rubrum* [red]) and other fungi, including human pathogens (green). The figure was kindly provided by Koichi Makimura, Teikyo University. The figure was adapted from a phylogenetic tree based on 18S ribosomal DNA sequences (Pathogenic Fungi Database [http://www.pfdb.net/]).

an α-box transcription regulator and a high-mobility-group domain. With the development of genome sequences for the dermatophytes, it will soon be possible to identify and characterize these mating features in dermatophyte species and to determine if species with no known mating cycle might be capable of mating.

CLINICAL IMPACT

Diagnosis. Gold standard diagnosis of dermatophyte infections is microscopic examination of the clinical specimen (nail, skin, and hair) with potassium hydroxide followed by culturing. The identification of species, which can be important for prognosis and for treatment, relies on culturing the organism, which may take up to 4 weeks, followed by microscopy, as well as nutritional tests (e.g., urease production). A handful of rapid diagnostic tests using current molecular methodology have been developed with the dermatophytes already (22, 29).

Treatment. Current therapy of fungal skin infections can take two forms. Topical treatments are appropriate only for early and/or mild skin infections, especially with *Trichophyton rubrum*, the major cause of tinea pedis. Systemic antifungal therapies are usually required for nail infections (onychomycosis) and for zoophilic dermatophyte infections (mainly tinea capitis/corporis). Especially for the treatment of onychomycosis, the rate of relapse or reinfection is approximately 20% within 3 years (49). Researchers lack robust genetic and molecular tools to monitor and evaluate treatment, although a recent survey of tinea capitis using the standardized test for mold susceptibilities indicated that in vitro resistance of the species involved (mainly *Trichophyton tonsurans* and *Trichophyton violaceum*) is not currently an issue, although reduced

azole susceptibility was described for *Trichophyton interdigitale* (16).

Epidemiology. Shedding of infected skin cells and hair is a frequent mode of transmission for tinea pedis (34). Direct transmission by contact is rare. Estimates suggest that 30 to 70% of adults are asymptomatic carriers of these fungi, and the incidence of symptomatic disease increases with age (4, 52). The fungi responsible are commonly spread in public facilities such as gyms and swimming pools. Tinea pedis infections are most commonly associated with warm and moist feet and are closely associated with wearing shoes or boots. The infections are more common in men, especially between 15 and 50 years of age, suggesting hormonal control. While the infection can be found in children as young as 2 years old, it is usually associated with teenagers as they reach puberty (34).

In younger children, tinea capitis is the predominant dermatophyte infection, with symptoms occurring in 3 to 8% of children, while rates can be as high as 50% in African American children in the United States and in underdeveloped and developing countries (2).

Molecular epidemiology. One important question regarding the epidemiology of dermatophytes is the issue of reactivation or reinfection. Is an infection the result of a reactivation of a past infection or a new infection from the environment? This question is most easily answered using molecular tools that have sufficient discrimination power to distinguish closely related species and strains. Appropriate species and strain typing in most organisms usually focuses on the rRNA gene cluster, using conserved regions of the rRNA or internal transcribed spacer (ITS). This type of analysis has been performed for many of the dermatophytes (14, 25–27, 43, 47, 59). However, the rRNA repeat is present in multiple copies in the genomes
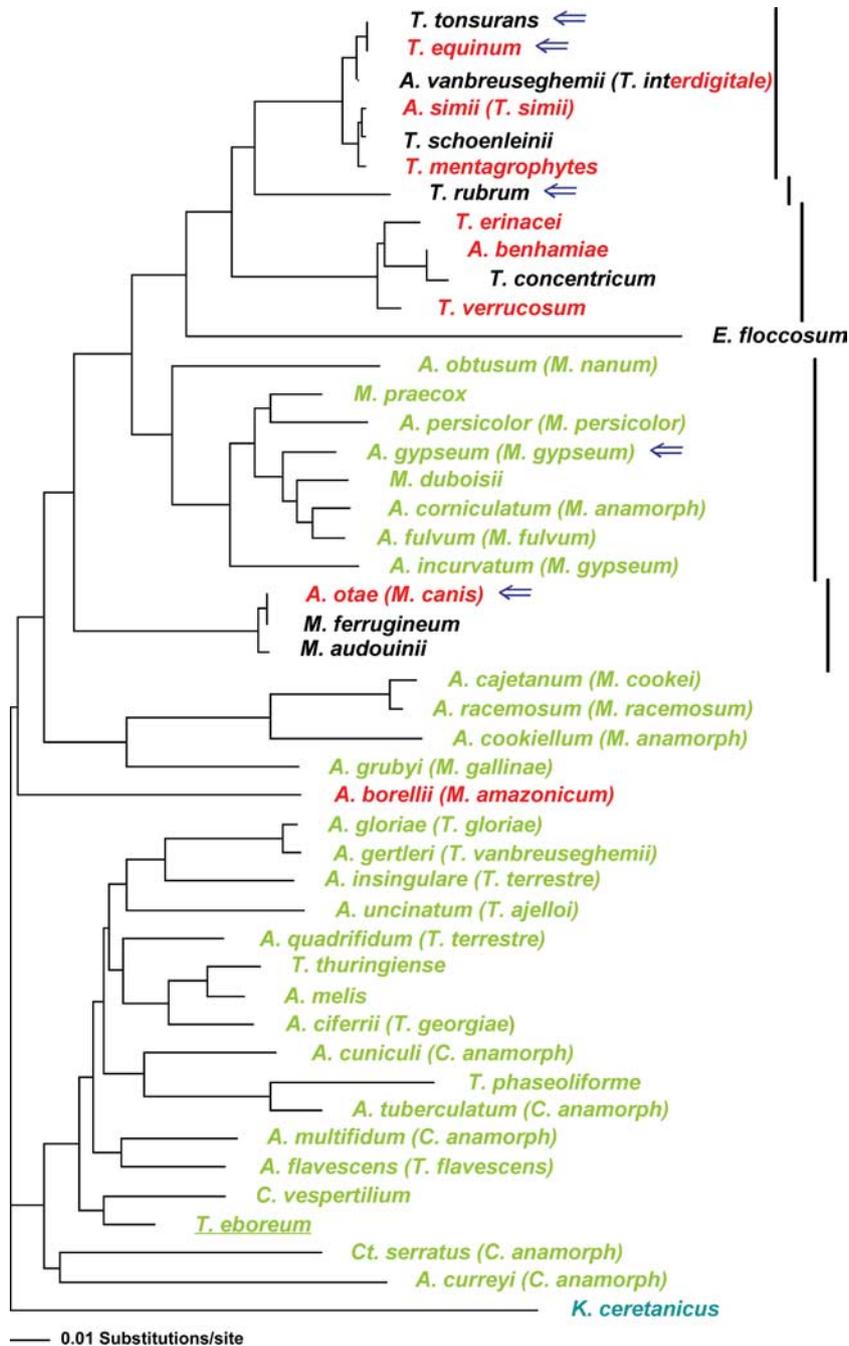
FIG. 2. Phylogeny of the dermatophytes using ITS sequences. The tree includes zoophilic (red), anthropophilic (black), and geophilic (green) dermatophytes. The horizontal length corresponding to 0.01 substitution/site is shown at the bottom. The five species to be sequenced are labeled with blue arrows. Vertical lines represent groupings of dermatophytes discussed in the text. *Keratinomyces ceretanicus* (dark green, at the bottom of the figure), another euascomycete, was used as an outgroup. This species is known to be the most phylogenetically distinct dermatophyte species and is not known to be pathogenic to humans (24). The figure was adapted from reference 22 with permission.

of most eukaryotes. Because of those repeats, PCR complications can occur associated with allelic variation between the repeats and mixing of alleles.

Recent molecular epidemiology using non-rRNA methods has been performed in *Trichophyton tonsurans*, the most common cause of tinea capitis (1, 2). In an initial study of preschool-aged children (2) using the rRNA locus and one additional gene locus, *T. tonsurans* infections were found to be endemic and disease appeared to be the result of activation of a single strain that persisted on the scalp, although multiple strain infections were observed. Analysis of the patient samples using two additional loci (1) further defined the mixed infections and demonstrated that mixed infections occur at a higher frequency than is commonly detected using cell morphology.

Taken together, the lack of effective diagnostic and treatment strategies, the sheer number of individuals that experi-

ence dermatophytic infections, and the economic consequences of these infections highlight deficiencies in the research efforts aimed at understanding this all-too-common group of fungal diseases. Genetic tools for the study of this important group of fungal pathogens are only beginning to be employed.

TOOLS FOR HYPOTHESIS GENERATION IN THE DERMATOPHYTES

ESTs and microarrays. Yang et al. (57) have recently established an extensive *T. rubrum* Expression Database (TrED) including expressed sequence tags (ESTs) and transcriptional profiles (www.mgc.ac.cn/TrED/). This database is one important component of the larger molecular toolbox needed for dermatophyte research. The database includes 40,617 ESTs, made from *T. rubrum* RNA extracted from spores, from cells postgermination, and from cells grown in collagen, elastin, and keratin. The ESTs provide evidence that the sequences do in fact encode expressed genes, and the ESTs will be useful in annotation of the genome sequences (see below).

The genes in the TrED data set are supplemented by data from several small-scale analyses with *T. rubrum*, including 28 genes identified by subtractive suppression hybridization analysis comparing growth in keratin versus glucose (39), 19 genes from representational difference analysis under the same conditions (3), and 39 genes from suppressive subtractive hybridization for genes overexpressed in the presence of antifungal drugs (42).

The TrED database also includes data from microarrays constructed with the cDNAs (and therefore not a complete gene set). The microarray data detail the transcriptional profile for conidial germination (38, 58) and for the cell's response to two antifungal drugs, ketoconazole and amphotericin B (61). The microarrays have also been used in comparative genomic hybridization analysis with 22 strains of dermatophytes, including *T. rubrum*, *T. tonsurans*, *Microsporum canis*, and *Microsporum gypseum* (57)—four out of five species that are currently being sequenced. The microarrays have also been used to investigate expression profiles in response to new antifungal drugs (60, 62), an important use of the technology against these important fungi. At the present time, additional transcriptome analyses using the microarrays are being performed to analyze the dermatophyte response to additional antifungals, and the dermatophyte response under conditions that simulate skin infections (Qi Jin, personal communication). In addition to transcriptome analyses, Jin and collaborators are studying the proteome of *T. rubrum*, currently focusing on the proteins within dormant conidia. As currently planned, each of these studies will be integrated on the TrED website (Jian Yang, personal communication) and will be available once published.

The TrED database has been analyzed for gene families (57). One of the most important gene families in dermatophytes is the proteinases. Dermatophytes have adapted to growth on nail, skin, and hair by using a variety of host proteins (especially keratin) as nutrients. Like several other fungi, dermatophytes secrete proteases that degrade skin and hair proteins, including elastase, collagenase, keratinase, and casein (34). TrED has detected gene families of metallo-peptidases,

serine peptidases, cysteine peptidases, and aspartic peptidases (37, 57). It has been shown already that a subset of these genes is important for fungus growth on keratinocytes (28). Current molecular techniques and databases will soon allow us to further characterize the proteinases important for pathogenesis.

Genome sequence project. At this time, there is no complete dermatophyte genome. However, two basidiomycete fungi associated with skin, *Malassezia globosa* and *Malassezia restricta*, have recently been sequenced (53). These species cause dandruff and seborrheic dermatitis on the skin and can cause systemic infections in patients, especially in those receiving total parenteral nutrition. The *Malassezia* genome lacks fatty acid synthase and contains several secreted lipases. These may explain the lipid dependence and skin association of the fungus. The *Malassezia* genome also contains a large number of secreted hydrolases that may be useful in adapting to growth on the skin. Several extracellular hydrolases are also present in the genome of *Candida albicans*, which occupies many of the same host niches. The *Mallassezia* genome sequence, with its lipases and hydrolases, sets expectations for the dermatophyte genomes that occupy a similar niche.

Determination of full genome sequences of five dermatophyte species is currently under way at The Broad Institute, supported by the National Human Genome Research Institute. That sequencing effort has been supervised by a steering committee including Susan Abdel-Rahman, Yvonne Gräser, Sarah Jane Gurr, Matthew Henn, Nilce Martinez-Rossi, Richard Summerbell, and Theodore White. The group includes researchers with clinical expertise, microbiological and taxonomical expertise, and molecular expertise. In the design of this project, the steering committee obtained input from the greater fungal research community and focused on species and strain selection.

Species selection. Five species were identified and prioritized based on their importance to disease, species host specificity, mating competence, and phylogenetic relationships (Fig. 2). High priority was given to sequencing of an anthropophile, a zoophile, and a geophile. Three of the species selected, *T. rubrum*, *M. canis*, and *M. gypseum*, are the most common geophile, zoophile, and anthropophile in human infections. These three species include mating-competent and -incompetent taxa, providing the opportunity to study sex determination at the genomic scale. An additional anthropophile and zoophile are being sequenced. These two species are separated by an appropriate phylogenetic distance for the use of comparative genomic techniques to identify regulatory motifs and to compare gene content and syntenic conservation across the species. The selected strains represent a critical evolutionary and phenotypic comparison with differences in host specificity. The species targeted for sequencing, presented in the order of their priority, are below:

Species 1, *Trichophyton rubrum*, is the most common dermatophyte species causing infections. *T. rubrum* is an anthropophile (human specific) and is not capable of mating (21). This fungus is the most frequent cause of fungal skin infections in humans worldwide and is found throughout the world.

Species 2, *Microsporum canis*, is the most commonly encountered zoophile in human infection. It is the most common cause of tinea capitis (fungal head infections) in Europe. Some

TABLE 1. Dermatophyte strains being sequenced

Species	Classification	Strain	Teleomorph	Sequence coverage	Current status[a]
T. rubrum	Anthropophile	CBS118892	No	8×	Sequence in progress, 10,572 traces
M. canis	Zoophile	CBS113480	Yes	8×	Sequence in progress, 243,936 traces
M. gypseum	Geophile	CBS118893	Yes	8×	Sequence complete, assembly in progress
T. tonsurans	Anthropophile	CBS112818	No	5×	Sequence in progress, 5,840 traces
T. equinum	Zoophile	CBS127.97	No	5×	Sequence in progress, 128,832 traces

[a] As of 20 May 2008. Sequence trace files can be obtained prior to assembly from the trace archive at http://www.ncbi.nlm.nih.gov/Traces/trace.cgi?cmd=stat&f=xml_list_species&m=obtain&s=species.

populations of *M. canis* are mating competent, and others reproduce clonally (45).

Species 3, *Microsporum gypseum*, is a mating-competent geophile (soil dwelling) found throughout the world in soil. In humans, it causes fungal skin infections of the head and torso, especially the arms. It is also known as gardener ringworm.

Species 4, *Trichophyton tonsurans*, is a mating-incompetent anthropophile. It is distributed worldwide but is the most common cause of scalp infections in children residing in the United States, Canada, and Latin America.

Species 5, *Trichophyton equinum*, is a zoophilic human pathogen, primarily associated with horses. Like *T. tonsurans*, there is no known mating in this species. Most important, there is very little divergence (0.2%) between *T. equinum* and *T. tonsurans*, despite their distinctive ecological niches (one is an anthropophile, and one is a zoophile). The sequencing of the *T. tonsurans* and *T. equinum* genomes offers a unique opportunity to investigate the evolutionary events that led to host specificity. Since *T. equinum* is a mating-incompetent zoophile, its inclusion provides a direct comparison to *M. canis*, a still mating-competent zoophile.

The five prioritized species represent four distinct phylogenetic groups, designated by vertical lines on the right of Fig. 2. The *T. rubrum*/*T. violaceum* group (the shortest vertical line) actually includes several morphotypes (in the past known as *Trichophyton yaoundei*, *Trichophyton soudanense*, and *Trichophyton gourvilii*) that are endemic in infections of the trunk and head in Africa and others that are distributed worldwide. Having a reference genome sequence for each dermatophyte clade will enable future targeted genetic analysis in closely related sister species.

Mating-competent species have an asexual type (anamorphs) and a sexual type (teleomorph). The teleomorph of *M. canis* is *Arthroderma otae*. The geophile *M. gypseum* has two teleomorphs, and the genome project will use an anamorphic strain that is able to generate the *Arthroderma gypseum* teleomorph. The other species, *T. rubrum*, *T. tonsurans*, and *T. equinum* do not have a teleomorph or known sexual cycle.

Strain selection. The following criteria were used for the selection of a specific strain for sequencing from each of the five species. The goals of these selection criteria were to ensure (i) that standard, clinically relevant strains are sequenced and (ii) that the sequenced strain is useful and appropriate for basic and molecular research efforts.

(i) Recent isolation and limited culture. The selected strains have been in culture for less than 10 years. The use of more recent culture limits the potential loss or modification of

genomic regions associated with virulence or other genetic determinants of disease, making the genome sequence more valuable for the design of diagnostics and the use in validation studies. It also ensures that the strain closely reflects a current clinical situation.

(ii) Human isolate. For the species that have reservoirs in soil and other animals, strains were identified that were known to have caused a human infection and thus have clinical significance.

(iii) Standard species classification. All strains were classified by experts using standard culture and morphological methods.

(iv) Molecular classification. All strains were classified by sequencing of the rRNA ITS (Fig. 2).

(v) Growth. All strains showed standard growth rates in culture.

(vi) Morphology and sporulation. The selected strains produce the appropriate cell types, including macroconidia and microconidia.

(vii) Protoplast formation. All strains form protoplasts for transformation at acceptable levels.

(viii) Drug susceptibility. Strains selected for sequencing are susceptible to standard drug therapies and to drugs used in molecular techniques, such as hygromycin and G418.

(ix) Availability. All strains are available at the Centraalbureau voor Schimmelcultures (CBS) in The Netherlands, which maintains a collection of living filamentous fungi, yeasts, and bacteria.

Criteria i through iv ensured that a standard, appropriate strain is being sequenced. Criteria v through ix ensured that the sequenced strain is useful and appropriate for basic and molecular research efforts.

The five strains selected for sequencing are listed in Table 1 with a current update on the status of the cloning effort.

Genome characteristics. Current knowledge of the dermatophyte genomes is limited. *T. rubrum* chromosomes have been separated by contour-clamped homogeneous electric field (CHEF) gel electrophoresis (6), detecting five chromosomes (sizes 5.8, 5.2, 4.6, 3.05, and 3.0 Mb). Therefore the genome is at least 22 Mb in size. The AT content of the genome is approximately 50% (based on analyses of sequenced genes), and 5 to 10% of the genome is repetitive DNA (G. Köhler, personal communication). These characteristics will be clarified with the completion of the genome sequences.

Annotation of the genome. Structural and functional annotation of the dermatophyte genomes will use available evidence and follow standard protocols at The Broad Institute.

For the identification of intron/exon boundaries, the EST database TrED will be used for training annotation gene prediction algorithms and for refining intron/exon boundaries of predicted genes across multiple taxa. Unfortunately the *T. rubrum* ESTs were not from the strain that was selected for the genome sequence. However, past annotation efforts have shown that ESTs from different taxa sharing at least 90% nucleotide identity are suitable for cross-species annotation using EST evidence (Chinnappa Kodira, personal communication).

Ploidy and heterokaryons. The ploidy of a dermatophyte nucleus is unclear. Issues of nuclear ploidy are complicated in dermatophytes and other filamentous fungi by the presence of heterokaryons—hyphae containing two genetically distinct nuclei. Heterokaryons can be resolved by the production of conidia, which usually contain a single nucleus.

The ploidy of a dermatophyte nucleus may be reflected in the mating ability. One assumption is that the mating-competent species are haploid, as they are capable of mating with strains of the opposite mating type. This is likely to hold true, although it is theoretically possible that mating-competent species are diploid and the result of mating is a tetraploid, as in *Candida albicans* (40). Thus the two mating-competent species, *M. canis*, and *M. gypseum*, are most likely to be haploid. The three other species being sequenced, *T. rubrum*, *T. tonsurans*, and *T. equinum*, are not mating competent, which may be an indication that the three species are diploid or that they are haploid but have lost mating function.

Evidence for haploid genomes comes from a gene deletion studies. In a strain of *M. canis*, a single homologous recombination at the *DNR1* gene resulted in a mutant phenotype (54). The single gene deletion resulted in complete loss of the gene sequence (as detected by Southern blotting and PCR) and resulted in a mutant phenotype (altered growth), strong evidence that the genome of this strain is haploid. Similarly, two recent gene deletions from *T. rubrum* also suggest that the strain used in that analyses is haploid (10, 12). This suggests that *T. rubrum* is haploid but not capable of mating.

The genome sequences should help to clarify the ploidy of the strains selected. Sequencing of a haploid organism rarely detects sequence differences—a nucleotide position that differs between sequenced clones (≪0.01%). These rare differences could be the result of PCR artifacts or recombination between clones. On the other hand, allelic differences in a diploid organism can be as high as 1% of the nucleotide positions (3 to 10% differences are usually considered indicative of species). Thus, the genome sequences should provide an indication of variation at nucleotide positions, which is an indication of allelic variation and an indication of ploidy.

The genome sequences of five dermatophytes, together with gene annotation and the current EST analysis, provide a strong foundation on which many researchers can explore deeper into the biology and pathogenesis of dermatophytes. Several additional hypothesis-generating tools may evolve from the genome sequence, including a microarray containing all open reading frames (based on the EST and predicted), and proteomics analyses in which all proteins can be identified using the genome sequence. These additional tools will allow a complete analysis of the genes and proteins involved in a particular process or expressed under certain conditions.

TOOLS FOR HYPOTHESIS TESTING IN THE DERMATOPHYTES

The genome sequences and the TrED study including EST and microarray data are major contributions to our understanding of dermatophyte pathogenesis and virulence. However, these two data sets are hypothesis generating. Observations from these databases will allow investigators to formulate hypotheses concerning the fungal pathogen and its interaction with the host. However, to test these new hypotheses, it is necessary to experimentally manipulate dermatophytes.

Transformation. One of the first steps in testing a hypothesis concerning a specific gene or DNA sequence is to create a mutant that is altered in expression of that gene. These mutants can include gene deletion, mutation, overexpression, or misexpression. To generate such mutants, it is necessary to introduce a foreign piece of DNA into a dermatophyte. DNA transformation of a dermatophyte was first reported in 1989 for *Trichophyton mentagrophytes* (18), in which a plasmid expressing hygromycin B phosphotransferase (*hph*) was introduced by protoplasting, resulting in transformation efficiencies of 0.004 to 6 transformants per μg of DNA and per 10^6 protoplasts. The plasmid integrated at multiple, random sites in the genome. Similarly, protoplast transformation of *hph* and green fluorescent protein (GFP) resulted in random integration within the genomes of *M. canis* and with *T. mentagrophytes* (56). Restriction enzyme-mediated integration enhances integration of plasmids containing *hph* and GFP three- to fourfold when used with protoplasting in *T. mentagrophytes* (32).

Most recently, gene deletions have been obtained using protoplasting in *M. canis* (54) and in *T rubrum* (10, 12). In the *M. canis* study, homologous recombination resulting in gene deletion was found in 2 out of 100 colonies screened—a low frequency of homologous recombination. The frequency of gene deletion in the two *T. rubrum* studies was not reported. There is a significant need to identify techniques to increase the frequency of homologous recombination.

A second selectable marker, the neomycin phosphotransferase gene (*nptII*) encoding G418 resistance has been successfully used to transform *T. mentagrophytes* using protoplasting (55). The vector that included GFP was integrated randomly, and GFP expression was observed.

These current gene deletion strategies provide the basics for gene deletion. To develop transformation as an effective and adaptable tool, several advancements are essential. They might include the use of additional selectable markers such as *SAT1* (44), the use of split marker transformation to increase homologous recombination without negative selection (5, 11), and the use of strains in which the KU genes important for non-homologous end joining have been deleted (7, 33). RNA silencing has already been reported in *M. canis* using current transformation capabilities, but more efficient transformation would increase the utility of this system (51).

It may be possible to perform large-scale mutant screens using DNA transformation, either with an adapted transformation system as described above or with the Ti plasmid in *Agrobacterium tumefaciens*. Ti-mediated transformation has been successfully used with several fungi, including the zygomycete *Rhizopus oryzae*, four basidiomycetes, and at least 31 ascomycetes. This includes several species closely related to

dermatophytes, including the dimorphics *Blastomyces*, *Histoplasma*, and *Coccidioides*, and at least five species of *Aspergillus*, including *A. fumigatus* (35, 36). Efficient transformation would enable researchers to perform effective genetic screens, which to date have not been reported.

Infection models. Once a dermatophyte has been experimentally manipulated and altered, it is then necessary to test its virulence and pathogenicity in an animal or ex vivo model. Selected animal models have been used with the dermatophytes. Historically, human volunteers were used to study dermatophytes (19). However, these models are not acceptable by today's ethical standards. Guinea pigs, mice, and rabbits are readily infected with zoophilic and geophilic dermatophytes, while anthropophilic species can occasionally induce a mild lesion. The most common model is the guinea pig (17, 23, 48). The basic procedure involves shaving the site of infection followed by heavily abrading or scarifying the area with a scalpel, razor blade, or sandpaper. The dermatophyte is then applied as a conidial (spore) and hyphal suspension, sometimes mixed with honey to create an ointment (50), and occluded on the skin for a few days to mimic the hot, wet conditions of shoes. Lesions develop within 1 week and resolve after 3 to 4 weeks; chronic infections are rare. The lesions do respond to oral or topical antifungal treatments (17). There is obviously a need for alternate vertebrate and nonvertebrate models for dermatophyte pathogenesis.

In recent years, human skin sections have been used as models for dermatophyte infection (9, 30, 31). One model used homogenized skin samples to provide a uniform growth medium (30). In a more representative ex vivo model, human skin explants were obtained following plastic surgery and infected at the surface with conidia. The resulting explant was then monitored for adhesion and invasion of the fungal cells. Initially, the spores adhere to the skin layers and germinate. Subsequently, the fungal hyphae penetrate and invade the layers of the skin. Invasion of the stratum corneum was accompanied by secretion of serine proteinases that are likely to be important for invasion of the deep layers of the skin (31). The explant model has recently been used to compare antifungal drug therapies (41).

CONCLUSIONS

Dermatophyte infections are the most common fungal infections worldwide. While they are not associated with significant mortality, they are associated with considerable morbidity, particularly affecting children and young adults, and individuals in lower socioeconomic settings, both in the United States and worldwide.

Despite the significance of these fungal infections, there is a dearth of attention from the research community. In general, pathogenic fungi receive less research focus than bacteria, viruses, or even parasites. Within the mycology research community, the dermatophytes have received little or no attention. This situation is reflected in research funding. In the last 10 years, NIH-supported research on the dermatophytes has been limited to a handful of grants, all focused on epidemiology or drug screens.

Dermatophyte research will experience a paradigm shift with the completion of the genome sequences for five dermatophytes

and the progress made with EST and microarray work as detailed on the TrED website. These major projects together represent a huge leap forward for researchers interested in addressing questions concerning dermatophyte pathogenesis.

The genome and TrED project give researchers major tools for generating hypotheses concerning the dermatophytes. However, researchers still lack up-to-date molecular tools for hypothesis testing in the dermatophytes. Molecular tools (including efficient and adaptable transformation) are needed to experimentally alter the dermatophyte cell. And infection models (including skin explants and nonvertebrate animal models) are needed to characterize its interactions with the host. With both sets of tools, researchers will be able to address questions concerning virulence and pathogenesis, to improve our understanding of tinea infections, and to work toward the development of new diagnostics, treatments, and prevention strategies for the dermatophytes.

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