Environmental Source of Candida dubliniensis

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We isolated Candida dubliniensis from a nonhuman source, namely, tick samples from an Irish seabird colony. The species was unambiguously identified by phenotypic and genotypic means. Analysis of the 5.8S rRNA gene showed that the environmental isolates belong to C. dubliniensis genotype 1.

The ever-increasing number of immunosuppressed humans has led to a marked rise in opportunistic infections, particularly those caused by fungi (1). Candida albicans is the yeast species most commonly associated with oropharyngeal and systemic candidiasis in immunocompromised persons. However, the last 2 decades have seen an increase in infections by other Candida species, including C. dubliniensis, which was first recognized as distinct from C. albicans in 1995 in Ireland (2,3). C. dubliniensis has been recovered mainly from the oral cavities of HIV-infected persons (4) but also from lungs, vaginas, blood, and feces; occasionally this organism causes fatal systemic infections (5). Isolates are assigned to 4 genotypes, defined by the sequence of the internal transcribed spacer regions of the rRNA gene (6).

C. dubliniensis is globally distributed. In HIV-infected patients, the oral prevalence is 1.5%–32% (5). In healthy persons not infected with HIV, C. dubliniensis is absent or rare, but 14% of healthy Caucasians had oral C. dubliniensis in a South African study (7). Like C. albicans, C. dubliniensis may be a member of the normal oral microbial flora of humans, and oral candidosis may result from overgrowth of resident strains. In contrast to other Candida species, some of which are associated with birds (8,9), C. dubliniensis has not been found to date in nonhuman environmental sources. This has led to speculation that the species may be restricted to humans, possibly occupying sites deep within the oropharynx or upper respiratory tract (5).

The Study

Fungal strains were obtained from Ixodes uriae ticks (as part of a National Environment Research Council–funded study of a tickborne virus) at a seabird breeding colony on Great Saltee Island, Ireland (52°07′N, 6°36′W), The ticks were taken from cracks in cliffs used by common guillemots (Uria aalge). Tissue cultures of tick homogenates generated for virus isolation were occasionally contaminated with yeastlike fungi.

To investigate this, individual adult ticks were homogenized in 1 mL minimum essential medium (MEM). After centrifugation (30 s, 10,000× g), 0.2 mL of supernatant was added to 4 mL MEM, 5% fetal bovine serum, and 100 μg/mL penicillin-streptomycin. Cultures incubated at 37°C were examined microscopically daily for up to 6 days. Positive cultures were plated twice on Sabouraud dextrose agar (SAB) with chloramphenicol (bioMérieux, Marcy l’Etoile, France) before phenotypic testing. Isolates were identified by using API identification kits (bioMérieux) and by conventional methods (10). Antifungal drug susceptibility was tested according to the Clinical Laboratory Standards Institute guidelines (11). The control strains were C. albicans ATCC 90028, C. glabrata ATCC 90030, C. krusei ATCC 6258, and C. dubliniensis NCPF 3949.

Internal transcribed spacer 1 and 2 regions (ITS1/ITS2) and the 5.8S rRNA gene were amplified with primers ITS1 and ITS4, described by White et al. (12). Template DNA was prepared by boiling single SAB-grown colonies in 50 μL ultrapure water for 10 min. After centrifugation (5 min, 10,000× g), 15 μL supernatant was added to 50 μL PCRs containing 1× reaction buffer, 1 μmol/L ITS primers, 1.5 mmol/L MgCl₂, 400 μmol/L deoxynucleoside triphosphates, and 2.5 U Immolase (Bioline Ltd., London, England, UK). Cycling parameters were 7 min at 95°C, 30 cycles of 1 min at 95°C, 1 min at 58°C, 1 min at 72°C, and a final extension of 5 min at 72°C. Products were purified (QiaQuick kit, QIAGEN Ltd., West Sussex, England, UK) and sequenced (BigDye kit and ABI 377 sequencer; Applied Biosystems, Foster City, CA, USA) by using the ITS primers. Sequences were assembled by using Lasergene 6, Seqman version II, and aligned by using BioEdit software (13).

Fungal isolation was undertaken on 2 separate days with samples from 2 distinct sites on the island (Table 1). On both days, Happy Hole West (HHW) ticks were processed immediately after Labour in Vain (LIV) ticks in the same class II microbiologic cabinet. No fungi were detected in HHW ticks, whereas 16.7%–27.6% of ticks sampled from 2 locations within LIV gave positive cultures (Table 1). Twenty-two isolates were obtained (Table 1); SL370–429 were from LIV-1 and SL495–531 from LIV-2 (SL = Saltee).

On SAB the colonies from positive cultures were a creamy white color with a glabrous appearance similar to C. albicans. SL375 had a mixed phenotype (large and small colonies, designated SL375–1 and SL375–2). Like C. albicans, all SL isolates were germ-tube positive and produced chlamydospores at 37°C on Corn Meal Tween 80.
agar (Oxoid Ltd, Basingstoke, England, UK) and Czapek Dox (1%) Tween 80 agar (Oxoid) (Figure 1A). None of the SL isolates grew at 43°C on SAB (Figure 1B), which suggested that they might be C. dubliniensis (14). This was confirmed by carbohydrate assimilation tests (Table 2) and by sequencing the 5.8S rRNA gene (Figure 2). With the API 20C AUX kit, all SL isolates yielded the same profile at 48 h, interpreted as 99.9% C. dubliniensis (Table 2). Eleven isolates from LIV-1 and the C. dubliniensis (NCPF 3949) reference strain were also tested with API 32C. All had an identical profile (7143100015), interpreted as 81.9% C. dubliniensis and 16.9% C. albicans.

ITS sequences of isolates SL375, SL397, SL407, SL410, SL411, SL417, and SL422 were identical to that of C. dubliniensis CD33 genotype 1 (Figure 2). Nevertheless, phenotypic variation among the SL isolate was evident. In addition to variation in trehalose assimilation rates, 3–4 distinct types were apparent in the germ tube test. Three independent inoculations (10^5 CFU/mL, mid log growth phase) of each isolate gave consistent morphologic differ-

Table 1. Male and female ticks positive for fungi in culture*

| Site                   | Male | Female | Both sexes |
|------------------------|------|--------|------------|
| Happy Hole West (HHW)-1| 0/26 | 0/25   | 0/51       |
| HHW-2                  | 0/17 | 0/23   | 0/40       |
| Labour in Vain (LIV)-1 | 5/30 | 8/29   | 13/59†     |
| LIV-2                  | 5/23 | 4/20   | 9/43†      |

*Adult Ixodes uriae ticks were collected from within 2 guillemot-breeding colonies on Great Saltee on August 25, 2004. The ticks were stored frozen and processed by M.A.N on November 11, 2005. (HHW-1 and LIV-1) and 20. 07.06 (HHW-2 and LIV-2).
†Isolates SL370, SL371, SL375, SL387, SL397, SL407, SL410, SL411, SL413, SL414, SL417, SL422, and SL429.
‡Isolates SL495, SL497, SL500, SL501, SL509, SL510, SL522, SL529, and SL531.

Figure 1. Phenotypic characteristics of environmental Candida dubliniensis isolates and reference strain of C. albicans. A) Morphology of pseudohyphal terminal chlamydoconidia of C. albicans (ATCC90028) and C. dubliniensis SL370 grown at 37°C on Corn Meal Tween 80 agar. Magnification × 50. B) Growth of representative Great Saltee (SL) isolates on Sabouraud agar after 48 h of incubation at 37°C and 43°C. The growth of the following isolates is shown: C. albicans (ATCC90028), C. dubliniensis (NCFP3949), and C. dubliniensis SL370, SL397, SL407, and SL410 (clockwise from the top in each petri dish).

Table 2. Substrate assimilation by Great Saltee fungi and Candida albicans

| Substrate          | SL407 | C. albicans (ATCC90028) |
|--------------------|-------|-------------------------|
| Pentoses           | –     | –                       |
| L-arabinose        | –     | –                       |
| D-xylene           | +     | –                       |
| Hexoses            | –     | –                       |
| D-glucose          | +     | –                       |
| D-galactose        | +     | –                       |
| α-methyl-D-glucoside| –   | +                       |
| Disaccharides      | –     | –                       |
| D-cellobiose       | –     | –                       |
| D-lactose          | –     | –                       |
| D-maltose          | +     | –                       |
| D-saccharose       | +     | –                       |
| D-trehalose†       | –     | +                       |
| Trisaccharides     | –     | –                       |
| D-melezitose       | –     | –                       |
| D-raffinose        | –     | –                       |
| Alcohols           | –     | –                       |
| Glycerol           | +     | –                       |
| Adonitol           | +     | +                       |
| Xylitol            | +     | –                       |
| Inositol           | –     | –                       |
| D-sorbitol         | –     | –                       |
| Organic acids      | –     | –                       |
| 2-keto-glucuronate | +     | +                       |
| Amino acids        | –     | –                       |
| N-acetylglucosamine| +     | –                       |
| Identification     | C. dubliniensis | C. albicans |
| API 20C AUX profile code | 6172134 | 2566174 |
| Predictive value   | 99.9%, excellent | 99.2%, very good |

*Results for 48 hours are shown. All 22 Great Saltee isolates gave similar profiles.
†By 72 hours all Saltee isolates showed some assimilation of trehalose; the degree of assimilation varied between isolates.
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