Isolation of *Candida dubliniensis* in an Aboriginal Community in Ontario, Canada

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This study reports the first isolation of *Candida dubliniensis* from North American Indians. Of 39 healthy human hosts sampled, two had *C. dubliniensis*. Genotypic analysis identified polymorphisms in these strains and differences from two reference strains. Our results suggest that yeast populations from indigenous communities in North America may be unique.

*Candida dubliniensis* is a newly described species with a close genetic similarity to the more common *Candida albicans* (9). Though *C. dubliniensis* has been recovered from several body sites in many human populations, it is most often recovered from the oral cavities of patients infected with human immunodeficiency virus (2, 3, 9). One hypothesis for the current global distribution of *C. dubliniensis* is the recent massive migrations of the human hosts, especially during the last century. If this hypothesis is correct, we expect that healthy human populations living in relative isolation should have no or very low percentages of *C. dubliniensis*. Furthermore, if such strains exist, their genotypes might be different from the genotypes found in other communities. Many aboriginal communities in North America are ideal populations to study to test this hypothesis.

We sampled yeast populations from healthy human hosts in the Six Nations and New Credit reserves in southern Ontario, Canada. These reserves were established in 1847 by the British government and currently occupy an area of about 20,000 ha with a population of about 17,000. The ancestors of the residents of these reserves were living in the Great Lakes region for thousands of years, long before the discovery of the New World about 500 years ago (5). These populations have remained relatively isolated, in human population genetics terms, from new immigrants and from surrounding nonnative communities, before and after the establishment of these reserves about 150 years ago (5). Participation in our survey was voluntary (parents gave consent for their children) and based on their availability from April to June in 2002. A total of 39 healthy individuals were recruited and agreed to participate. Their ages ranged from 4 to 75 years. Most individuals spent almost all their time on the reserves. Each individual was sampled three times in 2002: mid-April, mid-May, and mid-June. All hosts were healthy during the time of sampling, and none had human immunodeficiency virus infection before or during our sampling period. Sampling was done as described previously (4). A total of 351 samples were collected (three samples from three body sites from each of 39 hosts).

Swabs and nail samples were directly deposited into micro-centrifuge tubes containing 0.5 ml of sterile water. Each sample was vigorously vortexed, and liquid suspension was spread plated onto CHROMAgar Candida (CHROMAGar Company, Paris, France). Morphologically distinct green colonies from CHROMAgar plates were identified as either *C. dubliniensis* or *C. albicans* on the basis of carbohydrate metabolic profiles on API 20C strips (profiles typical of those for *C. albicans* were found for all green colonies isolated here). These colonies were then transferred to two sets of yeast extract-peptone-dextrose (YPED) (4) plates and incubated at 37 and 45°C, respectively. Colonies that grew at 37°C but not at 45°C were presumptively identified as *C. dubliniensis* (2, 3).

From the 351 samples, we obtained 22 strains of *C. albicans* and 4 putative strains of *C. dubliniensis*. The sample isolation rates for *C. albicans* and *C. dubliniensis* were 6.3% (22 of 351) and 1.1% (4 of 351), respectively. The calculated human host carriage rates for *C. albicans* and *C. dubliniensis* were 25% (10 of 39 hosts) and 5% (2 of 39 hosts), respectively. While the carriage and isolation rates for *C. albicans* in this community were comparable to those reported for other healthy human populations (4, 7), the rates for *C. dubliniensis* were higher than those in different communities in the same region or in different regions. For example, in a recent study, 24 healthy humans were sampled (for a total of 216 samples) for yeasts in the southern Ontario region of Hamilton and Toronto, a region close to the Native Indian community reported here. No strain of *C. dubliniensis* was found (4). In a study of other geographic regions, Xu and Mitchell reported that no *C. dubliniensis* was recovered from 239 healthy hosts from China and 483 healthy hosts from eastern North America (11) (note that no Native Indian population was sampled in that study). In a study of clinical samples, Odds et al. (8) found that *C. dubliniensis* constituted 2.1% of 2,489 isolates originally identified as *C. albicans* from a large yeast stock collection. Of the 55 isolates identified as *C. dubliniensis*, only 2 were from healthy individuals (8). In our current survey, 15% [4/(22 + 4)] of the isolates identified as *C. albicans* on the basis of results with API 20C strips and CHROMAgar medium were *C. dubliniensis*, and all were from healthy individuals.

All four strains of *C. dubliniensis* obtained here were from oral swabs: three were obtained from the same human host, one each at the three sampling periods, and the fourth was...
FIG. 1. Genotypic comparisons of four putative *C. dubliniensis* strains isolated in this study with two reference strains each of *C. dubliniensis* (CD36 and CM2) and *C. albicans* (CaLM1 and CaLM2). PCR products from primers CDG1 (A), OPA-17 (B), OPB-12 (C), and OPC-5 (D) are shown. In all four panels, lane 1 contains a 100-bp ladder and lanes 2 to 9 contain DNA from strains CdLM25-1, CdLM25-2, CdLM25-3, CdLM31-1, CD36, CM2, CaLM1, and CaLM2, respectively. The arrowheads in panel B indicate the presence of DNA fragments that were different in three strains (CdLM25-1, CdLM25-2, and CdLM25-3) isolated from the same host. The arrowheads in panels C and D indicate the presence of DNA fragments in the two reference *C. dubliniensis* strains that were different from the four *C. dubliniensis* strains isolated in this study.
from an unrelated host and was obtained in the first sampling period. As determined by the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution protocol (1, 6), all four strains were susceptible to two common antifungal drugs, amphotericin B and fluconazole, with identical MICs of 0.12 µg/ml for amphotericin B and 0.5 µg/ml for fluconazole. To confirm their identities and to study their genetic relationships, these strains were compared to two reference strains of *C. dubliniensis*, CD36 (originally isolated in Ireland) and CM2 (originally isolated in Australia) (2, 9), and two reference strains of *C. albicans* (CaLM1 and CaLM2) using genotypic tests as described below.

Genomic DNA was isolated from each of the eight isolates according to a previously described protocol (12) and stored at −20°C. Genotypes were determined by PCR using two types of primers. The first type, four primer pairs (CDG1, CDG2, CDG3, and CDG4), are specific for *C. dubliniensis* and genotype groups of *C. dubliniensis* (2). These primer pairs were recently developed on the basis of DNA sequence variations in strains in the internal transcribed spacer regions of the rRNA gene cluster (2). The second type is based on randomly amplified polymorphic DNA (RAPD). Three primers were used in this study: OPA-17 (5'-GACCGCTTG-3'), OPB-12 (5'-CCT TGACGCA-3'), and OPC-5 (5'-GATGACCGGC-3'). RAPD, agarose gel electrophoresis, ethidium bromide staining, and gel documentation were performed by the methods of Xu et al. (10, 12).

As in a previous report (2), *C. albicans* CaLM1 and CaML2 strains could not be amplified by the four *C. dubliniensis*-specific primer pairs. The two reference strains of *C. dubliniensis* (CD36 and CM2) were identified as belonging to genotype group 1 (Fig. 1A) (Fig. 1 in reference 2). Similarly, all four putative strains of *C. dubliniensis* isolated here could be amplified by the CDG1 primer pair (Fig. 1A). The PCR amplifications using primer pairs CDG2, CDG3, and CDG4 did not produce any product for these four strains or any other strains analyzed here (gels not shown, as there were no PCR products). The results indicated that our four strains belonged to genotype group 1 of *C. dubliniensis*.

The genetic relationships among these strains were further assessed by RAPDs using three primers. All three primers identified genetic differences between *C. albicans* and *C. dubliniensis* (Fig. 1B, C, and D). Among the three primers, OPA-17 revealed that all strains of *C. dubliniensis* had a different RAPD pattern (Fig. 1B). In contrast, primers OPB-12 and OPC-5 identified a single RAPD pattern among the four *C. dubliniensis* strains isolated here, but their genotype was different from those of the two reference strains (Fig. 1C and D). These results suggested that strains of *C. dubliniensis* from this auburn community were heterogeneous and genetically different from the two strains originally isolated from Australia and Ireland.

The genetic differences among strains CdLM25-1, CdLM25-2, and CdLM25-3 as revealed by RAPD primer OPA-17 were surprising. These and other RAPD banding pattern differences were reproducible in PCRs performed at different times and by different personnel in our laboratory (data not shown). Typically, yeast populations are relatively stable over a short time within individual hosts. However, temporal differences in oral yeast flora in hosts have been observed (2, 4). While the exact mechanism(s) for the observed differences in strains CdLM25-1, CdLM25-2, and CdLM25-3 is not known, there are several possibilities. The first possibility is that the original colonizing population in this host was highly mutable and novel genotypes could be generated within a short time. The second possibility is that the original colonizing population was genetically heterogeneous, and due to the sampling effect, different genotypes were isolated during each attempt. The third possibility is that there were high rates of immigration and emigration for yeast populations in this host, causing rapid population turnover. It should be noted that microevolution has been reported in populations of human pathogenic yeasts, including *C. dubliniensis* (2).

To our knowledge, this is the first report of the human pathogenic yeast *C. dubliniensis* from a North American aboriginal community. Though our host population size is relatively small (39 individuals), we attempted extensive sampling for each host during a 3-month period. Our study indicated a relatively high frequency of *C. dubliniensis* in this community. The genetic analysis identified that these strains were genetically different from the two reference strains, suggesting that the population of *C. dubliniensis* in this native community might be unique. However, large sample sizes from both native communities and nonnative communities are needed to firmly establish the genetic distinctness of *C. dubliniensis* populations in North American native communities. Our results are inconsistent with the hypothesis that the current global distribution of *C. dubliniensis* was due to recent migrations from one or a few geographic centers of human populations. This study also demonstrated that pathogen populations from indigenous communities could provide unique tests regarding pathogen distribution and evolution.

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