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Patterns of Human Oral Yeast Species Distribution on Hainan Island in China

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Abstract Infections by yeast strains of the genus *Candida* are among the most prevalent fungal infections of humans. These yeasts are common residents of the oral mucosa and other body surfaces. Since most yeast infections are due to endogenous strains and that species of *Candida* differ in virulence properties and in intrinsic susceptibilities to antifungal drugs, understanding the human commensal yeast flora can help designing effective treatment and prevention strategies against yeast infections. Here, we report the patterns of yeast species distributions in the oral cavities of 1,799 people from Hainan Island in southern China. Based on sequence information at the fungal barcode locus ITS regions, 368 of the 415 obtained oral yeast strains were identified as belonging to 26 yeast species, while the remaining 47 strains all showed significant sequence divergence to the currently described species. The four most common yeast species were *C. albicans* (42 %), *C. tropicalis* (20 %), *C. glabrata* (5.5 %), and *C. parapsilosis* (4.1 %) and 10 of the 26 yeast species were represented by only one strain each. Our analyses identified that the gender of hosts and ethnical background showed no contribution to oral yeast species distributions. However, the health status, place of birth, current residency, and the age of hosts all showed significant contributions to the distributions of the four dominant yeast species. We compared our results with those reported previously and discussed the potential mechanisms for the observed differences in oral yeast species distributions.

Keywords Oral yeasts · ITS sequences · *C. albicans* · *C. tropicalis* · Age effects · Geographic differences

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Introduction

Due to the large number of immuno-deficient and immuno-compromised hosts, infections caused by opportunistic pathogens are a serious problem worldwide, affecting millions of people each year [1]. Among the opportunistic pathogens, species in the genus *Candida* and other yeasts are among the most frequently found [2, 3]. Most of these opportunistic pathogens are common components of the normal commensal microflora on the oral mucosa and other

body surfaces. However, the applications of broad-spectrum antibiotics, infections by HIV, chemotherapy, and organ transplantation often cause these commensal yeasts to overgrow on the mucosal surfaces and result in mucosal membrane diseases. Furthermore, the yeasts can penetrate the mucosal membranes and cause systemic diseases [1, 2, 4]. Because most yeast infections are caused by endogenous strains, it is important to understand the commensal yeast flora of hosts at the population level [3, 4]. In addition, because species of *Candida* and other commensal yeasts differ in their susceptibilities to antifungal drugs and in other clinically important phenotypes [4, 5], understanding the commensal oral yeast flora could significantly increase our ability to design targeted prevention and treatment strategies. However, despite their clinical significance, relatively little is known about the factors influencing yeast species distributions in humans. The objective of this study is to investigate the potential contributions of host age, health status, and geography to yeast species distribution in oral cavities of humans on the tropical Hainan Island located in the South China sea.

Hainan Island is located in China's southernmost province (latitude 3°30′–20°10′ N; longitude 108°15′–120°15′ E) with a total area of 33,920 km^2. The Island was likely first colonized between 7,000 and 27,000 years ago by the Li people from southern China [6]. Starting around 1,000 years ago during the Song dynasty, large numbers of Han Chinese began to arrive the Island from the north and gradually pushed the Li people to the interior and southern parts of the island. Throughout most of its history, Hainan was relatively poor and underdeveloped compared with its northern neighbors in southern China. However, in 1988, the Chinese central government made Hainan a separate Province and designated it a special economic zone to attract financial investment and human capital. Since then, Hainan's infrastructure and demographics have changed significantly. At present, among the >8 million citizens on the Island, about 83 % are Han Chinese and 16 % are the Li people, with the remaining ~1 % belonging to the Miao and Zhuang minorities. The relatively well-documented human history of Hainan Island makes it an ideal place from which to examine the potential effects of historical and recent human demographic events on yeast species distributions.

Several previous studies have shown that oral yeast flora can differ significantly between geographically separated populations. In one survey, the oral yeast flora from the Chinese Mainlanders were found to be very different from those in North America [7]. For example, *C. albicans* was the dominant oral yeast species in North America [7, 8], but it was found in only about 10 % of the isolated yeasts from surveyed Chinese in Mainland China [7]. It was suggested that oral hygiene and diet might be associated with yeast species diversities. The oral hygiene hypothesis was supported by data from a latter study of university students at Hainan Medical College on Hainan Island, China that showed *C. albicans* as the dominant yeast species [9], similar to that found in North America but different from that in the general population on Mainland China [7]. However, the potential influences of other factors have not been examined.

In this study, we investigated the oral yeast species profiles on Hainan Island further by analyzing over 400 yeast strains from the oral cavities of a diversity of hosts throughout the main regions of the island. For each isolate, we obtained the sequence at the fungal barcode locus [10]: the internal transcribed spacer (ITS) regions of the nuclear ribosomal RNA gene cluster. The ITS sequences were then used to identify the species of each strain. The distributions of oral yeast species were then analyzed for potential contributions of age, current residence, ethnicity, and health status (healthy host or hospitalized patient). In addition, the roles of gender and host place of birth (Hainan or Mainland China) were also analyzed to determine if the results found in a previous study [9] among medical students also hold for the general population on Hainan Island. Our results identified significant contributions of host health status (normal vs. hospitalized patients), age, place of birth, and current residency on the patterns of oral yeast species distribution on Hainan Island. In contrast, gender and ethnicity were found to have no influence on oral yeast species distribution.

Materials and Methods

Samples

All samples were taken from residents on Hainan Island. None of the sampled hosts were short-term tourists, and each spent the majority of the time (more than 6 months per year) on Hainan Island. Many of the

hosts (especially those in the northwest and southeast Hainan, see below) lived their entire lives on the Island. The healthy hosts were all from local communities, while the hospitalized patients were from general public hospitals. At the time of sample collection, none of the healthy hosts had any symptoms of oral yeast infection. The samples from healthy hosts were taken following protocols used previously for collecting and isolating oral yeasts [7–9]. Briefly, sterile cotton swabs were used to sample the upper and lower outer gingiva of each person. After sampling, the tip of each swab was immediately cut off by a sterilized pair of scissors and submerged in a sterile cryogenic tube for selective yeast growth, storage, and transport. Each tube contained 0.5 ml of sterile enrichment YEPD broth composed of 2 % (weight/volume) yeast extract, 1 % bacto-peptone (BD), 2 % dextrose, 18 % glycerol, and the antibiotic chloramphenicol (50 μg/ml). After 2–3 days of incubation to enrich yeast population and minimize bacterial population in each sample, subcultures of the medium suspension were streaked onto Sabouraud glucose agar medium for isolation of yeasts, one plate for each tube. For plates with yeast colonies, a random yeast colony was picked from each plate for further streaking (for purification), storage, and subsequent analyses.

The patient samples were all collected from hospitalized hosts in general public hospitals (i.e., not specialized or private hospitals). The oral swab samples were obtained following the protocol described above for healthy hosts. The patients were hospitalized for a variety of reasons including childbirth, surgery, respiratory infections, diabetes, lung cancer, liver cancer, severe diarrhea, leukemia, high blood pressure, heart problems, high fever, severe headache, etc. For many patients, accurate clinical diagnoses of their underlying condition for hospitalization were not available. However, none of the sampled patients were hospitalized because of oral candidiasis. None of the hosts had HIV infection and none had obvious oral thrush at the time of sampling.

Our samples were from three main geographic areas: northeast Hainan (the city of Haikou, the provincial capital and the largest city on the Island, and its vicinities), northwest Hainan (Danzhou and Dongfang Counties), and southeast Hainan (Lingshui, Wuzhishan and Sanya cities). For each sampled host, we obtained their consent and recorded the following demographic information: ethnicity, place of birth, age, gender, current residency (northeast, northwest, and southeast Hainan), and health status (healthy host or hospitalized patient). The sampling and data collection followed the protocols established by the bioethics board at Hainan Medical College. A total of 1,799 individuals were sampled, including the 1,039 students at Hainan Medical College that we reported previously [9]. The demographics of all the sampled hosts are summarized in Table 1. In total, 415 oral yeast isolates were obtained with one isolate from each host. These yeast samples were obtained between 2006 and 2008, and pure cultures were stored permanently in a −80 °C freezer. These stored yeasts were then analyzed for species compositions among the different host groups.

Species Identifications

To identify the species status of the isolated yeast strains, we relied on sequence information at the ITS regions of the nuclear ribosomal RNA gene cluster. The ITS regions have been recommended as the fungal barcode [10], and there is a large number of yeast ITS sequences in GenBank (and other databases) for comparisons and species determination. Briefly, the ITS sequences were obtained for all 415 yeast isolates using primers ITS1 and ITS4, following protocols described in [9]. The obtained sequences were compared with those in the GenBank through the BLASTn search option. For species identifications, the primary criterion we used was the best match based on the full-length sequence to the type strain of a known taxon in the GenBank database. In addition, we used an E-score of $<10^{-10}$ and at least 97 % nucleotide sequence identity through the full-length of ITS (covering both regions 1 and 2 of the ITS) as cutoffs.

Data Analyses

To compare the rates of yeast carriage between and among different demographics groups, we used the chi-square contingency table test [11] embedded in the Microsoft Excel program. Comparisons were made for each of the six examined host features: age (10-year age groups: 0–9; 10–19; 20–29; 30–39; 40–49; 50–59; 60–69; 70–79; and 80+), sex (male or female), ethnicity (Han or Li), place of birth (Hainan or Mainland China), current residence (northeast,

Table 1 Relationships between human host features and oral yeast species distributions on Hainan Island, China

Host features	Host characteristics	Total no. of hosts sampled	Total no. of yeast strains (% hosts containing oral yeast)	*Ca*	*Cg*	*Cp*	*Ct*	Other species (no. of strains)	No. of unidentified yeast strains	Simpson's diversity index of known yeast species
Sex	Female	927	187 (20.1 %)	81	9	7	34	15 (34)	22	0.717
	Male	872	228 (26.1 %)	94	14	10	49	19 (36)	25	0.716
Place of birth	Hainan	1,118	370 (33.1 %)	151	22	13	77	22 (67)	40	0.726
	Mainland China	681	45 (6.6 %)	24	1	4	6	3 (3)	7	0.562
Residency during sampling	Northeast	1,236	182 (14.7 %)	104	13	6	29	12 (22)	8	0.607
	Northwest	191	78 (40.8 %)	13	1	2	37	5 (16)	9	0.652
	Southeast	372	155 (41.7 %)	58	9	9	17	16 (32)	30	0.750
Ethnicity	Han	1,433	343 (23.9 %)	154	20	14	74	19 (51)	30	0.695
	Li	366	72 (19.7 %)	21	3	3	9	12 (19)	17	0.810
Healthy	Yes	1,419	156 (11.0 %)	83	4	11	5	16 (28)	25	0.584
	No (patients)	380	259 (68.2 %)	92	19	6	78	14 (42)	22	0.732
Age group	0–9 (years)	60	28 (46.7 %)	9	0	1	5	6 (6)	7	0.743
	10–19 (years)	550	72 (13.1 %)	42	0	6	5	7 (11)	8	0.548
	20–29 (years)	710	47 (6.6 %)	28	1	0	4	5 (8)	6	0.514
	30–39 (years)	82	38 (46.3 %)	13	1	1	6	7 (8)	7	0.742
	40–49 (years)	81	33 (40.7 %)	10	1	1	8	5 (6)	5	0.743
	50–59 (years)	83	45 (54.2 %)	14	2	3	14	7 (9)	1	0.762
	60–69 (years)	71	39 (54.9 %)	15	0	1	9	8 (10)	2	0.738
	70–79 (years)	103	72 (69.9 %)	29	13	1	18	4 (7)	4	0.708
	80+ years	59	41 (69.4 %)	14	4	2	13	2 (4)	2	0.711
Total		1,799	415 (23.1 %)	175	23	17	83	22 (70)	47	0.714

Ca: Candida albican; Cg: Candida glabrata; Cp: Candida parapsilosis; Ct: Candida tropicalis

northwest, or southeast Hainan), and health status (healthy hosts or hospitalized patients).

To compare yeast species diversity among samples, we used Simpson's diversity index. This diversity index represents the probability that two randomly drawn individual strains from the specific sample belonged to two different yeast species. Here, only strains that we were able to identify to the species level were included in the calculations. To compare the potential differences in yeast species compositions among groups of hosts, we used the chi-square contingency table test [11] embedded in the Microsoft Excel program, similar to those for oral yeast carriage rate comparisons described above. Specifically, comparisons were made for each of the six examined host features with regard to the distribution of specific yeast species: age (10-year age groups: 0–9; 10–19; 20–29; 30–39; 40–49; 50–59; 60–69; 70–79; and 80+), sex (male or female), ethnicity (Han or Li), place of birth (Hainan or Mainland China), current residence (northeast, northwest, or southeast Hainan), and health status (healthy hosts or hospitalized patients). However, due to the small number of strains for many of the species in our samples (See below), for statistical robustness, only the four most common yeast species (*C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis*, see below) were included in the comparisons. In addition, when the expected numbers were smaller than five for certain species in a host group, the smallest categories were combined in the test as one group [11].

Results and Discussions

Oral Yeast Isolation Rates

A total of 1,799 people were sampled, including 1,256 from northeast Hainan, 191 from northwest Hainan

and 372 from southeast Hainan. The sample hosts were skewed in favor of the northeast because it was where our college was located, and it was easier to get volunteers to contribute samples. Among the 1,799 sampled individuals, the overall oral yeast isolation rate was 23.1 %. This rate was within the range of those reported previously in other surveys from different geographic regions and host groups [7–9, 12–15]. Interestingly, our study found significant contributions of five of the six examined demographic factors to oral yeast isolation rates (Table 1). Specifically, we found that males had an overall higher oral yeast carriage rate (26.1 %) than females (20.1 %) in Hainan ($p = 2.6 \times 10^{-3}$), that those who were borne in Hainan had a higher rate (33.1 %) than those borne on Mainland China (6.6 %) ($p = 2.88 \times 10^{-38}$), that the hospitalized patient group had a higher rate (68.2 %) than the healthy hosts (11.0 %) ($p = 4.856 \times 10^{-122}$), that those in the northwest (40.8 %) and the southeast (41.7 %) had similar rates ($p = 0.85$), but both were significantly higher than the northeast region (14.7 %) ($p < 3 \times 10^{-16}$), and that there was a significant rate heterogeneity among the age groups (an overall p value $= 4.75 \times 10^{-94}$). The only demographic factor that did not show a significant contribution was ethnicity, similar to that reported previously ($p = 0.084$) [7].

We would like to note that the very low rates of isolation for the 10–19 and the 20–29-year-old age groups, for the healthy hosts, for the northeast region, and for those borne outside of Hainan (Table 1) were all due to the low rate of isolation observed for the university students at Hainan Medical College as reported in a previous study [9]. The potential reasons for the low rate of oral yeast isolation for these students have been discussed in the previous study [9]. Excluding the university students from our analyses would significantly raise the oral yeast carriage rates for the remaining host groups in the respective demographic categories. However, though the differences in oral yeast isolation rates were smaller between/among the different demographic groups after the exclusion of the college students, those differences were still statistically significant for four of the six demographic factors except host gender and ethnicity (data not shown). Taken together, our observations suggest that oral yeast carriage rates are highly heterogeneous in Hainan, similar to the heterogeneity reported in previous studies [7–9, 12–15].

Yeast Species Composition

In this study, we obtained and analyzed a total of 415 yeast isolates from the oral cavities of people on Hainan Island in southern China. Among these, 368 isolates were identified to 26 yeast species based on their ITS sequences. The most common species was *C. albicans*, accounting for 42 % (175/415) of our strains. The second most common was *C. tropicalis* (20 %), followed by *C. glabrata* (5.5 %) and *C. parapsilosis* (4 %). Other species that were isolated from more than one host included *Kodamaea ohmeri* (11 strains), *Trichosporon asahii* (9 strains), *Candida krusei* (8 strains), *Candida metapsilosis* (7 strains), *Candida guilliermondii* (6 strains), *Candida dubliniensis* and *Candida orthopsilosis* (4 strains each), *Clavispora lusitaniae* and *Pichia anomala* (3 strains each), and *Candida intermedia, Rhodotorula mucilaginosa,* and *Saccharomyces cerevisiae* (2 strains each). The remaining 10 species (*Aureobasidium pullulans, Candida fukuyamaensis, Candida quercitrusa, Debaryomyces hansenii, Cryptococcus neoformans var. neoformans, Candida rugosa, Saccharomycopsis malanga, Trichosporon asteroides, Trichosporon faecale, and Yarrowia lipolytica*) were represented by one strain each.

The oral yeast species composition observed here differed from what had been reported in several previous studies [7–9, 12–15]. For example, in North America, oral yeasts were dominated by *C. albicans*, typically accounting for over 80 % of all oral yeast isolates [7, 8, 14]. On the other hand, *C. albicans* accounted for only about 10 % of the oral yeast flora on Mainland China, and there was a large diversity of yeast species for Chinese on Mainland China [7]. However, a survey of college students identified *C. albicans* as the most common oral yeast species (~80 %) at Hainan Medical College on Hainan Island in southern China [9]. Our analyses here showed that the prevalence of *C. albicans* (42 %) in the Hainan general population was intermediate between the medical students at Hainan Medical College (~80 %) and the Mainland Chinese population (~10 %). Our results thus reinforce the conclusion that geographic populations of humans can differ significantly in their oral yeast microbial flora.

Most of the yeast species found here have been previously reported as isolated from oral cavities of humans. However, several species such as *Cryptococcus*

neoformans and the three *Trichosporon* species found here are rarely isolated in human oral cavities. Furthermore, four hosts from southeast Hainan were found to have *Candida dubliniensis*, a species only recently reported in China [16]. All four hosts carrying *C. dubliniensis* were hospitalized patients. Also, of significant interests are the 47 strains ($\sim$11 % of the total strains) that showed significant ITS sequence differences to all currently known yeast species. Many of these yeasts likely represent novel species. Further morphological, physiological and DNA sequence analyses at multiple loci are need to firmly establish their taxonomic status.

Our species identification criterion based on >97 % ITS sequence identity was derived based on inter- and intra-species ITS sequence variations for the common *Candida* species [10]. A recent review also showed that the 3 % ITS sequence divergence corresponded well to reproductive isolation in basidiomycete fungi [17]. However, we would like to note that the 97 % ITS sequence identity cutoff for species identification might not be appropriate for many fungal groups. Indeed, most intra-specific ITS sequence variations are within 1 % [9, 10]. As a result, our inferred species number is likely conservative and an underestimate of the true number. To clearly differentiate our samples and those in databases, multi-locus sequence analyses of polymorphic single-copy genes would be needed to identify reproductively isolated units (phylogenetic species) in nature.

The distributions of yeast species for different demographic groups of hosts are summarized in Table 1. Below we describe and discuss the potential contributions of each of these factors to the diversity of yeast species observed in our samples.

Health Status of Hosts

Twenty known species of yeasts were found in the healthy host group while 18 known yeast species were identified in the hospitalized patient group (Table 1). However, the patient group had a higher species diversity (0.732) than the healthy host group (0.584) (Table 1). Overall, 12 species were shared between the two groups, while six were only found in the patient group, and eight were found only in the healthy host group. Of the six species found only in the patient group, two were represented by multiple strains each with four strains for *C. dubliniensis* and eight strains for *C. krusei*. Even for the species that were shared between the two groups, we found a significant difference in their relative prevalence. For example, the four dominant yeast species in our samples *C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis* showed significantly different distributions (Table 1, Chi square test, $p = 2.31 \times 10^{-11}$). Specifically, compared with the healthy host group, the hospitalized host group had significantly higher ratios of *C. parapsilosis* and *C. tropicalis* but lower ratios of *C. albicans* and *C. glabrata*. Among the four species, *C. tropicalis* showed the highest bias in the patient group, a 44 % higher than expected if both the healthy host group and the hospitalized host group had the same ratios (Table 1). It is known that *C. tropicalis* are broadly distributed in human populations [2–4, 7–9]. The significantly elevated prevalence of *C. tropicalis* to a level similar to that of *C. albicans* among the hospitalized patients suggest that the hospital environments in Hainan, the host conditions, or a combination of these factors have likely played a significant role for its common distribution among the patients.

Host Ethnicity

Two ethnic groups (Han and Li) were sampled in our surveys. These two groups accounted for over 98 % of the total population in Hainan. A total of 343 yeast isolates were obtained from Han Chinese, while 72 isolates were obtained from the Li people. The relative ratios of these two ethnic groups that we sampled (79.2 % for Han Chinese and 20.8 % for the Li minority) were similar to their relative ratios in the general Hainan population (83 % Han Chinese and 16 % Li minority, respectively). In total, 23 known species of yeasts were found in the Han group, while 18 known yeast species were identified in the Li group (Table 1). Overall, 15 species were shared between the two groups, while three were only found in the Li group, and eight were found only in the Han group. However, samples from the Li group had a higher species diversity (0.81) than the Han group (0.695) (Table 1). Different from the significant contributions by host health status to yeast species compositions, we found no significant difference in the relative proportions of the four dominant yeast species *(C. albicans, C. glabrata, C. parapsilosis,* and *C. tropicalis)* between the Han and the Li groups in Hainan ($p = 0.887$). This result is similar to what was found

among ethnic groups in North America where little difference in oral yeast species composition was found among Caucasians, African Americans, Asians, and Hispanics [7]. Thus, despite their differences in demographic history and other cultural differences between the Han and Li groups on Hainan Island, our results further support the conclusion that host ethnicity plays little role in determining oral yeast species composition.

Host Geography

Based on their geographic locations, our 415 strains were grouped into three regions northeast, northwest, and southeast with each containing 182, 78, and 155 strains, respectively. In total, the three regions had 16, 9, and 20 known yeast species each with species diversities of 0.61, 0.65, and 0.75, respectively (Table 1). Seven species were shared among all three regions (*C. albicans, C. glabrata, C. parapsilosis, C. tropicalis, C. krusei, K. ohmeri, and T. asahii*). In northwest Hainan, *C. tropicalis* was the dominant yeast species, accounting for 47 % of the total sample while *C. albicans* accounted for only 17 %. Different from the species compositions in the northwest region, *C. albicans* was the most common yeast species in both the northeast and southeast regions in Hainan, accounting for 57 and 37 % of the respective samples. Chi-square contingency table test showed that the four commonly found yeast species (*C. albicans, C. glabrata, C. parapsilosis, and C. tropicalis*) differed significantly in their distributions among the three geographic regions (Table 1, $p = 3.341 \times 10^{-10}$). As expected, the major geographic difference was found between northwest Hainan and the two regions from the east. In contrast, little difference was found between the northeast and southeast samples in the relative distributions of the four species (Table 1, $p = 0.319$). At present, the reasons for the large geographic differences between the northwest and east samples are unknown. However, climate differences could be a factor. For example, southeast Hainan receives about 1,400 mm of rainfall each year, significantly higher than that in northwest Hainan ($\sim$960 mm rainfall each year). The rainfall differences could have contributed to vegetation differences, yeast species differences associated with the vegetation and food and other environmental factors, all of which could have impacted human oral yeast flora. At present, the environmental yeast populations in Hainan are unknown.

Host Gender

Among the 415 yeast isolates, 187 were from female hosts and 228 were from male hosts. Fifteen of the 26 known species were found in both samples. Samples from the two host groups had very similar yeast species diversities of 0.717 and 0.716, respectively, for the female and male samples. In addition, the four main yeast species showed very similar distributions in the two groups (chi-square contingency table test, $p = 0.811$), with *C. albicans* being the most common species (41–43 %) in both groups followed by *C. tropicalis* (18–21 %), *C. glabrata* (4.8–6.1 %), and *C. parapsilosis* (3.7–4.4 %). However, of the 11 species found in only one of the two groups, three were found only in the male group while eight were found only in the female group. The species showing the biggest bias in distribution was *C. krusei*, with all 8 strains of this species found only in males and none in females. Interestingly, all eight isolates were from patients. *C. krusei* is an emerging fungal nosocomial pathogen primarily found in the immunocompromised patients and those with hematological malignancies [2–4]. Of further medical relevance is its natural resistance to fluconazole, a standard antifungal agent [5]. It is possible that the application of triazole antifungal drugs could have selected for intrinsically drug-resistant/tolerant yeast species such as *C. krusei* in hospital environments.

Place of Birth

Among the 415 yeast isolates, 370 were from hosts born within Hainan and 45 were from those born on Mainland China. Samples from the two host groups had different yeast species diversities, 0.562 for the sample from those born outside of Hainan and 0.726 for that from those born within Hainan. The different species diversity indices were also reflected by their species richness: seven known yeast species were identified for those born outside of Hainan and 25 for those born within Hainan, respectively.

Mainland China overall is geographically, culturally (including food culture) and climatically more heterogeneous than Hainan Island. As a result, we should expect that the yeast population from hosts

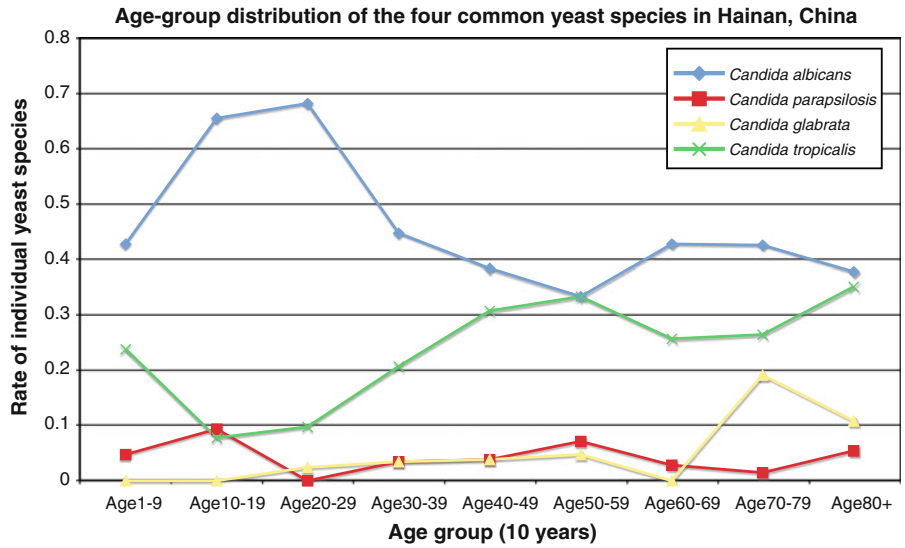

Fig. 1 Age-related distributions of the four most common yeast species in our total sample

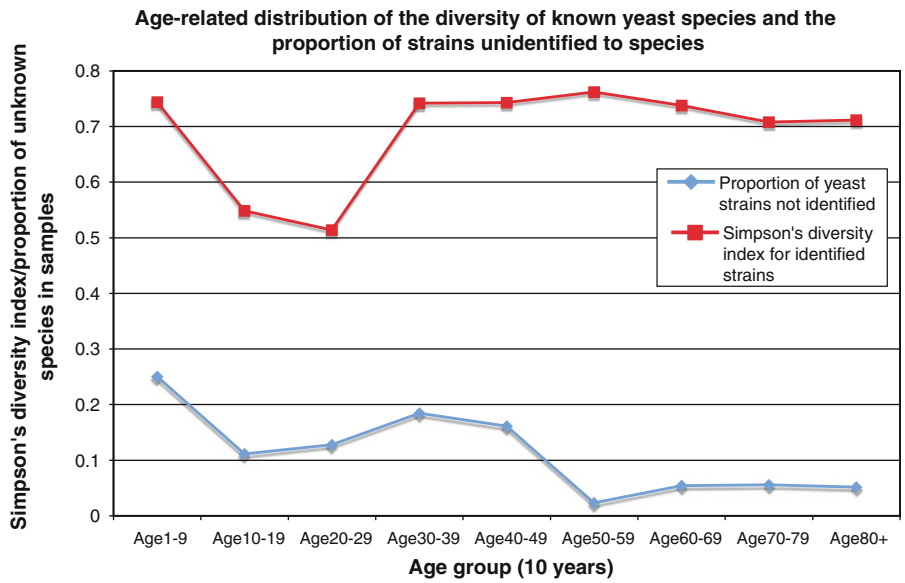

Fig. 2 Age-related distribution of Simpson's diversity index of known yeast species (*top line*) and the relative proportions of strains that were not identifiable to species levels (*bottom line*)

born on Hainan Island to be less diverse than that from hosts born on Mainland China. However, our data showed the opposite pattern. At present, the contributor(s) to the observed pattern is unknown. However, there are a couple of possibilities. The first is related to the abundant and high diversity of fruits on Hainan Island throughout the year due to its tropical climate. The diversity of fruits and the nutrients in these fruits could help maintaining the high yeast species diversity in the environment, and when consumed by humans, became part of the human oral microbial flora. Another possibility is related to sample sizes. The yeast population from those borne outside of Hainan is much smaller than that from hosts borne within Hainan. The small sample size for those borne outside of Hainan could make the species richness and species diversity estimates unreliable. Indeed, an earlier study found that oral yeast species diversity from Mainland

Chinese to be 0.791 [7], higher than for the sample from those borne on Hainan Island.

Of the seven known yeast species representing 38 of the 45 strains from those borne outside of Hainan, the most common was *C. albicans* (53 %), followed by *C. tropicalis* (13 %), *C. parapsilosis* (~9 %), and with *C. orthopsilosis, C. glabrata, P. anomala, and R. mucilaginosa* each represented by only one strain (2.2 % each; Table 1). For the sample from those born within Hainan, 330 of the 370 strains were distributed among 25 yeast species, with the four most common being *C. albicans* (41 %), followed by *C. tropicalis* (21 %), *C. glabrata* (6 %), and *C. parapsilosis* (3.5 %) (Table 1). Our statistical analyses showed that the two groups differed significantly in their distributions of these four main yeast species ($p = 0.039$). This result differs from that reported previously for college students in which no difference was found between samples from students born and raised in Mainland China and those born and raised in Hainan [9]. Two factors might have contributed to the different observations. First, the number of yeast strains for both groups in the previous study was much smaller than those in the current study. Larger sample sizes allow the detection of smaller differences between groups at statistically significant levels. Second, the host populations in the current study were much more heterogeneous than those in the previous survey.

Age

We found an overall significant difference in yeast species distributions among the 10-year age groups ($p = 1.32 \times 10^{-11}$). The significant difference is most noticeably reflected in the age-related patterns of the four dominant yeast species (Table 1; Fig. 1). For example, though *C. albicans* was the most commonly found yeast species across all age groups, it was the most dominant for the 10–29-year-old groups (Fig. 1). In contrast, while *C. tropicalis* was the second most common for all age groups, its prevalence increased with age, reaching a proportion similar to that of *C. albicans* for those 80 years and older (Fig. 1). Both *C. parapsilosis* and *C. glabrata* were relatively evenly distributed across the age groups. However, there was a high prevalence of *C. glabrata* in the 70+ year old groups. The differential species distributions were similarly reflected in Simpson's species diversity indices (Table 1; Fig. 2) in that age groups 10–19 and 20–29 with high percentages of *C. albicans* had the lowest species diversities. Interestingly, the number of strains that represented potential new species also showed an age-related distribution pattern (Fig. 2). A relatively high proportion (15 %) of the strains from hosts aged 49 years and younger could not be identified to species level based on their ITS sequences. In contrast, less than 5 % of the yeasts could not be identified for those from the 50 years and older age groups (Fig. 2). The reason for this pattern is not known at present, but it likely reflects yeast infection patterns and the intensity of our study. It is known that the older age groups were more likely to be susceptible to yeast infections and other diseases [2, 14–16, 18–22], resulting us having a better understanding of their oral yeast microflora.

Conclusion

This study investigated the potential influences of six demographic factors on oral yeast isolation rate and oral yeast species distributions from a geographically limited area, Hainan Island, in southern China. Five of the six factors were found to have significant influences on oral yeast isolation rates. However, the detailed mechanisms for how these factors might have contributed to the differential yeast isolation rates remained largely unknown. Among the six examined factors, the health status likely has the highest medical relevance where hospitalized patients are typically under greater physiological stress and are more likely to succumb to opportunistic yeast infections. It thus would be of great interest to further investigate the relationships between specific host health conditions and the yeasts that they carry in a larger scale survey and including more detailed information about host physiological conditions and underlying health problems.

A total of 26 known yeast species were found in our strains, with *C. albicans* being the most common (42 %), followed by *C. tropicalis* (20 %), *C. glabrata* (5.5 %), and *C. parapsilosis* (4.1 %). Interestingly, due to their significant ITS sequence divergences, we were unable to identify 47 of the 415 strains to the species level. Additional biochemical and gene sequence analyses will be needed in order to confirm whether these strains belong to existing species or new

species. Our analyses of the distributions of known yeasts revealed that neither host gender nor ethnicity impacted oral yeast species distribution. However, host residency, place of birth, health status, and age all showed significant effects on the distributions of the four most commonly isolated yeast species. *C. tropicalis* was the dominant species in northwest Hainan where it infected a large number of hospitalized patients aged 70 and above. The source(s) for the large number of *C. tropicalis* in this subpopulation remains to be investigated.

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