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Fruits as the vehicle of drug resistant pathogenic yeasts

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Summary *Objective:* We investigated the diversity and drug susceptibility of pathogenic yeasts on fruit surfaces.
Method: Fruits were purchased from supermarkets and washed with buffer. The pellets were re-suspended in medium after centrifugation. The cell suspensions were plated onto CHROMagar Candida medium. Yeasts were identified by ribosomal DNA sequencing and their drug susceptibilities were determined by broth microdilution assay.
Results: Of 184 isolates, comprised of 55 species, from 22 different types of fruits, 29 species, including *Candida famata*, *Candida fermentati*, *Candida guilliermondii*, *Candida intermedia*, *Candida krusei*, *Candida orthopsilosis*, *Candida parapsilosis*, *Candida pelliculosa*, *Candida tropicalis*, and others have been reported to cause diseases in humans. In addition to *C. krusei*, intrinsically resistant to fluconazole, all *Rhodotorula* and *Rhodosporidium* species were resistant to fluconazole. One each of *C. tropicalis* isolate was belonged to diploid sequence type (DST)149 and DST225, genotypes also detected in isolates from humans. Furthermore, the DST225 isolate was less susceptible to azole drugs. The susceptibilities to azole drugs for clinical and agricultural usage were associated to each other.

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Conclusion: It is important to be aware of the existence of pathogenic yeasts, especially drug-resistant ones, on the fruit surfaces, a potential route for pathogenic yeasts to be transmitted to humans.

Introduction

The prevalence of fungal infections has increased significantly in the past decades due to the increase of risk populations. Among the fungal pathogens causing morbidity and mortality in seriously immunocompromised hosts, *Candida* species are the most common ones. One emerging issue in managing fungal infection is that species causing nosocomial infections has shifted toward the more treatment-resistant non-albicans *Candida* species.[1–3] The prevalence of these species differed significantly in various geographic areas.[2–4] *Candida glabrata* was the most frequently isolated species in Western countries,[2,5] whereas *Candida tropicalis* predominated in Asia.[3,6,7] Furthermore, *C. tropicalis* develops drug resistance in the presence of fluconazole much more rapidly than other *Candida* species.[8]

Taiwan Surveillance of Antimicrobial Resistance of Yeasts (TSARY) was initiated in 1999 to monitor the trends of species distribution and drug susceptibilities of yeast pathogens.[9] Subsequent surveys were conducted in 2002, 2006, 2010, and 2014.[10–13] We found two genetically closely related DST types of *C. tropicalis* strains, DST140 (allele combination, 1, 3, 3, 17, 54, and 3 for *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a*, respectively) and DST98 (allele combination, 1, 3, 3, 17, 9, and 3), exhibiting reduced susceptibility to fluconazole in TSARY 1999 and 2006. There were also three DST149 (allele combination, 1, 44, 3, 7, 58, and 3) isolates exhibiting reduced susceptibility to fluconazole from three hospitals located in northern Taiwan. These results indicated that those DST strains exhibiting reduced susceptibility to fluconazole circulated widely in Taiwan from 1999 to 2006 and their presence was not a result of outbreaks in certain hospitals or geographic regions.[14]

Azole-resistant isolates can emerge following microbial exposure to drugs in either medical or agricultural settings. The existence of environmental routes for developing drug resistance in fungi has been further supported by the findings that azole-resistant *Aspergillus fumigatus* isolates recovered from soil and compost were genetically related to clinical resistant ones.[15,16] *Candida tropicalis* is prevalent in organically enriched soil, aquatic environments[17] and wild birds.[18] Previously, we found that approximately one third (18/56) of the isolates from soils collected in Taiwan exhibited reduced susceptibility to fluconazole. Furthermore, three were of DST140 and nine DST149,[14,19] genotypes also detected among isolates from humans.

Recent studies have revealed that fruits and vegetables, especially those consumed raw, can transmit microbial pathogens responsible for disease outbreaks. Although the significance of fresh produce to human health has been recognized, little is known about the transmission of microbial pathogens.[17] In addition to detecting pathogenic yeasts in environments, such as soil, it is important to investigate the presence of pathogenic yeasts on foods, especially those of *C. tropicalis* strains exhibiting reduced drug susceptibility. In this study, we isolated and characterized yeasts on the surface of fruits from supermarkets.

Materials and methods

Yeast isolation

Yeasts recovered from 60 samples comprised of 22 different kinds of fruits from 4 different supermarkets in northern Taiwan from late 2009 to early 2010 were characterized. Together, whole fruits from the same sampling were gently washed by 200 ml buffer (1% peptone, 0.5% NaCl) in a 10-liter sterilized bag. The water was then collected for centrifugation. The pellet was re-suspended in 0.5 ml YPD broth. An inoculation loop was used to transfer the cell suspension for plating onto CHROMagar Candida medium (BBL, Becton Dickinson Cockeysville, MD, USA). After 3-day incubation at 24 °C, representative colonies of each morphotype were picked for subsequent workup. One isolate per species per sample was analyzed. Due to availability and in individual supermarkets, there were different numbers of sampling for each type of fruit. In addition, due to the variation in fruit size, there were different numbers of fruit in each sampling, ranging from 2 to 100. Different samplings of similar kinds of fruits were grouped into one single type. For instance, citrus type included citrus, kumquat, murcott, orange, and tangerine; melon type included cantaloupe, champion melon, melon, watermelon; and pear type included honey pear, new high pear, new pear, and pear.

Identification

Cells were streaked onto CHROMagar Candida medium for single colony isolation. For identification, all isolates were subjected to ribosomal DNA (rDNA) sequencing. The internal transcribed spacer (ITS) region was amplified by the primers ITS1, 5′-TCCGTAGGTGAACCTGCGG-3′, and ITS4 5′-TCCTCCGCTTATTGATATGC-3′, and/or the D1/D2 region of rDNA was amplified by the primers NL1 5′-GCATATCAATAAGCGGAGGAAAAG-3′ and NL4 5′-GGTCCGTGTTTCAAGACGG-3′.[20] All novel sequences have been submitted to the website of National Center for Biotechnology Information (STable 1).

Drug susceptibility testing

Standard powder of fluconazole was kindly provided by Pfizer, and triadimenol (PS-1064, Chem Service) were dissolved in dimethyl sulfoxide (DMSO). The final concentrations of drugs were from 0.125 mg/l to 64 mg/l. Minimum inhibitory concentrations (MICs) were determined by the

Table 1 Distribution of yeast species and type of fruits.

Species	Total	Type of fruit (number of total fruit sampled/number of sampling)									
		Mango	Melon	Pear	Citrus	Grape	Tomato	Lemon	Peach	Star fruit	Wax apple
		(36/6)	(19/7)	(31/7)	(129/9)	(900/5)	(246/4)	(26/2)	(33/2)	(9/3)	(18/2)
Pichia kluyveri	24	4	4	1	3	0	2	0	1	2	1
Candida fermentati[a]	17	1	2	3	2	0	1	0	1	0	0
Hanseniaspora opuntiae	10	3	2	1	2	0	0	0	0	1	1
Hanseniaspora uvarum	10	1	2	0	1	1	0	1	1	0	0
Candida quercitrusa[a]	9	0	1	2	2	0	0	0	1	0	1
Candida famata[a]	7	1	0	2	1	0	0	0	0	1	0
Hanseniaspora thailandica	7	2	0	0	1	1	0	0	0	1	0
Sporidiobolus pararoseus	7	1	2	0	0	1	2	1	0	0	0
Lodderomyces elongisporus[a]	6	1	2	0	0	0	0	1	0	0	0
Candida oleophila	5	0	0	0	1	2	0	0	0	0	0
Candida natalensis	4	0	0	0	0	1	1	0	0	0	0
Candida railenensis	4	0	0	0	2	1	0	0	0	0	0
Candida sorboxylosa	4	0	0	0	0	0	1	0	0	2	0
Rhodotorula mucilaginosa[a]	4	1	0	0	0	1	0	1	1	0	0
Aureobasidium melanogenum[a]	3	1	0	0	0	0	1	1	0	0	0
Candida krusei[a]	3	1	1	0	0	0	1	0	0	0	0
Candida pulcherrima[a]	3	0	0	2	0	0	0	0	0	0	0
Candida tropicalis[a]	3	0	1	0	0	0	0	0	0	0	1
Debaryomyces nepalensis[a]	3	1	0	0	0	0	1	0	1	0	0
Issatchenkia occidentalis	3	0	0	0	0	0	0	0	0	0	2
Rhodosporidium paludigenum	3	0	2	0	0	0	1	0	0	0	0
Rhodotorula glutinis[a]	3	0	0	1	0	0	0	1	0	0	0
Candida catenulata[a]	2	0	0	0	1	0	1	0	0	0	0
Candida conglobata[a]	2	0	0	0	0	0	0	1	0	0	0
Candida orthopsilosis[a]	2	0	0	1	1	0	0	0	0	0	0
Candida valida[a]	2	0	0	2	0	0	0	0	0	0	0
Cryptococcus flavescens[a]	2	1	0	0	0	0	0	0	0	0	0
Issatchenkia terricola[a]	2	0	0	0	0	2	0	0	0	0	0
Pichia aff. Fermentans Y153	2	0	0	0	0	0	0	0	0	0	1
Rhodotorula diobovat	2	0	1	0	0	1	0	0	0	0	0
Trichosporon asahii[a]	2	0	0	0	0	0	0	1	0	0	0
Aureobasidium pullulans[a]	1	0	0	0	0	0	0	0	0	0	0
Candida akabanensis	1	1	0	0	0	0	0	0	0	0	0
Candida diversa	1	0	0	0	0	1	0	0	0	0	0
Candida guilliermondii var. membranifaciens[a]	1	0	0	0	0	0	0	0	0	0	1
Candida guilliermondii[a]	1	0	0	1	0	0	0	0	0	0	0
Candida intermedia[a]	1	0	0	0	0	0	0	0	0	0	0
Candida lipolytica[a]	1	0	0	1	0	0	0	0	0	0	0
Candida nonsorbophila	1	0	1	0	0	0	0	0	0	0	0
Candida parapsilosis[a]	1	0	0	1	0	0	0	0	0	0	0
Candida pelliculosa[a]	1	0	0	1	0	0	0	0	0	0	0
Candida xylopsoci	1	0	0	0	0	0	0	0	0	0	0
Cyberlindnera xylosilytica	1	1	0	0	0	0	0	0	0	0	0
Hanseniaspora guilliermondii	1	0	0	0	0	0	0	0	0	0	0
Pichia mexicana[a]	1	1	0	0	0	0	0	0	0	0	0
Pichia pijperi	1	0	0	0	0	0	0	0	0	0	0
Pseudozyma fusiformata	1	0	0	0	0	0	0	0	0	0	0
Pseudozyma hubeiensis	1	0	0	0	0	0	0	0	0	0	0
Rhodosporidium babjevae	1	0	0	1	0	0	0	0	0	0	0
Rhodotorula dairenensis[a]	1	0	0	0	0	0	0	0	0	0	0
Saccharomyces bulderi	1	0	0	0	0	0	0	0	1	0	0
Saccharomyces cerevisiae[a]	1	0	0	0	0	0	0	0	1	0	0
Torulaspora delbrueckii	1	0	0	0	0	0	0	0	0	0	0
Trichosporon jirovecii[a]	1	0	0	0	1	0	0	0	0	0	0
Wickerhamomyces pijperi	1	0	0	0	0	0	0	0	0	1	0
Total	184	22	21	20	18	12	12	8	8	8	8

[a] Has been reported to cause diseases in humans.

Type of fruit (number of total fruit sampled/number of sampling)											
Jujube	Banana	Guava	Coconut	Kiwi	Loquat	Papaya	Plum	Salted olive	Shakya	Persimmon	Salted plum
(73/2)	(11/2)	(10/2)	(3/1)	(10/1)	(30/1)	(4/2)	(100/1)	(24/1)	(4/1)	(3/1)	(26/1)
1	0	2	0	0	0	0	1	1	1	0	0
1	1	1	1	1	1	0	0	0	1	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	1	0	0	1
1	0	0	0	0	0	0	0	0	1	0	0
0	1	0	0	0	0	1	0	0	0	0	0
0	0	0	0	0	1	1	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	1	0	0	0	0	0
0	0	0	0	0	0	0	1	1	0	0	0
0	0	0	0	0	0	0	1	0	0	0	1
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	1	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	1	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	1	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	1	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	1	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
1	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	1	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	1	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	1	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
0	0	0	0	0	0	0	0	0	0	0	0
7	5	5	4	4	4	4	4	3	3	2	2

same *in vitro* antifungal susceptibility testing established in our laboratory according to the guidelines of M27-A3 recommended by the Clinical and Laboratory Standards Institute.[10,21] RPMI medium 1640 (31800-022, Gibco BRL) was used for the dilution and growth of the yeast culture. Strains from American Type Culture Collection (ATCC), including *Candida albicans* (ATCC 90028), *Candida krusei* (ATCC 6258), and *Candida parapsilosis* (ATCC 22019), were used as the standard controls. Growth of each isolate was measured by the Biotrak II plate spectrophotometric reader (Amersham Biosciences, Biochrom Ltd., Cambridge England) after incubation at 35 °C for 24 h and 48 h.

MICs were defined as the concentration of drugs capable of reducing the turbidity of cells to greater than 50%. The newly defined species-specific breakpoints for common *Candida* species were applied in the present study.[22] There is no breakpoint interpretation for *C. krusei* since it is assumed to be intrinsically resistant to fluconazole. The clinical breakpoints of *C. tropicalis* and *C. parapsilosis* are: MICs $\leq$2 mg/l were considered to be susceptible, $\geq$8 mg/l resistant, and 4 mg/l susceptible-dose dependent (SDD). For the species of which clinical breakpoints have not been established, we applied epidemiological cutoff values instead.[22] For *Candida guilliermondii* MICs >8 mg/l, *Candida orthopsilosis* >2 mg/l, and *Candida pelliculosa* >4 mg/l were considered non-wild-type. In the present study, for the *Candida* species of which clinical breakpoints and epidemiological cutoff values have not been established, we applied the clinical breakpoints of *C. tropicalis* instead. Furthermore, the breakpoints of fluconazole were also applied to triadimenol. The MICs of 50% and 90% of the total population were defined as MIC_{50} and MIC_{90}, respectively.

Multilocus sequence typing of *C. tropicalis*

Based on our previous report,[23] the DNA fragments of six genes of *C. tropicalis*: *ICL1*, *MDR1*, *SAPT2*, *SAPT4*, *XYR1*, and *ZWF1a* were sequenced for the analyses. The resulted sequences were aligned with BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium) and compared with those in the database of *C. tropicalis* (http://pubmlst.org/ website) to obtain the identities (DST).

Results

Distribution of yeasts

A total of 184 yeast isolates (Table 1) were recovered from 60 fruit samples from 4 supermarkets. Due to availability of fruits, there were different numbers of sampling for each type of fruit, ranging from 1 to 9. The average number of different species recovered from one sample was 3. There were 6, 5, 4, 3, 2, and 1 species recovered from 3, 4, 16, 15, 15, and 7 individual samples, respectively. There were six species isolated from a sample of lemon (*Aureobasidium melanogenum*, *Candida conglobate*, *Hanseniaspora uvarum*, *Lodderomyces elongisporus*, *Rhodotorula glutinis*, and *Trichosporon asahii*), tomato (*Candida catenulata*, *Candida fermentati*, *Candida natalensis*, *Pichia kluyveri*, *Rhodosporidium paludigenum*, and *Sporidiobolus pararoseus*), and mango (*C. fermentati*, *P. kluyveri*, *S. pararoseus*, *Cryptococcus flavescens*, *Hanseniaspora opuntiae*, and *Hanseniaspora thailandica*). There were five species isolated from a sample of new pear (*C. fermentati*, *C. guilliermondii*, *C. orthopsilosis*, *Candida quercitrusa*, and *Candida valida*), new high pear (*C. fermentati*, *R. glutinis*, *Candida famata*, *Candida lipolytica*, and *C. pelliculosa*), kumquat (*C. fermentati*, *C. orthopsilosis*, *C. quercitrusa*, *H. opuntiae*, and *H. thailandica*), and wax apple (*C. guilliermondii var. membranifaciens*, *C. quercitrusa*, *C. tropicalis*, *H. opuntiae*, and *Issatchenkia occidentalis*).

Mango was the most common source (12%), followed by melon (11.4%), pear (10.9%), citrus (9.8%), grape (6.5%), tomato (6.5%), lemon (4.3%), peach (4.3%), star fruit (4.3%), wax apple (4.3%), jujube (3.8%), banana (2.7%), guava (2.7%), and 9 others including coconut, kiwi, loquat, papaya, persimmon, plum, salted olive, salted plum, and shakya (Table 1).

The 184 isolates were classified into 55 different species (Table 1). A total of 24 *P. kluyveri* isolates were recovered and it was the most common species, followed by 17 *C. fermentati*, 10 each of *H. opuntiae* and *H. uvarum*, 9 *C. quercitrusa*, 7 each of *C. famata*, *H. thailandica* and *S. pararoseus*, 6 *L. elongisporus*, 5, *Candida oleophila*, and other 45 species. A total of 86 isolates of 29 pathogenic yeast species were recovered. There were 17 *C. fermentati*, 9 *C. quercitrusa*, 7 *C. famata*, 6 *L. elongisporus*, 4 *Rhodotorula mucilaginosa*, 3 each of *A. melanogenum*, *C. krusei*, *Candida pulcherrima*, *C. tropicalis*, *Debaryomyces nepalensis*, and *R. glutinis*, 2 each of *C. catenulata*, *Candida conglobata*, *C. orthopsilosis*, *C. valida*, *C. flavescens*, *Issatchenkia terricola*, and *T. asahii*, 1 each of *Aureobasidium pullulans*, *C. guilliermondii*, *C. guilliermondii var. membranifaciens*, *Candida intermedia*, *C. lipolytica*, *C. parapsilosis*, *C. pelliculosa*, *Pichia mexicana*, *Rhodotorula dairenensis*, *Saccharomyces cerevisiae*, and *Trichosporon jirovecii*. Among the commonly isolated *Candida* species in clinical settings, there were three *C. krusei* from melon, mango, and tomato, three *C. tropicalis* from banana, melon and waxed apple, two *C. orthopsilosis* from citrus and pear, and one *C. parapsilosis* from pear (Table 1). Neither *C. albicans* nor *C. glabrata* was recovered.

Antifungal drug susceptibilities

Of the 184 isolates, some had growth defect in the conditions for determining drug susceptibility. For example, *Hanseniaspora* spp., *I. terricola*, *Sporidiobolus paraoseus*, and *Torulaspora delbrueckii* failed to grow in RPMI medium. Furthermore, *C. oleophila*, *R. paludigenum*, *C. flavescens*, *Pichia pijperi*, *Pseudozyma hubeiensis*, *R. glutinis*, and *Saccharomyces bulderi* failed to grow at 35 °C. Thus, the drug susceptibilities of 115 isolates were determined (Table 2).

The MIC_{50} fluconazole and triadimenol were the same as 2 mg/l. MIC_{90} fluconazole and triadimenol were 64 mg/l and 16 mg/l, respectively. There were 45 (39.1%) isolates with fluconazole MIC $\geq$ 8 mg/l (Table 2). They included 24 *P. kluyveri*, 4 *R. mucilaginosa*, 3 each of *C. krusei* and *I. occidentalis*, 2 each of *C. quercitrusa*, *Rhodotorula diobovat*,

Table 2 Antifungal susceptibilities of 114 isolates.

Species	MIC (mg/l)												Total
	Fluconazole						Triadimenol						
	2	4	8	16	32	64	2	4	8	16	32	64	
Total	59	11	5	3	7	30	65	19	19	8	1	3	115
Pichia kluyveri	0	0	0	1	6	17	5	5	12	2	0	0	24
Candida fermentati[a]	15	1	1	0	0	0	5	10	1	1	0	0	17
Candida quercitrusa[a]	6	0	2	0	0	0	7	1	0	0	0	0	8
Lodderomyces elongisporus[a]	6	0	0	0	0	0	6	0	0	0	0	0	6
Candida famata[a]	4	0	0	0	0	0	4	0	0	0	0	0	4
Rhodotorula mucilaginosa[a]	0	0	0	0	0	4	0	0	2	1	0	1	4
Candida natalensis	3	0	0	0	0	0	2	1	0	0	0	0	3
Candida krusei[a]	0	0	1	2	0	0	3	0	0	0	0	0	3
Candida pulcherrima[a]	3	0	0	0	0	0	3	0	0	0	0	0	3
Candida tropicalis[a]	2	1	0	0	0	0	2	0	0	1	0	0	3
Issatchenkia occidentalis	0	0	0	0	0	3	0	0	0	3	0	0	3
Candida oleophila	2	0	0	0	0	0	2	0	0	0	0	0	2
Candida railenensis	1	1	0	0	0	0	2	0	0	0	0	0	2
Candida sorboxylosa	0	2	0	0	0	0	2	0	0	0	0	0	2
Debaryomyces nepalensis[a]	1	1	0	0	0	0	2	0	0	0	0	0	2
Rhodotorula glutinis[a]	0	0	0	0	1	1	0	0	1	0	1	0	2
Candida catenulata[a]	2	0	0	0	0	0	2	0	0	0	0	0	2
Candida conglobata[a]	2	0	0	0	0	0	1	1	0	0	0	0	2
Candida orthopsilosis[a]	2	0	0	0	0	0	2	0	0	0	0	0	2
Rhodotorula diobovat	0	0	0	0	0	2	0	0	1	0	0	1	2
Aureobasidium melanogenum[a]	0	0	1	0	0	0	1	0	0	0	0	0	1
Rhodosporidium paludigenum	0	0	0	0	0	1	1	0	0	0	0	0	1
Cryptococcus flavescens[a]	0	1	0	0	0	0	1	0	0	0	0	0	1
Trichosporon asahii[a]	1	0	0	0	0	0	1	0	0	0	0	0	1
Candida akabanensis	1	0	0	0	0	0	1	0	0	0	0	0	1
Candida guilliermondii var. membranifaciens[a]	0	1	0	0	0	0	1	0	0	0	0	0	1
Candida guilliermondii[a]	1	0	0	0	0	0	0	0	1	0	0	0	1
Candida intermedia[a]	1	0	0	0	0	0	1	0	0	0	0	0	1
Candida nonsorbophila	1	0	0	0	0	0	1	0	0	0	0	0	1
Candida parapsilosis[a]	1	0	0	0	0	0	1	0	0	0	0	0	1
Candida pelliculosa[a]	0	1	0	0	0	0	1	0	0	0	0	0	1
Candida xylopsoci	1	0	0	0	0	0	1	0	0	0	0	0	1
Pichia mexicana[a]	1	0	0	0	0	0	0	1	0	0	0	0	1
Pichia pijperi	0	1	0	0	0	0	1	0	0	0	0	0	1
Pseudozyma fusiformata	1	0	0	0	0	0	1	0	0	0	0	0	1
Rhodosporidium babjevae	0	0	0	0	0	1	0	0	0	0	0	1	1
Rhodotorula dairenensis[a]	0	0	0	0	0	1	0	0	1	0	0	0	1
Saccharomyces bulderi	1	0	0	0	0	0	1	0	0	0	0	0	1
Saccharomyces cerevisiae[a]	0	1	0	0	0	0	1	0	0	0	0	0	1

[a] Has been reported to cause diseases in humans.

and *R. glutinis*, 1 each of *A. melanogenum*, *C. fermentati*, *Rhodosporidium babjevae*, *R. paludigenum*, *R. dairenensis*. A total of 11 (9.6%) isolates had fluconazole MICs 4 mg/l. These consisted of two *Candida sorboxylosa*, and one each of *C. fermentati*, *C. guilliermondii var. membranifaciens*, *C. pelliculosa*, *Candida railenensis*, *C. tropicalis*, *C. flavescens*, *D. nepalensis*, *P. pijperi*, and *S. cerevisiae*.

For triadimenol, there were 31 (27%) isolates with MIC $\geq$ 8 mg/l (Table 2). These included 14 *P. kluyveri*, 4 *R. mucilaginosa*, 3 *I. occidentalis*, 2 each of *C. fermentati*, *R. diobovat*, and *R. glutinis*, 1 each of *C. guilliermondii*, *C. tropicalis*, *R. babjevae*, and *R. dairenensis*. There were 19 isolates (16.5%) have triadimenol MIC 4 mg/l, comprised of 10 *C. fermentati*, 5 *P. kluyveri*, 1 each of *C. conglobata*, *C. natalensis*, *C. quercitrusa*, and *P. mexicana*. The susceptibilities to azole drugs for clinical and agricultural usage were associated to each other (Table 3). Of the 114 isolates, 59 (51.3%) and 65 (56.5%) isolates were with MICs $\leq$ 2 mg/l to fluconazole and triadimenol, respectively. Of the 59 isolates with fluconazole MIC $\leq$2 mg/l, 2, 12 and 45 had triadimenol MIC $\geq$8 mg/l, 4 mg/l, and $\leq$2 mg/l, respectively. There were 28 isolates, including 14 *P.*

Table 3 Association of susceptibilities to fluconazole and triadimenol.

	Triadimenol										
Fluconazole	0.125	0.25	0.5	1	2	4	8	16	32	64	Total
0.125	7	0	1	0	0	0	0	0	0	0	8
0.25	4	1	0	0	2	0	0	0	0	0	7
0.5	2	0	1	0	1	1	0	0	0	0	5
1	2	2	4	3	3	2	0	0	0	0	16
2	1	4	3	2	2	9	2	0	0	0	23
4	1	3	1	3	1	1	0	1	0	0	11
8	0	0	1	1	1	1	0	1	0	0	5
16	0	0	1	0	2	0	0	0	0	0	3
32	0	0	0	0	3	2	2	0	0	0	7
64	0	0	0	0	2	3	15	6	1	3	30
Total	17	10	12	9	17	19	19	8	1	3	115

kluyveri, 4 *R. mucilaginosa*, 3 *I. occidentalis*, 2 each of *R. diobovat* and *R. glutinis*, 1 each of *C. fermentati*, *H. opuntiae*, *R. babjevae*, and *R. dairenensis*, with both fluconazole and triadimenol MICs $\geq$8 mg/l.

A total of three *C. tropicalis* were isolated in the present study. The isolate F85 was from banana and had fluconazole MIC 4 mg/l and triadimenol MIC 16 mg/l after 24-h incubation and fluconazole MIC 16 mg/l and triadimenol MIC 16 mg/l after 48-h incubation. The isolate F91 was from wax apple and had fluconazole MIC 0.25 mg/l and triadimenol MIC 0.5 mg/l after 24-h and MICs increased to 64 mg/l for both drugs after 48-h incubation. And the isolate F283 was from melon and had fluconazole MIC 1 mg/l and triadimenol MICs 0.5 mg/l after 24-h incubation. The fluconazole and triadimenol MICs did not change after 48-h incubation. Since both F85 and F91 exhibited reduced susceptibility to fluconazole, the MLST types of both isolates were determined and they were belonged to DST225 and DST149, respectively.

Discussion

In the present study, we identified isolates of *C. tropicalis* with DST types also detected in humans. In addition, several common *Candida* species causing diseases in humans,[3,24] such as, *C. famata*, *C. guilliermondii*, *C. krusei*, *C. orthopsilosis*, and *C. parapsilosis* were also detected on fruit surfaces. Among the 86 yeast pathogens recovered in the present study, 14 isolates of 7 different species have fluconazole MIC $\geq$8 mg/l after 24-h incubation. In addition to *C. krusei* intrinsically resistant to fluconazole,[25] all *Rhodotorula* spp. were with high fluconazole MICs. These results suggest a possible route for transmitting the fungi along with the drug resistance from environments to humans. In the study of Török and King, they have identified 239 isolates from partially and fully processed produces.[26] Based on morphological, physiological, and biochemical characterizations, they classified those isolates into 36 species. Among the 127 isolates from fruit produces in their study, *S. cerevisiae* has the highest frequency of 7.5% (18/239), followed by *C. tropicalis* 4.2% (10/239). In the present study, we have applied rDNA sequencing to classify the 184 isolates into 55 species. There were three *C. tropicalis* and one *S. cerevisiae* detected. The differences may be due to the methods of isolation, climates, environmental factors, agricultural practices, and whether the produces were processed.

Long duration of exposure to drugs and high numbers of reproducing microorganisms contribute to the selection and/or enrichment of drug-resistant individuals. In fungi, drug resistance is more likely to be the outcome of sequential accumulation of adaptive mutations in the chromosomes.[27,28] Previously, we have reported that *C. tropicalis* isolates with fluconazole MICs $\geq$64 mg/l after 48-h incubation were recovered from human and soil. Albeit from different geographic regions of Taiwan, they shared indistinguishable PFGE patterns and belonged to the same DST type. Furthermore, we found that approximately one third (18/56) of isolates had fluconazole MIC 64 mg/l after 48-h incubation and 9 of them belonged to one MLST type, DST149.[29] The F91 isolates was also belonged to DST149. Moreover, F85 was belonged to DST225, another genotype found in humans in our other survey (data not shown).

A recent report has contributed the development of drug-resistant *A. fumigatus* to the usage of agricultural azoles.[30] In addition, previous study has shown that in human immunodeficiency virus infected patients, *C. albicans* and other yeast species were cross-resistant to medical and agricultural azole drugs.[31] However, no *C. tropicalis* was recovered in that study. According to the data from "Domestic Manufacturers Production & Sale of Pesticides " published by the Taiwan Crop Protection Industry Association, there is an increased usage of azole-type compounds in the agriculture in Taiwan, up from approximately 100 tons in 2005 to 145 tons in 2009 (about 45% increase). Since both effective reproduction and spreading of resistant fungi in the environment are to be anticipated, we are confronted with the major challenge of drug-resistance development in environmental setting on a global scale. Hence, to reduce and prevent patients from acquiring azole-resistant *C. tropicalis* and other pathogenic fungal species, it is advisable to take precaution on the use of azoles in not only clinical but also agricultural settings. The mechanisms contributing to the reduced susceptibility to azole drugs are under investigation.

Fluconazole has become one of the most commonly prescribed drugs among clinically available antifungal drugs due to low cost and less side effects. Exposure to azole compounds paves the way for the selection and enrichment of fungal isolates exhibiting reduced susceptibility to drugs, which may occur in patients receiving azole treatments. However, the development of isolates exhibiting reduced susceptibility to fluconazole in person to person transmission during medical treatment is unlikely. Alternatively, the use of azole compounds in the environment may select organisms exhibiting reduced susceptibility to drugs, which may then find their ways to humans. Since we purchased fruits from supermarkets in the present study, our data cannot rule out the possibility that the pathogenic yeasts on the fruit surfaces may come from supermarket employees and/or customers. Nonetheless, our findings demonstrate that pathogenic yeasts, including those with reduced susceptibility or even resistant to drugs, exist and survive on the surface of fruits regardless of their original sources. Thus, we should be cautious when providing fruits and/or juice to severely immunocompromised patients.

Contributions

Conceived and designed the experiments and wrote the manuscript: HJL, YLY. Performed the experiments: SHT, WLC, YZC, ZLZ, HFC, CFL. Analyzed the data: CFL, YLY, HJL.

Conflict of interest

The authors declare that they have no conflict of interest.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.jinf.2017.06.005.

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