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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.
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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

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