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LETTER TO THE EDITOR

Capability of Fluorescent Capillary Electrophoresis To Distinguish Species of the *Candida parapsilosis* Complex

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Candida parapsilosis complex (psilosis complex) has three genetically distinct but not easily phenotypically distinguishable cryptic species, *C. parapsilosis sensu stricto*, *Candida metapsilosis*, and *C. orthopsilosis*. It has been reported as one of the most important non-*albicans* agents commonly detected in invasive candidiasis (1–3). Each psilosis complex species manifests a unique epidemiology, virulence, and antifungal susceptibility (4); thus, precise identification can be of clinical interest, and various molecular methods are being developed (5–7). Our previous studies (5, 8) reported the capability of *ITS2* ribosomal DNA (rDNA) amplification followed by fluorescent capillary electrophoresis (f-*ITS2*-PCR-CE) to distinguish between various *Candida* species, including psilosis complex, by a small but constant 0.4-bp difference between *C. parapsilosis* and *Candida orthopsilosis*. The actual positions of the size standard fragments are plotted against the expected size of each size standard fragment, which defines the function of these variables. The size (bp) of an analyzed fragment is then calculated according to this function; therefore, decimal values are being reported. The 0.4-bp size difference is presumably caused by the different mobilities of single-stranded DNA (ssDNA) fragments, resulting from their different primary structures.

To address f-*ITS2*-PCR-CE reliability, a large collection of 342 clinical isolates identified as psilosis complex species by matrix-assisted laser desorption ionization–time of flight mass spectrometry (MALDI-TOF MS) (Biotyper, Bruker, Germany), using formic acid for sample semi-extraction (9) and α-cyano-4-hydroxycinnamic acid as a matrix, was analyzed. All discrepant results were confirmed by panfungal PCR followed by sequencing (10).

From 339 isolates (99.1%) concordantly identified by MALDI-TOF MS and f-*ITS2*-PCR-CE, 305 (89.1%) were identified as *C. parapsilosis sensu stricto*, 19 (5.6%) were identified as *C. metapsilosis*, and 15 (4.4%) were identified as *C. orthopsilosis*. Two isolates (0.6%) were identified as *C. metapsilosis* and *C. orthopsilosis* by MALDI-TOF MS, while f-*ITS2*-PCR-CE identified both isolates as *C. parapsilosis sensu stricto*, which was subsequently confirmed by sequencing. Reanalyzing MALDI-TOF MS using full extraction protocol according to the manufacturer's instructions resulted in the identification of *C. parapsilosis sensu stricto* in both cases. Further, one isolate (0.3%) was identified as a *C. parapsilosis sensu stricto* and *C. metapsilosis* mixed culture by f-*ITS2*-PCR-CE. Sequencing confirmed the identification of *C. parapsilosis sensu stricto* together with another fungus, while MALDI-TOF MS detected *C. metapsilosis* exclusively.

While *C. metapsilosis* has a different length and is easily distinguishable from any other species (304 bp), *C. parapsilosis sensu stricto* and *C. orthopsilosis* have the same sequenced *ITS2* region length (300 bp). However, this study confirmed that little difference in size is determined by capillary electrophoresis (297.4 and 297.8 bp in *C. parapsilosis* and *C. orthopsilosis*, respectively). This 0.4-bp size difference is stable and sufficient enough to reliably identify these psilosis complex species, although not in one sample simultaneously (Fig. 1). Superior to MALDI-TOF MS, f-*ITS2*-PCR-CE could

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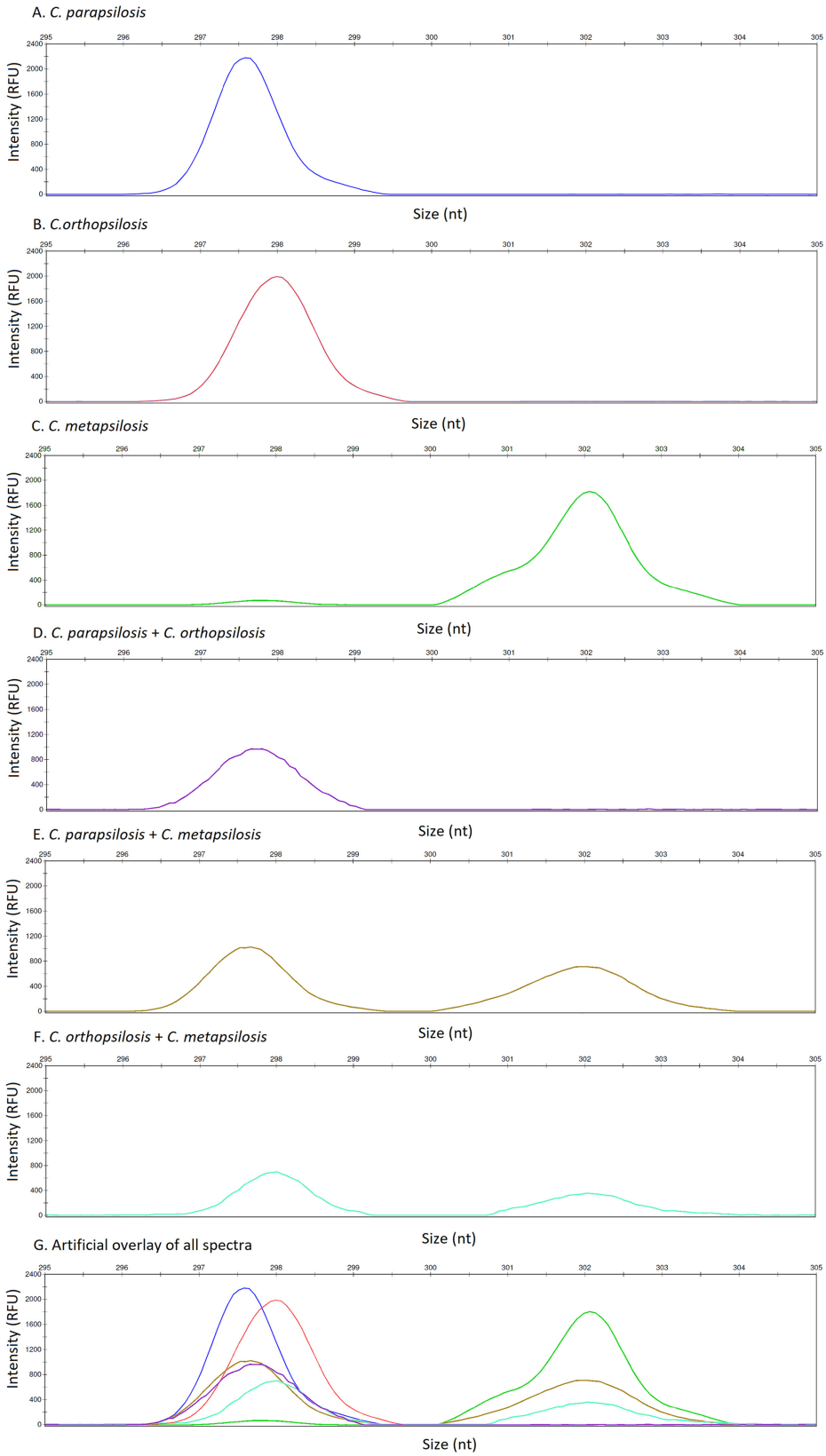

FIG 1 f-*ITS2*-PCR-CE spectra of psilosis complex species. Unique f-*ITS2*-PCR-CE psilosis complex species spectra. It is apparent that a 0.4-nt difference between *C. parapsilosis* (blue) (A) and *C. orthopsilosis* (red) (B) is sufficient to distinguish between them (spectra overlay) (G), although not if present in one sample (violet) (D). *C. metapsilosis* (green) (C) is easily distinguishable from either *C. parapsilosis* or *C. orthopsilosis* if presents in a mixture (E, F, G).

distinguish *C. parapsilosis*/*C. orthopsilosis* in a mixed sample with *C. metapsilosis* (Fig. 1) if appropriate template ratios entered PCR (5). Unlike other psilosis complex-specific methods (6, 7), f-*ITS2*-PCR-CE can also identify other *Candida* spp. (5). Moreover, f-*ITS2*-PCR-CE is applicable to detect the PCR products of DNA extracted directly from clinical samples (8). Thus, no culture is needed, and identification is fastened. The DNA fragments of floating or dead fungi cells can be detected by PCR-based molecular techniques. This can be advantageous if nondividing cells are present in a sample but can generate false-positive results.

In conclusion, parallel analysis of the large clinical sample collection by MALDI-TOF MS and f-*ITS2*-PCR-CE proved the latter technique suitable to precisely discriminate psilosis complex species. This technique can be used as a cheap and quick alternative to sequencing. Moreover, it was proved to be beneficial when isolates of low MALDI-TOF MS identification score and mixed cultures are analyzed. However, more comprehensive multicenter studies with a larger number of *C. metapsilosis* and *C. orthopsilosis* isolates are necessary to further validate this method.

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