



Letter to the Editor

SSCP is not suitable as screening technique to detect mutations in *ERG11* gene from *Candida* species



El método molecular SSCP no permite la detección de mutaciones en el gen *ERG11* de *Candida*

To date, many molecular mechanisms of fluconazole (FLZ) resistance in *Candida* species have been described and may occur simultaneously in resistant isolates.⁴ One of the most studied mechanisms is characterized by missense mutations in *ERG11* gene, leading to a decrease in the affinity of the FLZ for its enzyme target, 14 α -demethylase. The identification of these mutations is based on the sequencing of the *ERG11* open reading frame (ORF) followed by computational analysis of translated codons. However, the cost of the sequencing for a large number of samples is high and expensive for many laboratories. The single-strand conformational polymorphism (SSCP) is a mutation-screening technique widely used for its simplicity and high resolution, detecting changes even at a single base level. This ability is because a single-stranded DNA folds differently from another if it differs by a single base, resulting in different mobility in electrophoresis gels.² In this light, we evaluated the usefulness of SSCP as a screening method to detect mutations in *ERG11* gene.

In this study, a total of 87 *Candida* clinical isolates (32 *Candida albicans*, 34 *Candida tropicalis*, 11 *Candida parapsilosis* complex, six *Candida glabrata* and four *Candida krusei*) were evaluated. Molecular identification and susceptibility testing were previously performed.¹ To improve the SSCP efficiency, we amplified the *ERG11* gene of each species in four PCR products around 400 bp using previously described primers.¹ These four PCR products cover together the entire ORF. Once amplified, each PCR product was denatured by 15 min at 95 °C after addition of 1:1 running buffer (formamide 98%, EDTA 10 mM, bromophenol blue 0.05% and xylene cyanol 0.05%), chilled by 5 min in ice and then loaded in SSCP gels. The electrophoresis were performed in 6% polyacrylamide gels (glycerol 5% added) under the following conditions: 45 mA at 10 °C by 5.5 h in 1 $\times$ TBE buffer (tris–borate–EDTA). Gels were fixed in ethanol 10% for 30 min and then submitted to silver staining.³ The same PCR products were further purified, and both strands sequenced and compared to their respective migration patterns on SSCP gels.

Because the SSCP is able to detect mutations in a single base (point mutations), we expected the isolates with mutated sequences to show different SSCP patterns when compared to the wild sequence isolates. However, the results observed were not the expected, and we could not draw a correlation between SSCP patterns and the presence of mutations. Indeed, we observed that some PCR products with the same SSCP pattern exhibited different number of mutations or even their absence (Fig. 1). In the same way,

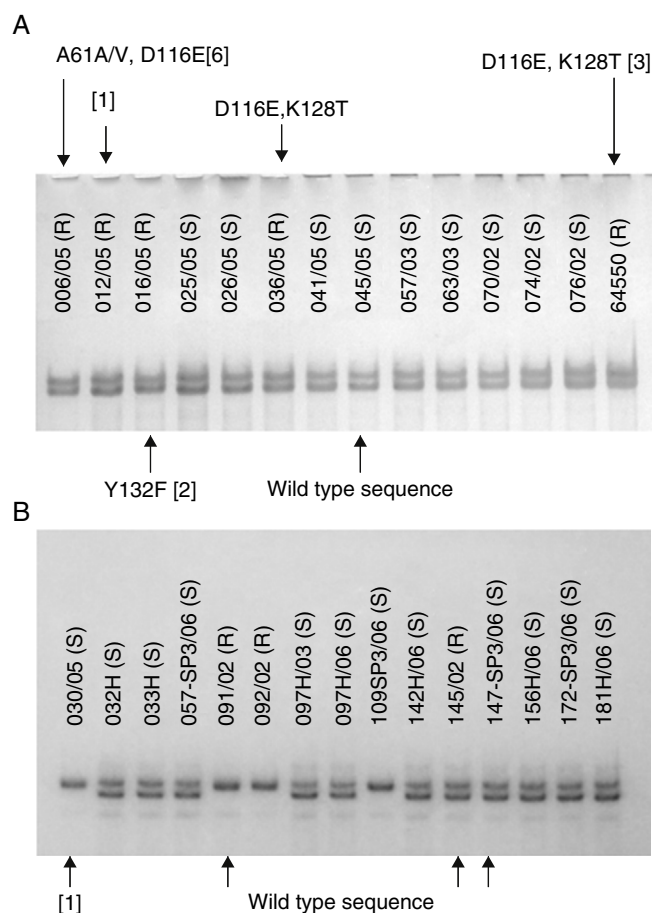


Fig. 1. (A) SSCP gel of the first ~400 bp of *ERG11* gene of *Candida albicans* clinical isolates showing different mutations (indicated by arrows) at the same electrophoresis pattern. (B) SSCP gel of the last ~400 bp of *ERG11* gene of *Candida tropicalis* clinical isolates showing different electrophoresis patterns with the same wild type sequence. Silence mutations are presented in brackets and heterozygosity by a slash.

some different SSCP patterns showed the same wild type sequence. The susceptibility data could not be either linked to SSCP patterns. In other words, susceptible and resistant isolates shared the same patterns. These results were observed in all the four PCR products from all five species, suggesting the SSCP is not a reliable screening technique to detect mutations in *ERG11* gene from *Candida* clinical isolates. On the other hand, different results might be reached when analyzing shorter PCR products (<400 bp) once the SSCP sensitivity is highly influenced by both length of the DNA amplicon as well as the GC content.

References

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