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OPEN PEER REVIEW

Development of Short-term Membrane-based Cultivation Combined with Dual-target Melting Analysis for Rapid Differentiation of Common *Candida* Species

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1 Original submission

2 comments · 24 May 2023 – 24 May 2023

Referee 1 Reviewer

MINOR REVISION 24 May 2023

The genus and species names of the microorganisms should be italicized.
Other than faster diagnosis, no other benefit has been reported. What is the cost of the test, and can it be done in any laboratory?
What was the origin of the clinical samples of Candida spp., and did the growth rate differ among samples from different body sites?
Given that there was a difference in the growth of Candida in the two environments only in the first 10 hours and no difference was observed in the next 10 hours or afterward, is there any conceivable reason for this? Pay more attention to this point in the discussion.

Associate Editor

MAJOR REVISION 24 May 2023

Reviewer 1:
Public Comment:
English editing is required.
The discussion needs to be revised. The results of the present study should be analyzed alongside those of other studies with similar and conflicting findings.
The sensitivity and specificity of color tests and molecular tests should be mentioned.
The results of the statistical analysis are not mentioned.
The importance of using this method to identify common Candida species is not mentioned in the discussion.
The origin of the 70 Candida clinical isolates should be mentioned.
Decision:
Major revision
Reviewer 2:
The genus and species names of the microorganisms should be italicized.
Other than the speed of diagnosis, no other benefit has been stated. What is the cost of the test, and can it be performed in any laboratory?
What is the origin of the clinical samples of Candida spp., and did the growth rate differ between these samples from different body sites?
Given that there was a difference in the growth of Candida in the two environments only in the first 10 hours and no difference was observed in the next 10 hours or thereafter, is there any conceivable reason for this? Pay more attention to this point in the discussion.
Decision:
Minor revision

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Revision 1

AUTHORS' RESPONSE TO REVIEWERS

Authors' reply to reviewers

The next pages contain the authors' point-by-point reply to the comments of this revision round, as submitted with the revised manuscript. It is published as the authors wrote it.

Reviewer 1:

Question: 1. English edition is required.

Reply:

We would like to thank you for the comments. The revised manuscript was edited for English language by LetPub (https://www.letpub.com/editorial-certificate) with regard to grammar, punctuation, spelling, and clarity. The language editor is a native English speaker with long-term experience in editing scientific and technical manuscripts. A certificate of English language editing was provided. In the revised manuscript, the English edition was highlighted in green text, as were the responses to other comments below.

Question: 2. The discussion needs to be revised. The results of the present study should be analyzed with other similar and opposite studies.

Reply: The reviewer's comment is reasonable. Following the suggestion, we have revised the discussion. In the revised manuscript, the comparison of membrane-based cultivation in this study and the previous study about a membrane technology for the detection of virulent Bacillus anthracis and Yersinia pestis was updated. Please see lines 262 to 265 of the revised manuscript for details. Moreover, the melting analysis results of CALB and CAND primer sets for common *Candida* species were compared with those of existing studies, and the results of studies that had not been previously reported were also indicated. Please see lines 289 to 296, and lines 304 to 307 of the revised manuscript for details.

Question: 3. The sensitivity and specificity of color tests and molecular tests should be mentioned.

Reply: Thank you for your suggestion. In this study, 72 *Candida* clinical isolates, as shown in the revised Table 1, were identified by color tests and ITS sequencing. In the revised manuscript, the comparison of the color test and sequencing identification of clinical isolates is presented in Table 1; please see Table 1 at line 347 of the revised manuscript for details. In addition, the agreement between the results of the dual-target melting analysis and the sequencing results was provided, which is presented in Table 5. Please see lines 429 to 431 of the revised manuscript for details. These tables are used to present the sensitivity and specificity of the color reaction and molecular assays.

Question: 4. The results of statistical analysis are not mentioned.

Reply: According to your suggestion, the analysis of significant differences between the Tm values of different *Candida* species was added to the revised manuscript. Please see lines 142 to 143, and 182 to 185 of the revised manuscript for details.

At the same time, a comparison of the results of dual-target melting analysis and sequencing was also performed in a tabular analysis. Please see lines 429 to 431 of the revised manuscript for details.

Question: 5. The importance of using this method to identify common *Candida* species is not mentioned in the discussion.

Reply: Following your suggestion, we have further revised the discussion on the importance of technique in this study. The culture method has always been a reliable method for microbial identification, but it is time-consuming. The technology involved in this research consists of two parts. The first part was short-term membrane-based cultivation, which aimed to improve the efficiency of the culture method. The importance and necessity of the culture method for fungal detection were retained by enriching the samples through short-term cultivation, resulting in the identification time of *Candida* species advanced and the overall detection time being shortened; the second part is to improve the identification resolution of *Candida* species through dual-target melting analysis. By combining the two, it is expected to achieve both rapid and accurate identification. As we all know, the clinical treatment of *Candida* infection, especially severe infection, is a race against time, and the results of this study are undoubtedly of great significance for improving the current situation in this regard. Inappropriate empiric therapy can be minimized through early identification of *Candida* species. Please see lines 224 to 229, and lines 233 to 235 of the revised manuscript for details.

Furthermore, compared to other PCR-based techniques, there was no post-operation required after amplification, and the strain was identified directly by the contour of the melting curve without electrophoresis or enzyme digestion, which reduces the contamination of external nucleic acids. Please see lines 271 to 275 of the revised manuscript for details.

In addition, in previous studies on the identification of *Candida* species by melting curve analysis, there were more single melting peaks. Considering the fact that the difference between the Tm values of some detection targets was small (a difference of approximately 1°C), and this small difference often became less obvious due to the slight change of gene sequence caused by species phylogeny and evolution, thus reducing the discrimination between strains based on Tm values. However, the application of dual-target melting curve analysis in this study increased the resolution of strain identification. Please see lines 289 to 298, and lines 304 to 307 of the revised manuscript for details.

In summary, the technology developed in this study can demonstrate its importance in identification and differentiation of common *Candida* species in a faster, more convenient, and more accurate manner.

Question: 6. The origin of 70 *Candida* clinical isolates should be mentioned.

Reply: Thank you for raising this important question; we had neglected to account for this information in our previous manuscript submission. Firstly, it should be noted that we also added two clinical isolates of *C. parapsilosis* that were not correctly identified by the color test, so the total number of clinical isolates in the revised manuscript was 72. The sources of all clinical isolates in this study (as seen

in newly added Table 1) were provided in the revised manuscript. Please see line 347 of the revised manuscript for further details.

Reviewer 2:

Question: 1. The name of the genus and species of the microorganisms should be italic.

Reply: At first, we thank you for your comments. We have carefully checked the name of the genus and species of the microorganisms involved in the revised manuscript and confirmed that they are italicized. Please see line 288 of the revised manuscript for details.

Question: 2. Other than the speed in diagnosis, no other benefit has been announced, and what is the cost of the test and whether it can be done in any laboratory?

Reply: Thank you for raising this important question. Improving detection speed is one of the benefits of this study. In addition, the importance and necessity of the culture method for fungal detection were retained by enriching the samples through short-term culture. Please see lines 224 to 229 of the revised manuscript for further details.

Furthermore, compared to other PCR-based techniques, there was no post-operation required after amplification, and the strain was identified directly by the contour of the melting curve without electrophoresis or enzyme digestion, which reduces the contamination of external nucleic acids. Please see lines 272 to 275 of the revised manuscript for details.

Additionally, in previous studies on the identification of *Candida* species by melting curve analysis, there were more single melting peaks. Considering the fact that the difference between the Tm values of some detection targets was small (a difference of approximately 1°C), and this small difference often became less obvious due to the slight change of gene sequence caused by species phylogeny and evolution, thus reducing the discrimination between strains based on Tm values. However, the application of dual-target melting curve analysis in this study increased the resolution of strain identification. Please see lines 289 to 298, and lines 304 to 307 of the revised manuscript for details.

In summary, the technology developed in this study can demonstrate its importance in identification and differentiation of common *Candida* species in a faster, more convenient, and more accurate manner.

As for the cost of each test, we calculated the test cost according to the retail price. The detection of each sample mainly involves two steps: membrane-based culture and dual-target melting analysis, and the costs are 0.85 USD/test and 2.01 USD/test respectively. The total cost is approximately 2.86 USD/test. In addition to the incubator and fluorescent quantitative PCR instrument, no other special

equipment is required during the entire detection process, so it can be widely used in routine laboratories.

Question: 3. Where the origin of Clinical is samples of *Candida* spp. and did the growth rate differ between these samples from different body sites?

Reply: Thank you for raising this important question; we had neglected to account for this information in our previous manuscript submission. Firstly, it should be noted that we also added two clinical isolates of *C. parapsilosis* that were not correctly identified by the color test, so the total number of clinical isolates in the revised manuscript was 72. The sources of all clinical isolates in this study (as seen in newly added Table 1) were provided in the revised manuscript. Please see line 347 of the revised manuscript for further details.

The results of the growth curves of strains from each source (newly added Figure 2) were provided in the revised manuscript. The comparison of the growth curves of *Candida* isolates from different sources showed that the growth characteristics of *Candida* from the different sources tended to be similar. As the curves showed, the lag, log, and stationary phases were clearly exhibited. No matter which part of the body the sample was taken from, it took about 8 to 10 hours for the yeasts to adapt to the normal growth environment before they were ready to proliferate and enter the log phase. Please see lines 456 to 474 of the revised manuscript for further details.

Question:4. According to there was a difference in the growth of *Candida* in the two environments only in the first 10 hours and no difference was observed in the next 10 hours and after that, is there any conceivable reason for that? Pay more attention to this point in the discussion.

Reply: According to your suggestion, we have added further clarification to this issue in the discussion section. The nutritional status of *Candida* in membrane-based culture was consistent with conventional SDA culture. When cultured with conventional SDA in the first 10 hours of growth, the generated colonies were small, and the contrast between the natural white colonies and the light-colored background of the culture medium was correspondingly low, making it difficult to distinguish them by visual inspection. When using membrane-based culture combined with thiazolyl blue plate, the contrast between the dark blue-stained colonies and the white background of the membrane was significantly increased, making it convenient for observation by the naked eye. After culturing for 10 hours, the colony size was sufficient for observation, even though it was grown on a conventional SDA plate. Membrane-based cultivation was no longer necessary at this point. It can be seen that the advantage of membrane-based culture lies in the early observation and early sampling of *Candida* culture. Please see lines 240 to 254 of the revised manuscript for further details.

Reviewer 3:

Question:

Reply:

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2 Revision 1

0 comments

No public comments in this round.