Rapid *Candida* Species Identification; Is MALDI-TOF MS The Answer?

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Abstract

Background: The incidence of superficial and deep fungal infections have been on the increase over the last decade, so much so that *Candida* species are now the fourth most common cause of bloodstream infections in the United States, it is paramount in these cases that a rapid identification is possible to ensure an appropriate effective course of treatment is made available to the patient. Traditional fungal and yeast identification methods usually involve biochemical, morphological and physiological tests, that can be time consuming and labour intensive. These techniques also require a high level of skill, training and often the personal judgement/experience of a clinical mycologist. Commercial kits are available for the more clinically relevant yeasts and fungi including *Candida* species, these however are mainly based on biochemical tests and/or colour interpretation, often only covering a narrow range of species and/or require a 24-72h following culture to obtain an identification.
MALDI-TOF MS has recently shown great success in bacterial identification and this paper aims to explore the possibility of developing the technique to include yeasts, in particular *Candida species*. The use of MALDI-TOF MS has the advantage of reducing culture time to 24 hours, and identification to within minutes.
Method: Different species of *Candida* were cultured on a variety of culture medium; intact *Candida* cells were transferred directly from the medium plate to a MALDI target plate and overlaid with MALDI matrix. The co-crystallised sample was then irradiated with a N_2 laser and the resulting plume of positive ions separated using time-of-flight mass spectrometry, which produces a mass spectral fingerprint.
Results: The mass spectral fingerprint patterns produced were interrogated for characteristic properties, to assess the most appropriate culture medium and culture conditions suitable for identification purposes.
Conclusions: Using MALDI-TOF MS it is possible to obtain characteristic fingerprints for *Candida* species on a variety of media, which could provide the clinical mycologist with a simple rapid tool for *Candida* species identification.

Introduction

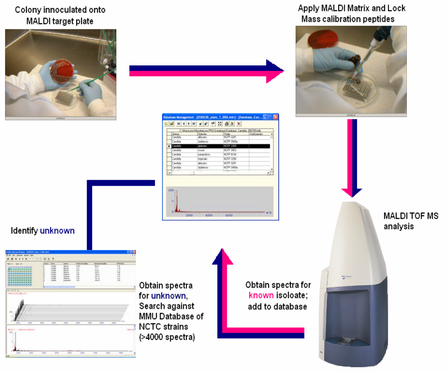
Fungal infections both superficial and deep have been on the increase over the past decade. These have been correlated with an increase in the number of immunocompromised patients as a result of the human immunodeficiency virus (HIV) pandemic and the increased use of immunosuppressive therapy for cancer treatment/organ transplantation patients[1].

The British Society for Medical Mycology (BSMM) currently recommends that yeast isolates should be identified to species level, as the correct antifungal treatment can usually be selected based upon the species identification. This also allows the mycologist to gain a better understanding of the epidemiology and possibly pin-point outbreak situations[2].

Current methods of identification rely heavily on sugar assimilation and fermentation tests, together with colony and microscopic morphology. There are several commercial kits available for yeast identification from pure culture. They can be time consuming and labour intensive, requiring between 24-72 hours for identification. CHROMagar is an alternative method based on colony colour. The plates are read at 48h & 72 hours and depend upon colour changes e.g. light green (*C.albicans*) and dark green (*C.dubliniensis*) colonies. This can lead to errors due to such subtle differences. More recently molecular methods (e.g. PCR, RAPD, AFLP) have been reported as useful when identifying *Candida* to species level and can reduce the time to identification to around 8 hours from pure culture. These techniques require a high level of skill, are expensive and not suitable to cope with a large sample through put.

It is becoming essential to rapidly identify *Candida* species to ensure appropriate antifungal therapy and hospital control measures can be put in place. In this paper we intend explore the possibility of using MALDI-TOF MS as a method to rapidly identify *Candida* species in minutes rather than hours.

Methods



Culture Medium:

- Sabouraud dextrose agar (**SAB**; CM0041,Oxoid, Basingstoke, UK)
- Chromogenic Candida agar (**Chromo**; CM1002,Oxoid, Basingstoke, UK)
- Columbia agar with horse blood (**CBA**; PB1022,Oxoid, Basingstoke, UK)

Incubation Conditions:

- 30°C, 24 hours aerobically
- 37°C, 24 hours aerobically

MALDI Matrix:

- 5-chloro-2-mercaptobenzothiazole (CMBT) 3mg/mL
- Matrix solvent; acetonitrile:methanol:water (1:1:1), 0.1% formic acid, 0.01M 18-crown-6.

All six *Candida* strains used in this study were supplied as freeze dried ampoules by the National Collection of Pathogenic Fungi (NCPF, Bristol, UK). All strains were cultured on all media in both incubation conditions.

The mass spectral fingerprint patterns obtained were interrogated for characteristic properties, to assess the most appropriate culture medium and culture conditions suitable for identification purposes.

Results

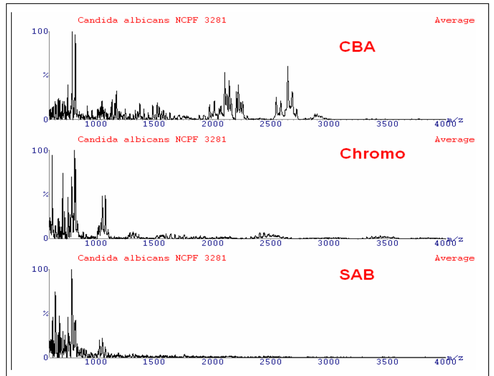


Figure 1: Comparison of the spectral fingerprint patterns for *Candida albicans* NCPF 3281, from three different media types: CBA; Chromo; SAB

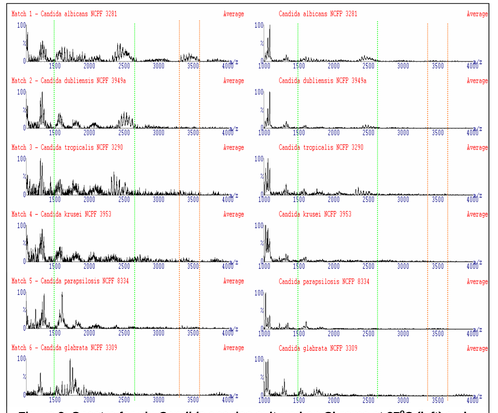


Figure 3: Spectra for six *Candida* species cultured on Chromo at 37°C (left) and 30°C (right), highlighting differences between each species. Spectral pattern remains constant when cultured at 37°C compared to 30°C although spectra at 37°C contains more intense peaks (m/z 1000-4000Da). Candida glabrata also contains two further small, but discriminatory high mass peaks between 4500-6000Da (data not shown)

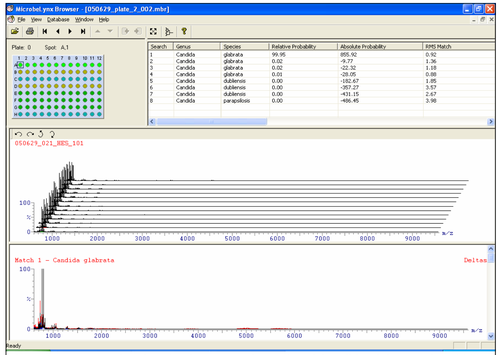


Figure 5: Browser showing database search results of Candida glabrata NCPF 3309. The browser shows the 8 best database matches; Search 1-4 correctly identify the strain to species and Search 5-8 correctly identify the strain to genus.

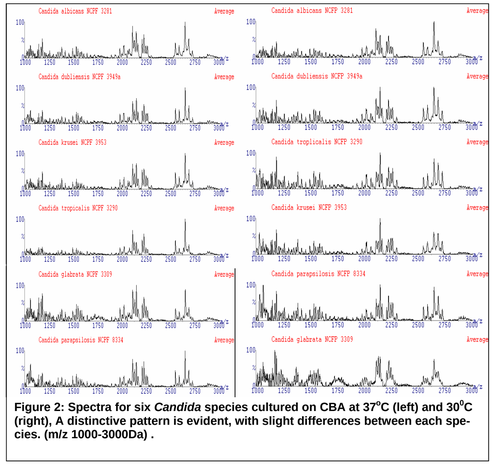


Figure 2: Spectra for six *Candida* species cultured on CBA at 37°C (left) and 30°C (right), A distinctive pattern is evident, with slight differences between each species. (m/z 1000-3000Da) .

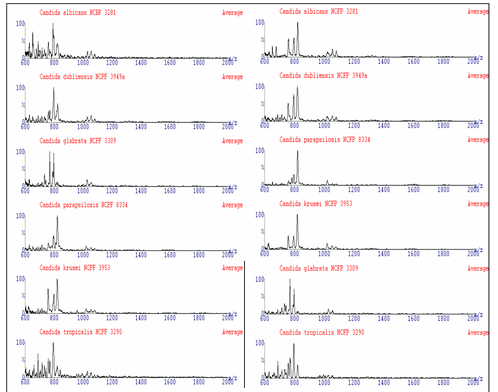


Figure 4: Spectra for six *Candida* species cultured on SAB at 37°C (left) and 30°C (right), A distinctive pattern is evident, with slight differences between each species, (m/z 600-2000Da)

Conclusions

- ***Candida* species produce MALDI-TOF MS fingerprint patterns.**
- **A distinctive fingerprint for the *Candida* genus is evident, although culture dependent (Fig 1).**
- **Slight spectral differences between *Candida* species are observed when cultured on CBA and SAB (Fig 2 & 4).**
- **Spectral differences between *Candida* species are enhanced when cultured on Chromo agar (Fig 3).**
- **Database searching produced identification at the species level (Fig 5).**

Further Work

- **Extend work to include:**
  - **More strains**
  - **More species**
  - **More yeasts/moulds**

References

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3. Keys C, Dare D, Sutton H, Wells G, Lunt M, McKenna T, McDowall M, Shah H., *Compilation of a MALDI-TOF mass spectral database for the rapid screening and characterisation of bacteria implicated in human infectious diseases*, Infection, Genetics and Evolution; 2004, Volume 4; Issue 3, 221 – 242.