Review Article

Old and Modern Identification Techniques of medically important yeasts

Sayan Bhattacharyya

From, Professor, Department of Microbiology, AIIH&PH, BN campus, JC- 27 and 27B, Sector 3, Kolkata- 700106, India

ABSTRACT

Yeasts are unicellular fungal elements. Yeasts may exist as such, or may be found at 37 C in thermally dimorphic fungi like Histoplasma capsulatum and Talaromyces marneffei. They are often encountered in the clinical microbiology setting from various kind of clinical specimens like urine, cerebrospinal fluid (CSF), and blood. Medically important yeasts are Candida albicans, Candida auris, Malassezia furfur and Cryptococcus spp. They are incriminated in various infections like oral thrush, fungemia, meningitis and cystitis. Many a times they are also encountered as normal inhabitants of the human skin and mucosa. Various degrees of immunosuppression in people, in the form of neutropenia or low CD4 T cell count may predispose a susceptible host to develop infections caused by yeasts. They need to be identified correctly for epidemiological purposes and also for institution of correct empirical antifungal therapy. Many old and new techniques have hence been described in the scientific literature for yeast identification. Old techniques like observation of shape and size, Germ tube test using pooled human serum, observing microscopic morphology by Dalmau slit culture, thermotolerance, CHROM agar, lecithinase and lipase production on egg yolk agar, sugar fermentation and sugar assimilation may need a lot of time and expertise, and may yield non-specific results, too. Modern technologies for yeast identification, like MALDI-TOF and Whole genome sequencing, are, hence fast replacing existing ones in this regard, by dint of their rapidity, low cost at times and precision. In this review we have tried to collate, assimilate and present available techniques for yeast identification.

Key words: Assimilation, Fermentation, Identification, Techniques, Yeasts.

Accurate and rapid identification of microorganisms is crucial in clinical diagnostics, food safety, environmental monitoring, and research. Identification of yeasts is also important in this regard, and can be carried out by conventional and new methods. Yeasts are unicellular fungi that divide by budding (1). Some medically important yeasts are Candida albicans, Candida glabrata, Candida auris, Malassezia furfur and Cryptococcus spp. They are incriminated in various infections like oral thrush, UTI, nosocomial infections and deep-seated infections, skin and sebaceous gland infections, and meningitis, respectively. Traditional methods such as morphology and culture-based techniques may not be so reliable, since they often tend to be time-consuming and limited in precision. Morphology may be confusing, as for instance, both Cystobasidium spp and Cryptococcus neoformans may have capsule (1). In contrast, modern identification techniques, particularly molecular methods like PCR and sequencing, and Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) mass spectrometry, have revolutionized microbial identification by offering rapid, specific, and reliable results. Identification of yeasts up to species level is important from epidemiological viewpoint and also from the angle of need for correct empirical chemotherapy. Hence both old and new methods need to be stressed upon, as because both may be necessary, depending upon the settings and circumstances. Laboratory professionals need to know the pros and cons of both types of methods. This review article hence attempts to address the issue of traditional and new, breakthrough methods for identification of medically important yeasts.

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OLD TRADITIONAL METHODS

a. Appreciation of shape and size: - Cryptococcus spp. are large (6-10 µm diameter) round yeasts while Candida spp. are oval yeasts [1]. Candida glabrata are small, while Malassezia furfur are also small (2-2.5 µm in size) lipophilic yeasts. Appreciation of shape and size helps a lot in identification of medically important yeasts.
b. Germ tube test: - Germ tube test is done from 2-3 yeast colonies incubated in 0.5 ml of pooled human serum. In place of serum, tryptone soya broth and even peptone water can also be used safely [2]. The basic idea is that

Correspondence to: Dr. Sayan Bhattacharyya, Department of Microbiology, AIIH&PH, BN campus, JC- 27 and 27B, Sector 3, Kolkata- 700106.

Email: sayantheboss@yahoo.co.in, drsayanb@aiihph.gov.in

some yeasts like C. albicans and C. dublinensis produce immature or primitive hyphae, also called germ tubes, in presence of media which are nutritionally poor. It is to be remembered that serum, in spite of being rich in fatty acids, is made up mainly of long-chain fatty acids and is hence nutrient-poor for the yeasts. Cryptococcus spp., Trichosporon spp. and other yeasts are Germ tube negative.

c. Thermotolerance: - Both C. albicans and C. dublinensis are germ tube positive. They can be differentiated based on thermotolerance, carried out in water bath at 42◦C - 44 ◦C for 24 hours [3]. C. albicans grows at this temperature while C. dublinensis cannot. Temperature-based identification can also be used for identifying Cryptococcus spp. Only C. neoformans can grow at 37 ◦C while other Cryptococcus spp. cannot.

d. Dalmau technique on Corn meal agar: - Dalmau technique was invented by Dalmau, and is based on typical microscopic morphology of the yeasts on slit inoculation in nutrient-poor media like Corn meal agar and Rice extract agar under partial anaerobiosis [4]. Tween-80 can be added to Corn meal agar to yield better results. The test gives better results in glass petri dishes as compared to plates made of other materials. Glucose peptone agar may also be used as a substitute of Corn meal agar for this purpose. Yeasts with pseudohypahe and single round terminal chlamydospores are produced by C. albicans while multiple round terminal chlamydospores at tip of pseudohyphae are produced by C. dublinensis. C. glabrata produces only small yeasts and no pseudohyphae. Trichosporpon spp. produce yeasts with arthroconidia while Geotrichum spp. produce only arthroconidia in Dalmau technique.

e. Sugar fermentation test: - This test is easy and precise, and rests on the principle of hydrolysis of sugar (2% weight/volume) fermentatively by the sugar-breaking enzymes present in yeasts; Durham's tube can also be used here to check for gas production [5]. To check for acid production, Andrade's indicator can be employed. Cryptococcus neoformans generally does not break down glucose, sucrose, maltose, or lactose. The algorithm for yeast identification based on Dalmau technique, sugar fermentation, thermotolerance and germ tube test is appended in the table that follows: -

Table 1. Scheme for yeast identification based on traditional tests

Yeast species	Dalmau micromorphology	Sugar fermentation test result	Germ tube test	Thermotolerance	Other features
C. albicans	Single terminal chlamydospores	Glucose, maltose fermented, acid and gas produced.	Positive after 2 hours	Positive at 44 ◦C	--
C. dublinensis	Multiple terminal chlamydospores	Glucose, maltose fermented, acid and gas produced.	Positive after 2 hours	Negative at 44 ◦C	Often fluconazole resistant
C. krusei	Matchstick-like appearance of yeasts and pseudohyphae	Glucose, maltose, sucrose fermented, acid and gas produced, pellicle seen.	Negative	Negative at 44 ◦C	Dry, flat colonies
C. parapsilosis	Yeasts and pseudohyphae	Only glucose fermented to yield acid and gas	negative	Negative at 44 ◦C	---
C. tropicalis	Yeasts and pseudohyphae in tree-like branching pattern	Glucose, maltose, sucrose fermented, acid and gas produced	Negative (false positive; constriction at base)	Negative at 44 ◦C	Often fluconazole resistant

f. Sugar assimilation test: - This test is more specific than sugar fermentation for yeast identification but more difficult to do. It uses yeast nitrogen base mixed with molten agar and yeast colonies which then solidifies to pour plates and sugars in form of disks having 10% of the sugar are added [6]. Up to 6 different sugar disks may be added in 1 plate. A zone of haziness around the sugar disks implies positive assimilation of that particular sugar. It is also known as Auxanogram.

g. CHROM agar Candida is a simple ready-made medium used to differentiate yeasts on the basis of colour of colonies. For instance, C. albicans produces green

colonies and *C. glabrata* mauve colonies [7]. However, it may have some subjective variations.

h. Ability to grow at 37°C: - This test is specific for C. neoformans and distinguishes it from other Cryptococcus spp. which cannot grow at this temperature [8].
i. Other than these, fungal metabolites like mannitol and arabinitol can also be measured and gives an idea of invasive fungal infections, like invasive candidiasis. Elevated D-Arabinitol/L-Arabinitol or D-Arabinitol/creatinine ratio in serum or urine have been found in immunocompromised and neutropenic, patients suffering from invasive candidiasis [9].
j. Cryptococcal latex agglutination test can be used to safely detect Cryptococcus neoformans from serum and CSF. It is a rapid test and takes only a few minutes.

MOLECULAR METHODS

PCR-based Assays Targeting ITS and D1/D2 Regions

Polymerase Chain Reaction (PCR) is a cornerstone of molecular diagnostics. In fungal and bacterial identification, PCR assays often target conserved genetic markers such as the Internal Transcribed Spacer (ITS) region and the D1/D2 domain of the 28S ribosomal RNA gene. These regions contain species-specific sequences that enable accurate identification.

For fungi, the ITS region is considered the universal DNA barcode due to its high variability among species and high conservation within species. In yeast identification, the D1/D2 region of the large subunit ribosomal RNA is frequently targeted. PCR amplification of these regions followed by gel electrophoresis or sequencing enables identification at the species level with high sensitivity.

Real-Time PCR and Multiplex PCR for Rapid Detection

Real-time PCR (qPCR) improves upon conventional PCR by allowing real-time monitoring of amplification using fluorescent dyes or probes. This technique enables quantification of microbial load and provides results within hours. Real-time PCR is highly sensitive and specific and is particularly useful in detecting pathogens directly from clinical specimens without the need for culturing.

Multiplex PCR allows simultaneous amplification of multiple target sequences in a single reaction. By using multiple sets of primers, it can detect various organisms or multiple genes from a single pathogen. This is highly valuable in situations requiring the detection of co-infections or comprehensive pathogen panels, such as in respiratory tract or gastrointestinal infections.

DNA Sequencing for Species-Level Identification

DNA sequencing, particularly of the ITS and D1/D2 regions, provides definitive identification by comparing obtained sequences against reference databases such as GenBank or UNITE. Sequencing is especially useful for identifying rare, novel, or atypical organisms that are not easily distinguishable using phenotypic methods or PCR alone.

Next-generation sequencing (NGS) further enhances this capability by allowing simultaneous sequencing of multiple organisms from complex samples, enabling metagenomic analysis and detection of unculturable species. Although more resource-intensive, sequencing offers unmatched accuracy in microbial identification and classification

Matrix-Assisted Laser Desorption Ionization–Time of Flight (MALDI-TOF) Mass Spectrometry

Principle and Workflow

MALDI-TOF mass spectrometry is a proteomic technique which identifies microorganisms based on their unique protein "fingerprints." In this method, microbial cells are mixed with a chemical matrix and ionized using a laser. The resulting ions are accelerated through a vacuum tube, and their time of flight is measured. The mass-to-charge ratio of the proteins creates a spectrum that is compared to a reference database for identification. Today, MALDI-TOF MS has become a mainstream platform in most clinical laboratories for bacterial identification. However, its application in clinical mycology for yeast and mold identification has been lagging, which is mostly attributable to the lack of standardized processes and poor fungal database representation [10].

The typical workflow includes: (a) isolation of the organism from a culture, (b) application of a small sample to a MALDI plate with matrix solution, (c) ionization and detection, and (d) comparison of the mass spectrum with a database to identify the organism. Results are typically available within minutes.

Advantages: Speed, Accuracy, Cost-Effectiveness

MALDI-TOF MS is renowned for its speed—offering species-level identification within minutes after organism isolation. It is also highly accurate for a wide range of bacteria, yeasts, and fungi, assuming a comprehensive reference database is available. Once the system is set up, the cost per test is significantly lower than molecular or biochemical methods, making it an attractive option for high-throughput laboratories [11].

Furthermore, MALDI-TOF requires minimal reagents and labour, contributing to its cost-efficiency. Its ability to identify closely related species with high precision makes it especially valuable in clinical microbiology and hospital settings.

Limitations: Database Dependency and Initial Setup Cost

Despite its advantages, MALDI-TOF has certain limitations. The accuracy of identification heavily relies on the quality and completeness of the reference database. Rare or newly discovered organisms may not be accurately identified if their spectra are not included in the library. In such cases, additional testing such as sequencing may be necessary.

Molds are more challenging to identify than yeasts by MALDI-TOF, due to limitations in the number of spectra included in commercial libraries [12].

Another significant limitation is the high initial investment required for MALDI-TOF equipment and database licensing [13]. Additionally, the technique generally requires pure cultures, which may delay diagnosis if rapid identification from clinical specimens is needed [14].

LAMP or Loop-mediated Isothermal amplification of DNA: - LAMP is an isothermal DNA amplification method which depends on 4 or 6 pairs of primers to amplify miniscule quantities of DNA within a very short period with simple operation, rendering it more suitable for low-resource areas [15]. It has produced good results for identification of Candida spp. and Aspergillus spp. For the detection of fungi by LAMP, sputum and blood may be considered as the most appropriate samples.

Immunoassay and beta-glucan assay: - They have been used but have low specificity [15]. Mannan and Antibody to Mannan are quite useful for diagnosis of Invasive Candidiasis. The performance of combined Mannan and Antibody to Mannan testing has been found to be superior to either Mannan or Anti-Mannan testing [16].

ELISA: - The hyphal form of Candida albicans is known to add to its pathogenicity during invasive candidiasis. Candidalysin, which is a highly expressed hyphal-specific extracellular peptide of C. albicans, has been identified as a critical virulence factor in C. albicans infections. This indirect ELISA assay for detecting serum anti-candidalysin IgG shows higher sensitivity and specificity as compared to fungal culture and β-D-glucan assays, respectively. Furthermore, it also helps discriminate colonization and invasive infection. However, it is recommended to look for additional pan-candida biomarkers, like mannan, along with anti-candidalysin IgG antibodies for a better and precise diagnosis [17]. Detecting serum anti-candidalysin IgG by indirect ELISA has been reported to be useful for invasive candidiasis.

Fluorescent in-situ hybridization (FISH): - Fluorescence in situ hybridisation (FISH) has been successfully used in clinical microbiology for the identification of various pathogens, including fungi. The hybridisation of fixed fungi with fluorescently labelled oligonucleotide probes which are complementary to unique target sites on the ribosomal RNA permits direct microscopic visualisation with no need of prior amplification steps. It can even be done from blood culture smears. As an alternative to DNA-based FISH probes, peptide nucleic acid (PNA) probes having a neutral backbone may be employed, although these probes are much costlier [18].

Electrophoretic karyotyping: - The technique of pulsed field gradient gel electrophoresis, also known as orthogonal field alternation gel electrophoresis (OFAGE), has been used to separate and typify intact chromosomal deoxyribonucleic acid (DNA) molecules of the yeast Saccharomyces cerevisiae. This is very important from taxonomic viewpoint.

CONCLUSION

Modern identification techniques, including molecular methods and MALDI-TOF MS, have significantly enhanced the speed and accuracy of microbial diagnostics and yeast identification. PCR-based assays, real-time PCR, and DNA sequencing allow precise identification and quantification, even in complex samples. Newer methods like MALDI-TOF MS and FISH offer rapid, cost-effective identification directly from cultures, making it indispensable in clinical microbiology. Together, these technologies form a powerful toolkit that continues to evolve, shaping the future of microbial diagnostics and public health surveillance. Hence both the traditional or old and new techniques can be combined to improve yeast identification from various clinical specimens.

REFERENCES

1. Borman AM, Johnson EM.. Candida, Cryptococcus, and Other Yeasts of Medical Importance. Manual of Clinical Microbiology. First published: 11 August 2023. Section VI. Mycology. https://doi.org/10.1002/9781683670438.mcm0121
2. Arora DR, Saini S, Aparna, *et al*. Evaluation of germ tube test in various media. Indian J Pathol Microbiol. 2003; 46(1):124-6.
3. Peltroche-Llacsahuanga H, Schmidt S, Seibold M, *et al*. Differentiation between Candida dubliniensis and Candida albicans by fatty acid methyl ester analysis using gas-liquid chromatography. J Clin Microbiol 2000; 38(10):3696-704. doi: 10.1128/JCM.38.10.3696-3704.2000.
4. Beena MS. Characterization and Virulence of Candida Isolated from Children with Dental Caries and Its susceptibility to various antimicrobial agents. DOI: http://dx.doi.org/10.5772/intechopen.91912.
5. Kali A, Srirangaraj S, Charles PMV. A cost-effective carbohydrate fermentation test for yeast using microtitre plate. Indian J Med Microbiol 2015; 33(2): 293-295. https://doi.org/10.4103/0255-0857.154884.
6. Devadas SM, Ballal M, Prakash PY, *et al*. Auxanographic Carbohydrate Assimilation Method for Large Scale Yeast Identification. J Clin Diagn Res 2017; 11(4):DC01-DC03. doi: 10.7860/JCDR/2017/25967.9653.
7. CHROMagarTM Candida. Available from: https://www.chromagar.com/en/product/chromagar-candida/ last accessed 22.08.25.
8. Cryptococcus neoformans. Available from:- https://www.sciencedirect.com/topics/neuroscience/cryptococcus-neoformans. Last accessed 22.08.25.
9. Christensson B, Sigmundsdottir G, Larsson L. D-arabinitol--a marker for invasive candidiasis. Med Mycol. 1999; 37(6):391-6. doi: 10.1046/j.1365-280x.1999.00249.x.
10. Lau AF. Matrix-Assisted Laser Desorption Ionization Time-of-Flight for Fungal Identification. Clin Lab Med 2021; 41(2):267-283. doi: 10.1016/j.cll.2021.03.006.
11. Tran A, Alby K, Kerr A, *et al*. Cost Savings Realized by Implementation of Routine Microbiological Identification by Matrix-Assisted Laser Desorption Ionization-Time of Flight

Mass Spectrometry. J Clin Microbiol 2015; 53(8):2473-9. doi: 10.1128/JCM.00833-15.
12. Identification of yeasts and moulds – MALDI-ToF MS. Available from:- https://en.fungaleducation.org/maldi-tof-ms-identification/ last accessed 22.8.25.
13. Fissel JA. Enter the Matrix: An Update on MALDI-ToF MS Advancements through 2024. Clin Microbiol Newsletter 2024; 46:22-26. https://doi.org/10.1016/j.clinmicnews.2024.05.001.
14. Topić Popović N,Kazazić S.P., Bojanić K., Strunjak-Perović I., &Čož-Rakovac R. Sample preparation and culturecondition effects on MALDI-TOF MS identificationof bacteria: A review. Mass Spec Rev. 2023; 42:1589-1603. https://doi.org/10.1002/mas.21739
15. Bumbrah GS, Jain S, Singh S, *et al*. Diagnostic Efficacy of LAMP Assay for Human Fungal Pathogens: a Systematic Review and Meta-analysis. Curr Fungal Infect Rep 2023:1-11. doi: 10.1007/s12281-023-00466-0.
16. Mikulska M, Calandra T, Sanguinetti M, *et al*; Third European Conference on Infections in Leukemia Group. The use of mannan antigen and anti-mannan antibodies in the diagnosis of invasive candidiasis: recommendations from the Third European Conference on Infections in Leukemia. Crit Care. 2010; 14(6):R222. doi: 10.1186/cc9365.
17. Luo T, Li X, Yan H, *et al*. Detection of serum anti-candidalysin IgG by indirect ELISA: a novel auxiliary tool for diagnosing invasive candidiasis in a preliminary pediatric study. Microbiol Spectr 13:e03245-24. https://doi.org/10.1128/spectrum.03245-24.
18. Da Silva RM Jr, Da Silva Neto JR, Santos CS, *et al*. Evaluation of fluorescence in situ hybridisation (FISH) for the detection of fungi directly from blood cultures and cerebrospinal fluid from patients with suspected invasive mycoses. Ann Clin Microbiol Antimicrob. 2015; 14:6. doi: 10.1186/s12941-015-0065-5.

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