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Brief report

SCA Medium: A New Culture Medium for the Isolation of all *Candida auris* Clade

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Abstract: *Candida auris* is an emerging multidrug resistant yeast causing nosocomial infections and associated with high mortality in immunocompromised patients. Rapid identification and characterisation is necessary for its diagnosis and containing spread. In this study, we present a selective culture medium for all *C. auris* clades. This medium is sensitive with a limit of detection of 10^2 CFU/ml. The 100% specificity of SCA (Specific *C. auris*) medium is confirmed on a set of 134 *Candida* strains, 50 bacterial species and 200 human stool samples. Thus, this medium specifically selects for *C. auris* isolation from clinical samples, and allows studying its phenotypic profile.

Keywords: *Candida; Candida auris; Culture; Emerging fungus; Isolation; Specific medium*

1. Introduction

Candida auris is an emerging multidrug-resistant pathogen that was first isolated in 2009 [1] and is now known to have four geographical clades: South Asia: India, East Asia: Japan, Southern Africa and South America: Venezuela [2]. However, the fifth clade (Clade V) has recently been described in Iran [3]. *C. auris* is associated with significant mortality in immunocompromised patients with multiple comorbidities such as diabetes mellitus, renal failure and cardiovascular disease [2,4]. *C. auris* is a biofilm-forming, halotolerant, thermo-resistant yeast [1] that can grow at temperatures ranging between 30ºC and 42ºC and can tolerate up to 10% salinity [5]. This renders *C. auris* a nosocomial pathogen capable of causing a wide range of nosocomial infections due to its colonization of medical equipment, plastic surfaces, and nosocomial environment [2,3]. Since *C. auris* is a highly resistant to almost all antifungal agents [6] that is associated with fatal infections [2], its rapid identification and characterization is necessary to optimize clinical outcome and attempt containing its nosocomial and worldwide spread.

C. auris poses many diagnostic challenges. Most commercially available, biochemical, phenotypic and spectrometry, misidentify *C. auris* as other *Candida* species such as *Candida famata, Candida haemulonii, Candida duobushaemulonii, Candida sake, Candida lustaniae, Candida guilliermondii* etc… [7,8]. This often leads to delays in appropriate management and treatment. However, the use of Matrix-Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry (MALDI-TOF-MS) is promising [7-9], since the spectrum of *C. auris* is available in the reference database. In addition, the corresponding identified colonies are carefully selected and isolated among other fungal colonies grown on a given medium by the end-user. In 2017, a modified Sabouraud medium was suggested by Welsh et al. for isolation of *C. auris* [5]. However, some *Candida* isolates were still misidentified

[7,8]. For this purpose, we developed a specific medium that enable to isolate all *C. auris* clades from clinical samples.

2. Materials and Methods

We collected 134 *Candida* strains (27 different species) (Table 1). We cultured them on the modified Sabouraud broth suggested by Welsh *et al*, containing 20g/l Mannitol as a carbon source (in order to inhibit *C. glabrata* growth) in liquid and solid phase. For the liquid phase, we controlled for growth by spectrophotometric measurement at 24, 48 and 72 hours of incubation, for 3 days at 40ºC. For the solid phase we added autoclaved bacterial agar (15 g/l) and adjusted for a pH=7.

We also cultured, all *Candida* strains on the different culture media used in our diagnostic laboratories, for yeast on (Sabouraud Dextrose Agar, CHROMagar *Candida*, Buffered Charcoal Yeast Extract) and for bacterial isolation on (Chocolate Agar, Tryptic Soy Agar, Columbia agar, Mannitol Salt Agar 2, MacConkey agar) (BioMérieux, Marcy-l'Etoile, France) and on LBJMR (Lucie Bardet - Jean-Marc Rolain) medium [10].

Finally, we cultured the 70 *Candida* strain on a combination of the Welsh *et al*. broth and MacConkey agar: MCK (a selective medium for Gram negative bacteria) [11]. We prepared several media with different concentrations of bile salts (1.5 g/l, 1 g/l, 0.75 g/l) and/or crystal violet (1 mg/l, 0.5 mg/l) (used as inhibitors in MCK) [11] to the initial broth composition of Welsh *et al*., in liquid and solid phase.

To study the specificity of our designated medium, we cultured for 3 days at 40ºC a panel of various gram positive and gram negative bacteria, different *C. auris* clades (DSM 21092, and 7 strains which were kindly provided by Dr. Jacques F Meis, Canisius Wilhemina Hospital - Department of Medical Microbiology and Infectious Diseases – Netherlands), other *Candida* strains including the most closely *Candida* species to *C. auris* (*C. haemulonii* and *C. duobushaemulonii*) [2,4], and 200 fecal samples that are negative by real-time PCR for *C. auris* collected at the Marseille Hospital (AP-HM, Assistance Publique-Hôpitaux Marseille), [12] **(Table 1)**. To determine the sensitivity of our *C. auris* specific medium, we cultured a series of ten-fold dilution of 0.5 McFarland of *C. auris* DSM 21092. We also cultured a mixture of the same ten-fold dilutions of 0.5 McFarland of *C. auris* DSM 21092 with a stool sample (10^6/ml to 10^1/ml). We did this as an attempt to determine whether the natural presence of microbes in stools would affect the growth of *C. auris* on our designated medium.

3. Results and discussion

Regarding the medium of Welsh *et al*., only *C. auris* and *C. tropicalis* grew among all tested *Candida* spp. In addition, on MCK agar, there was no growth of all tested *C. tropicalis* strains, but we observed growth of all *C. auris* clades. The growth of *C. tropicalis* was inhibited by adding crystal violet (an inhibitor in MCK) at 0.5 mg/l to the initial broth suggested by Welsh *et al*, with no effect on the growth of the 8 *C. auris* strains in solid and liquid phase. The specificity of this medium was also confirmed after the cultivating of the above-mentioned bacterial spp. *Candida* spp. and fecal samples; no growth was noted after 3 days of incubation at 40ºC. Concerning the sensitivity of our medium, after cultivation of several dilutions on our medium, the limit of detection (LOD) of *C. auris* was 10^2 CFU/ml in both serial dilutions of *C. auris* with physiological water and fecal sample. Thus, the presence of other microbes in the tested fecal sample does not inhibit its growth. All growing colonies have been confirmed by MALDI-TOF-MS and real time PCR [12].

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Table 1. Strains and samples tested on the SCA medium

Type	Strain/Sample	Number	Source	Origin
Gram positive bacteria	*Bacillus cereus*	1	Clinical	Marseille, France
	Corynebacterium amycolatum	1	Clinical	Marseille, France
	Corynebacterium jeikeium	1	Clinical	Marseille, France
	Corynebacterium propinquum	1	Clinical	Marseille, France
	Corynebacterium striatum	1	Clinical	Marseille, France
	Enterococcus faecalis	1	Clinical	Marseille, France
	Enterococcus faecium	1	Clinical	Marseille, France
	Micrococcus luteus	1	Clinical	Marseille, France
	Staphylococcus aureus	1	Clinical	Marseille, France
	Staphylococcus capitis	1	Clinical	Marseille, France
	Staphylococcus cohnii	1	Clinical	Marseille, France
	Staphylococcus epidermidis	1	Clinical	Marseille, France
	Staphylococcus haemolyticus	1	Clinical	Marseille, France
	Staphylococcus lugdunensis	1	Clinical	Marseille, France
	Staphylococcus pasteuri	1	Clinical	Marseille, France
	Staphylococcus saprophyticus	1	Clinical	Marseille, France
	Staphylococcus simulans	1	Clinical	Marseille, France
	Staphylococcus warneri	1	Clinical	Marseille, France
	Streptococcus agalactiae	1	Clinical	Marseille, France
	Streptococcus dysgalactiae	1	Clinical	Marseille, France
	Streptococcus equinus	1	Clinical	Marseille, France
	Streptococcus mitis	1	Clinical	Marseille, France
	Streptococcus pneumoniae	1	Clinical	Marseille, France
	Staphylococcus hominis	1	Clinical	Marseille, France
	Streptococcus salivarius	1	Clinical	Marseille, France
Subtotal		**25**		
Gram negative bacteria	*Achromobacter xylosoxidans*	1	Clinical	Marseille, France
	Acinetobacter baumannii	1	Clinical	Marseille, France
	Bacteroides fragilis	1	Clinical	Marseille, France
	Citrobacter braakii	1	Clinical	Marseille, France
	Citrobacter freundii	1	Clinical	Marseille, France
	Citrobacter koseri	1	Clinical	Marseille, France
	Enterobacter aerogenes	1	Clinical	Marseille, France
	Enterobacter asburiae	1	Clinical	Marseille, France
	Enterobacter cloacae	1	Clinical	Marseille, France
	Enterobacter kobeii	1	Clinical	Marseille, France
	Escherichia coli	1	Clinical	Marseille, France
	Haemophilus influenzae	1	Clinical	Marseille, France
	Haemophilus parainfluenzae	1	Clinical	Marseille, France
	Hafnia alvei	1	Clinical	Marseille, France

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	Klebsiella oxytoca	1	Clinical	Marseille, France
	Klebsiella pneumoniae	1	Clinical	Marseille, France
	Moraxella catarrhalis	1	Clinical	Marseille, France
	Morganella morganii	1	Clinical	Marseille, France
	Pasteurella multocida	1	Clinical	Marseille, France
	Proteus mirabilis	1	Clinical	Marseille, France
	Proteus vulgaris	1	Clinical	Marseille, France
	Providencia stuartii	1	Clinical	Marseille, France
	Pseudomonas aeruginosa	1	Clinical	Marseille, France
	Raoultella ornithinolytica	1	Clinical	Marseille, France
	Stenotrophomonas maltophilis	1	Clinical	Marseille, France
Subtotal		**25**		
	Candida albicans	73	Clinical	Marseille, France
	Candida glabrata	8	Clinical	Marseille, France
	Candida krusei	4	Clinical	Marseille, France
	Candida parapsilosis	6	Clinical	Marseille, France
	Candida lusitaniae	3	Clinical	Marseille, France
	Candida tropicalis	6	Clinical	Marseille, France
	Candida zelanoides	1	Clinical	Marseille, France
	Candida lipolytica	1	Clinical	Marseille, France
	Candida inconspicua	1	Clinical	Marseille, France
	Candida intermedia	1	Clinical	Marseille, France
	Candida guilliermondii	3	Clinical	Marseille, France
	Candida bracarensis	1	Clinical	Marseille, France
	Candida utilis	1	Clinical	Marseille, France
Yeast	*Candida bovina*	1	Clinical	Marseille, France
	Candida dubliniensis	2	Clinical	Marseille, France
	Candida norvegensis	1	Clinical	Marseille, France
	Candida kefyr	2	Clinical	Marseille, France
	Candida beverwijkiae	1	Clinical	Marseille, France
	Cryptococcus diffluens	1	Clinical	Marseille, France
	Cryptococcus uniguttulatus	1	Clinical	Marseille, France
	Cryptococcus neoformans	2	Clinical	Marseille, France
	Saccharomyces cerevisiae	2	Clinical	Marseille, France
	Rhodotorula mucilaginosa	1	Clinical	Marseille, France
	Yarrowia lipolitica	1	Clinical	Marseille, France
	Candida haemulonii	1	Clinical	Netherlands
	Candida duobushaemulonii	1	Clinical	Netherlands
	Candida auris	8	Clinical	DSMZ/ Netherlands
Subtotal		**134**		
Human samples	Stool samples	200	Clinical	Marseille, France
Total		**384**		

The final composition of our medium in 1l of deionized water was: 5 g of pancreatic digest of casein, 5 g of peptic digest of animal tissue, 100 g of NaCl, 20 g of mannitol, 0.5 mg of crystal violet, 50 mg/l of chloramphenicol and 50 mg/l of gentamicin with pH=7 (± 0.2) at 40ºC **(Table 2).**

Table 2: Final composition of SCA (Specific *Candida auris*) medium

	Pancreatic digest of casein	Peptic digest of animal tissue	NaCl	Mannitol	Crystal violet	Agar	pH	Chloramphenicol	Gentamicin
Welsh et al broth	5g	5g	100g	20g	-	-	5.6	50 mg/l	50 mg/l
SCA medium	5g	5g	100g	20g	0.5 mg	15g	7	50 mg/l	50 mg/l

As previously described, *C. auris, C. krusei* and *C. parapsilosis* produce pink colonies on the CHROMagar medium [2,4]. Moreover, the enrichment broth (Salt SAB Broth with mannitol as a carbon source) for *C. auris* isolation, developed by Welsh *et al.*, was not evaluated against *C. tropicalis* strains [5]. In our work, we show that this enriched broth supports the growth of *C. tropicalis* as well as that of *C. auris*. This finding emphasizes the need to develop a specific medium for *C. auris* to yield more accurate identification.

Among tested media, MCK agar, and more specifically the crystal violet dye, prevented the growth of *C. tropicalis* strains without disrupting *C. auris* strains' growth. SCA (Specific *C. auris*) medium is a modified version of the above-mentioned broth whereby crystal violet powder is added to inhibit the growth of *C. tropicalis*. According to our results, crystal violet supplementation, along with high salinity (10%) and elevated temperature (40°C) incubation, increased the specificity of SCA medium. Thus, we have successfully developed a specific diagnostic tool: the SCA medium to isolate all *C. auris* clades from complex microbial communities.

Moreover, spiking a stool sample with a *C. auris* strain did not affect neither the specificity nor the sensitivity of this medium. However, further evaluations of both specificity and sensitivity, by testing more clinical *C. auris* isolates and other closely related yeasts (such as *C. famata* and *C. sake*), are required.

4. Conclusion

The use of our developed medium enables the rapid, specific isolation of *C. auris* strains and helps in timely management of patients and resources to limit the occurrence of *C. auris* outbreaks. We propose an implementation of SCA medium in routine clinical mycology for screening skin, urine, vaginal and blood samples, especially in high-risk populations [13,15]. This SCA medium will further enhance our understanding of the phenotypic characteristics of *C. auris* and future isolates, most importantly allowing an accurate study of their antifungal resistance profiles.

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