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ORIGINAL ARTICLE

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Evaluation of CHROMagar™ Candida Plus chromogenic agar for the presumptive identification of *Candida auris*

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Abstract

Skin colonization by the emerging pathogen *Candida auris* is common in outbreaks within medical settings. Culture-based screening of patients is an effective management strategy to control the pathogen, and the newly developed CHROMagar™ Candida Plus medium is claimed to enable the presumptive identification of *C. auris*. Here, we evaluated the use of this medium with 63 *C. auris* strains comprising its four well-established clades, as well as genetically related comparators, including species from the *Metschnikowia* clade. The colors and halos of both confluent growth and discrete colonies of all the tested strains were compared. It was found that on CHROMagar™ Candida Plus, *C. auris* formed characteristic white colonies with blue-green halos that were more evident after 72 hr of incubation at 35°C than after 48 hr. However, distinguishing between closely related species such as *Candida haemulonii*, *Candida pseudohaemulonii*, and *Candida duobushaemulonii* required the consideration of parameters other than color, including colony size and growth ability at 35°C. In conclusion, the novel chromogenic medium CHROMagar™ Candida Plus constitutes an easy screening tool for *C. auris*.

KEYWORDS

Candida auris, CHROMagar™ Candida Plus, screening

INTRODUCTION

Candida auris is a recently emerged fungus that was first isolated in Japan in 2005 from human otorrhea and reported in 2009.[1] This yeast may cause invasive infections associated with high mortality[2–5] and is often resistant to multiple antifungal drugs.[6–11] *C. auris* falls into at least four phylogenomic clades.[12] Strains from Clades I, III, and IV are more pathogenic, while populations of Clade II (East Asia) have, thus far, not been linked to any outbreaks. Skin colonization by the yeast has been a prominent feature in *C. auris* outbreaks, and the thorough screening of patients is crucial to prevent further transmission of the pathogen and to ensure effective management.[10,12–15]

Until recently, the only available rapid culture-based screening tool for *Candida* species was the chromogenic agar medium CHROMagar™ Candida.[16] However, the chromogenic appearance of *C. auris* greatly resembles that of *Candida parapsilosis* on this medium, making it difficult to distinguish between the two species. This has prompted the development of a new chromogenic agar medium for *Candida* species distinction – namely, CHROMagar™ Candida Plus.[17,18] In a previous study, it was concluded that CHROMagar™ Candida Plus was useful for the rapid isolation and identification of *C. auris*; however, only confluent growth was analyzed.[17] In the current study, the new medium was further evaluated using our stock of *C. auris* strains representing all four clades, as well as other main *Candida* species. In addition, both the confluent growth and discrete colonies were compared.

MATERIALS AND METHODS

A total of 63 *C. auris* strains were tested, including 16 from Clade I, 26 from Clade II, 10 from Clade III, and 11 from Clade IV (Table 1). In addition, 28 strains of non-*auris* species, including *Candida albicans* ($n = 3$), *C. parapsilosis*

Abbreviations: CG, confluent growth; DC, discrete colonies; H, halo; R, reverse; S, surface; SDA, Sabouraud dextrose agar.

TABLE 1 *Candida* strains tested in the study

Candida species	Clade	Strains
C. auris	Clade I	LSEM3214[a]—LSEM3225[a], LSEM3684[a], LSEM3685[a]
		NCPF8971, NCPF8985
	Clade II	JCM15448
		CBS12372, CBS12373, NCPF8984
		LSEM3540[b], LSEM3541[b], LSEM4151[b], LSEM4152[b], LSEM3649[b]—LSEM3660[b], LSEM3769[b], LSEM3770[b], LSEM3980[b], LSEM3981[b]
		LSEM3682[a], LSEM3683[a]
	Clade III	LSEM3662[a]—LSEM3671[a]
	Clade IV	NCPF8977
		LSEM3672[a]—LSEM3681[a]
C. albicans	N/A	ATCC90028, ATCC10231, TIMM1768
C. parapsilosis	N/A	ATCC22019, ATCC90018, NBRC1396
C. tropicalis	N/A	ATCC750, TIMM0313, NBRC0006
C. glabrata	N/A	ATCC90030, CBS138
C. krusei	N/A	ATCC6258, ATCC2258, TIMM3378
C. haemulonii	N/A	JCM3762
C. pseudohaemulonii	N/A	JCM12453
C. duobushaemulonii	N/A	CBS6915, CBS7098, CBS7798, CBS7799, CBS7800, CBS9754
C. lusitaniae	N/A	NBRC10059, NBRC1019
C. kefyr	N/A	NBRC1777, NBRC0432
C. guilliermondii	N/A	ATCC9058, LSEM1953

N/A, not applicable.

[a]Strains are a generous gift from the US Centers for Disease Control and Prevention.

[b]Strains are Japanese clinical isolates, and their clades were determined as described previously.[19]

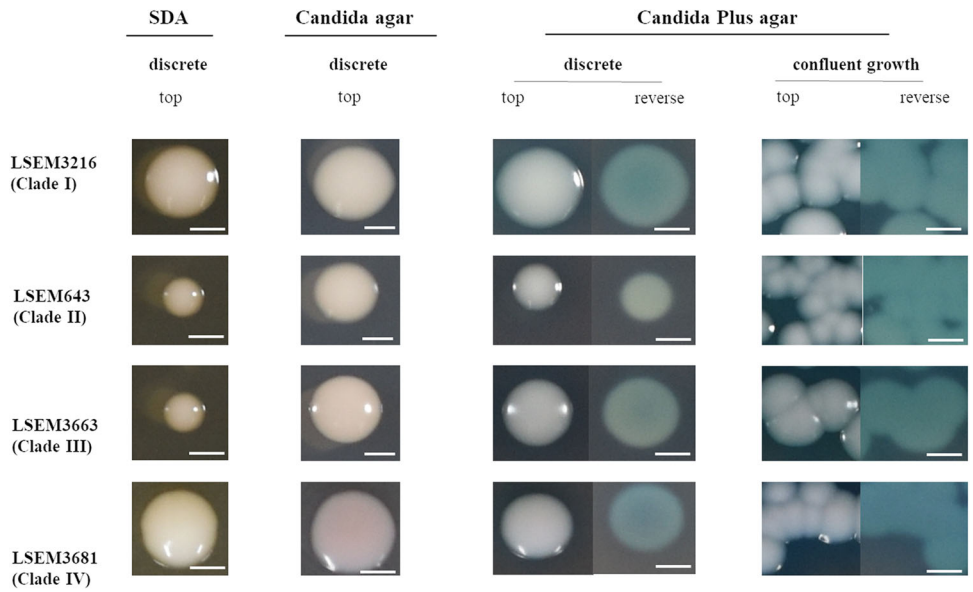

FIGURE 1 Comparison of *Candida auris* appearance on tested media. Strains representing four clades of *C. auris* were streaked out onto Sabouraud dextrose agar (SDA), CHROMagar™ Candida (Candida agar), and CHROMagar™ Candida Plus (Candida Plus agar). All cultures were observed after incubation at 35°C for 48 hr. Scale bar = 1 mm

(*n* = 3), *Candida tropicalis* (*n* = 3), *Pichia kudriavzevii* (syn. *Candida krusei*; *n* = 3), *Candida glabrata* (*n* = 3), *Candida haemulonii* (*n* = 1), *Candida pseudohaemulonii* (*n* = 1), *Candida duobushaemulonii* (*n* = 6), *Clavispora* (syn. *Candida) lusitaniae* (*n* = 2), *Kluyveromyces marxianus* (syn. *Candida kefyr*; *n* = 2), and *Meyerozyma* (syn. *Candida*) *guilliermondii* (*n* = 2). The yeast was routinely maintained on Sabouraud dextrose agar (SDA) at 35°C. The correct identification of *C. auris* strains was confirmed by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (BioTyper, Bruker Daltonics).

For chromogenic feature analyses, 2-day-old cultures on SDA at 35°C were used. The tested species were inoculated onto the CHROMagar™ Candida Plus media (Kanto Chemical Co.), as well as onto CHROMagar™ Candida (Kanto Chemical Co.) and SDA for comparison. Inoculations were performed using the streak dilution method to allow the formation of both confluent growth and discrete colonies. The results were read after 48 and 72 hr of incubation at 35°C.

RESULTS AND DISCUSSION

CHROMagar™ Candida is a widely used selective medium for the presumptive identification of some *Candida* species, including *C. albicans*, *C. tropicalis*, and *C. krusei*. Although it supports the isolation of the emerging yeast *C. auris*, it does not produce distinctive coloring for this species. Indeed, it was observed that isolates representing the four *C. auris* clades formed colonies with ivory to pale pink colors, largely resembling those of *C. parapsilosis* and *C. pseudohaemulonii* (Figures 1 and 2). Similar results were obtained on the nonselective SDA medium (Figure 1). CHROMagar™ Candida Plus medium was developed as a fast tool for the specific presumptive identification of *C. auris* isolates.

The appearance of the confluent growth and discrete colonies of *C. auris* on CHROMagar™ Candida Plus is summarized in Table 2. The confluent growth for 57 of the 63 *C. auris* strains tested was white after incubation for 48 hr, whereas the six remaining strains were colored in indigo, cream, or dark cream (Table 2). Moreover, all these confluent growths had blue-green halos in the agar immediately surrounding them (Table 2). On the other hand, the discrete colonies were white in 60 strains, with the three remaining strains displaying cream to dark-cream colors (Table 2). While the halos of these discrete colonies were blue green in 36 strains and cream color in one strain, no halos appeared in 26 strains. Surprisingly, in all *C. auris* clades, discrete colonies had blue-green halos in about 90% of the strains after 48 hr of incubation, except for Clade II, where only about 11% of the strains displayed such halos (Table 2), indicating weaker enzymatic activity to produce blue green in this clade. However, when incubated for 72 hr, discrete colonies for all but four strains of Clade II produced blue-green halos, indicating that the color transition over a 72 hr incubation time could presumptively differentiate it from the other clades. Moreover, *C. auris* strains incubated for up to 72 hr formed confluent growth and discrete colonies with white to pale-pink coloring, the majority of which were surrounded by blue-green halos (Table 2). It is noteworthy that no differences were observed among susceptible and resistant strains (data not shown). Hence, on CHROMagar™ Candida Plus medium, *C. auris* generally forms white colonies with blue-green halos that are more evident after 72 hr versus 48 hr of incubation, particularly with strains from Clade II.

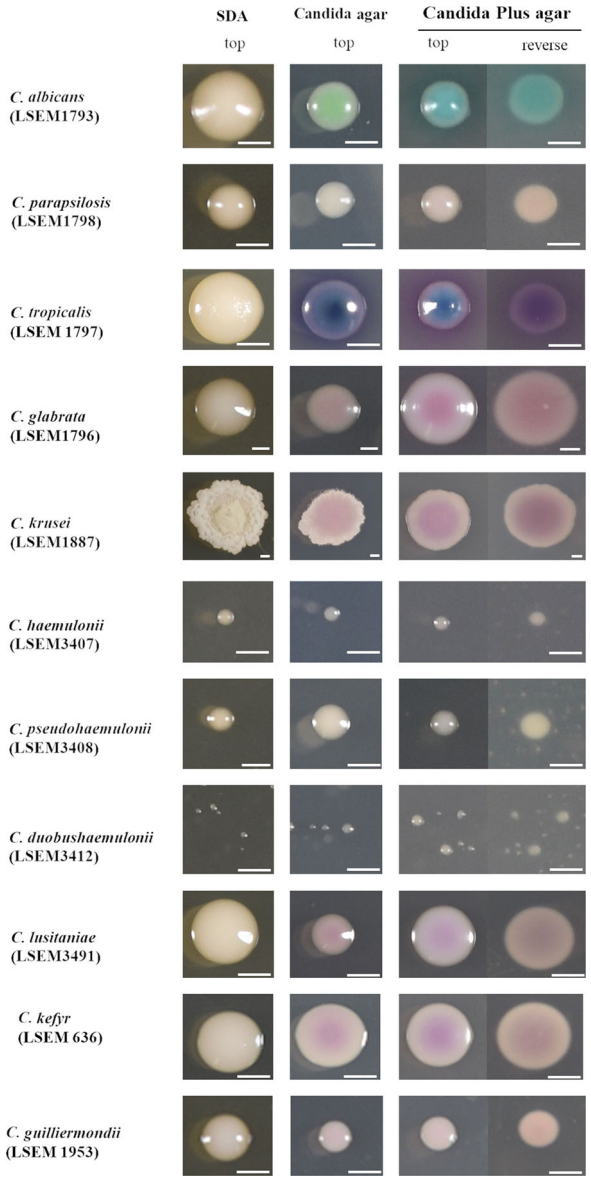

FIGURE 2 The appearance of non-*auris* yeast species on tested media. Yeast species were streaked out onto Sabouraud dextrose agar (SDA), CHROMagar™ Candida (Candida agar), and CHROMagar™ Candida Plus (Candida Plus agar). All cultures were observed after incubation at 35°C for 48 hr. Scale bar = 1 mm

Previous studies using CHROMagar™ Candida Plus have shown slightly different results. Borman et al.[17] found that

TABLE 2 The appearance of *C. auris* on CHROMagar™ Candida Plus

				Incubation time			
				48 hr		72 hr	
Clade	Strain no.	Colony		Color	Strain no.	Color	Strain no.
I	16	CG	C	White	16	White	12
						Pale pink	4
			H	Blue green	16	Blue green	16
		DC	C	White	16	White	15
						Pale pink	1
			H	Blue green	14	Blue green	16
				——	2		
II	26	CG	C	White	20	White	17
				Cream	2	Pale pink	8
				Dark cream	3	Indigo	1
				Indigo	1		
			H	Blue green	26	Blue green	26
		DC	C	White	23	White	26
				Cream	2		
				Dark cream	1		
			H	Blue green	3	Blue green	22
				Cream	1	——	4
				——	22		
III	10	CG	C	White	10	White	2
						Pale pink	8
			H	Blue green	10	Blue green	10
		DC	C	White	10	White	3
						Pale pink	7
			H	Blue green	9	Blue green	10
				——	1		
IV	11	CG	C	White	11	Pale pink	11
			H	Blue green	11	Blue green	11
		DC	C	White	11	White	4
						Pale pink	7
			H	Blue green	10	Blue green	11
				——	1		

C, colony; CG, confluent growth; DC, discrete colonies; H, halo.

C. auris formed pale-cream colonies with blue halos, regardless of the clonal linage of the tested strains. However, the results were read after 36 hr, and the study used only a limited number of *C. auris* strains, particularly in Clade II (only JCM15448 and CBS12373). The performances of these two strains in the current study were similar only in confluent growth observations; both of them formed blue-green halos even after 48 hr. A later study showed that *C. auris* strains produced light-blue colonies with blue halos when they were incubated at 37°C for 48 hr.[18] The elevated temperature may explain the light-blue coloring of *C. auris*, consistent with a study showing that the hues of some

TABLE 3 The appearance of non-*auris* on CHROMagar™ Candida Plus

			Incubation time			
			48 hr		72 hr	
Species	Growth		Color	Strain no.	Color	Strain no.
C. albicans	CG	S	Blue green	3	Blue green	3
		R	Blue green	3	Blue green	3
	DC	S	Blue green	3	Blue green	3
		R	Blue green	3	Blue green	3
C. parapsilosis	CG	S	White	3	White	2
					Violet	1
		R	Mauve	1	Violet	2
			Blue green	2	Blue green	1
	DC	S	White	2	Dark cream	3
			White	1		
		R	White	1	Mauve	3
			White	2	White	3
C. tropicalis[b]	CG	S	Metallic blue	3	Metallic blue	3
		R	Metallic blue	3	Metallic blue	3
	DC	S	Metallic blue	3	Metallic blue	3
		R	Violet	3	Violet	3
		H	Violet	3	Violet	3
C. glabrata	CG	S	Mauve	2	Mauve	2
		R	Mauve	2	Mauve	2
	DC	S	Mauve	2	Mauve	2
		R	Mauve	2	Mauve	2
C. krusei	CG	S	Mauve	3	Pink	3
		R	Mauve	3	Mauve	3
	DC	S	Pink	3	Pink	3
		R	Mauve	3	Mauve	3
C. haemulonii	CG	S	White	1	Mauve	1
		R	Mauve	1	Mauve	1
	DC	S	White	1	Mauve	1
		R	Mauve	1	Mauve	1
C. pseudohaemulonii[b]	CG	S	White	1	White	1
		R	Blue green	1	Blue green	1
	DC	S	White	1	White	1
		R	White	1	Blue green	1
		H			Blue green	1
C. duobushaemulonii	CG	S	White	5	White	4
			Violet	1	Mauve	2

TABLE 3 (Continued)

Species	Growth		Incubation time 48 hr Color	48 hr Strain no.	72 hr Color	72 hr Strain no.
		R	Blue green	3	Violet	6
			Violet	3		
	DC	S[a]	White	1	White	6
		R[a]	White	1	White	6
C. lusitaniae	CG	S	Indigo	1	Metallic blue	1
			White	1	White	1
		R	Indigo	1	Metallic blue	1
			Violet	1	Violet	1
	DC	S	Violet	2	Indigo	1
					Pale pink	1
		R	Violet	2	Violet	2
C. kefyr	CG	S	Mauve	1	Violet	1
			Pink	1	Purple	1
		R	Mauve	1	Violet	2
			Pink	1		
	DC	S	Mauve	1	Purple	1
			Pink	1	Violet	1
		R	Mauve	1	Violet	2
			Pink	1		
C. guilliermondii	CG	S	Violet	2	Blue	2
		R	Violet	2	Blue	2
	DC	S	Mauve	1	Violet	1
			White	1	White	1
		R	Mauve	1	Violet	1
			White	1	Blue	1

CG, confluent growth; DC, discrete colonies; H, halo; R, reverse; S, surface.

[a]Only 1 strain formed colonies up to 48 hr.

[b]Species with halos.

Candida species were deeper at 37°C than at lower temperatures when tested on CHROMagar™ Candida.[20]

In our study, confluent growth and discrete colonies displayed similar colorations, except in Clade II strains, for which confluent growth had blue-green halos in all tested 26 strains from the clade (Table 2) at 48 hr, while discrete colonies in the majority of the strains did not show halos until 72 hr. Accordingly, prolonged incubation is recommended for a more accurate presumptive identification of *C. auris*.

The colony appearance of non-*auris* yeast on CHROMagar™ Candida Plus is summarized in Table 3 and Figure 2. Clearly, on this medium, *C. auris* can be distinguished from *C. albicans*, which forms blue-green colonies, *C. lusitaniae* with its indigo/violet colonies, *C. tropicalis* with its metallic blue colonies and violet halos, *C. parapsilosis* with their white or mauve colors without halo, *C. glabrata* with its mauve color, *C. krusei* or *C. kefyr* with their mauve/pink color, or *C. guilliermondii* with its violet/mauve colorations.

In addition, our study included comparators from the *Metschnikowia* clade, which includes, besides *C. auris*, species such as *C. haemulonii*, *C. pseudohaemulonii*, and *C. duobushaemulonii*. *C. haemulonii* could be differentiated from *C. auris* by its mauve surface/reverse color, as well as the colony sizes. The colony size of *C. auris* on

CHROMagar™ Candida Plus is about 1.2 to 2.7 mm, while *C. haemulonii* generates colonies of 0.2 to 0.3 mm, and *C. pseudohaemulonii* forms white colonies smaller than 0.2 mm, making the distinction from *C. auris* even easier, though the yeast can produce blue-green halos at 72 hr incubation. Likewise, *C. duobushaemulonii* produces colonies with white/violet color and shows a poor growth ability at 35°C as colonies were confirmed in only one of six strains after 48 hr of incubation. Moreover, the growth of *C. auris*, *C. haemulonii*, *C. pseudohaemulonii*, and *C. duobushaemulonii* at 42°C using CHROMagar™ Candida Plus was tested. Only *C. auris* produced colonies after 72 hr (data not shown), in agreement with a previous study in which *C. haemulonii*, *C. haemulonii* var. *vulnera*, and *C. duobushaemulonii* could not grow at 42°C using CHROMagar™ Candida.[21] Thus, it is possible to distinguish *C. auris* from other *Candida* species using CHROMagar™ Candida Plus, particularly when incubated at 42°C.

In conclusion, CHROMagar™ Candida Plus can be used for the rapid presumptive identification of *C. auris* isolates, which generally form white colonies with blue-green halos that are more evident after 72 hr of incubation.

CONFLICT OF INTEREST

"CHROMagar™ Candida Plus" and "CHROMagar™ Candida" have received research grants from Kanto Chemical Co., Inc.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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