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Evaluation of the CHROMagar Candida Plus medium for presumptive identification of yeasts and MALDI-TOF MS identification

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Abstract

Rapid and accurate yeasts species identification in clinical laboratories is important for appropriate and timely antifungal treatment. We evaluate the performance of the new medium CHROMagar™ Candida Plus for presumptive identification of yeasts species and MALDI-TOF identification. We identify 303 strains belonging to 60 clinically relevant yeasts species by using the new medium. Presumptive identification was correct at the *Candida albicans* complex, *Candida tropicalis* and *Pichia kudriavzevii* (*Candida krusei*) species. However, although this medium was able to identify all *Candida auris* and *Candida glabrata* strains, other species were misidentified as *C. auris* or *C. glabrata*. A total of 215 strains were identified by using MALDI-TOF and evaluated two incubation temperatures (30°C and 37°C) and two incubation times (24 h and 72 h). Most strains (94%; 202/215) were correctly identified at the species (n:190) or complex level (n:12) at both temperatures and incubation times. However, we observed that the time of incubation (24 h vs. 72 h) affects the score values when yeasts are incubated at 37°C, but does not affect score values when yeasts are incubated at 30°C. In conclusion, the new medium has a good performance in the presumptive identification of the *C. albicans* complex, *C. tropicalis* and *P. kudriavzevii* (*C. krusei*). In addition, this medium is useful for the screening of *C. auris* and *C. glabrata* isolates, but identification should be confirmed by other more specific techniques, like MALDI-TOF.

KEYWORDS
Candida, Candida auris, CHROMagar Candida, CHROMagar Candida Plus, MALDI-TOF

1 | INTRODUCTION

Identification of yeasts species has become crucial for yeasts infections treatment. Rapid and accurate species identification is important for appropriate and timely antifungal treatment.[8,14,15] CHROMagar Candida is a chromogenic selective medium widely used in the presumptive identification of yeasts species commonly isolated in clinical samples.[5,13,16] Recently, a new version of this medium has been released, the CHROMagar™ Candida Plus (CCP).[2] This is the first chromogenic isolation medium to detect and differentiate *Candida auris*, an emerging multidrug-resistant species, in addition to other major clinical *Candida* species such as *Candida albicans*, *Candida tropicalis*, *Candida glabrata* and *Pichia kudriavzevii* (*Candida krusei*). Also, chromogenic media are capable of detecting

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easily mixed infections unlike non-selective yeasts growth media such as Sabouraud Dextrose Agar (SDA). Therefore, many clinical laboratories use chromogenic media to culture their clinical samples. However, Sasoni et al have recently reported some misidentifications of *Candida parapsilosis* complex strains as *C. auris* by using the new CCP medium,[19] so more extensive performance analyses are necessary.

On the other hand, the MALDI-TOF MS technique has revolutionised the whole microbial identification, including yeast species identification, as it is easier, faster and more accurate than conventional techniques.[4,18,23] However, although a cost-effective technique, it is not yet economically viable for all clinical laboratories as they may not have a large number of samples. Even so, these laboratories can refer samples for MALDI-TOF MS identification to another laboratory when necessary.

The objective of this work was to evaluate the performance of the new CCP medium by challenging against a large number of regional strains belonging to several yeasts species, including various strains belonging to the *Candida haemulonii* complex and several strains belonging to the *C. parapsilosis* complex. Here, we also evaluate the use of this medium in MALDI-TOF MS identification.

2 | MATERIALS AND METHODS

2.1 | Strains

A total of 303 strains belonging to 60 species were evaluated in the study, including 12 ATCC reference strains, 45 strains from a yeasts MALDI-TOF panel, and a *C. auris* panel (both gently provided by the Centers for Disease Control and Prevention from the United States), and 246 regional strains. These strains were previously identified by DNA-based methods; including ribosomal DNA sequencing, *ACT1* sequencing and the restriction fragment length polymorphism of the orotidine monophosphate pyrophosphorylase gene (*URA5*-RFLP) for *Cryptococcus* genotyping.[20–22] All strains are deposited at the culture collection of the Mycology Department of the National Institute of Infectious Diseases 'Dr. Carlos G. Malbrán' (INEI).

2.2 | CHROMagar™ Candida Plus presumptive identification

CHROMagar™ Candida Plus was prepared according to manufacturer instructions and the reference strains *C. albicans* ATCC10231, *C. tropicalis* ATCC1369, *C. glabrata* ATCC2001, *P. kudriavzevii* (*C. krusei*) ATCC6258 and *C. auris* CDC80 were used as quality control. All strains were cultured on Sabouraud (SDA) (Oxoid) during 24–48 h at 28°C. From this culture, a loop of fresh colonies was deposited onto CCP plates, and isolated by the exhaustion method. A total of 303 strains were incubated at both 30°C and 37°C for 72 h, including 103 strains belonging to the *C. parapsilosis* complex. Presumptive identification was performed according to manufacturer instructions at 48 h of incubation. All plates were interpreted by at least two different technicians with previous training in CHROMagar Candida identification.

2.3 | MALDI-TOF MS identification

The CCP plates were also used to perform MALDI-TOF MS identification. All 215 strains were identified at both temperatures, 30°C and 37°C, and at both incubation times, 24 h and 72 h, to determine if these conditions can affect the performance of MALDI-TOF MS identification. Protein extraction was performed by using the direct extraction method. Briefly, a single colony was taken with a wood stick and smeared by duplicate onto a steel target. Then 1 µL of 100% formic acid was added and let dry. Finally, 1 µL of HCCA was added and let dry. All samples were identified by using the Bruker Daltonics database (BDAL) MBT-8468 and a previously validated *in-house* database named 'LevDMic' version 2.[23,24] We consider as 'correct identification at species level' those results with a score value ≥1.700 and the presence of only one species at the TOP 10 of best scores with a score value ≥1.700. Also, we consider as 'correct identification at complex level' those results with a score value ≥1.700 and the presence of closely related species (belonging to the species complex) at the TOP 10 of best scores with a score value ≥1.700.

2.4 | Statistical analysis of MALDI-TOF MS best score values

The bests score values obtained with the different temperatures and incubation times of the 215 strains cultured in CCP were analysed. Also, the best score values of all isolates cultured in SDA were included. The groups were compared using the Kruskal–Wallis test and then pairwise comparisons by the Mann–Whitney test. Statistically significant differences (p-values <.05) were determined by using the program Epi Info™ version 7.2 (Atlanta, GA, USA).

3 | RESULTS

3.1 | CHROMagar™ Candida Plus presumptive identification

Table 1 summarises identification results and compares presumptive identification at 30°C and 37°C using the CCP medium with the reference identification. Both temperatures were analysed to determine if the temperature would affect the performance. All 58 strains belonging to the species *C. albicans*, *C. tropicalis*, *P. kudriavzevii* (*C. krusei*), *C. auris* and *C. glabrata* were correctly identified by using the CCP medium. To note, *C. auris* colonies, including those from reference strains, were cream to light pink with shades of light blue and a blue halo at inverse, and blue colour at reverse. Also, *C. glabrata* colonies showed colonies from mauve to lilac colour. Therefore, identification was made by comparing with reference strains.

TABLE 1 Presumptive identification results using CHROMagar Candida Plus at both temperatures versus reference identification.

Reference identification[a]	Presumptive identification at 37°C		Presumptive identification at 30°C	
	N/N total strains	Result	N/N total strains	Result
Candida albicans	10/10	*Candida albicans*	10/10	*Candida albicans*
Candida dubliniensis	5/5		5/5	
Candida tropicalis	10/10	*Candida tropicalis*	10/10	*Candida tropicalis*
Pichia kudriavzevii (*Candida krusei*)	10/10	*Pichia kudriavzevii* (*Candida krusei*)	10/10	*Pichia kudriavzevii* (*Candida krusei*)
Candida auris	23/23	*Candida auris*	23/23	*Candida auris*
Candida haemulonii	0/9		1/9	
Candida parapsilosis	6/50		3/50	
Candida orthopsilosis	20/30		20/30	
Candida metapsilosis	13/23		11/23	
Candida vulturna	0/1		1/1	
Starmerella sorbosivorans (*Candida sorbosivorans*)	0/1		1/1	
Magnusiomyces clavatus (*saprochaete clavata*)	1/1		1/1	
Wickerhamiella pararugosa (*Candida pararugosa*)	0/4		1/4	
Candida glabrata	5/5	*Candida glabrata*	5/5	*Candida glabrata*
Candida intermedia	4/4		4/4	
Clavispora lusitaniae	2/6		2/6	
Cyberlindnera fabianii	2/3		2/3	
Cyberlindnera jadinii (*Candida utilis*)	1/2		1/2	
Saccharomyces cerevisiae	4/5		4/5	
Wickerhamomyces anomalus (*Candida pelliculosa*)	2/5		2/5	
44 yeast species	185	No identification	186	No identification

[a]Reference identification by DNA-based methods; including ribosomal DNA sequencing, *ACT1* sequencing and the *URA5*-RFLP for *Cryptococcus*.

A total of 59 strains belonging to 15 species were misidentified as *C. albicans*, *C. auris* and *C. glabrata* using the CCP medium at 30°C. On the other hand, a total of 60 strains belonging to 11 species were misidentified as *C. albicans*, *C. auris* and *C. glabrata* using the CCP medium at 37°C. Table 1 shows the species and the number of misidentified strains. No misidentification was observed for the *C. tropicalis* and *P. kudriavzevii* species at both temperatures.

All strains misidentified as *C. albicans* belonging to the closely related species *Candida dubliniensis*, indicating that the CCP medium correctly identifies at the *C. albicans* species complex level.

The CCP medium was able to distinguish *C. auris* from most strains belonging to the closely related *C. haemulonii* complex, showing better performance at 37°C (100%, 30/30) than at 30°C (93%, 28/30) (Table S1). Only two strains belonging to the closely related species *C. haemulonii* and *Candida vulturna* were misidentified at 30°C. On the other hand, a high number of *C. parapsilosis* complex strains were misidentified as *C. auris* at both temperatures, being higher for *C. orthopsilosis* (67% at both temperatures) and *C. metapsilosis* (57% at 37°C and 48% at 30°C) than for *C. parapsilosis* (12% at 37°C and 6% at 30°C). In addition, other strains belonging to the species *Starmerella sorbosivorans*, *Magnusiomyces clavatus* and *Wickerhamiella pararugosa* were misidentified as *C. auris*. Figure 1A shows the colonies of *C. auris* and *C. orthopsilosis*.

Additionally, the presumptive identification of the 103 *C. parapsilosis* complex strains was recorded after 72 h of incubation at both temperature. In this case, we observe an increase in the percentage of misidentification as *C. auris*, being 44% at 37°C and 34% at 30°C for *C. parapsilosis*, 90% at both temperatures for *C. orthopsilosis*, and finally 100% at 37°C and 96% at 30°C for *C. metapsilosis*. To note, some *C. parapsilosis* complex strains developed rough colonies, so they were considered as 'no identification' even if colony colour was light blue with blue halo.

Fifteen strains were misidentified as *C. glabrata* at both temperatures. These strains belong to six species: *Candida intermedia*, *Clavispora lusitaniae* (*C. lusitaniae*), *Cyberlindnera fabianii*, *Cyberlindnera jadinii*, *Saccharomyces cerevisiae* and *Wickerhamomyces anomalus*. Figure 1B shows the colonies of *C. glabrata* and *S. cerevisiae*. On the other hand, strains belonging to the closely related species *Candida bracarensis* and *Candida nivariensis* developed cream-coloured colonies. Therefore,

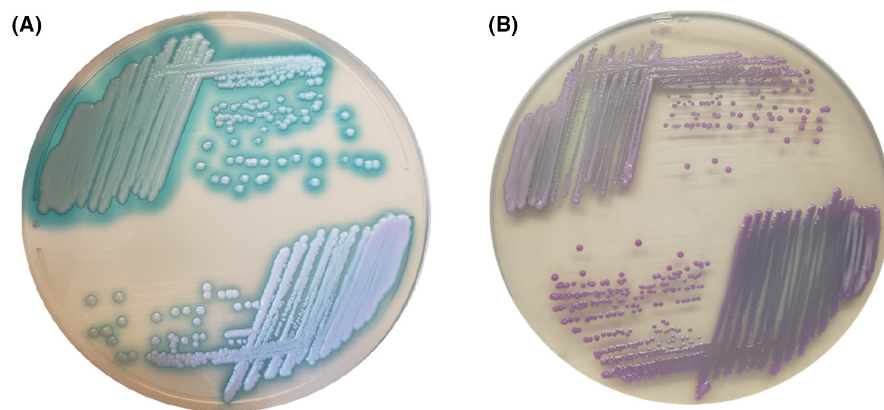


FIGURE 1 (A) *Candida auris* (top) and *Candida orthopsilosis* (bottom) colonies on CHROMagar Candida Plus. (B) *Candida glabrata* (top) and *Saccharomyces cerevisiae* (bottom) colonies on CHROMagar Candida Plus.

the CCP medium was able to distinguish *C. glabrata* sensu stricto from the cryptic species *C. bracarensis* and *C. nivariensis*.

3.2 | MALDI-TOF MS identification

Most strains (94%; 202 out of 215) were correctly identified at the species (n:190) or complex level (n:12) at both temperatures and both incubation times (Table S1). The number of identified strains at the different growth conditions was as follows: 205 for 37°C-24 h, 204 for 37°C-72 h, 208 for 30°C-24 h and 209 for 30°C-72 h.

Four strains were not able to grow at 37°C but they were able to grow at 30°C being correctly identified at the species level (3 *Debaryomyces hansenii* and 1 *Debaryomyces tyrocola*). One strain of *C. auris* was unable to obtain a protein spectrum at 37°C, but it was correctly identified at 30°C. Six strains were not identified at both temperatures and both incubation times. These strains belong to four species not represented in the databases (*Candida melibiosica*, *Cyberlindnera rhodanensis*, *Trichomonascus ciferrii* and *Wickerhamomyces onychis*). We were unable to obtain a protein spectrum of one strain belonging to the species *Candida palmioleophila* at 30°C after 24 h of incubation and at 37°C after 72 h of incubation. Finally, one strain of *C. vulturna* was misidentified as *Candida pseudohaemulonii* at both temperatures and incubation times. This species was not represented in databases.

Of the twelve strains identified at the complex level, four were identified at *C. haemulonii* complex (two *C. duobushaemulonii* and two *C. pseudohaemulonii*), four were identified at *Meyerozyma guilliermondii* complex (three *M. caribbica* and one *M. carpophila*), three were identified at *Cryptococcus neoformans* complex (one *C. neoformans*, one *C. deneoformans* and one intervarietal hybrid *C. neoformans* x *C. deneoformans*) and one was identified at the *Cryptococcus gattii* complex (one *Cryptococcus gattii*).

3.3 | Statistical analysis of MALDI-TOF MS best score values

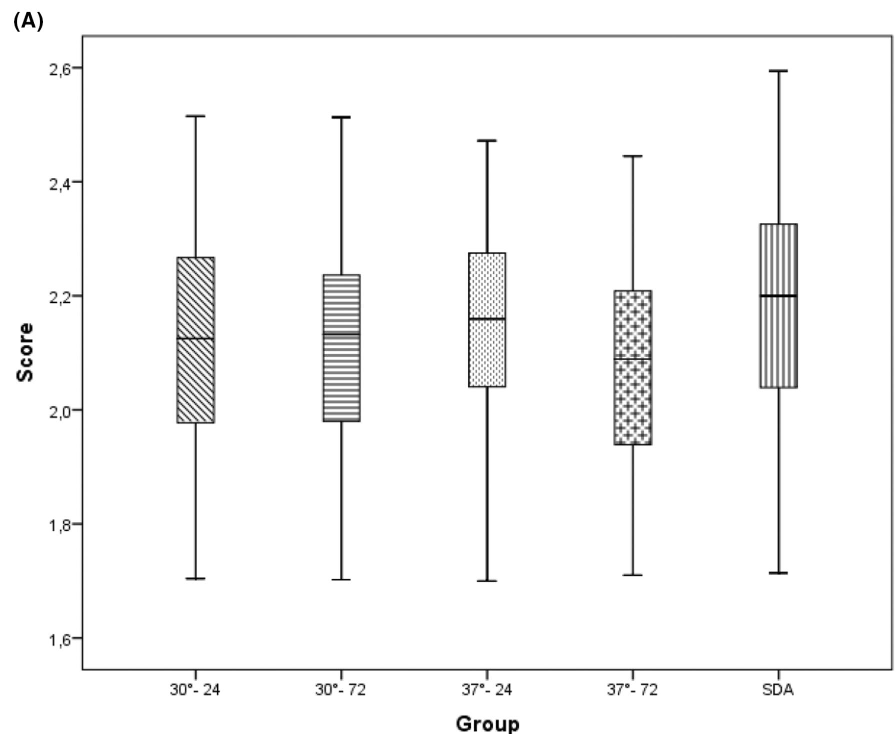
We have found almost no difference in the number of correctly identified strains at both temperatures and both incubation times, except for the four strains that were unable to grow at 37°C and the strain that was unable to obtain its protein spectrum at 37°C.

Even so, we performed a statistical analysis to determine if the temperature or incubation time may affect the score values when using CCP as a growing medium. A boxplot of the score values by temperature (30°C and 37°C) and incubation time (24 h and 72 h) are shown in Figure 2A. The highest scores values were obtained with an incubation time of 24 h at both 30°C and 37°C; no significant difference was observed between both temperatures at this incubation time (Figure 2B). However, results showed that the score values at 72 h are lower than at 24 h, and there are significant differences between the score values when comparing incubation times (groups 37°C – 24 h vs. 37°C – 72 h, 37°C – 24 h vs. 30°C – 72 h and 30°C – 24 h vs. 37°C – 72 h), except for the group 30°C – 24 h vs. 30°C – 72 h (Figure 2B). Also, we compared the score values obtained with the medium CCP and with the SDA medium. The highest score values were obtained with the SDA medium, showing significant differences with all groups except for the group 37°C – 24 h.

4 | DISCUSSION

As the emergence of *C. auris*, it becomes necessary for clinical laboratories to have methods to detect and identify rapidly this species. In this context, a new version of the CHROMagar Candida has recently been released. The CHROMagar Candida Plus is a new chromogenic medium suitable for the isolation and presumptive identification of *C. albicans*, *C. tropicalis*, *P. kudriavzevii*, *C. glabrata* and *C. auris*.[12] Some studies have already evaluated this medium showing that it is useful to detect *C. auris* in surveillance and environmental samples.[2,10–12] However, our study is a challenging evaluation of the CCP medium to detect possible misidentifications. Here we analyse a large number of yeasts species, including several species rarely or uncommonly isolated from clinical samples. Our results showed some possible misidentifications which are analysed below.

C. albicans misidentifications were due to the CCP medium cannot differentiate this species from *C. dubliniensis*, which has also been observed for the previous version of CHROMagar™ Candida and reported by other authors.[6,17] Therefore, we recommend that the presumptive identification of *C. albicans* by using these media



(B)

Group	Test U de Mann-Whitney [P value]
37 °C 24 h vs. 37 °C 72 h	U=16066 [*P*: 0,000***]
37 °C 24 h vs. 30 °C 24 h	U=19373 [*P*: Ns]
37 °C 24 h vs. 30 °C 72 h	U=18680,5 [*P*: 0,03*]
37 °C 72 h vs. 30 °C 24 h	U=18360,0 [*P*: 0,022*]
37 °C 72 h vs. 30 °C 72 h	U=19086,5 [*P*: Ns]
30 °C 24 h vs. 30 °C 72 h	U=21009,0 [*P*: Ns]
37 °C 24 h vs. SDA	U=19241,5 [*P*: NS]
37 °C 72 h vs. SDA	U=14891,5 [*P*: 0,000***]
30 °C 24 h vs. SDA	U=17962 [*P*: 0,03*]
30 °C 72 h vs. SDA	U=17247 [*P*: 0,000***]

FIGURE 2 (A) Boxplot of the score values using the medium CCP (CHROM Candida Plus) at 30°C and 24 h of incubation (group 30°-24), 30°C and 72 h of incubation (group 30°-72), 37°C and 24 h of incubation (group 37°-24), 37°C and 72 h of incubation (group 37°-72), and the score values using the medium Sabouraud Dextrose Agar at 28–30°C and 24–48 h of incubation (SDA). (B) The groups were compared using the Kruskal–Wallis test and then pairwise comparisons by the Mann–Whitney test. Statistically significant differences (*p*-values <.05) were determined by using the program Epi Info™ version 7.2. NS: no significant.

should be informed at the complex level (*C. albicans* species complex) instead of at the species level. On the other hand, presumptive phenotypic tests like chlamydospore formation on tobacco agar or sunflower seed agar can be performed to differentiate *C. albicans* from *C. dubliniensis*.[1,3,7]

Candida auris was differentiated from closely related species by using the CCP medium in agreement with previous studies.[6] However, the misidentification of only one strain of *C. haemulonii* and *C. vulturna* species was observed. In this sense, other authors also reported the misidentification of *C. pseudohaemulonii* and *C. vulturna* strains as *C. auris*.[6,25] Moreover, strains belonging to unrelated species have also been misidentified as *C. auris*, including several strains of the *C. parapsilosis* complex, which has also been reported by other authors.[10,19] Therefore, the presumptive identification of *C. auris* by using the CCP medium should be confirmed by other more specific methods, like MALDI-TOF MS or DNA sequencing. Nevertheless, all *C. auris* isolates were correctly identified, indicating this medium is useful for the screening of *C. auris* colonies. To note, we recommend observing the morphology and colour from isolated colonies and do not identify isolates after the 36–48 h of incubation determined by the manufacturer.

Candida glabrata accounted for the highest number of misidentifications, including species like *C. intermedia*, *C. lusitaniae*, *C. fabianii* and *S. cerevisiae*. In this sense, other authors have also reported the misidentification of *C. lusitaniae* as *C. glabrata* by using the CCP medium.[6,12] Therefore, the presumptive identification of

C. glabrata by this medium should be confirmed by other more specific methods like MALDI-TOF MS or DNA sequencing. Also, *C. glabrata* can be presumptively identified by using the rapid trehalose test.[9]

MALDI-TOF MS identification by using the CCP medium had a similar performance than identification by using the SDA medium, 94.0% vs. 94.3% of correct identifications, respectively.[23] However, the statistical analysis showed that, although there is no difference in the number of correctly identified strains (score >1.700) as mentioned above, the MALDI-TOF MS score values using the CCP medium are lower than using the SDA medium. This is probably because the reference spectra database was created with strains cultured in the SDA medium. Regarding the incubation time and temperature using the CCP medium, we observed that the time of incubation (24 h vs. 72 h) affects the score values when yeasts are incubated at 37°C but does not affect score values when yeasts are incubated at 30°C. Moreover, some strains were not able to grow at 37°C. Therefore, incubation at 30°C during 24–72 h is desired.

The limitations at species level identification were the same as reported previously.[23,24] In this sense, our laboratory, in its role as the National Reference Center in Medical Mycology, has recently published a guideline of recommendation about how to inform the MALDI-TOF MS results taking into account the limitations of the technique in differentiating some closely related species.[24]

In conclusion, the CCP is a good medium for the presumptive identification of *C. albicans* complex, *C. tropicalis* and *P. kudriavzevii* (*C. krusei*). This medium can be used for the screening of *C. auris* isolates but we recommend confirming its presumptive identification by other more specific methods. Also, we do not recommend the presumptive identification of *C. glabrata* by using solely this medium due to the high number of species that can be misidentified as *C. glabrata*. Finally, the CCP medium has good performance compared with the SDA medium for MALDI-TOF MS identification. These results allow the implementation of MALDI-TOF MS identification algorithms using the CCP as a growth medium instead of SDA. We also recommend checking purity and the concordance between MALDI-TOF results and the morphology of cultures to inform definitive identification.

AUTHOR CONTRIBUTIONS

Constanza Giselle Taverna: Conceptualization; investigation; writing – original draft; formal analysis; methodology; visualization. **Matías Ezequiel Vivot:** Investigation; methodology; visualization. **Bárbara Abigail Arias:** Investigation; visualization; methodology. **Lucia Irazu:** Formal analysis; software; writing – review and editing. **Cristina Elena Canteros:** Writing – review and editing; conceptualization; supervision; resources; project administration.

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CONFLICT OF INTEREST STATEMENT

The authors confirm that they have NO affiliations with or involvement in any organisation or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

DATA AVAILABILITY STATEMENT

The data that support the findings will be available in DOI:10.1111/MYC.13633 following an embargo from the date of publication to allow for commercialization of research findings.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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