

SHORT COMMUNICATION

Tomato juice as a potential replacement for human serum in germ tube test assay

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Received: 21/06/2019 ; Accepted: 06/04/2020

Abstract: *Candida albicans* (*C. albicans*) is the most common opportunistic fungal pathogen often isolated from clinical samples. Traditionally, the differentiation of *C. albicans* from other *Candida* species is performed through the germ tube test using human serum. The aim of this study was to assess the use of tomato juice of “Thilina” variety for the differentiation of *C. albicans* from other non *albicans* species. This study was carried out using 66 *Candida* isolates including five *Candida* standards. Candidal suspension (100 µl) equals to 0.5 McFarland was mixed with 1 ml of fresh human serum and 1 ml of tomato juice, separately. Test tubes were incubated at 37 °C and the observations were made at 15 minute intervals for two hours. The tomato juice exhibited 97% sensitivity, 100% specificity, 97% positive predictive value, 100% negative predictive value and higher positive correlation ($R^2 > 0.6$) in producing results. There was no statistically significant difference ($P = 0.05$) in the meantime of initial germ tube production when tomato juice was compared with the routine serum medium. For the first time the study strongly suggests that human serum can be replaced by juice of “Thilina” variety of tomato for the differentiation of *C. albicans* from non *albicans* species.

Keywords: Tomato juice; *Candida albicans*; Differentiation; Germ tube formation; Human serum.

INTRODUCTION

Candida albicans (*C. albicans*) is one of the most frequently isolated yeasts in clinical laboratories (Mayer *et al.*, 2013). The ability of *C. albicans* to infect diverse host niches is supported by a wide range of virulent factors (Calderone *et al.*, 2001; yang, 2003; Kumamoto and Marcelo, 2005). This requires the organism to be differentiated from other *Candida* species in clinical specimens (Isibor *et al.*, 2005; Cornelius *et al.*, 2018). Although various methods are available for the identification of *C. albicans*, routine identification is based on a few criteria such as growth characteristics, morphology and fermentation (Brooks *et al.*, 2013; Ameen *et al.*, 2017; Sanchez *et al.*, 2017). Traditionally, the preliminary identification of *C. albicans* is made through the use of a germ tube test (Sheppard *et al.*, 2008; Greenwood *et al.*, 2012). 95% of *C. albicans* is positive while 5% is negative for germ tube test performed using fresh human serum (Jan *et al.*, 2018). However, *C.*

dubliniensis is also capable of producing germ tubes in serum germ tube assay (Moran *et al.*, 2012).

The use of human serum in the traditional germ tube test is cheap but presents a hazard for transmission of infectious diseases and there is also a possibility for false negative results due to the biological inhibitors present in the human serum (Mattei *et al.*, 2004; Atlay *et al.*, 2017; Mehta *et al.*, 2018). Commercially prepared germ tube medium, which is safe is also available, yet could be unaffordable for countries with limited resources (Sudbery *et al.*, 2004). This warrants investigations for an economically feasible alternative medium for identification of *C. albicans*.

Tomato fruit contains beta-carotene, niacin, calcium, lycopene, derivatives of hydroxycinnamic acid, flavonoids, high amount of water and vitamins, specifically A, C, and E. Water, nutrient content and acidic environment (pH 4.9 – 6.5) enhance microbial growth, especially of fungi, which degrade the nutrients through enzyme production (Bello *et al.*, 2016; Terna and Simon, 2017). Ghosh *et al.*, (2009) reported that fungi were more active than bacteria in tomato fruit spoilage.

Induction of germ tube production under slightly acidic pH and the high susceptibility of tomato to fungal diseases as reported in previous studies suggest the possibility of using tomato juice (pH = 5) as a medium for germ tube production by *C. albicans* (Bello *et al.*, 2016). This study investigates the possibility of using tomato juice from the “Thilina” variety as a low cost, less hazardous alternative to human serum for germ tube test for *C. albicans*.

MATERIALS AND METHODS

Tomato (*Tomato lycopersicum*) variety

Young, fresh, undamaged fruits of “Thilina” variety of tomato were obtained from Horticultural Crop Research and Development Institute, Gannoruwa, Peradeniya, Sri Lanka. The fresh fruits were collected once a week at the time of harvesting and stored in the refrigerator (20°C) until used.

Collection of human serum

Fresh human serum was collected from the Biochemistry

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Laboratory, Teaching Hospital Peradeniya, Kandy, Sri Lanka. Human serum samples (n=50) were collected into a sterile bottle and the fresh serum pool was prepared every day (Matara *et al.*, 2017).

Strain selection

Standard *C. albicans* isolate ATCC 10291 was obtained from the Microbiology unit, Medical Research Institute, Colombo, Sri Lanka. Other required *Candida* isolates were obtained from the culture collection in the Microbiology Laboratory, Division of Microbiology, Faculty of Dental Sciences, University of Peradeniya. Sixty six *Candida* isolates were used for this study (Standard *Candida* isolates: *C. albicans* (ATCC 10231), *Candida parapsilosis* (ATCC 22019), *Candida krusei* (ATCC 6258), *Candida glabrata* (ATCC 90030), *Candida tropicalis* (ATCC 13803); Clinical *Candida* isolates: *C. albicans* – 32, Non *C. albicans* – 29).

Preparation of tomato (*Solanum lycopersicum*) juice

Tomatoes were washed thoroughly with sterile distilled water and wiped with 70% Ethanol. Tomato juice was obtained using the 3cc sterile needle and syringes and transferred to a sterile glass bottle.

Preparation of *Candida* cell suspensions

Candida cell suspensions were prepared immediately before the germ tube assay. A screw-capped test tube with 8 ml distilled water was sterilized by autoclaving at 121 °C and 15 lb pressure for 15 to 20 minutes. Two to three isolated colonies from a 24-hour *Candida* culture were smeared in the inner wall of the test tube. Then the colonies were dispersed in distilled water and it was mixed further using a vortex machine (Vortex mixer, Lab Tech). The turbidity of the suspension was made equal to that of the 0.5 McFarland standard by adding more inoculum or diluting with sterile distilled water (final cell density ca. 10^8 cells/ml).

Germ tube assay

A suspension (100 µl) of *C. albicans* clinical isolate with turbidity made equal to that of 0.5 McFarland standard was mixed with 1 ml of human serum as the positive control, with 1 ml of tomato juice as the test and 1 ml of distilled water as the negative control. The test tubes were incubated at 37 °C for the production of germ tubes. The observations were recorded at 15 minute intervals for

two hours. One drop from each test tube was placed on a sterile glass slide and covered with a clean coverslip. Each slide was observed under light microscope ($\times 40$ objective). The presence of germ tubes was noted and the time taken for the initial appearance of germ tubes was noted. Further, germ tube assay was performed on standard *C. albicans* (ATCC 10231), standard non *albicans* such as *C. parapsilosis* (ATCC 22019), *C. krusei* (ATCC 6258), *C. glabrata* (ATCC 90030), *C. tropicalis* (ATCC 13803) and non-*Candida* clinical isolates. Each experiment was duplicated and repeated three times.

Statistical analysis

Statistical analysis was done using SPSS version 23 to perform the Paired t test (95% confidence interval) and Pearson correlation. The sensitivity, specificity, positive predictive value and negative predictive value were also calculated.

$$\text{Sensitivity} = \frac{\text{True positive}}{\text{True positive} + \text{False negative}} \times 100$$

$$\text{Specificity} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100$$

$$\text{Positive predictive value} = \frac{\text{True positive}}{\text{True positive} + \text{False positive}} \times 100$$

$$\text{Negative predictive value} = \frac{\text{True negative}}{\text{True negative} + \text{False positive}} \times 100$$

RESULTS AND DISCUSSION

The results showed that 32 out of 33 *C. albicans* isolates including the standard isolate (ATCC 10231) produced germ tubes in tomato juice while all 33 *C. albicans* isolates produced germ tubes in the human serum (Figure 1). All the 33 non *albicans* isolates and four non *albicans* standards, namely *C. parapsilosis* ATCC 22019, *C. krusei* ATCC 6258, *C. glabrata* ATCC 90030 and *C. tropicalis* ATCC 13803 failed to produce germ tubes in tomato juice, consistent with the routine human serum method. The mean time taken for the formation of germ tubes for *C. albicans* clinical isolates in human serum and tomato juice was and minutes, respectively.

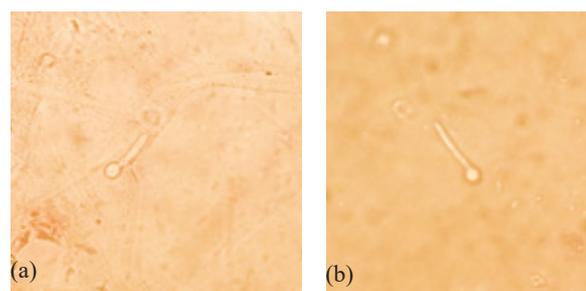


Figure1: Photomicrographs ($\times 40$) showing germ tube production in tomato juice (a) and in human serum (b).

Table 1: Germ tube test result for clinical and standard *Candida* isolates in human serum and tomato juice.

	Number of isolates that produced germ tubes in human serum	Number of isolates that failed to produce germ tubes in human serum	Total number
Number of isolates that produced germ tubes in tomato juice	32 (True positive)	0 (False positive)	32
Number of isolates that failed to produce germ tubes in tomato juice	1 (False negative)	33 (True negative)	34
Total number	33	33	66

Table 2: Intra species variation in germ tube production of *C. albicans* in human serum and tomato juice.

	Initial percentage detection of germ tubes (%)	
	Human serum	Tomato
15 minutes	0%	0%
30 minutes	6.3 %	3.1%
45 minutes	18.8%	12.5%
60 minutes	62.5%	68.8%
75 minutes	0%	0%
90 minutes	3.1%	3.1%
105 minutes	3.1%	6.3%
120 minutes	6.3%	6.3%

The results showed that, tomato juice alone can induce germ tube formation of *C. albicans* yeast cells with 97% sensitivity and 100% specificity (Table 1). There is no significant difference in the mean time for the induction of germ tubes between human serum and tomato juice ($P = 0.129$). According to the statistical analysis the positive predictive value was 97% and the negative predictive value was 100%.

The time taken for the initiation of germ tube formation varied between 30 minutes and 120 minutes in both pooled human serum and tomato juice. Further, 62.5% and 68.8% of *C. albicans* clinical isolates showed germ tube production after one hour in human serum and in tomato juice, respectively. *C. albicans* isolates have taken different times to produce germ tubes and at the end of 120 minutes all the clinical isolates started to produce germ tubes. This highlights intra species variation in germ tube production within *C. albicans* (Table 2).

Statistical analysis between the mean time of germ tube production of pooled human sera and tomato juice expressed a P value of 0.129 ($P > 0.05$) indicating that there was no statistically significant difference in the meantime of germ tube production. This suggests that tomato juice can be used for demonstrating the germ tube production with similar turnaround time as human serum. Pearson correlation ($R^2 > 0.834$, $P = 0.01$) reveals that there is a higher positive correlation between the mean time of human serum and tomato juice in germ tube assay. Hence, according to the Pearson correlation results, human serum can be replaced by tomato juice in the germ tube assay.

CONCLUSION

Over the years, studies have been conducted to discover a new source for the differentiation of *C. albicans* from non *albicans*. In the current study, tomato juice assay was used to differentiate *C. albicans* from other *Candida* species using 61 clinical isolates and 5 *Candida* standards including *C. albicans*, *C. parapsilosis*, *C. krusei*, *C. glabrata* and *C. tropicalis*. The result of tomato juice study showed 97% sensitivity, 100% specificity, 100% positive predictive value, 97% negative predictive value and higher positive correlation ($R^2 > 0.6$) There was no statistically significant difference ($P = 0.129$) in the meantime taken for initial germ tube production between the tomato juice and the routine human serum medium. The results of the current study revealed that the human serum used in the routine germ tube assay can be replaced by the tomato juice of “Thilina” variety for the differentiation of *C. albicans* from non *albicans* species.

ACKNOWLEDGEMENT

The authors wish to acknowledge the support received from Faculty of Allied Health Sciences and Division of Microbiology, Faculty of Dental Sciences, University of Peradeniya, Sri Lanka. The authors are also grateful to Dr. Pasan Jayasinghe for guidance with the statistical analyses.

DECLARATION OF CONFLICT OF INTEREST

Authors declare no conflict of interest.

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