



RESEARCH NOTE

REVISED *Saccharomyces cerevisiae* show low levels of traversal across human endothelial barrier *in vitro* [version 2; referees: 2 approved]

Previously titled: *Saccharomyces cerevisiae* show low levels of traversal across the human blood brain barrier *in vitro*

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Abstract

Background: *Saccharomyces cerevisiae* is generally considered safe, and is involved in the production of many types of foods and dietary supplements. However, some isolates, which are genetically related to strains used in brewing and baking, have shown virulent traits, being able to produce infections in humans, mainly in immunodeficient patients. This can lead to systemic infections in humans.



Methods: In this work, we studied *S. cerevisiae* isolates in an *in vitro* human endothelial barrier model, comparing their behaviour with that of several strains of the related pathogens *Candida glabrata* and *Candida albicans*.


Results: The results showed that this food related yeast is able to cross the endothelial barrier *in vitro*. However, in contrast to *C. glabrata* and *C. albicans*, *S. cerevisiae* showed very low levels of traversal.

Conclusions: We conclude that using an *in vitro* human endothelial barrier model with *S. cerevisiae* can be useful to evaluate the safety of *S. cerevisiae* strains isolated from foods.

Open Peer Review

Referee Status:  

	Invited Referees	
	1	2
REVISED		
version 2		report
published		
12 Sep 2017		
version 1		
published	report	report
20 Jun 2017		

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- Felipe H. Santiago-Tirado** , Washington University School of Medicine, USA

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Comments (0)

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Author roles: **Pérez-Torrado R:** Conceptualization, Data Curation, Formal Analysis, Investigation, Methodology, Software, Supervision, Validation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; **Querol A:** Conceptualization, Formal Analysis, Funding Acquisition, Investigation, Project Administration, Resources, Supervision, Validation, Visualization, Writing – Review & Editing

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Grant information: This work was supported by grants AGL2012-39937-C02-01 and AGL2015-67504-C3-1-R from the Spanish Government and ERDF (European Regional Development Fund) and by grant PROMETEO (project PROMETEOII/2014/042) from Generalitat Valenciana to AQ. *The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.*

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REVISED Amendments from Version 1

In this new version, we have considered HUVEC cells as a model of endothelial cells instead of a blood brain barrier.

See referee reports

Introduction

Saccharomyces cerevisiae is generally considered safe, and is involved in the production of a variety of foods and dietary supplements. Several types of food and beverage still contain viable yeast cells¹⁻⁵. However, in the last years human infections with *Saccharomyces cerevisiae* have increased⁶⁻⁸. Consequently, *S. cerevisiae* is considered an emerging pathogen⁹⁻¹¹. Different parts of the body can be affected in immunocompromised¹²⁻¹⁵ and healthy patients¹⁶⁻¹⁸. The potential virulence of this yeast has been analysed with different methods *in vitro*¹⁹⁻²² and *in vivo*²³⁻²⁷, for example by measuring epithelial barrier traversal²⁸. These reports have suggested that certain strains can cause disease and death in murine models. However, the bio-therapeutic agent Ultraleuvre (*S. cerevisiae* var. *boulardii*) and other supplements are consumed in high doses, ranging from 10⁷ to 10¹⁰ live yeast cells per day and for long periods.

The study of yeast virulence includes studying their behaviour when they encounter endothelial barriers. Opportunistic pathogenic yeasts such as *C. glabrata* and *C. albicans* are able to pass the intestinal barrier^{29,30} and generate systemic infections³¹⁻³³. Also, *C. albicans* can cross the blood-brain barrier (BBB) to reach the brain^{34,35}. Regarding *S. cerevisiae*, infections after oral ingestion¹⁶ or digestive translocation^{12,14,36} show that it can reach brain in murine models²⁵. However, few studies

have investigated the behaviour of *S. cerevisiae* when they reach endothelial barriers²⁸.

Methods

Yeast strains and growth media

The yeast strains are described in Table 1. Strains were propagated in YPD media (1% glucose, 1% BactoPeptone, 0.5% yeast extract) for 24 h at 30°C.

Growth of mammalian cells

Human umbilical endothelial cells (HUVECs) (Clonetics®) were grown in minimum essential medium (Earle's salt, 25 mM HEPES and GlutaMAX™, Invitrogen) supplemented with 10% foetal bovine serum (FBS, Cambrex Bio Science), 1% nonessential amino acids (Invitrogen) and 50 µg mL⁻¹ gentamicin (Invitrogen). The cells were grown in 150 cm² culture flasks (TPP) at 37°C in a humidified atmosphere of 5% CO₂ and 95% air until a confluence. Culture medium was changed every second day.

Trans-epithelial electrical resistance (TEER) assay

HUVEC cells (1×10⁵ cells/cm²) were seeded on Transwell® filter inserts (8 µm, Corning Incorporated) in 24-well plates (Corning Incorporated). A volume of 200 µL cell growth medium was added to the apical compartment and 1250 µL to the basolateral compartment. The TEER was measured using the Millicell-ERS Electrical Resistance System (Millipore). The net value of the TEER (Ωcm²) was corrected for background resistance by subtracting the contribution of the cell-free filter and the medium (110 Ωcm²). The TEER was measured before the addition of yeasts.

Determination of permeability coefficient

1 µg/mL of fluorescein (Sigma) was added to the media in the apical compartment of the transwell, with or without

Table 1. Yeast strains used in this study.

Strain	Species	Source
W303	<i>S. cerevisiae</i>	From our collection
102	<i>S. cerevisiae</i>	Vall d'Hebron Hospital (Barcelona, Spain) ¹⁹
60	<i>S. cerevisiae</i>	Vall d'Hebron Hospital (Barcelona, Spain) ¹⁹
Cb	<i>S. cerevisiae</i>	Vall d'Hebron Hospital (Barcelona, Spain) ¹⁹
Co	<i>C. glabrata</i>	Vall d'Hebron Hospital (Barcelona, Spain)
C2	<i>C. glabrata</i>	Provided by B. Hube (Friedrich Schiller University; Jena, Germany)
C4	<i>C. glabrata</i>	Provided by B. Hube (Friedrich Schiller University; Jena, Germany)
C5	<i>C. glabrata</i>	Provided by B. Hube (Friedrich Schiller University; Jena, Germany)
CA-1	<i>C. albicans</i>	Statens Serum Institute (Copenhagen, Denmark)
SC5314	<i>C. albicans</i>	Provided by A. Yañez ²² (Universitat de Valencia, Spain)
ATCC26555	<i>C. albicans</i>	Provided by A. Yañez ²² (Universitat de Valencia, Spain)
CBS562	<i>C. albicans</i>	From our collection

established HUVEC monolayers, and fluorescence was measured over time in the media of the apical and basolateral compartment. The apparent permeability, P_{app} , was defined as (Hilgers *et al.*, 1990):

$$P_{app} = (\Delta A_R / \Delta t) / C_{D,0} \quad (1)$$

$(\Delta A_R / \Delta t)$ is the rate of drug appearance in the receiver side, S is the surface area of the Transwell (0.33 cm² for Transwell® inserts (8 μm pore size, Corning) of 6.5-mm insert diameter), and $C_{D,0}$ is the initial drug concentration in the donor side at time = 0. Values are expressed in cm/s.

Ability to cross the endothelial barrier

HUVEC cells were seeded on Transwell® filter as described above. Yeasts grown overnight at 30°C in YPD were resuspended (10⁶ cells mL⁻¹) in the apical compartment and incubated at 37°C in a humidified atmosphere of 5% CO₂ and 95% air. After 12 h, the basolateral compartment medium was replaced. Colony forming units were counted in YPD plate triplicates after two days. Control wells used to evaluate yeast growth showed no significant growth after 12 h. Negative control HUVEC Transwells without adding cells were performed to control TEER stability across the experiment.

Results

Evaluation of the endothelial barrier integrity

To establish an *in vitro* human endothelial barrier, we used HUVEC monolayers, a methodology that has been widely used^{37,38}. Monolayers were formed in transwells and two different methods were used to determine the robustness, consistency and integrity of the barrier. First, we studied the TEER, indicative of physical separation. After seeding the HUVECs, TEER was measured and we observed increased values over time that were overcoming 450 Ωcm², which correlates with the establishment of a monolayer barrier. Second, we studied the monolayer permeability. The value obtained was 1.82±0.13 (10⁻⁶ cm/s) on average, which indicates an integral barrier with low permeability³⁹.

Study of the ability of yeast species to cross the human endothelial barrier *in vitro*

To determine whether *S. cerevisiae* is able to cross the human endothelial barrier, we used an *in vitro* model of the endothelium with HUVECs⁴⁰. The number of cells in the basolateral compartment was measured 12 hours after addition of *S. cerevisiae*, *C. albicans* and *C. glabrata* strains to the apical compartment (Figure 1). The results showed that all yeast strains were able to cross the endothelial barrier. While elevated number of cells from *C. glabrata* and *C. albicans* strains were able to cross the endothelial barrier, *S. cerevisiae* values were low. Furthermore, while the *S. cerevisiae* control strain W303 showed the lowest levels of yeast transcytosis, the other opportunistic pathogenic strains presented higher levels.

To compare the different species, the average level of cell transcytosis for all strains of each species was calculated (Figure 2). After 12 h, *Candida* species showed a high number of cells in

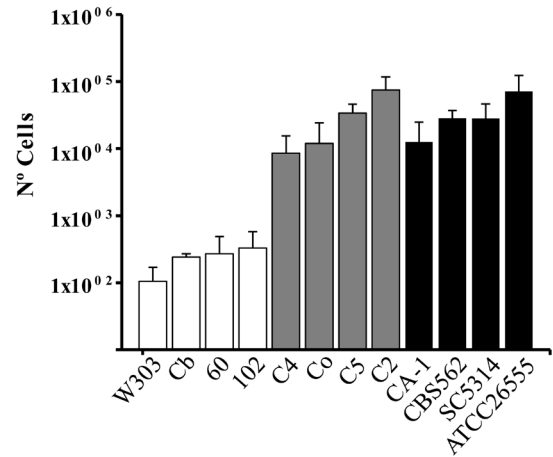


Figure 1. Number of yeast cells that were able to cross the endothelial barrier. To perform this assay we established HUVEC monolayers in Transwell® filter inserts in 24 well plates. 24 hours after apical addition of various strains of *S. cerevisiae*, *C. albicans* and *C. glabrata*, yeast cells from the basolateral compartment were incubated on YPD plates and colonies were counted after one day of growth. Values were obtained after plating several dilutions of the basolateral compartment media. Average of three experiments and standard deviation is shown. To determine statistically significant data, Student *t*-tests were performed in Excel with 0.05 as the *p*-value.

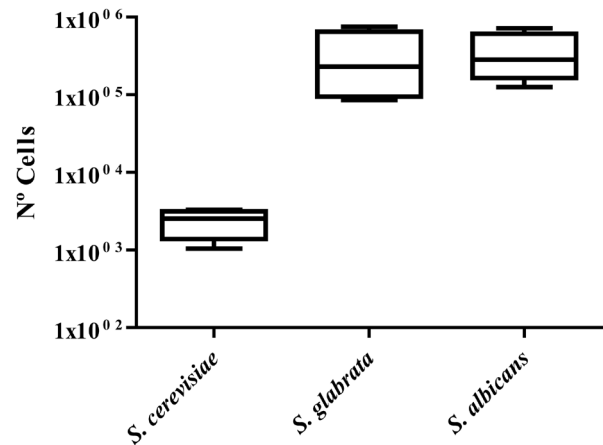


Figure 2. Box graph comparing the number of cells able to cross the endothelial barrier in the three yeast species.

the basolateral chamber (4.9–5.7 Log₁₀ units). On the contrary, we observed that *S. cerevisiae* showed significantly lower levels (1.0–3.3 Log₁₀ units) than the *Candida* species.

Dataset 1. Raw data of permeability measurements and cell counts for endothelium traversal

<http://dx.doi.org/10.5256/f1000research.11782.d177554>

Discussion

A model for traversal across the *e in vitro* has been used to study behaviour and pathogenicity mechanisms of yeast strains such as *C. albicans*^{34,35}. Here, we have shown that *S. cerevisiae* strains are able to cross the endothelial barrier. This data is in accordance with previous studies, where *S. cerevisiae* cells were observed in the brain after systemic infections in murine models²⁵. When comparing to other well-known yeast pathogens such as *C. glabrata* and *C. albicans*, none of the *S. cerevisiae* strains were able to cross the endothelial barrier at high levels. Despite *S. cerevisiae* pathogenicity levels being lower than other opportunistic yeasts, we recommend

the potential risk of new *S. cerevisiae* strains to be evaluated before using them in food production.

Data availability

Dataset 1: Raw data of permeability measurements and cell counts for endothelium traversal. DOI, [10.5256/f1000research.11782.d177554](https://doi.org/10.5256/f1000research.11782.d177554)⁴¹

Competing interests

No competing interests were disclosed.

Grant information

This work was supported by grants AGL2012-39937-C02-01 and AGL2015-67504-C3-1-R from the Spanish Government and ERDF (European Regional Development Fund) and by grant PROMETEO (project PROMETEOII/2014/042) from Generalitat Valenciana to AQ.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

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[Data Source](#)

Open Peer Review

Current Referee Status:  

Version 2

Referee Report 19 September 2017

doi:10.5256/f1000research.13710.r25924



Felipe H. Santiago-Tirado 

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By stating that their model is an endothelial barrier *in vitro* model rather than a blood-brain barrier model, the authors have addressed my main concern. Considering that they submitted this work as a 'Note', defined as a small, often preliminary study, I believe it is suitable for indexing at F1000. I, nevertheless, encourage them to consider my previous minor comments on their follow up studies.

Competing Interests: No competing interests were disclosed.

Referee Expertise: Fungal pathogenesis, membrane trafficking

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.

Version 1

Referee Report 01 September 2017

doi:10.5256/f1000research.12728.r25622



Felipe H. Santiago-Tirado 

Department of Molecular Microbiology, Washington University School of Medicine, St. Louis, MO, USA

Although the article is interesting and the data clear, I believe the authors are overstating the findings. First, HUVECs are not considered a good model for blood-brain barrier anymore. They used to be a favorite one because they are a human cell line, however, they are not of cerebral origin, and deviate considerably from the behavior of cerebral endothelial cells. They could fix this by calling their model an "endothelial" monolayer instead, or repeat the experiment using "real" BBB cell lines (i.e. hCMEC/D3, which is commercially available). Also, they report the TEER values (which by the way the correct units should be resistance (ohms) times area (cm²) rather than dividing by it) before the start of the experiment, but they should also measure the integrity of the monolayer at the end of the experiment, to rule out that the amount of *S. cerevisiae* crossing is due to rupture of the monolayer. This assay is also hard to interpret in the absence of a negative control - in fact, *S. cerevisiae* has been traditionally used as a negative control on this type of assays! Would inert beads also cross? Would any other organisms cross

at the same rate? Maybe they can check this by using fluorescent beads and measuring fluorescence on the bottom. Or if easier to do by CFUs, they could add another organism known to not been able to cross and count CFUs. Overall, it is a nice preliminary report, one worth the time pursuing. Considering this was submitted as a Research Note, I believe is appropriate for indexing once they address my comments above.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Partly

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Partly

Competing Interests: No competing interests were disclosed.

Referee Expertise: Fungal pathogenesis

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Referee Report 14 August 2017

doi:[10.5256/f1000research.12728.r24594](https://doi.org/10.5256/f1000research.12728.r24594)



Rosa de Llanos

School of Applied Sciences, Edinburgh Napier University, Edinburgh, UK

1. Introduction:

I would consider to change the sentence “Consequently, *S. cerevisiae* is considered an emerging pathogen” with “Consequently, *S. cerevisiae* is considered an emerging pathogen **of low virulence**”

2. Origin of isolation of the yeast strains could be included in Table 1.

3. Methods:

Abbreviation of BBB should be added in the title Ability to cross the blood-brain barrier.

4. Results:

In Figure 1 there are different colours but not information about the meaning of it has been included. In Figure 2, there is a mistake for *C. glabrata* and *C. albicans*, they are named as *S.glabrata* and *S.albicans*.

Is the work clearly and accurately presented and does it cite the current literature?

Yes

Is the study design appropriate and is the work technically sound?

Yes

Are sufficient details of methods and analysis provided to allow replication by others?

Yes

If applicable, is the statistical analysis and its interpretation appropriate?

Yes

Are all the source data underlying the results available to ensure full reproducibility?

Yes

Are the conclusions drawn adequately supported by the results?

Yes

Competing Interests: No competing interests were disclosed.

Referee Expertise: Fungal pathogenesis, food microbiology, environmental microbiology

I have read this submission. I believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard.
