**RESEARCH NOTE**

*Saccharomyces cerevisiae* show low levels of traversal across the human blood brain barrier *in vitro* [version 1; referees: 1 approved, 1 approved with reservations]

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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 blood brain 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 blood brain 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 blood brain barrier model with *S. cerevisiae* can be useful to evaluate the safety of *S. cerevisiae* strains isolated from foods.

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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

**Competing interests:** No competing interests were disclosed.

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**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 cells\(^1\)–\(^5\). However, in the last years human infections with _S. cerevisiae_ have increased\(^6\)–\(^8\). Consequently, _S. cerevisiae_ is considered an emerging pathogen\(^9\)–\(^11\). Different parts of the body can be affected in immunocompromised\(^12\)–\(^15\) and healthy patients\(^16\)–\(^18\). 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 traversal\(^28\). These reports have suggested that certain strains can cause disease and death in murine models. However, the bio-therapeutic agent Ultralevure (_S. cerevisiae_ var. _boulardii_) and other supplements are consumed in high doses, ranging from 10\(^7\) to 10\(^10\) 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 infections\(^1\)–\(^3\). Also, _C. albicans_ can cross the blood-brain barrier (BBB) to reach the brain\(^31\)–\(^33\). Regarding _S. cerevisiae_, infections after oral ingestion\(^16\) or digestive translocation\(^12\),\(^14\),\(^36\) show that it can reach brain in murine models\(^25\). However, few studies have investigated the behaviour of _S. cerevisiae_ when they reach endothelial barriers\(^28\).

**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 cm\(^2\) culture flasks (TPP) at 37°C in a humidified atmosphere of 5% CO\(_2\) and 95% air until a confluence. Culture medium was changed every second day.

**Trans-epithelial electrical resistance (TEER) assay**

HUVEC cells (1×10\(^5\) cells cm\(^{-2}\)) 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 (Ωcm\(^{-2}\)) was corrected for background resistance by subtracting the contribution of the cell-free filter and the medium (110 Ωcm\(^{-2}\)). The TEER was measured before the addition of yeasts.

**Table 1. Yeast strains used in this study.**

<table>
<thead>
<tr>
<th>Strain</th>
<th>Species</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>W303</td>
<td><em>S. cerevisiae</em></td>
<td>From our collection</td>
</tr>
<tr>
<td>102</td>
<td><em>S. cerevisiae</em></td>
<td>Vall d’Hebron Hospital (Barcelona, Spain)(^19)</td>
</tr>
<tr>
<td>60</td>
<td><em>S. cerevisiae</em></td>
<td>Vall d’Hebron Hospital (Barcelona, Spain)(^19)</td>
</tr>
<tr>
<td>Cb</td>
<td><em>S. cerevisiae</em></td>
<td>Vall d’Hebron Hospital (Barcelona, Spain)(^19)</td>
</tr>
<tr>
<td>Co</td>
<td><em>C. glabrata</em></td>
<td>Vall d’Hebron Hospital (Barcelona, Spain)</td>
</tr>
<tr>
<td>C2</td>
<td><em>C. glabrata</em></td>
<td>Provided by B. Hube (Friedrich Schiller University; Jena, Germany)</td>
</tr>
<tr>
<td>C4</td>
<td><em>C. glabrata</em></td>
<td>Provided by B. Hube (Friedrich Schiller University; Jena, Germany)</td>
</tr>
<tr>
<td>C5</td>
<td><em>C. glabrata</em></td>
<td>Provided by B. Hube (Friedrich Schiller University; Jena, Germany)</td>
</tr>
<tr>
<td>CA-1</td>
<td><em>C. albicans</em></td>
<td>Statens Serum Institute (Copenhagen, Denmark)</td>
</tr>
<tr>
<td>SC5314</td>
<td><em>C. albicans</em></td>
<td>Provided by A. Yañez(^22) (Universitat de Valencia, Spain)</td>
</tr>
<tr>
<td>ATCC26555</td>
<td><em>C. albicans</em></td>
<td>Provided by A. Yañez(^22) (Universitat de Valencia, Spain)</td>
</tr>
<tr>
<td>CBS562</td>
<td><em>C. albicans</em></td>
<td>From our collection</td>
</tr>
</tbody>
</table>
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 established HUVEC monolayers, and fluorescence was measured over time in the media of the apical and basolateral compartment. The apparent permeability, Papp, was defined as (Hilgers et al., 1990):

\[ P_{\text{app}} = \frac{\Delta A_{R}/\Delta t}{C_{D,0}} \]  

(\(\Delta A_{R}/\Delta t\)) 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 blood-brain 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.

Results

Evaluation of the blood-brain barrier integrity

To establish an in vitro BBB, we used HUVEC monolayers, a methodology that has been widely used. 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 Ω cm⁻², 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 permeability.

Study of the ability of yeast species to cross the human BBB in vitro

To determine whether S. cerevisiae is able to cross the human BBB, we used an in vitro model of the human BBB with HUVECs. 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 BBB. While elevated number of cells from C. glabrata and C. albicans strains were able to cross the BBB, 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 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.

Discussion

A model for traversal across the BBB in vitro has been used to study behaviour and pathogenicity mechanisms of yeast strains such as C. albicans. Here, we have shown that S. cerevisiae strains are able to cross the BBB. This data is in accordance with...
previous studies, where \( S.\ cerevisiae \) cells were observed in the brain after systemic infections in murine models\(^3\). 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 BBB 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 BBB traversal. DOI, 10.5256/f1000research.11782.d165113\(^4\)

Competition of 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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    Publisher Abstract | Publisher Full Text
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Open Peer Review

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 (cm$^2$) 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

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.