**Saccharomyces cerevisiae** show low levels of traversal across human endothelial barrier *in vitro* [version 2; referees: 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.
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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 Saccharomyces 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\(^31\)–\(^33\). Also, C. albicans can cross the blood-brain barrier (BBB) to reach the brain\(^34\),\(^35\). Regarding S. cerevisiae, infections after oral ingestion\(^36\) or digestive translocation\(^12\),\(^14\),\(^36\) show that it can reach brain in murine models\(^35\). 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 (\(\Omega\)cm\(^2\)) 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.

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

| Strain | Species     | Source                                      |
|--------|-------------|---------------------------------------------|
| W303   | S. cerevisiae | From our collection                         |
| 102    | S. cerevisiae | Vall d’Hebron Hospital (Barcelona, Spain)\(^13\) |
| 60     | S. cerevisiae | Vall d’Hebron Hospital (Barcelona, Spain)\(^13\) |
| Cb     | S. cerevisiae | Vall d’Hebron Hospital (Barcelona, Spain)\(^13\) |
| Co     | C. glabrata  | Vall d’Hebron Hospital (Barcelona, Spain)   |
| C2     | C. glabrata  | Provided by B. Hube (Friedrich Schiller University; Jena, Germany) |
| C4     | C. glabrata  | Provided by B. Hube (Friedrich Schiller University; Jena, Germany) |
| C5     | C. glabrata  | Provided by B. Hube (Friedrich Schiller University; Jena, Germany) |
| CA-1   | C. albicans  | Statens Serum Institute (Copenhagen, Denmark) |
| SC5314 | C. albicans  | Provided by A. Yañez\(^22\) (Universitat de Valencia, Spain) |
| ATCC26555 | C. albicans  | Provided by A. Yañez\(^22\) (Universitat de Valencia, Spain) |
| CBS562 | C. albicans  | From our collection                         |
established HUVEC monolayers, and fluorescence was measured over time in the media of the apical and basolateral compartment. The apparent permeability, \( P_{\text{app}} \), 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 cm\(^2\) 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 (10\(^6\) cells mL\(^{-1}\)) in the apical compartment and incubated at 37°C in a humidified atmosphere of 5% CO\(_2\) 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 Ωcm\(^2\), 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\(^{39}\).

**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 HUVECs\(^{40}\). 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
the basolateral chamber (4.9–5.7 Log_{10} units). On the contrary, we observed that S. cerevisiae showed significantly lower levels (1.0–3.3 Log_{10} units) than the Candida species.

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. 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 models. 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. DOL 10.5256/f1000research.11782.d177554

Competing interests
No competing interests were disclosed.

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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.

References

1. Fröhlich-Wyder MT: 8 – Yeasts in dairy products. In: Yeasts in Food. Beneficial and Detrimental Aspects. Bookhout T, Robert V, Editors. Behr, Hamburg. 2003; 209–237. Publisher Full Text
2. Jacques N, Casaregola S: Safety assessment of dairy microorganisms: the hemiascomycetous yeasts. Int J Food Microbiol. 2008; 126(3): 321–326. PubMed Abstract | Publisher Full Text
3. Deak T, Beuchat L: Handbook of spoilage yeasts. CRC, Boca Raton; 1996. Reference Source
4. Loureiro V, Malfeito-Ferreira M: Spoilage yeasts in the wine industry. Int J Food Microbiol. 2003; 86(1–2): 23–50. PubMed Abstract | Publisher Full Text
5. Loureiro V, Querol A: The prevalence and control of spoilage yeasts in foods and beverages. Trends Food Sci Technol. 1999; 18(11): 356–365. PubMed Abstract | Publisher Full Text
6. Herbrecht R, Nivoix Y: Saccharomyces cerevisiae fungemia: an adverse effect of Saccharomyces boulardii probiotic administration. Clin Infect Dis. 2005; 40(11): 1635–1637. PubMed Abstract | Publisher Full Text
7. Montiineri A, Lacabba C, Larocca L, et al.: [Saccharomyces cerevisiae fungemia associated with multifocal pneumonia in a patient with alcohol-related hepatic cirrhosis]. Infez Med. 2008; 16(4): 227–229. PubMed Abstract
8. Swingwe N, Norðd N, VAN Rooij P, et al.: Bloodstream yeast infections: a 15-month survey. Epidemiol Infect. 2009; 137(7): 1037–1040. PubMed Abstract | Publisher Full Text
9. Hazen KC: New and emerging yeast pathogens. Clin Microbiol Rev. 1995; 8(4): 462–478. PubMed Abstract | Free Full Text
10. Murphy A, Kavanagh K: Emergence of Saccharomyces cerevisiae as a human pathogen. Implications for biotechnology Review. Enzyme Microb Technol. 1999; 25(7): 551–597. Publisher Full Text
11. Ponton J, Rüchel R, Clemons KV, et al.: Emerging pathogens, Med Mycol. 2000; 38(Suppl 1): 225–26. PubMed Abstract | Publisher Full Text
12. Enache-Angoulvant A, Hennquin C: Invasive Saccharomyces infection: a comprehensive review. Clin Infect Dis. 2005; 41(11): 1559–1568. PubMed Abstract | Publisher Full Text
13. Lolis N, Veldhois D, Moraitou H, et al.: Saccharomyces boulardii fungemia in an intensive care unit patient treated with caspofungin. Crit Care. 2008; 12(2): 414. PubMed Abstract | Publisher Full Text | Free Full Text
14. Muñoz P, Bouza E, Cuenca-Estrella M, et al.: Saccharomyces cerevisiae fungemia: an emerging infectious disease. Clin Infect Dis. 2005; 40(11): 1625–1634. PubMed Abstract | Publisher Full Text
15. Williams JS, Mufli GJ, Powell S, et al.: Saccharomyces cerevisiae emboli in an immunocompromised patient with relapsed acute myeloid leukemia. Clin Exp Dermatol. 2007; 32(4): 385–387. PubMed Abstract | Publisher Full Text
16. Jensen DP, Smith DL: Fever of unknown origin secondary to brewe’s yeast ingestion. Arch Intern Med 1976; 136(3): 332–333. PubMed Abstract | Publisher Full Text
17. Smith D, Metzgar D, Wils C, et al.: Fatal Saccharomyces cerevisiae aortic graft infection. J Clin Microbiol. 2002; 40(7): 2691–2692. PubMed Abstract | Publisher Full Text | Free Full Text
18. Sobel JD, Schmitt CA, Lynch M, et al.: Emerging problem of vaginitis due to Saccharomyces cerevisiae. Clin Infect Dis. 1993; 16: 93–94.
19. de Llanos R, Fernández-Espinar MT, Querol A: A comparison of clinical and food Saccharomyces cerevisiae isolates on the basis of potential virulence factors. Antonie van Leeuwenhoek. 2006; 90(3): 221–231. PubMed Abstract | Publisher Full Text
20. Klingberg TD, Leinik U, Amelberg N, et al.: Comparison of Saccharomyces cerevisiae strains of clinical and nonclinical origin by molecular typing and determination of putative virulence traits. FEMS Yeast Res. 2008; 8(4): 631–640. PubMed Abstract | Publisher Full Text | Free Full Text
21. McCusker JH, Clemens KV, Stevens DA, et al.: Saccharomyces cerevisiae virulence phenotype as determined with CD-1 mice is associated with the ability to grow at 42 degrees C and form pseudohyphae. Infect Immun. 1994; 62(12): 5447–5455. PubMed Abstract | Free Full Text
22. Yáñez A, Murciano C, Llopis S, et al.: In vivo and in vitro studies on virulence and host responses to Saccharomyces cerevisiae clinical and non-clinical isolates. Open Mycol J. 2009; 3: 37–47.
23. Byron JK, Clemens KV, McCusker JH, et al.: Pathogenicity of Saccharomyces cerevisiae in complement factor five-deficient mice. Infect Immun. 1995; 63(2): 478–485. PubMed Abstract | Free Full Text
24. Clemens KV, McCusker JH, Davis RW, et al.: Comparative pathogenesis of clinical and nonclinical isolates of Saccharomyces cerevisiae. J Infect Dis. 1994; 169(6): 859–867. PubMed Abstract | Publisher Full Text
25. de Llanos R, Llopis S, Molerito G, et al.: In vivo virulence of commercial Saccharomyces cerevisiae strains with pathogenicity-associated phenotypic
traits. Int J Food Microbiol. 2011; 144(3): 393–399.
PubMed Abstract | Publisher Full Text

26. McCullough MJ, Clemons KV, McCusker JH, et al.: Species identification and virulence attributes of Saccharomyces boulardii (nom. Inval.). J Clin Microbiol. 1998; 36(9): 2613–2617.
PubMed Abstract | Free Full Text

27. Llopis S, Querol A, Heyken A, et al.: Transcriptomics in human blood incubation reveals the importance of oxidative stress response in Saccharomyces cerevisiae clinical strains. BMC Genomics. 2012; 13: 419.
PubMed Abstract | Publisher Full Text | Free Full Text

28. Pérez-Torrado R, Llopis S, Jespersen L, et al.: Clinical Saccharomyces cerevisiae isolates cannot cross the epithelial barrier in vitro. Int J Food Microbiol. 2012; 157(1): 59–64.
PubMed Abstract | Publisher Full Text

29. Li L, Redding S, Dongari-Bagtzoglou A: Candida glabrata: an emerging oral opportunistic pathogen. J Dent Res. 2007; 86(3): 204–15.
PubMed Abstract | Publisher Full Text

30. Naglik JR, Moyes DL, Wächtler B, et al.: Candida albicans interactions with epithelial cells and mucosal immunity. Microbes Infect. 2011; 13(12-13): 963–76.
PubMed Abstract | Publisher Full Text | Free Full Text

31. Brun S, Dalle F, Saulnier P, et al.: Biological consequences of petite mutations in Candida glabrata. J Antimicrob Chemother. 2005; 56(2): 307–14.
PubMed Abstract | Publisher Full Text

32. Dalle F, Wächtler B, L'Ollivier C, et al.: Cellular interactions of Candida albicans with human oral epithelial cells and enterocytes. Cell Microbiol. 2010; 12(2): 248–271.
PubMed Abstract | Publisher Full Text

33. Hoarau G, Kendrick O, Lagre M, et al.: Detection of (1,3)-β-D-glucans in situ in a Candida albicans brain granuloma. J Infect. 2013; 67(6): 622–4.
PubMed Abstract | Publisher Full Text

34. Jong AY, Stins MF, Huang SH, et al.: Traversal of Candida albicans across human blood-brain barrier in vitro. Infect Immun. 2001; 69(7): 4536–44.
PubMed Abstract | Publisher Full Text | Free Full Text

35. Jong AY, Chen SH, Stins MF, et al.: Binding of Candida albicans enolase to plasminogen results in enhanced invasion of human brain microvascular endothelial cells. J Med Microbiol. 2003; 52(Pt 8): 615–22.
PubMed Abstract | Publisher Full Text

36. Lherm T, Monet C, Nougère B, et al.: Seven cases of fungemia with Saccharomyces boulardii in critically ill patients. Intensive Care Med. 2002; 28(6): 797–801.
PubMed Abstract | Publisher Full Text | Free Full Text

37. Langford D, Hurford R, Hashimoto M, et al.: Signalling crosstalk in FGF2-mediated protection of endothelial cells from HIV-gp120. BMC Neurosci. 2005; 6: 8.
PubMed Abstract | Publisher Full Text | Free Full Text

38. Wilhelm I, Fazakas C, Krizbai IA: In vitro models of the blood-brain barrier. Acta Neurobiol Exp (Wars). 2011; 71(1): 113–128.
PubMed Abstract

39. Gaillard PJ, de Boer AG: Relationship between permeability status of the blood-brain barrier and in vitro permeability coefficient of a drug. Eur J Pharm Sci. 2000; 12(2): 95–102.
PubMed Abstract | Publisher Full Text

40. Sun SW, Zu XY, Tuo QH, et al.: Caveolae and caveolin-1 mediate endocytosis and transcytosis of oxidized low density lipoprotein in endothelial cells. Acta Pharmacol Sin. 2010; 31(10): 1336–42.
PubMed Abstract | Publisher Full Text | Free Full Text

41. Pérez-Torrado R, Querol A: Dataset 1 in: Low levels of in vitro human blood brain barrier traversal of the food related emerging pathogen Saccharomyces cerevisiae. F1000Research. 2017.
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

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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

**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.