

Response to Reviewers- Manuscript #28894

Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility

Christine West, Andrew M. Stanisz, Annette Wong, & Wolfgang A. Kunze

Firstly, we would like to thank all of the reviewers for their time in reviewing our paper and for the positive and encouraging feedback. We have made our revisions accordingly. As per the editor's comments, we have provided an audio core tip and separate figures/images.

Reviewer's code: 02770241

Comments

"The authors investigated the possible effects of *Saccharomyces boulardii* or *Saccharomyces cerevisiae* on reducing the stress-related intestinal dysmotility. The study is well designed and well implemented. The manuscript should be accepted for publication with the following revision. 1. The biggest confusion of the manuscript is the inconsistent use of statistical terms. In the abstract and Tables, P-value was used, while the probability of superiority (PS) was used in the results section. It is difficult for any reader to match these two values. The authors should make this consistent by removing the use of PS, or adding p-values in the results section. 2. The use of the yeast species should be consistent. When first introduced, they should be used as "*Saccharomyces boulardii* and *Saccharomyces cerevisiae*", rather than "*Saccharomyces boulardii* and *cerevisiae*". Later, the species should be used as "*S. boulardii* and *S. cerevisiae*", rather than "*S. boulardii* and *cerevisiae*". 3. The term "post-incubation yeast solution" is not clear. How was this condition achieved? This could significantly affect the composition of the Snt. 4. P-values should be provided in Figure 1A and 1C."

1. P-values have been added to the results section to provide consistency in the use of statistical terms.
2. The names of the yeast species have been made consistent throughout the manuscript.
3. The term "post-incubation yeast solution" has been described. The yeasts were diluted and incubated as previously described in the manuscript. The supernatant was then separated in the centrifugation process that is also described in the methods.
4. P-values have been provided in Figure 1A and 1C.

Reviewer's code: 03317031

Comments

This study aims to investigate the capacity of *Saccharomyces cerevisiae* and *boulardii* yeasts to reverse or to treat acute stress-related intestinal dysmotility in mice. I have just few comments listed below: 1. In order to improve the readability of the introduction section it needs to be shortened. Maybe the first 2 paragraph can be shortened and a bit more information about the rational and potential significance of the study in the last paragraph. 2. How many mice were enrolled in each arm of the study? Please clarify. 3. Implications for application in humans should be more discussed.

1. The first paragraph of the introduction has been shortened. The second last paragraph of the introduction was moved to the end to improve readability and to better create a lead in to the body of the manuscript. This paragraph better represents the ration and potential significance of the study.
2. The number of mice in each part of the study was clarified in the methods and can be found in the tables as mean \pm SD (N), where N denotes the number of mice used.
3. Implications for application to humans were added to the discussion. We made reference to cited clinical trials in humans in our introduction in the final paragraph of the discussion to support human implications.

Reviewer's code: 02255435

Comments

West et al. used an ex vivo perfusion method to examine effects of the yeasts *S. boulardii* and *S. cerevisiae* on stress induced jejunal and colonic dysmotility in mice. They found that *S. boulardii*, and to a lesser extent, *S. cerevisiae* was able to mitigate restraint stress induced reduction in jejunal transit velocity and frequency as well as increase in colonic transit velocity and frequency. These results are novel and intriguing as *Saccharomyces* yeasts had not been previously shown to counteract the effect of stress on gut motility. Moreover, the authors also provided evidence that yeast supernatant per se had a similar effect as yeast cells on gut dysmobility induced by stress. Data presented in the manuscript are straightforward and support the author's conclusions. The work points to potential usage of *Saccharomyces* yeasts for treatment of gut dysmotility. The authors should speculate on mechanisms underlying the effect of yeast on gut motility despite that finding the bioactive agent(s) in yeast supernatant may be difficult as they mentioned. p.7, line 4 from bottom: "... 27% (PS=93%) for colon" is most likely incorrect as Fig. 1C shows no change in PCC peak value for colon. p. 10, 1st paragraph: The authors consider "indirect modes of action involving immune cells" less likely than pharmacological mode of action. What is the reason for this notion?

1. To speculate on mechanisms underlying the effect of yeast on gut motility, we made reference to the findings of Al-Nedawi et al. (2015) in which application of *Lactobacillus rhamnosus* JB-1TM microvesicles to gut epithelium had similar

effects to application of the whole bacteria. In a similar fashion, it may be of interest to further isolate the yeast supernatant into individual components and test them for their effect in the same model.

2. The error in p.7, line 4 has been rectified. PCC peak value increased by 1% with stress in the colon.
3. Our results are consistent with other findings showing a short latency in onset in the effect of microbes on intestinal propulsive motility (Massi et al. 2006; Wang et al. 2010). The region-specific and local effect on the intestine is consistent with a drug-like pharmacological mode of action. Based on this evidence, we consider pharmacological mode of action more likely than very fast indirect modes involving immune cells.

Reviewer's code: 02840182

Comments

The authors investigated the effects of *Saccharomyces boulardii* and *Saccharomyces cerevisiae* on the stress-related intestinal dysmotility in mice. This is a well designed study. The effects of probiotic microorganisms were compared in different doses.

The manuscript was well written. I believe that it will contribute to the literature. I think it can be accepted without revision.

1. Thank you for the positive response and acceptance of our manuscript.

Reviewer's code: 00055213

Comments

In their paper, "Effects of *Saccharomyces cerevisiae* or *boulardii* yeasts on acute stress induced intestinal dysmotility," Christine West and co-authors, describe their studies in mice that explore the impact of yeast on altered gut function after acute psychological stress. After a 1 hour stress that altered gut motility, the authors killed the mice, and exposed ex-vivo intestinal preps to yeast or yeast supernatants after which they assessed gut motility; results were compared to unstressed gut preps. The authors report that yeast treatment improved stress-induced changes in gut motility, which included effects with opposite valence in jejunal compared with the colonic segments. Most convincingly, this study demonstrates, by assessing ex vivo preps and, in addition, by testing yeast supernatants, that the impact of yeast is mediated locally and may not require an active fungal application. I feel that this paper is suitable for publication and that the representation of data in figures and tables is helpful and suitable for publication. The paper is well-written.

1. Thank you for the positive response and acceptance of our manuscript.

