Reviewer’s report

Title: Oral treatment with Lactobacillus Rhamnosus attenuates behavioral deficits and immune changes in chronic social stress

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Reviewer: Peter Holzer

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This manuscript reports on the effects of Lactobacillus rhamnosus (strain JB-1) on chronic social defeat (CSD)-evoked alterations of anxiety-related, social and aggressor avoidance behavior, immune cell phenotype, faecal microbial community and faecal metabolite profile in male C57BL/6 mice. The authors present findings to show that exposure to a single microbial strain for 28 days conveys partial protection from CSD-induced behavioral disturbances and systemic immune alterations while CSD-evoked changes in the faecal microbial community remain unaffected. Interestingly, CSD-induced faecal dysbiosis persisted for 3 weeks and was associated with a disturbance of the faecal profile of metabolites. The authors conclude that JB-1 alters CSD-induced disturbances of behaviour independently of an effect on gut microbiota. The mechanism of this interaction with the brain and the possible role of changes in many faecal metabolites remain unexplored. The authors’ claim that JB-1 facilitates the recovery from CSD-evoked dysbiosis is not directly supported by the data of the study.

General comments

Although the effect of stress on the gut microbiota-brain axis is already well studied (see below), the current study presents a number of interesting findings including (1) the absence of JB-1 effects on baseline behavior in male C57BL/6 mice, (2) the absence of an effect of JB-1 on CSD-induced gut dysbiosis, (3) the persistence of CSD-induced dysbiosis for at least 3 weeks, (4) the partial effect of JB-1 in preventing some of the CSD-evoked behavioural disturbances, and (5) the behavioral benefit exerted by JB-1 being independent of the faecal microbiota. However, the manuscript as it is also raises a number of questions that require the attention of the authors.

Specific issues

1. Introduction. The statement that it is not known “whether bottom-up signalling along the gut-brain axis regulates stress-related changes in behaviour and neural function” neglects some of the pertinent findings, including those of the authors of this manuscript themselves (reference # 10), let alone other authors (e.g., Sudo et al. J Physiol 2004;558:263-275; Ait-

2. The experimental procedures relating to splenocyte isolation and processing need be detailed.

3. Figure 3A and 3B. It seems as if the same data are presented twice, in the left panel of Figure 3A without significant difference between VEH and JB-1, but with a significant difference in Figure 3B because here only two groups are compared with each other. Please explain.

4. Figure 4F and related text. In contrast to what is claimed by the authors, this Figure does not show that JB-1 facilitates the recovery of the dysbiotic microbiota. This could only be claimed if there were a significant difference between Defeat/VEH and Defeat/JB-1, but such a difference does obviously not exist.

5. The authors invested quite some efforts into analysing the faecal metabolome and found many alterations following exposure to CSD. Unfortunately, these findings are hardly dealt with in the Discussion, although it has to be admitted that alterations in the faecal metabolite concentrations may be of limited relevance to gut-brain communication. The plasma metabolome would provide much more valid information on metabolites that may play a role in gut-brain interaction.

6. JB-1 was without appreciable effect on CSD-evoked faecal dysbiosis. This finding fits largely with the literature showing that probiotic treatment has inconsistent effects on the faecal microbiota profile in humans and mammals. An important question is whether JB-1 was at all detectable in the faecal samples. This touches the issue whether JB-1 was at all able to reach the large intestine. If not, a primary effect in the small intestine may be envisaged, as alluded by the authors in the Discussion.

7. The bibliographic information for references # 20, # 32, # 61, # 62 and # 64 is incomplete or in need of amendment.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
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No

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