Author's response to reviews

Title: The host response to the probiotic Escherichia coli strain Nissle 1917: Specific up-regulation of the proinflammatory chemokine MCP-1

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Author's response to reviews: see over
Dear Editor,

enclosed please find the revised version of our manuscript entitled „The host response to the probiotic *Escherichia coli* strain Nissle 1917: Specific up-regulation of the proinflammatory chemokine MCP-1”.

All comments of the reviewer have been carefully addressed as outlined below in detail. We feel that they have significantly improved and clarified our paper and we hope that the revised manuscript is now suitable for publication. As suggested by the editor we would be very happy to change our submission to the sister journal BMC Medical Genetics if this does not require a new review process.

Yours sincerely,
Sya Ukena and Florian Gunzer
Reply to the reviewers comments

Major Compulsory Revisions

Reviewer:
1) Abstract: This needs to be re-written as in the current form it is too general for the research conducted. For example....

Reply:
As suggested by the reviewer, the abstract has been completely re-written and the results concerning the 2nd cell line and the mice findings have now been included. The final sentence has been removed.

Reviewer:
2) Background: There is no proof in humans that bacteriocins or microcins are important in bacterial interference but merely speculated.

Reply:
As requested, this part has been replaced by more profound information concerning proposed mechanisms by which probiotics may mediate their effects in vivo.

Reviewer:
3) Background: The last sentence “counteraction of inflammatory processes by stabilization of the gut microbial environment and the intestine’s permeability barrier” does not make sense. Clarification of what is trying to be said is needed.

Reply:
We agree with the reviewer that this statement is misleading. We have therefore removed this sentence together with the changes introduced in response to comment 2 by the reviewer.

Reviewer:
4) Methods: Were pH determinations made before and after the bacterial incubation period? If there was a pH change, were the alterations in gene expression on the basis of the pH change?

Reply:
We have carefully considered the influence of pH upon planing of our experiments. However, due to the design of our study it can be ruled out that changes in pH are solely responsible for the observed alterations in gene expression modulated by EcN. On the one hand, the buffer capacity of the cell culture medium was not exhausted neither for Caco-2 cells cocultured with EcN nor for cells grown together with E. coli MG1655 as displayed by the media color indicator. Furthermore, since E. coli MG1655 was used as control in all validation experiments, gene regulation observed was truly EcN specific. Moreover, CBA measurements of MCP-1 protein expression levels exhibited similar results for untreated Caco-2 cells and cells cocultured with E. coli MG1655, indicating that potential pH changes in bacterial coculture did not influence protein secretion in this study.
Reviewer:
5) Methods: DTT treatment of intestinal pieces would be expected to do far more than remove excess mucus. Was simple washing with PBS not enough?

Reply:
Preparation of tissue pieces was performed according to modified published protocols [1-4]. In each of these publications DTT has been used in order to remove the mucus layer. Certainly, the protective mucus layer plays an essential role particularly for the inhibition of adherence of enteric pathogens, but we wanted to confirm the data obtained from cell culture with human intestinal epithelial cell lines by tissue coculture. Therefore, we had to remove the mucus layer in order to ensure direct contact between the bacteria and the epithelial cells.

Reviewer:
6) Methods: Explain why the 10 genes were of major interest? Were they of major interest at the outset of the experiments? Did they gain interest as they had some of the most prominent changes among the different genes whose expression levels were modulated by EcN?

Reply:
Unfortunately, there was a typing error in the original version of the manuscript. 9 genes instead of 10 genes were investigated further in the study. The selection of these genes was based on both, prominent changes in their gene expression profiles and their putative biological implication at the outset of our study. This issue has now been clarified in the results section of the revised manuscript. However, table 2 provides data on additional genes that might be of interest for researchers in the field, far beyond this study.

Reviewer:
7) Conclusions: Page 18, last paragraph. The authors speculate that upregulation of MCP-1, MIP-2α and MIP-2β upon contact with the probiotic EcN might reflect being part of the host defense process against pathogenic bacteria through the establishment of a protective immunologic barrier.....

Reply:
This postulation has been modified and clarified in the discussion of the revised version of the manuscript.

Reviewer:
8) Abstract: Conclusion. As valid a speculation as the last sentence currently in the abstract is that the use of EcN could lead to worsening of mucosal inflammation in those with a reduced mucosal barrier. While the authors may choose to include this speculation in the discussion neither the current last sentence of the current abstract nor the speculation in this point should be part of the abstract.

Reply:
As requested by the reviewer, this sentence has been removed.
Minor Essential Revisions

Reviewer:
9) Background: It is not clear what the authors mean by “probiotic genes”. The sequence of some microbes used as probiotics have been delineated.

Reply:
This part in the background section has been modified together with the changes proposed by the reviewer in comment 2 and 3.

Reviewer:
10) Background: The authors should include the dose off mesalamine (1,500 mg) used in the comparative trial and the recurrence rate (approx. 35%) as the former is quite low and the latter is high.

Reply:
According to the reviewers suggestion this information has been included in the background section of the revised manuscript.

Reviewer:
11) Background: “a semi-rough … known protein toxins” is written awkwardly and should be re-written.

Reply:
As requested, this part has been re-written. Semirough is a somewhat confusing description assigned to the LPS phenotype of Ecn by Blum et al. [5] who identified and described a point mutation for the O-antigen polymerase leading to only one repeating unit in the side chain of the lipopolysaccharide sugar.

Reviewer:
12) Methods: Caco cells are well known to have significant alterations depending on the passage number. Details of the cells should be included.

Reply:
Detailed information on the passage number is now given in the methods section of the revised manuscript.

Reviewer:
13) Methods: What specifically was the inoculum corresponding to the low bacterial MOI?

Reply:
As requested, the specific inoculum has been calculated and included in the materials section of the revised manuscript.

Reviewer:
14) Methods: It is not clear why lower bacterial numbers were required with intestinal pieces if they were already colonized? Please clarify.

Reply:
We have decided to use a lower bacterial inoculum in order to ensure a coincubation time of 6 hours as in all other coculture experiments of this study. Because of the already colonized intestine a higher bacterial inoculum would have led to rapid acidification of the media thus making a coincubation time of 6 hours impossible. As pointed out earlier by the reviewer, the pH changes of the media might then lead to alterations in gene expression. Interestingly, even a low inoculum of Ecn was sufficient to up-regulate MCP-1 gene expression in mouse intestinal tissue.
Reviewer:  
15) Methods: It should be described that CM stands for conditioned media.

Reply:  
The missing information has been provided.

Discretionary Revisions

Reviewer:  
16) Background: It is suggested the authors use the WHO/FAO definition.

Reply:  
The WHO/FAO definition of the term “probiotic” has been included in the revised manuscript together with a new reference.

Reviewer:  
17) Background: First paragraph, 3rd sentence “To be treated” could be removed since probiotics are not always used to treat conditions but also used to maintain 'health'.

Reply:  
The authors fully agree with the reviewers view and have removed this sentence.

Reviewer:  
18) Methods: What difference are photomicrographs A I, II, and III trying to depict? Are they really needed?

Reply:  
In our opinion the photomicrographs nicely visualize the interaction between confluent Caco-2 cells and the bacteria. They demonstrate the non-invasiveness and the lack of adherence (e. g. attaching and effacing) of the organisms under investigation in this study.

References


