

Format for ANSWERING REVIEWERS



November 16, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 6419-review.doc).

Title: Combined Probiotic Bacteria Promotes Intestinal Epithelial Barrier Function in IL-10 KO Mice

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Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 6419

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated.

2 Revision has been made according to the suggestions of the reviewer:

Response to NO 00007966 reviewer:

- (1) We have use colitis to instead of IBD except that cited from the reference.
- (2) The figure 2A and 2B showed mild inflammatory cell infiltration and mucosal damage in Bifico treated IL-10 KO mice when compared with IL-10 KO mice that no treated with Bifico. The "mild..." means "less inflammatory cell infiltration and mucosal damage". However, the colitis in treated IL-10 KO mice is still rather significant when compared with WT mice. In the process of our experiment, we found that oral administration of Bifico for four weeks only decreased the severity of colitis in IL-10 KO mice but could not reach cure of colitis. As reviewer's mentioned, the effects of Bifico on colonic inflammation was partial and treated mice still had evidence of substantial inflammation. In the clinical, the Bifico is only used in the adjuvant treatment of intestinal diseases, such as UC, CD, Diarrhea . We have highlighted that the effects of Bifico on colonic inflammation was partial and treated mice still had evidence of substantial inflammation in abstract and discussion section.

Response to NO 00504764 reviewer:

- (1) Probiotics given orally by gavage have been used in many studies.
- (2) Although gastric acidification and digestion may be influence the survive of probiotics, the prebiotics produced by probiotics were functional in gut.
- (3) IL-10 KO mice were not kept in germ-free conditions. They were housed under specific pathogen-free conditions in Shanghai Jiao Tong University Medical School as mentioned in MATERIALS AND METHODS.
- (4) Maybe 0.4 micron is better in Caco-2 cell experiment, but a part of studies also use 3 micron pore filters to support the CACO-2 cells, especially when measured the permeability of Caco-2 monolayers.
- (5) EIEC and the probiotics are only placed on upside of the CACO-2 cell layers (in the transwell).
- (6) We have use colitis to instead of IBD except that cited from the reference.
- (7) We have discussed that the epithelial cells may respond directly to certain probiotic bacteria. Certain strains of Lactobacillus, for example, release surface-active components, which inhibit adhesion of pathogenic bacteria. Thus, probiotic bacteria may protect epithelium by receptor competition, whereby probiotics compete with microbial pathogens for a limited number of receptors present on the surface

epithelium.

(8) Indeed, Caco-2 cell death is being caused by the EIEC. It is one of reason that induced the increased permeability when CACO-2 cell layers treated with EIEC.

(9) There are several studies have obtained such high resistances for CACO-2.

Minor Comments:

(1) VCC MC8 is model of multi-channel voltage current clamp.

(2) We have changed "often induces" to "occurs with".

(3) We have changed "ameliorated" to "improved".

3 References and typesetting were corrected.

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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