



**State Key Laboratory of Food Science and Technology
& School of Food Science and Technology
Jiangnan University**

October 12, 2017

Cover letter

Dear editor,

Thank you very much for comments and correction from you and reviewers on our manuscript entitled "Composition and Immunostimulatory Properties of Extracellular DNA from Mouse Small Intestine Flora " (Manuscript NO.: 36350). The comments were insightful in improving our manuscript and valuable for our further study.

We have revised the manuscript in accordance with the comments and suggestion, and carefully proof-read the manuscript as highlighted. Meanwhile, the detailed corrections are listed in the "Response to review's comments" point by point.

Once again, thank you very much for your attention to our manuscript.

Yours sincerely,

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Response to review's comments

Question and Revision for Reviewer #02440884:

1 The title gives the focus on small intestine. The title does not reflect intestinal tissues and colon are used. The title should be changed.

Re: The title was revised as “Composition and Immunostimulatory Properties of Extracellular DNA from Mouse gut Flora”.

2 Page 1: abbreviation DC is given prior to the term: "dendritic cells"

Re: "dendritic cells" was used here.

3 Primers 338F/806R are not mentioned in the Materials and Methods.

Re: 338F/806R was added to Materials and Methods in part of Terminal restriction fragment length polymorphism (T-RFLP) analysis.

4. Morphological characterization of mucus layers is not convincing. Electronmicrocopy or multiimmunofluorescence should be regarded as additional techniques.

Re: Alcian blue pH 2-5-PAS (AB/PAS) is commonly used to identify acid and neutral mucins in mucus layer (Matsuo et al. 1997). We used this classic staining to locate mucus layer. Electronmicrocopy or multiimmunofluorescence was also widely used in this recently. It was more powerful method. Unfortunately, it was not available now. we would develop this method in our lab.

Matsuo K, Ota H, Akamatsu T, et al. Histochemistry of the surface mucous gel layer of the human colon.[J]. Gut, 1997, 40(6):782.

5. Figure 1, legend: in the Figure colon and small intestine should be marked/annotated.

Re: colon and small intestine was annotated in Figure 1.

Reviewer #02803865

1 "It has demonstrated": it has been demonstrated

Re: revised as suggestion.

2 "It's Worth noting" to note would be.

Re: revised as suggestion.

3 "in mucus layer" in the mucus layer

Re: revised as suggestion.

4 "one weeks" one week

Re: revised as suggestion.

5 Sentence "And the DNA //"

This sentence was revised as “Ethanol precipitation was used to concentrate DNA.”

6 Table 1: use of cap letters for Index/index; use of cap letters for table,

Re: Use of cap letters was revised in Table 1

7 Figure "because approximatively" delete because

Re: “because” was deleted here.

8 "This agreed with the finding" this is in agreement with the finding

Re: revised as suggestion.

9 "It was reported that" can be deleted from the sentence
Re: "It was reported that" can be deleted from the sentence
10 "little eDNA."
Re: it was revised as trace eDNA.

Reviewer # 02536349

1) AIM

The present study was aimed to demonstrate that specific bacteria might release bacterial extracellular DNA (eDNA) to exert ~~immunomodulatory~~ **immunomodulatory** (typographic error)

Re: revised as suggestion.

2) RESULTS

TOTO-1 iodide staining confirmed existence of eDNA in loose mucus layer of mouse colon and thin surface mucus layer of small intestine. Illumina sequencing analysis and T-RFLP revealed that the composition of the eDNA in the small intestine mucus was significantly different from that of the ~~intracellular DNA (iDNA)~~ **iDNA** of the small intestine mucus bacteria

(The abbreviation was already stated in methods section)

Re: revised as suggestion.

3) The eDNA induced ~~significant~~ **significantly** lower TNF- α /IL-10 and IL-6/IL-10 ratios than iDNA, suggesting the ~~importance~~ predominancy of eDNA for maintaining immune homeostasis of gut.

Re: revised as suggestion.

4) Our results indicated that degraded bacterial genomic DNA's ~~were~~ mainly released by gram-negative bacteria, especially by ~~the~~ *Bacteroidales-S24-7* and *Stenotrophomonas* genus ~~in-mucus-of-mice-small-intestine~~ **in gut mucus of mice**.

Re: revised as suggestion.

5) Composition and ~~Immunostimulatory~~ **Immuno-stimulatory** Properties of Extracellular DNA from Mouse ~~Small-Intestine~~ **Gut** Flora
(The title)

Re: revised as suggestion.

6) They ~~showed~~ decreased pro-inflammatory activity compared ~~with to~~ total gut flora genomic DNA.

Re: revised as suggestion.

7) Key word: bacterial extracellular DNA; small intestine; flora; ~~immunestimulatory~~ **immune-stimulatory** property; mouse; **gut microbiota**.

Re: revised as suggestion.

8) Core tip: Our results revealed that degraded bacterial genomic DNA's ~~were~~ mainly released by gram-negative bacteria, especially by ~~the~~ *Bacteroidales-S24-7* and *Stenotrophomonas* genus in mucus of mice ~~small-intestine~~ **gut**. They ~~showed~~ decreased pro-inflammatory activity compared ~~with to~~ **genomic DNA of total gut flora**. Our study highlights the ~~bacterial-bacteria~~ **derived DNAs** play ~~an potential~~ **an important** role in modulating local immune response in ~~mice gut small-intestine~~ **mice gut**.

Re: revised as suggestion.

9) Because in the mucosal environment, dendritic cells (DCs), ~~DC~~ and enterocyte permanently monitor the bacterial burden and structure in the gut ^[5], it is conceivable that this physiologic interaction significantly contributes to gut homeostasis. It has demonstrated that extracted DNA of gut lumen flora limited potently regulatory T cell (Treg) induction by ~~lamina propria dendritic cells (DCs)~~ DCs of lamina propria, thus controlling the balance between Treg and effector T cell frequency and function ^[6].

Re: revised as suggestion.

10) This step was repeated ~~for~~ twice and the supernatant was pooled.

Re: revised as suggestion.

11) ~~And Then~~ the DNA is concentrated by alcohol precipitation

Re: this sentence was revised to “Then ethanol precipitation was used to concentrate DNA”.

12) ~~mucus bacterial genomic DNA~~ genomic DNA of mucoid bacteria.

Re: revised as suggestion.

13) As shown in Figure 1, AB-PAS staining ~~show~~ showed apparent differences

Re: revised as suggestion.

14) (23 °C, 12h/12h light/dark, 50% humidity, *ad libitum* access to normal chow and water) for one ~~weeks~~ week prior to experimentation.

Re: revised as suggestion.

15) Table 1: ~~the Analysis~~ The analysis of diversity index

Re: revised as suggestion.

16) Analysis at the genus level (Figure 3 and ~~table S3~~ Table 3) provided more detailed information.

Re: Table S3 should be Table S1.

17) In the present study, we extracted eDNA from mice ~~small-intestinal~~ small intestinal and colon established a suitable PCR-TRFLP protocol to distinguish the microorganism diversity, which consisted of eDNA and iDNA.

Re: revised as suggestion.

18) These eDNAs might also be released by bacteria killed by antimicrobial peptides (~~AMPs~~) secreted by Paneth cells residing at the base of the small intestinal crypts (just one usage, no need for abbrev.)

Re: revised as suggestion.

19) Analysis at the genus level (Figure 3 and table S3) provided more detailed information.

(Table 2 and 3 are missing)

Re: Table S3 should be Table S1.

20) ~~It's worth~~ It is worth noting that intestinal epithelial cells do not respond equally to bacterial DNA.

Re: revised as suggestion.