

January 6, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 7642-review.doc).

Title: Emerging roles of lactic acid bacteria in protection against colorectal cancer

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Name of Journal: *World Journal of Gastroenterology*

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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 reviewers.

A "core tip" section has been added as suggested. All other editorial changes have been addressed as follows:

Reviewer 1: Dear Authors Your review manuscript contains results of recent studies about probiotics and colon cancer. I think it that the contents are easy to understand for beginners and are also suitable for the journal. However, the manuscript, particularly in names of bacteria and references, has many typos. --- in Epigenetic Targeting Produces by LAB You mentioned about effects of SCFAs. However, main end products of LABs are lactic acid and/or ethanol+CO₂. Please add more explanation about it. If you can, please read and add the bellow papers to your discussion and references. Jounai K, et al. (2012). PlosOne 7: e32588. Segawa S. et al. (2011). PlosOne 6: e23278. Yan, F. et al. (2011). J Clin Invest 121, 2242. (Excuse me, my English is not good. So I cannot check language evaluation,) Thank you.

Response: The reviewer raised very good points. We agreed with the reviewer that main end products of LABs are lactic acid and/or ethanol + CO₂. In addition, according to the published data by Duncan in *Appl Environ Microbiol* in 2004, lactic acid is also a type of short chain fatty acids (SCFAs), which are also a well-known class of epigenetic drugs known as histone deacetylase inhibitors (HDACi) that have a central role as anti-cancer agents with strong anti-proliferative effects on tumour cells.

As for the papers the reviewer suggested, we also added the suggested reference, i.e. Jounai K, et al. (2012). Plos One 7: e32588 in our references (39). Thank you.

Reviewer 2: Overall the review covers multiple aspects of LAB biology. However I feel a more in-depth discussion of LAB and innate signals that drive immune responses and discussion on induction of Th2 versus foxp3+ treg induction. The Microbiome is an immensely popular topic and in the news a lot recently, Can the authors address and review studies that show dysbiosis of gut bacteria after LAB administration and how this impacts disease.

Response: The reviewer raised great points. In this review, we discussed about the DCs immunoreactions through Toll-like receptor 2, IL-12 and TNF- α signals changes during innate and adaptive pathogenic immune responses. The innate immune response was related to lipoteichoic acid (LTA). In term of Th2 versus foxp3+ treg induction, we were unable to find useful information that is associated with the roles of LAB in colorectal cancer.

Based on available research and clinical data, there are four general causes of intestinal dysbiosis: putrefaction, fermentation, deficiency and sensitization. I am sure that LAB would have some roles in affecting dysbiosis of gut bacteria. This would be very interesting topic to discuss, however, which perhaps exceed the scope of the current review.

Reviewer 3: This is a review on the emerging roles of lactic acid bacteria in colorectal cancer. My comments on this paper are: (1) This is not a systematic review. The authors failed to do a systematic search on all the published articles related to the topic under review. There is a potential that the authors' potential biases are being introduced into the paper. (2) The Abstract contains very little information. (3) The authors used the term 'colon' sometimes to mean the colon but sometimes to mean the colorectal ".... known as 'gut microbiota' plays an critical role in colon carcinogenesis [4, 5]". When I referred to references 4 and 5, these studies were conducted on colorectal cancer instead of colonic cancer. (4) There are occasional grammatical mistakes. (5) Reference 3 is incomplete.

Response: We really appreciate the comments of reviewer. Our responses are as follows: (1) We have made corresponding changes to make it more systematic. (2) The reviewer raised a very good point and we really appreciate. As for this abstract, we were trying to explain the significance of LAB in protection against colorectal cancer and show the brief introduction of what we were going to discuss. (3) We appreciate such an important point. We have change it in the revised manuscript. (4) Thank you very much for reminding us. We have carefully checked our manuscript and corrected those grammatical mistakes. (5) Reference 3 has been completed.

Reviewer 4:

Critique 1: This manuscript presents a very interesting review of all of the different manners in which LABs can exert protective effects against cancer. The topic is clearly relevant and the review informative. However, there are several errors in the text that need to be corrected and small issues that should be clarified or improved. My specific comments are:

-I would suggest to change the title to something like "Emerging roles of lactic acid bacteria in protection against colorectal cancer", otherwise it seems like LABs are a cause of cancer

Response: We greatly appreciate the reviewer's suggestion, and have changed the title in the revised manuscript. The new title becomes: "Emerging roles of lactic acid bacteria in protection against colorectal cancer".

Critique 2: the way in which the authors employ the term "probiotic" is not always correct. The FAO/WHO definition of probiotics is "live microorganisms which when administered in adequate amounts confer a health benefit on the host". It also states that a probiotic needs to be taxonomically defined (at the genus, species and strain level), and that safety assessment and efficacy research in

humans needs to be available. Therefore the term should not be applied to "LABs" in a generic manner, as in the Abstract sentence stating "For example, lactic acid bacteria (LAB), the most common probiotics in gut microbiota...", and in several other sentences of the introduction. LABs in general should rather be treated less categorically as a group of organisms which overall seem to have positive effects on the host, and the term "probiotic" should be reserved for specific strains with demonstrated, concrete benefits.

on the other hand, LABs are not defined by their beneficial effects, but rather by their metabolic capacity to produce lactic acid. Therefore the sentence "LAB are defined as useful probiotics which produce lactic acid as their major fermentation product" is incorrect and should be changed.

Response: The reviewer raised a great point. We also agreed with the reviewer about the definition of probiotics and LAB and thank for pointing out the confusion about this definition. We have changed the definition of LAB to ' As the most common types of microbes used as probiotics, Lactic acid bacteria (LAB) are comprised of an ecologically diverse group of microorganisms united by formation of lactic acid as the primary metabolite of sugar metabolism.'

Critique 3: the sentence "exopolysaccharides (EPS) of *L. acidophilus* and *L. rhamnosus* were antitumorigenic against HT-29 colon cancer cell death and that this activity was due to the activation of autophagic cell death" is unclear. The word "death" after "colon cancer cells" should probably be removed.

Response: We greatly appreciate the reviewer for pointing out the unclear sentence. We have removed the word 'death' from the sentence.

Critique 4: -"NADH oxidase/ preoxidase system" should be "NADH oxidase/ peroxidase system"

Response: We thank the reviewer for pointing out the error. We have corrected it.

Critique 5: the paragraph regarding LTA-related inflammation is confusing regarding inflammatory vs anti-inflammatory effects. In particular, IL-10 has an anti-inflammatory role, so the sentence stating "regulatory IL-10 inflammatory cytokines" is incorrect and should be changed. Moreover, the title of the paragraph, "LTA related inflammation protection during tumor development", is misleading, as the authors actually report that LTA is pro-inflammatory and that it is its modification or suppression that confers beneficial effects.

Response: We greatly appreciate the reviewer for pointing out the error and impropriety in our review. We have changed them in the revised manuscript. "regulatory IL-10 inflammatory cytokines" has been corrected to " regulatory IL-10 anti-inflammatory cytokines " and the title has been changed to "LTA related pro-inflammation protection during tumor development".

Critique 6: in the sentence stating "inhibiting tumor growth and increased survival", "increased" should be "increasing".

Response: We thank the reviewer for pointing out the error. We have corrected it.

Critique 7: in the sentence "Taken together, these studies provide convincing evidence demonstrating the important role of LAB and their byproducts in carcinogenesis processes.", as in the title, the reader would think that LAB cause carcinogenesis. It should be changed to something like "in the protection against carcinogenesis processes".

Response: It is really grateful for the reviewer to point out the impropriety. We have changed this sentence into " Taken together, these studies provide convincing evidence demonstrating the

important role of LAB and their byproducts in the protection against carcinogenesis processes "

Critique 8: the sentence "It is worth noting that some progress has been made in identification of "bacterial" biomarkers for cancer detections" is also misleading as the described biomarker is not bacterial but encoded in human cells.

Response: We greatly appreciate the reviewer for pointing out this impropriety. We have changed this sentence into "It is worth mentioning that some progress has been made in identification of "bacterial biomarkers" for cancer detections ". We used the personification here to explain the importance of those bacteria.

Critique 9: the sentence "Nevertheless, LABs' demonstrated functions on repairing defective apoptotic processes or controlling cell proliferation in cancer has made them an attractive tool for treating gut dysbiosis associated with colon cancers" is also puzzling. Gut dysbiosis refers to an unbalanced bacterial community in the gut, whereas the beneficial effects of LAB alluded to in the sentence refer to antitumoral activities, and not to restructuring of the microbiota.

Response: We greatly appreciate the reviewer for pointing out this impropriety. We have changed this sentence into "Nevertheless, LABs' demonstrated functions on repairing defective apoptotic processes or controlling cell proliferation in cancer has made them an attractive tool for helping treat colorectal cancers"

Reviewer 4: I would congratulate with the Authors for this manuscript. It is well written, easy to read, in good english style and with very useful figures.

Response: We thank the reviewer very much for the certainty of our work. We really appreciate it.

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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