We recently read the paper entitled “Prevention of enteric bacterial infections and modulation of gut microbiota with conjugated linoleic acids producing Lactobacillus in mice,” by Peng et al. published in Gut Microbes with interest. We were very interested in the descriptions of some of the recombinant Lactobacillus casei strains in the manuscript and, in particular, those containing the mcra gene (myosin-cross-reactive protein, MCRA) which were described as those which produce conjugated linoleic acid (CLA), however, from previous lines of evidence these strains do not produce CLA but rather the hydroxyl fatty acid.

In the paper, Peng et al. characterized a genetically modified L. casei strain and evaluated its health-associated benefits on the host in murine model. The research is valid and important for probiotic development. Unfortunately, the overexpressed gene, myosin cross-reactive antigen gene (mcra) from L. rhamnosus GG, was claimed as linoleate hydratase, which converts linoleic acid to 10-hydroxy-cis-12-octadecenoic acid (10-HOE), and 10,13-dihydroxyoctadecanoic acid (10,13-DiHOA). Subsequently, a number of publications has confirmed the presence of MCRA in numerous bacteria (especially in lactic acid bacteria such as L. plantarum, L. acidophilus, L. rhamnosus, Bifidobacterium breve, etc), and that it is an oleate hydratase or linoleate hydratase and not a linoleate isomerase.

Indeed, CLA production in Lactobacillus, at least in L. plantarum, is generally a multiple-step reaction with multiple intermediates, such as 10-HOE, 10-oxo-cis-12-octadecenoic, 10-oxo-trans-11-octadecenoic acid, and 10-hydroxy-trans-11-octadecenoic acid. The reactions are catalyzed by a three component enzyme complex, including MCRA, short-chain dehydrogenase/oxidoreductase (DH) and acetoacetatedecarboxylase (DC). The source of the L. rhamnosus mcra gene for these experiments in the paper was L. rhamnosus GG which itself is not a CLA producer. As such it lacks the genetic determinants necessary for CLA production. Moreover, the L. rhamnosus mcra gene used in the paper has been previously identified as linoleate hydratase, where detailed information was presented including the products formed from oleic acid and linoleic acid, respectively. In contrast, in the original paper published by the authors, where the recombinant L. casei was originally constructed, neither CLA nor fatty acid products were presented. Therefore, description of MCRA as a “linoleate isomerase” is not appropriate in the two publications. Indeed, this expressing clone would more likely make 10-HOE rather than CLA. If, however, the
recombinant strain does produce CLA, it would mean that the L. casei host strain would have to have DH and DC as well which could further metabolize the 10-HOE generated by MCRA. I would suggest that the authors take these comments into consideration.

In defense of the work, we would like to emphasize that 10-HOE is a novel functional fatty acid and has some protective effects on the host.14,15 In this respect, the results presented are valid and meaningful for 10-HOE and its producer but not for CLA in our opinion based on the evidence presented above.

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**References**

1. Kil KS, Cunningham MW, Barnett LA. Cloning and sequence analysis of a gene encoding a 67-kilodalton myosin-cross-reactive antigen of Streptococcus pyogenes reveals its similarity with class II major histocompatibility antigens. Infect Immun. 1994;62(6):2440–2449. doi:10.1128/IAI.62.6.2440-2449.1994.

2. Rosson RA, Grund AD, Deng MD, Sanchez-Riera F Linoleate isomerase. United States. Patent 6743609. 2004.

3. Volkov A, Liavonchanka A, Kamneva O, Fiedler T, Goebel C, Kreikemeyer B, Feussner I. Myosin cross-reactive antigen of Streptococcus pyogenes M49 encodes a fatty acid double bond hydratase that plays a role in oleic acid detoxification and bacterial virulence. J Biol Chem. 2010;285(14):10353–10361. doi:10.1074/jbc.M109.081851.

4. Kishino S, Takeuchi M, Park SB, Hirata A, Kitamura N, Kusinawa J, Kiyono H, Iwamoto R, Isobe Y, Arita M, et al. Polysaturated fatty acid saturation by gut lactic acid bacteria affecting host lipid composition. Proc Natl Acad Sci USA. 2013;110 (44):17808–17813. doi:10.1073/pnas.1312937110.

5. Yang B, Chen H, Gu Z, Tian F, Ross RP, Stanton C, Chen YQ, Chen W, Zhang H. Synthesis of conjugated linoleic acid by the linoleate isomerase complex in food-derived lactobacilli. J Appl Microbiol. 2014;117 (2):430–439. doi:10.1111/jam.12524.

6. Chen YY, Liang NY, Curtis JM, Günzle MG. Characterization of linoleate 10-hydratase of Lactobacillus plantarum and novel antifungal metabolites. Front Microbiol. 2016;7:1561. doi:10.3389/fmicb.2016.01561.

7. Yang B, Qi H, Gu Z, Zhang H3, Chen W, Chen H, Chen YQ. Characterization of the triple-component linoleic acid isomerase in Lactobacillus plantarum ZS2058 by genetic manipulation. J Appl Microbiol. 2017;123(5):1263–1273. doi:10.1111/jam.13570.

8. Yang B, Chen H, Song Y, Chen YQ, Zhang H, Chen W. Myosin-cross-reactive antigens from four different lactic acid bacteria are fatty acid hydratases. Biotech Lett. 2013;35(1):75–81. doi:10.1007/s10529-012-1044-y.

9. Volkov A, Khoshnevis S, Neumann P, Herrfurth C, Wohlwend D, Ficner R, Feussner I. Crystal structure analysis of a fatty acid double-bond hydratase from Lactobacillus acidophilus. Acta Crystallogr D. 2013;69 (Pt4):648–657. doi:10.1107/S0907444913000991.

10. Rosberg-Cody E, Liavonchanka A, Göbel C, Ross RP, O’Sullivan O, Fitzgerald GF, Feussner I, Stanton C. Myosin-cross-reactive antigen (MCRA) protein from Bifidobacterium breve is a FAD-dependent fatty acid hydratase which has a function in stress protection. BMC Biochem. 2011;12:9. doi:10.1186/1471-2091-12-9.

11. O’Connell KJ, Motherway MO, Hennessey AA, Brodhun F, Ross RP, Feussner I, Stanton C, Fitzgerald GF, van Sinderen D. Identification and characterization of an oleate hydratase-encoding gene from Bifidobacterium breve. Bioengineered. 2013;4 (5):313–321. doi:10.4161/bioe.24159.

12. Xu H, Lee HY, Hwang B, Nam JH, Kang HY, Ahn J. Kinetics of microbial hydrogenation of free linoleic acid to conjugated linoleic acids. J Appl Microbiol. 2008;105(6):2239–2247. doi:10.1111/j.1365-2672.2008.04200.x.

13. Peng M, Tabashsum Z, Patel P, Bernhardt C, Biswas D. Linoleic acid overproducing Lactobacillus casei limits growth, survival and virulence of Salmonella typhimurium and enterohaemorrhagic Escherichia coli. Front Microbiol. 2018;9:2663. doi:10.3389/fmicb.2018.02663.

14. Ikeguchi S, Izumi Y, Kitamura N, Kishino S, Ogawa J, Akaie A, Kume T. Inhibitory effect of the gut microbially produced linoleic acid metabolites, 10-oxo-trans-11-octadecenoic acid and 10-hydroxy-cis-12-octadecenoic acid, on BV-2 microglial cell activation. J Pharmacol Sci. 2018;138(1):9–15. doi:10.1016/j.jspham.2018.06.015.

15. Miyamoto J, Igarashi M, Watanabe K, Karaki SI, Mukoyama H, Kishino S, Li X, Ichimura A, Irie J, Sugimoto Y, et al. Gut microbiota confers host resistance to obesity by metabolizing dietary polysaturated fatty acids. Nat Commun. 2019;10(1):4007. doi:10.1038/s41467-019-11978-0.