

Accepted Manuscript

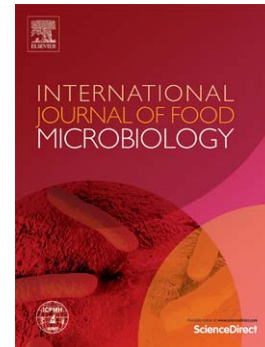
Letter to the editor on 'Enhancing vitamin B₁₂ content in soy-yogurt by *Lactobacillus reuteri*, IJFM. 206:56–59'

Pekka Varmanen, Paulina Deptula, Bhawani Chamlagain, Vieno Piironen

PII: S0168-1605(16)30145-3
DOI: doi: [10.1016/j.ijfoodmicro.2016.03.029](https://doi.org/10.1016/j.ijfoodmicro.2016.03.029)
Reference: FOOD 7182

To appear in: *International Journal of Food Microbiology*

Received date: 2 February 2016
Accepted date: 28 March 2016



Please cite this article as: Varmanen, Pekka, Deptula, Paulina, Chamlagain, Bhawani, Piironen, Vieno, Letter to the editor on 'Enhancing vitamin B₁₂ content in soy-yogurt by *Lactobacillus reuteri*, IJFM. 206:56–59', *International Journal of Food Microbiology* (2016), doi: [10.1016/j.ijfoodmicro.2016.03.029](https://doi.org/10.1016/j.ijfoodmicro.2016.03.029)

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Dear Editor,

A recent paper in IJFM (Gu et al., 2015) describes that the vitamin B₁₂ content of soy-yogurt can be enhanced through fermentation by *Lactobacillus reuteri*. The authors suggest that *L. reuteri* fermented soy product could be served as an alternative food for the aged and vegetarians with a high risk of low vitamin B₁₂ content in serum. However, we have concerns about this study.

In humans, diet is considered the most likely source of vitamin B₁₂, but the presence of B₁₂ in various foods is misinterpreted due to obscurity in the methodology used to measure the compound and particularly in the ability to discriminate between active forms of B₁₂ and other corrinoids (Degnan et al. 2014). While earlier studies reported that *L. reuteri* produces vitamin B₁₂ (Taranto et al. 2003; Santos et al. 2008), recent studies using mass spectrometry to accurately differentiate between different cobamides have revealed that *L. reuteri* produces only pseudovitamin form of B₁₂ (Santos et al. 2007; Crofts et al. 2013; Degnan et al. 2014). From the perspective of human metabolism, the difference between vitamin and pseudovitamin is crucial, as the transporter protein in the human gastrointestinal tract, the intrinsic factor, has very low affinity to pseudovitamin (Stupperich and Nexø, 1991), making it virtually unavailable to humans.

While the HPLC/UV (Gauch et al., 1992) or UHPLC/UV (Chamlagain et al., 2015) methods can be used for separation of the vitamin-B₁₂-like compounds, Gu et al. (2015) do not document the distinction between the vitamin B₁₂ and the pseudovitamin. As production of active B₁₂ vitamin by *L. reuteri* would be surprising and contradict current knowledge based on molecular studies (Santos et al. 2007; Crofts et al. 2013), the distinction between active and pseudo forms is crucial for the assessment of this study.

Furthermore, based on the results obtained with microbiological method (Denter and Bisping, 1994) Gu et al. report production (10%) of vitamin analogs by *L. reuteri*. By definition "analogs" give positive result in bioassay but lack vitamin B₁₂ activity and include other corrinoids, such as pseudovitamin B₁₂ and factor A, as well as non-corrinoid compounds, such as DNA, deoxyribonucleotides and deoxyribonucleosides (Ball 2006). In the protocol by Denter and Bisping (1994) all the corrinoid compounds, including vitamin B₁₂ and pseudovitamin, are destroyed by heating, and the "analogs" supporting growth of the test organism are accordingly non-corrinoid compounds (Ball 2006). Thus, in our opinion the term "vitamin analogs" is used in a misleading context by Gu et al., since here it cannot include pseudovitamin B₁₂.

L. reuteri has a long history of safe use and could be potentially used for in situ fortification of foods with vitamin B₁₂. However, for this purpose it is of vital importance to make a distinction between strains producing the active and the pseudo form of the vitamin.

Yours sincerely,

Pekka Varmanen, Paulina Deptula, Bhawani Chamlagain and Vieno Piironen

Department of Food and Environmental Sciences,
University of Helsinki

References:

Ball, G.F.M.(2006). Chapter 18: Microbiological Methods for the Determination of the B-Group Vitamins in Vitamins in foods: Analysis, bioavailability and stability. Boca Raton: CRC Press.

- Chamlagain, B., Edelmann, M., Kariluoto, S., Ollilainen, V., & Piironen, V. (2015). Ultra-high performance liquid chromatographic and mass spectrometric analysis of active vitamin B₁₂ in cells of *Propionibacterium* and fermented cereal matrices. *Food chemistry*, 166, 630-638.
- Crofts, T. S., Seth, E. C., Hazra, A. B., and Taga, M. E. (2013). Cobamide structure depends on both lower ligand availability and CobT substrate specificity. *Chemistry & Biology*, 20(10), 1265-1274.
- Denter, J., Bisping, B., 1994. Formation of B-vitamins by bacteria during the soaking process of soybeans for tempe fermentation. *Int. J. Food Microbiol.* 22, 23–31.
- Gauch, R., Leuenberger, U., Mueller, U., 1992. Bestimmung der wasserloeslichen Vitamine B1, B2, B6 und B₁₂ in Milch durch HPLC. *Z. Lebensm.-Unters. Forsch.* 195, 312–315.
- Gu, Q., Zhang, C., Song, D., Li, P., & Zhu, X. (2015). Enhancing vitamin B 12 content in soy-yogurt by *Lactobacillus reuteri*. *International journal of food microbiology*, 206, 56-59.
- Santos, F., Vera, J. L., Lamosa, P., de Valdez, G. F., de Vos, W. M., Santos, H., Sesma, F. & Hugenholtz, J. (2007). Pseudovitamin B₁₂ is the corrinoid produced by *Lactobacillus reuteri* CRL1098 under anaerobic conditions. *FEBS letters*, 581(25), 4865-4870.
- Santos, F., Vera, J. L., van der Heijden, R., Valdez, G., de Vos, W. M., Sesma, F., & Hugenholtz, J. (2008). The complete coenzyme B₁₂ biosynthesis gene cluster of *Lactobacillus reuteri* CRL1098. *Microbiology*, 154(1), 81-93.
- Stupperich, E., & Nexø, E. (1991). Effect of the cobalt-N coordination on the cobamide recognition by the human vitamin B₁₂ binding proteins intrinsic factor, transcobalamin and haptocorrin. *European Journal of Biochemistry*, 199(2), 299-303.
- Taranto, M. P., Vera, J. L., Hugenholtz, J., De Valdez, G. F., & Sesma, F. (2003). *Lactobacillus reuteri* CRL1098 produces cobalamin. *Journal of bacteriology*, 185(18), 5643-5647.
- Degnan, P. H., Taga, M. E., & Goodman, A. L. (2014). Vitamin B₁₂ as a Modulator of Gut Microbial Ecology. *Cell metabolism*, 20(5), 769-778.