The Mechanistic Effects of Human Digestion on Magnesium Oxide Nanoparticles: Implications on Probiotics *Lactobacillus rhamnosus GG* and *Bifidobacterium bifidum VPI 1124*

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The gastrointestinal (GI) microbiota is linked to intestinal homeostasis and crucial for overall host health. When the microbiota is impaired or altered, common inflammatory and metabolic disorders are more likely to be developed. Exogenous factors such as diet have been reported to highly impact microbial dysbiosis. Because of their unique physicochemical properties, metal oxide nanoparticles (NPs) are widely used as food additives. MgO NPs are an EU-approved food additive (E 530) present in milk, canned food, and dietary supplements, and can be used as an anti-acid and laxative. In high doses, MgO act as antimicrobial and antibiofilm. The relationship between MgO NPs and the human intestinal microbiota, however, has not been thoroughly investigated.

The aim of this study is to investigate the impact of physiologically relevant concentrations (267 mg/day) of food additive MgO NPs (65 nm) on the viability, growth, and biofilm development of two human gut-derived commensal bacteria: the Gram-positives *Lactobacillus rhamnosus GG* and *Bifidobacterium bifidum VPI 1124*. Considering the biological implications of human digestion, MgO NPs were subjected to an *in vitro* digestion (with gastric enzymes; inorganic salts; changing pH and temperature), then diluted in bacterial growth medium at low (L-MgO; 4.3×10^{-5} mg/mL), medium (M-MgO; 4.3×10^{-4} mg/mL) and high (H-MgO; 4.3×10^{-3} mg/mL) physiologically-relevant concentrations to treat both planktonic (free cells) and biofilms (sessile bacterial communities) of *L. rhamnosus* and *B. Bifidum*.

The obtained results indicate that after *in vitro* digestion and due to pH oscillations, MgO NPs partially dissociate into Mg^{2+}, but some particles remain as crystal structures in the nm range. The presence of M- and H-MgO significantly increased cell growth of both *L. rhamnosus* and *B. bifidum* when growing as biofilms but not as planktonic cells. Although no differences in biofilm morphology were observed by confocal imaging, COMSTAT2® analysis detected a 1.5-fold increase in biomass of H-MgO samples compared to control. Interestingly, M- and H-MgO maximized the biofilm development of *L. rhamnosus,* but reduced *B. bifidum*. Hence, we suggest that the presence of released Mg^{2+} could be selectively used as a nutrient playing a major role in the described results. Moreover, no detrimental effects were detected after short-term exposures as interactions of MgO NPs-bacteria are not favored since both structures are negatively charged.