

SUPPLEMENTARY INFORMATION

Table S1. Blank measurements. Average of the hydrodynamic size (nm) measured with Nanosight and Nano Zetasizer, polydispersity index (Pdl), zeta potential values (mV) and electrophoretic mobility ($\mu\text{m}\cdot\text{cm}\cdot\text{V}\cdot\text{s}^{-1}$) of the different media used in the study. Data shown as mean \pm SEM.

Blanks	Primary particle diameter (nm)	Hydrodynamic size (Nanosight)	Hydrodynamic size (Nano Zetasizer)	Pdl	Zeta Potential (mV)	Electrophoretic mobility ($\mu\text{m}\cdot\text{cm}/\text{V}\cdot\text{s}$)
Water	N/A	N/A	N/A	N/A	N/A	N/A
BHI+0.1% Agar	N/A	230.6 \pm 194.3	943.57 \pm 263.48	0.54 \pm 0.10	-509.8 \pm 104.8	-0.41 \pm 0.09
Stomach Digesta	N/A	185.92 \pm 5.86	-767.11 \pm 108.5	0.69 \pm 0.07	-3.67 \pm 1.12	-0.28 \pm 0.09
Intestine Digesta	N/A	207.33 \pm 9.25	266.4 \pm 39.2	0.57 \pm 0.04	-65.3 \pm 0.95	-2.39 \pm 0.03

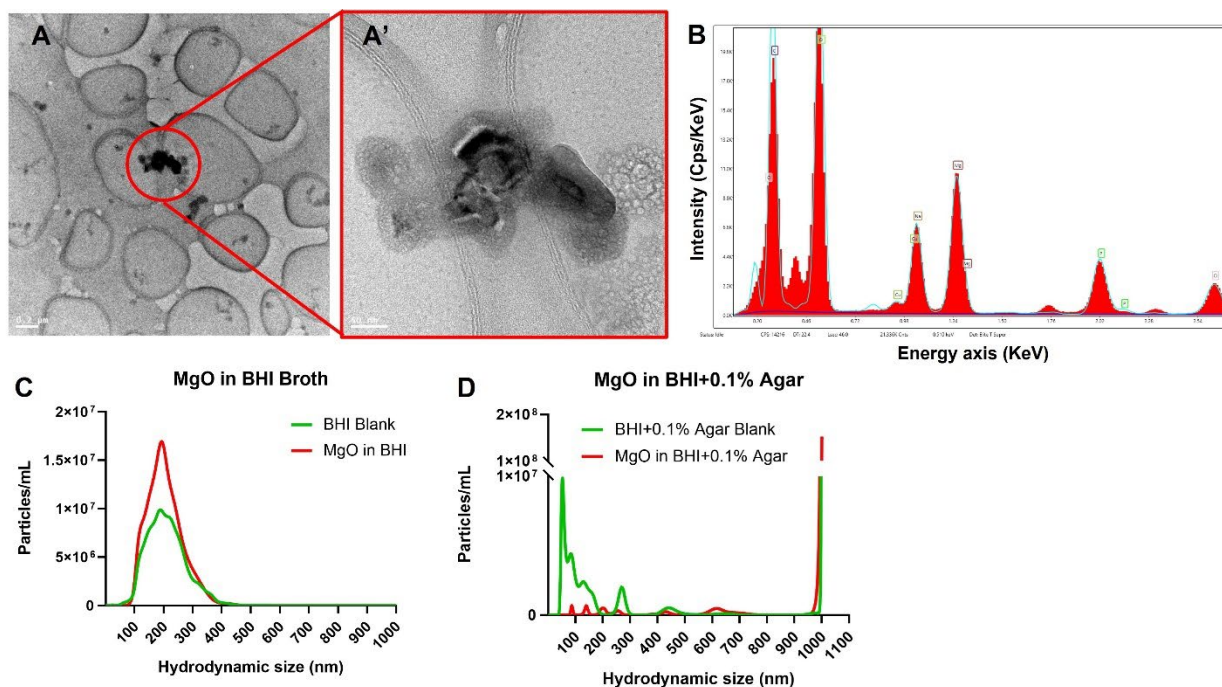


Figure S1. Effects of BHI and agar on food additive MgO. (A) TEM images of food additive MgO in BHI+0.1% Agar. (A') Zoom from the previous TEM image. (B) EDS analysis from image A' showing the presence of Mg. (C) Hydrodynamic size distribution of food additive MgO in BHI broth and (D) BHI+0.01% Agar analyzed with Nanosight.

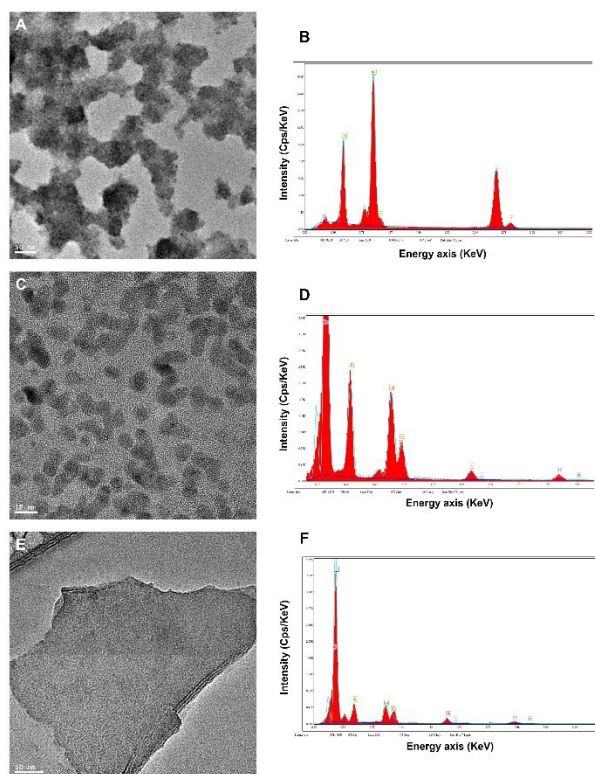


Figure S2. TEM-EDS analysis of blanks. TEM images of (A) stomach digesta; (C) intestine digesta; and (E) BHI+0.1% Agar. EDS analysis of the respective images containing (B) stomach digesta; (D) intestine digesta; (F) BHI+0.1% agar showing no Mg detectable.

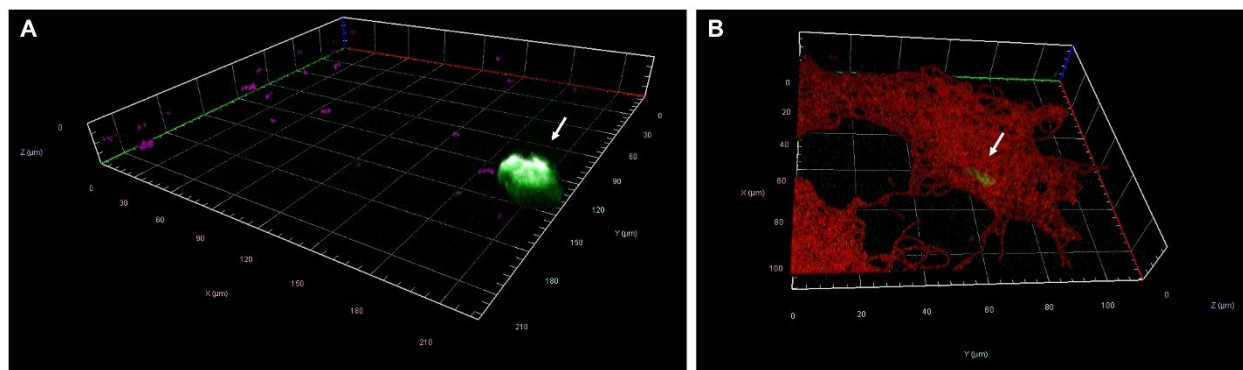


Figure S3. Confocal Images. 3D images of (A) *B. bifidum* (violet) and (B) *L. rhamnosus* (red) biofilms exposed to high concentrations of *in vitro* digested food additive MgO-NPs (bright green) for 24 hours. White arrows indicate aggregates of NPs.

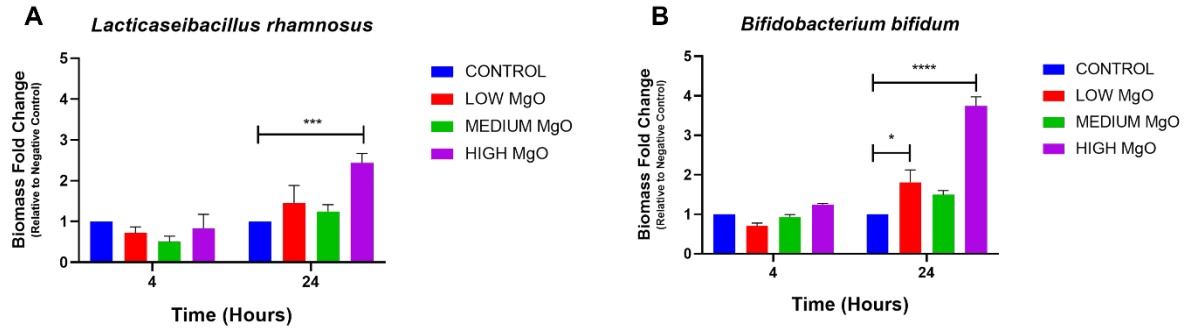


Figure S4. Comstat analysis of the confocal images. The biofilm biomass of (A) *L. rhamnosus* and (B) *B. bifidum* were imaged with confocal microscopy and measured using Comstat2 after exposures to low, medium and high concentrations of digested food additive MgO for 4 and 24 hours. Data is shown as mean±SEM and analyzed using Two-way ANOVA with Dunnett's post-test. (*) $p < 0.05$; (**) $p < 0.01$; (***) $p < 0.001$; (****) $p < 0.0001$.

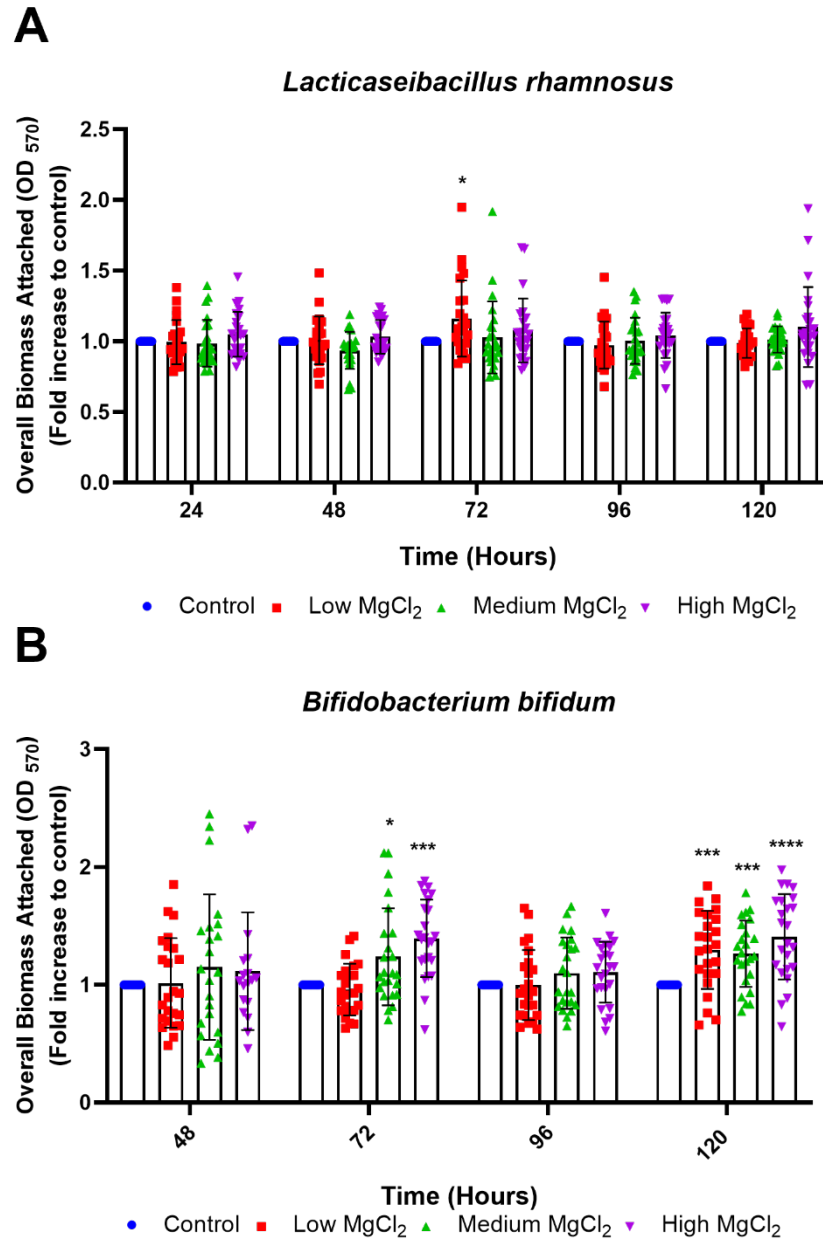


Figure S5. Fold increase of the overall biomass attached. The initial attachment and biofilm development of (A) *L. rhamnosus* and (B) *B. bifidum* were assessed using the Crystal Violet Assay after exposing both bacterial strains to low, medium and high concentrations of MgCl₂ up to 5 days. Data is shown as mean±SD and analyzed using Two-way ANOVA with Dunnett's post-test. (*) $p<0.05$; (**) $p<0.01$; (***) $p<0.001$; (****) $p<0.0001$.