Impact of food grade and nano-TiO2 on human gut microbiota

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Titanium dioxide is a white metal oxide employed as a pigment, which is commonly used in coatings of candies and chewing-gum. Food-grade TiO_2, referred to as E171 in Europe and INS171 in North America, includes a nano-sized fraction, representing up to 36% of the particles [1]. Due to concerns about TiO_2 nanoparticles (NPs) as potentially hazardous, at least by inhalation [2], the toxicity of ingested TiO_2 NPs are currently under investigation. However, the impact of confectionary titania additives has yet to be determined.

As a model of the human digestive system, we used a defined gut bacterial community, MET-1 (microbial ecosystem therapeutic-1), which contains 33 different bacterial species established from the collected stool of a healthy donor. The anaerobic consortium was batch cultured (n=30) for 48 h at 37°C in a starch-based medium [3]. To establish that the consortia was responsive to amendment, pancreatic amylase (37.5U/ml) was added to 9 of the cultures. Food-grade TiO_2 from several suppliers were used to amend the cultures at two realistic concentrations (based on a single unit of gum or popular confectionary; 100-250 ppm). In addition, purchased TiO_2 NPs (25 nm; P25) were used. All experiments were done in triplicate. The assessment of the impact of additives to the model digestive system employed assays to measure physiological, biochemical and molecular responses. Gas production was monitored using gas chromatography, and fatty acid methyl ester (FAME) analysis used the MIDI Sherlock Microbial Identification System protocol. DNA analysis included polymerase chain reaction denaturing gradient gel electrophoresis (PCR-DGGE) and 16S ribosomal RNA gene fragment 454-pyrosequencing.

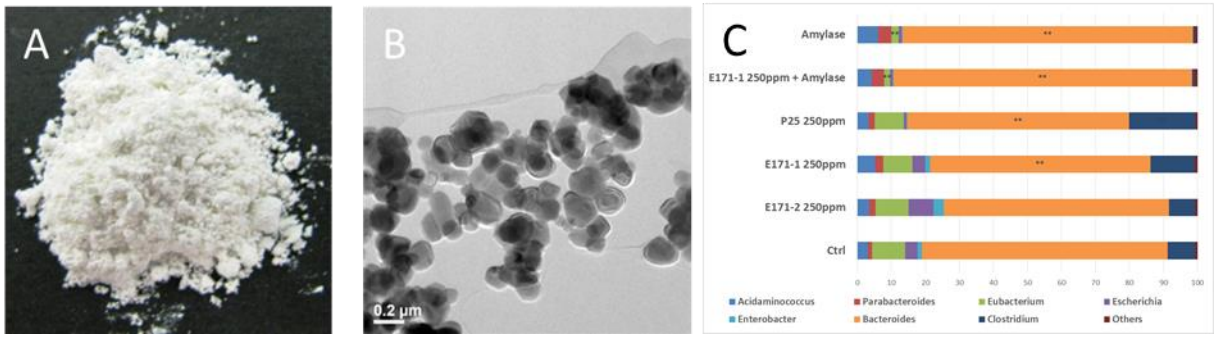

Fig. 1. A: Food grade TiO2 / B: TEM image of food grade TiO_2 / C: 454 pyrotag sequencing results

Our results showed that the consortium was clearly responsive to amendment since the addition of pancreatic amylase, which would have hydrolyzed a portion of the starch substrate, shifted fatty acid profiles, reducing some Gram negative and positive signatures, compared to controls. DNA analysis also demonstrated the significant reduction of sequences corresponding to *Eubacterium sp*. and *Clostridium cocleatum*, bacteria known for their starch-utilization pathways. In contrast to these results, at both concentrations, the tested TiO_2

particles had no impact on the model gut microbiota gas production nor on fatty acid composition. Only the food grade sample n°1 induced a small variation in gas composition, when tested at 250 ppm ($p<.05$) and this as well as P25 particle controls resulted in a limited shift in the saturated fatty acid composition (12:00 and 14:00, $p<.05$). PCR-DGGE profiles and phylogenetic distributions obtained from 454 pyrotag 16S rRNA gene sequencing confirmed the modest impact on the bacterial community by food grade n°1 and P25, with a significant decrease in sequences corresponding to the dominant *Bacteroides ovatus* (-10%) in favor of *C. cocleatum* (+10%; $p<0.05$).

Despite these minor shifts in the relative abundance of two members of the model gut consortium, taken together, we believe that food grade titania and TiO_2 NPs particles do not have a major impact on the human gut microbiota when tested at realistic concentrations. These results will be welcome news to consumers.

References

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