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► **To cite this version:**

William Dufefoi, Kristy Moniz, Emma Allen-Vercoe, Marie-Hélène Ropers, Virginia L. Walker. Impact of food grade and nano-TiO<sub>2</sub> on human gut microbiota. 13. Congrès international sur les matériaux nanostructurés "nano 2016", Aug 2016, Québec, Canada. hal-02793695

**HAL Id: hal-02793695**

**<https://hal.inrae.fr/hal-02793695>**

Submitted on 5 Jun 2020

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## Impact of food grade and nano-TiO<sub>2</sub> 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<sub>2</sub>, 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<sub>2</sub> nanoparticles (NPs) as potentially hazardous, at least by inhalation [2], the toxicity of ingested TiO<sub>2</sub> 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<sub>2</sub> 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<sub>2</sub> 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.

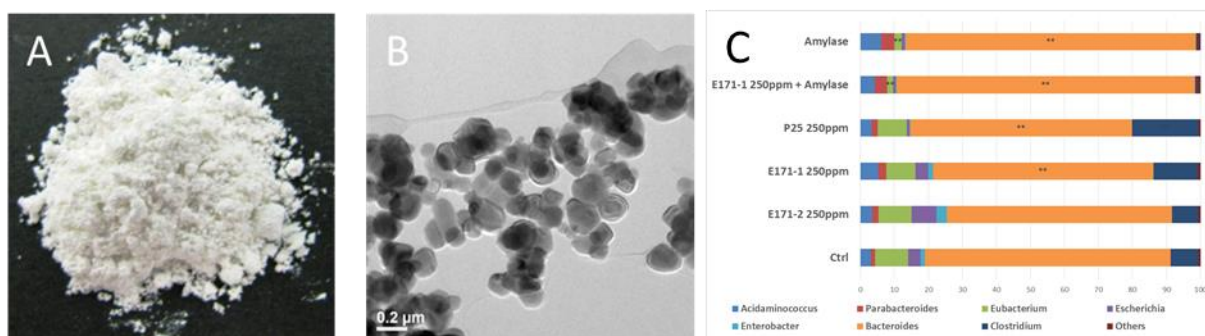


Fig. 1. A: Food grade TiO<sub>2</sub> / B: TEM image of food grade TiO<sub>2</sub> / C: 454 pyrotag sequencing results

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<sub>2</sub>

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<sub>2</sub> 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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### **Acknowledgements**

This work has been carried out in the framework of the Labex Serenade (ANR-11-LABX-0064) and of the A\*MIDEX project (ANR-11-IDEX-0001-02), funded by the «Investissements d’Avenir» French Government program managed by the French National Research Agency (ANR) and by the Natural Sciences and Engineering Research Council (Canada) to VKW.

We thank G. Cairns (Analytical Services Lab, Queen’s University), C. Carlucci and P. Das, for providing the initial help with the gas chromatography, the MET-1 community, and helpful assistance.