Unrevealing the effects of food-related engineered nanoparticles on the intestinal biofilm

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INTRODUCTION

The use of **nanoparticles** (**NPs**, material that is at least one dimension below 100 nm) (Fig. 1) in food and agricultural applications has raised in the last years a number of safety, environmental, ethical and policy **regulatory issues**[1].

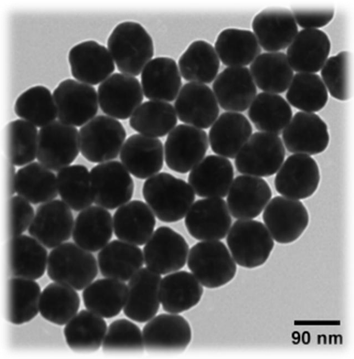

Figure 1 .Silver nanoparticles used in food-packaging application. http://nanocomposix.com/

At present, the major current food-related area of NPs application is **packaging**, and it has been estimated that the average person in a developed country consumes **over a trillion man-made food-related NPs every day**[2].
In addition, the growing awareness of the importance of the **gut microbiome** in health and disease, and the recognition that intestinal microflora exists as **biofilm**, highlights the need to consider how deeply NPs affect the gut biofilm-structures ecology and thus human health.

AIM

My PhD research, which is part of a bigger project **(NANOGUT)** supported by **Fondazione Cariplo**, aims to elucidate the effects of sub-lethal concentrations of food-related **nanoparticles (NPs)** on the **gut ecosystem,** potential toxicity mechanisms, and create the scientific know-how to develop leading edge methodologies for the nanosafety assessment.

METHODS

My project will develop an interactive in vitro gut ecosystem model composed of four interdependent components:

1. CACO-2 INTESTINAL EPITHELIAL CELL
2. ANAEROBIC MONO- AND MULTI-SPECIES INTESTINAL BIOFILM
3. SILVER NANOPARTICLES (AgNPs), chosen as the more representative NPs used in food packages.
4. THE PROBIOTIC BACTERIUM *Bacillus subtilis* subsp. *subtilis* (*natto*).

Acute and chronic effects of sub-lethal concentrations of AgNPs on the gut ecosystem are investigated.

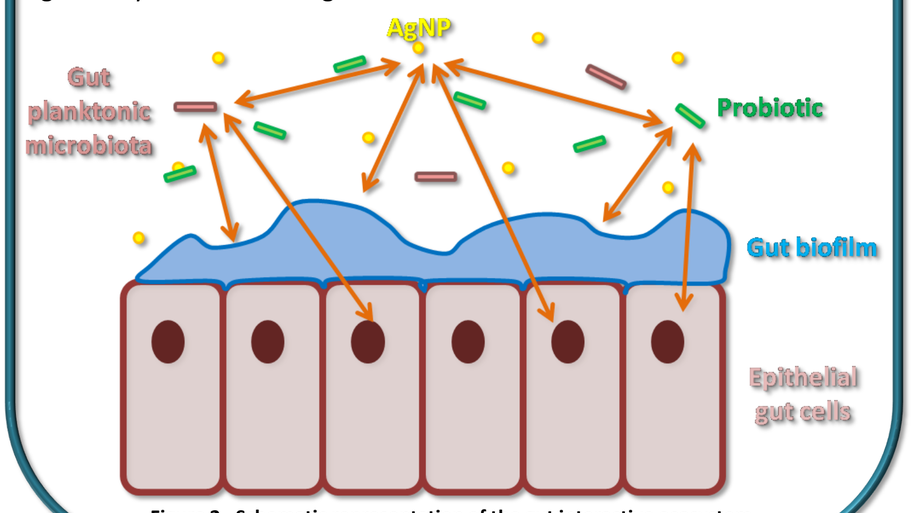

Figure 2 . Schematic representation of the gut interactive ecosystem.

SELECTION OF THE BEST MEDIUM

AgNPs concentration stability within three different microbiological media was investigated for 24 hours to establish the best medium that could guarantee the most reliable **AgNP bioavability** in liquid cultures[3].

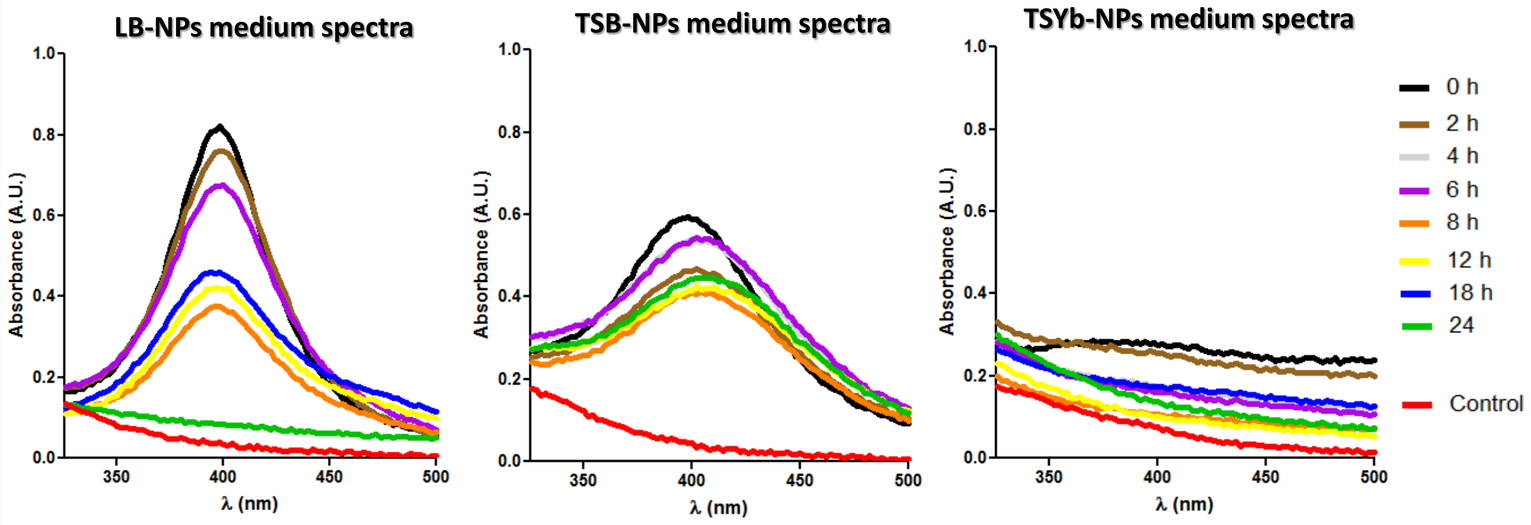

Figure 3 . Spectra of the three compared media acquired during the first 24 hours after AgNPs were added.

The expected **peak at 390 nm of AgNPs** in solution was compared with those of the negative control without nanoparticles. Tripric Soy Broth (TSB) was the medium where NPs concentration was more stable in time and was chosen to perform further experiments.

NPs EFFECTS ON PLANKTONIC GROWTH

Three different concentrations of AgNPs were added to planktonic cultures of *E. coli* (gut representative bacterium – Fig 4) and *B. subtilis* (probiotic) under **anaerobic conditions** to find out the highest sub-lethal concentration between 0.1 and 0.01 µg/mL to be used in the gut ecosystem model. Bacterial cellular activity with and without the chosen AgNPs concentration was than evaluated by measuring ATP bioluminescence.

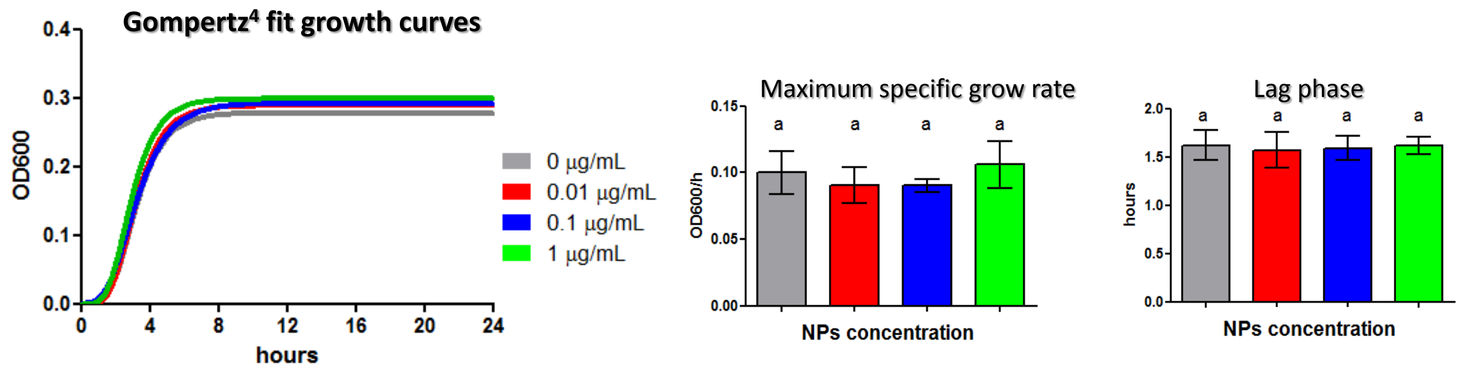

Figure 4 . On the left Growth curves of *E. coli* with different concentrations of AgNPs fitted with Gompertz model; on the right maximum specific growth rate and lag phase data analyzed with ANOVA test.

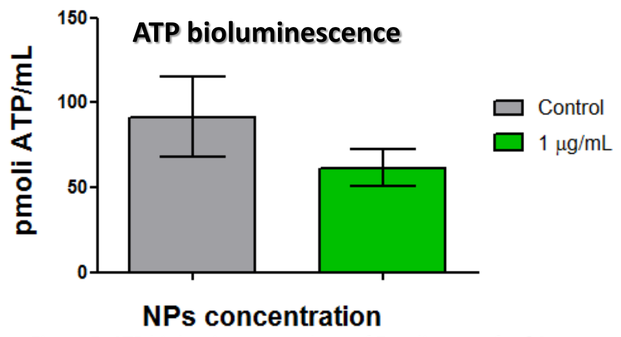

Figure 5. ATP bioluminescence data of E. coli grown for 24 hours in anaerobic conditions within an AgNPs concentration of 1µg/mL.

Results showed **no significative differences in planktonic growth** among the samples and control, so 1 µg/mL was the chosen concentration to be used. Bioluminescence assay values confirmed the choice.

GROWING A BIOFILM UNDER ANAEROBIC CONDITIONS

The next step of my PhD project was aimed to develop a **new method** to grow *Escherichia coli* biofilms under anaerobic conditions.
The method will be used in my future work to:

- Test nanoparticles effects on mono-species biofilms.
- Growing multispecies biofilms in anaerobic conditions.
- Reproduce the complex interactions of a gut interactive ecosystem.

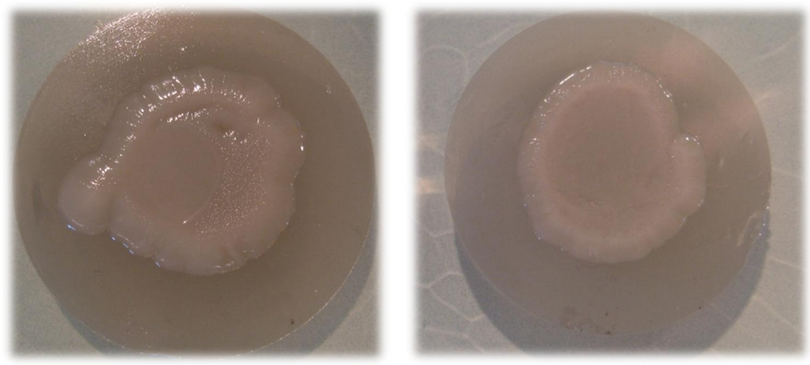

Figure 6 . Pictures of *E. coli* biofilms obtained under anaerobic conditions.

CONCTACTS AND ACKNOWLEDGEMENTS

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