Four Types of TiO$_2$ Reduced the Growth of Selected Lactic Acid Bacteria Strains

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Abstract: Food-grade titanium dioxide (TiO$_2$) containing a nanoparticle fraction (TiO$_2$ NPs) is widely used as a food additive (E171 in the EU). In recent years, it has increasingly been raising controversies as to the presence or absence of its harmful effects on the gastrointestinal microbiota. The complexity and variability of microbiota species present in the human gastrointestinal tract impede the assessment of the impact of food additives on this ecosystem. As unicellular organisms, bacteria are a very convenient research model for investigation of the toxicity of nanoparticles. We examined the effect of TiO$_2$ (three types of food-grade E171 and one TiO$_2$ NPs, 21 nm) on the growth of 17 strains of lactic acid bacteria colonizing the human digestive tract. Each bacterial strain was treated with TiO$_2$ at four concentrations (60, 150, 300, and 600 mg/L TiO$_2$). The differences in the growth of the individual strains were caused by the type and concentration of TiO$_2$. It was shown that the growth of a majority of the analyzed strains was decreased by the application of E171 and TiO$_2$ NPs already at the concentration of 150 and 300 mg/L. At the highest dose (600 mg/L) of the nanoparticles, the reactions of the bacteria to the different TiO$_2$ types used in the experiment varied.

Keywords: TiO$_2$ NPs; nanoparticles; E171; bacterial; microbiome

1. Introduction

Food additives are widely used in the food industry to improve the flavor, smell, color, and shelf life of food [1]. Food-grade TiO$_2$ (E171) is a white pigment and brightening agent used in substantial amounts in confectionery, white sauce, and frosting [2,3]. It should be noted that E171 contains different sized TiO$_2$ particles, including nanoparticles <100 nm. Dudefoi et al. [4] detected a level of 17–36% of TiO$_2$ NPs (NPs—nanoparticles) in seven purchased food samples. Similarly, in their research, other authors showed the content of the <100 nm TiO$_2$ nanoparticle fraction in the range from 10 to 49% [3,5,6], whereas a recent a study conducted by Geiss et al. [7] demonstrated that the level of nanoparticles exceeded 50%. It has been reported that the pigment is contained in over 900 food products worldwide. The daily consumption of the compound depends on the age, body weight, and place of residence. For instance, 0.2–0.7 mg and 1 mg TiO$_2$/kg body weight (b.w.) are consumed per day in the USA and Great Britain, respectively. However, due to their lower body weight and the higher consumption of sweets, children under 10 years of age have a higher daily consumption of TiO$_2$.
age may ingest from 1–2 mg to 2–3 mg TiO$_2$/kg per day in the USA and Great Britain, respectively [2,8]. In turn, 0.5–0.7 mg TiO$_2$/kg b.w./day [9] and 0.2–0.4 mg TiO$_2$/kg b.w./day are consumed in the Netherlands and Europe, respectively [10].

In recent years, the use of titanium dioxide as a food additive (E171) has raised considerable controversy [11–13]. For instance, France was the first country to prohibit the use of this food additive for fear of its potential harmfulness [11]. Its presence is increasingly often associated with disorders of the intestinal barrier, including intestinal dysbiosis [4,14,15]. Exposure to the compound may induce chronic changes in the composition and/or metabolic activity of commensal bacteria (intestinal dysbiosis) that exert an effect on the immune system [16]. The bacteria may come into contact with TiO$_2$ NPs through both food consumption and intestinal passage, which may affect the host’s microbiota and health [17,18]. Changes in the intestinal microbiota can be induced by stress or inadequate diet and may be associated with such diseases as obesity, inflammatory bowel disease, and diabetes [19].

The microbiota in the gastrointestinal tract plays an especially important role as a basis of the health of the host. The commensal microbial community not only contributes to the digestion of dietary fiber but also interacts strongly with epithelial cells to maintain an effective gut barrier separating the organism from the external environment of the host [16,20]. The small size of NPs allows them to cross the cell barrier in the gastrointestinal tract or the mucus layer [21,22].

Determination of the toxicity of TiO$_2$ NPs may be influenced by interfacial electrostatic interactions and the physicochemical parameters of the medium (pH, size, ionic strength, temperature, electrolyte composition, and light exposure) [23,24]. There are only few studies assessing the interactions between NPs and gut microbiota; they are mainly focused on direct interactions with intestinal epithelial cells [25,26].

The aim of the present study was to assess the response of selected lactic acid bacteria strains to E171/TiO$_2$ NPs, depending on their concentration, size, and applicability.

2. Materials and Methods

2.1. Nanoparticles

The investigations were carried out with the use of four types of TiO$_2$. Food-grade TiO$_2$ (E171) was purchased from three suppliers from Poland: Warchem Sp z o.o., Marki; Biomus, Lublin; and Food Colors, Piotrków Trybunalski (No. 1, 2, and 3, respectively). For comparison, TiO$_2$ NPs were purchased from Sigma-Aldrich (CAS Number: 718467-100G. Titanium (IV) oxide, nanopowder, 21 nm) (No. 4) (Figure 1).

2.2. Sample Preparation

Aqueous solutions of each type of TiO$_2$ were prepared in glass bottles with deionized water at the concentrations of 60, 150, 300, and 600 mg/L (a, b, c, and d, respectively). Subsequently, each sample was sonicated for 30 min. in an ultrasonic bath (25 °C, 250 W, 50 Hz). Fresh solutions were prepared before each experiment.

2.3. NPs Characterization

2.3.1. Zeta-Potential Measurements

The zeta potential (ζ) of the titanium oxide samples (1–4) was measured using the light scattering method with a Zetasizer 3000 instrument (Malvern, UK). The Zeta Potential measurements were conducted at a pH range from 2.0 to 10.0 with a fixed scattering angle of 90°. Smoluchowski’s equation was used to convert the electric mobility into ζ. Before analysis, water suspensions of the examined samples were prepared by sonification of 5 mg/mL of distilled water for 30 min.
Figure 1. TEM images of TiO$_2$. E171 (No. 1, 2, 3) and TiO$_2$ NPs (No. 4).

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2.3.2. Microscopy Analysis

The size (morphology) of the nanoparticles was examined using a TEM transmission electron microscope (FEI Tecnai G2 T20 X-Twin Ltd., Japan). The following procedure was employed for preparation of samples for the determinations: a nanoparticle suspension (sonicated to re-suspend the sediment) was pipetted onto formvar/carbon film-coated TEM grids. After 5 min, a drop of the preparation was drained with a piece of filter paper, and the mesh was dried. The entire process was carried out in a Petri dish with a piece of parafilm as a pure medium. Morphometric measurements were made using the iTEm/AnalySIS software.

The morphometry was performed manually due to the aggregation of the particles. The images were recalibrated based on the scale mark. A few images with the highest magnification and good quality were selected from each set. Since the particles had an oval or slightly elongated polygonal shape, their largest size was measured. Up to 20 distinguishable particles (if that many were available) were randomly selected from each processed image. The mean, standard deviation, and the smallest and largest particle sizes were calculated from the collected data (STATISTICA 13.0, StatSoft, Krakow, Poland).
2.4. Bacterial Cultures

The growth curves of 17 strains of lactic acid bacteria on nanoparticle-supplemented media (Table 1) were determined with the use of Bioscreen C (Labsystem, Helsinki, Finland) as in Gustaw et al. [27]. MRS media with the addition of the nanoparticles were prepared. In the study, four types of TiO\textsubscript{2} from different manufacturers were used in four concentration variants (a, b, c, d), in triplicate. The experiment was performed for 72 h by measuring OD\textsubscript{600} nm every 2 h. A control was performed in each experiment. The comparison of these values showed the inhibitory properties of the particular types of nanoparticles and their concentrations. On the basis of the results obtained, growth kinetics values and statistics were determined using the PYTHON script for individual strains and each medium variant used (Supplementary Materials, Figures S1–S17) [28].

Table 1. List of bacterial strains under study.

| Species and Strain |
|--------------------|
| Lacticaseibacillus rhamnosus B-1445 |
| Lactiplantibacillus plantarum IB |
| Bifidobacterium bifidum B 41410 |
| Lactococcus lactis PCM 2678 |
| Bifidobacterium adolescentis DSM 20086 |
| Lactobacillus acidophilus DSM 20079 |
| Bifidobacterium longum B-41409. ATCC 15707. DSM 20,219 (intestine of adult human) |
| Pediococcus pentosaceus |
| Lactobacillus johnsonii DSM 10553 |
| Lacticaseibacillus casei Lby |
| Lactobacillus delbrueckii sp. bulgaricus |
| Lactobacillus gasseri PCM 2500 |
| Limosilactobacillus fermentum PCM 491 |
| Lactobacillus helveticus DSM 20075 |
| Lactobacillus intermedicus B 3693 |
| Levilactobacillus brevis B 1139 |
| Lactobacillus plantarum B 4496 |

3. Results

3.1. Characterization of E171/TiO\textsubscript{2} NPs

3.1.1. Zeta Potential of E171/TiO\textsubscript{2} Nanoparticles

The zeta potential was noticed to be pH dependent (Figure 2). The curves had a similar course in all tested samples, analogous to those described in the literature [29,30]. The ζ values ranged from +40 mV (pH = 2) to −17 mV (pH = 10) for samples 1–3. In the case of sample 4, this range was slightly wider, i.e., from +44 mV to −22 mV, which suggested higher stability of dispersion. The determined isoelectric points (IEP) were pH = 7.8 for samples 1–3 and pH = 7.6 for TiO\textsubscript{2} sample 4. The ζ values were positive below these pH values and negative above these values.

3.1.2. Transmission Electron Microscopy (TEM) Analysis of the Samples

The particle size distribution was determined with the TEM technique, which also showed that all samples were in the range of nanomaterials. The distribution of the sizes of the three tested E171 samples (No. 1, 2, 3) were similar and ranged from 40 to 283 nm. Approximately 25–40% of the particles were smaller than 100 nm (Figure 3). The TiO\textsubscript{2} NPs particle fraction (No. 4) was entirely in the nano-range (10–50 nm).
3. Results

3.1. Characterization of E171/TiO\(_2\) NPs

3.1.1. Zeta potential

The zeta potential of the E171/TiO\(_2\) nanoparticles as a function of pH; E171 (No. 1, 2, 3), TiO\(_2\) NPs (No. 4).

![Zeta potential vs pH graph](image)

3.1.2. Size distribution

Size distribution of all tested TiO\(_2\) materials [%]; E171 (No. 1, 2, 3), TiO\(_2\) NPs (No. 4).

![Size distribution graph](image)

3.2. Bacteria

To check the impact of the nanoparticles, the growth of the 17 selected strains of lactic acid bacteria was monitored for 72 h using Bioscreen C.

The growth of a majority of the bacterial strains was inhibited on the medium supplemented with E171/TiO\(_2\) NPs, compared to the control (MRS medium). The differences in the growth inhibition between some strains were dependent on the type and concentration of TiO\(_2\) (Figure 4, Supplementary Materials: Figures S1–S18). The percentage of inhibition relative to the control was directly proportional to the increase in the concentration of the nanoparticles.
Figure 4. Growth of selected bacteria after application of four types of TiO$_2$ at the concentration of 600 mg/L; E171 (No. 1, 2, 3), TiO$_2$ NPs (No. 4).
The smallest differences were found at the lowest concentration (60 mg/L) in most of the 17 strains tested. The growth curve on the medium supplemented with the lowest concentration of all TiO₂ types (1, 2, 3, 4) was similar to the control in 12 strains, whereas the strains of *B. adolescentis* (Figure S18O), *L. delbrueckii* (Figure S18AO), *L. gasseri* (Figure S18AT), *L. fermentum* (Figure S18BA), and *L. intermedius* (Figure S18BH) exhibited growth inhibition even at such low concentrations of the nanoparticles. The differences in the growth curves between the E171/TiO₂ NPs variants (1, 2, 3, 4) at this concentration were insignificant. Additionally, in the case of *L. delbrueckii* sp. *bulgaricus* (TiO₂ NPs 4) (Figure S18AO), the onset of the exponential growth phase was significantly delayed, in comparison with the control.

At the two successive concentrations of 150 mg/L (b) and 300 mg/L (c), all strains were characterized by a certain degree of growth inhibition. Interestingly, both these concentrations sometimes induced a similar degree of bacterial growth inhibition, and the growth curves often overlapped. The higher concentrations also contributed to differences between the applied TiO₂. This was evident in *L. rhamnosus*, *B. bifidum*, *L. acidophilus*, and *L. brevis* at the concentration of 150 mg/L (Figure S18B,H,T,BL) and in *B. longum*, *L. rhamnosus*, and *L. acidophilus* at the concentration of 300 mg/L (Figure S18A,B,C,U).

Significant differences in bacterial growth were found in the majority of the tested strains cultured on the MRS medium supplemented with the four different types of TiO₂, at the concentration of 600 mg/L (d) (Figure 4, Supplementary Materials: Figures S1–S18) (Table 2). The highest growth inhibition was determined in *L. plantarum* and *B. adolescentis*, where virtually complete growth inhibition was demonstrated, and in *L. intermedius*, *L. fermentum*, *L. brevis*, *L. casei* Lby, *L. plantarum* IB, *B. bifidum*, and *L. rhamnosus* (Figure 4). It was also observed that the *B. adolescentis* and *L. helveticus* strains (at the concentration of 600 mg/L) were characterized by a delayed onset of the log phase, in comparison with the control (Figure 4 and Figure S18BG). Interesting findings were also obtained for the *L. gasseri*, *L. plantarum* IB, *L. rhamnosus*, and *L. helveticus* strains (Figure S18AZ,BG, and Figure 4), as the application of the different TiO₂ types at the concentration of 600 mg/L resulted in differences in the growth of these bacteria, depending on the type of E171/TiO₂ NPs.

The bacterial growth kinetics was calculated using the PYTHON script (Table 2). The highest inhibition of growth was caused by the concentration of 600 mg/L (d). In the case of the maximum OD, the lowest values (to 0.2 OD) were found at this concentration. Additionally, TiO₂ No. 3 was found to cause the highest inhibition of bacterial growth in 11 strains, likewise TiO₂ No. 2 in four strains (Table 2). In turn, in the case of max OD, the lowest inhibition was caused by nanoparticle No. 4. The longest cell doubling time was observed in six strains supplemented with TiO₂ NPs (No. 4) and in five strains growing in the presence of TiO₂ No. 2. The values of the Max Specific Growth Rate (1/h) indicate the length of the "exponential growth" phase. The shortest phase or its absence (no growth) was determined in the case of TiO₂ No. 2 and 3. The analysis of the calculated values of the lag phase revealed prolonged duration of this growth stage even up to 51 h in 11 strains in the TiO₂ NPs variant (No. 4), disregarding the lack of these data related to the absence of growth. The lag phase is usually extended, as the bacteria have to adapt to the new unfavorable environment, i.e., the nanoparticle-supplemented medium. However, in a few cases where the growth curves were relatively flattened, there were only slight differences in comparison with the control.
Table 2. Bacterial growth parameters (600 mg/L); E171 (No. 1, 2, 3), TiO$_2$ NPs (No. 4).

| Species | Types of TiO$_2$ | Lag Time (h) | Max Specific Growth Rate (h$^{-1}$) | Doubling Time (h) | Max OD | Min OD | R2 |
|---------|-----------------|--------------|----------------------------------|------------------|--------|-------|-----|
| 4       | control         | 4.70         | 0.15                             | 4.48             | 1.86   | 0.05  | 1.00|
| 2       | 1.36            | 0.04         | 16.65                            | 0.64             | 0.02   | 0.99  |
| 3       | 0.00            | 0.03         | 24.79                            | 0.47             | 0.03   | 0.98  |
| 4       | 9.94            | 0.05         | 14.08                            | 0.49             | 0.05   | 1.00  |
| L. plantarum | control         | 31.24        | 0.05                             | 12.89             | 0.92   | 0.01  | 0.99|
| 2       | 34.21           | 0.07         | 10.11                            | 0.65             | 0.00   | 1.00  |
| 3       | n.g             | n.g          | n.g                              | 0.10             | 0.01   | n.g   |
| 4       | n.g             | n.g          | n.g                              | 0.15             | 0.01   | n.g   |
| B. adolescentis | control        | 16.21        | 0.16                             | 4.45             | 1.67   | 0.01  | 1.00|
| 1       | 0.10            | 0.01         | 56.67                            | 0.30             | 0.06   | 0.98  |
| 2       | 0.79            | 0.06         | 11.92                            | 0.59             | 0.05   | 0.98  |
| 3       | 51.19           | 0.02         | 34.48                            | 0.43             | 0.00   | 1.00  |
| 4       | 20.55           | 0.01         | 83.28                            | 0.44             | 0.05   | 1.00  |
| L. intermedius | control       | 7.39         | 0.10                             | 6.95             | 1.73   | 0.07  | 1.00|
| 1       | 1.38            | 0.07         | 9.33                             | 0.60             | 0.02   | 0.99  |
| 2       | 0.00            | 0.04         | 17.38                            | 0.49             | 0.01   | 0.98  |
| 3       | n.g             | n.g          | n.g                              | 0.12             | 0.01   | n.g   |
| 4       | n.g             | n.g          | n.g                              | 0.14             | 0.01   | n.g   |
| L. fermentum | control         | 7.90         | 0.04                             | 16.11             | 0.63   | 0.03  | 0.99|
| 1       | 5.91            | 0.17         | 4.05                             | 1.73             | 0.02   | 1.00  |
| 2       | 6.51            | 0.05         | 13.50                            | 0.52             | 0.01   | 1.00  |
| 3       | 7.65            | 0.03         | 23.27                            | 0.29             | 0.00   | 1.00  |
| 4       | 9.83            | 0.03         | 20.44                            | 0.31             | 0.01   | 0.91  |
| L. brevis | control         | 9.94         | 0.05                             | 14.08             | 0.49   | 0.05  | 1.00|
| 2       | 2.85            | 0.16         | 4.21                             | 1.75             | 0.05   | 1.00  |
| 3       | 1.91            | 0.05         | 13.75                            | 0.72             | 0.05   | 0.97  |
| 4       | 0.00            | 0.05         | 13.07                            | 0.62             | 0.02   | 0.98  |
| 3       | 11.68           | 0.03         | 22.35                            | 0.46             | 0.06   | 0.98  |
| 4       | 13.22           | 0.06         | 10.67                            | 0.78             | 0.01   | 0.99  |
| L. casei Lby | control         | 14.55        | 0.24                             | 2.84             | 1.83   | 0.01  | 1.00|
| 1       | 11.72           | 0.12         | 6.01                             | 0.92             | 0.00   | 0.99  |
| 2       | 10.47           | 0.13         | 5.15                             | 1.32             | 0.10   | 1.00  |
| 3       | 11.30           | 0.12         | 5.63                             | 1.05             | 0.03   | 0.99  |
| 4       | 9.93            | 0.11         | 6.09                             | 1.18             | 0.00   | 1.00  |
| L. plantarum IB | control     | 26.82        | 0.15                             | 4.58             | 1.79   | 0.07  | 1.00|
| 1       | 8.83            | 0.03         | 24.79                            | 0.53             | 0.03   | 0.96  |
| 2       | 1.16            | 0.03         | 22.35                            | 0.46             | 0.06   | 0.98  |
| 4       | 13.22           | 0.06         | 10.67                            | 0.78             | 0.01   | 0.99  |
| B. bifidum | control         | 9.24         | 0.16                             | 4.21             | 1.75   | 0.05  | 1.00|
| 1       | 1.91            | 0.05         | 13.75                            | 0.72             | 0.12   | 1.00  |
| 2       | 0.00            | 0.05         | 13.07                            | 0.62             | 0.02   | 0.98  |
| 3       | 0.00            | 0.03         | 21.36                            | 0.43             | 0.03   | 0.99  |
| 4       | 2.85            | 0.08         | 9.10                             | 0.92             | 0.03   | 1.00  |

n.g.—no growth.
4. Discussion

The cultivation of the lactic acid bacteria in the presence of the nanoparticles showed the inhibition of bacterial growth; however, the concentration at which the minimal effect was noted was strain dependent. We showed that the lowest concentration that caused the growth inhibition in all strains was 150 or 300 mg/L. Interestingly, at these doses of the nanoparticles, there were evident differences in the bacterial response to the different E171/TiO$_2$ NPs types used in the experiment. The number of works discussing this scientific topic is rather limited. Dudefai et al. [4] reported that food-grade TiO$_2$ particles did not significantly affect the human intestinal microbiota and showed a slight decline in the percentage of Gram-negative B. ovatus and an increase in the number of Gram-positive C. cobleatum strains. As reported by Ripolles-Avila et al. [31], depending on the dose, TiO$_2$ NPs exhibit antibacterial activity against Gram-positive bacteria (S. aureus, B. cereus, L. casei, L. bulgaricus, L. acidophilus, and L. lactis). They showed that the optimal content of TiO$_2$ nanoparticles in a bacterial culture suspension (100 µg/mL) reduced the amount of 2–3 log bacterial populations assessed after 24 h of incubation. Radziwił et al. [17] investigated interactions between TiO$_2$ NPs (food-grade E171 and TiO$_2$—P25) and bacteria ingested with food (e.g., L. lactis). They reported inhibition of bacterial growth (L. lactis, L. rhamnosus) induced especially by food-grade TiO$_2$, as described in the present study as well. These authors suggested that E171 may have been trapped by food-borne bacteria in the intestine, which may have induced physiological changes in the most sensitive species. Lately, Mukherjee et al. [32] anatase (50 nm, 98% pure, hydrophilic) at 1 ppm significantly increased the growth of Bacillus coagulans after 14–18 h of incubation in the absence of light. No such effect in the presence of 0.1 ppm of TiO$_2$ NPs was observed, up to 18 h of incubation, as compared with the control without NPs. The highest TiO$_2$ concentration applied in the cited study (10 ppm) showed less pronounced effect than 1 ppm concentration, and the highest concentration was more or less similar to control. Authors also studied this NP at higher concentrations but reported efficient aggregation of nanoparticles that could result in the lack of interactions with bacteria.

Authors point out that various factors can influence the interactions between NPs and intestinal bacteria, e.g., the surface charge of bacteria and nanoparticles, the surface charge of ingested food, the composition of the chemical substance, and the diet [33]. As reported by Pagnout et al. [23], the toxicity of TiO$_2$ NPs is related to electrostatic interactions between bacteria and nanoparticles, leading to the adsorption thereof on the cell surface. Planchon et al. [34] supported the concept of the heterogeneity of bacterial populations. In their research, they evidenced that, after exposure to TiO$_2$ NPs, some bacteria were fully coated by the compound, whereas a substantial part of the bacterial population was free from the nanoparticles, which resulted in differences in the metabolome and proteome. Similarly, Radziwił et al. [17] demonstrated that part of the bacterial population was free from TiO$_2$ NPs, while some bacteria interacted strongly with NPs.

Exposure of tissue to nanoparticles can have far-reaching consequences ex vivo as well as in vivo. In the ex vivo study involving a gastrointestinal tract model, Limage et al. [21] showed that the presence of commensal Gram-positive L. rhamnosus bacteria and nanoparticles changed the thickness and composition of the mucus layer. This is particularly disadvantageous, as it has been shown that Lactobacillus spp. can increase the production of mucins MUC2 and MUC3. With the mucus layer strengthened in this way, the attachment of enteropathogenic Escherichia coli is hampered, which provides protection against pathogen invasion [21,35].

An imbalance in the composition of the gut bacteria can cause some health disturbances as shown in several animal studies. Li et al. [36] reported changes in the intestinal microbiota composition and a significant decline in the Bifidobacterium count number in male mice receiving TiO$_2$ NPs (1 mg/kg/day for 7 days). Pignet et al. [37] showed that orally administered TiO$_2$ NPs (2 and 10 mg TiO$_2$/kg b.w./day and 50 mg TiO$_2$/kg b.w./day) had minimal effect on the composition of the intestinal microbiota in the mouse colon and small intestine but caused the reduced expression of the colonic mucin gene, increased ex-
pression of the β-defensin gene, colonic inflammation (decreased crypt length, infiltration of CD8+ T cells, increased macrophages, increased expression of inflammatory cytokines).

Mu et al. [38] reported that administration of TiO$_2$ NPs (10 and 50 nm) to young weaned mice for 2–3 months in the diet reduced the numbers of Bifidobacterium and Lactobacillus, which led to weight loss. In in vivo study conducted by Cao et al. [11], oral administration of TiO$_2$ (E171, 112 nm) and TiO$_2$ NPs (33 nm) to obese and non-obese mice (0.1% w/w in the diet for 8 weeks) resulted in a significant reduction in the intestinal amounts of Bifidobacterium and Lactobacillus bacteria count number. The authors found that TiO$_2$ NPs induced more severe colon inflammation than TiO$_2$ (E171), especially in the more susceptible obese mice, which was also associated with their high-fat diet. Authors suggested that TiO$_2$ exposure of mice with reduced levels of Bifidobacterium and Lactobacillus may result in increased susceptibility to such diseases as irritable bowel syndrome [11].

5. Conclusions

Due to their unique physicochemical properties, titanium dioxide nanoparticles are produced all over the world. The high level of production and wide application of these nanoparticles create hazards to the environment and humans. Despite their widespread use as food additives, the risk of ingestion thereof has not been fully documented to date. Current research provides conflicting evidence of the effects of inorganic nanoparticles on the human microbiome. The application thereof as food additives and their subsequent impact on the function of the gastrointestinal tract, including a direct effect on the microbiota, require elucidation. The present study showed that bacterial growth was inhibited by both food-grade E171 and TiO$_2$ NPs in most of the strains tested. This may suggest that the antimicrobial properties of NPs may alter the gut microbiota; therefore, further research is necessary in this field to understand the toxicity of NPs to the human microbiome.

Supplementary Materials: The following are available online at https://www.mdpi.com/article/10.3390/foods10050939/s1, Figure S1: Regression curves of selected data. B. adolescentis (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S2: Regression curves of selected data. B. bifidum (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S3: Regression curves of selected data. B. longum (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S4: Regression curves of selected data. L. acidophilus (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S5: Regression curves of selected data. L. brevis (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S6: Regression curves of selected data. L. casei (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S7: Regression curves of selected data. L. delbrueckii sp. bulgaricus (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S8: Regression curves of selected data. L. fermentum (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S9: Regression curves of selected data. L. gasseri (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S10: Regression curves of selected data. L. helveticus (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S11: Regression curves of selected data. L. intermedius (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S12: Regression curves of selected data. L. johnsoni (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S13: Regression curves of selected data. L. lactis (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S14: Regression curves of selected data. L. plantarum (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S15: Regression curves of selected data. L. plantarum IB (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S16: Regression curves of selected data. L. rhamnosus (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S17: Regression curves of selected data. P. pentosaceus (1, 2, 3, 4—types of TiO$_2$; a, b, c, d—concentration of TiO$_2$: 60, 150, 300, and 600 mg/L). Figure S18: Growth of selected bacteria after application of four types of TiO$_2$ at the concentration of 600 mg/L; E171 (No. 1, 2, 3), TiO$_2$ NPs (No. 4).
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