Electronic Supplementary Material (ESI) for Environmental Science: Nano.

Supplementary Information

Food-related engineered nanoparticles and food-grade TiO_2 impact the metabolism of a human commensal bacterial strain in physiologically relevant conditions

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Table S1. Primers used in this study.

Target gene	Forward and reverse primers	Amplicon (bp)
salA	F: 5′- AAGGGAGAATGATTGCCATGAA-3′ R: 5′GAGTTTGGACAGTCATCAGTAATAGTTG- 3′	144
sboA	F:5′-TCTCGTTGAGATTGAAGCAATGA-3′ R:5′-TGCGACATTCGTGTGAAATGG-3′	112
sboG	F:5′- GCGGTCACGGAATTTGATAG-3′ R:5′-TTTTTAGCATACCTTTTTATGGATCA-3′	290
sboK	F:5′- GGCTGGGCAGGGTATAAGAT-3′ R:5′-TTTGCAAGTGTCCAAAATGAA-3′	134
gyrA	F:5′- GGCAATCAACCCATTTTGACG -3′ R:5′- CGAAGGTGAGACCATTCAAACAG -3′	148

Note: SalA[36], SalB (*sboA, sboG, sboK*)[35].

Table S2. Polydispersity indices (PDI) of dynamic light scattering (DLS) measurements of NPs in different media.

Nanoparticles	PDI in ultrapure water	PDI in rich growth medium (THB)	PDI in artificial saliva	PDI in "spent" artificial saliva
Ag	0.4 ± 0.06	0.3 ± 0.04	0.7 ± 0.15	1.0 ± 0
SiO_2	0.5 ± 0.08	0.4 ± 0.05	0.2 ± 0.06	0.4 ± 0.03
TiO_2	0.7 ± 0.27	0.9 ± 0.10	0.6 ± 0.03	0.9 ± 0.23
Food-grade TiO_2	0.2 ± 0.01	0.1 ± 0.1	0.1 ± 0.04	0.8 ± 0.19

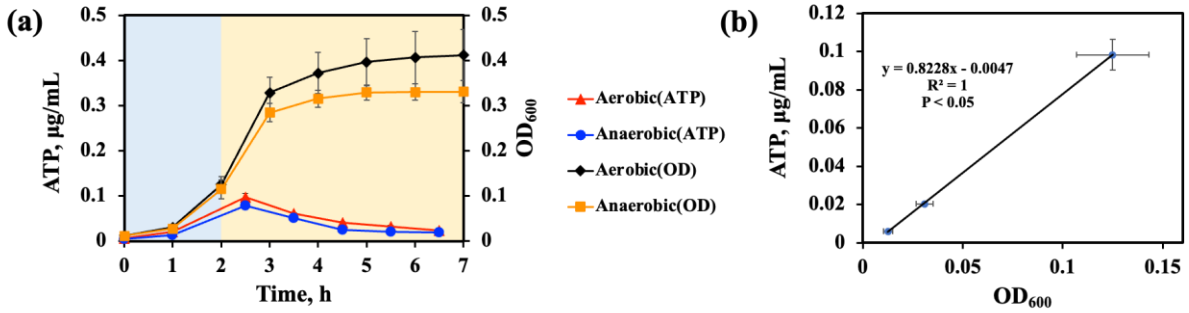

Figure S1. Comparison of growth of *S. salivarius* K12 in rich growth medium (THB) measured by optical density at 600 nm (OD_{600}) and ATP quantification. (a) Aerobic and anaerobic growth of *S. salivarius* K12 in THB, measured either by OD_{600} or ATP quantification. The data points within the left-side blue area were used to calculate specific growth rates after transforming the values to natural logarithms (Table S3). (b) Plot of ATP concentration and OD_{600} of *S. salivarius* K12 after growth in THB, demonstrating significant positive correlation between ATP and cell concentration in bacterial cultures over the exponential growth phase. The data points are the average of 3 replicates and the error bars indicate standard deviations. When error bars are not visible, the standard deviations were small and the error bars are hidden by the data points.

Table S3. Specific growth rates (h^{-1}) of *S. salivarius* K12 calculated based on the optical density (OD_{600}) or ATP concentrations during growth in rich growth medium (THB) in aerobic or anaerobic conditions. The Student's t-test analysis indicated no statistically significant differences in the specific growth rates ($p > 0.05$).

Method	Aerobic	Anaerobic
OD_{600}	1.18 ± 0.05	1.17 ± 0.06
ATP content	1.05 ± 0.05	1.16 ± 0.11

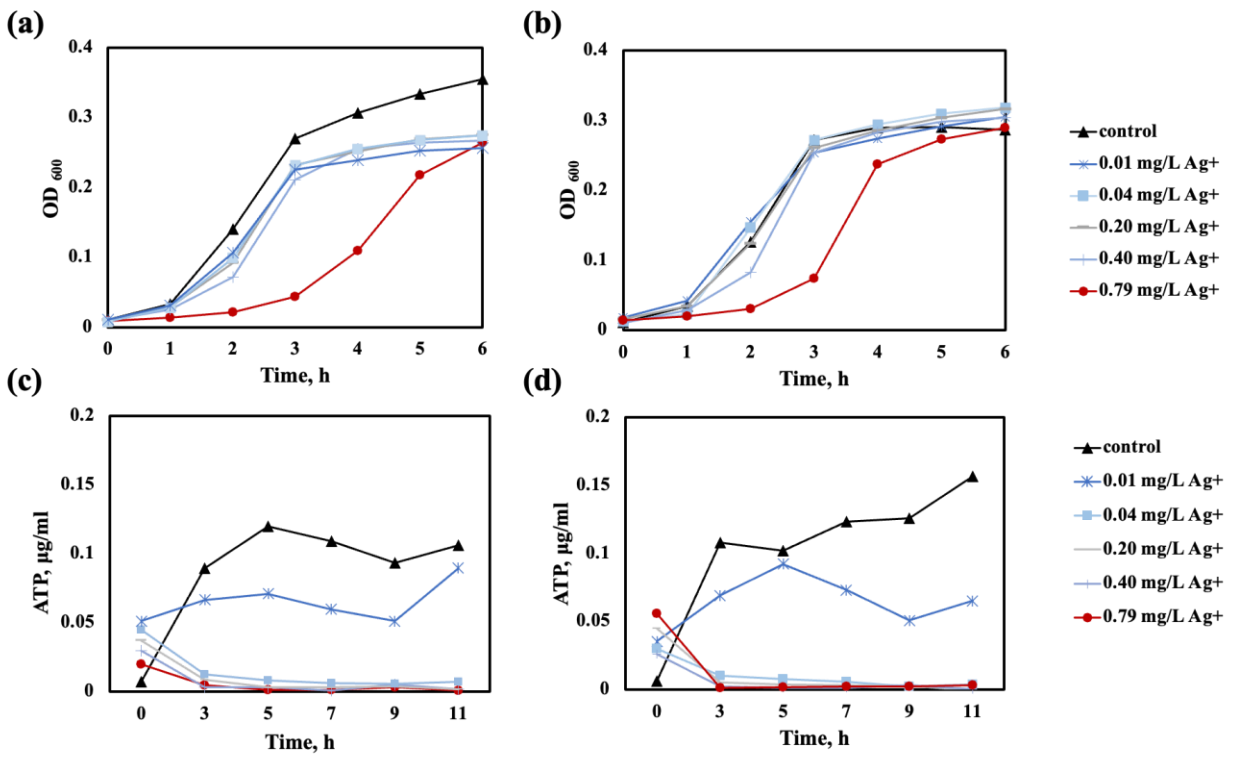

Figure S2. Growth of *S. salivarius* K12 with and without $AgNO_3$ (at 0.0125, 0.0625, 0.3125, 0.625, 1.25 mg/L; corresponding Ag concentrations: 0.01, 0.04, 0.20, 0.40, 0.79 mg/L) in (a, b) rich growth medium (THB) based on optical density and (c, d) artificial saliva based on ATP concentrations in (a, c) aerobic or (b, d) anaerobic conditions.

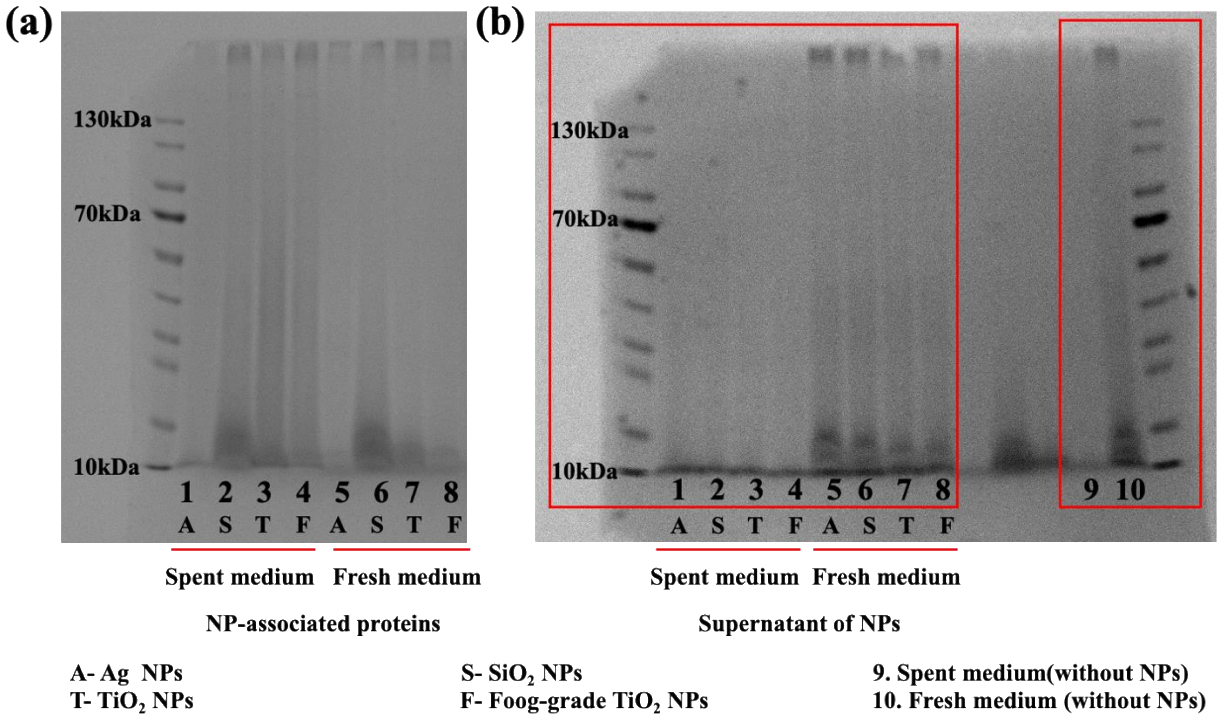

Figure S3. SDS-PAGE of (a) NP-associated proteins and (b) proteins in the supernatants of NPs after incubation in spent and fresh artificial saliva at 37℃, 200 rpm for 24 h. "Spent" artificial saliva refers to the medium that was inoculated with *S. salivarius* K12, incubated at 37°C, 200 rpm for 18 h, then centrifuged at 12,000 g for 10 min to remove bacteria. "Fresh" artificial saliva refers to the control medium in preparation of spent artificial saliva (i.e., artificial saliva incubated at 37°C, 200 rpm for 18 h, then centrifuged at 12,000 g for 10 min).

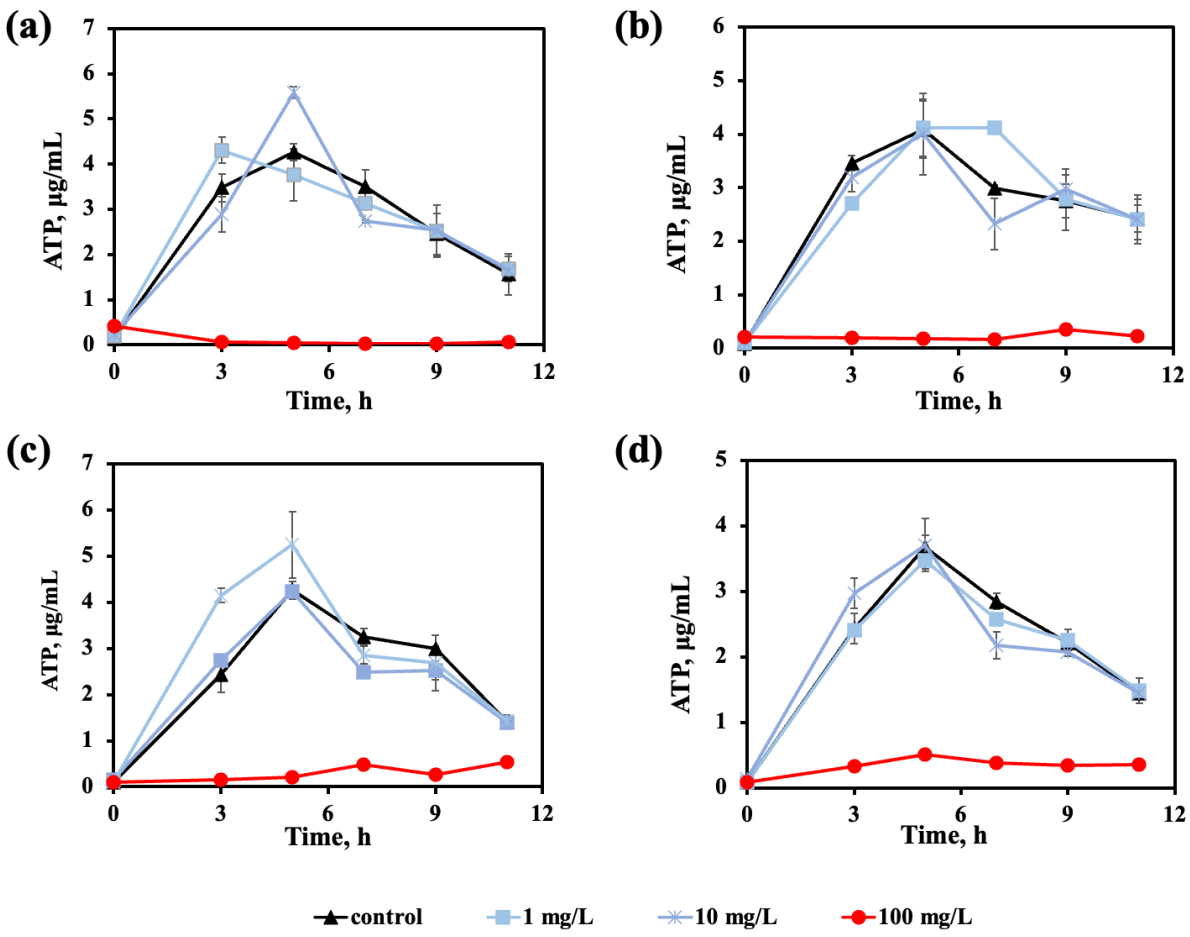

Figure S4. Growth of *S. salivarius* K12 with and without transformed (a) Ag NPs, (b) SiO_2 NPs, (b) TiO_2 NPs, and (d) food-grade TiO_2 at 1, 10 or 100 mg/L. Bacteria were grown aerobically at 37°C and ATP was used as a proxy for cell numbers during the exponential growth phase.

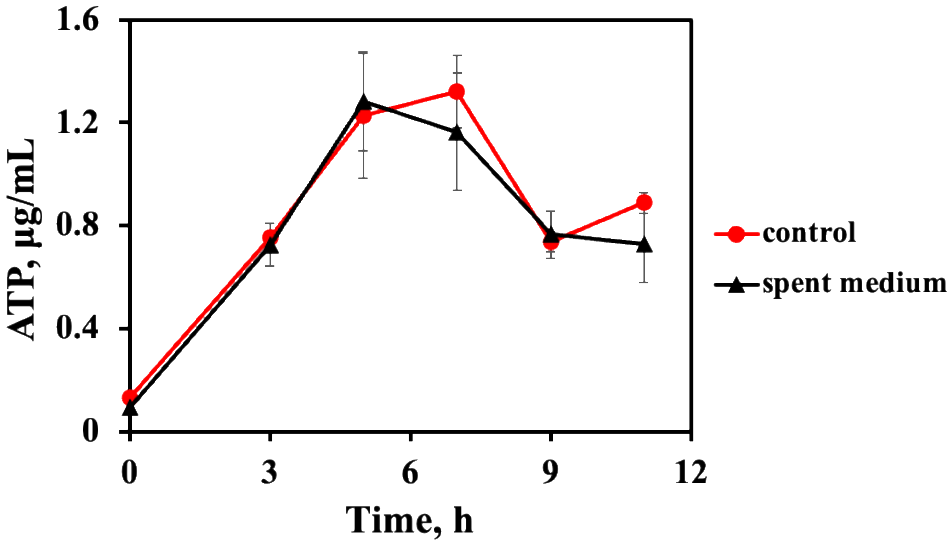

Figure S5. Growth of *S. salivarius* K12 in spent artificial saliva (spent medium) and fresh artificial saliva (control). Bacteria were grown aerobically at 37°C and ATP was used as a proxy for cell numbers. The overlapping growth curves during the exponential growth phase (0-5h) indicate similar growth rates in spent and fresh artificial saliva, suggesting that there was a sufficient amount of nutrients in the spent medium to support growth of *S. salivarius* fresh inoculum.