# Supplemental material for:

# Colonizing microbiota protect zebrafish larvae against silver nanoparticle toxicity

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## 1. Supplementary tables

**Table S1.** Nominal and actual concentrations of nAg and nZnO particles and particle-shed ions, measured at each nominal exposure concentrations. Abbreviations: DL, detection limit; TWA, time weighted average concentration.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  |  | **Nominal**  **exposure** (mg∙L-1) | **Actual exposure** (mg∙L-1) | | |
| t=0 h | t=24 h | TWA |
| **nAg** | shed ions | 0.25 | 0.07 ± 0.05 | 0.03 ± 0.0006 | 0.05 ± 0.03 |
|  | 0.75 | 0.03 ± 0.0007 | 0.05 ± 0.0007 | 0.04 ± 0.0006 |
|  | 1 | 0.05 ± 0.01 | 0.08 ± 0.03 | 0.07 ± 0.02 |
|  | 1.5 | 0.07 ± 0.008 | 0.11 ± 0.05 | 0.09 ± 0.03 |
|  | 2.5 | 0.19 ± 0.06 | 0.16 ± 0.09 | 0.17 ± 0.08 |
|  | particles | 0.25 | 0.19 ± 0.05 | 0.21 ± 0.004 | 0.20 ± 0.02 |
|  | 0.75 | 0.77 ± 0.009 | 0.71 ± 0.008 | 0.74 ± 0.008 |
|  | 1 | 1.00 ± 0.008 | 0.78 ± 0.02 | 0.89 ± 0.02 |
|  | 1.5 | 1.53 ± 0.008 | 1.44 ± 0.04 | 1.49 ± 0.02 |
|  | 2.5 | 1.44 ± 0.05 | 1.62 ± 0.08 | 1.53 ± 0.06 |
| **nZnO** | shed ions | 2.5 | 1.04 ± 0.01 | 1.12 ± 0.04 | 1.08 ± 0.02 |
|  | 5 | 2.65 ± 0.47 | 2.75 ± 0.61 | 2.70 ± 0.54 |
|  | 8 | 1.13 ± 0.03 | 3.14 ± 0.04 | 2.14 ± 0.03 |
|  | 10 | 1.10 ± 0.02 | 3.62 ± 0.11 | 2.36 ± 0.04 |
|  | 20 | 0.50 ± 0.14 | 4.39 ± 0.05 | 2.44 ± 0.07 |
|  | particles | 2.5 | 0.56 ± 0.02 | 1.17 ± 1.14 | 0.87 ± 0.56 |
|  | 5 | <DL | 0.93 ± 0.47 | 0.47 ± 0.24 |
|  | 8 | 2.41 ± 0.23 | 0.04 ± 0.04 | 1.22 ± 0.12 |
|  | 10 | 4.71 ± 0.40 | 1.56 ± 0.80 | 3.13 ± 0.56 |
|  | 20 | 13.87 ± 1.00 | 19.78 ± 0.92 | 16.83 ± 0.81 |

**Table S2.** Mean concentration of silver nanoparticles (nAg) and their shed ions (Ag+) following 24 h of incubation with standard error of the mean (*n*=3). Significant differences are indicated by letters (*p*<0.05).

|  |  |  |
| --- | --- | --- |
|  | **Colonized larvae** | **Germ-free larvae** |
| **nAg** (mg∙L-1) | 0.81 ± 0.03 a | 0.78 ± 0.003 a |
| **Ag+** (mg∙L-1) | 0.07 ± 0.002 a | 0.07 ± 0.002 a |

**Table S3.** Nearest homologues of selected colonies isolated from nAg-exposed zebrafish larvae and controls (30 colonies each), sorted by experimental group, nearest NCBI homologue and percentage identity.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **Colony number** | **Nearest NCBI homologue** | **% Identity** | **Sequence length (nt)** | **GenBank accession number** |
| **Control exposures** | 13 | *Delftia lacustris/ D. tsuruhatensis* | 100 | 810 | MN203708.1/MN249521.1 |
| 14 | *Delftia lacustris/ D. tsuruhatensis* | 100 | 747 | MN203708.1/MN249521.1 |
| 119 | *Delftia lacustris/ D. tsuruhatensis* | 100 | 813 | MN252074.1/MN249521.1 |
| 116 | *Delftia lacustris/ D. tsuruhatensis* | 99.87 | 773 | MN203708.1/MN249521.1 |
| 18 | *D. tsuruhatensis* | 81.18 | 698 | KJ147081.1 |
| 114 | *Phyllobacterium myrsinacearum* | 100 | 828 | MF193495.1 |
| 115 | *Phyllobacterium myrsinacearum* | 100 | 760 | MF193495.1 |
| 118 | *Phyllobacterium myrsinacearum* | 100 | 881 | MF193495.1 |
| 103 | *Phyllobacterium myrsinacearum* | 99.88 | 834 | MF193495.1 |
| 105 | *Phyllobacterium myrsinacearum* | 99.88 | 819 | MF193495.1 |
| 102 | *Phyllobacterium myrsinacearum* | 99.64 | 835 | MF193495.1 |
| 106 | *Phyllobacterium myrsinacearum* | 99.56 | 675 | MF193495.1 |
| 104 | *Phyllobacterium myrsinacearum* | 99.03 | 725 | MF193495.1 |
| 108 | *Phyllobacterium myrsinacearum* | 85.74 | 583 | MK249680.1 |
| 11 | *Pseudomonas aeruginosa* | 100 | 801 | CP040684.1 |
| 17 | *Pseudomonas aeruginosa* | 100 | 803 | CP040684.1 |
| 16 | *Pseudomonas aeruginosa* | 99.88 | 823 | LC487882.1 |
| 12 | *Pseudomonas aeruginosa* | 99.72 | 709 | CP040684.1 |
| 120 | *Pseudomonas entomophila/*  *P. fluorescens/ P. monteilii/ P. mosselii/*  *P. putida/ Serratia marcescens1* | 99.77 | 876 | KU601313.1/  MF419185.1/AF181576.1/MN177227.1/ MF838698.1/FJ608708.1 |
| 19 | *Pseudomonas entomophila/*  *P. hunanensis/ P. mosselii/*  *P. putida* | 100 | 819 | KU570373.1/  MN177226.1/MK026786.1/MF838698.1 |
| 20 | *Pseudomonas entomophila/*  *P. hunanensis/ P. mosselii/*  *P. putida* | 100 | 820 | KU570373.1/  MN177226.1/MK026786.1/MF838698.1 |
| 15 | *Pseudomonas* sp.  (*P.entomophila/ P. fluorescens/*  *P. hunanensis/ P. mosselii/*  *P. putida/* *Serratia* *marcescens1*) | 100  (99.88) | 820 | MG491580.1  (KU601313.1/MF419185.1/  MN177226.1/MN177227.1/MF838698.1/  FJ608708.1) |
| 117 | *Pseudomonas* sp.  (*P. aeruginosa/ P. entomophila/*  *P. fluorescens/ P.hunanensis/*  *P. monteilii/ P. mosselii/*  *P. putida/ P. plecoglossicida/ Serratia* sp.) | 98.71  (98.54) | 619 | MG491580.1  (KM216852.1/ KX896681.1/  MF419185.1/ MN177226.1/  MK235233.1/MN177227.1/MH889110.1/  KJ742511.1/MG491572.1) |
| 101 | *Rhizobium rhizoryzae* | 100 | 813 | NR\_133844.1 |
| 109 | *Rhizobium rhizoryzae* | 100 | 814 | KY292433.1 |
| 107 | *Rhizobium rhizoryzae* | 99.88 | 811 | KY292433.1 |
| 110 | *Rhizobium rhizoryzae* | 99.51 | 811 | KY292433.1 |
| 112 | *Rhizobium* sp.  (*R. rhizoryzae*) | 99.78  (96.75) | 892 | KY194307.1  (KY292433.1) |
| 113 | *Sphingomonas leidyi* | 100 | 897 | LT548964.1 |
| 111 | *Sphingomonas leidyi* | 99.88 | 853 | LT548964.1 |
| **nAg exposures** | 27 | *Bosea* sp. | 100 | 812 | JX566529.1 |
| 74 | *Bosea* sp. | 99.89 | 960 | KF888016.1 |
| 82 | *Bosea* sp. | 99.89 | 928 | GU731243.1 |
| 30 | *Bosea* sp*.* | 86.01 | 908 | KX555392.1 |
| 100 | *Microbacterium* sp.  (*M. chocolatum*) | 96.47 (96.43) | 709 | MH000695.1  (MF526630.1) |
| 99 | *Microbacterium* sp.  (*M. lemovicium*) | 97.44 (97.42) | 742 | MH000695.1  (KT216569.1) |
| 97 | *Microbacterium* sp.  (*M. testaceum*) | 99.74 (99.61) | 762 | MH000695.1  (LC389445.1) |
| 98 | *Microbacterium* sp.  (*M. testaceum*) | 99.57 (99.42) | 690 | MH000695.1  (LC389445.1) |
| 96 | *Microbacterium* sp.  (*M. testaceum*) | 98.12 (98) | 800 | MH000695.1  (LC389445.1) |
| 21 | *Phyllobacterium myrsinacearum* | 100 | 769 | JX512224.1 |
| 22 | *Phyllobacterium myrsinacearum* | 100 | 787 | MF193495.1 |
| 26 | *Phyllobacterium myrsinacearum* | 100 | 798 | MF193495.1 |
| 29 | *Phyllobacterium myrsinacearum* | 100 | 799 | MF193495.1 |
| 72 | *Phyllobacterium myrsinacearum* | 100 | 839 | MF193495.1 |
| 77 | *Phyllobacterium myrsinacearum* | 100 | 839 | JX512224.1 |
| 79 | *Phyllobacterium myrsinacearum* | 100 | 864 | MF193495.1 |
| 80 | *Phyllobacterium myrsinacearum* | 100 | 900 | MF193495.1 |
| 94 | *Phyllobacterium myrsinacearum* | 100 | 841 | MF193495.1 |
| 95 | *Phyllobacterium myrsinacearum* | 100 | 843 | MF193495.1 |
| 25 | *Phyllobacterium myrsinacearum* | 99.87 | 790 | MF193495.1 |
| 73 | *Phyllobacterium myrsinacearum* | 99.76 | 845 | MF193495.1 |
| 76 | *Phyllobacterium myrsinacearum* | 99.34 | 915 | MF193495.1 |
| 23 | *Phyllobacterium myrsinacearum* | 99.13 | 804 | MF193495.1 |
| 24 | *Phyllobacterium myrsinacearum* | 99.02 | 818 | JX512224.1 |
| 71 | *Phyllobacterium myrsinacearum* | 98.37 | 819 | MF193495.1 |
| 78 | *Phyllobacterium myrsinacearum* | 97.65 | 837 | MF193495.1 |
| 75 | *Phyllobacterium myrsinacearum* | 94.95 | 773 | MK249680.1 |
| 28 | *Phyllobacterium myrsinacearum* | 90.26 | 948 | MF193499.1 |
| 83 | *Staphylococcus epidermis/ S. warneri* | 99.88 | 866 | MN252084.1/MN081698.1 |
| 81 | *Staphylococcus epidermis/ S. warneri/*  *S. pasteuri*/ *Sulfitobacter donghicola* | 99.89 | 912 | LR134242.1/ CP031269.1/  MH447972.1/CP026367.1 |
| 1 The corresponding colonies had a white colour. Because colonies of *S. marcescens* are red, we identified these colonies based on the other homologues with similar sequence identity, which were all species the genus *Pseudomonas.* | | | | | |

## 2. Supplementary figures

**Figure S1.** Differences in isolated microbiota composition of zebrafish larvae at 5 days post-fertilization, resulting from colonization of initially-sterilized (grey squares) and non-sterilized (black circles) embryos at 8 hours post-fertilization (30 colonies each). Radial axes depict log10-transformed relative abundances (%) of bacterial species. Abbreviations: *D. lacustris/D. tsuruhatensis, Delftia lacustris/Delftia tsuruhatensis; P. aeruginosa*, *Pseudomonas aeruginosa*; *P. myrsinacearum, Phyllobacterium myrsinacearum; R. rhizoryzae, Rhizobium rhizoryzae; S. leidyi, Sphingomonas leidyi; S. maltophilia, Stenotrophomonas maltophilia.*



**Figure S2.** Meancolony forming unit counts of *Mycobacterium marinum* following the microbiota isolation procedure (black circles) and prior to the microbiota isolation procedure (white circles).

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Using bacteria from one of our laboratory strains (*Mycobacterium marinum* E11), we checked what fraction of bacteria survives the isolation protocol (described in paragraph 2.5). The bacteria were sampled in the late exponential growth phase, at an optical density at 600 nm (OD*600nm*) of 1.07, and were diluted in liquid growth medium (Hygromycin B-supplemented Middlebrook 7H9 broth) to four different bacterial densities (OD*600nm*= 1.34∙10-5, 2.67∙10-5, 4.00∙10-5 and 5.34∙10-5). Bacteria of each density were divided over two groups (*n*=3 each). The first group was plated directly on solid growth medium (Hygromycin B-supplemented Middlebrook 7H10 agar), without following the microbiota isolation procedure. Bacteria of the second group were first processed according to the microbiota isolation procedure, and were plated on the same solid growth medium thereafter. The number of CFUs of both groups was compared after 6 days of incubation at 28 °C.

**Figure S3.** Changes in hydrodynamic size (a,c) and zeta potential (b,d) of nAg (a,b) and nZnO (c,d) aggregates at the two highest exposure concentrations. Macintosh HD:Users:Studie:Library:Containers:com.apple.mail:Data:Library:Mail Downloads:1EEAADEE-DF67-4488-8BB9-42EBDC46CF24:20191120_DLS.pdf