SUPPLEMENTARY TABLES

**TABLE S1. Bacterial pathogens used to screen for potential lethal effects in axenic zebrafish larvae.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Origin** | **Reference** |
| *Aeromonas hydrophila sp. anaerogenes* CIP 76.15 | Used oil-emulsions | CRBIP |
| *Aeromonas hydrophila sp. dhakensis* CIP 107500 | Human feces, Bangladesh | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP 52.94 | Frog | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP 76.14 | Tin of milk with a fishy odor | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP 103561 | Unknown | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP 103697 | Unknown | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP107274 | Human feces | CRBIP |
| *Aeromonas hydrophila sp. hydrophila* CIP 200522 | Fish isolate | CRBIP |
| *Aeromonas hydrophila sp. ranae* CIP 107985 | Frog, Thailand | CRBIP |
| *Aeromonas salmonicida sp. achromogenes* CIP 104001 | Trout, *Salmo trutta*, UK | CRBIP |
| *Aeromonas salmonicida sp. masoucida* CIP 103210 | Fish blood, *Oncorhynchus masou* | CRBIP |
| *Aeromonas salmonicida sp. pectinolytica* CIP 107036 | River water, Argentina | CRBIP |
| *Aeromonas salmonicida sp. salmonicida* CIP 63.4 | Fish, trout | CRBIP |
| *Aeromonas salmonicida sp. salmonicida* CIP 107106 | Diseased fish, Denmark | CRBIP |
| *Aeromonas veronii* CIP 109836 | Fish | CRBIP |
| *Edwardsiella ictaluri* CIP 81.96 | Catfish enteric septicemia, USA | CRBIP |
| *Edwardsiella tarda* CIP 78.61 | Human, feces, USA | CRBIP |
| *Listonella anguillara* CIP 63.36 | Ulcerated cod, *Gadus morhua*, Norway | CRBIP |
| *Listonella anguillara* CIP 64.14 | Ulcerous lesion in plaice, *Pleuronectes platessa*, UK | CRBIP |
| *Listonella anguillara* CIP 73.4 | Diseased rainbow trout | CRBIP |
| *Photobacterium damselae sp. piscicida* CIP 103910 | White perch, USA | CRBIP |
| *Vibrio parahaemolyticus* CIP 71.1 | Sea fish isolate | CRBIP |
| *Vibrio parahaemolyticus* CIP 71.2 | Sea fish isolate | CRBIP |
| *Vibrio ichthyoenteri* CIP 104815 | Japanese flounder fish isolate | CRBIP |
| *Yersinia ruckeri* CIP 82.80 | Rainbow trout, red mouth disease, USA | CRBIP |

**CRBIP:** *Institut Pasteur, Centre de Resources Biologiques de l’Institut Pasteur.*

**TABLE S2. Commensal and probiotic bacteria used to screen for potential protective effect in axenic zebrafish larvae.**

|  |  |  |
| --- | --- | --- |
| **Name** | **Origin** | **Reference** |
| *Aeromonas veronii* CIP 109836 | Fish | CRBIP |
| *Bacillus cereus* CIPA28 | Lactic ferment | CRBIP |
| *E. coli* BW25113 | Laboratory strain | [[1](#_ENREF_1)] |
| *E. coli* ED1a | Human feces from healthy man (France), Probiotic | [[2](#_ENREF_2)] |
| *E. coli* ED1a-sm | Spontaneous streptomycin-resistant mutant of ED1a | This study |
| *E. coli* ED1a-sm F’*tet* | Laboratory strain | This study |
| *E. coli* K-12 MG1655 | Laboratory strain | [[3](#_ENREF_3)] |
| *E. coli* K-12 MG1655 *attB::gfp-bla* (F’*tet ∆traD::apra ∆tetR::zeo tetA::*Tn*luxCDABE*-Km) | Laboratory strain | This study |
| *E. coli* 10.22 | Commensal strain from hospitalized adult faeces | [[4](#_ENREF_4)] |
| *E. coli* 10.94 | Commensal strain isolated from healthy adult faeces | [[4](#_ENREF_4)] |
| *E. coli* 11.25 | Commensal strain isolated from a healthy adult | [[4](#_ENREF_4)] |
| *E. coli* Nissle 1917 - DSM6601 | Human feces, Commensal strain used as probiotic | [[5](#_ENREF_5)] |
| *E. coli* 083 | Commensal strain used as probiotic | Lab collection |
| *Enterobacter cloacae* 10.91 | Commensal strain isolated from a healthy adult | C. Le Bougennec |
| *Lactobacillus casei* CIP103137 T-ATCC393 | Cheese | CRBIP |
| *Lactobacillus casei sp. rhamnosus* | Human isolate used as probiotic | Lab collection |
| *Lactobacillus delbruecki sp bulgaricus* CIP 101027 | Bulgarian yogurt | CRBIP |
| *Lactobacillus paracasei sp. paracasei* CIP103918 T-ATCC25302 | Unknown | CRBIP |
| *Lactobacillus paracasei* CIP 109805 | Human feces, The Netherlands | CRBIP |
| *Lactobacillus plantarum* WCFS1 | Human saliva, Probiotic | [[6](#_ENREF_6)] |
| *Lactobacillus rhamnosus* A157T-ATCC7449 | Unknown | CRBIP |
| *Lactobacillus rhamnosus* GG – ATCC53103 | Probiotic human isolate | [[7](#_ENREF_7)] |
| *Pediococcus acidilactici* CIP 103408 | Neotype strain | CRBIP |
| *Phaeobacter inhibens* CIP 109852 | Fish, *Scophthalmus maximus*, Spain | CRBIP |
| *Pseudomonas fluorescens* CIP 109851 | Unknown | CRBIP |
| *Vibrio parahaemolyticus* CIP 109835 | Human feces | CRBIP |
| *Escherichia coli* H19 | EHEC UK Human isolate | [[8](#_ENREF_8)] |
| *Escherichia coli* DAEC 7 | Human isolate (Brazil)- Diffusely adhering (DAEC) | [[8](#_ENREF_8)] |
| *Escherichia coli* DAEC18 | Human isolate (Brazil)- Diffusely adhering (DAEC) | [[8](#_ENREF_8)] |
| *Escherichia coli* iai 44 | Human isolate (France)- Urinary infection | [[8](#_ENREF_8)] |
| *Escherichia coli* iai 73 | Human isolate (France)- septicemia | [[8](#_ENREF_8)] |
| *Escherichia coli* G001 | Human isolate (French Guyana) | [[8](#_ENREF_8)] |
| *Escherichia coli* Ec111 | Commensal, Roe-Deer, *Capreolus capreolus,* France | [[8](#_ENREF_8)] |
| *Escherichia coli* Ec029 | Commensal Impala, *Aepyceros melampus* Gabon | [[8](#_ENREF_8)] |
| *Escherichia coli* Ec248 | Commensal, Horse, *Equus caballus*, France | [[8](#_ENREF_8)] |
| *Escherichia coli* Ec300 | Commensal, Dog, *Canis familiaris*, France | [[8](#_ENREF_8)] |
| *Escherichia coli* Ec212 | Commensal, Horse, *Equus caballus*, France | [[8](#_ENREF_8)] |

**CRBIP:** *Institut Pasteur, Centre de Resources Biologiques*

**References**

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**TABLE S3. CFU quantification at 9 dpf of germ-free larvae pretreated with selected probiotics at 4 dpf.** Means and standard deviations of the number of CFU recovered from larvae are reported (n=4).

|  |  |
| --- | --- |
|  | **Cfu/larvae** |
| Germ free | 0 ± 0 |
| *E. coli* K-12 MG1655 F’ | 2.2 x103 ± 6.11x102 |
| *E. coli* ED1a | 1.93x104 ± 1.04x103 |
| *V. parahaemolyticus* | 7.15x103 ± 1.08 x103 |
|  |  |

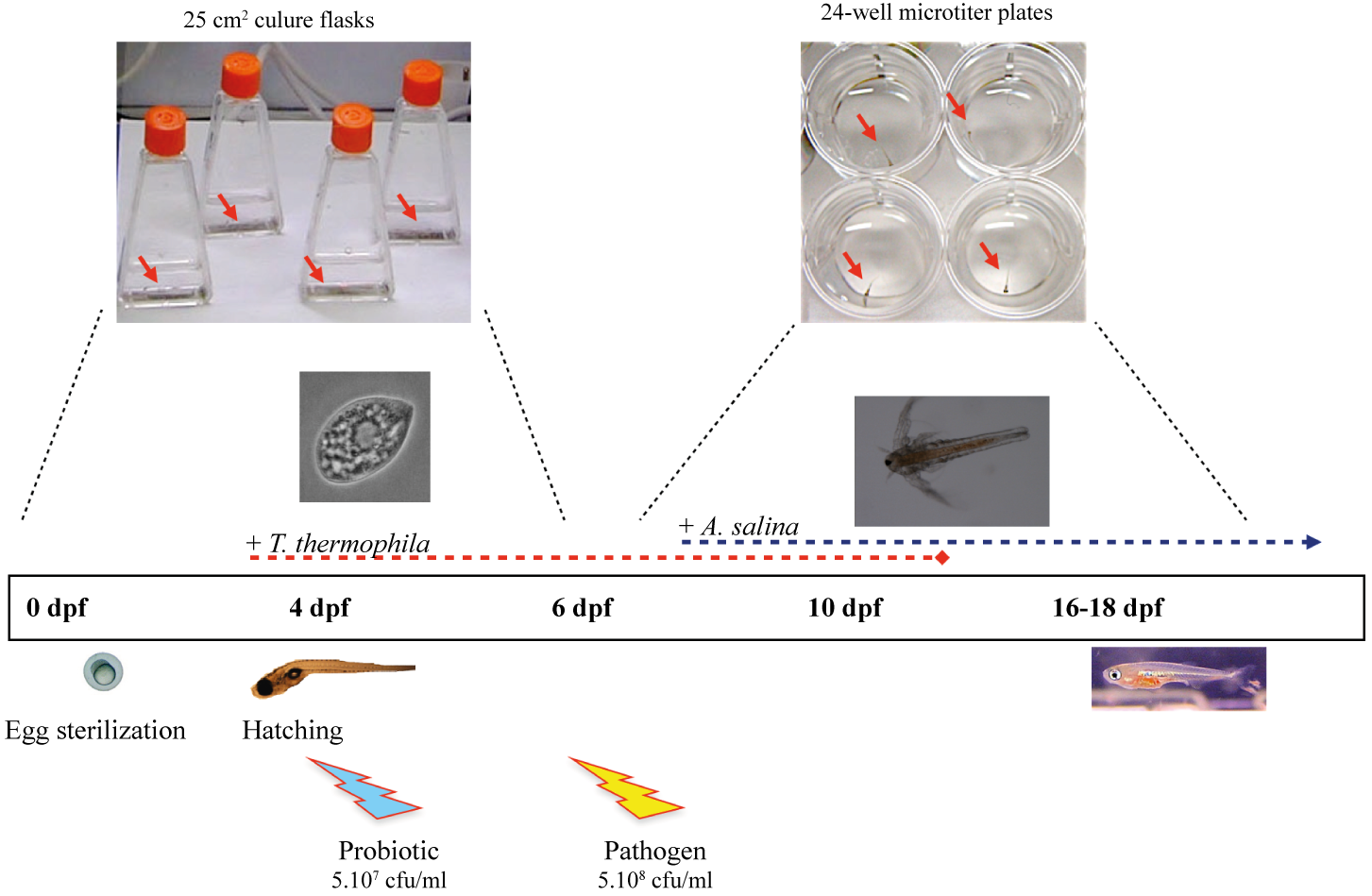
**TABLE S4.  qPCR quantification of colonization by E. ictaluri in germ-free and probiotic-preteated  larvae .** Shown are mean ± SEM from three pools of three larvae each; data have been normalized to one of the samples from high-dose *E. ictaluri*.

|  |  |  |
| --- | --- | --- |
|  | ***E. ictaluri gDNA / fish gDNA*** | |
| *E. ictaluri* 2.106 3dpi | | 0.22 ± 0.04 |
| *E. ictaluri* 2.1073dpi | | 0.85 ± 0.31 |
| *E. ictaluri* 2.108 3dpi | | 1.10 ± 0.19 |
| *E. coli* MG1655 + *E. ictaluri* 2.108 3dpi | | 1.24 ± 0.49 |
| *E. coli* MG1655F’ + *E. ictaluri* 2.108 3dpi | | 1.62 ± 0.45 |

**TABLE S5. Primers used in this study***.*

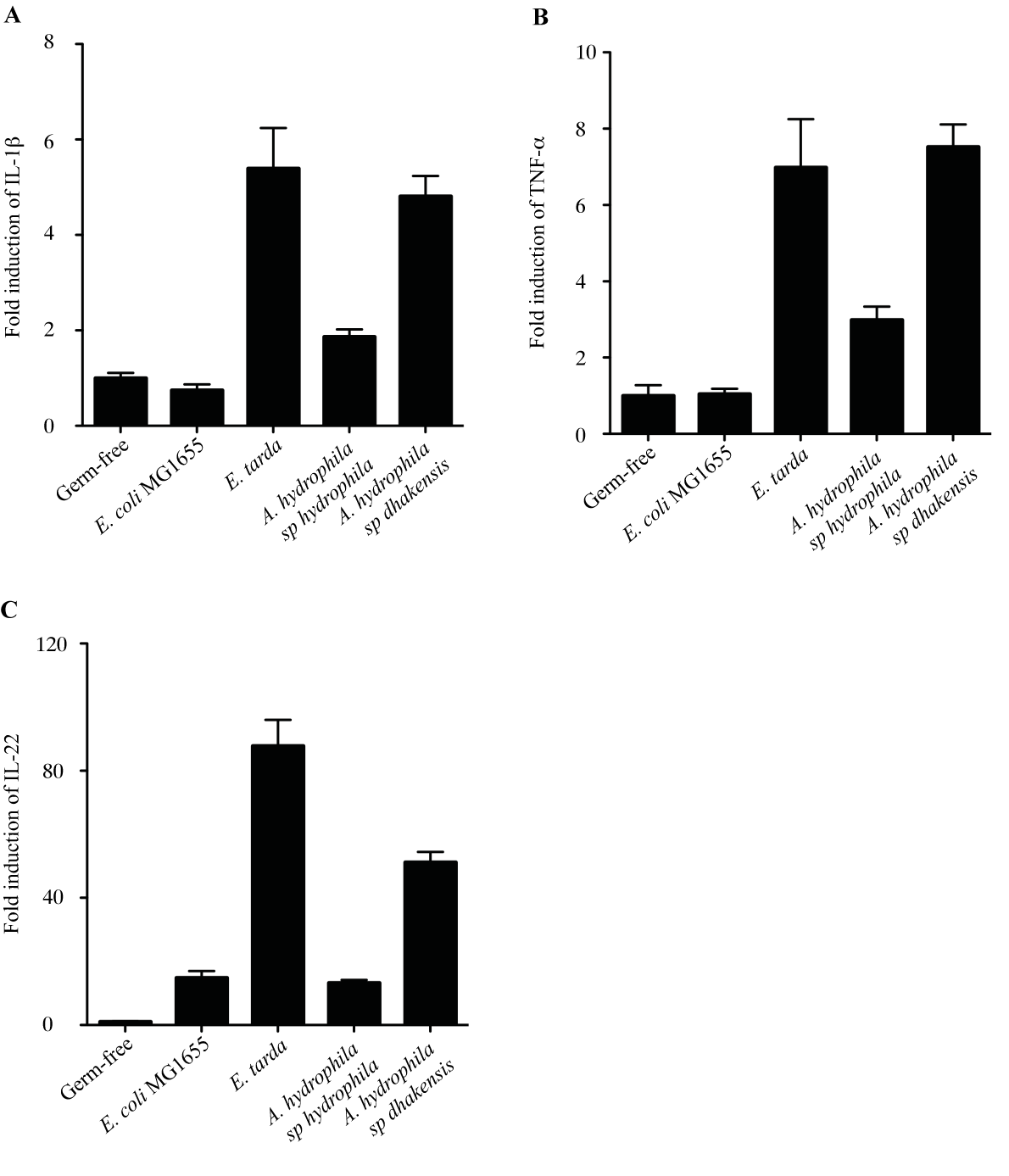
|  |  |  |
| --- | --- | --- |
| **Procedure** | **Name** | **Sequence** |
| *Sterility-16S* | B27 forward | 5’-AGAGTTTGATCCTGGCTCAG -3’ |
|  | B1492 reverse | 5’-GGTTACCTTGTTACGACTT -3’ |
|  |  |  |
| *IL-1*β *antisense probe* | IL-1β forward | 5’-ATGGCATGCGGGCAATATGA-3’ |
|  | IL-1β reverse | 5’-GAATTCATTAACCCTCACTAAAGGGAGGCCAGGTACAGGTTACTTT-3’ |
|  |  |  |
| *RT-PCR* | TNF-α forward | 5’-CAGAGTTGTATCCACCTGTTA -3’ |
|  | TNF-α reverse | 5’-TTCACGCTCCATAAGACCCA -3’ |
|  |  |  |
|  | IL-1β forward | 5’-GAGACAGACGGTGCTGTTTA -3’ |
|  | IL-1β reverse | 5’-GTAAGACGGCACTGAATCCA -3’ |
|  |  |  |
|  | IL-10 forward | 5’-AGAGCAGGAGAGTCGAATGC -3’ |
|  | IL-10 reverse | 5’-GTACCTCTTGCATTTCACCA -3’ |
|  |  |  |
|  | IL-22 forward | 5’-TTGGAATCAGACGAGCACAC-3’ |
|  | IL-22 reverse | 5’-GGCCAAATCCATAATTGCAC-3’ |
|  |  |  |
| *genomic qPCR* | Eictaluri-forward  forward | 5’-AGCGCCACCTTTGTGGATAA-3’ |
|  | Eictaluri-reverse | 5’-TACGCTTTCCTCAGTGAGTG-3’ |
|  | Csf1r-forward | 5’-TGGACTTCACAGGAACATACAAG-3’ |
|  | Csf1r-reverse | 5’-TCGGAGAAACAAAGAGAACTCG-3’ |

SUPPLEMENTARY FIGURES

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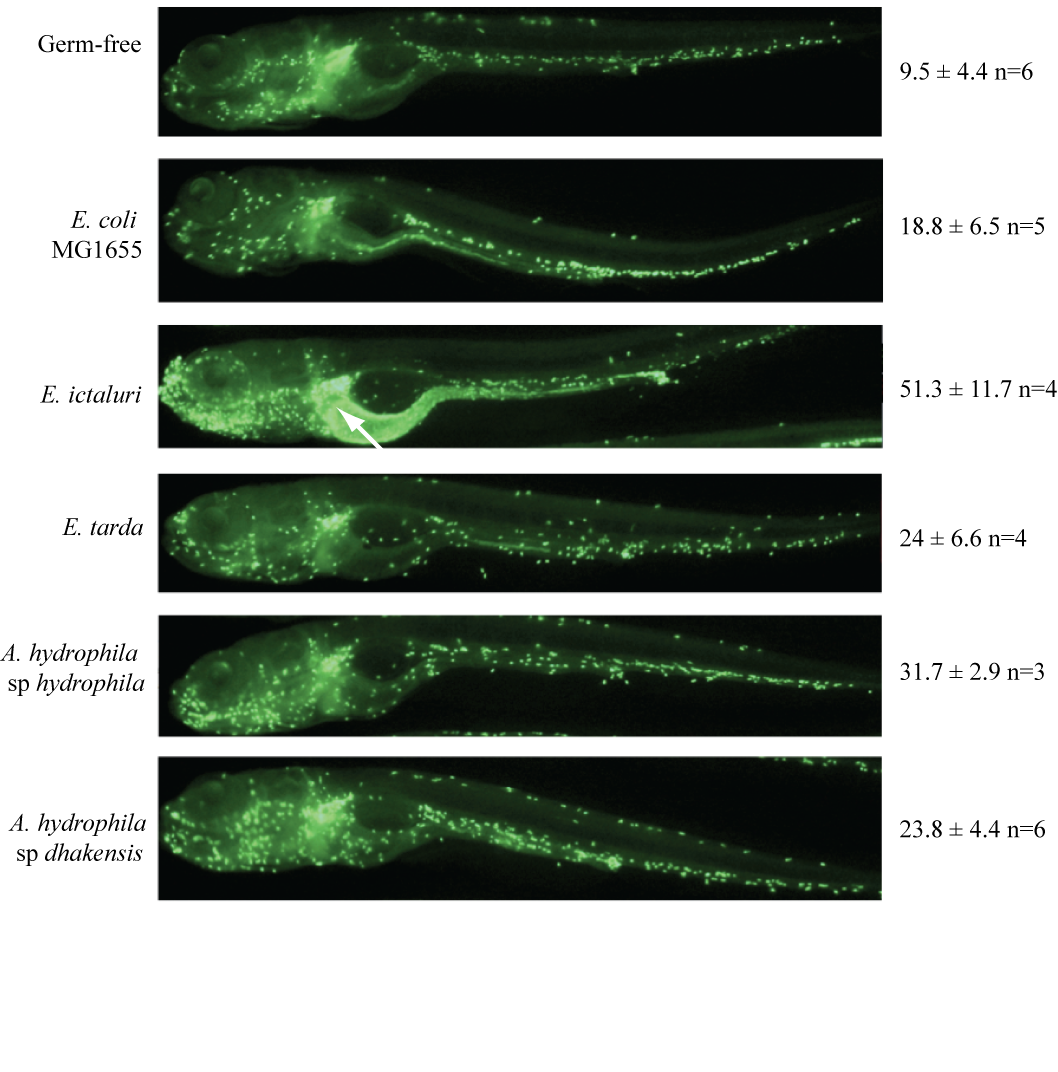
**Figure S1,** Rendueles *et al*

**Figure S1. Protocol and timeline of axenic zebrafish infection and co-infection used in this study.** After fertilization, eggs are sterilized and kept in sterile, autoclaved mineral water at 28°C in vented cap cell culture flasks until 6 dpf. Zebrafish larvae are then transferred one-by-one into 24-well microtiter plates containing 2 ml of water per well. Starting at 4 dpf, larvae are fed every 2 days with axenic *T. thermophila* till day 15. For longer experiments, in addition to *Tetrahymena*, larvae were also fed axenic *A. salina* from 10 dpf onwards. Pathogenic bacteria are added to the water at 6 dpf for 6 h and then larvae are transferred to fresh water. To test the protective effect of potentially probiotic strains, larvae were pre-colonized by commensal bacteria diluted in water at 4 dpf, after hatching.



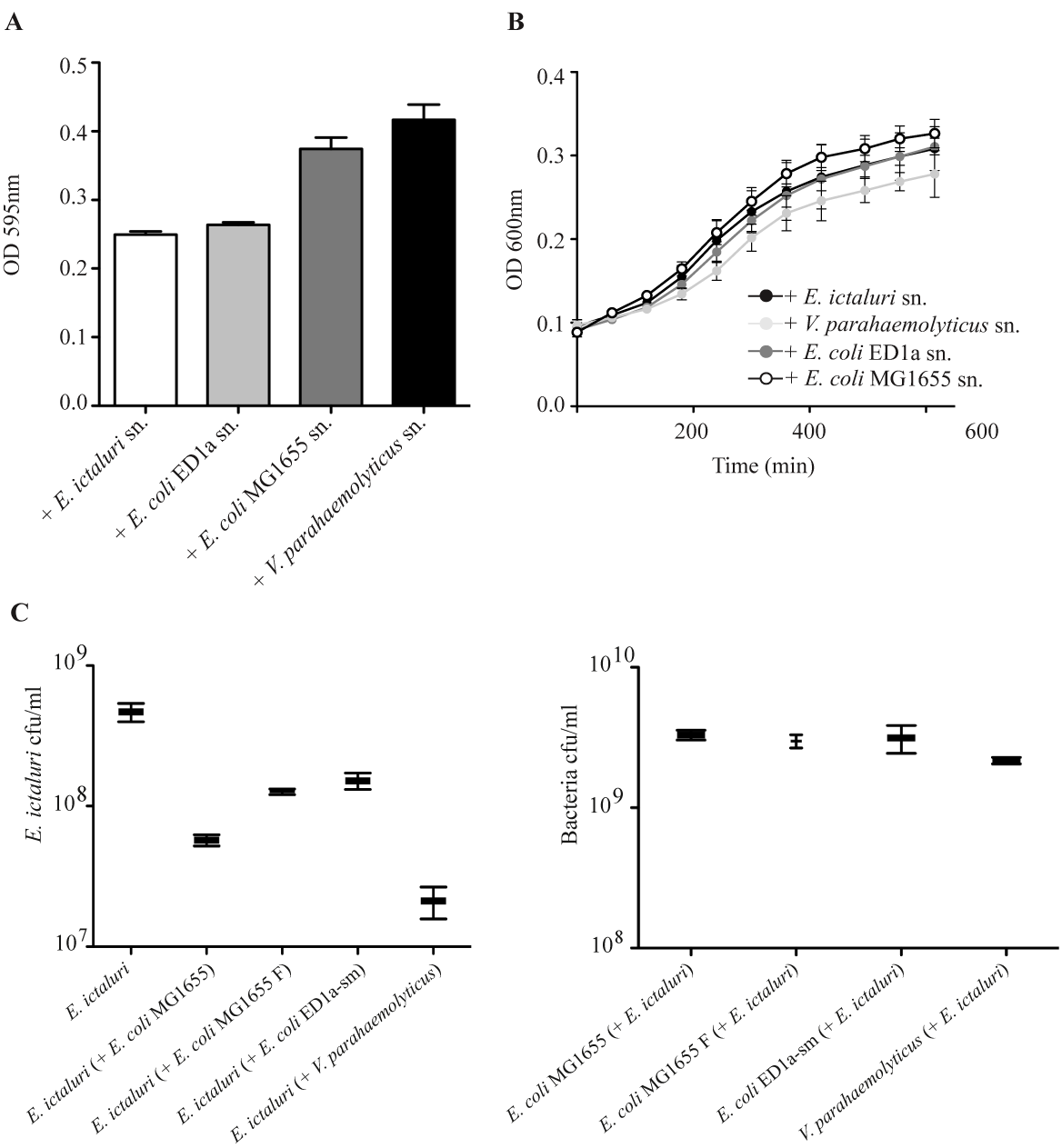
**Figure S2,** Rendueles *et al*

**Figure S2. Inflammation marker expression of gnotobiotic zebrafish larvae upon infection by mild pathogens.**qRT-PCR was performed using primers specific to *il1b* (**A**), *tnfa* (**B**), and *il22* (**C**) (inflammation markers) on RNA extracted from pools of 5 larvae at 3 dpi from germ-free larvae or larvae exposed to *E. coli* MG1655 (control), *E. tarda, A. hydrophila* sp. hydrophilaor *A hydrophila* sp. *dhakensis* at 4 dpf. Levels are expressed relative to the germ-free larvae. Error bars represent 95% confidence intervals from three technical replicates; one representative experiment out of two.



**Figure S3,** Rendueles *et al*

**Figure S3. Neutrophil localization upon pathogen infection**. Neutrophil infection in germ-free *mpx::gfp* larvae or *mpx::gfp* larvae infected with *E. coli* MG1655 (control) or different pathogens. At 4 days post-infection, larvae were fixed and analyzed by whole-mount immunofluorescence. Neutrophils were detected as GFP-expressing leukocytes (green). A quantification of gut-associated neutrophils in infected larvae is indicated on the right for each group of larvae. Note also GFP-expressing enterocytes as indicated by white arrow.

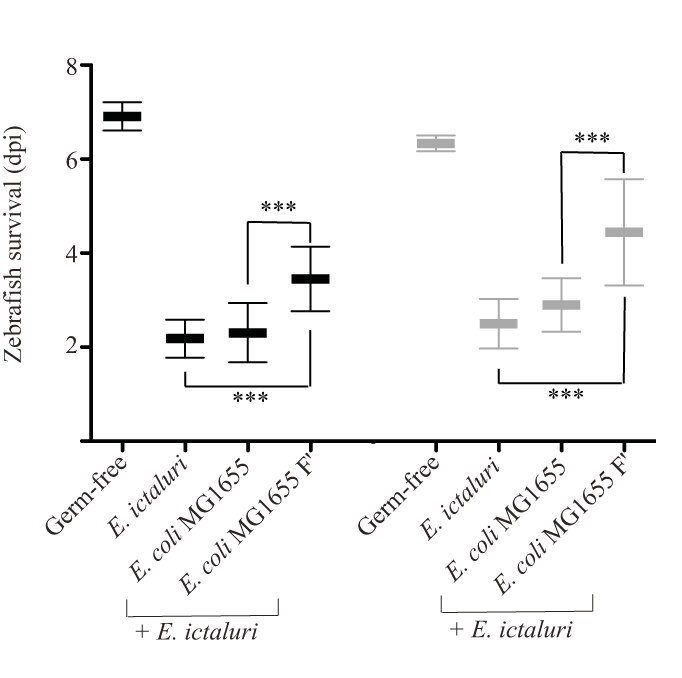


**Figure S4,** Rendueles *et al*

**Figure S4.** **Impact of identified protective strains on *E. ictaluri* growth and biofilm formation**  (**A**) Biofilm assay: *E. ictaluri* was mixed in a 1:1 ratio with filtered supernatants of probiotic strains and grown in 96-well microtiter plates at 28°C for 48 h. Microtiter plates were then washed 3 times with water and stained with crystal violet. Biofilm formation was quantified by dissolution of crystal violet and measurement at OD 595 nm. Addition of *E. ictaluri*’s own supernatant was included as a control. (**B**) *E. ictaluri* growth in presence of probiotic supernants: *E. ictaluri* inoculum was mixed in a 1:1 ratio with filtered supernatant (sn.) from *E. coli* MG1655*,E. coli* ED1a-sm, and *V. parahaemolyticus* and allowed to grow at 28°. OD 600 nm measurements were taken every 30 minutes. Growth of *E. ictaluri* with its own supernatant was included as a control. The assay was performed twice in microtiterplates, and 12 different wells were monitored for each condition. (**C**) Broth co-cultures of *E. ictaluri* with the three identified protective strains. 3ml of BHI medium was inoculated with *E. ictaluri* alone or with probiotic strain and co-cultures were incubated at 30°C with agitation. Serial dilutions of over-night resulting co-cultures were spotted on BHI+catalase plates in order to obtained isolated colonies (*E. ictaluri* forms patches rather than individualized colonies in absence of catalase). Plates were incubated at 30°C overnight and *E. ictaluri* and *E. coli* MG1655*,E. coli* ED1a-sm, and *V. parahaemolyticus* cfu were counted. *E. ictaluri* was distinguished from co-cultivated bacteria based on its characteristic yellowish colony morphotype. **Left panel:** *E. ictaluri*  cfu in corresponding co-cultures. **Right panel:** Corresponding protective bacteria cfu in corresponding co-cultures with *E. ictaluri*. Results are expressed as mean±SD of three co-cultures for each condition.

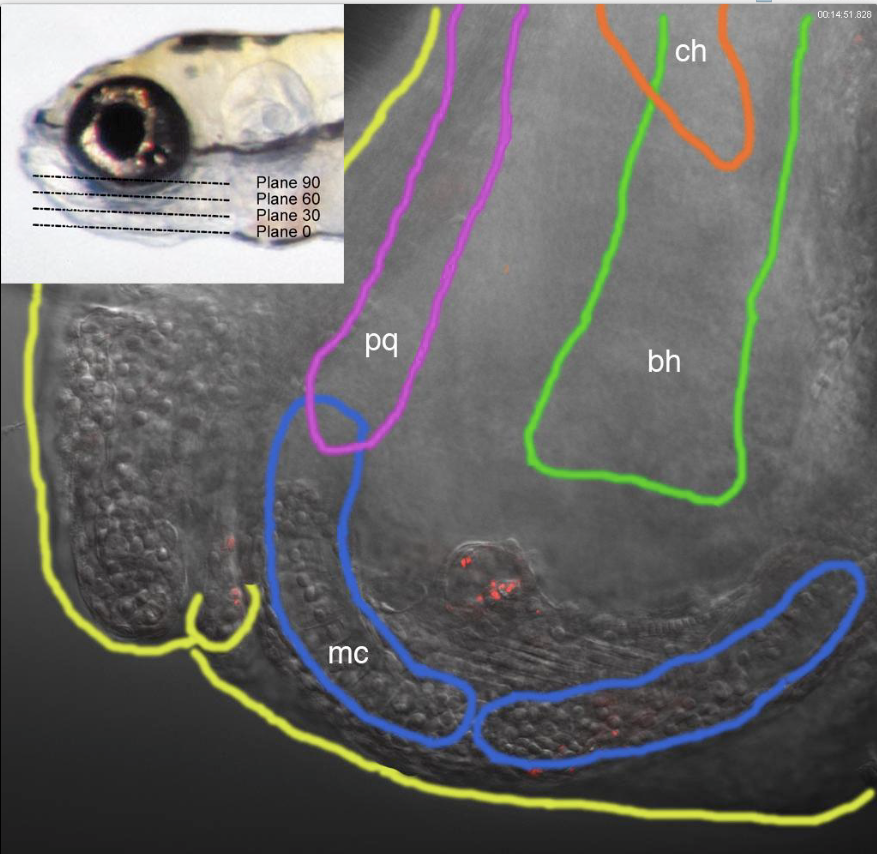
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**Figure S5,** Rendueles *et al*

**Figure S5*.*Neutrophils redistribution in head and gut.** Neutrophils redistribution was quantified by calculating the ratio (neutrophils counts in hematopoietic sites/neutrophils counts in the head and gut) for each larvae. Results are presented as mean+SEM. Statistical significance was calculated between the corresponding pretreated larvae infected and non-infected by *E. ictaluri* using unpaired two-tailed t-test with Welch's correction (\*p<0.05, \*\*p< 0.01, \*\*\*p<0.001). One larvae infected by *E. ictaluri* with a high ratio value was considered as an outlier and excluded from this analysis.

**Figure S6,** Rendueles *et al*

**Figure S6*.*  Compared life expectancy upon *E. ictaluri* infection in germ-free and conventional larvae pre-colonized with various *E. coli*.** Mortality of germ-free (black) and conventional (grey) zebrafish larvae pre-colonized at 4 dpf with *E. coli* MG1655 and *E. coli* MG1655 F’, and infected at 6 dpf with *E. ictaluri*. Mean survival is represented by a large hyphen. Standard deviations are also indicated ( \*\*\*p<0.001).

****

**Video S1,** Rendueles *et al*

**Video S1.** ***Edwardsiella ictaluri* colonizes both sides of the lower jaw of zebrafish larvae.** Larva analyzed 3 days post-infection by whole-mount immunofluorescence, using an antibody staining bacteria; fluorescence image (red) superimposed to transmission images (gray). Larva Z-stack taken with a confocal microscope and 40x objective. Ventral view with some lateral tilt, anterior to bottom. The first image of the movies provides  a visual help on the top left corner (over the eye) roughly indicating the planes of observation throughout the movie, and a coloured scheme of the cartilages visible in the stack. mc: Meckel's cartilage; pq: palatoquadrate; bh: basihyal; ch: ceratohyal (see [Kimmel CB](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Kimmel%20CB%22%5BAuthor%5D); [Miller CT](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Miller%20CT%22%5BAuthor%5D) and [Moens CB](http://www.ncbi.nlm.nih.gov/pubmed?term=%22Moens%20CB%22%5BAuthor%5D). (2001),Specification and morphogenesis of the zebrafish larval head skeleton. Dev Biol. 15;233(2):239-57.) The yellow line depicts the contour of the fish. Note that figure 3D corresponds to a maximal projection of planes 61 to 75 of the whole stack.