The Responses of Germ-Free Zebrafish (*Danio rerio*) to Varying Bacterial Concentrations, Colonization Time Points and Exposure Duration

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Running title: Bacteria mono-association of germ-free zebrafish

|  |  |  |  |
| --- | --- | --- | --- |
| Gene name | Forward (5′-3′) | Reverse (5′-3′) | Sequence Numbers |
| In zebrafish (*Danio rerio*)  |  |
| **Elongation factor 1α (*ef-1α*)** | CTGGAGGCCAGCTCAAACAT | ATCAAGAAGAGTAGTACCGCTAGCATTAC | FJ915061.1 |
| **Glyceraldehyde-3-phosphate dehydrogenase(*gapdh*)** | CGCTGGCATCTCCCTCAA | TCAGCAACACGATGGCTGTAG | NM\_213094.2 |
| **Bata-actin(*β-actin*)** | CGAGCTGTCTTCCCATCCA | TCACCAACGTAGCTGTCTTTCTG | XM\_028814403.1 |
| **Signal transducer and activator of transcription 3(*state3*)** | TAACCTCTTACTCATCCTCCA | AAGAGGTTGTAGAAGTAGA | CR847978.18 |
| **Cell division cycle 25A (*cdc25a*)** | TCGCTCTCCTGCCTTCAAGA | GACAGCGAATGACAGGCGAA | EU921896.1 |
| **Fasting-inducing adipose factor (*fiaf*)** | CGAGCGCATCAAGCAACA | TCGCTCGTTTTTCATCGTAATCT | NM\_001256203.1 |
| **Carnitine palmitoyltransferase 1a (*cpt1a*)** | GCAGATGACGGTTATGGTGTTTC | CCGTCTCAGGACTGGAGAACTT | NM\_001005940.1 |
| **X-box binding protein 1 (*xbp-1*)** | TGCGAGACAAGACGAGTGAT | CAGTACCTGAACCTGCTCCTT | BC164779.1 |
| **Activating transcription factor 6 (*Atf6*)** | CTTCAGACGCCTCAGTCAATG | GACCACAGGAGATGTTGTTACC | NM\_001110519.1 |
| **Serum amyloid a (*saa*)** | CGCAGAGGCAATTCAGAT | CAGGCCTTTAAGTCTGTATTTGTTG | NM\_001005599.1 |
| **Complement factor b (*bf*)** | GCCACAGTGCTACGCTGATTT | GTTGAACTGTTAGAGTTGTCGTTAGAGAATT | XM\_021468751.1 |
| **Proliferating cell nuclear antigen (*pcna*)** | GATGGTAGTTTGGGCCTTAGCTTT | AAAAACAAGACTGAATGTGGAGTATGTAC | NM\_131404.2 |
| **Heat-shock protein (*hsp70*)** | ACCAGGGCAACAGAACAACA | GTCATCAAACCTCCTGCCGA | NM\_001113589 |
| **Myeloiddifferentiationfactor88 (*myd88*)** | TCACGTACCTGGAGATCAAAAACTTCGAG | CCACTGGAACCTGAAGCGGTTTCCTC | NM\_212814.2 |
| **Solute carrier family 6 member 4 (*Slc4a6a*)** | AGTGGACCTGGGCAATG | AGAAGATACGGCAAGAGAAG | DQ285098 |
| **Monoamine oxidase (*mao*)** | GCAGTCAGAGCCCGAATC | CACACCCATAAACTTGAGGAATC | NM\_212827 |
| **Glucokinase (*gk*)** | GAGAGGACTGCGTGTGGAGACA | TCACCAACCTCGGAGCCTTCAG | NM\_001045385.2 |
| **5-hydroxytryptamine (serotonin) receptor 1A****(*htr1a*)** | AGAGCAGCGAGGTGAC | GAGCCGATGATTTGGTAAC | EH441641 |
| **Tryptophan hydroxylase 1a (*tph1a*)** | CAGTTCAGTCAGGAGATTGG | GACAGTGCGTGCTTCAG | AF548566 |
| **Brain derived neurotrophic factor (*bdnf*)** | GCTCAGTCATGGGAGTCC | ATAGTAACGAACAGGATGG | U42489 |
| **Interleukin-10 (*il-10*)** | ATTTGTGGAGGGCTTTCCTT | AGAGCTGTTGGCAGAATGGT | NM\_001020785.2 |
| **Interleukin-1β (*il-1β*)** | GAGACAGACGGTGCTGTTTA | GTAAGACGGCACTGAATCCA | NM\_212844.2 |
| **Tumor Necrosis Factor α （*tnf-α*）** | CAGAGTTGTATCCACCTGTTA | TTCACGCTCCATAAGACCCA | NM\_212859.2 |

**Supplementary Table S1.**

The primer sequences used for qPCR in the present study

**Supplementary Table S2.**

The results of normality test for measured genes in the present study

|  |  |  |
| --- | --- | --- |
|  | ***E. coli***  |  ***B. subtilis*** |
| **Genes** | **Statistic** | **P-value**  | **Statistic** | **P-value**  |
| *GK* | 0.951  | \*0.812 | 0.971  | \*0.970 |
| *state3* | 0.722  | \*\*0.002  | 0.807 | 0.838 |
| *cdc25a* | 0.983  | 0.103  | 0.875 | 0.565 |
| *xbp-1* | 0.862  | 0.851 | 0.943  | \*0.365 |
| *atf-6* | 0.981  | 0.314 | 0.932  | \*0.138 |
| *bf* | 0.955  | 0.339  | 0.9221  | \*\*0.021 |
| *pcna* | 0.728  | \*0.052 | 0.921  | \*0.137  |
| *hsp70* | 0.985  | 0.078  | 0.956  | 0.606  |
| *myd88* | 0.864  | 0.596  | 0.790  | \*\*0.014 |
| *saa* | 0.981  | \*0.877 | 0.931  | 0.055  |
| *fiaf* | 0.953  | 0.166  | 0.784  | 0.388  |
| *cpt1a* | 0.724 | \*0.064 | 0.710  | \*0.098 |
| *il-10* | 0.987  | \*0.061 | 0.871  | \*0.461  |
| *tnf-a* | 0.860  | 0.682  | 0.986  | 0.684  |
| *il-1b* | 0.981  | 0.081  | 0.761  | 0.304 |
| *htr1aa* | 0.953 | 0.282  | 0.954  | 0.282  |
| *tph1a* | 0.723  | \*\*0.006 | 0.721  | \*0.073  |
| *mao* | 0.986  | 0.861  | 0.985  | \*0.858 |
| *slc4a6a* | 0.862  | \*0.491 | 0.863  | \*0.491 |
| *bdnf* | 0.981  | 0.816  | 0.981  | 0.816 |

Numbers without a symbol for p values were normally distributed (p > 0.05). The symbol \* indicates the results of p values for data which were normally distributed after log transformation, while \*\* shows the p values results of parameters that were not normally distributed even after log transformation.

 **Supplementary Figure S1.**



Dissolved oxygen level in the media and the expression level of hypoxia inducible factors 3α (*hif3α*)when zebrafish were inoculated with *E. coli* DH5α*.* (**A**) Dissolved oxygen level of media of GF group and all mono-associated groups (inoculated with *E. coli* DH5α ranging from 102 CFU/ml to 108 CFU/ml at 3 dpf and immersed for 24 h or 48 h). (**B**) The expression level of *hif3α* of GF group and all mono-associated groups (inoculated with *E. coli* DH5α ranging from 102 CFU/ml to 108 CFU/ml at 3 dpf and immersed for 24 h). All data (A, B) are presented as means ± SEM, n = 45 per group.

**Supplementary Figure S2.**



Dissolved oxygen level in the media and the mRNA expression level of hypoxia inducible factors 3 alpha (*hif3α*)when zebrafish were inoculated with *B. subtilis* WB800N*.* (**A**) Dissolved oxygen level of media of GF group and all mono-associated groups (inoculated with *B. subtilis* WB800N ranging from 102 CFU/ml to 108 CFU/ml at 3 dpf and immersed for 24 h or 48 h). (**B**) The expression level of *hif3α* of GF group and all mono-associated groups (inoculated with *B. subtilis* WB800N ranging from 102 CFU/ml to 108 CFU/ml at 3 dpf and immersed for 24 h). All data are presented as means ± SEM, n = 45 per group.

**Supplementary Figure S3**



Comparisons of gene expression levels among GF zebrafish, CONR zebrafish and mono-associated zebrafish (inoculated with *E. coli* DH5α ranging from 102 CFU/ml to 106 CFU/ml at 3 dpf and immersed for 24 h). (**A**) The expression levels of immunity related genes *saa*, (**B**) *bf* and(**C**) *myd88.* All results are shown as means ± SEM of six replicates for each experiment, n = 90, per group. Different letters indicate statistically significant differences among groups (p < 0.05).

**Supplementary Figure S4.**



Comparisons of gene expression levels among GF zebrafish, CONR zebrafish and mono-associated zebrafish (inoculated with *B. subtilis* WB800N ranging from 102 CFU/ml to 106 CFU/ml at 3 dpf and immersed for 24 h). **(A)** The expression levels of immunity related genes *saa*, **(B)** *bf* and **(C)** *myd88*. The results are shown as means ± SEM of six replicates for each experiment, n = 90, per group. Different letters indicate statistically significant differences among groups (p < 0.05).