Exposure to yeast shapes the intestinal bacterial community assembly in zebrafish larvae

Prabhugouda Siriyappagouder[1], Jorge Galindo-Villegas[2], Jep Lokesh[1],

Victoriano Mulero[2], Jorge Fernandes*[1] and Viswanath Kiron*[1]

[1]*Faculty of Biosciences and Aquaculture, Nord University, 8049 Bodø, Norway*

[2]*Department of Cell Biology and Histology, Faculty of Biology, Institute of Biomedical Research of Murcia-Arrixaca. Campus Universitario de Espinardo. University of Murcia, 30100 Murcia, Spain*

Supplementary figure legends

FIGURE S1. Rarefaction curves for the species richness of the intestinal bacteria in zebrafish larvae. The curves were generated by plotting the number of species against the number of sequences per sample. The shaded portion around each line represents the 95% confidence interval. Colour codes and the respective samples are: Royal blue – CRC (n = 7), yellow green – CRD (n = 9), orange red – CRP (n = 9), navy blue – GFC (n = 7), yellow – GFD (n = 6), maroon – GFP (n = 7) and sky blue – water samples (n = 17, from all the treatments and zebrafish facility).

FIGURE S2. Relative abundance of the core microbiota in the intestine of zebrafish larvae. Prevalence and detection thresholds of 0.97 and 0.2, respectively were used to determine the core microbiota. Conventionally-raised control– CRC, conventionally-raised *Debaryomyces*-exposed– CRD, conventionally-raised *Pseudozyma*-exposed– CRP, germ-free control– GFC, germ-free *Debaryomyces*-exposed– GFD, and germ-free *Pseudozyma*-exposed– GFP.

FIGURE S3. Relative abundance of the top 20 abundant taxa (OTUs) among the intestinal bacterial communities of zebrafish larvae. Classes (A), orders (B) and families (C). Shades of purple and pink are given for the phylum Proteobacteria, and green for Bacteriodetes. Conventionally-raised control– CRC, conventionally-raised *Debaryomyces*-exposed– CRD, conventionally-raised *Pseudozyma*-exposed– CRP, germ-free control– GFC, germ-free *Debaryomyces*-exposed– GFD, and germ-free *Pseudozyma*-exposed– GFP.

FIGURE S4. Composition and diversity of the intestinal bacterial communities of the conventionally-raised (CR) and germ-free (GF) zebrafish larvae from the control group. Principal coordinate analysis plot based on weighted UniFrac distance metric (A). Ellipses are drawn include 95% of samples from a normally distributed data. Species richness (B), Shannon diversity (C), Simpson diversity (D), phylogenetic diversity (E) and core abundance (F).

FIGURE S5. Relative abundance of bacterial classes in the intestine of zebrafish larvae and water samples. CRCW, CRDW and CRPW represent the water samples collected from their respective flasks/tanks on day 2, 3, 7 and 14. GFCW, GFDW and GFPW represent the water samples collected only on day 14. ZSW represents the water samples collected from the zebrafish facility on day 7 and 14.

Figure S1.

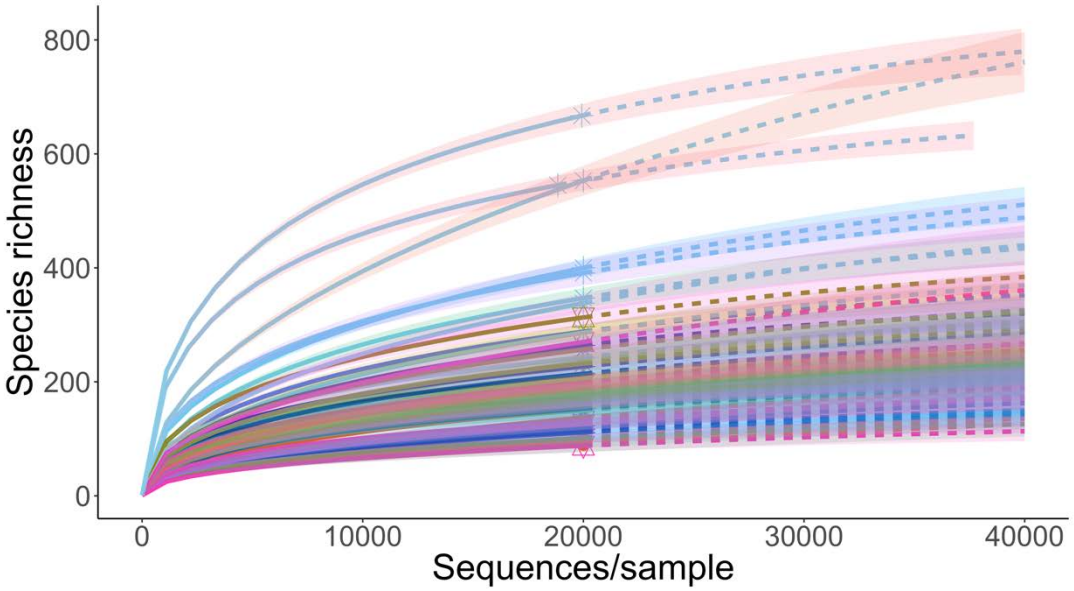

Species richness
800
600
400
200
0
0
10000
20000
30000
40000
Sequences/sample

Figure S2.

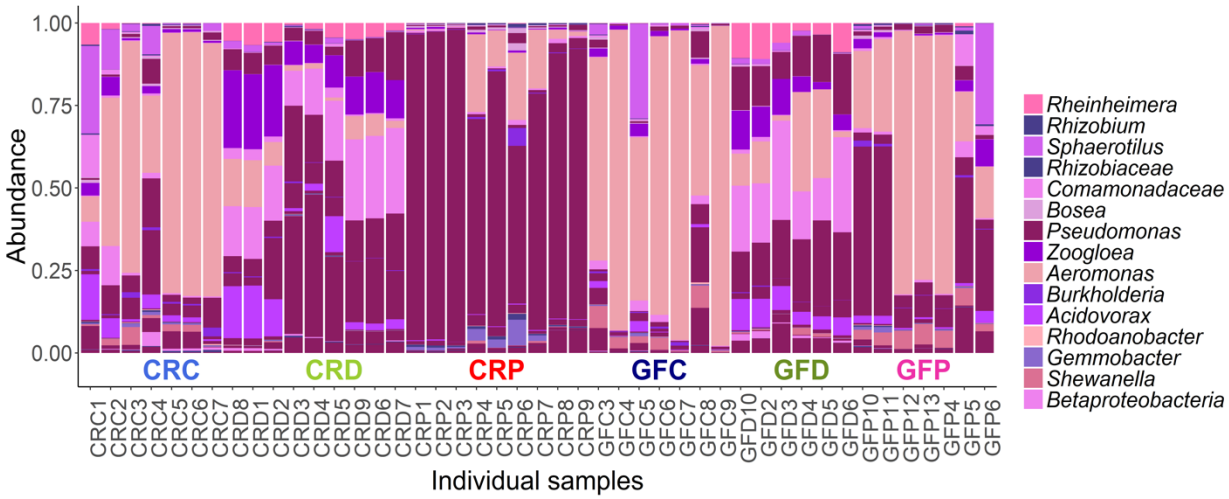

1.00
0.75
0.50
0.25
0.00
Abundance
CRC
CRD
CRP
GFC
GFD
GFP
CRC1 CRC2 CRC3 CRC4 CRC5 CRC6 CRC7 CRD8 CRD1 CRD2 CRD3 CRD4 CRD5 CRD9 CRD6 CRD7 CRP1 CRP2 CRP3 CRP4 CRP5 CRP6 CRP7 CRP8 CRP9 GFC3 GFC4 GFC5 GFC6 GFC7 GFC8 GFC9 GFD10 GFD2 GFD3 GFD4 GFD5 GFD6 GFP10 GFP11 GFP12 GFP13 GFP4 GFP5 GFP6
Individual samples
Rheinheimera
Rhizobium
Sphaerotilus
Rhizobiaceae
Comamonadaceae
Bosea
Pseudomonas
Zoogloea
Aeromonas
Burkholderia
Acidovorax
Rhodoanobacter
Gemmobacter
Shewanella
Betaproteobacteria

Figure S3.

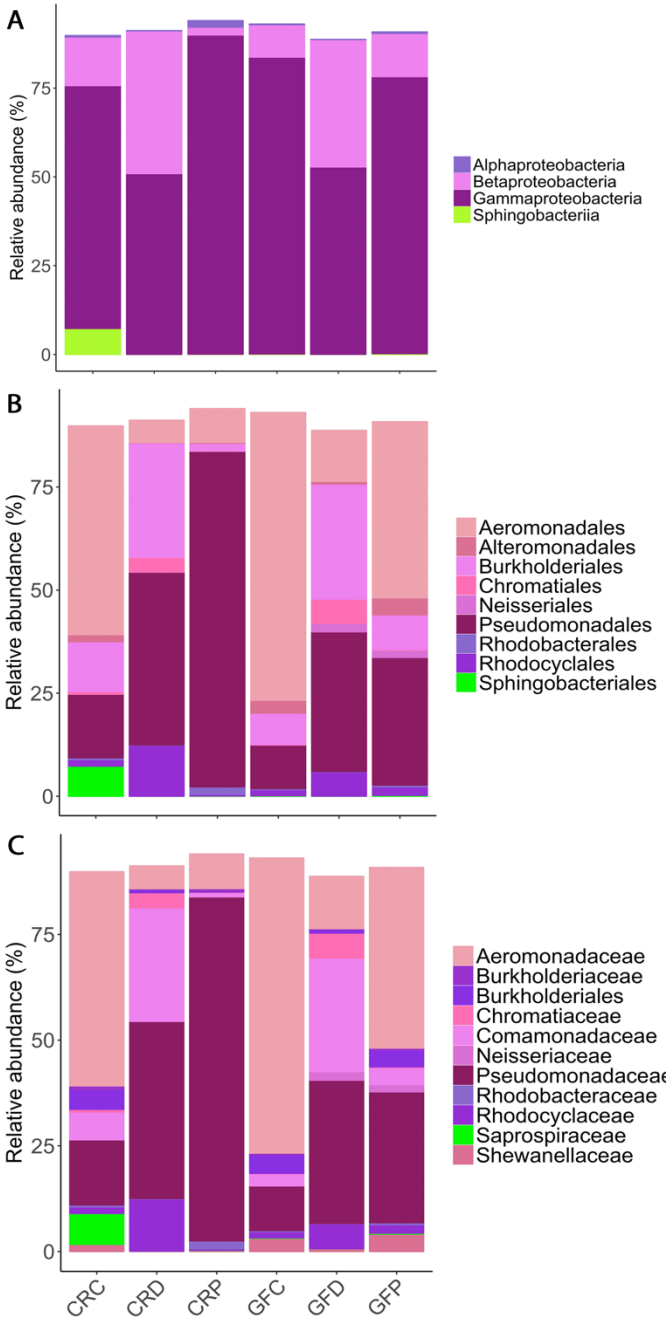

A
Relative abundance (%)
0
25
50
75
Alphaproteobacteria
Betaproteobacteria
Gammaproteobacteria
Sphingobacteriia
B
Relative abundance (%)
0
25
50
75
Aeromonadales
Alteromonadales
Burkholderiales
Chromatiales
Neisseriales
Pseudomonadales
Rhodobacterales
Rhodocyclales
Sphingobacteriales
C
Relative abundance (%)
0
25
50
75
Aeromonadaceae
Burkholderiaceae
Burkholderiales
Chromatiaceae
Comamonadaceae
Neisseriaceae
Pseudomonadaceae
Rhodobacteraceae
Rhodocyclaceae
Saprospiraceae
Shewanellaceae
CRC
CRD
CRP
GFC
GFD
GFP

Figure S4.

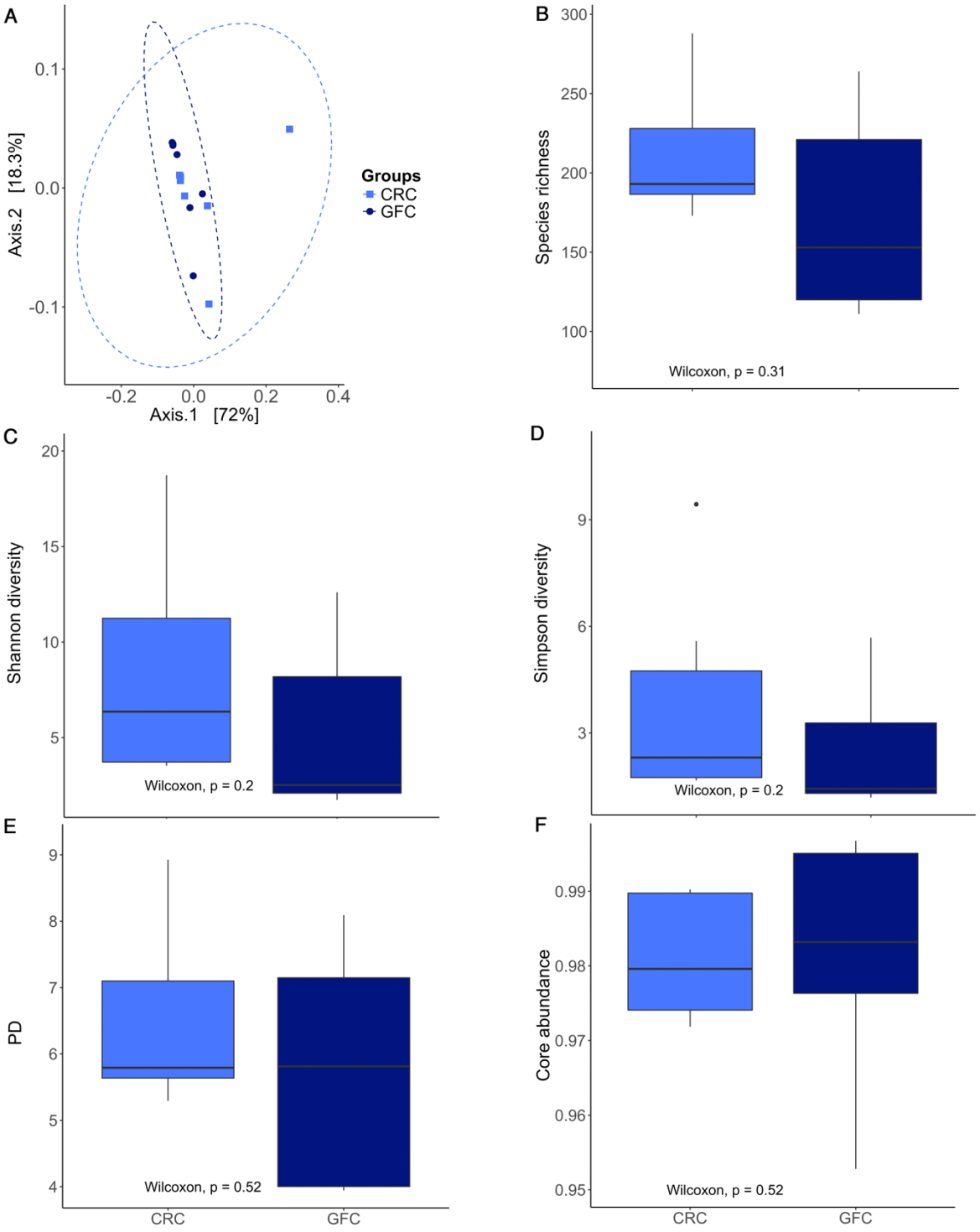
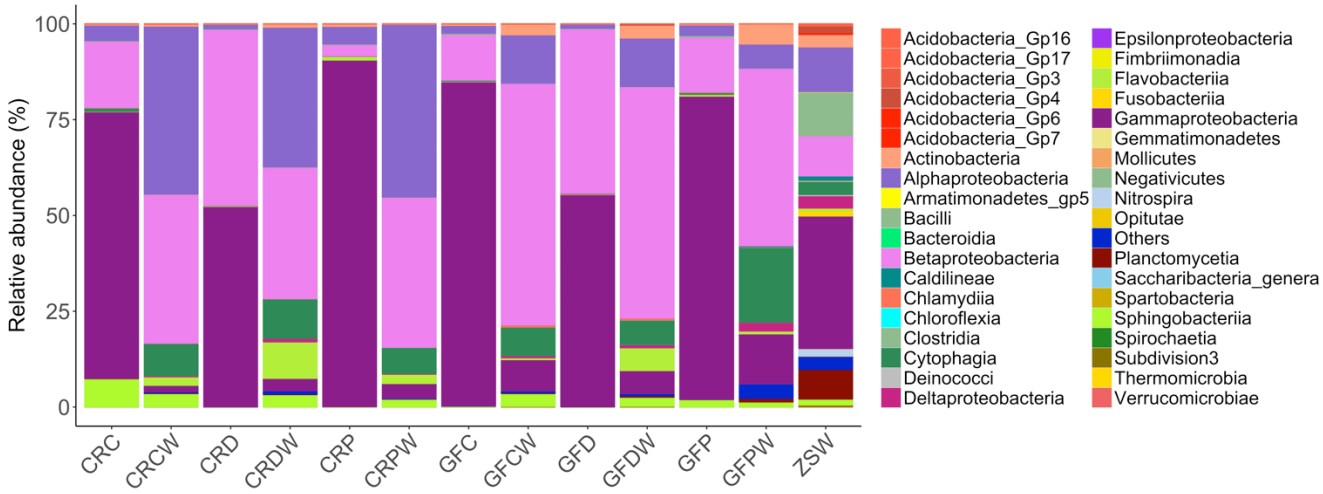

A
0.1
0.0
-0.1
Axis.2 [18.3%]
-0.2
0.0
0.2
0.4
Axis.1 [72%]
Groups
CRC
GFC
B
300
250
200
150
100
Species richness
Wilcoxon, p = 0.31
C
20
15
10
5
Shannon diversity
Wilcoxon, p = 0.2
D
9
6
3
Simpson diversity
Wilcoxon, p = 0.2
E
9
8
7
6
5
4
PD
Wilcoxon, p = 0.52
CRC
GFC
F
0.99
0.98
0.97
0.96
0.95
Core abundance
Wilcoxon, p = 0.52
CRC
GFC

Figure S5.


Relative abundance (%)
100
75
50
25
0
CRC
CRCW
CRD
CRDW
CRP
CRPW
GFC
GFCW
GFD
GFDW
GFP
GFPW
ZSW
Acidobacteria_Gp16
Acidobacteria_Gp17
Acidobacteria_Gp3
Acidobacteria_Gp4
Acidobacteria_Gp6
Acidobacteria_Gp7
Actinobacteria
Alphaproteobacteria
Armatimonadetes_gp5
Bacilli
Bacteroidia
Betaproteobacteria
Caldilineae
Chlamydiia
Chloroflexia
Clostridia
Cytophagia
Deinococci
Deltaproteobacteria
Epsilonproteobacteria
Fimbriimonadia
Flavobacteriia
Fusobacteriia
Gammaproteobacteria
Gemmatimonadetes
Mollicutes
Negativicutes
Nitrospira
Opitutae
Others
Planctomycetia
Saccharibacteria_genera
Spartobacteria
Sphingobacteriia
Spirochaetia
Subdivision3
Thermomicrobia
Verrucomicrobiae