

Supplementary Material

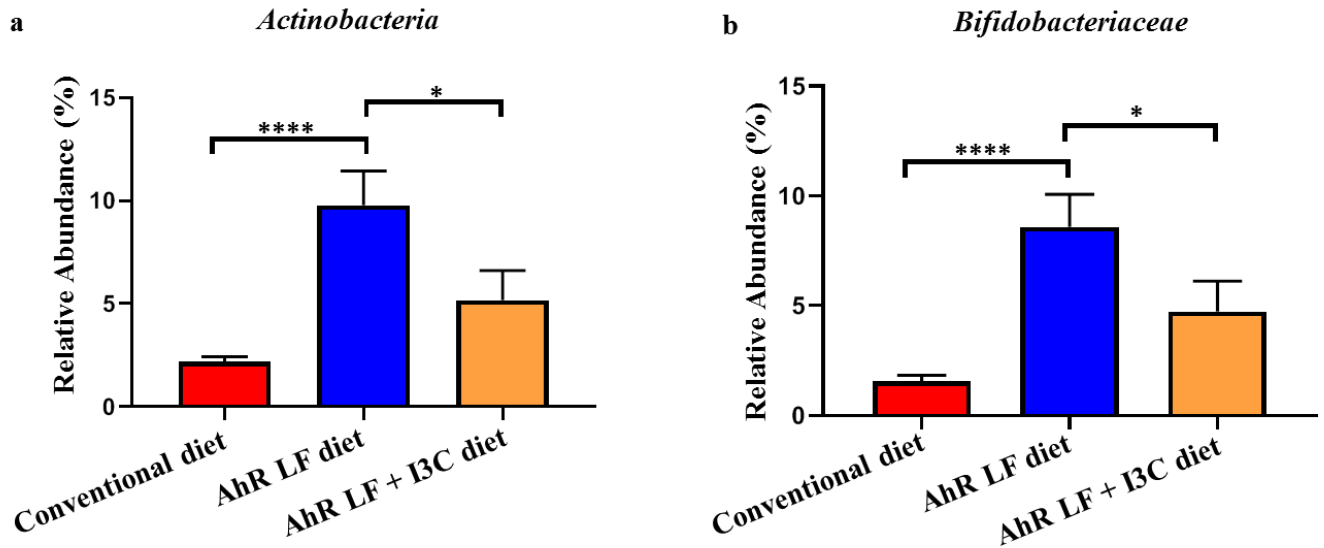
Depletion of dietary aryl hydrocarbon receptor ligands alters microbiota composition and function

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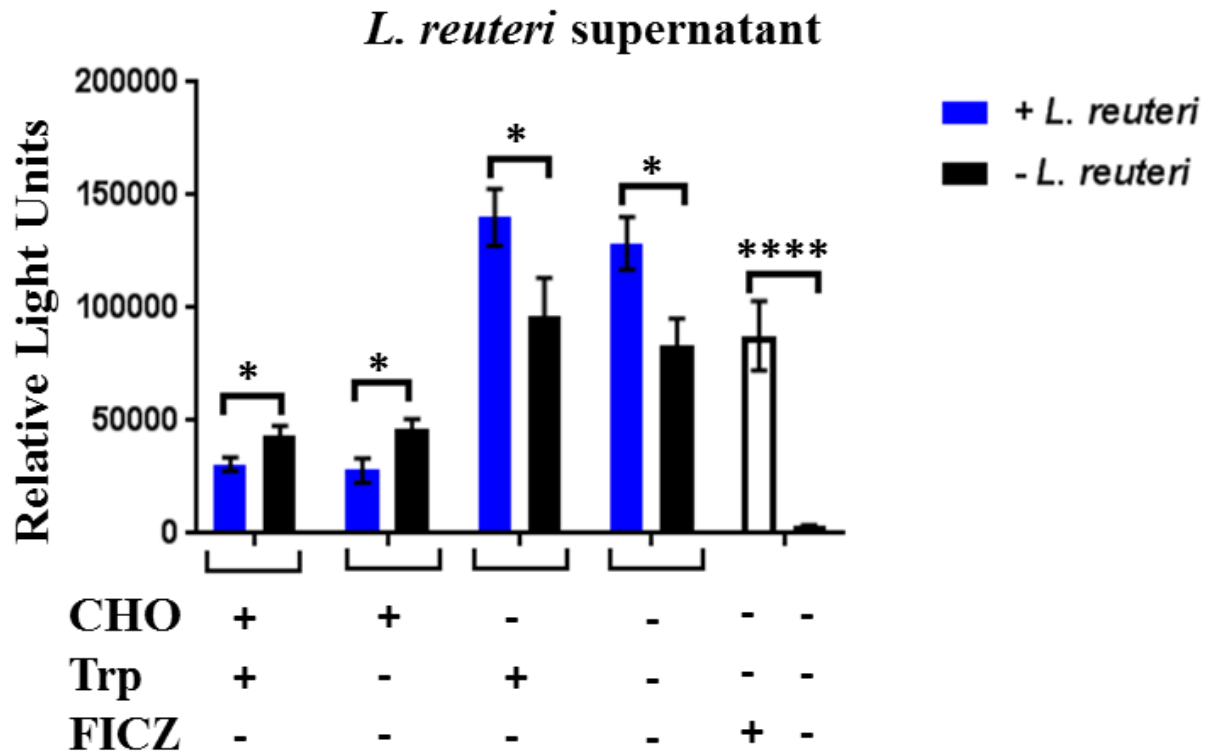
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Supplementary Figure 1



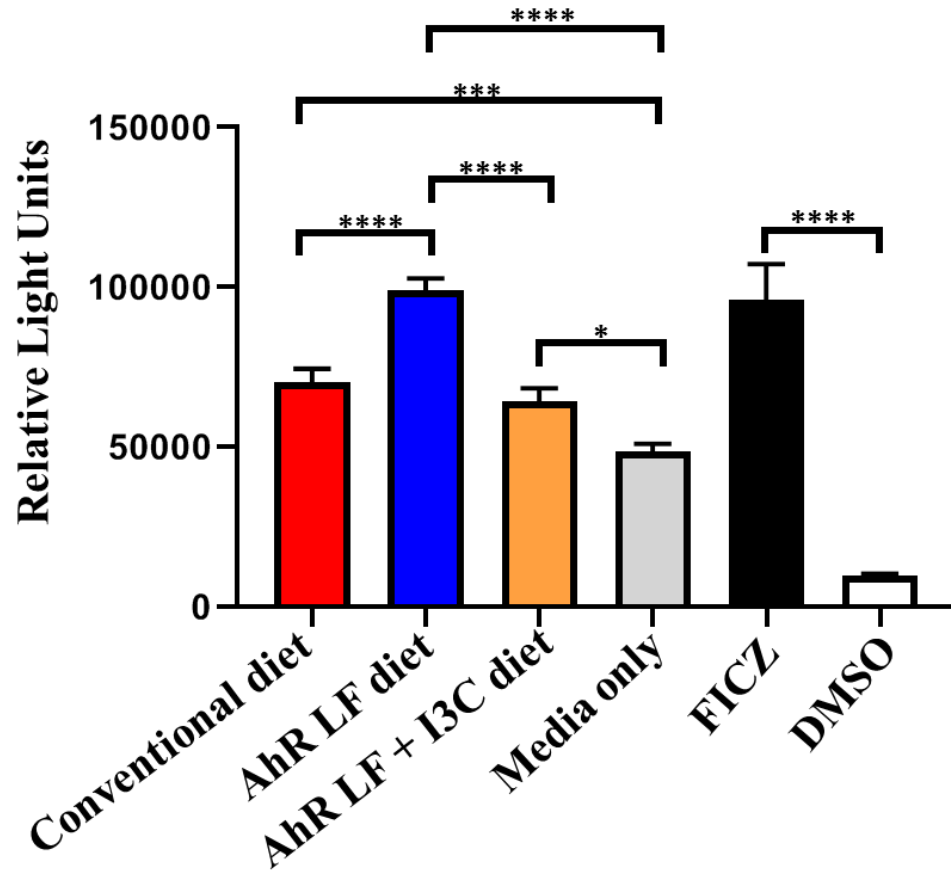
Relative abundance of (a) *Actinobacteria* and (b) *Bifidobacteriaceae* from mice on the conventional diet (n=12), mice on the AhR ligand-free (LF) diet (n=7), and mice on the AhR LF diet supplemented with I3C (AhR LF + I3C diet) (n=6). Error bars show mean \pm SEM. *p < 0.05 ****p < 0.0001, one-way ANOVA with Tukey's multiple comparisons test.

Supplementary Figure 2



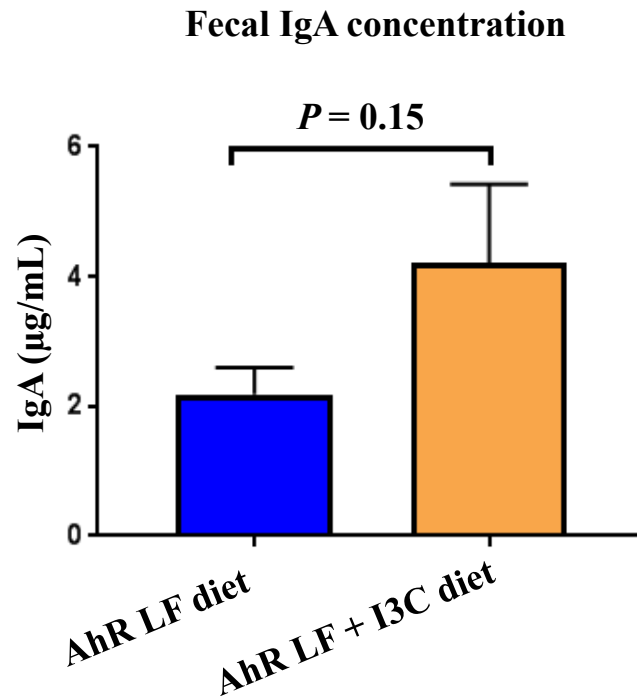
HCT116 cells engineered to express luciferase upon AhR ligation were treated with bacterial conditioned supernatants from *Lactobacillus reuteri* cultured under the indicated conditions. Cells were also treated with non-conditioned broth to establish baseline levels of any AhR ligands present. As a positive control, some cells were treated with 100nM of 6-formylindolo[3,2-b]carbazole (FICZ), a known AhR ligand. Dimethyl sulfoxide (DMSO), the FICZ vehicle, was used as a negative control. n=9 per group. Error bars show mean \pm SEM. *p < 0.05 ****p < 0.0001, Student's t test.

Supplementary Figure 3



Culture supernatants of fecal samples obtained from mice on the indicated diet were cultured with HCT116 cells that express luciferase when AhR is activated. HCT116 cells were also treated with sterile chopped meat broth (media only) to evaluate how initial levels of AhR ligands in the broth are altered following fecal culture. As a positive control, some cells were treated with 100nM of 6-formylindolo[3,2-b]carbazole (FICZ), a known AhR ligand. Dimethyl sulfoxide (DMSO), the FICZ vehicle, was used as a negative control. n=7 or 8 per group. Error bars show mean \pm SEM. *p<0.05 ***p<0.001 ****p<0.0001, one-way ANOVA with Tukey's multiple comparisons test.

Supplementary Figure 4



Fecal samples from mice on the AhR ligand-free (LF) diet and AhR LF diet supplemented with I3C (AhR LF + I3C diet) were collected and IgA levels were determined by ELISA. $n=5$ for each group. Error bars show mean \pm SEM. P value was obtained from a two-tailed, unpaired Student's t test.

Supplementary Table 1

Relative abundance of bacterial families among each diet and in all mice combined

	CD average	LF average	I3C average	Overall average
k_Bacteria;p_Firmicutes;c_Erysipelotrichi;o_Erysipelotrichales;f_Erysipelotrichaceae	0.176450108	0.464700659	0.247591048	0.274234088
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_S24-7	0.199449395	0.123568204	0.128024563	0.161060702
k_Bacteria;p_Verrucomicrobia;c_Verrucomicrobiae;o_Verrucomicrobiales;f_Verrucomicrobiaceae	0.057919947	0.130534882	0.319514684	0.141034866
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Lachnospiraceae	0.128443678	0.025282454	0.037914195	0.077831459
k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Lactobacillaceae	0.128367083	0.029214056	0.020014263	0.074599559
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;Other	0.038259267	0.023601923	0.081191576	0.044458965
k_Bacteria;p_Actinobacteria;c_Actinobacteria;o_Bifidobacteriales;f_Bifidobacteriaceae	0.015458435	0.085793387	0.047286109	0.042790863
k_Bacteria;p_Proteobacteria;c_Deltaproteobacteria;o_Desulfovibrionales;f_Desulfovibrionaceae	0.022562838	0.034865496	0.026801647	0.027024897
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f__	0.04845347	0.006001074	0.007001933	0.02661843
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Ruminococcaceae	0.02074361	0.023537134	0.031336934	0.024068194
k_Bacteria;p_Firmicutes;c_Bacilli;o_Turicibacteriales;f_Turicibacteraceae	0.048257014	0.001901098	7.73806E-05	0.023714245
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Clostridiaceae	0.036399596	6.28506E-05	0.000146797	0.017524636
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Prevotellaceae	0.022596605	0.000283726	0.001100646	0.011189969
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Rikenellaceae	0.0140724	0.002557747	0.011112188	0.010137846
k_Bacteria;p_Firmicutes;Other;Other;Other	0.016681076	0.003885122	0.001619814	0.009483506
k_Bacteria;p_Actinobacteria;c_Coriobacteriia;o_Coriobacteriales;f_Coriobacteriaceae	0.006404491	0.012066181	0.004457819	0.007522563
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Bacteroidaceae	0.007953848	0.006178197	0.006041413	0.006997682
k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;f_Streptococcaceae	0.000717262	0.003815807	0.009694182	0.003739316
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_Rickettsiales;f__	0.001562048	0.003075664	0.003880468	0.002542281
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_Porphyrimonadaceae	0.00017968	0.00700729	0.000463006	0.002159409
k_Bacteria;Other;Other;Other;Other	0.000308934	0.004205754	0.001449977	0.001673894
k_Bacteria;p_Firmicutes;c_Clostridia;Other;Other	0.000196381	0.002377021	0.00335733	0.001565588
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Odoribacteraceae]	0.00010789	0.001431372	0.004161956	0.001451441
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Moraxellaceae	0.001897397	0.000737582	2.16084E-05	0.00112246
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_[Mogibacteriaceae]	0.001446969	0.000246997	0.00040885	0.000861828
k_Bacteria;p_Proteobacteria;c_Alphaproteobacteria;o_RF32;f__	0.00075304	0.00034592	0.000973969	0.000692069
k_Bacteria;p_Cyanobacteria;c_4C0d-2;o_YS2;f__		0.6.88777E-05	0.002253691	0.000560172
k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Alcaligenaceae	0.000786575	0.000104741	0.000135577	0.000439422
k_Bacteria;p_Firmicutes;c_Bacilli;Other;Other	0.00080704	6.58215E-05	2.71165E-05	0.000412317
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Dehalobacteriaceae	0.00034323	0.000182849	0.000758441	0.000397974
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f__	2.83688E-06	0.000874319	1.05299E-05	0.000248698
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Enterobacteriales;f_Enterobacteriaceae	0.000331185	5.859E-05	0.000235146	0.000231809
k_Bacteria;p_Firmicutes;c_Bacilli;o_Lactobacillales;Other	0.000326591	0.000166859	4.88147E-05	0.0002152
k_Bacteria;p_Proteobacteria;c_Betaproteobacteria;o_Burkholderiales;f_Comamonadaceae	0.000297337	0.000147422	0.000103714	0.000208891
k_Bacteria;p_Tenericutes;c_Mollicutes;o_RF39;f__	0.000172895	0.000349109	1.05299E-05	0.000183267
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Veillonellaceae	0.000232961	5.15748E-05	0.00014622	0.000161355
k_Bacteria;p_Proteobacteria;c_Gammaproteobacteria;o_Pseudomonadales;f_Pseudomonadaceae	0.000194941	9.67035E-05	9.19042E-05	0.000142706
k_Bacteria;p_Firmicutes;c_Bacilli;o_Bacillales;f_Staphylococcaceae	0.000138951	0.000126464	6.55179E-05	0.000117831
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Eubacteriaceae	0.000230757	9.06483E-06	1.64793E-05	0.000117257
k_Bacteria;p_Tenericutes;c_Mollicutes;o_Anaeroplasmatales;f_Anaeroplasmataceae	0.00023811		1.08243E-05	0.000116891
k_Bacteria;p_Bacteroidetes;c_Bacteroidia;o_Bacteroidales;f_[Barnesiellaceae]	0.000211246	2.34583E-05	1.06807E-05	0.00011053
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Christensenellaceae	2.8577E-06	0.00033745		0.9.58576E-05
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptococcaceae	4.00236E-05		0.000262141	8.21252E-05
k_Bacteria;p_Firmicutes;c_Clostridia;o_Clostridiales;f_Peptostreptococcaceae		0.5.91006E-05	0.000168317	5.69443E-05

Families are ordered from the highest to lowest overall relative abundance average (the average relative abundance of all mice in the study). Also shown is the relative abundance for each diet. CD, conventional diet (n=12). LF, AhR ligand-free diet (n=7). I3C, AhR ligand-free diet supplemented with I3C (n=6).

Supplementary Table 2

Characterization of the top 100 most abundant *Erysipelotrichaceae* operational taxonomic units (all 3 groups combined)

OTU abundance rank	Consensus lineage	Average relative abundance (all samples, %)	Top BLAST result (percent match)
1	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	20.7	Faecalibaculum rodentium strain ALO17 (88%)
6	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	3.7	Faecalibaculum rodentium strain ALO17 (93%)
11	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	1.67	Faecalibaculum rodentium strain ALO17 (100%)
64	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.138	Faecalibaculum rodentium strain ALO17 (90%)
75	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.109	Faecalibaculum rodentium strain ALO17 (92%)
80	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.101	Faecalibaculum rodentium strain ALO17 (97%)

Supplementary Table 3

Characterization of the top 100 most abundant *Erysipelotrichaceae* operational taxonomic units within the conventional diet group

OTU abundance rank	Consensus lineage	Average relative abundance (all samples, %)	Top BLAST result (% match)
1	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	12.6	Faecalibaculum rodentium strain ALO17 (88)
8	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	3.4	Faecalibaculum rodentium strain ALO17 (93)
31	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.43	Faecalibaculum rodentium strain ALO17 (100)
56	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.18	Faecalibaculum rodentium strain ALO17 (92)
78	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.074	Faecalibaculum rodentium strain ALO17 (90)
92	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.00719	Faecalibaculum rodentium strain ALO17 (97)

Supplementary Table 4

Characterization of the top 100 most abundant *Erysipelotrichaceae* operational taxonomic units within the AhR ligand-free diet group

OTU abundance rank	Consensus lineage	Average relative abundance (all samples, %)	Top BLAST result (% match)
1	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	37.6	Faecalibaculum rodentium strain ALO17 (88)
5	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	3.6	Faecalibaculum rodentium strain ALO17 (93)
6	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	3.4	Faecalibaculum rodentium strain ALO17 (100)
21	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.29	Faecalibaculum rodentium strain ALO17 (90)
23	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.27	Faecalibaculum rodentium strain ALO17 (97)
75	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.014	Faecalibaculum rodentium strain ALO17 (92)

Supplementary Table 5

Characterization of the top 100 most abundant *Erysipelotrichaceae* operational taxonomic units within the AhR ligand-free + I3C diet group

OTU abundance rank	Consensus lineage	Average relative abundance (all samples, %)	Top BLAST result (% match)
2	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	16.9	Faecalibaculum rodentium strain ALO17 (88)
5	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	4.4	Faecalibaculum rodentium strain ALO17 (93)
8	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	2.1	Faecalibaculum rodentium strain ALO17 (100)
56	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.0914	Faecalibaculum rodentium strain ALO17 (97)
59	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.0802	Faecalibaculum rodentium strain ALO17 (90)
60	k__Bacteria; p__Firmicutes; c__Erysipelotrichi; o__Erysipelotrichales; f__Erysipelotrichaceae	0.0802	Faecalibaculum rodentium strain ALO17 (92)