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Dale Bumpers College of Agricultural, Food & Life Sciences
Animal Science

DIVISION OF AGRICULTURE
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University of Arkansas System

UNIVERSITY OF MINNESOTA
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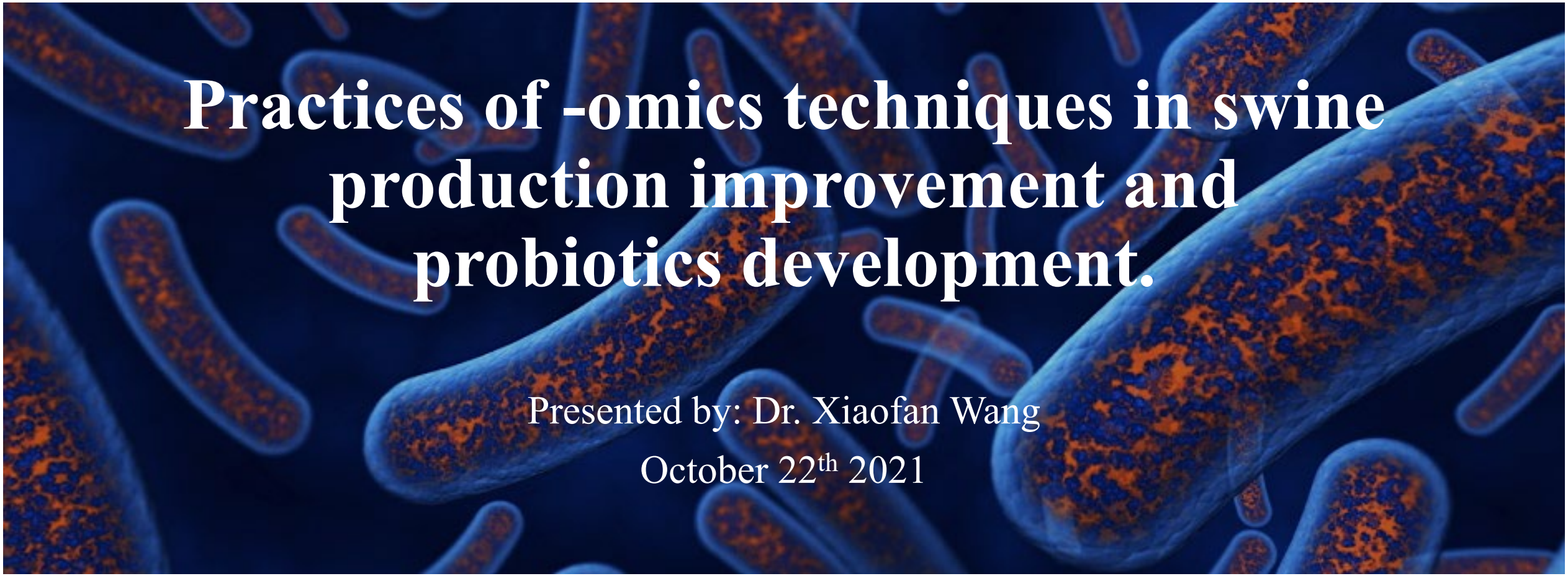


Practices of -omics techniques in swine production improvement and probiotics development.

Presented by: Dr. Xiaofan Wang

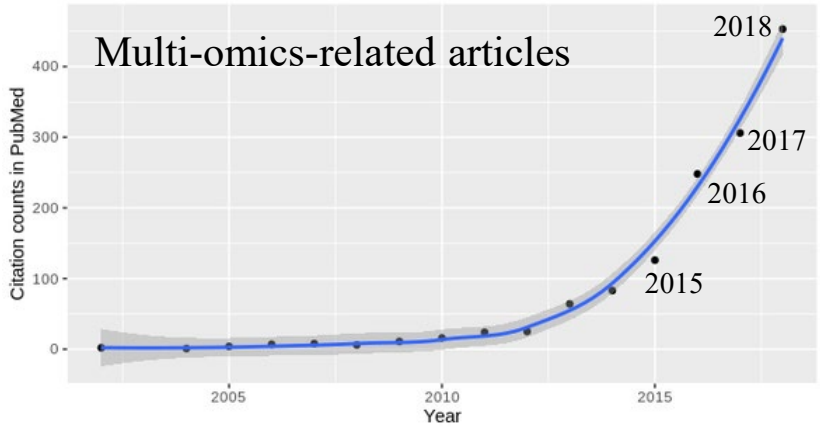
October 22th 2021

Content

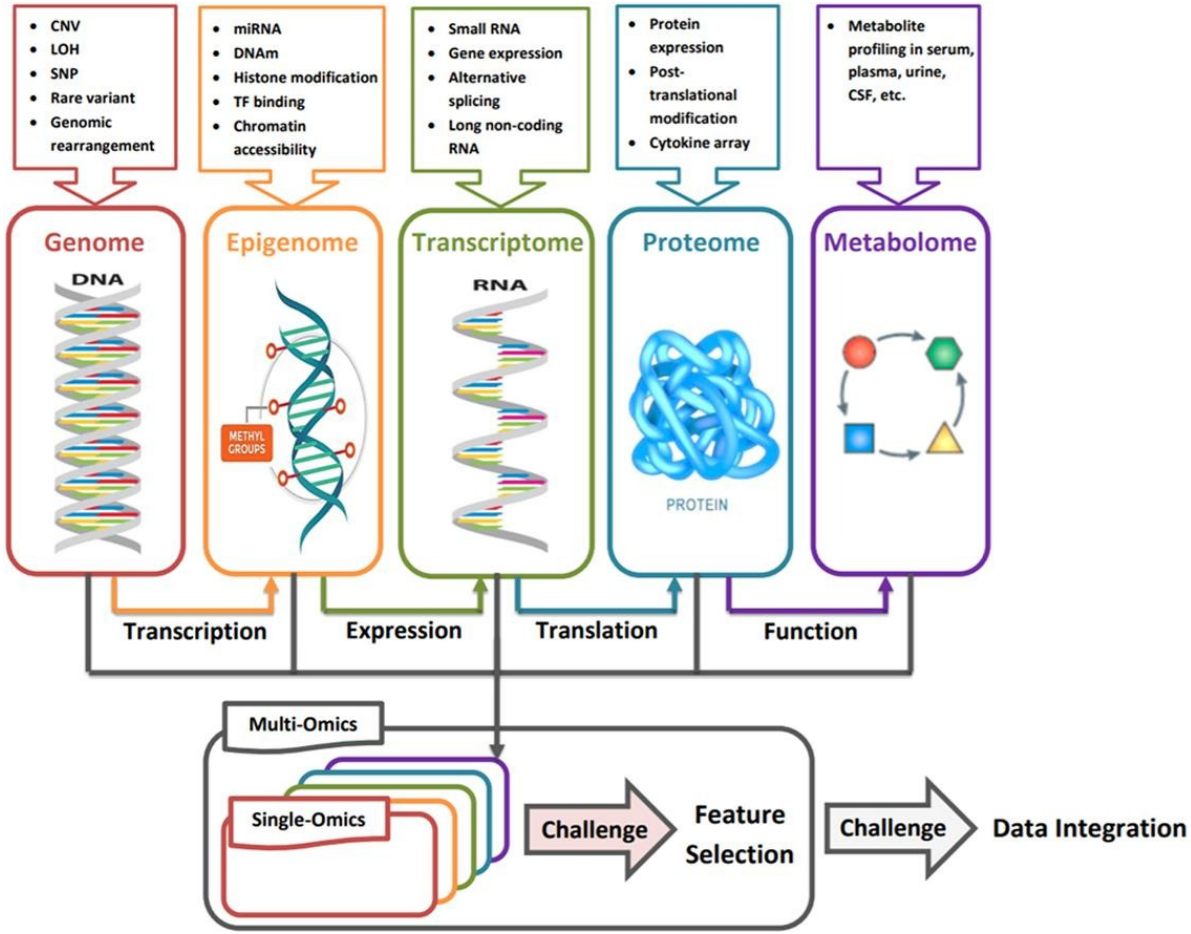
- Introduction of multi-omics and their applications in gut microbiome study
- Swine probiotics: market size and economic potential
- Characterization of longitudinal swine gut microbiome using culture dependent and independent approaches
- Probiotics identification, isolation, and validation using multi-omics methods

Multi-omics and their applications in gut microbiome study

- a high-throughput sequencing based biological analysis approach consisting of microbiomics (16s rRNA, ITS, culturomics, and metagenomics), genomics, transcriptomics, epigenomics, proteomics, metabolomics, and patholomics etc.
- quantitatively track changes in biological processes in unprecedented detail (Ali Ebrahim et al., 2016)
- disclose relationships between biological entities and identify biomarkers characterizing biological systems.
- rapid growth in size and complexity of multi-omics data sets indicated the advent of big-data era in biological and medical fields



https://en.wikipedia.org/wiki/Multiomics#List_of_softwares_for_multi-omic_analysis

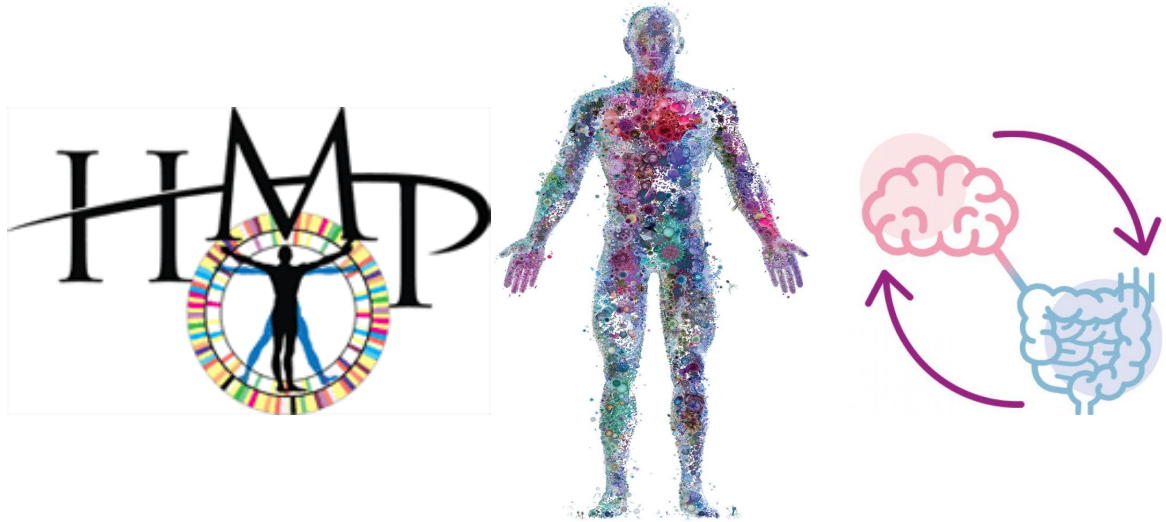


(Momeni et al., 2020)

Multi-omics and their applications in gut microbiome study

❖ Applications

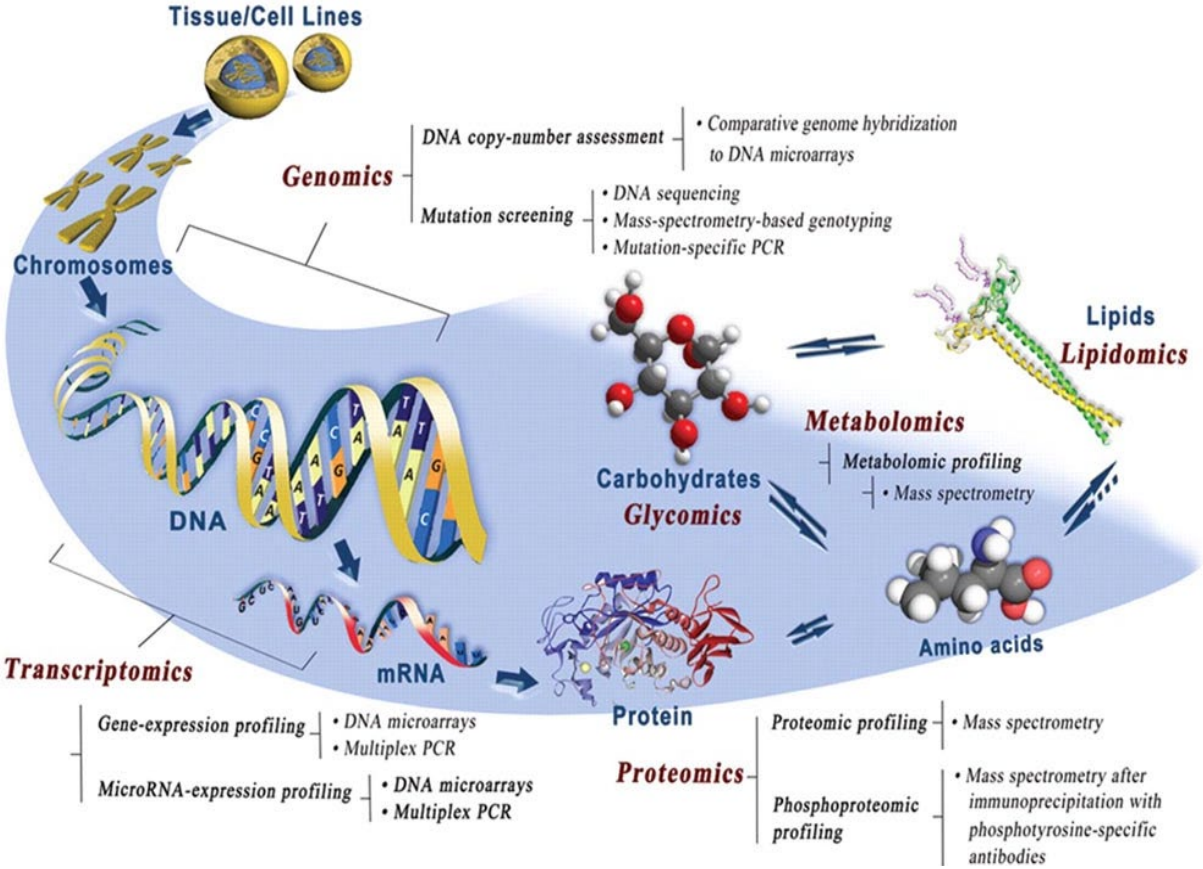
- Human Microbiome Project
  - Phase 2: integration of multi-omic data from host/microbiome to human disease
  - Gut/nasal microbiome and type 2 diabetes
  - Gut microbiomes and inflammatory bowel disease
  - Vaginal microbiomes and pre-term birth
  - Brain-gut axis
- Systems Immunology
  - collect omics data that related to immune response to infectious diseases such as pediatric chikungunya
  - effectiveness and side effects of vaccines (transcriptome and plasma metabolomics)
- Cancer
  - multi-omics data repositories (Subramanian et al., 2020)
- Precision medicine



DATA REPOSITORY	WEB LINK	DISEASE	TYPES OF MULTI-OMICS DATA AVAILABLE
The Cancer Genome Atlas (TCGA)	https://cancergenome.nih.gov/	Cancer	RNA-Seq, DNA-Seq, miRNA-Seq, SNV, CNV, DNA methylation, and RPPA
Clinical Proteomic Tumor Analysis Consortium (CPTAC)	https://cptac-data-portal.georgetown.edu/cptacPublic/	Cancer	Proteomics data corresponding to TCGA cohorts
International Cancer Genomics Consortium (ICGC)	https://icgc.org/	Cancer	Whole genome sequencing, genomic variations data (somatic and germline mutation)
Cancer Cell Line Encyclopedia (CCLE)	https://portals.broadinstitute.org/ccle	Cancer cell line	Gene expression, copy number, and sequencing data; pharmacological profiles of 24 anticancer drugs
Molecular Taxonomy of Breast Cancer International Consortium (METABRIC)	http://molonc.bccrc.ca/aparicio-lab/research/metabric/	Breast cancer	Clinical traits, gene expression, SNP, and CNV
TARGET	https://ocg.cancer.gov/programs/target	Pediatric cancers	Gene expression, miRNA expression, copy number, and sequencing data
Omics Discovery Index	https://www.omicsdi.org	Consolidated data sets from 11 repositories in a uniform framework	Genomics, transcriptomics, proteomics, and metabolomics

Multi-omics and their applications in gut microbiome study

❖ Technologies and data analysis methods



https://www.pratiks.info/research/harvard/novel-multiomics-technologies-to-decipher-molecular-signatures-of-infectious-diseases

❖ A community-maintained list of software packages for multi-omics data analysis.

Website: https://github.com/mikelove/awesome-multi-omics

- Multi-omics correlation or factor analysis
- Ecology multi-table literature
- Chemometrics multi-table literature
- Behavioral research multi-table literature
- Multi-omics clustering / classification / prediction
- Multi-omics networks
- Single cell multi-omics
- Multi-study correlation or factor analysis
- Multi-omics reviews / evaluations
- Multi-omics application papers
- Multi-omics data management

Multi-omics networks

- 2018 - MolTi-DREAM - Didier - identifying communities from multiplex networks, and annotated the obtained clusters article
- 2019 - RWR-MH - Valdeolivas - Random walk with restart on multiplex and heterogeneous biological networks article

Animal probiotics and future market size

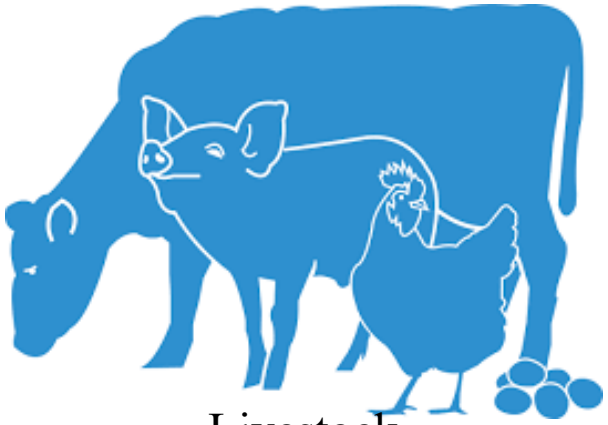
Probiotics
Live microorganisms that confer a health benefit on the host when administered in adequate amounts.

Prebiotics
Substrates that are selectively utilized by host microorganisms, conferring a health benefit.

Synbiotics
Combination of both probiotics and prebiotics

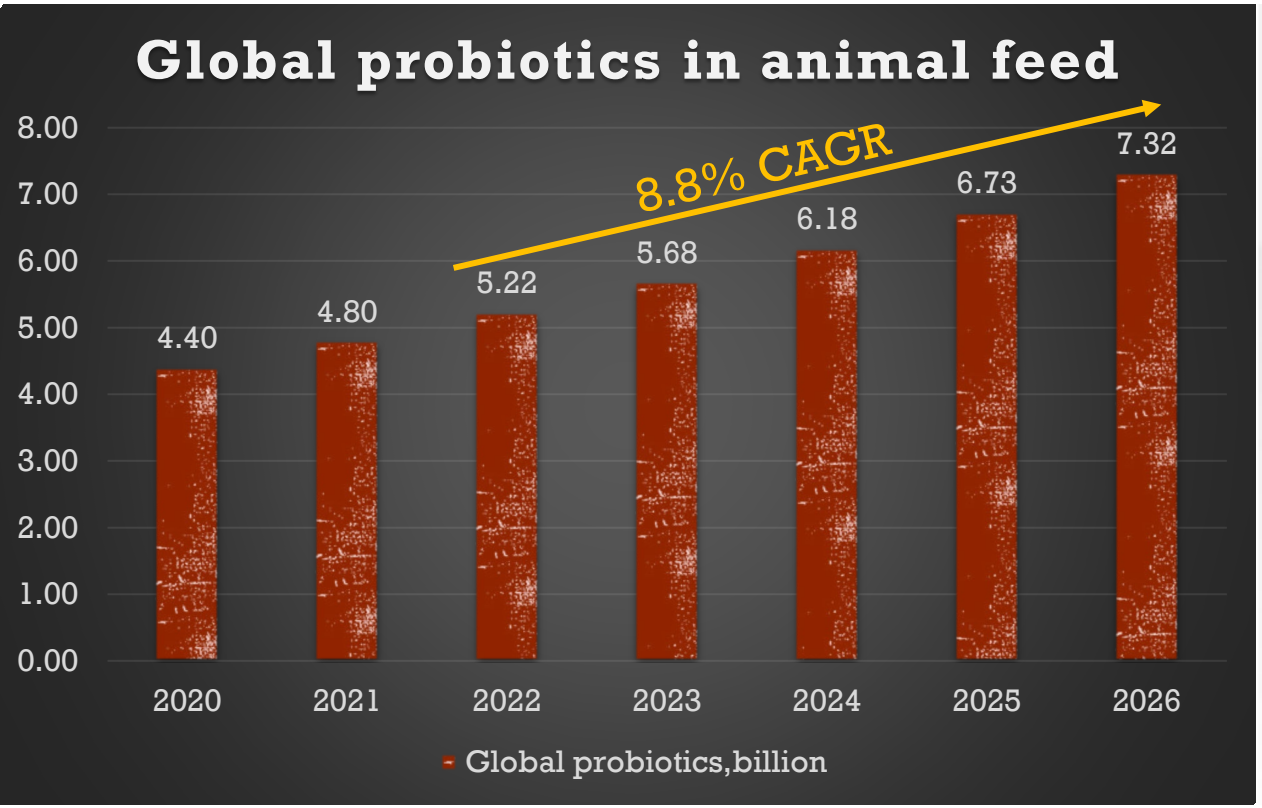
Postbiotics
Compounds produced by microorganisms, released from food components or microbial constituents, including non-viable cells that when administered in adequate amounts, promote health and well-being.

Campanian animals

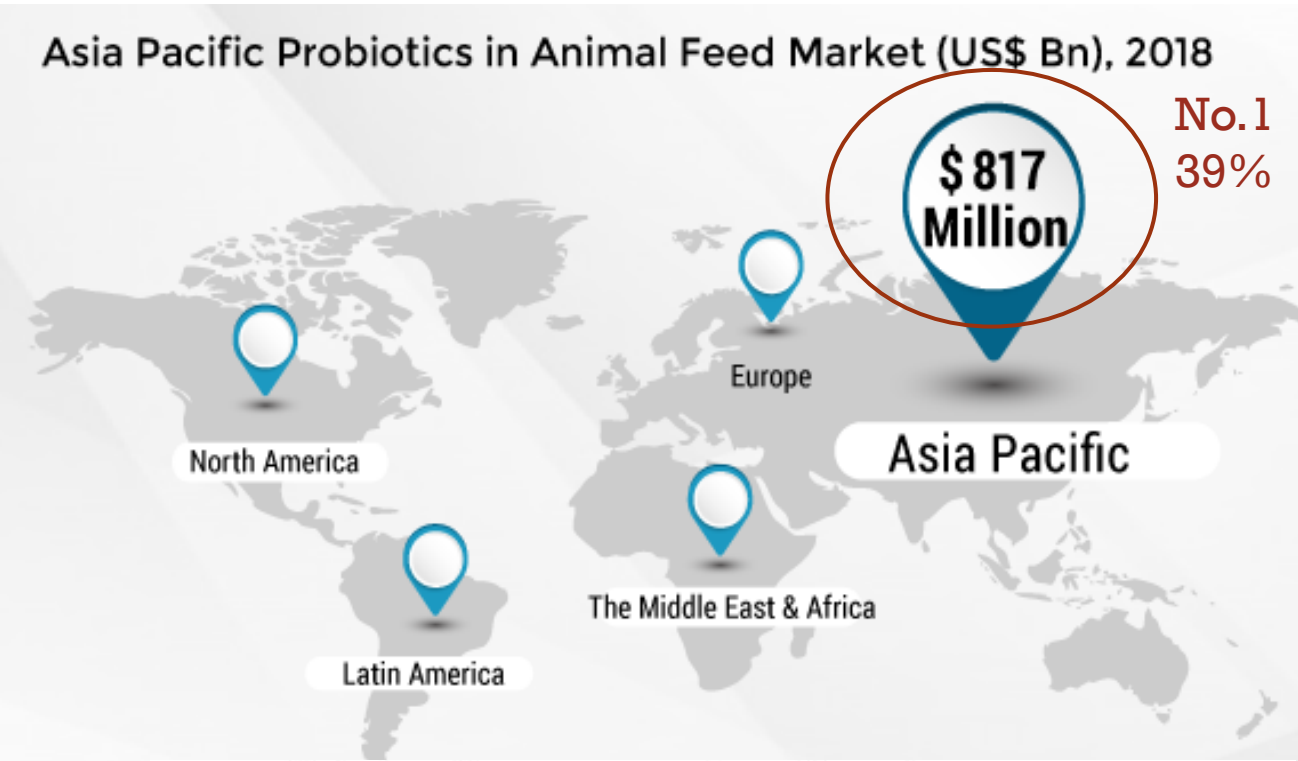


Livestock

Animal probiotics and future market size

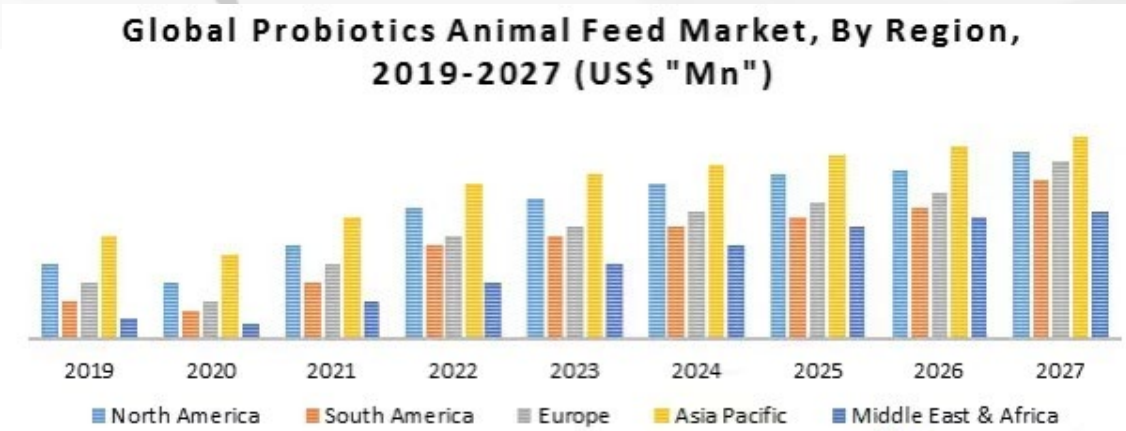


https://www.prnewswire.com/news-releases/probiotics-in-animal-feed-market-worth-7-3-billion-by-2026--exclusive-report-by-marketsandmarkets-301354278.html



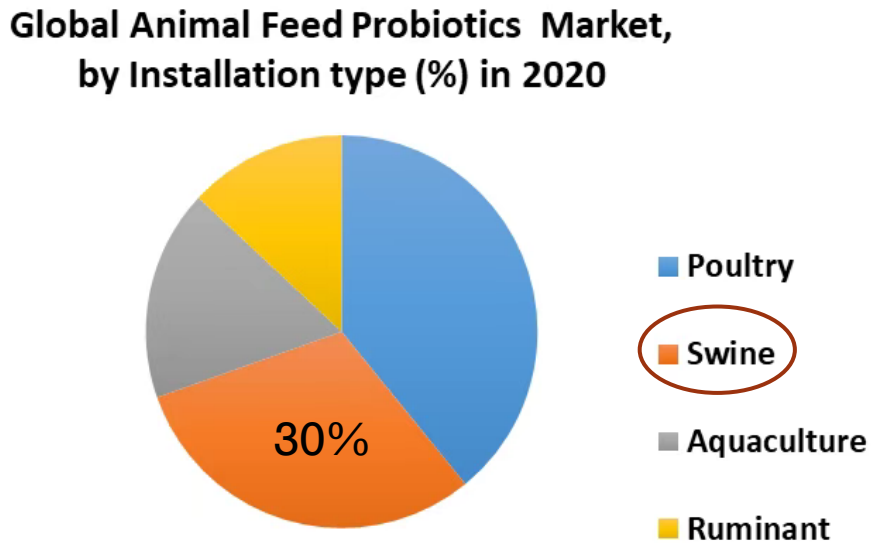
- The China Ministry of Agriculture and Rural Affairs released the ban of antibiotics use in animal feed from 2020.

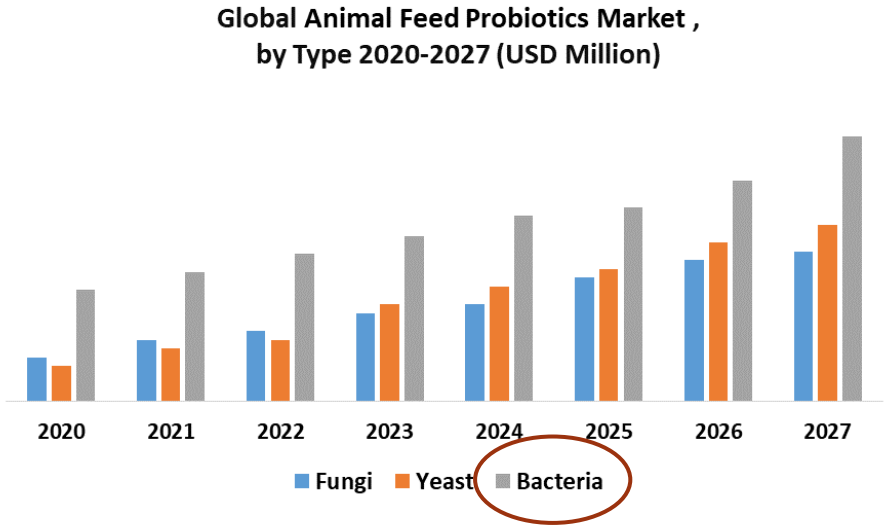
- The Asia Pacific is projected to account for the largest market size during the forecast period.



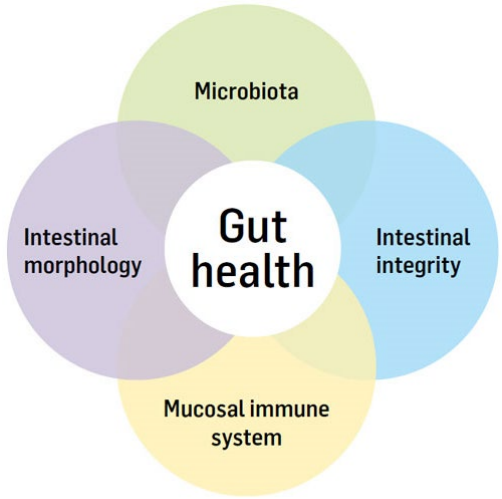
MAXIMIZE MARKET RESEARCH PVT. LTD.

Swine probiotics





- Three traditional swine probiotics: *Lactobacillus*, *bacillus*, and yeast
  - Benefits:
  - improve gut homeostasis, alleviate stress, and preventing diarrhea
  - Immune protective and pathogen prevention
  - Improve feed efficiency and meat production



  - Drawbacks:
  - Inconsistent or poor growth promoting performance
  - Limited probiotics options (GRAS list)
  - Un- or inadequately characterized biological functions and delivery mechanism

A workflow combining culture-dependent and culture-independent approaches using multi-omics techniques was suggested for revolutionary probiotic selection in swine industry.

Characterization of longitudinal swine gut microbiome using culture dependent and independent approaches

KEY BIOLOGICAL AND ECOLOGICAL QUESTIONS

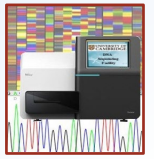
- How does the swine gut microbiome change from birth to market?
- Whether adult pigs' microbiota can colonize in young piglets?
- What are the major drivers shaping the swine gut microbiome?
- Who are the residents and who are passengers?
- Is there a core microbiome?
- Are probiotics stage-related?
- What is the dynamics of pathogens in swine gut?

Chapter I. Dynamics

TEST

- A total of seventeen pigs (PIC29*380) born from three sows in parity 2 were raised in University of Arkansas-Division of Agriculture Swine Research Unit in Fayetteville, Arkansas.
- All pigs were fed the corn, soybean meal based standard diets.
- Body weight and rectal swabs were collected on each collection date.

V4 region of 16s rRNA



Deblur

qiime2

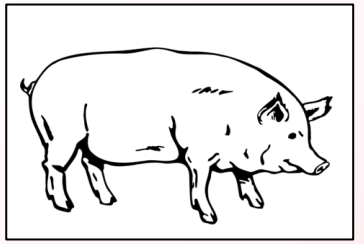
Day 0 11 20 27 33 41 56 61 76 90 104 116 130 146 159 174

End_Lactation

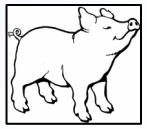
End_Nursery

End_Growing

End_Finishing

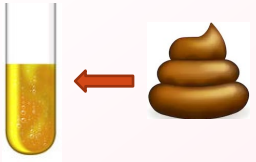


x 17



x 24

gavage



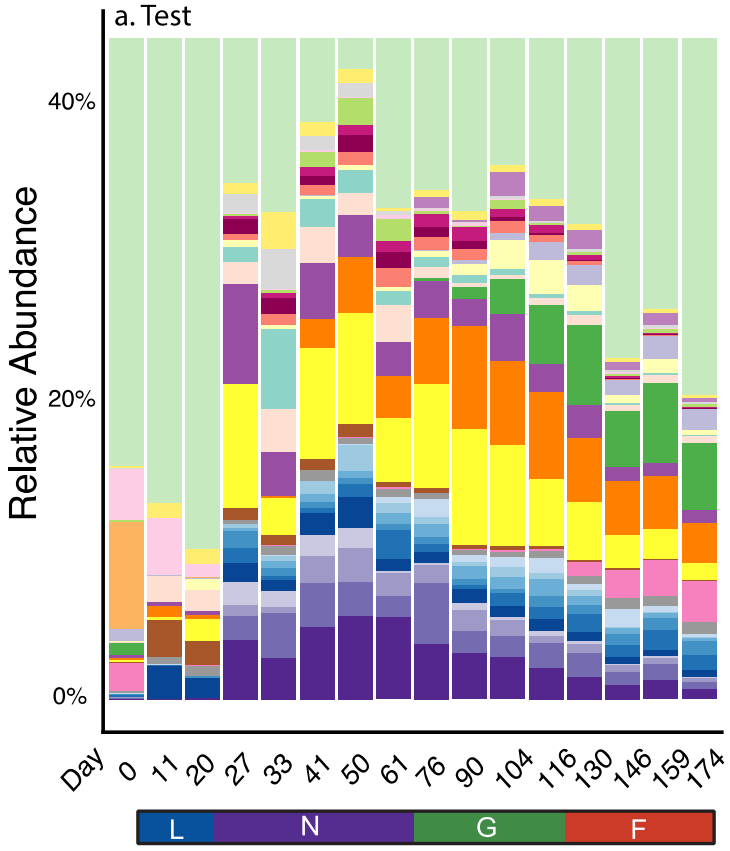
Donor pig:
Growing phase 2

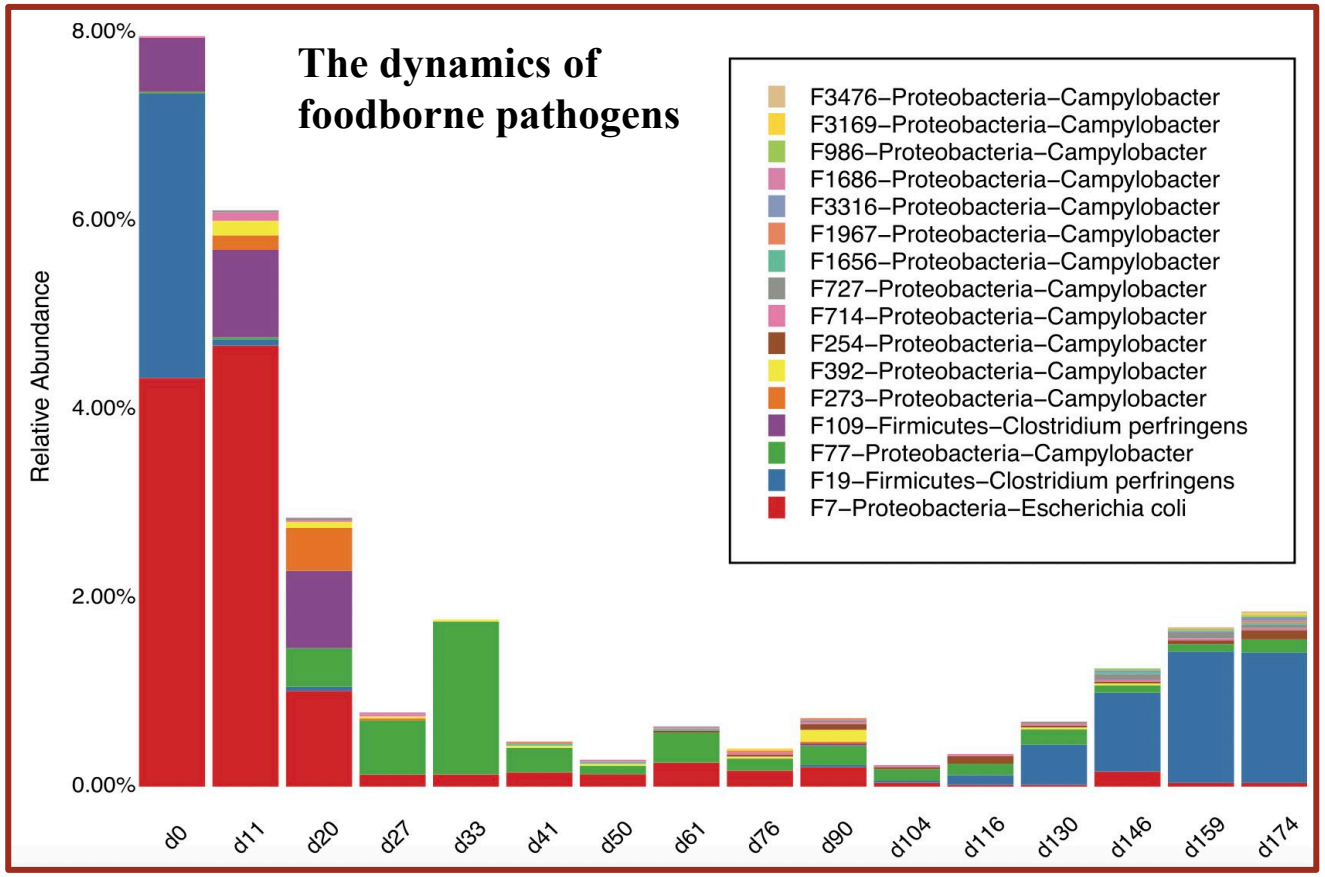
- A total of twenty-four weaned pigs were selected and gavaged with one of treatments: 10 ml of 20% glycerol (Con) or 10 ml of 20% glycerol with extracted fecal bacteria (FMT) from grower phase 2 pig donor.
- Pigs were drenched two consecutive days d 21 and 22. Swabs were collected on d 21, 22, 23 and at each subsequent phase change.

VALIDATION

Wang et al, 2019, Microbiome

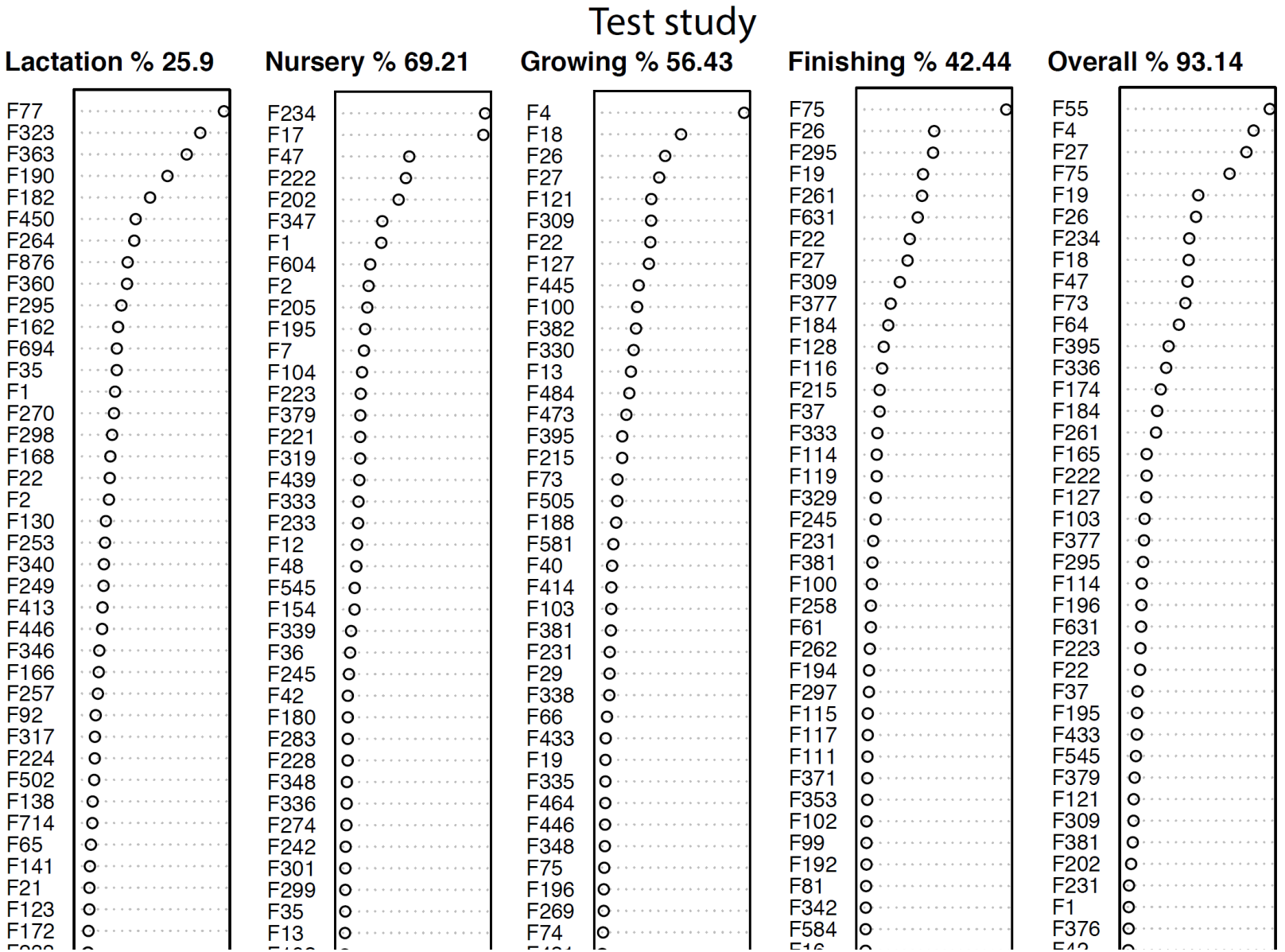
Dynamics of the swine gut microbiome from birth to market: composition





Others
F17–Actinobacteria–Collinsella aerofaciens
F32–Proteobacteria–Succinivibrionaceae–unclassified
F33–Proteobacteria–Succinivibrio
F7–Proteobacteria–Escherichia coli
F42–Firmicutes–Veillonellaceae–unclassified
F37–Firmicutes–Faecalibacterium prausnitzii
F40–Firmicutes–Faecalibacterium prausnitzii
F11–Firmicutes–Rummeliibacillus
F31–Firmicutes–Ruminococcaceae–unclassified
F18–Firmicutes–SMB53
F13–Firmicutes–Anaerovibrio
F16–Firmicutes–Blautia
F8–Firmicutes–Phascolarctobacterium
F4–Firmicutes–Clostridiaceae–unclassified
F5–Firmicutes–Lactobacillus
F2–
F1–Firmicutes–Megasphaera
F20–Bacteroidetes–Prevotella stercorea
F10–Bacteroidetes–YRC22
F23–Bacteroidetes–S24–7–unclassified
F29–Bacteroidetes–Prevotella
F43–Bacteroidetes–Prevotella
F25–Bacteroidetes–Prevotella
F21–Bacteroidetes–Prevotella
F12–Bacteroidetes–Prevotella
F9–Bacteroidetes–Prevotella
F36–Bacteroidetes–Prevotella copri
F14–Bacteroidetes–Prevotella copri
F6–Bacteroidetes–Prevotella copri
F3–Bacteroidetes–Prevotella copri

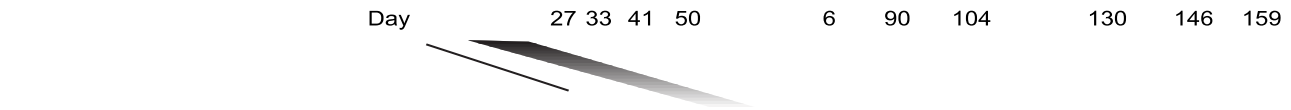
Growth performance associated bacteria at different growth stages



Wang et al, 2019, Microbiome

Chapter I. Dynamics

a



"Core" microbiome

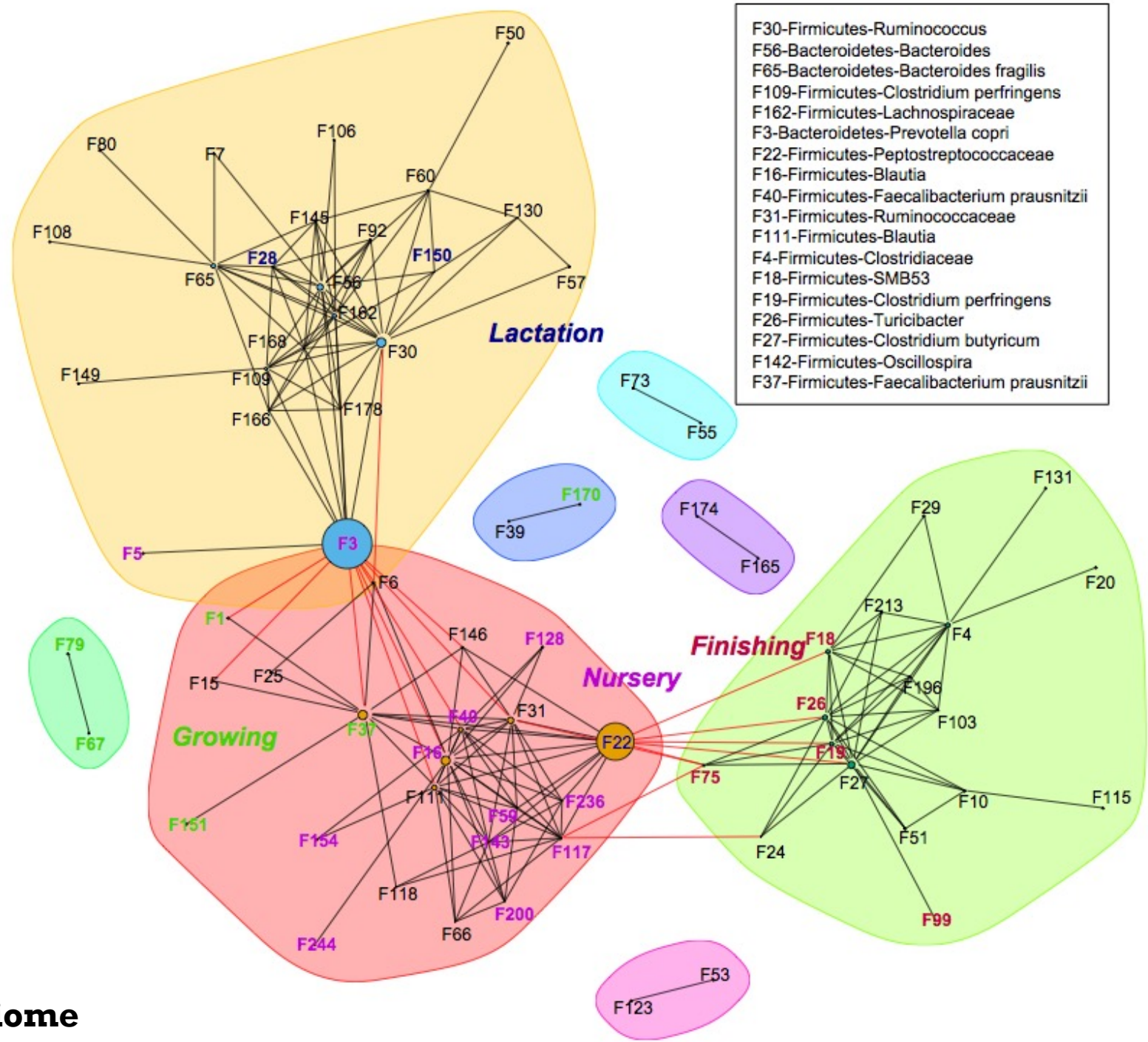
b

"Passenger"

Wang et al, 2019, Microbiome

Chapter I. Dynamics

Co-occurred bacteria/ networks



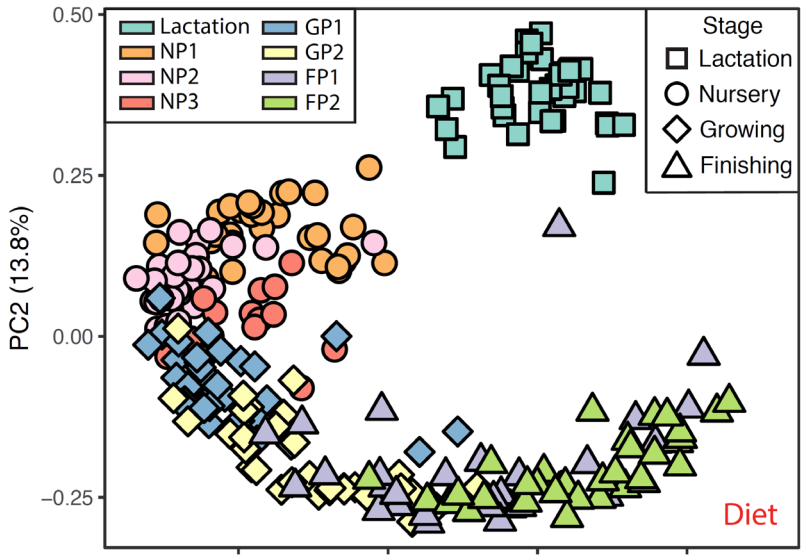
Wang et al, 2019, Microbiome

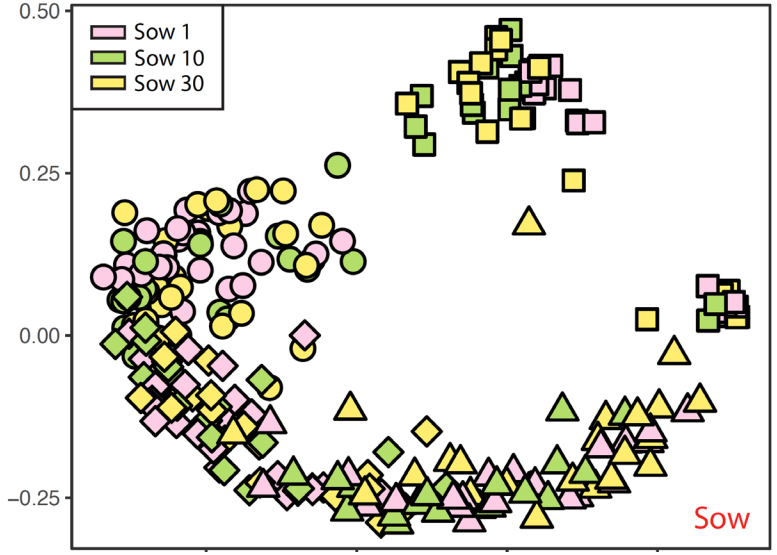
Chapter I. Dynamics

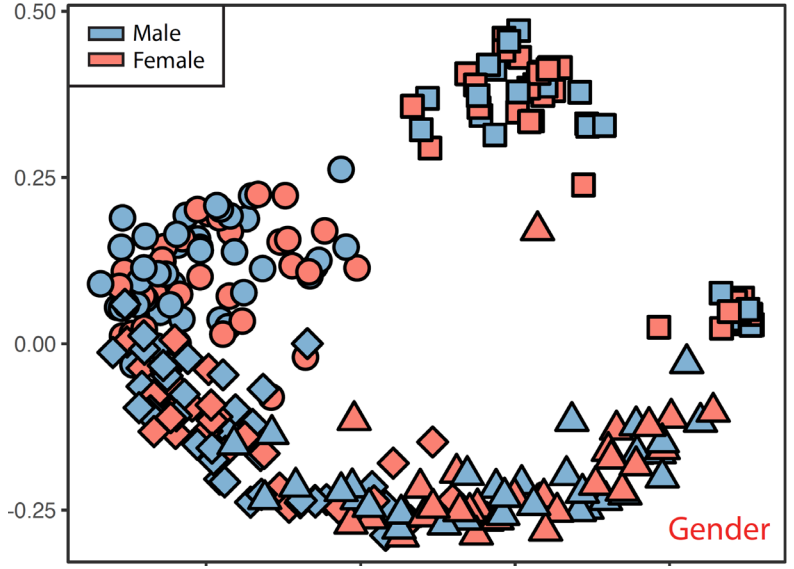
What are the drivers of gut microbiome?

a

	Df	SumsOfSqs	MeanSqs	F	R^2	P	Residuals
Diet	6	17.17	2.86	19.07	0.34	0.001	0.57
Age	1	0.48	0.48	3.20	0.01	0.004	
Gender	1	0.23	0.23	1.52	0.00	0.113	
Sow	2	0.64	0.32	2.13	0.01	0.006	



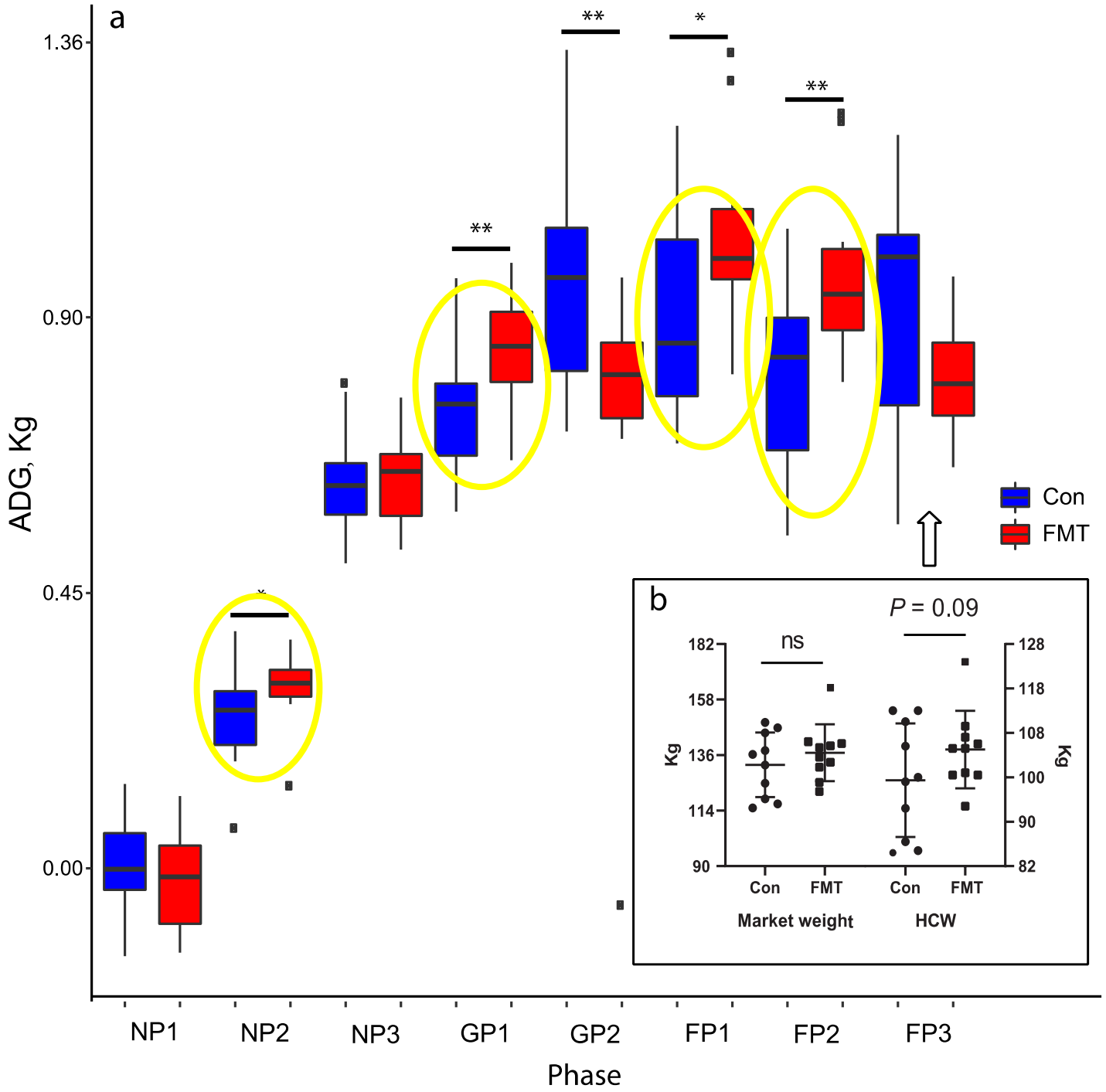




Wang et al, 2019, Microbiome

Chapter I. Dynamics

Growth performances

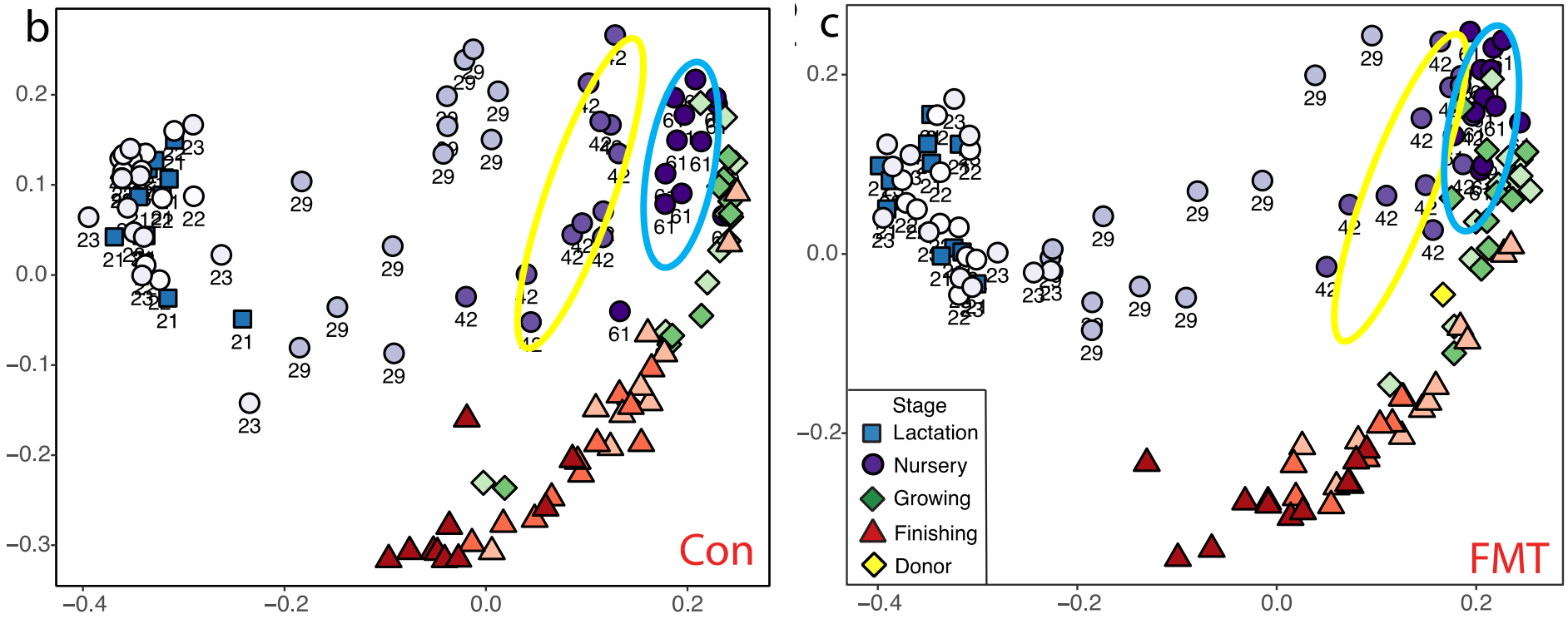


Wang et al, 2019, Microbiome

Chapter I.
Dynamics

Jaccard

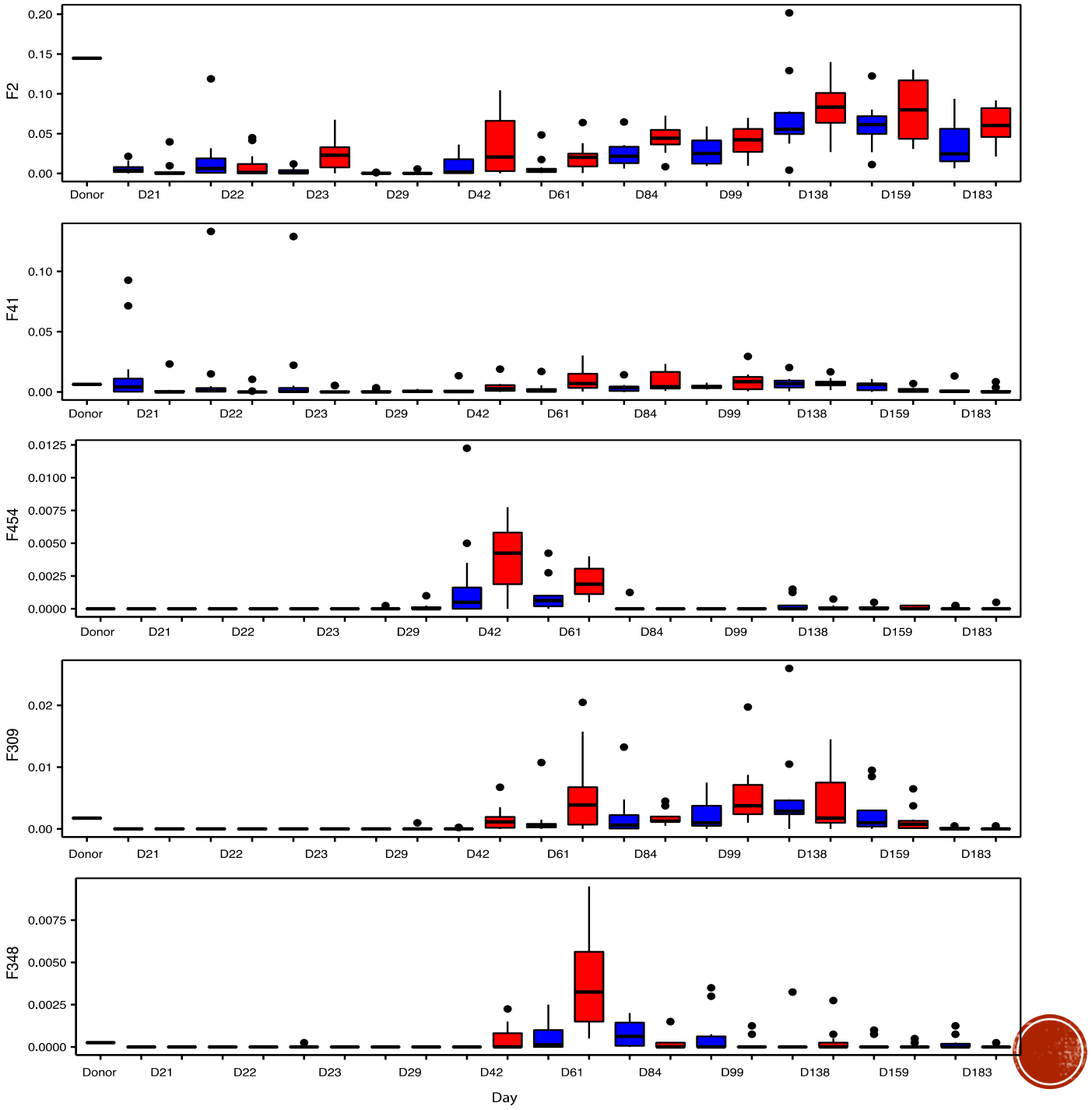
Different microbiome structures on D42 and D61



Wang et al, 2019, Microbiome

Chapter I. Dynamics

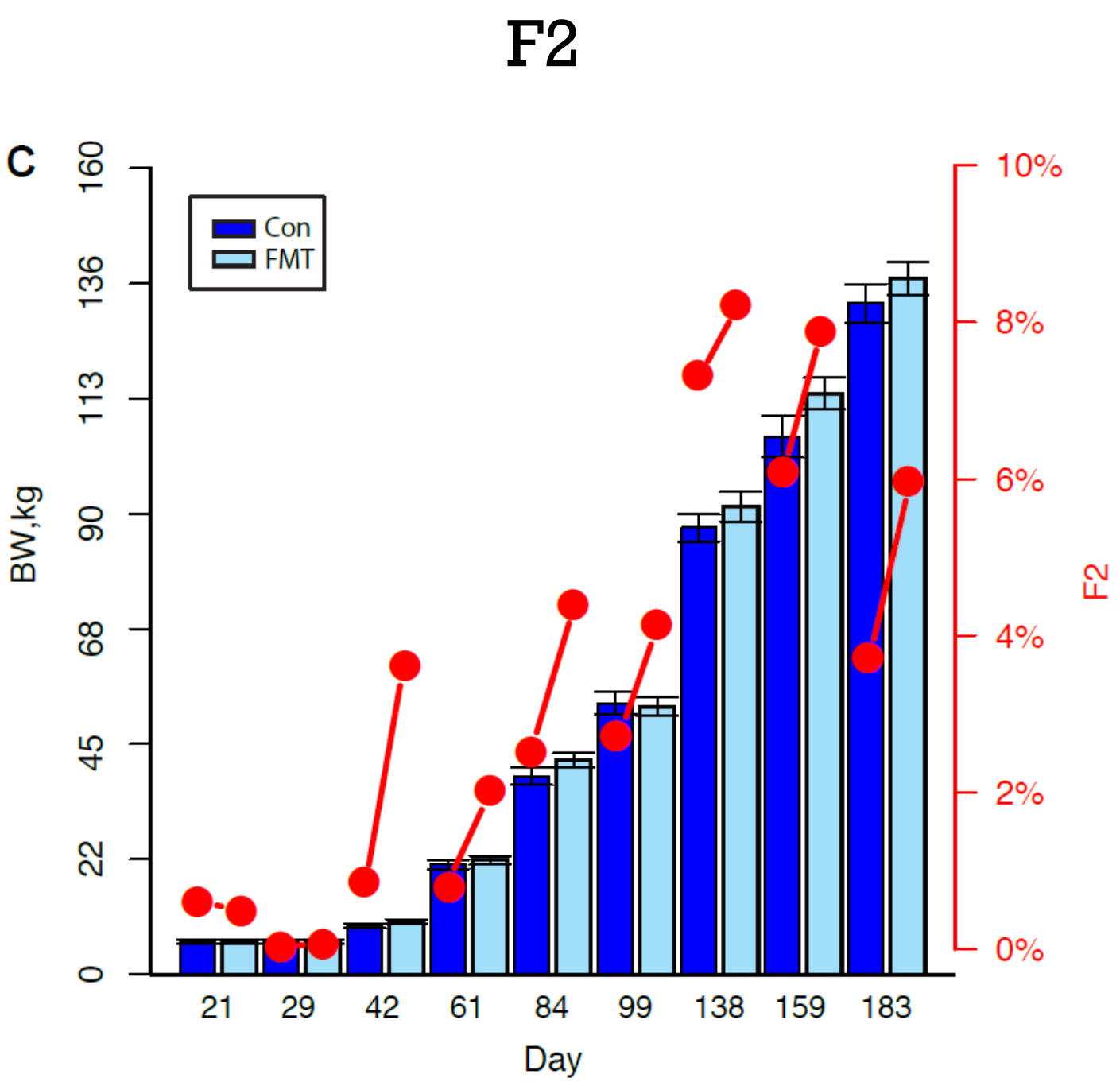
“Colonized” in FMT group



Wang et al, 2019, Microbiome

Chapter I. Dynamics

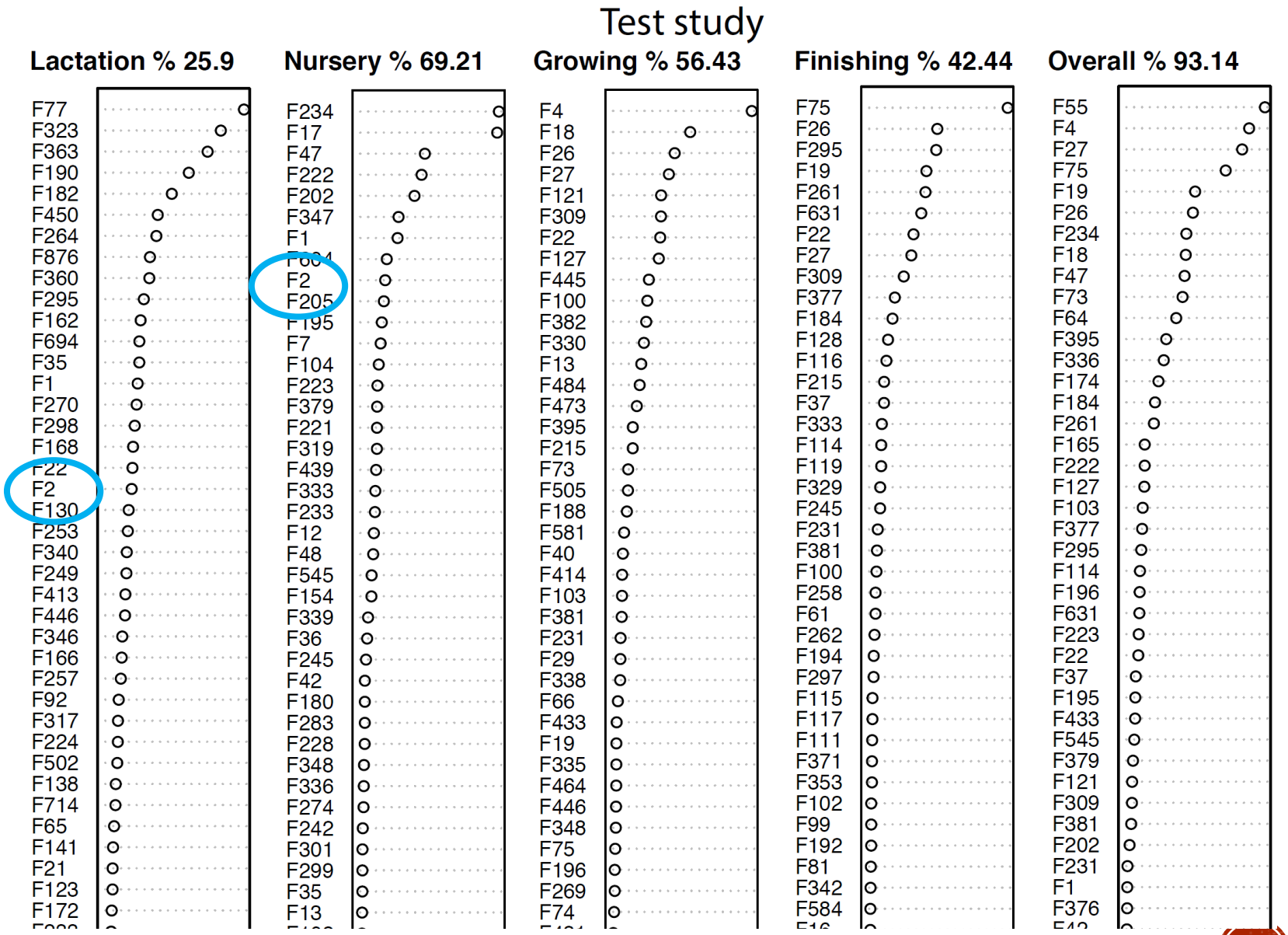
Potential probiotic



Wang et al, 2019, Microbiome

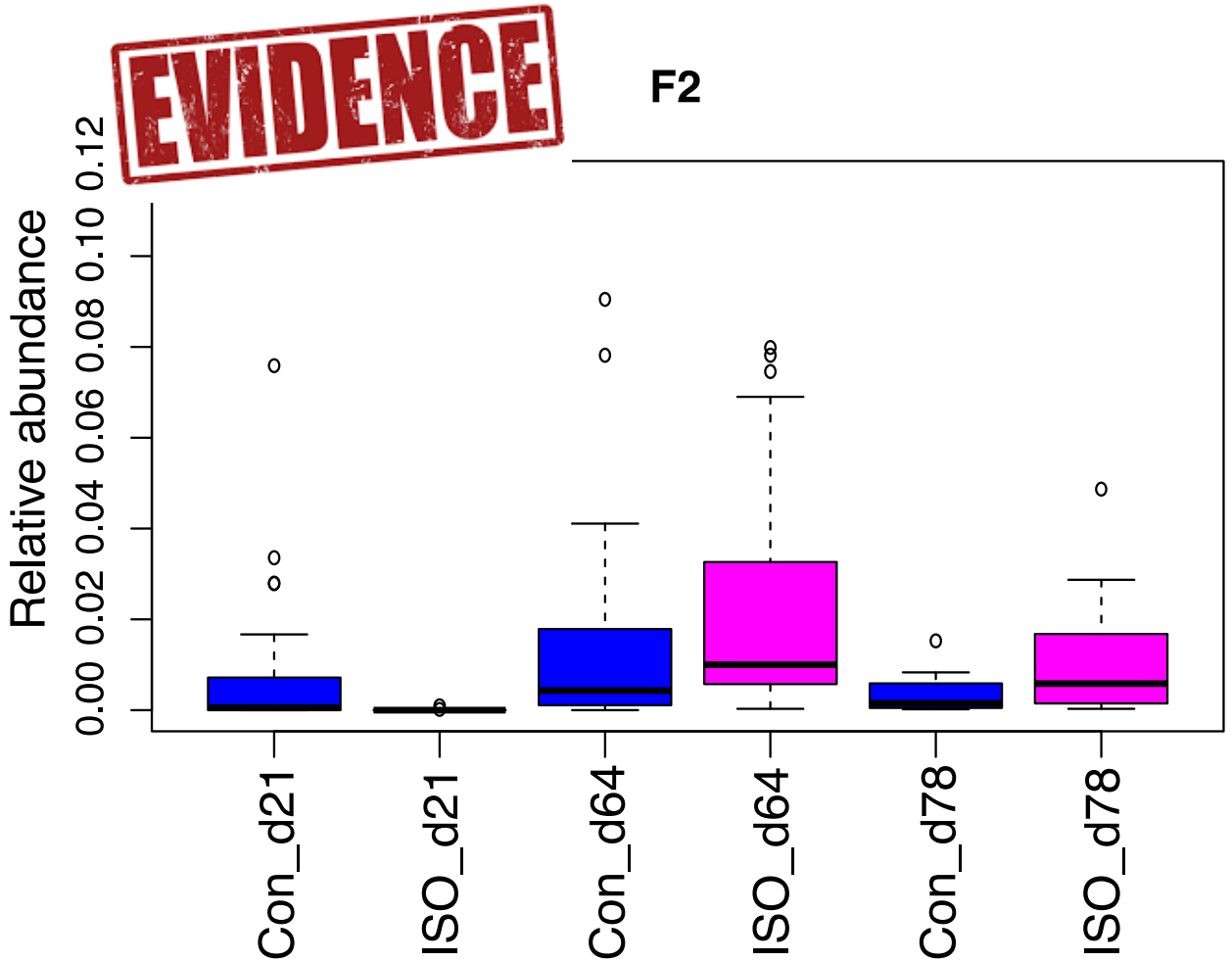
Growth performance associated bacteria at different growth stages

Potential probiotic



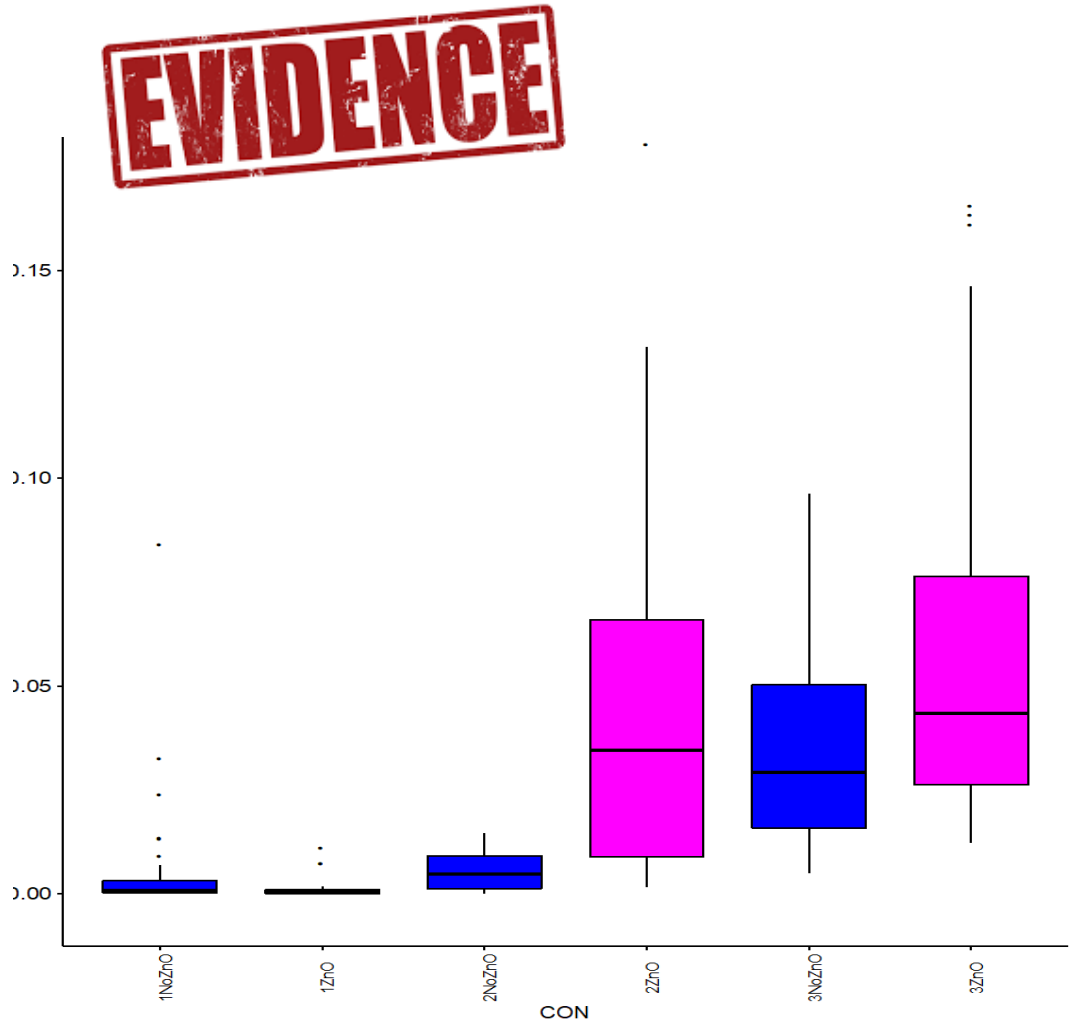
Wang et al, 2019, Microbiome

Relative abundances of F2 in the isolator trial



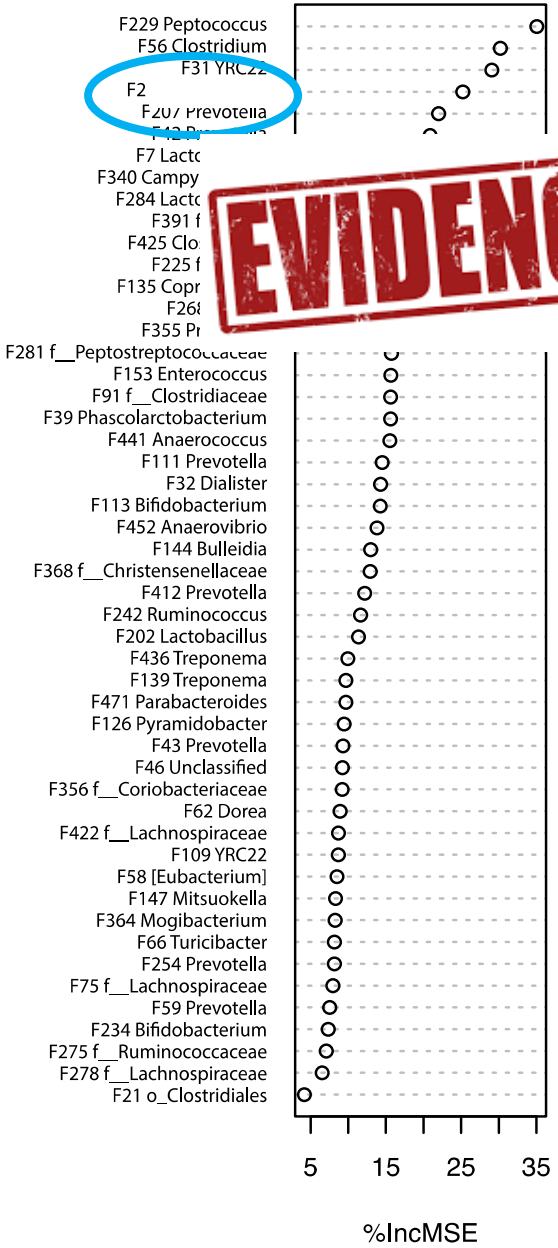
Tsai et al., 2018, Frontiers in Microbiology

Relative abundances of F2 in pigs fed with or without ZnO

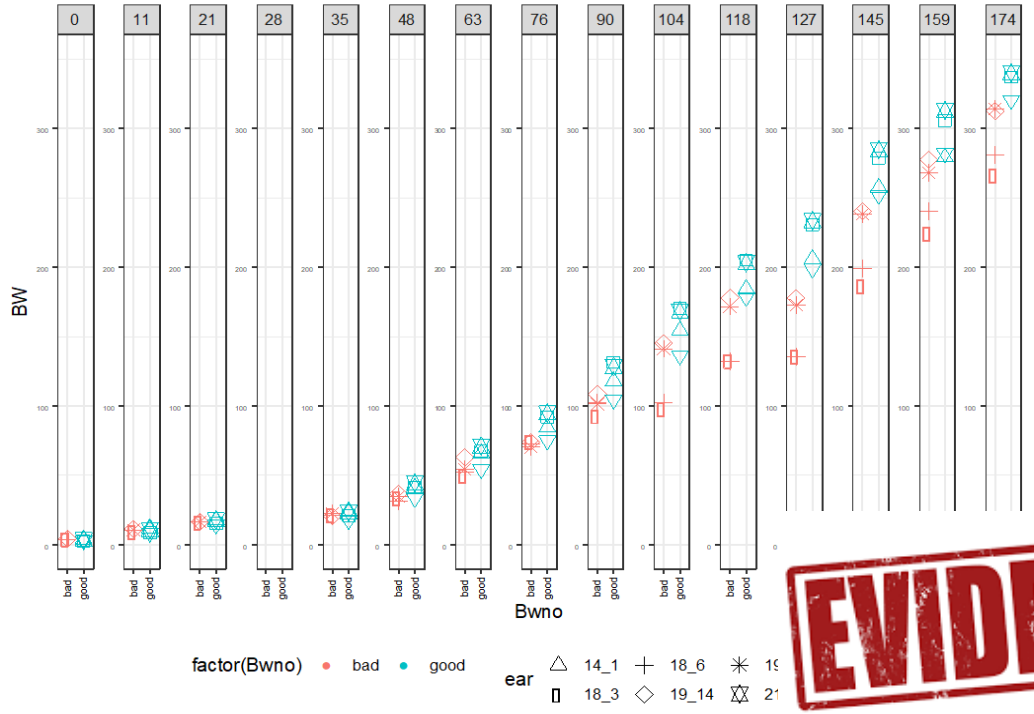


Wei et al., 2020, microorganisms

The top 50 estimated age (EMA)-associated features isolated by the regression based random forest.



EVIDENCE



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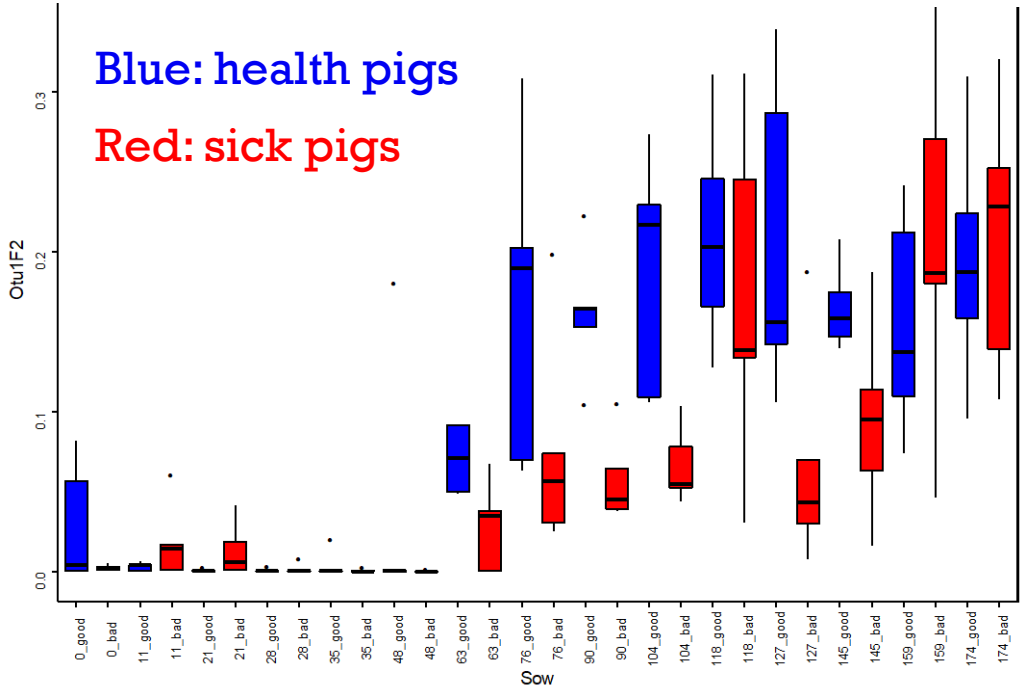
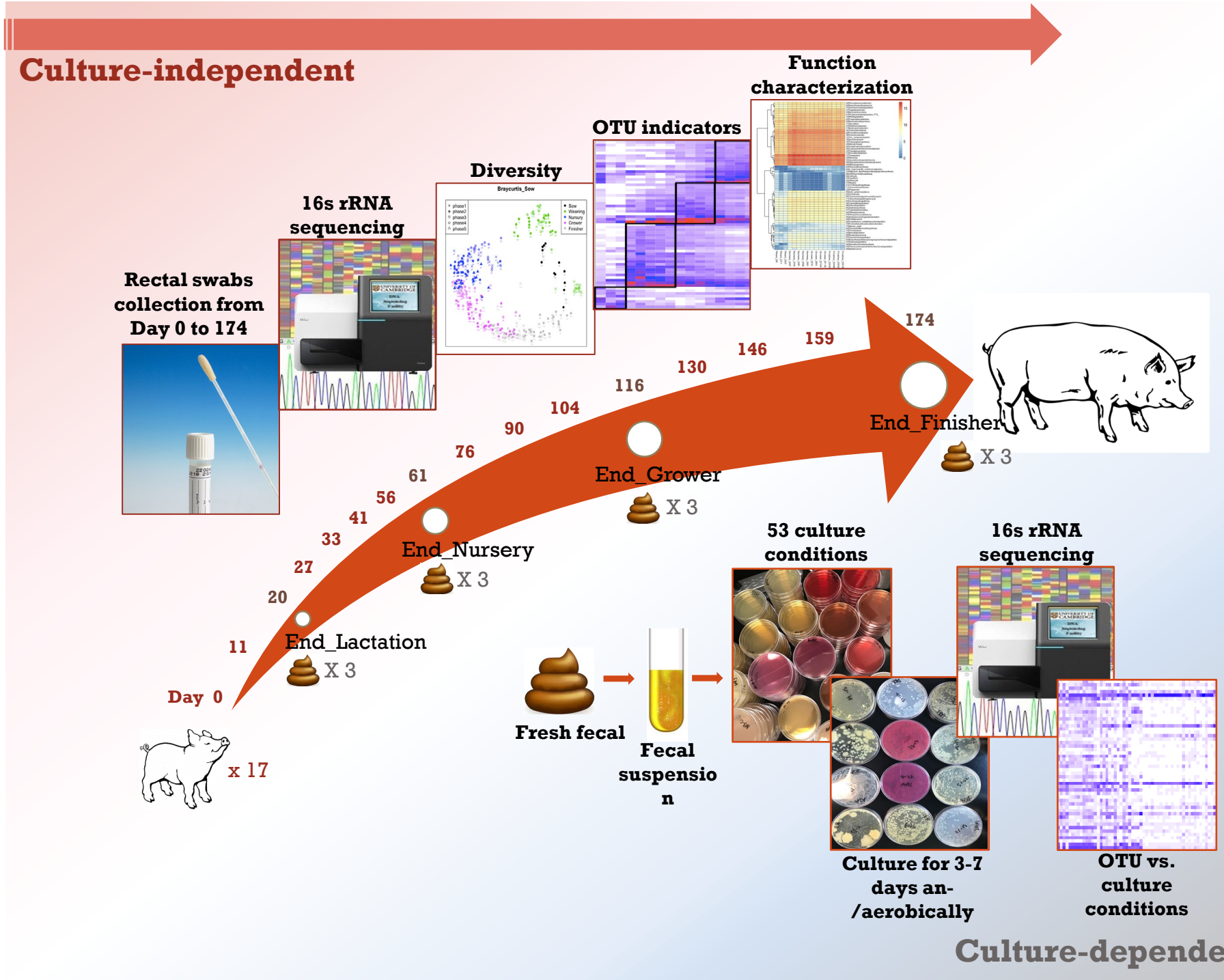
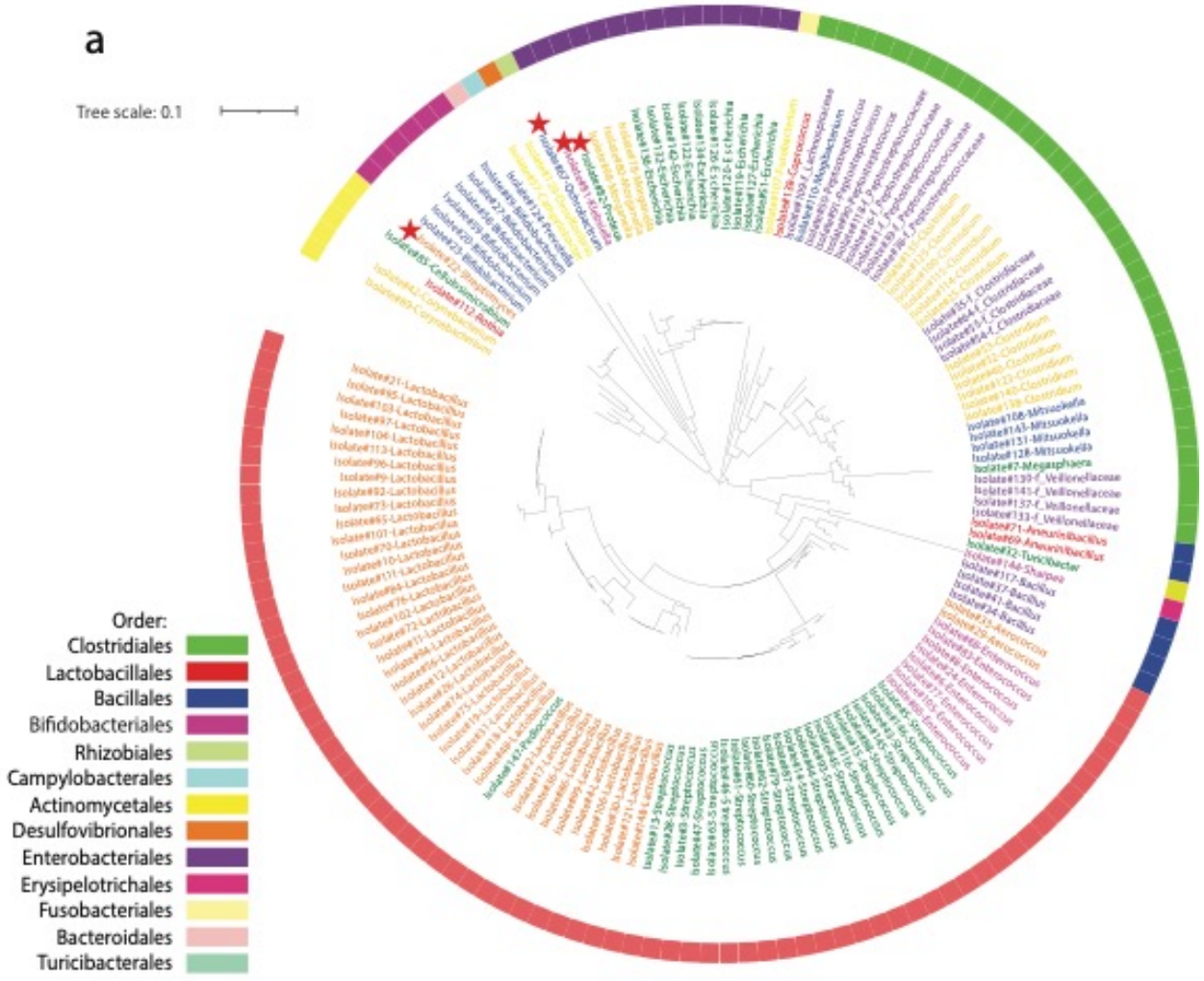


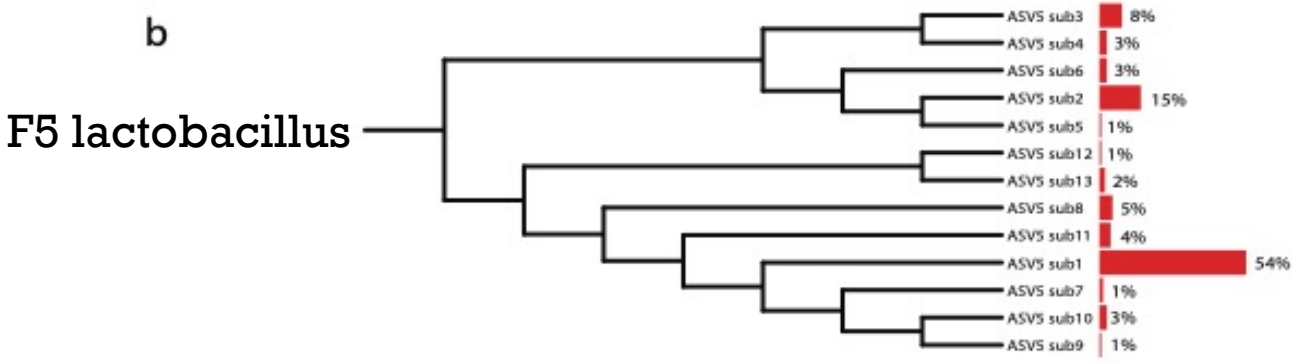
Culturomics

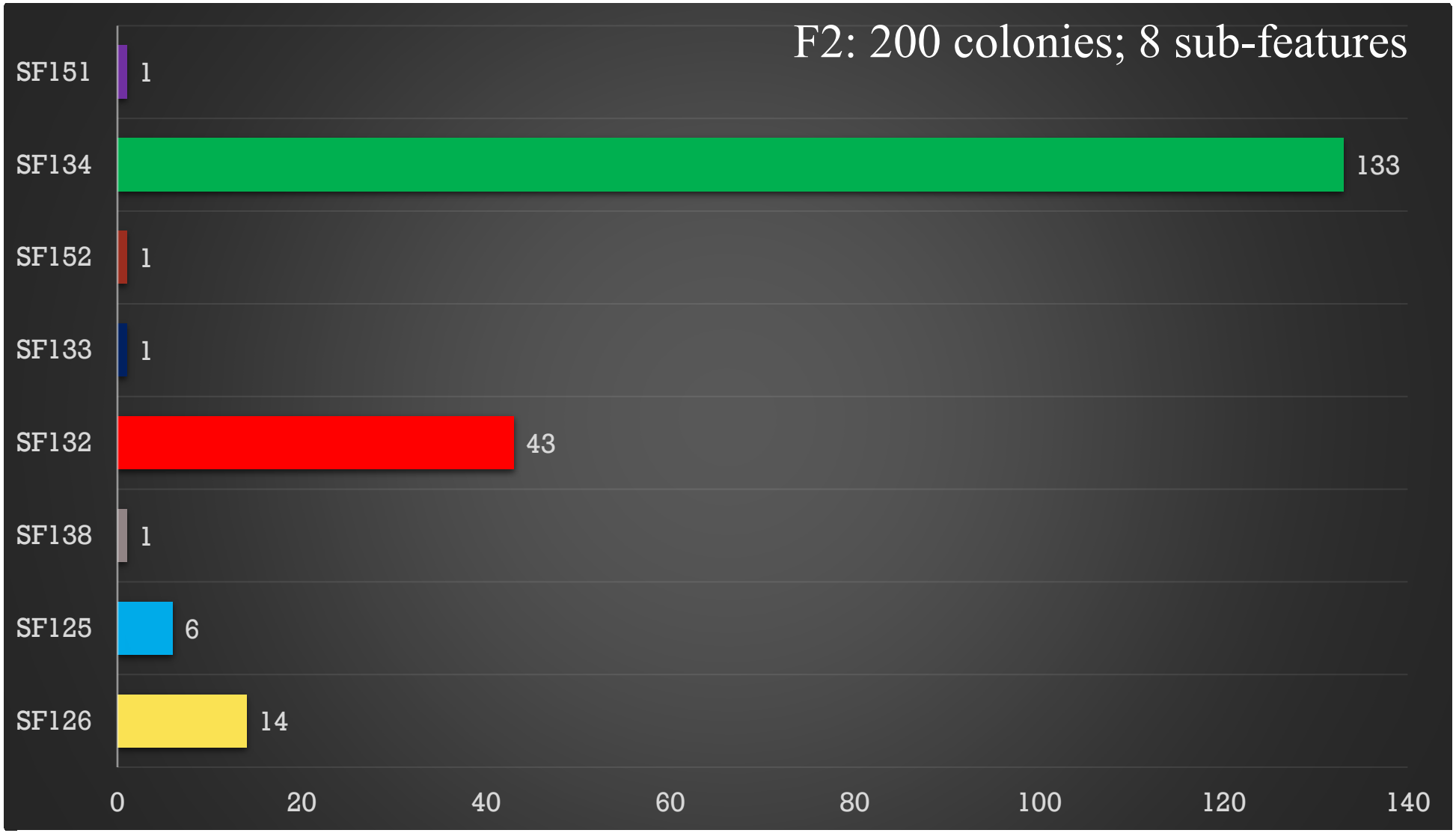
Culturomics experimental design

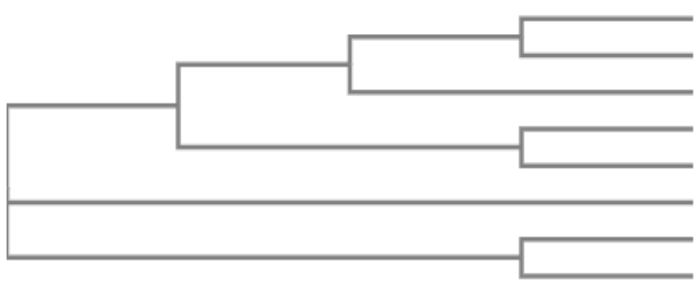


ASV-culture method heatmap



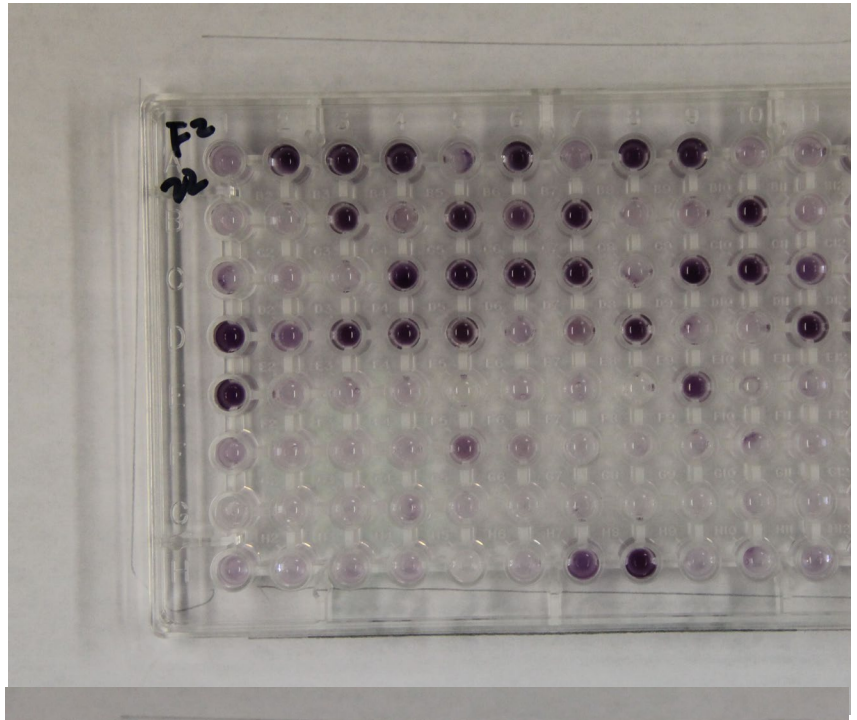


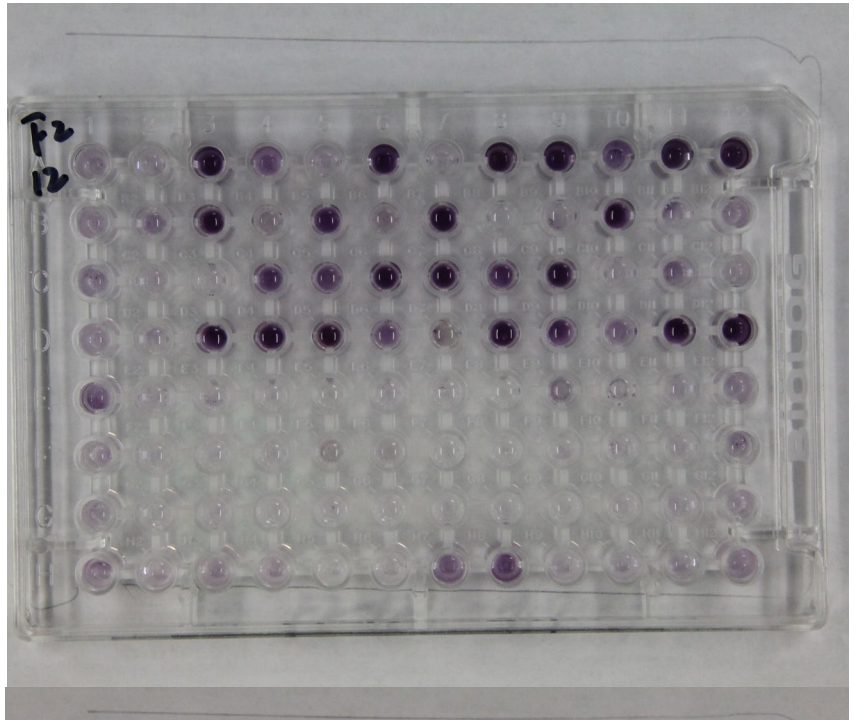


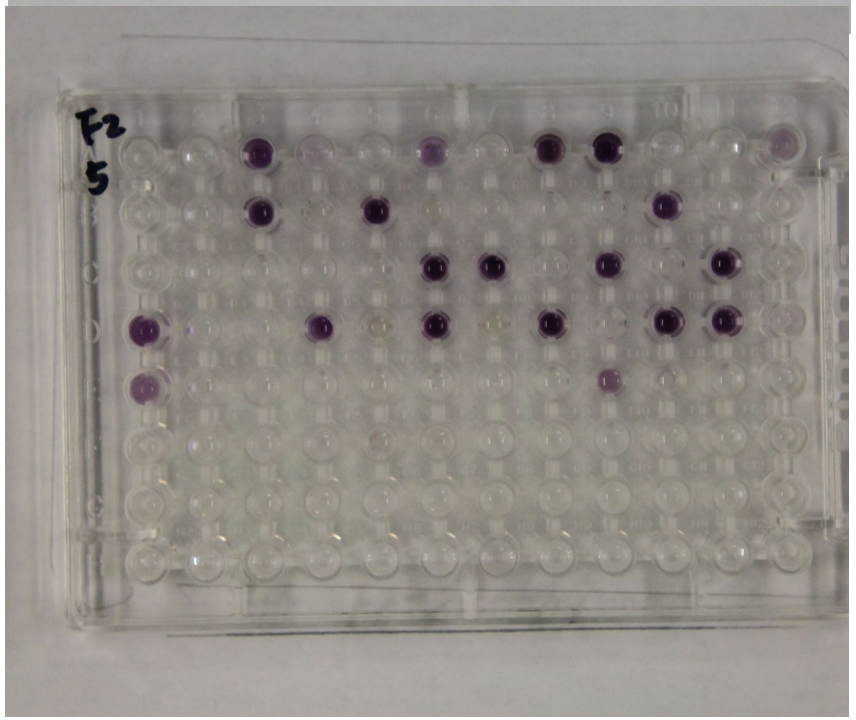


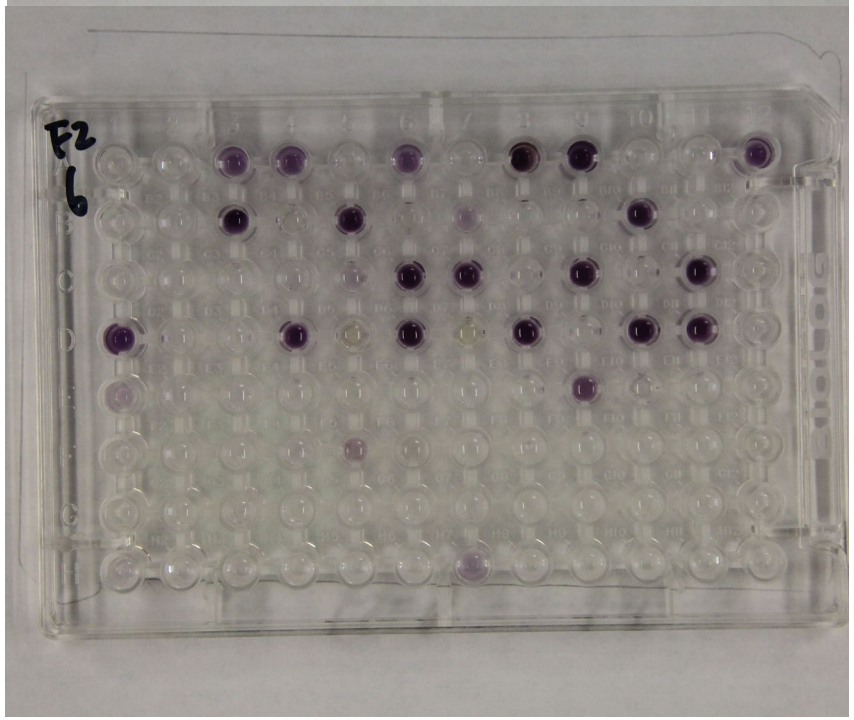
Sub-feature151|F2-26 0.00107
Sub-feature134|F2-3_F2-20_F2-25_F2-#6_#F2-17_F2-16_F2-15_F2-13_F2-18_F2-19_F2-8 0
Sub-feature152|F2-24 0.00107
Sub-feature133|F2-9 0.00107
Sub-feature132|F2-1_F2-11_F2-21_#F2-5_F2-6_#F2-7_F2-10_F2-2 0
Sub-feature138|F2-14 0.00107
Sub-feature125|F2-12_F2-#36 0.00107
Sub-feature126|F2-22_F2-23 0.00107

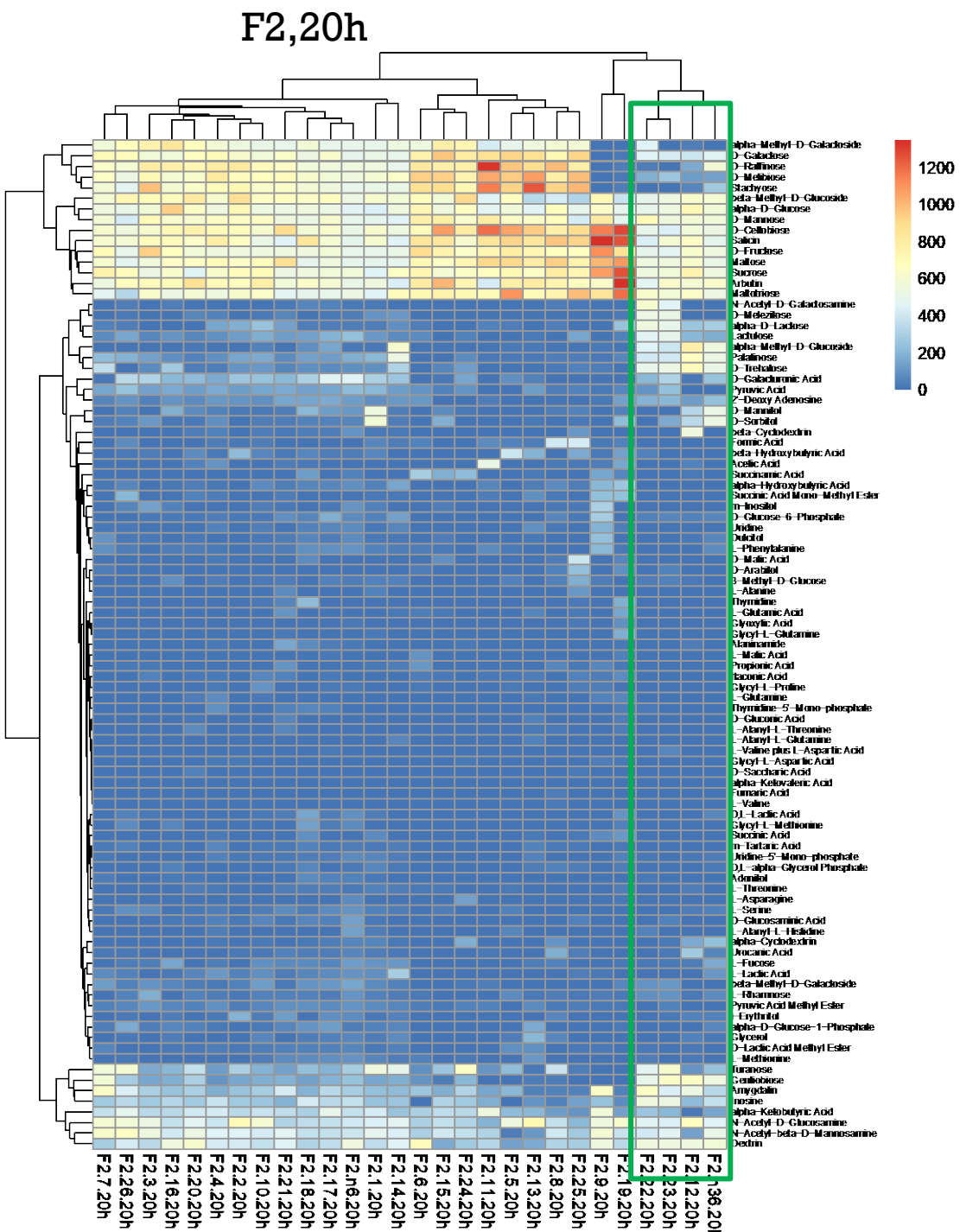
Metabolic profile

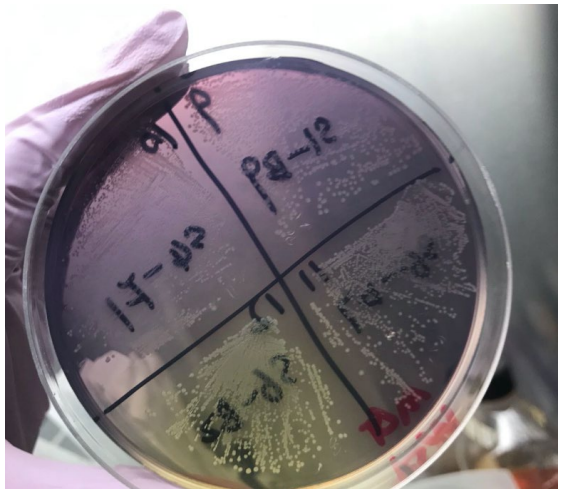


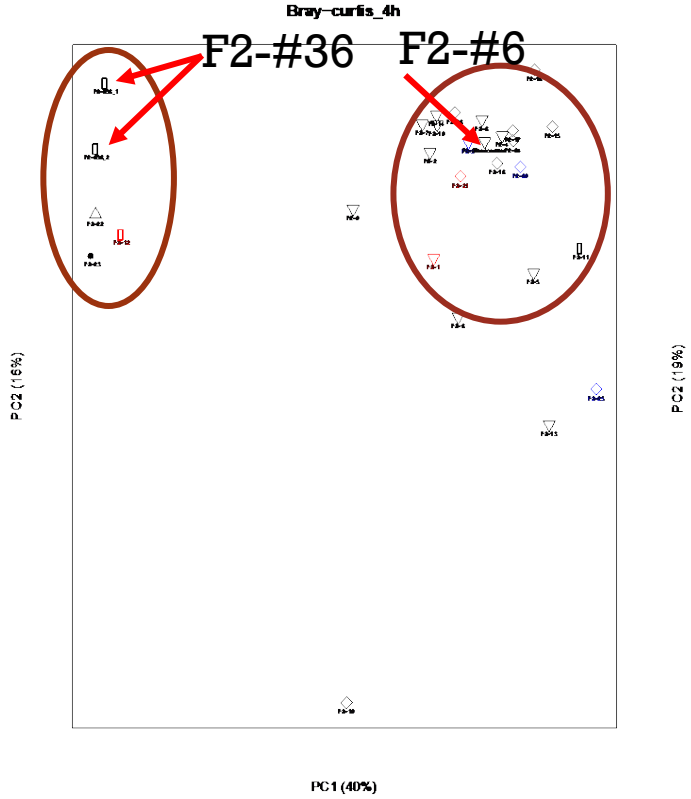


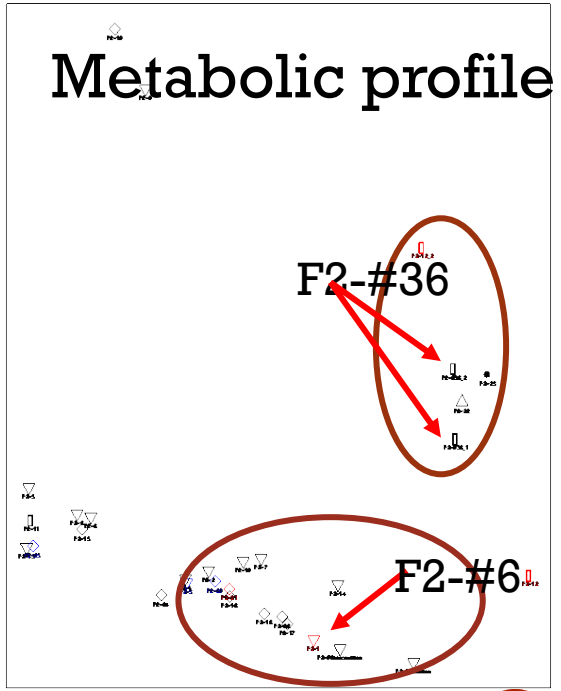




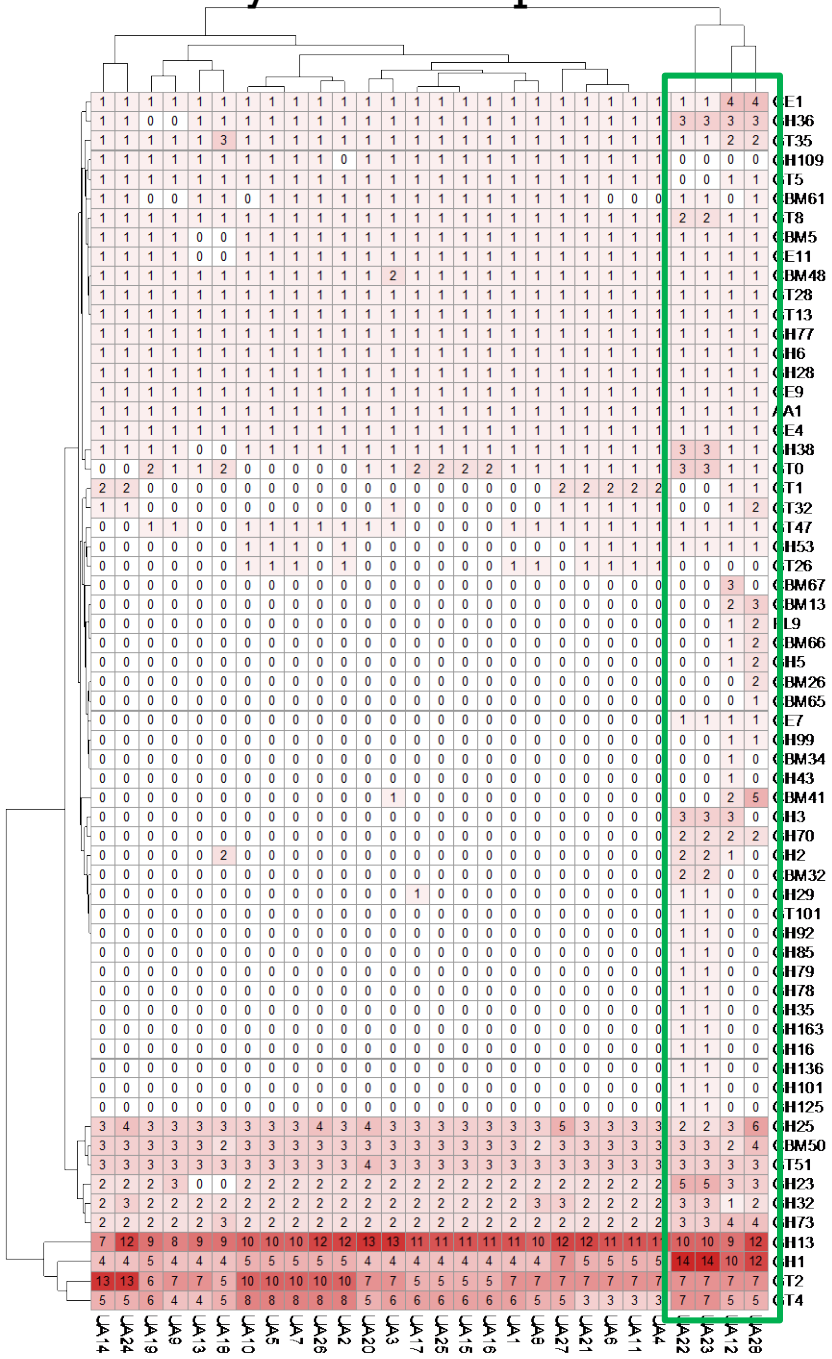

F2,20h
1200
1000
800
600
400
200
0
F2.7.20h
F2.26.20h
F2.3.20h
F2.16.20h
F2.20.20h
F2.4.20h
F2.2.20h
F2.10.20h
F2.21.20h
F2.18.20h
F2.17.20h
F2.6.20h
F2.1.20h
F2.14.20h
F2.15.20h
F2.24.20h
F2.11.20h
F2.5.20h
F2.13.20h
F2.8.20h
F2.25.20h
F2.9.20h
F2.19.20h
F2.22.20h
F2.23.20h
F2.12.20h



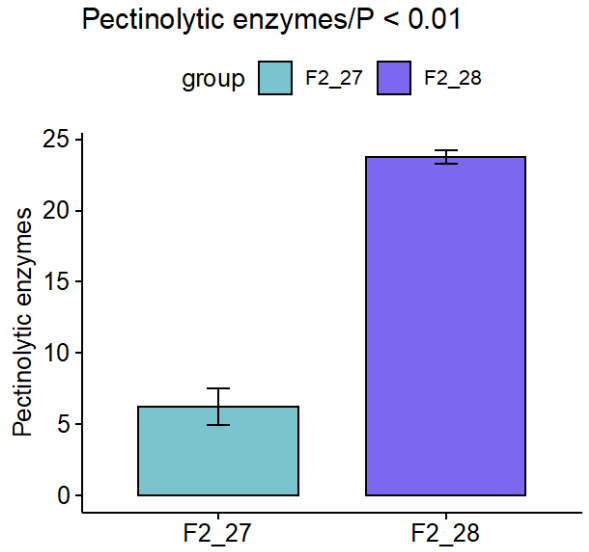

Bray–curtis_4h
F2-#36
F2-#6
PC2 (19%)
PC1 (40%)

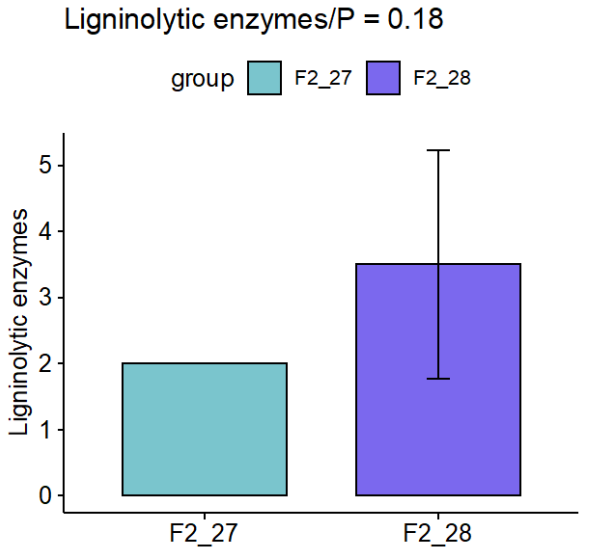

Bray–curtis_20h
Metabolic profile
F2-#36
F2-#6
PC2 (19%)
PC1 (40%)

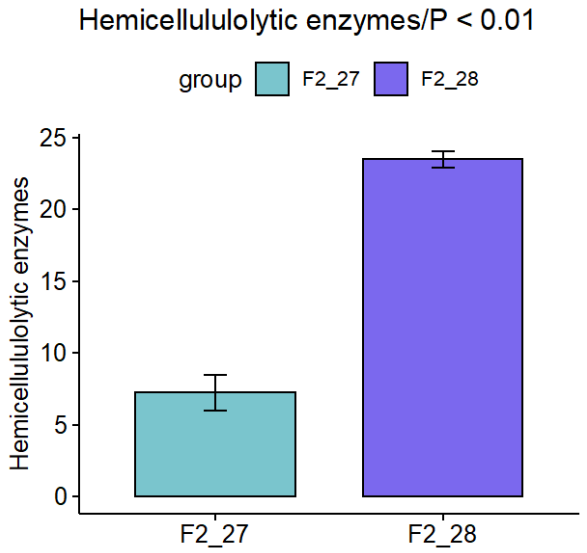
Cazyme heatmap

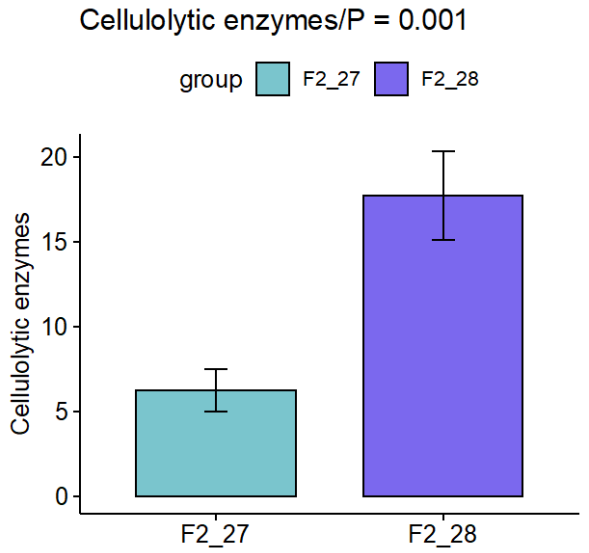


Whole genome sequencing





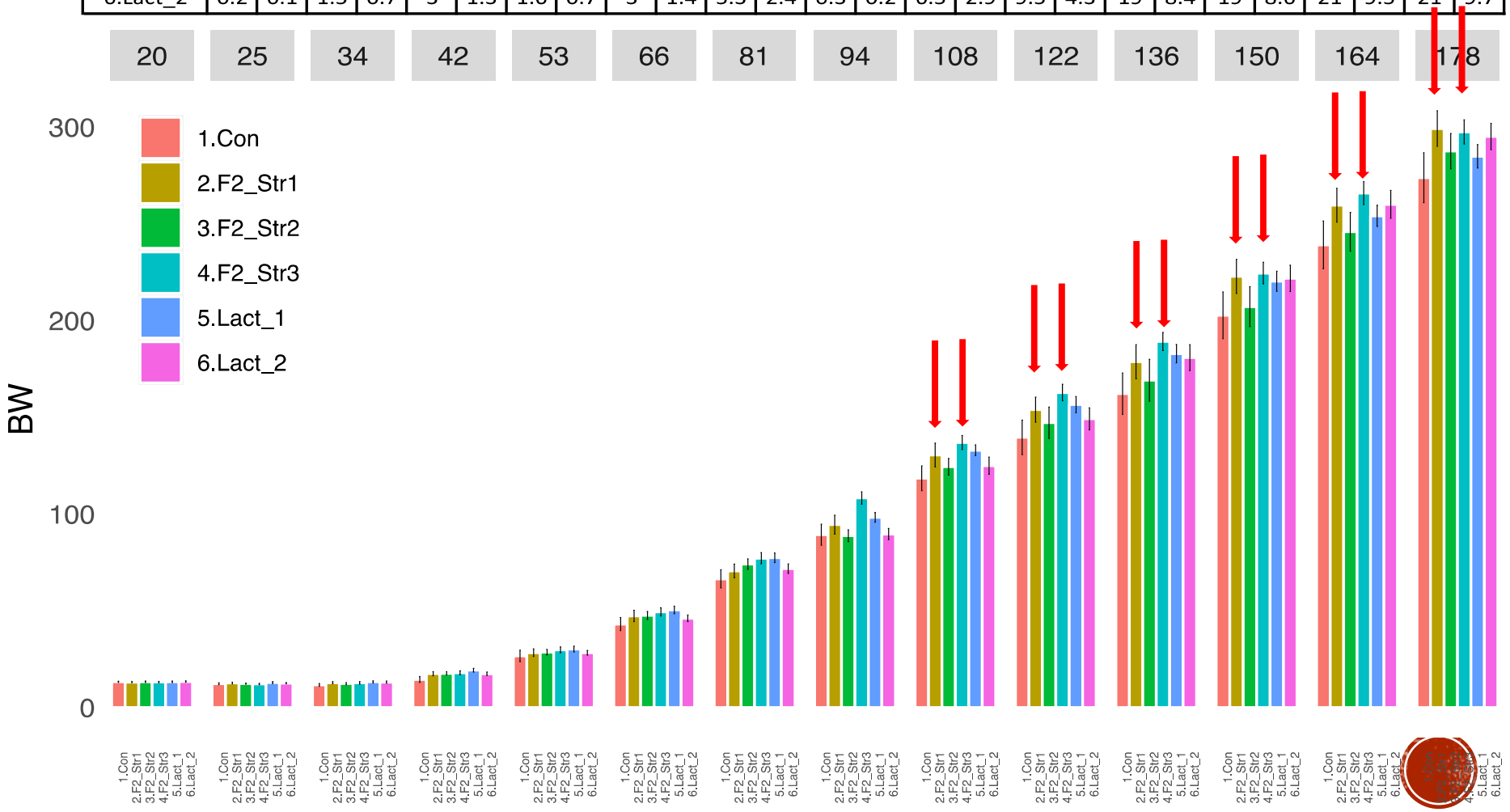




F2 oral inoculation trial 1:

Body weight of pigs from all six treatments

Age	25		34		42		53		66		81		94		108		122		136		150		164		178	
	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg	lb	kg
2.F2_Str1	0.3	0.2	1.2	0.5	3	1.4	1.7	0.8	4.2	1.9	4.1	1.8	5.2	2.4	12	5.4	14	6.4	17	7.5	20	9.1	21	9.3	25	12
3.F2_2	0	0	0.7	0.3	3.2	1.4	1.9	0.9	4.4	2	7.7	3.4	-1	-0	6	2.7	7.5	3.4	7	3.1	4.4	2	6.8	3.1	14	6.3
4.F2_Str3	-0	-0	1.1	0.5	3.5	1.6	3.1	1.4	6.3	2.8	11	4.8	19	8.7	19	8.3	23	11	27	12	22	9.9	27	12	24	11
5.Lact_1	0.5	0.3	1.6	0.7	4.8	2.2	3.6	1.6	7.3	3.3	11	4.9	8.9	4.1	15	6.5	17	7.6	21	9.4	18	8	15	6.8	11	5.1
6.Lact_2	0.2	0.1	1.5	0.7	3	1.3	1.6	0.7	3	1.4	5.3	2.4	0.3	0.2	6.5	2.9	9.5	4.3	19	8.4	19	8.6	21	9.5	21	9.7



F2 oral inoculation trial 2: F2-#28

- 16S rRNA gene sequencing
- Metagenome
- Metabolom

D0 and D1: F2 gavage

D21: F2 gavage

Nursery

Growing

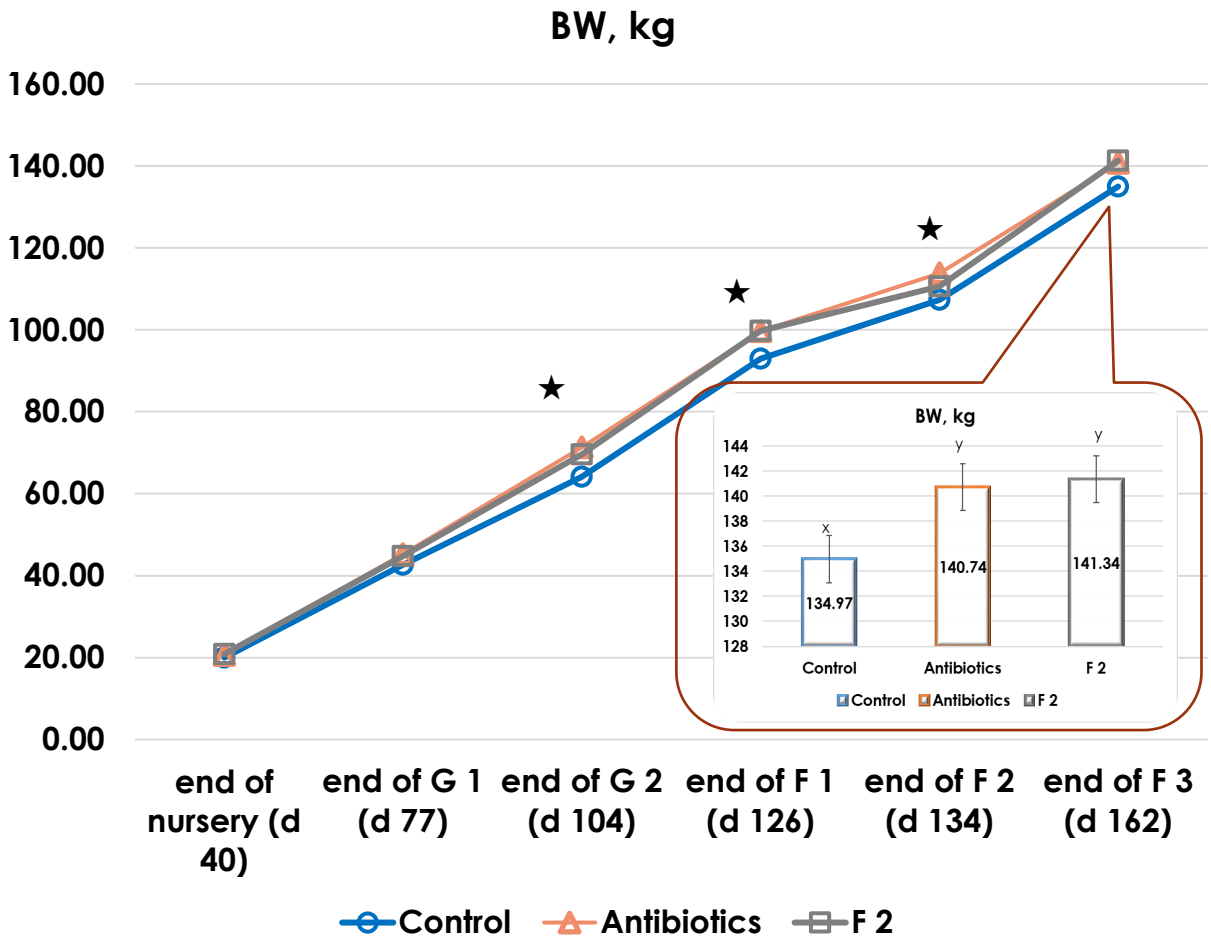
Finishing

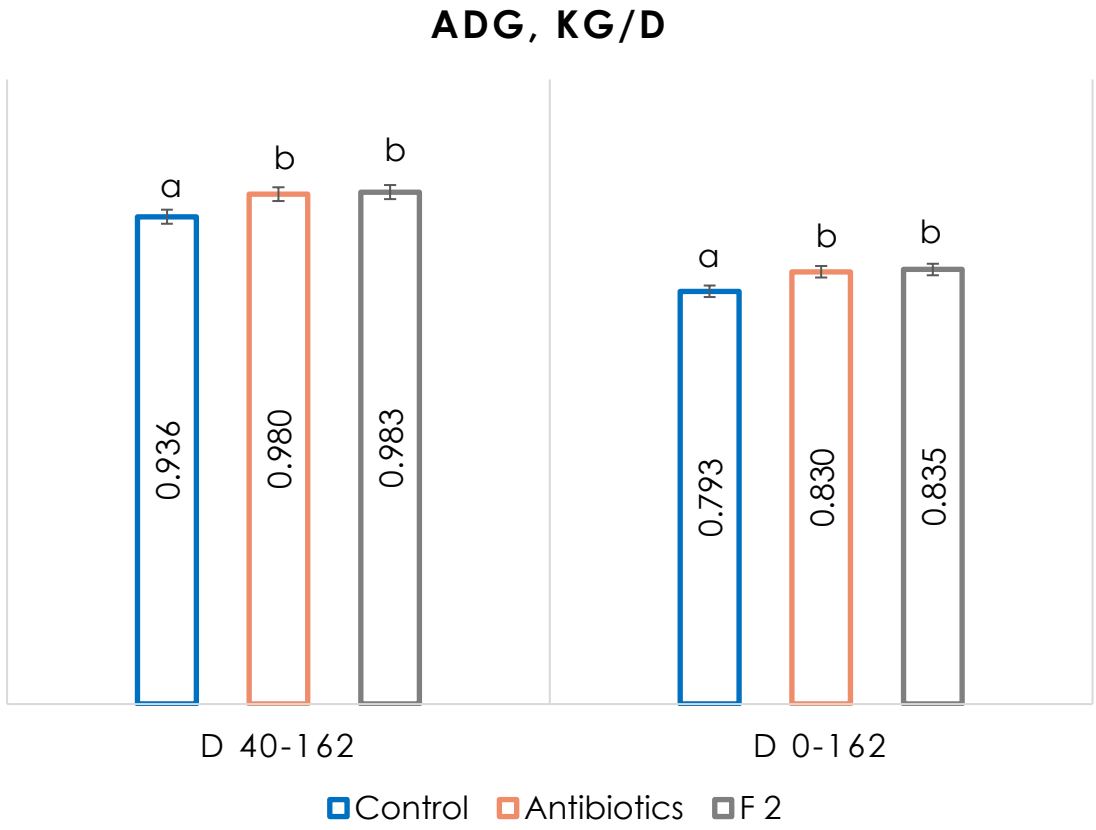
	D0	D7	D14	D21	D28	D40	D77	D104	D126	D134	D162
	11/11/20	11/18/20	11/25/20	12/2/20	12/9/20	12/21/20	1/27/21	2/23/21	3/17/21	3/25/21	4/22/21
Trt 1 Con	13.7	13.73	14.8		26.18	43.34	42.63	64.11	92.95	107.33	134.97
Trt 2 F2	13.57	13.78	15.12		29.03	46.08	44.74	69.60	99.79	110.62	141.34
Trt 3 Bacillus	13.73	13.75	14.4		25.66	41.14					
Trt 4 F2+B	13.48	13.68	14.79		28.45	46.35					
Trt 5 B2	13.64	13.59	14.61		26.8	43.48					
Trt 6 Antibiotics	13.68	14.19	14.86		28.82	45.5	45.24	71.36	99.68	113.82	140.74

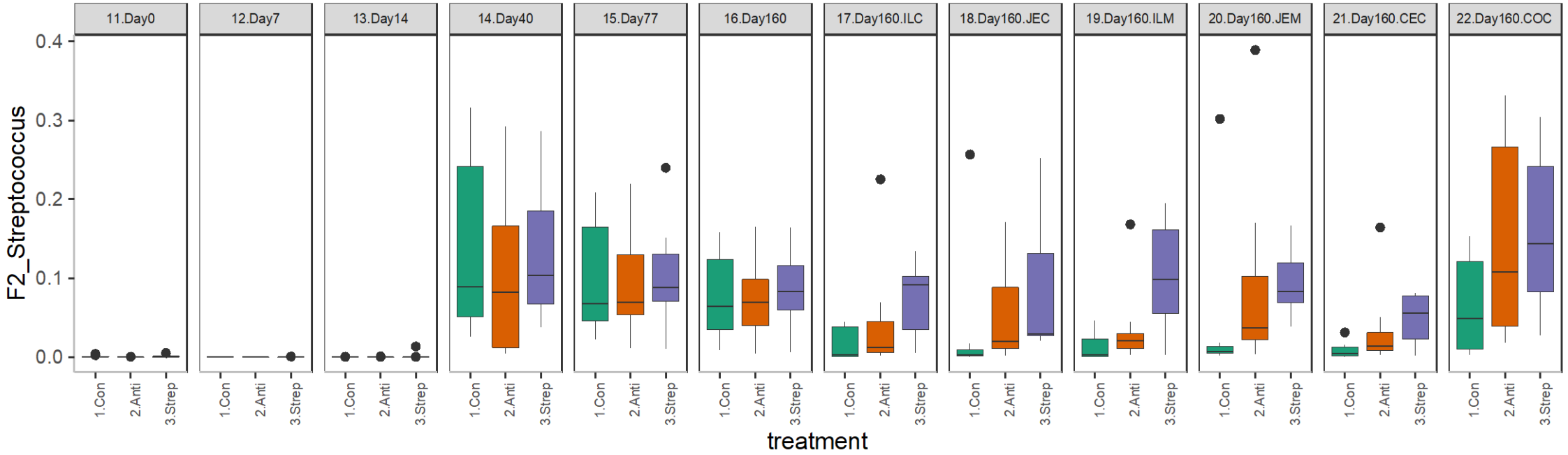
BW and rectal swabs

1. Carcass performance (Belly, loin)
2. Tissue collection
   - Pancreas tissue,
   - Ileum and jejunum tissue, mucosa, and digesta content
   - Cecum and colon tissue, digesta content

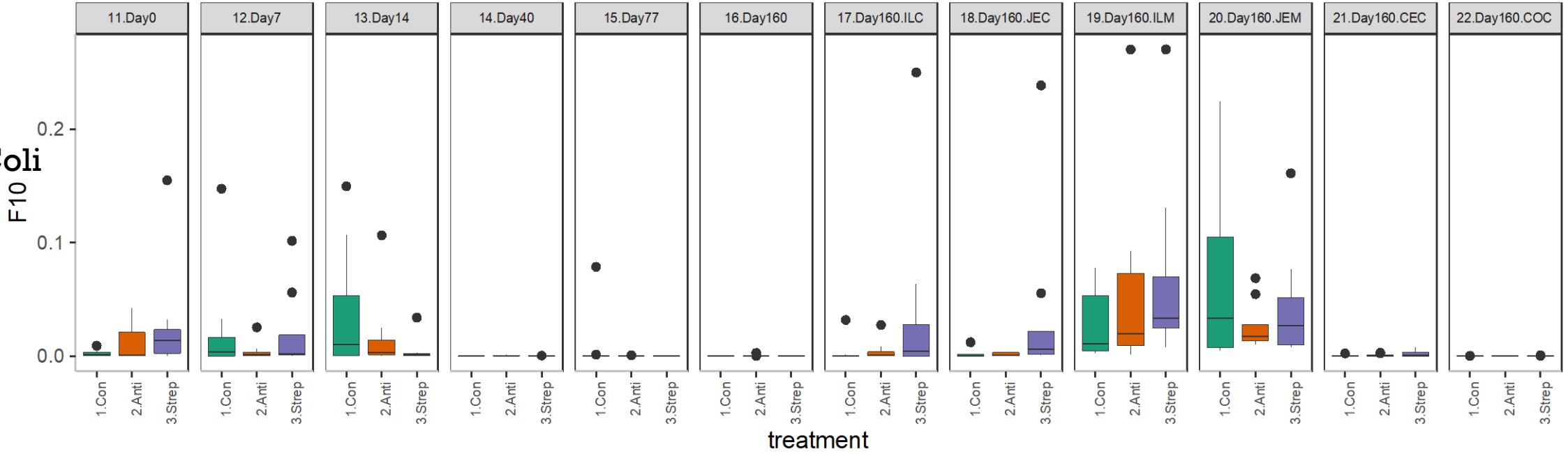
- 16S rRNA
- Enzyme activity


BW, kg
160.00
140.00
120.00
100.00
80.00
60.00
40.00
20.00
0.00
end of nursery (d 40)
end of G 1 (d 77)
end of G 2 (d 104)
end of F 1 (d 126)
end of F 2 (d 134)
end of F 3 (d 162)
Control
Antibiotics
F 2
BW, kg
144
142
140
138
136
134
132
130
128
x
y
y
134.97
140.74
141.34
Control
Antibiotics
F 2
Control Antibiotics F 2

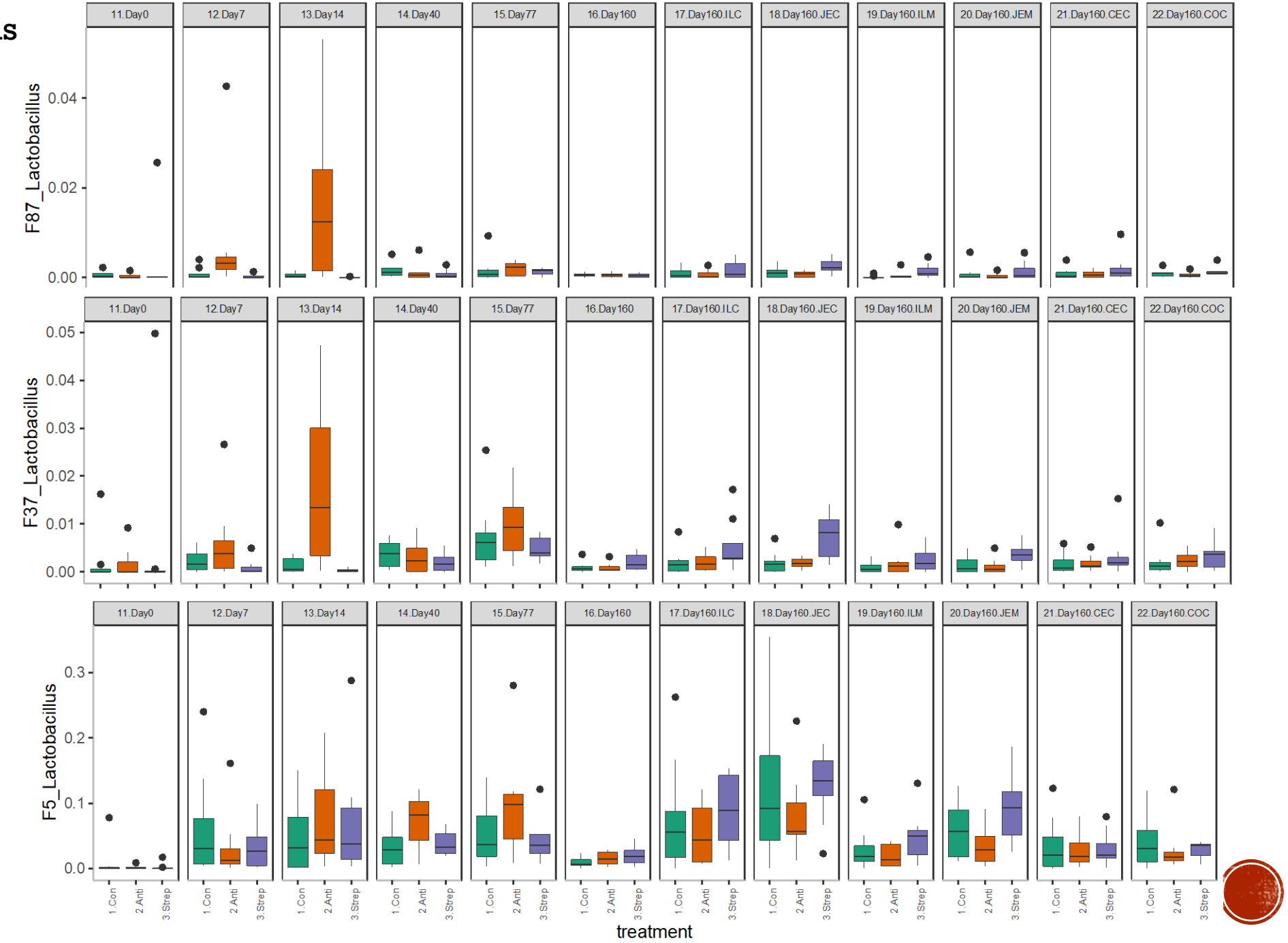

ADG, KG/D
a
b
b
0.936
0.980
0.983
a
b
b
0.793
0.830
0.835
D 40-162
D 0-162
Control
Antibiotics
F 2


11.Day0
12.Day7
13.Day14
14.Day40
15.Day77
16.Day160
17.Day160.ILC
18.Day160.JEC
19.Day160.ILM
20.Day160.JEM
21.Day160.CEC
22.Day160.COC
F2_Streptococcus
0.0
0.1
0.2
0.3
0.4
1.Con
2.Anti
3.Strep
treatment

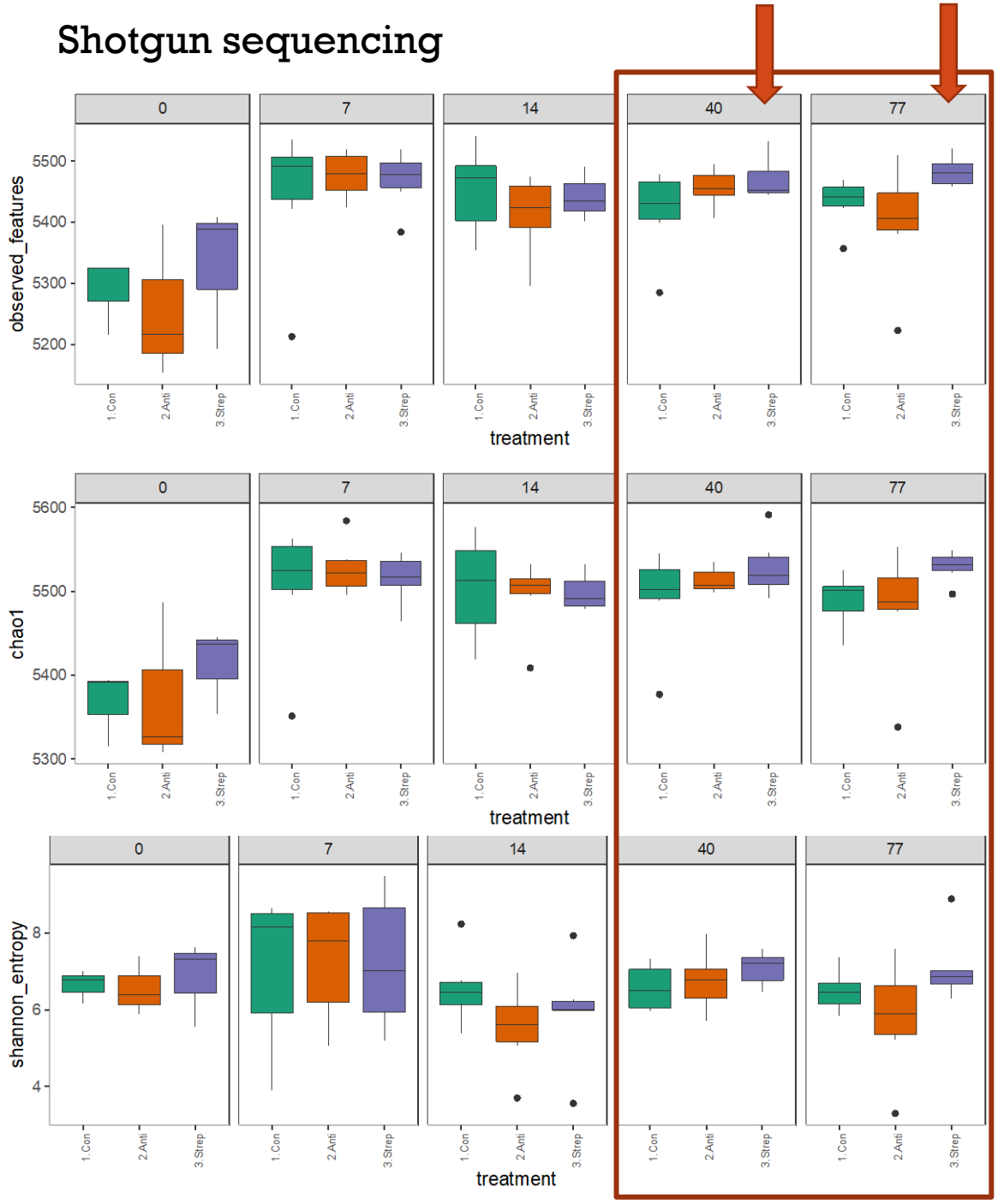
E.Coli


11.Day0
12.Day7
13.Day14
14.Day40
15.Day77
16.Day160
17.Day160.ILC
18.Day160.JEC
19.Day160.ILM
20.Day160.JEM
21.Day160.CEC
22.Day160.COC
F10
0.0
0.1
0.2
1.Con
2.Anti
3.Strep
treatment

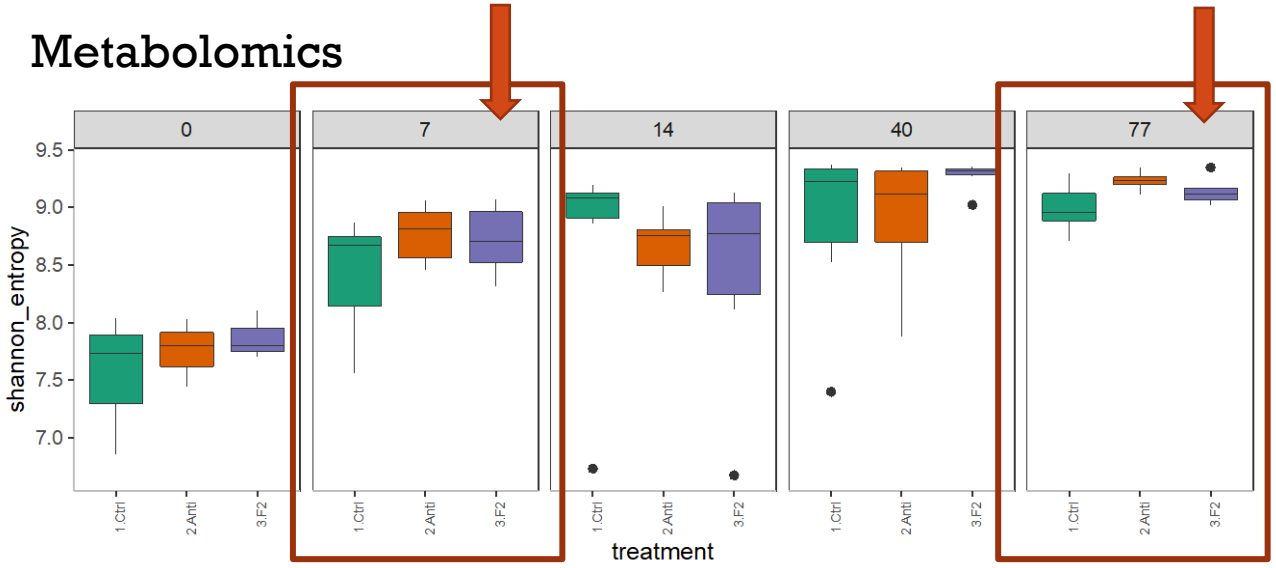
Modulation of lactobacillus in the gut

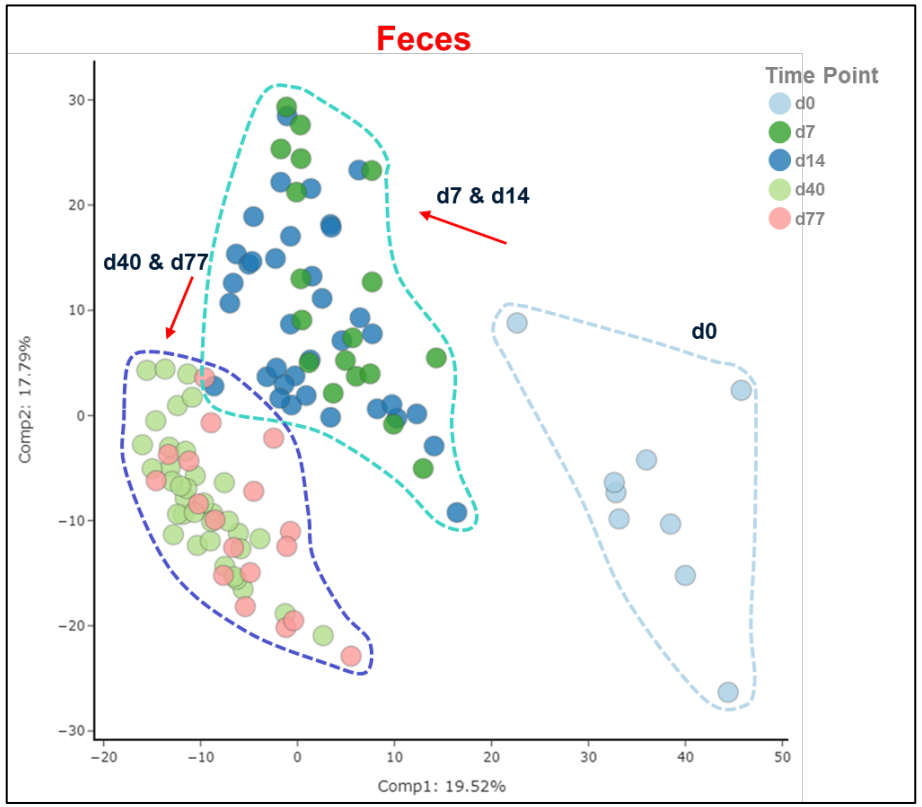


Shotgun sequencing



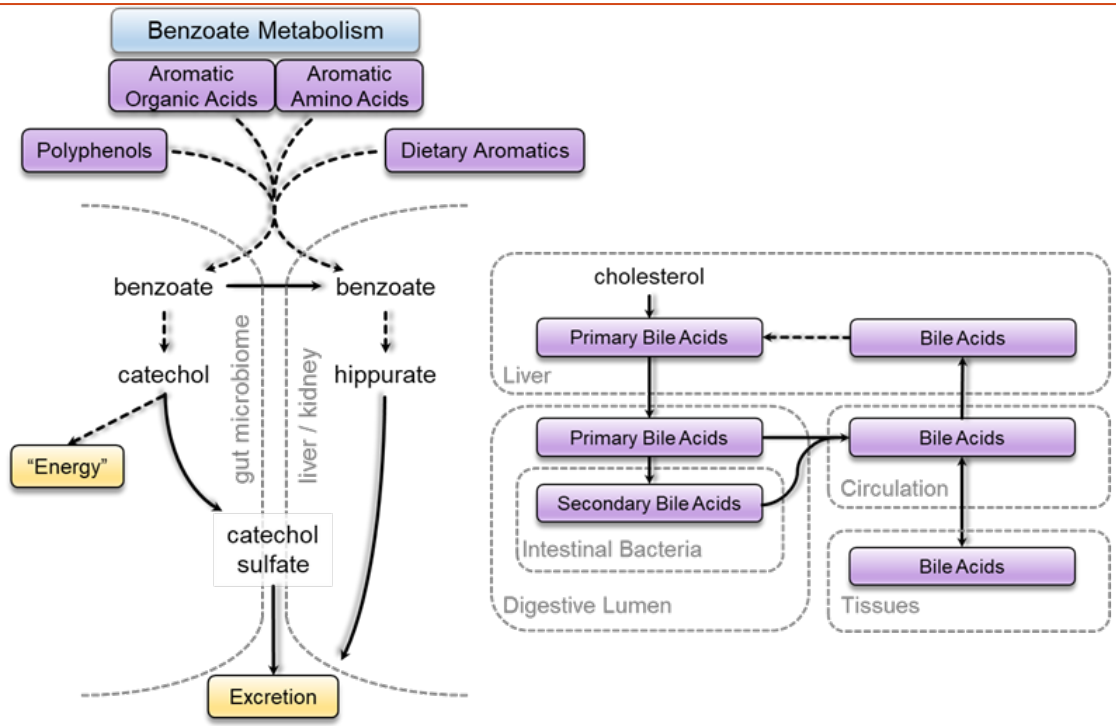
Metabolomics

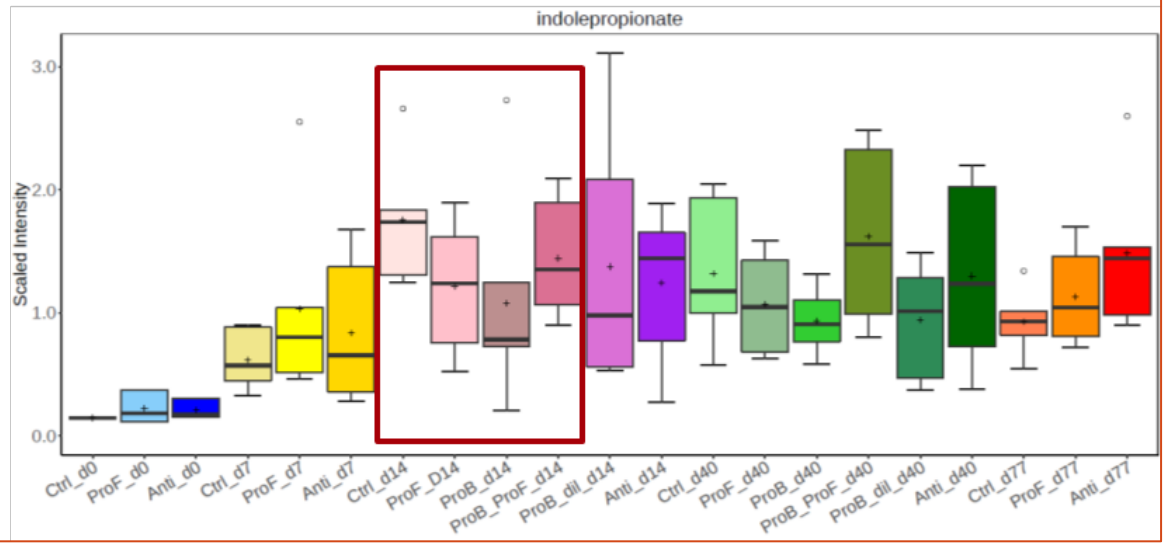




Metabolomics-d14

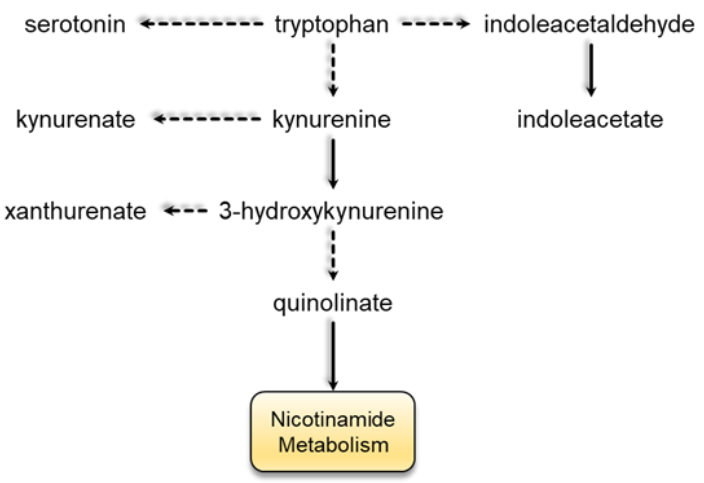
Sub Pathway	Biochemical Name	Fold of Change		
		Welch's Two Sample t-Test	Two Way Repeated Measures ANOVA	
		PROB D14 / CTRL D14	PROBIOTICF D14 / CONTROL D14	ANTIBIOTIC D14 / CONTROL D14
Phenylalanine Metabolism	phenylalanine	0.90	0.96	1.07
	N-acetylphenylalanine	1.23	0.83	2.22
	N-methylphenylalanine	1.90	1.96	1.14
	2-hydroxyphenylacetate	1.28	1.58	1.22
Tyrosine Metabolism	tyrosine	0.86	0.92	1.03
	tyramine	0.70	0.18	0.45
	tyrosol	1.25	0.52	0.86
	vanillactate	0.39	0.59	0.82
	gentisate	0.30	0.40	0.70
Tryptophan Metabolism	tryptophan	1.04	0.93	0.92
	N-acetyltryptophan	1.25	1.21	1.31
	oxindolylalanine	1.59	1.37	1.32
	kynurenine	1.14	1.18	1.41
	kynurenate	0.54	0.50	0.78
	anthranilate	1.43	1.32	1.37
	tryptophol	0.50	0.60	1.20
	indolepropionate	0.61	0.69	0.71
	skatol	1.54	1.12	0.36
Secondary Bile Acid Metabolism	6-beta-hydroxylithocholate	1.82	1.68	0.50
	lithocholate	1.37	1.39	0.47
	tauroursodeoxycholate	0.74	0.08	0.49
	dehydrolithocholate	1.57	0.89	0.80
	hyocholate	0.56	1.53	0.90
	glycohyocholate	0.40	0.16	0.27
	taurohyocholate*	0.93	0.11	1.20
	hyodeoxycholate	0.76	0.95	0.59
	taurohyodeoxycholic acid	0.13	0.09	0.16
	glycohyodeoxycholate	0.12	0.18	0.05
	glycocholenate sulfate*	0.83	0.25	0.31
	7alpha-hydroxycholestenone	1.73	1.79	1.26

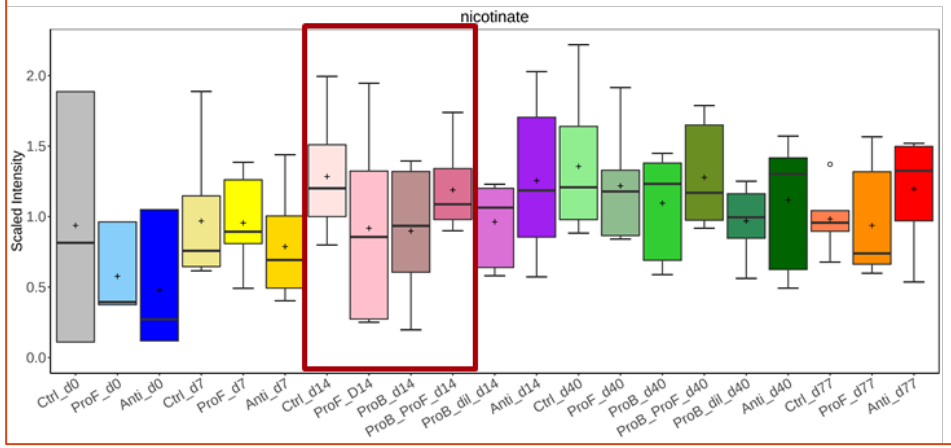


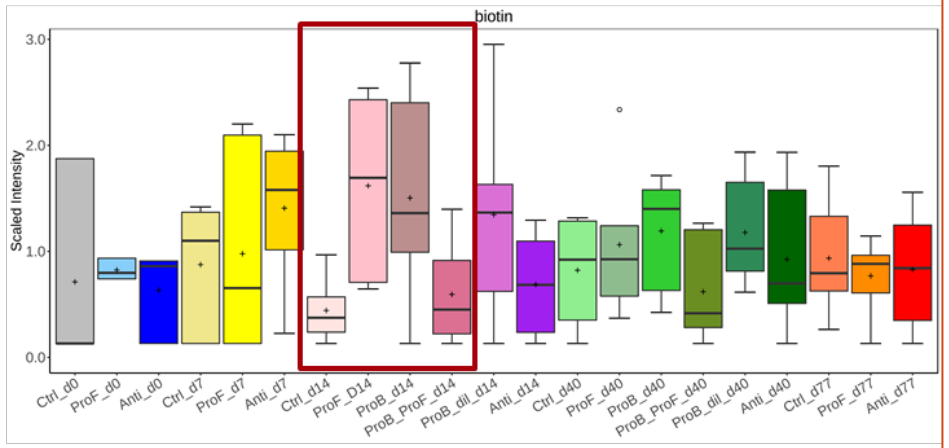


Metabolomics-d14

Sub Pathway	Biochemical Name	Fold of Change		
		Welch's Two Sample t-Test	Two Way Repeated Measures ANOVA	
		PROB D14 / CTRL D14	PROBIOTICF D14 / CONTROL D14	ANTIBIOTIC D14 / CONTROL D14
Nicotinate and Nicotinamide Metabolism	nicotinate	0.70	**0.71**	0.98
	nicotinamide	1.20	0.82	0.64
	1-methylnicotinamide	0.29	**0.17**	0.42
	2-hydroxynicotinate	0.43	0.54	0.38
	trigonelline (N'-methylnicotinate	0.02	0.02	0.04
Pantothenate and CoA Metabolism	pantoate	2.73	1.14	**2.80**
	pantothenate	0.86	0.73	1.31
Ascorbate and Aldarate Metabolism	threonate	0.64	**0.39**	1.16
	oxalate (ethanedioate)	0.70	0.83	1.02
Tocopherol Metabolism	alpha-tocopherol	**1.25**	**1.39**	1.07
	alpha-tocopherol acetate	**1.65**	**1.81**	1.33
	delta-tocopherol	**1.47**	**1.59**	1.26
	alpha-tocotrienol	1.17	**1.47**	1.01
	gamma-tocotrienol	**1.26**	**1.40**	1.01
Biotin Metabolism	biotin	**3.40**	**3.66**	1.55
Thiamine Metabolism	thiamin (Vitamin B1)	1.79	1.34	0.91
Vitamin A Metabolism	retinol (Vitamin A)	**3.01**	**2.07**	**2.14**
Vitamin B6 Metabolism	pyridoxine (Vitamin B6)	1.32	0.95	0.84
	pyridoxamine	1.37	1.17	1.14
	pyridoxate	1.19	**0.33**	1.64







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