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Drawing back the veil:
a metagenomic study of species diversity in UK food industry

Rob Limburn, Campden BRI

Rob.limburn@campdenbri.co.uk

Tel: +44 (0)1386 842493

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Introduction

- A range of UK food manufacturing facilities were sampled to gain insights into the species composition of food industry biofilms.
- Used in support of a research project on biofilm inactivation/removal.
- Factories were visited over a 1-year period.
- Biofilms were detected, sampled and analysed using traditional methods and metagenomic analysis to determine species composition.

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Sampling of biofilm from food facilities - overview

- Sampling was carried out at 7 processing facilities:
  - Meat (Cooked & Raw facilities)
  - Ready meals (2 facilities)
  - Smoked RTE fish
  - Dairy desserts
  - Ambient retorted products

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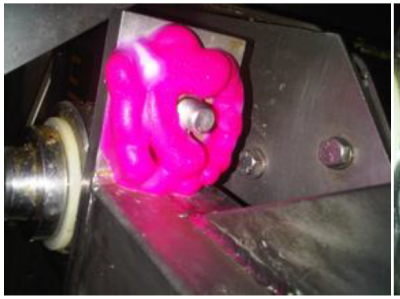
Sampling of biofilm from food facilities – finding biofilms!

- Use of two commercially available products designed for visualisation of biofilm-associated cells (Biofinder spray, TBF300)



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Sampling of biofilm from facilities

- Visited during routine hygiene shutdown
  - Sampled post-hygiene
- Sample sites were identified using Biofinder/TBF300
  - Food contact and non-food contact areas sampled
- Up to 20 cell scraper/ flocked swab samples taken post-cleaning/disinfection
  - Method used to improve recovery of biofilm from surfaces
  - Flocked swabs to improve recovery of biofilm from swabs into solution

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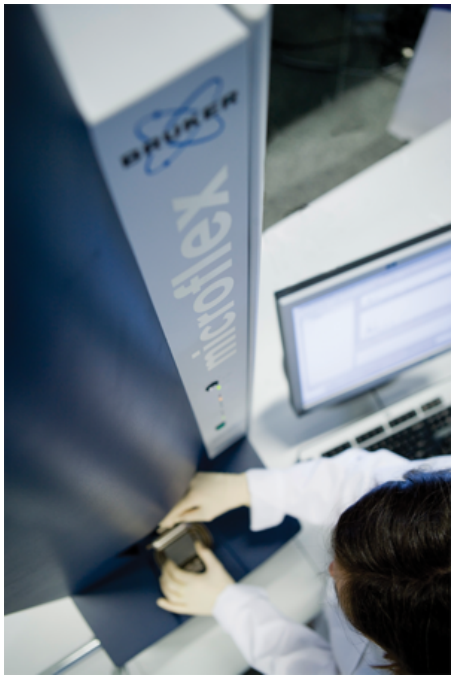
Sampling of biofilm from food facilities

- Swabbing/scraping from areas of food production
- Swab recovery through vortexing/sonication
- Swabs tested for TVC (30°C, aerobic)
  - Isolates identified by MALDI and kept for future use



Sample ID	Organism (best match)	Score Value	Organism (second-best match)	Score value
10A	Chryseobacterium shigense	2.13	Chryseobacterium elymi	1.99
10B	Serratia liquefaciens	2.01	Serratia liquefaciens	1.92
10C	No organism id possible	1.52	No id possible	1.45
8A	Pseudomonas antarctica	2.21	Pseudomonas extremorientalis	2.18
8B	Pseudomonas flavescens	1.81	No organism id possible	1.41
8C	Pseudomonas tolaassii	2.10	Pseudomonas libanensis	2.05
8D	Pseudomonas stutzeri	2.30	Pseudomonas stutzeri	2.17

Meaning of Score Values

Range	Interpretation	Colour
2.00 – 3.00	High-confidence identification	Green
1.70 – 1.99	Low-confidence identification	Yellow
0.0– 1.69	No Organism Identification Possible	Red

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Sampling of biofilm from food facilities

- Isolates tested for biofilm forming potential by using crystal violet microplate assay – ranked weak, medium or strong dependent on absorbance.
- Swabs tested for carbohydrate, protein and uronic acids using microplate assays (Maes et al, 2017)
- Metagenomic analysis of swab suspensions and comparison of microbial profile from different factory areas.

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Metagenomic analysis of biofilms – advanced microbial profiling (AMP)

- Natural microflora has traditionally been a difficult area to assess.

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Metagenomic analysis of biofilms – advanced microbial profiling (AMP)

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  - Traditional cultural analysis of microorganisms selects for specific types, e.g a standard total count test will select for microorganisms that will grow:
    - aerobically
    - at 30°C over 2/3 days
    - in the nutrients available in the media used
    - Will provide estimated counts of microorganisms

Metagenomic analysis of biofilms – advanced microbial profiling (AMP)

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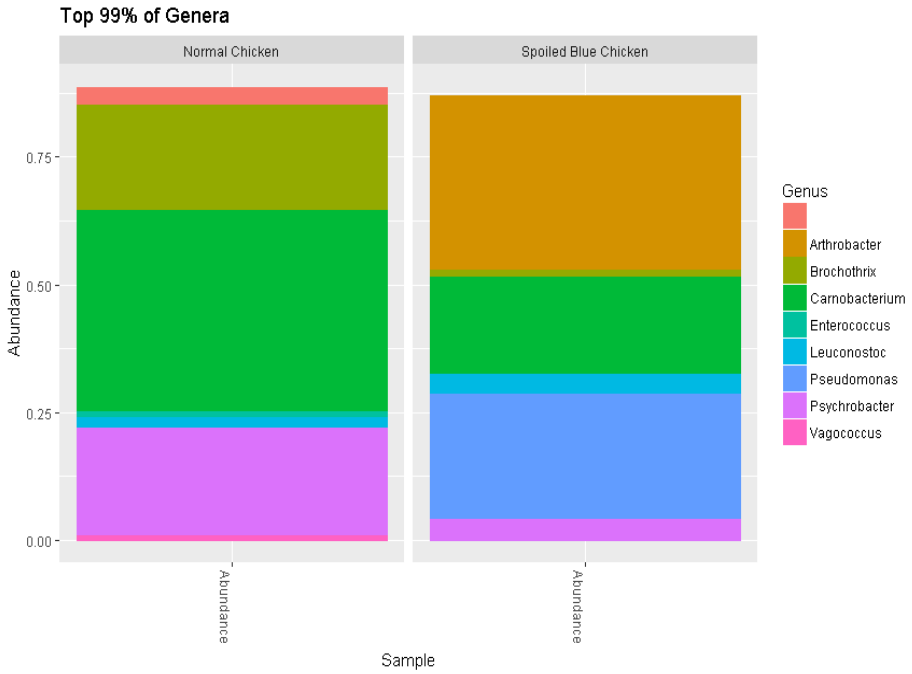
    - in the nutrients available in the media used

    Will provide levels of microorganisms found
  - AMP looks for the bacterial DNA in a sample, will provide analysis on types of microorganisms present
    - Gene encoding 16S ribosomal subunit used in this study.
    - Gives organisms present and their relative abundances.
    - Provides a "microbial profile" for each sample

Food industry and metagenomics: Spoilage investigations
Example

- MAP cooked chicken
- Some packs were spoiling with blue discolouration.
- AMP was run on spoiled and not spoiled samples.



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Metagenomic analysis

- DNA extracted and 16s rDNA sequenced using Illumina MiSeq system
- Bacterial species present in each sample identified by comparing sequences to a public database
- Data analysed using R software (Phyloseq package) to compare bacterial species composition of each sample

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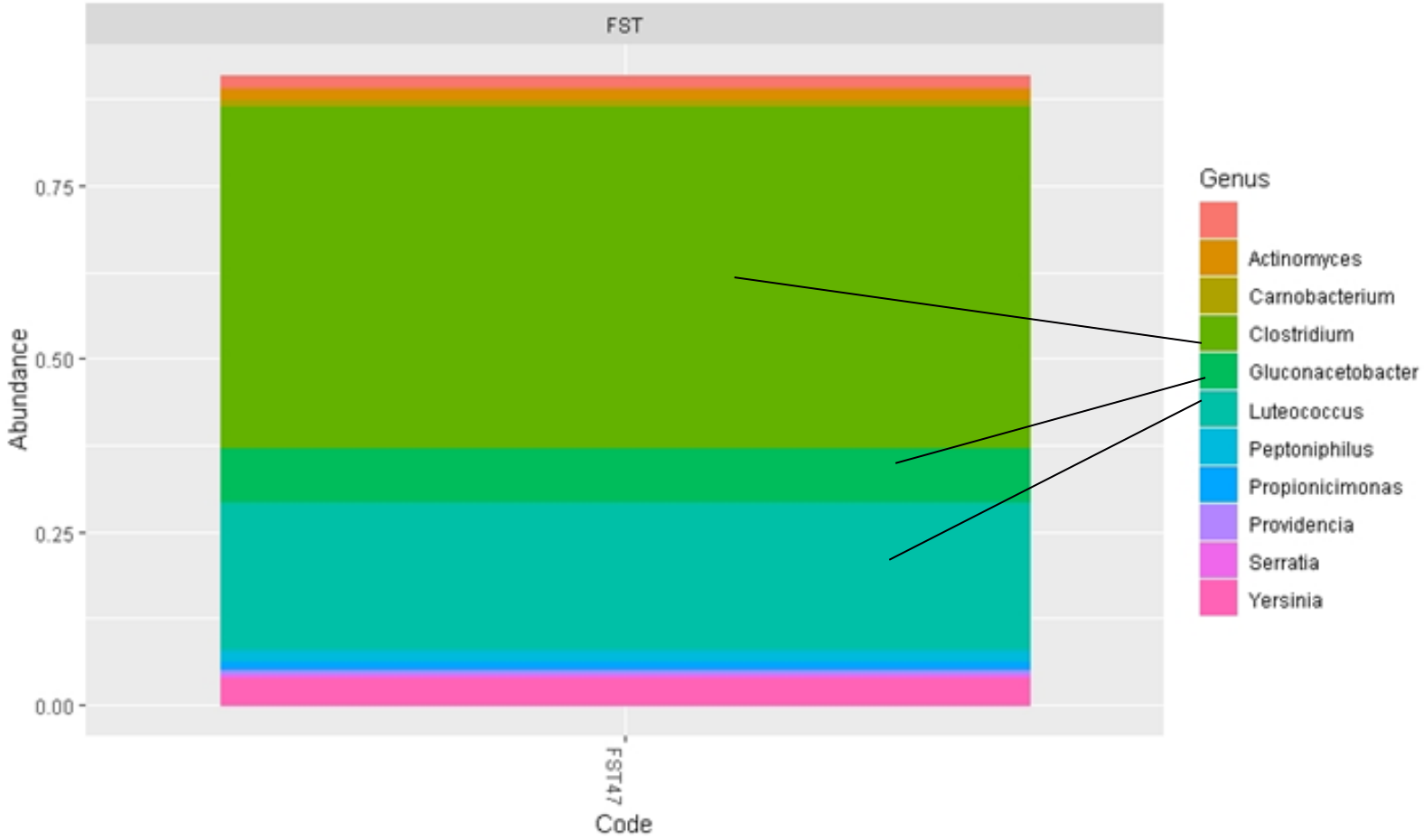
Most abundant Genera, Biofilm sample from behind a seal in Rheon forming tool, RTE fish factory



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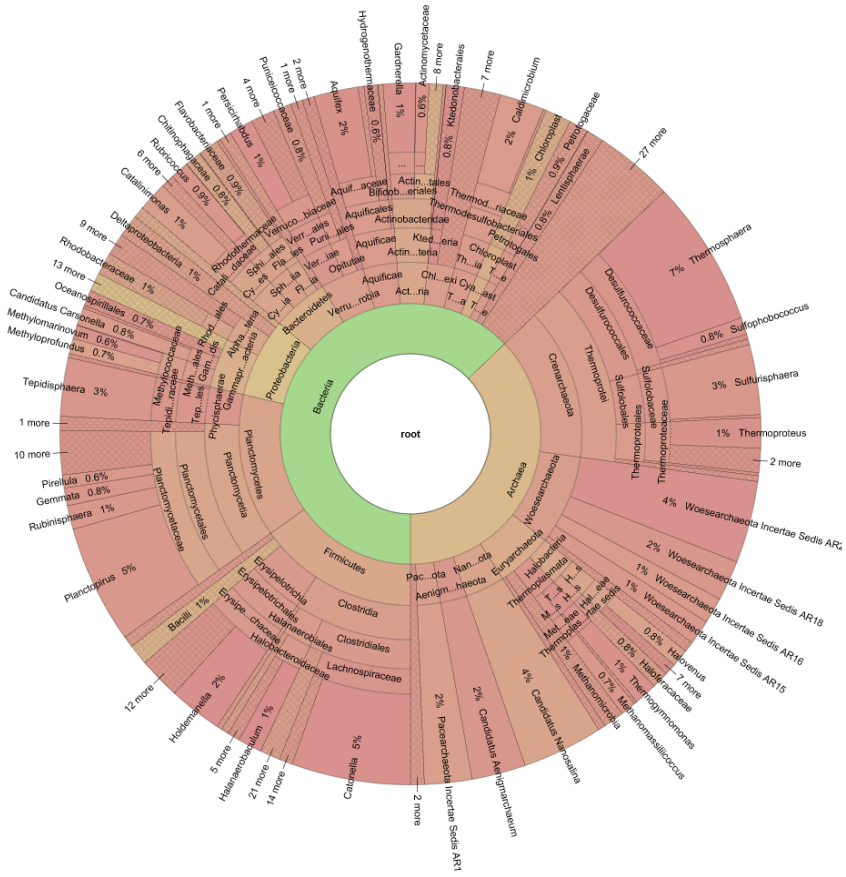
Profiles


root
Bacteria
Archaea
Proteobacteria
Crenarchaeota
Euryarchaeota
Firmicutes
Planctomycetes
Bacteroidetes
Thermoprotei
Thermoplasmata
Halobacteria
Clostridia
Planctomycetia
Planctomycetales
Planctomycetaceae
Thermoproteales
Thermoproteaceae
Sulfolobales
Desulfurococcales
Desulfurococcaceae
Thermoproteus
Thermosphaera
Sulfurisphaera
Sulfophobococcus
Woesearchaeota
Pacearchaeota
Candidatus Aenigmarchaeum
Candidatus Nanosalina
Methanomicrobia
Methanomassiliicoccus
Thermogymnomonas
Haloarcula
Haloterrigena
Halobacteriaceae
Halobacteriales
Haloferacaceae
Haloferax
Natronomonas
Halorubrum
Natrialba
Natronococcus
Natronolimnobius
Natronorubrum
Halogeometricum
Halosimplex
Halovenus
Halopiger
Halobaculum
Halonotius
Halostagnicola
Haloplanus
Halorhabdus
Halosarcina
Halosarcinaceae
Haloarchaeobius
Halococcus
Haloferax
Haloterrigena
Halobacteroidaceae
Lachnospiraceae
Clostridiales
Haloanaerobiales
Erysipelotrichaceae
Erysipelotrichales
Erysipelotrichia
Bacilli
Planctopirus
Gemmata
Pirellula
Rubinisphaera
Tepidisphaera
Methylomarinovum
Methyloprofundus
Candidatus Caldarchaeum
Oceanospirillales
Rhodobacteraceae
Rhodothermaceae
Cytophagia
Flavobacteriaceae
Chitinophagaceae
Rubricoccaceae
Catalinimonas
Deltaproteobacteria
Aquificae
Aquificales
Aquificaceae
Hydrogenothermaceae
Aquifex
Gardnerella
Actinomycetaceae
Actinobacteridae
Bifidobacteriales
Ktedonobacterales
Thermodesulfobacteriales
Thermodesulfobacteriaceae
Petrotogales
Petrotogaceae
Chloroplast
Chloroplast
Calothrix
Lentisphaerae
Lentisphaerae
Pedosphaeraceae
Pedosphaerae
Caldimicrobium
Thermodesulfobacteriaceae
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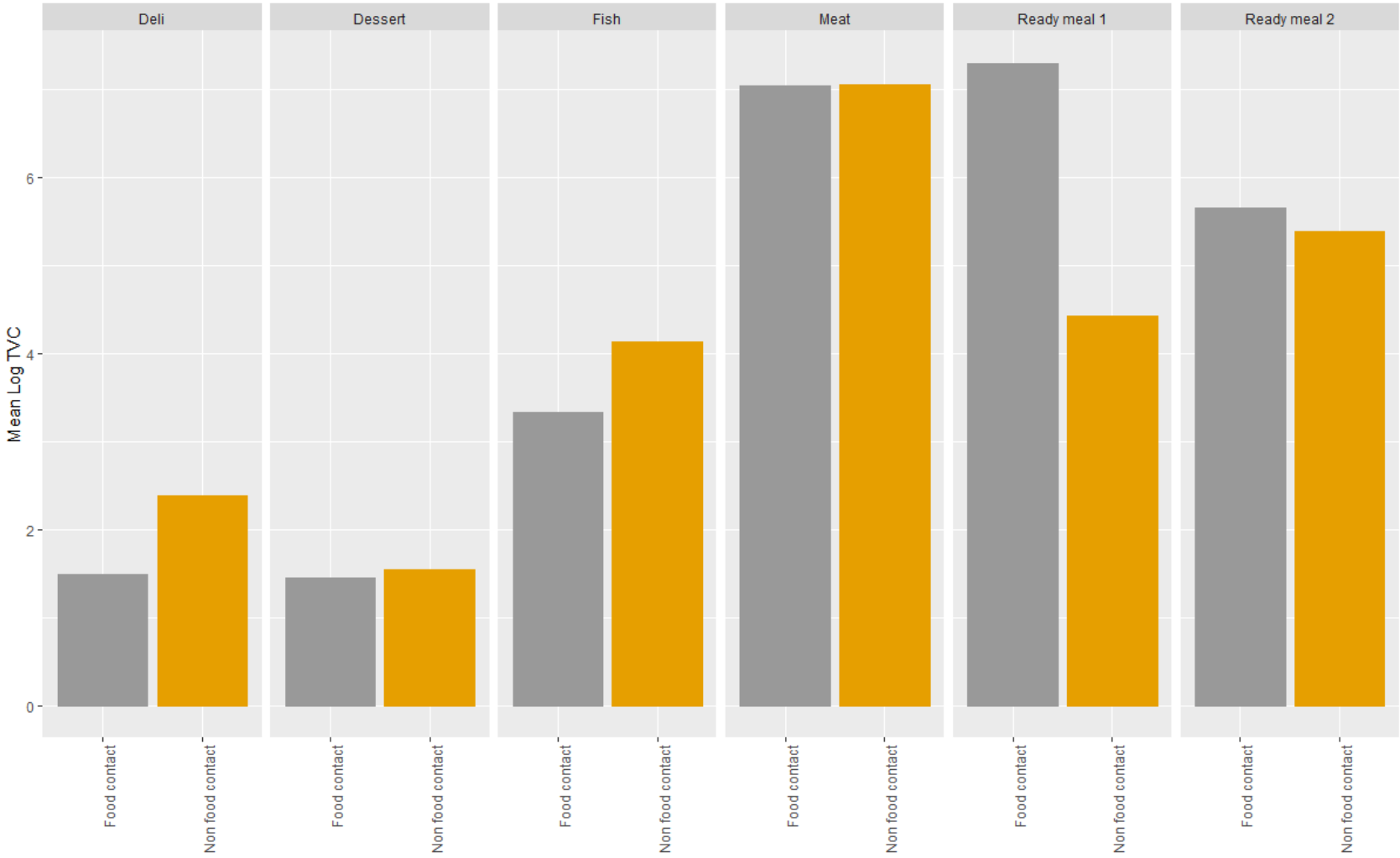
TVCs of factory post-clean biofilm samples



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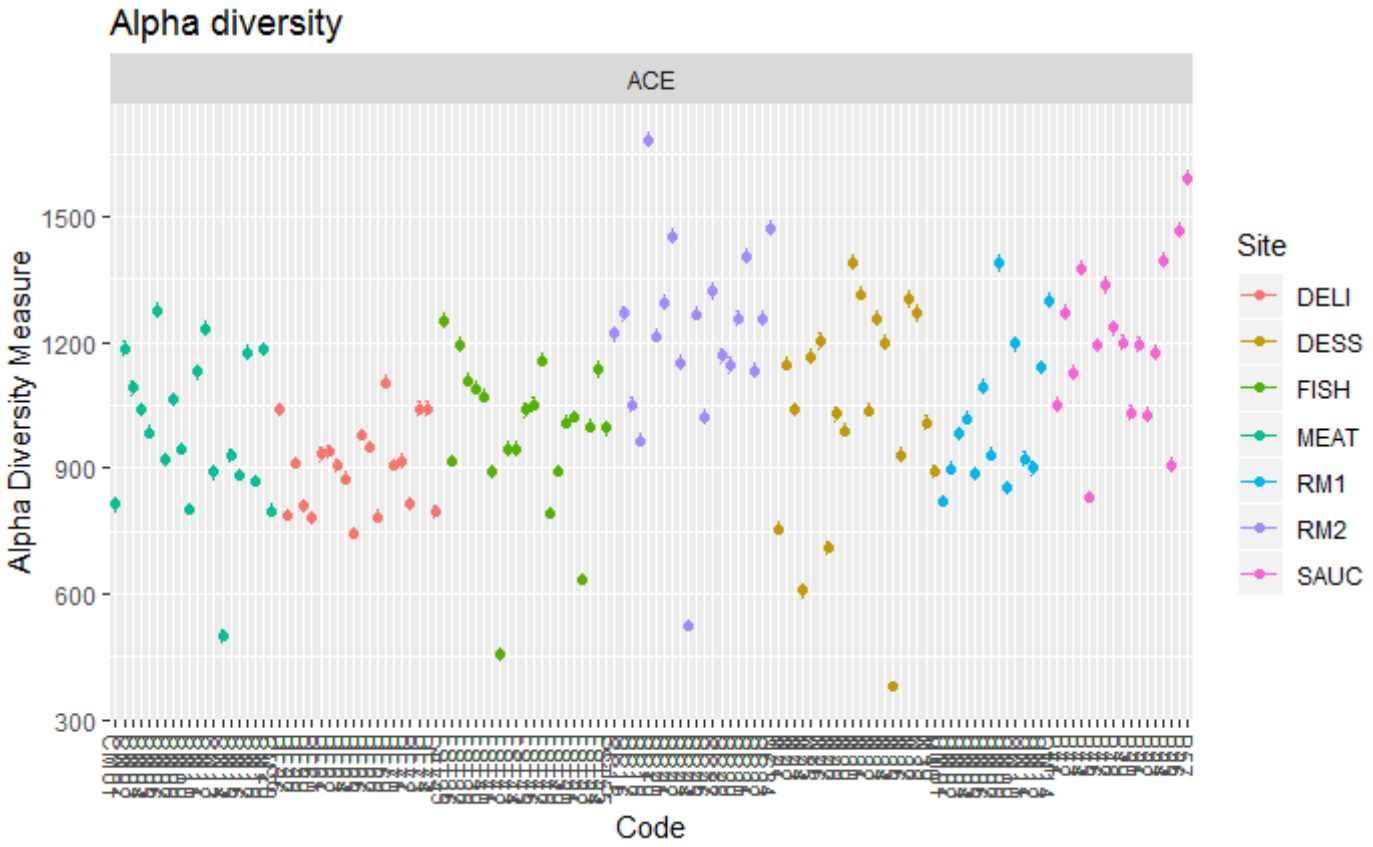
Alpha diversity plot for food industry biofilms (Illumina)



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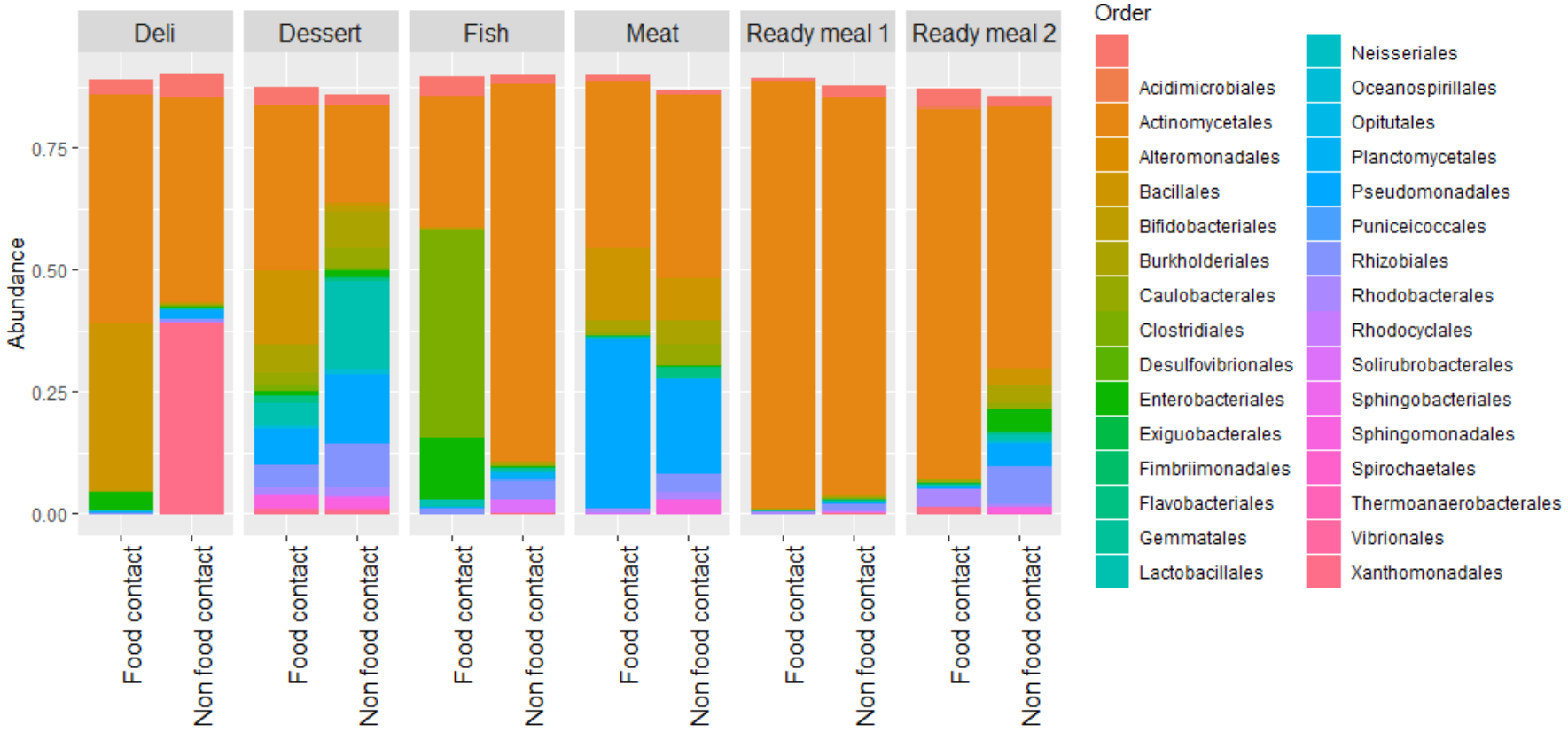
Advanced Microbial Profiling – Order level



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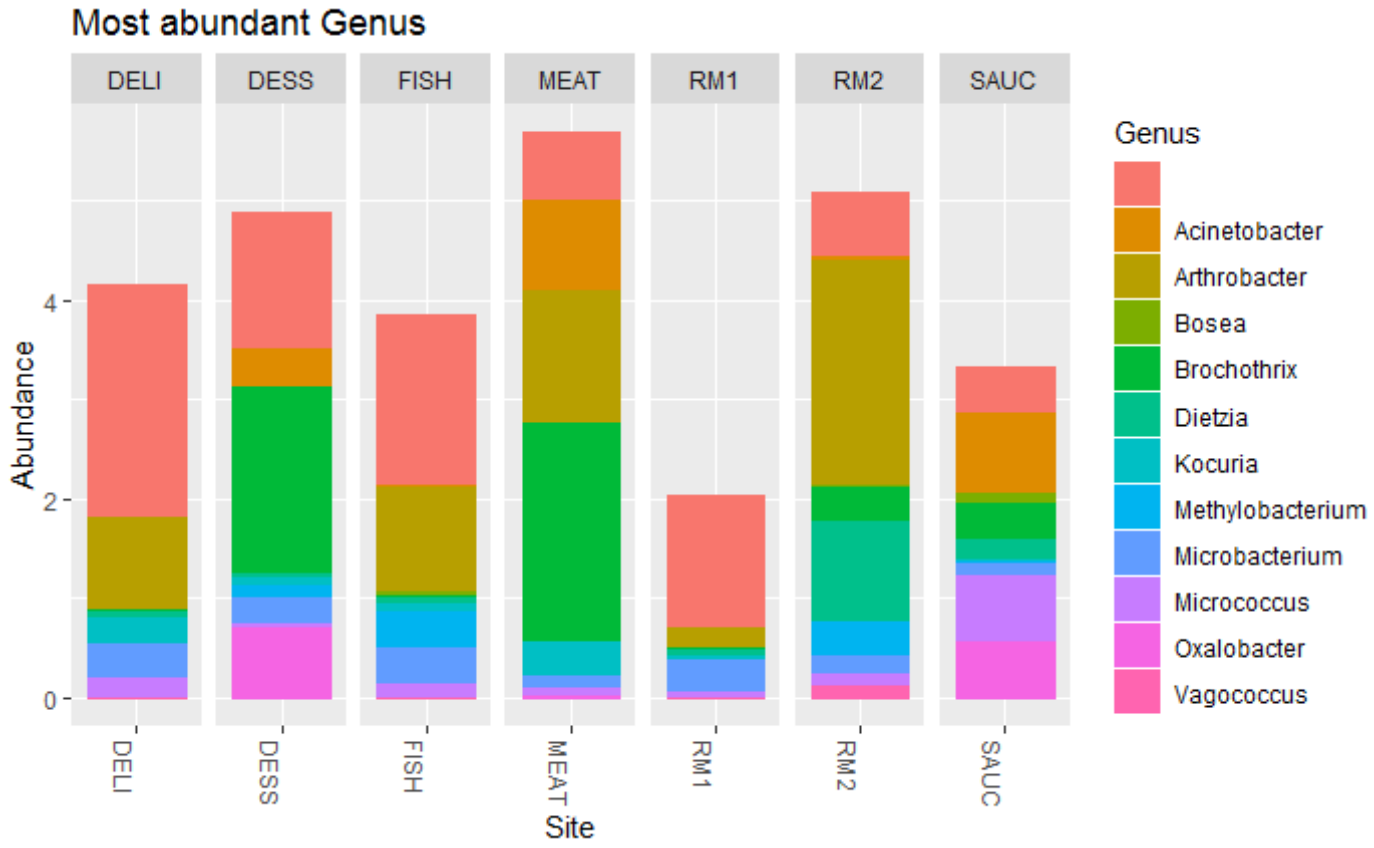
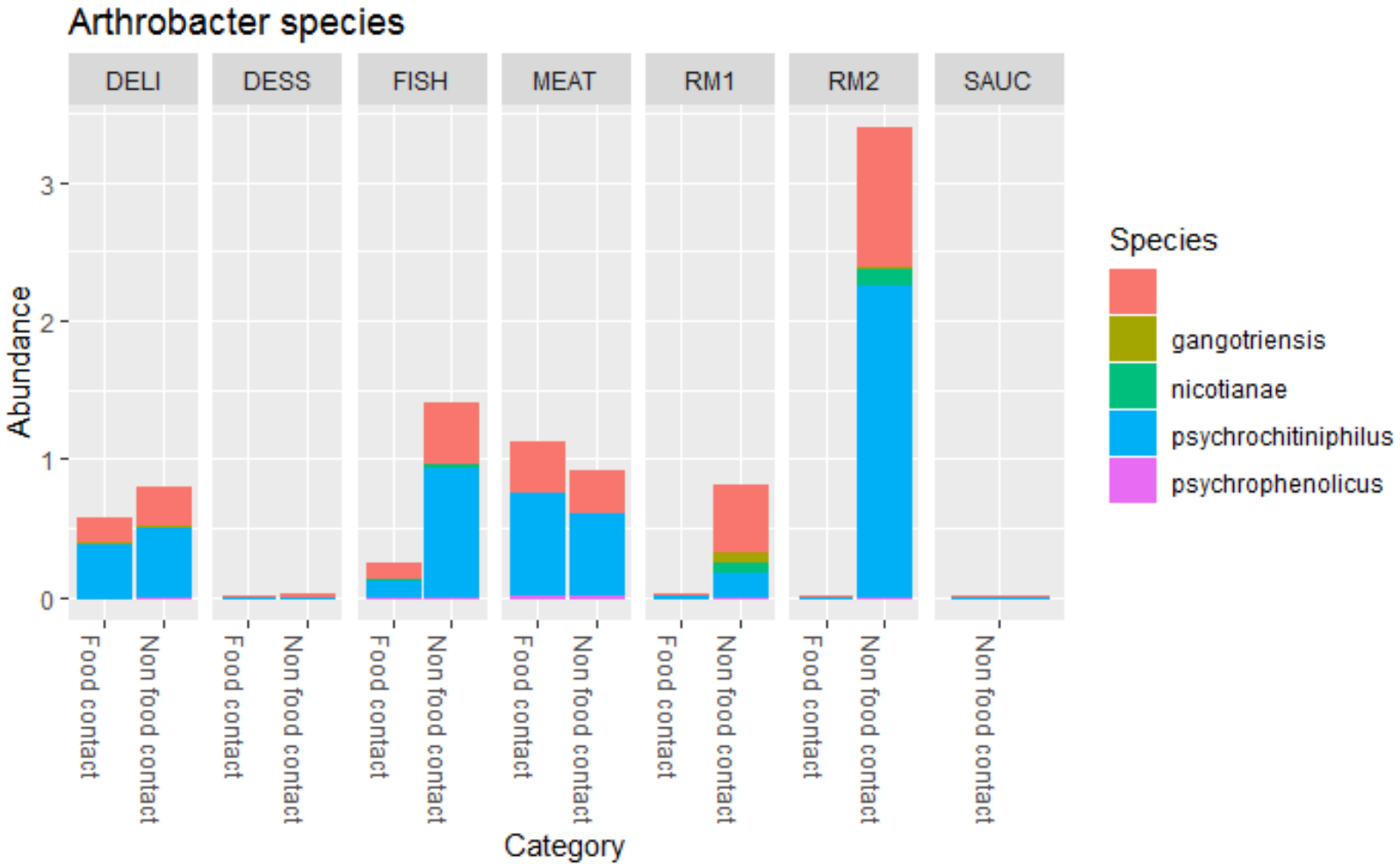
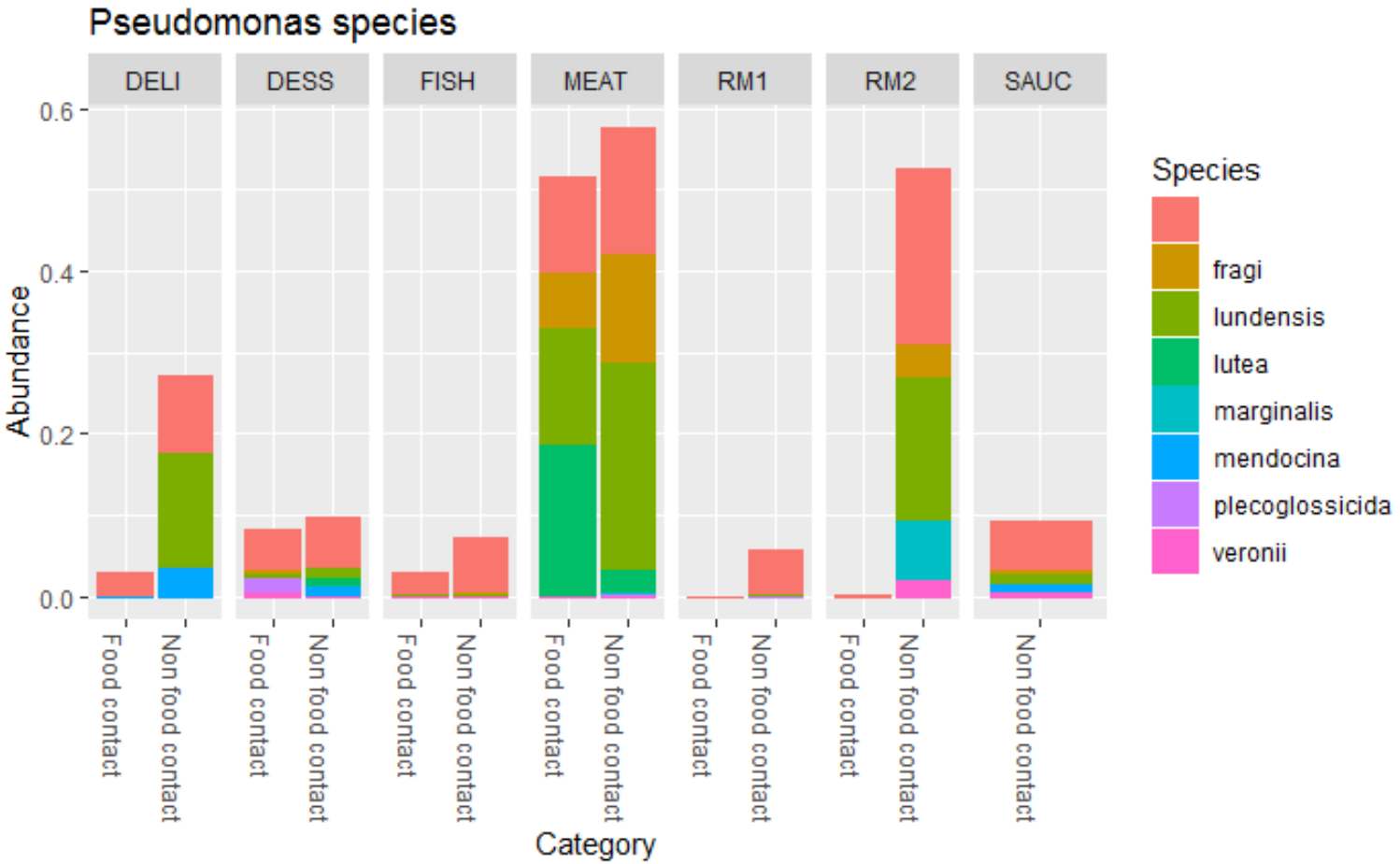
Most abundant genera





- Psychrotrophic bacteria (Actinomycetales) very common in soil, water
- Hygiene indicator organisms in foods



- Species commonly associated with spoilage of meats, milk, vegetables
- Slime formers (*P. lundensis, P. fragi*)

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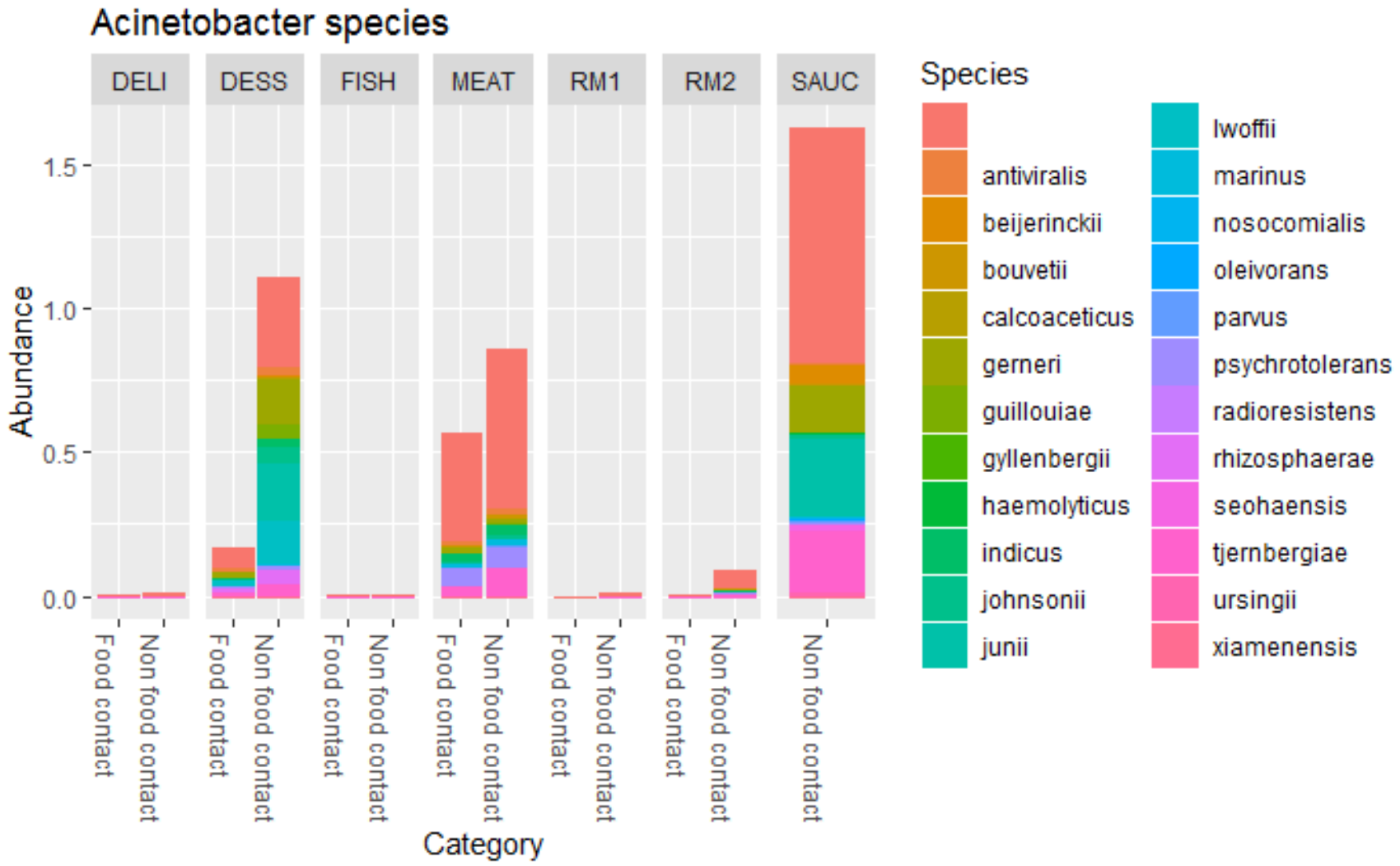

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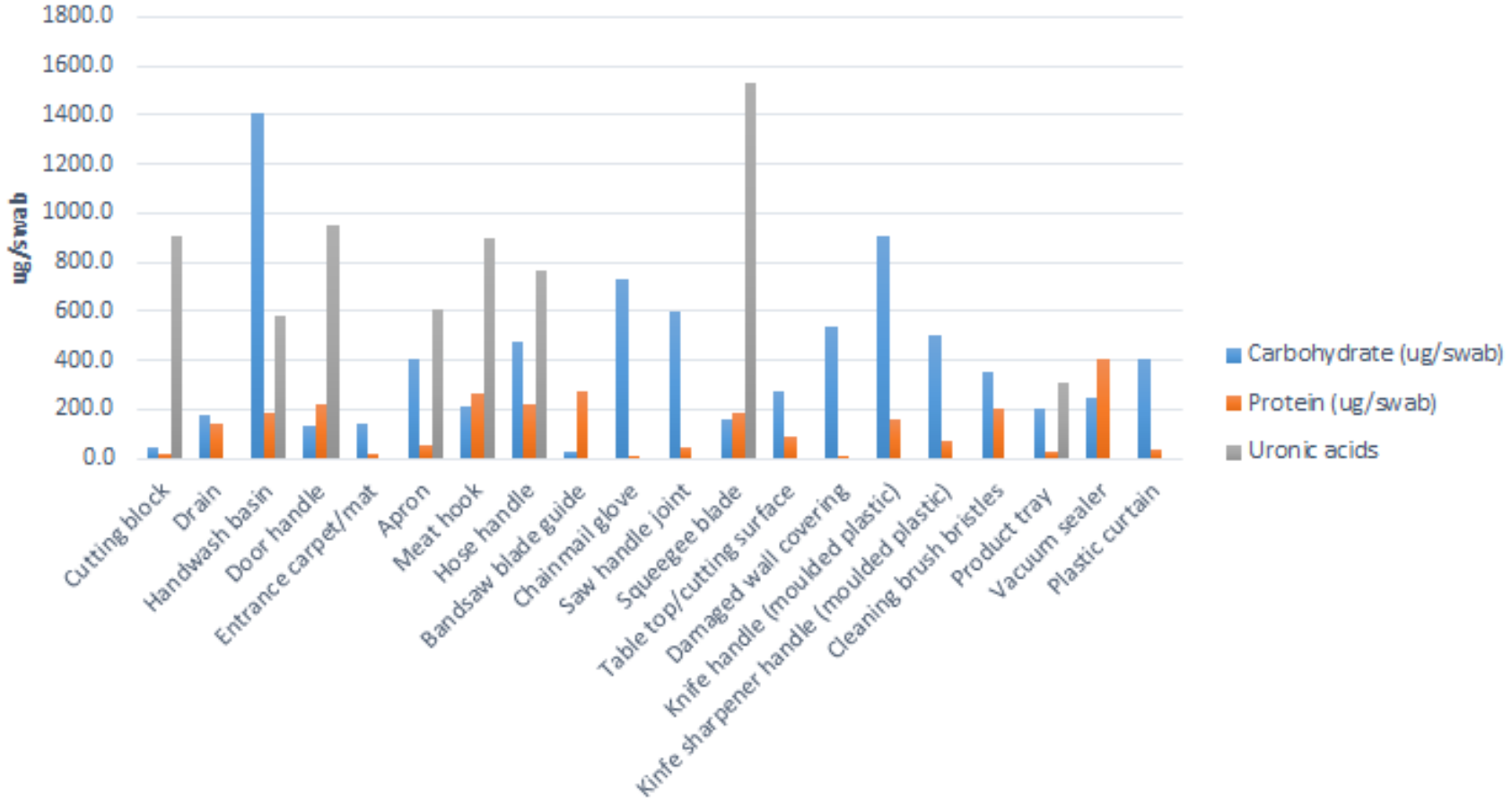
Factory biofilm results

example: raw meat facility

Site	Description	log CFU/swab count	Predominant isolate (MALDI)	Crystal violet assay (biofilm formation)
1	Cutting block	7.54	No ID possible	Weak
2	Drain	8.90	*Microbacterium lacticum*	Medium
3	Handwash basin	8.47	*Pseudomonas koreensis*	Strong
4	Door handle	7.03	*Kocuria rhizophila*	Weak
5	Entrance carpet/mat	6.42	No ID possible	Weak
6	Apron	6.97	*Kocuria salsicia*	Strong
7	Meat hook	6.92	*Pseudomonas lundensis*	Strong
8	Hose handle	6.88	*Kocuria rhizophila*	Strong
9	Bandsaw blade guide	6.12	*Kocuria carniphila*	Weak
10	Chainmail glove	6.99	*Microbacterium maritypicum*	Weak
11	Saw handle joint	7.32	*Kocuria rhizophila*	Strong
12	Squeegee blade	7.81	*Carnobacterium maltaromaticum*	Weak
13	Table top/cutting surface	7.13	*Enterococcus devriesei*	Weak
14	Damaged wall covering	5.95	*Microbacterium maritypicum*	Strong
15	Knife handle (moulded plastic)	7.70	*Pseudomonas lundensis*	Strong
16	Knife sharpener handle (moulded plastic)	7.45	*Dermacoccus nishinomiyaensis*	Weak
17	Cleaning brush bristles	7.00	*Acinetobacter parvus*	Strong
18	Product tray	6.41	No ID possible	Medium
19	Vacuum sealer	7.29	*Kocuria rhizophila*	Weak
20	Plastic curtain	4.76	*Kocuria rhizophila*	Weak

Raw meat facility - Levels of carbohydrate, protein and uronic acids



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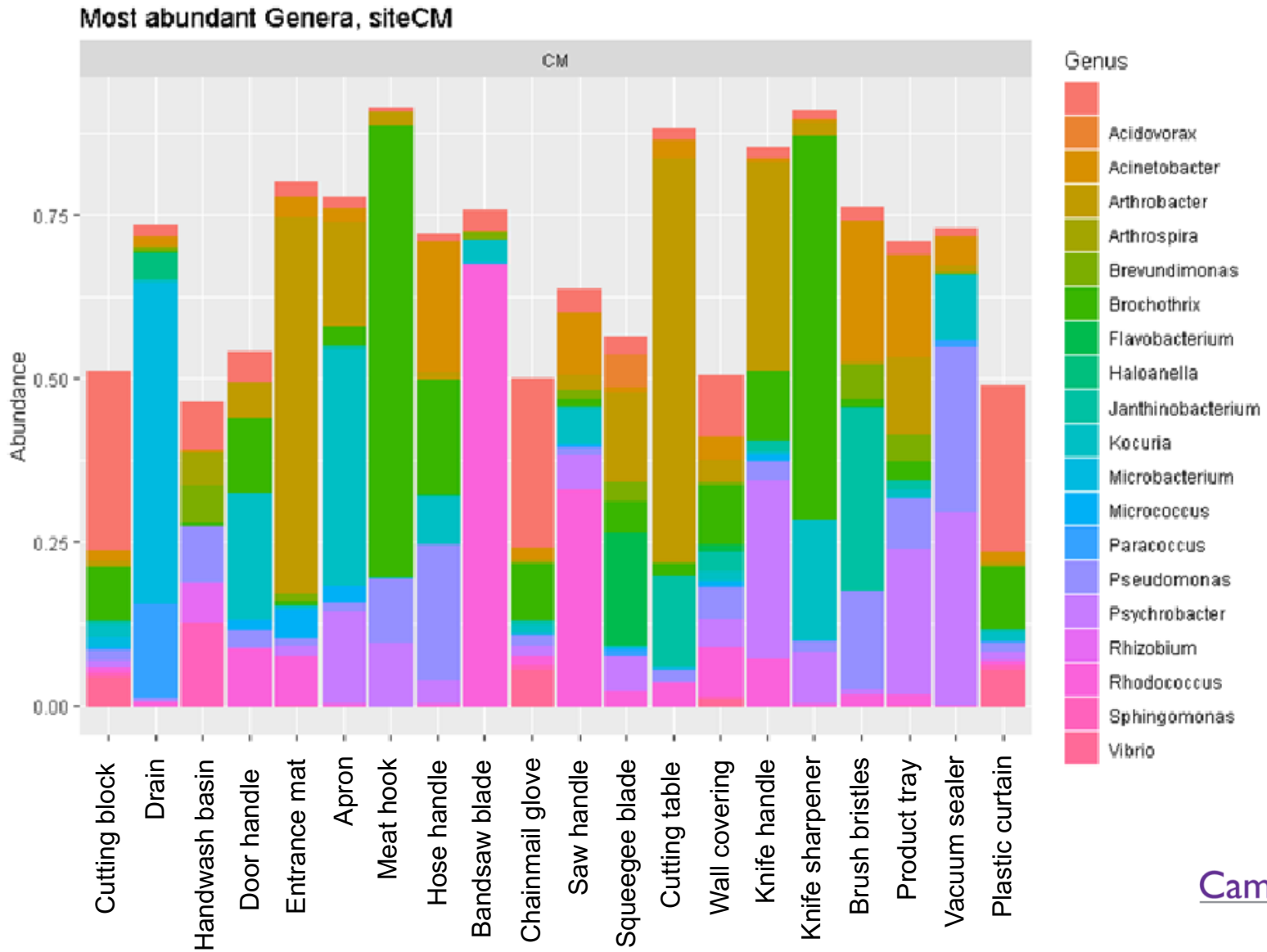
Raw meat facility - metagenomic analysis



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Changes in species composition over time – meat factory

- Meat processing facility re-visited weekly
- 9-week period
- 10 sample points were analysed each week after cleaning.
- Metagenomic profiles generated (Thermo S5)
- Sequences compared against Thermo Scientific curated sequence database.

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Metagenomic analysis

- Thermo Scientific Ion Genestudio S5 Food Protection System
- Thermofisher curated database

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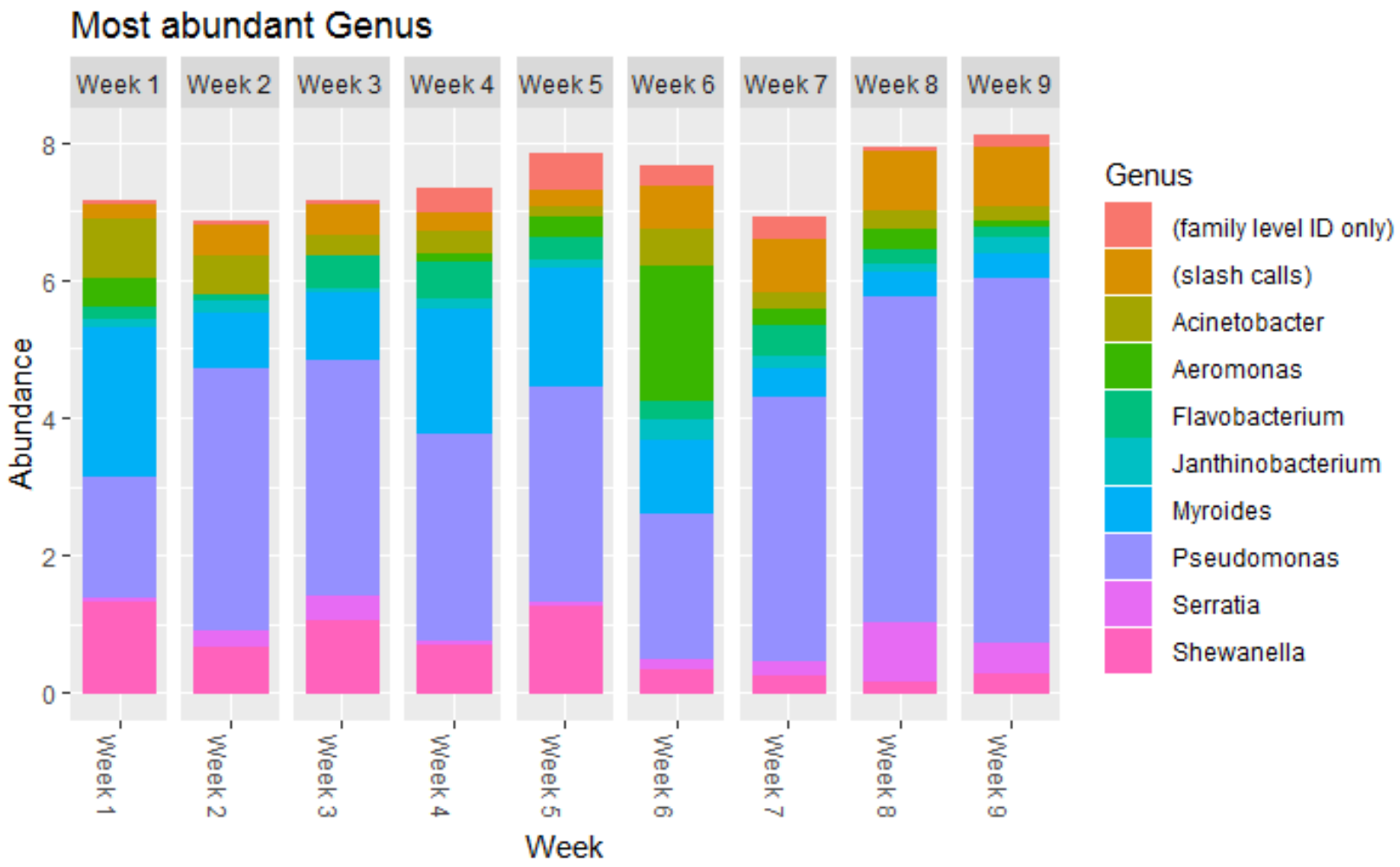

- Relative abundances were fairly stable
- Changes in species composition can be monitored over time

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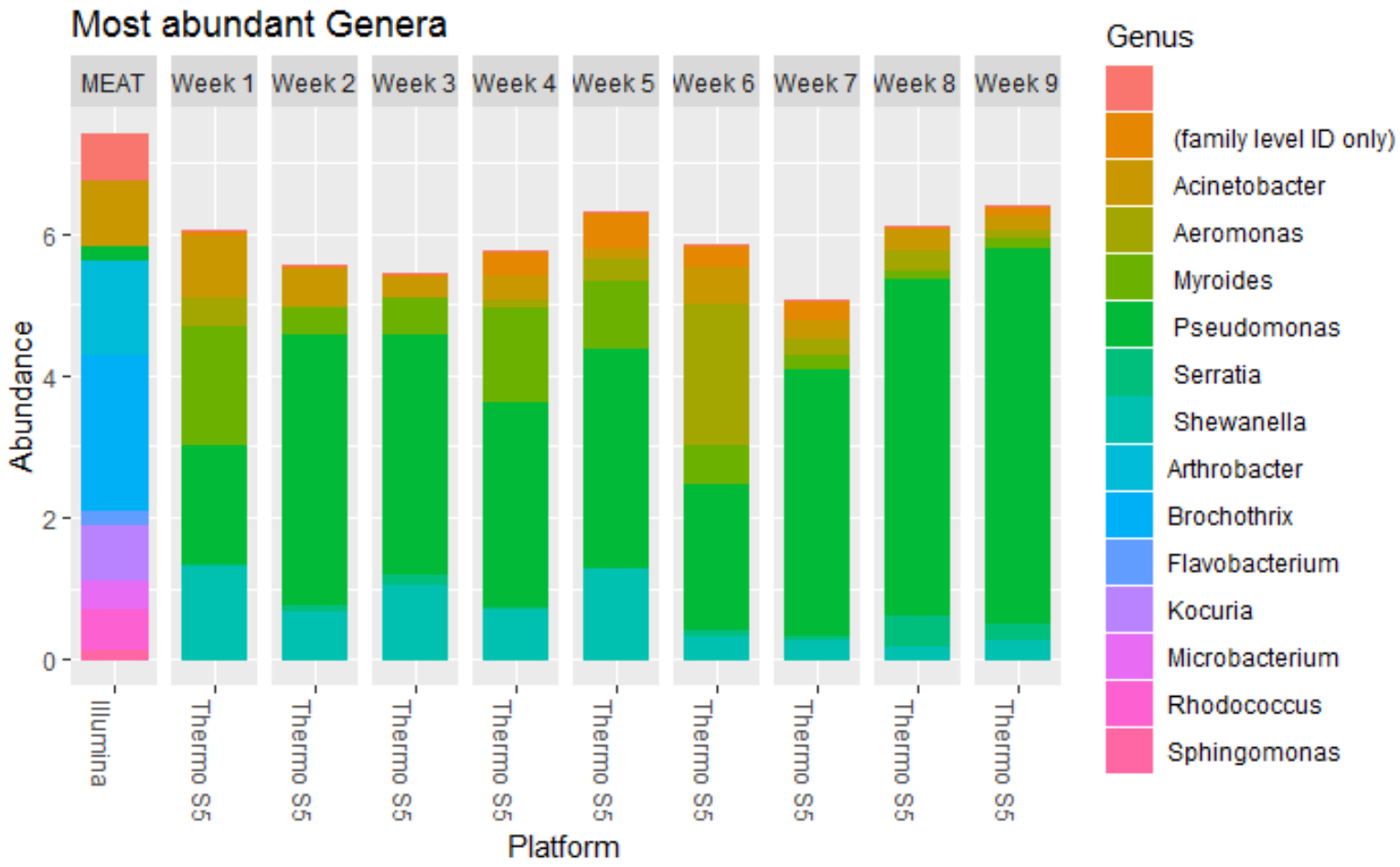
Same factory, different sequencing platforms/databases…



- Very different profiles seen using different platforms for swabs from the same factory
- Differences in databases (curated vs non-curated)?
- Careful interpretation required!

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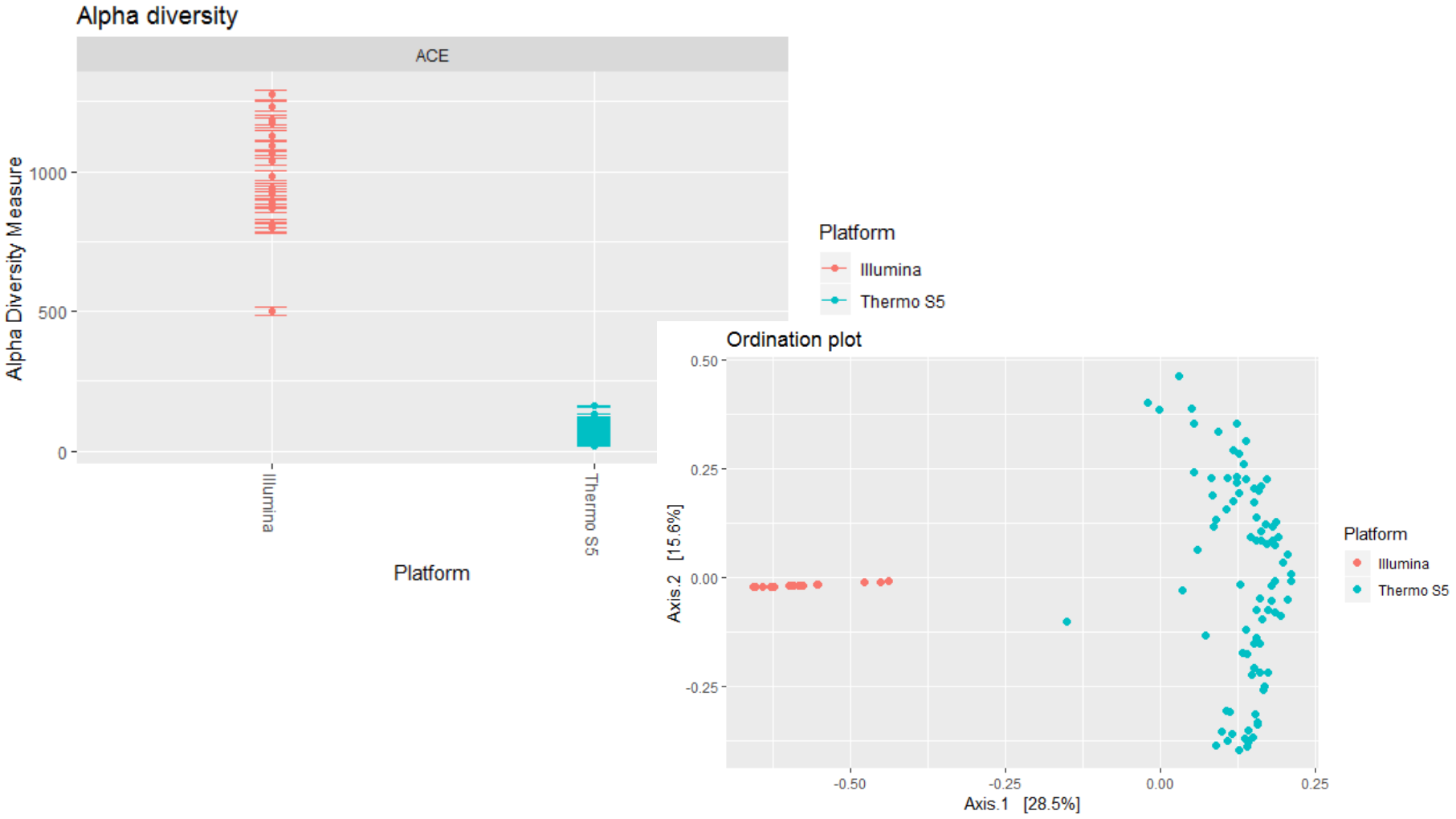
Same factory, different sequencing platforms/databases…



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Summary – biofilm diversity

- Scraper/swab samples **from biofilms** showed high TVC counts post-cleaning in some areas – AFTER standard cleaning/disinfection procedures had been applied.
- ALL biofilm samples assessed were found to be highly diverse (100's of species).
- Many species could not be identified (probably due to short reads/database limitations).
- Metagenomic analysis is a powerful tool to help us understand the microbiome within food factory biofilms and how this affects products.

The Future

- AMP is being used more and more widely in the food industry. For example:
  - Hygiene (including biofilms!)
  - Shelf Life
  - Spoilage Investigations
  - Maturation of fermented products (flavour profile)
  - Air Filters
  - …

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Thanks for listening!

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