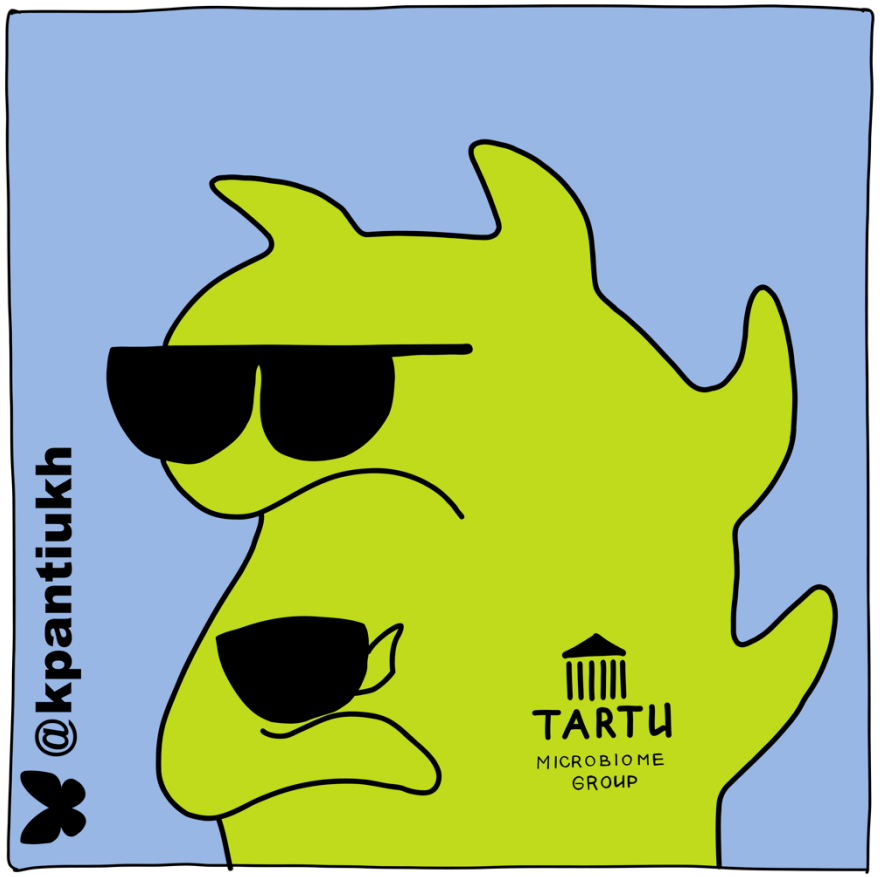

Metagenomics
Lecture 3

MWAS – METAGENOME WIDE ASSOCIATION STUDY

Kateryna Pantiukh

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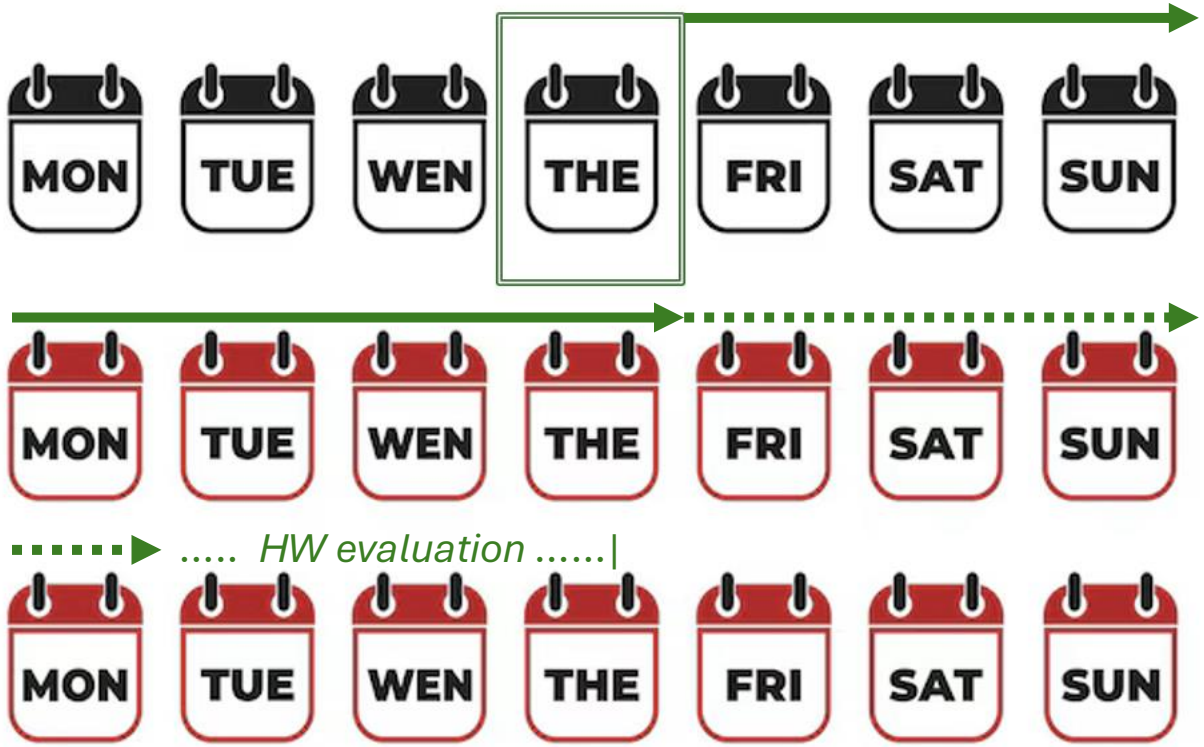
GitHub

Revision of homework deadline



Microbiome

A community of
microorganisms that lives in
a specific environment

- *Bacteria*
- *Archaea*
- *Viruses*
- *Microeucarites*

- Primar degrades
- Primary fermenters
- Secondary fermenters
- Sinks

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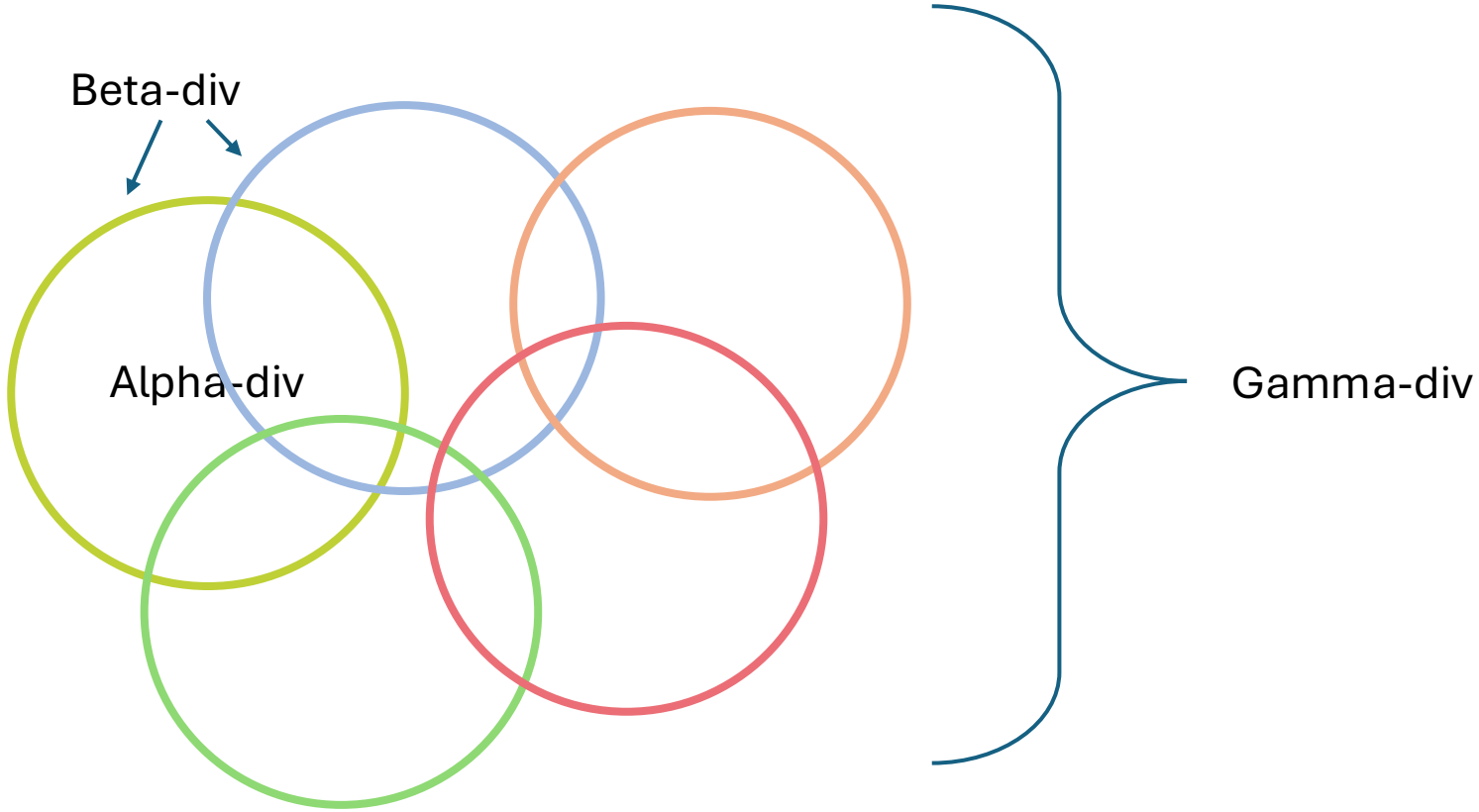
Community may have different level of complexity

… may be evaluated with
Community **diversity indexes**

alpha-, beta-, gamma-

Microbiome



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Community may have different level of complexity

… may be evaluated with
Community **diversity indexes**

alpha-, beta-, gamma-

… the first and simplest measures that can reveal differences between communities

Microbiome

A community of
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*Associations between **community complexity** and the feature of interest*

Microbiome

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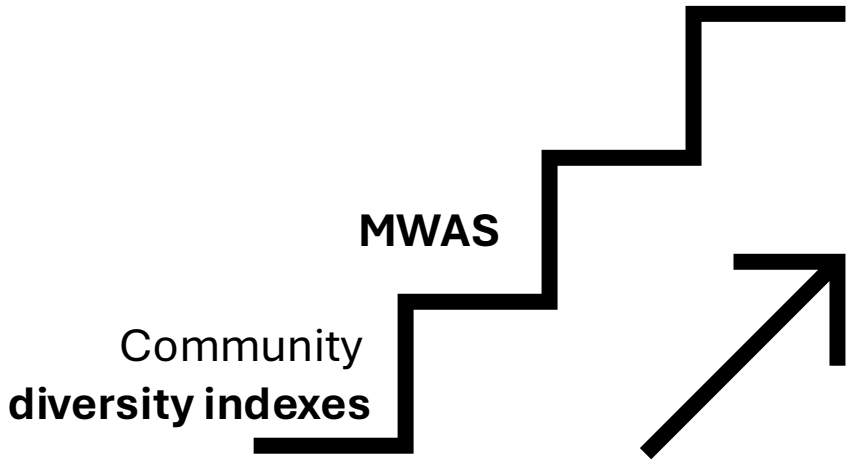
- Sinks

MWAS – microbiome wide association study

*Associations between **specific species***
and the feature of interest



MWAS

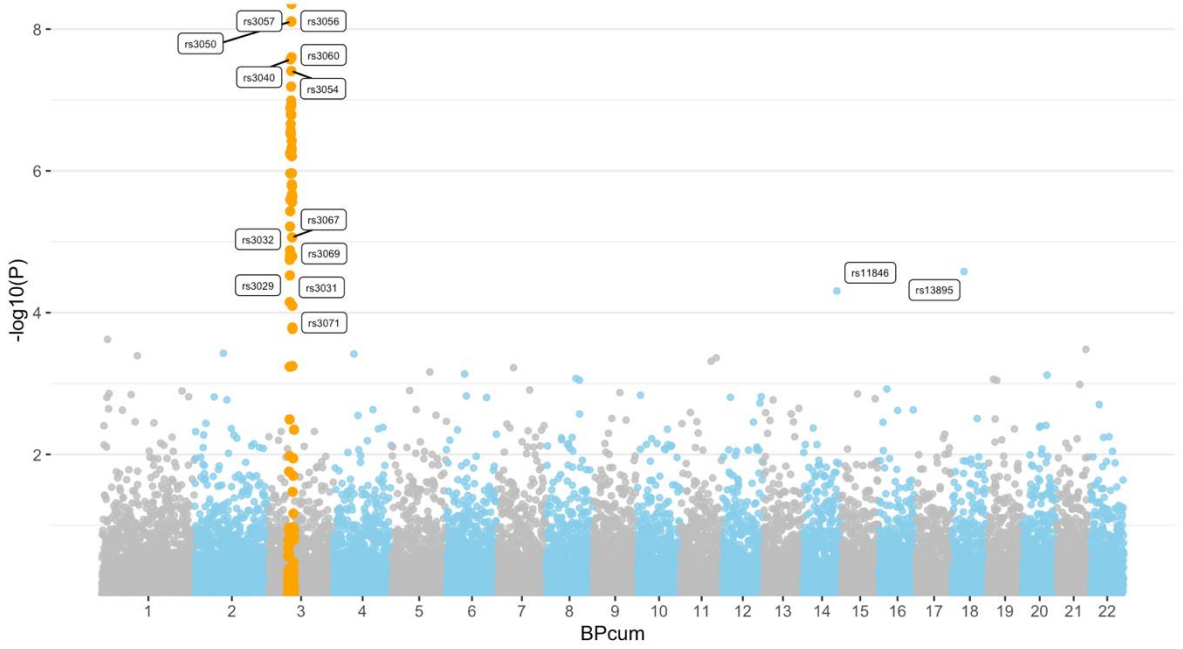
MWAS – microbiome wide association study

GWAS – genome wide association study

*Associations between **specific genome variations** (SNP or indel) and the feature of interest*

Genotyping

Manhattan plot

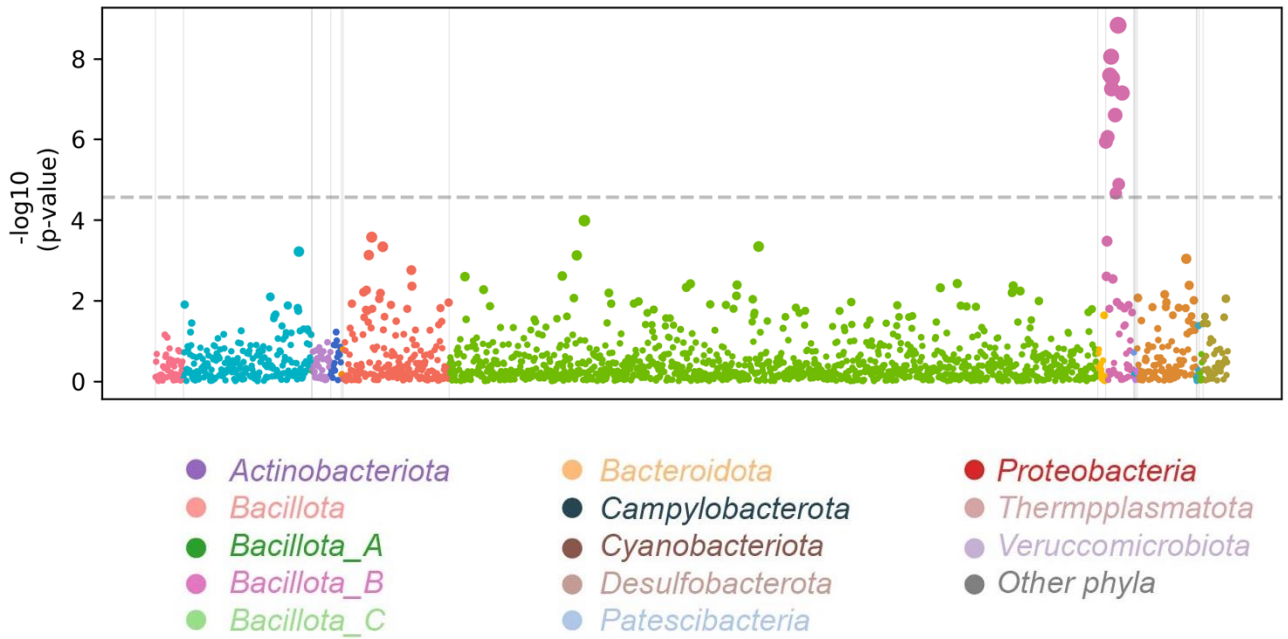


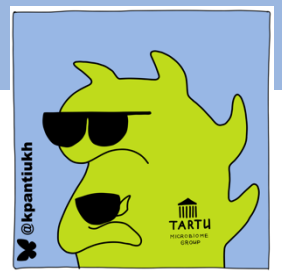
MWAS

MWAS – microbiome wide association study

Bacteria sp. instead of SNP

Manhattan plot





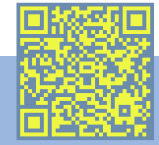
MWAS

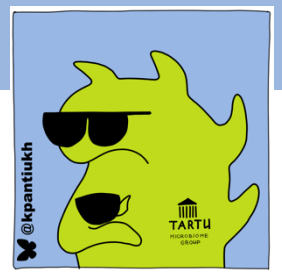
MWAS – microbiome wide association study

$$Y_i = \beta_0 + \beta_1 X_i + \sum_{j=2}^{p} \beta_j C_{ij} + \epsilon_i$$

Where:

- Y_i is the phenotype (e.g., disease status, quantitative trait) for sample i
- X_i is the abundance (or presence/absence) of a microbial feature in sample i
- C_{ij} are covariates (age, sex, sequencing batch, etc.)
- β_0 is the intercept
- β_1 is the effect size of the microbial feature
- ϵ_i is the residual error





MWAS

MWAS – microbiome wide association study

Heart Desease status = Intersept coeficient + ∑(effect of species × abundance of species) + ∑(effect of covariates × covariates) + error

{ *Age, sex, BMI, Stool type* }

MWAS

MWAS – microbiome wide association study

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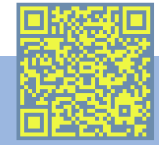
→ !! Correction for multiple testing



MWAS

MWAS – microbiome wide association study

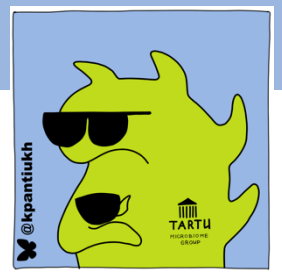
!! Correction for multiple testing

Bonferoni correction

Significance level / number of tests

0.05 / 3 = 0.0166

Corrected significance level = 0,0166



MWAS

MWAS – microbiome wide association study

Heart Desease status = Intersept coeficient + ∑(effect of species × abundance of species) + ∑(effect of covariates × covariates) + error

Age, sex, BMI, Stool type

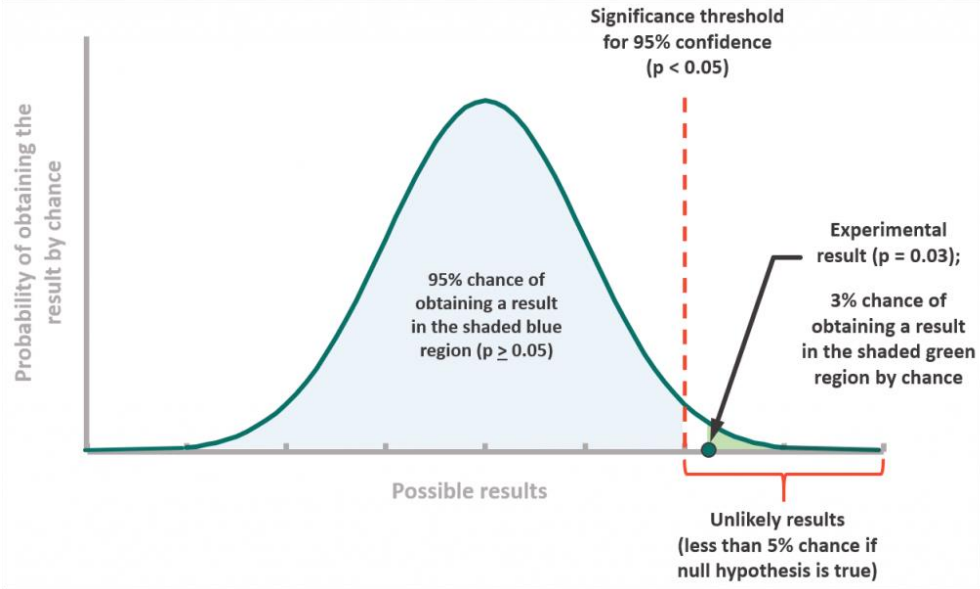
Main outcome: p-value and beta coeficient

p-value

MWAS – microbiome wide association study

null hypothesis: the true effect is zero

The p-value is the probability of observing a result **at least as extreme as the one you got**, assuming that null hypothesis is true.



p-value

MWAS – microbiome wide association study

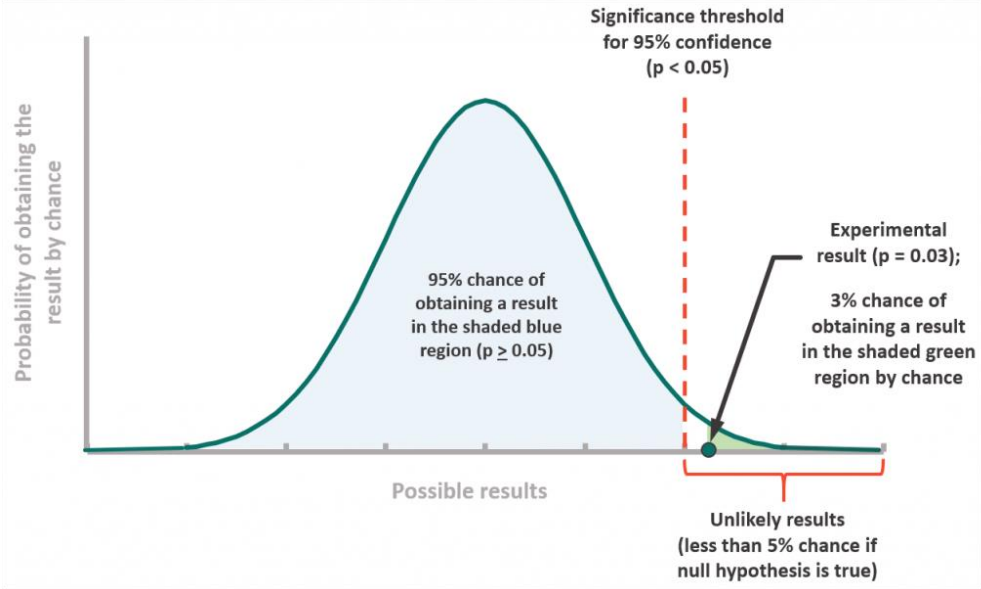
What it tells you:

- A **small p-value** means your result would be unlikely if the effect were truly zero.
- A **large p-value** means your data are quite compatible with no effect.

What it does *not* tell you:

- It is **not** a measure of effect size or importance!!!

* - Effect size - beta



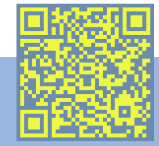
MWAS

MWAS – microbiome wide association study

If p-value < level of significance
We consider the association
Statistically significant

- Positive betta – positive correlation
- Negative betta – negative correlation

pheno	name	bacteria	p-value	betta
N97	Female infertility	A0002_Methanobrevibacter_A_smithii_A	8,53E-06	0,00073
N97	Female infertility	H0023_Alistipes_communis	2,84E-07	0,003378
K21	Gastro-esophageal reflux disease	H0092_CAG-41_sp900066215	2,09E-06	0,000291
M13	Other arthritis	H0117_Scatosoma_sp900555925	9,53E-07	0,001968
H40	Glaucoma	H0220_Ruminiclostridium_E_sp900539195	7,09E-06	0,001756
G43	Migraine	H0224_Merdimorpha_sp002314265	1,43E-06	0,000376
I48	Atrial fibrillation and flutter	H0237_Dysosmobacter_welbionis	1,58E-06	0,000956
M13	Other arthritis	H0262_UMGS692_sp900544545	8,77E-06	0,006968
F41	Other anxiety disorders	H0280_Enterocloster_sp000431375	2,32E-06	0,000527



MWAS

MWAS – microbiome wide association study

level of significance = 5 * 10-6

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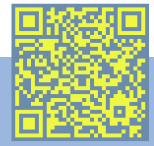
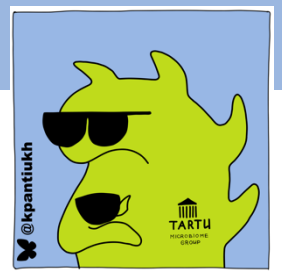
Alistipes putredinis is positively associated with female infertility status, indicating higher relative abundance in diagnosed individuals compared with controls





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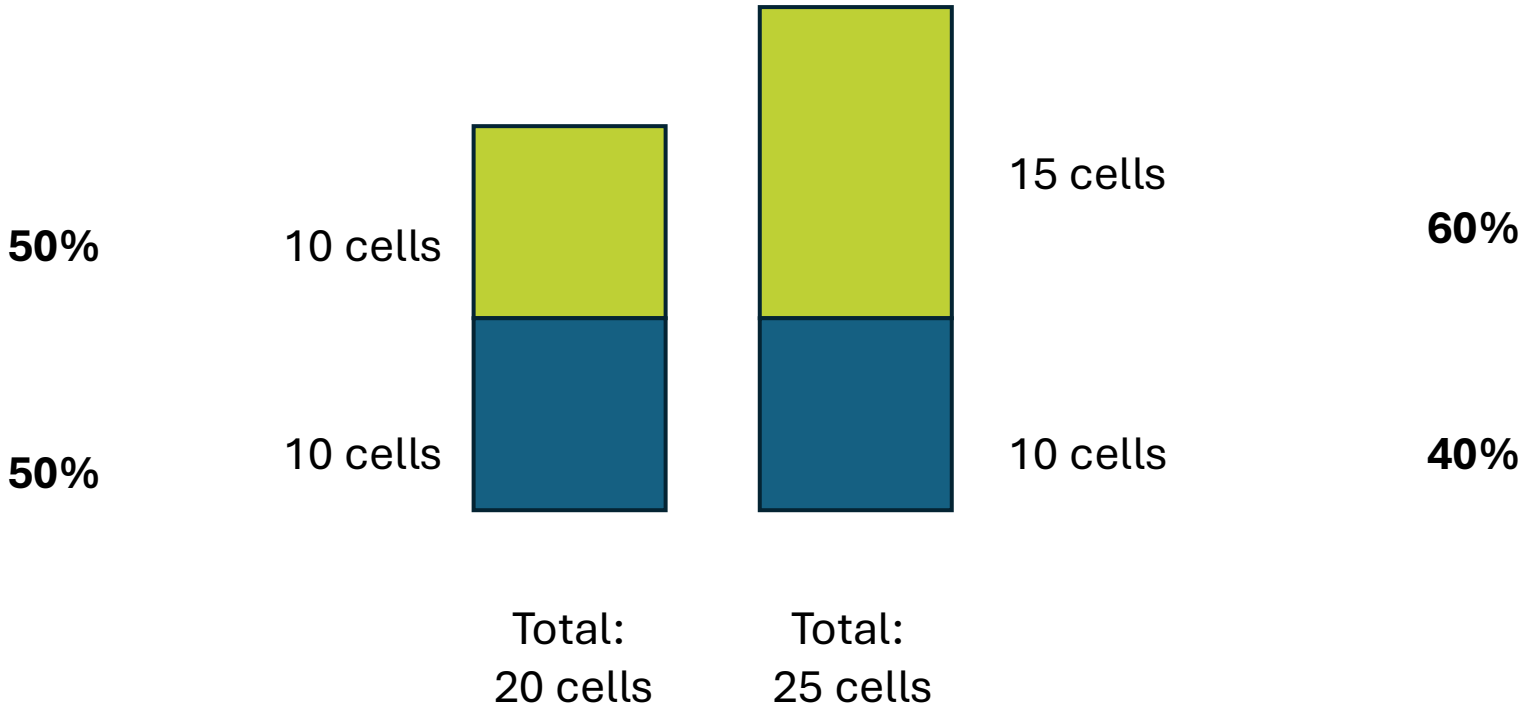
Alistipes putredinis is positively associated with female infertility status, indicating higher relative abundance in diagnosed individuals compared with controls, however the estimated effect size is small

Abundance metrics

relative abundance PROBLEM



MWAS

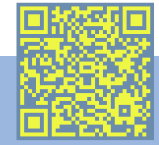
MWAS – microbiome wide association study

INPUT:
relative abundance

	Tom	Mary
Species 1	0.1	3.7
Species 2	0.0	0.2
Species 3	2.3	0.0

INPUT:
presence-absence

	Tom	Mary
Species 1	1	1
Species 2	0	1
Species 3	1	0

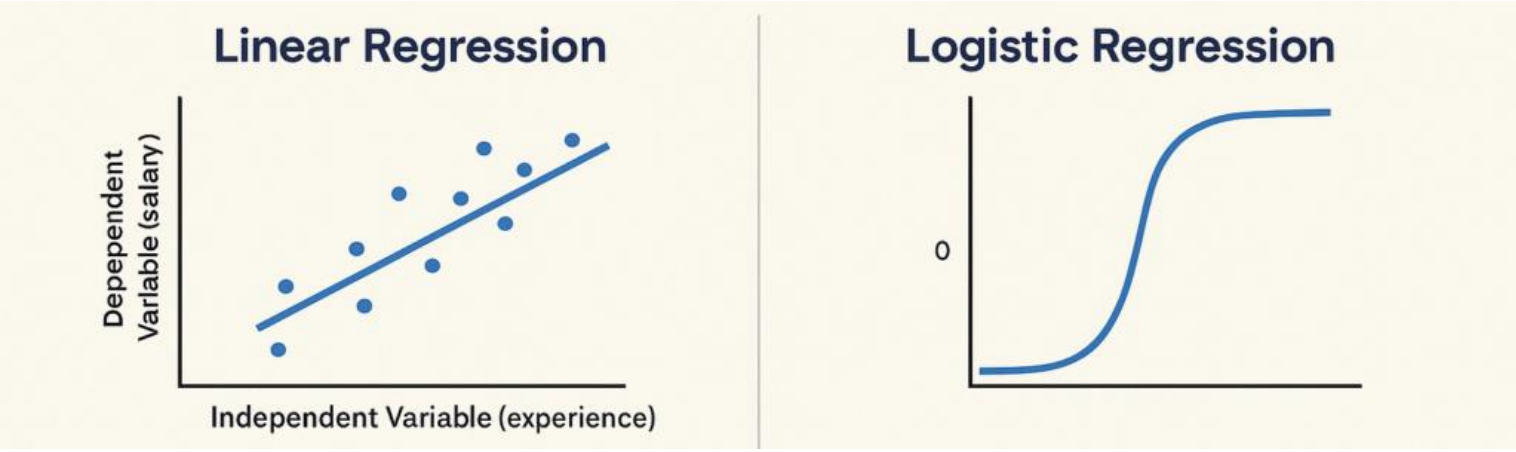


MWAS

MWAS – microbiome wide association study

INPUT:
relative abundance

INPUT:
presence-absence



Effect size - odds ratios

MWAS – microbiome wide association study

Example:

- Predictor: Alistipes putredinis present vs absent
- Outcome: Female infertility
- Logistic regression gives OR = 2.0

Interpretation:

Individuals with Alistipes putredinis present have twice the odds of being diagnosed with female infertility compared to individuals without this bacterium, holding other variables constant.

If OR = 0.5, the interpretation flips:
Individuals with Alistipes have half the odds of infertility compared to those without it.

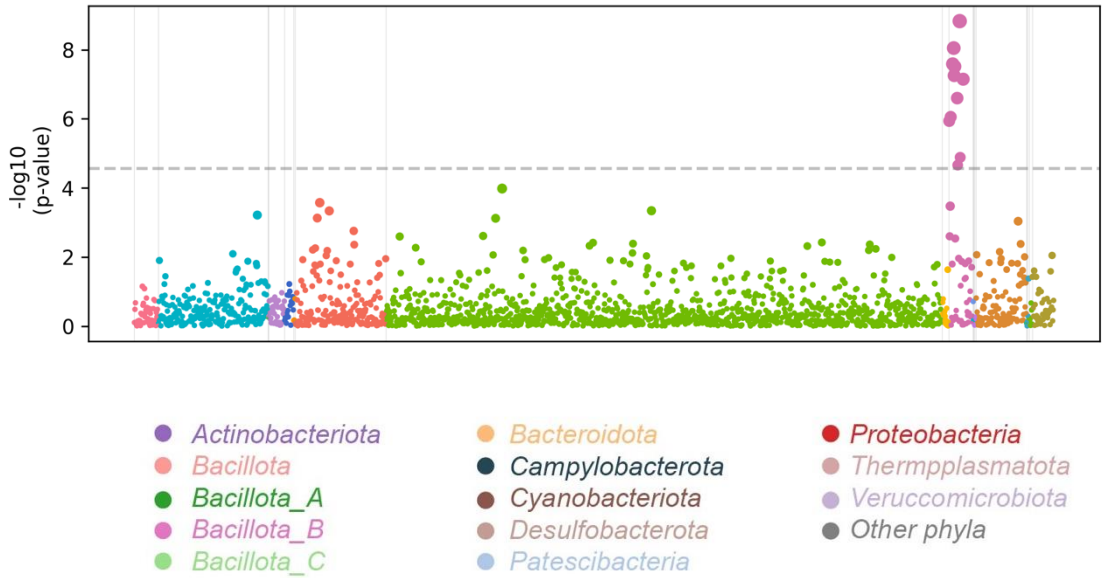
MWAS

Visualisation example

Linear regression

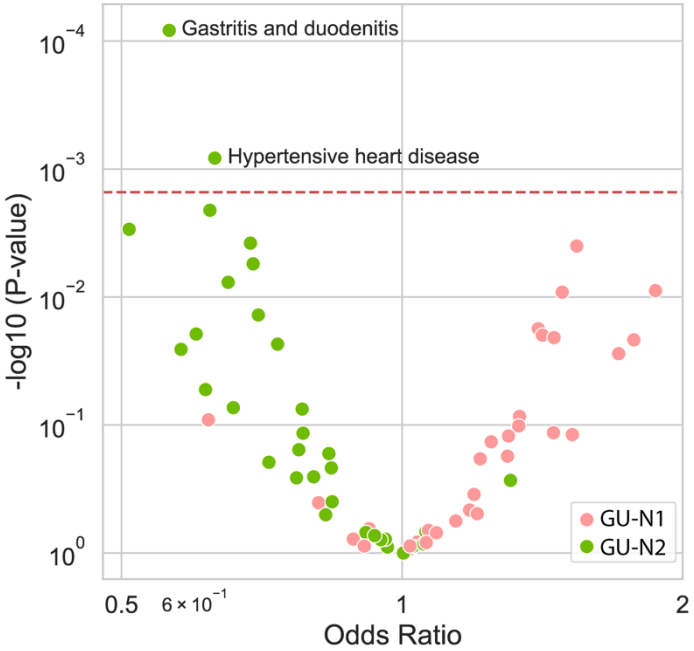
(relative abundance input)

Manhattan plot



Logistic regression

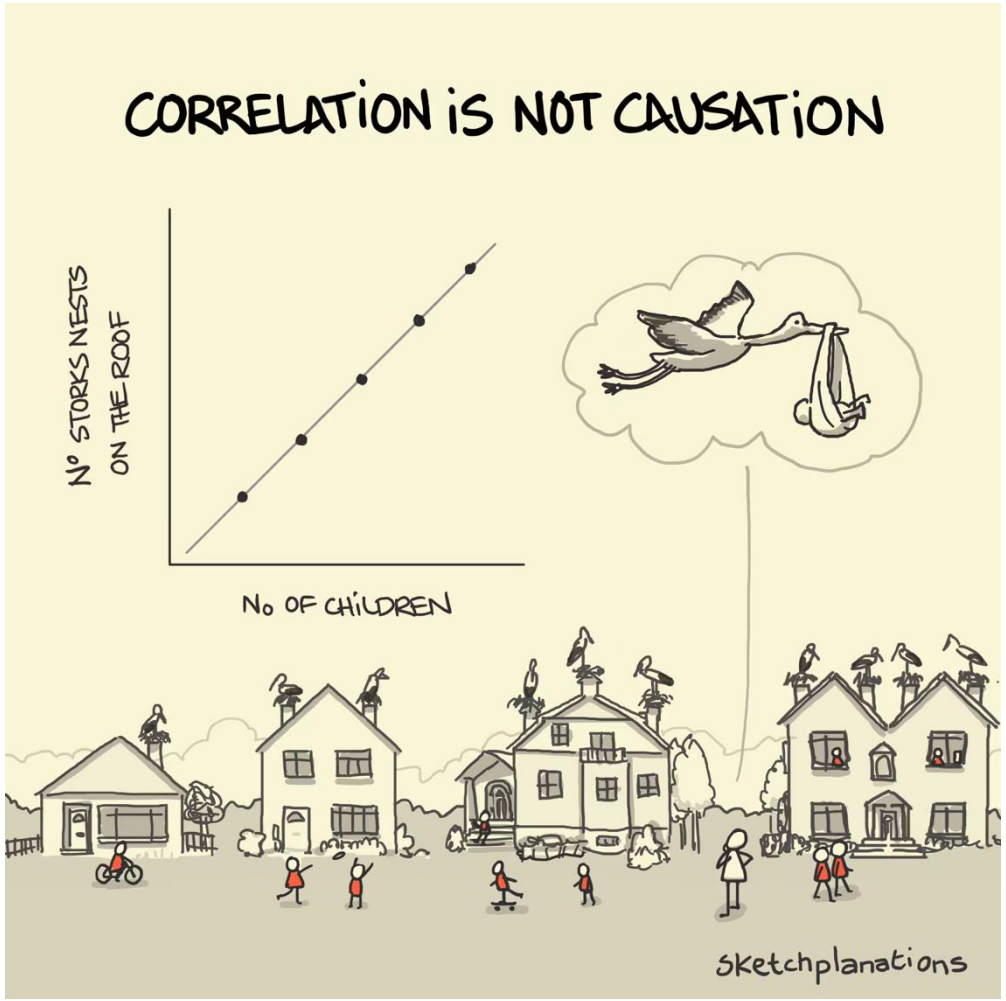
(presence-absence input)



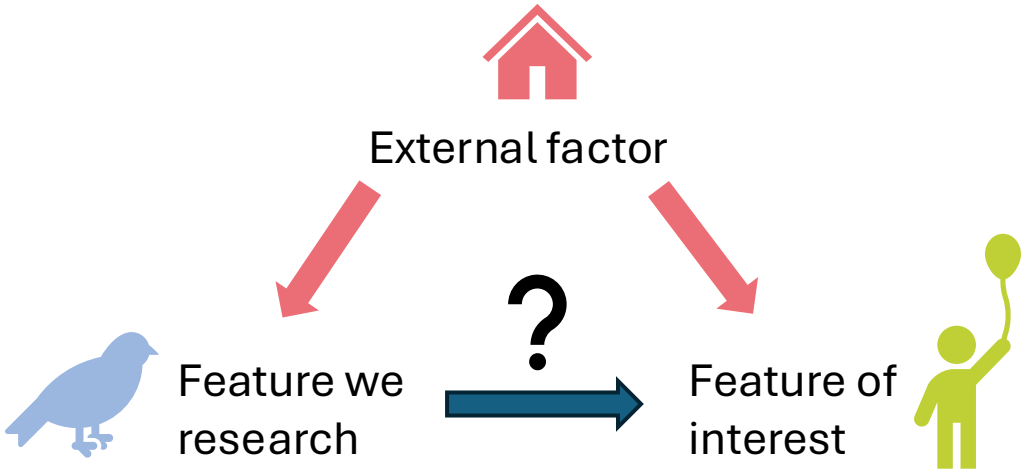
Causation issue

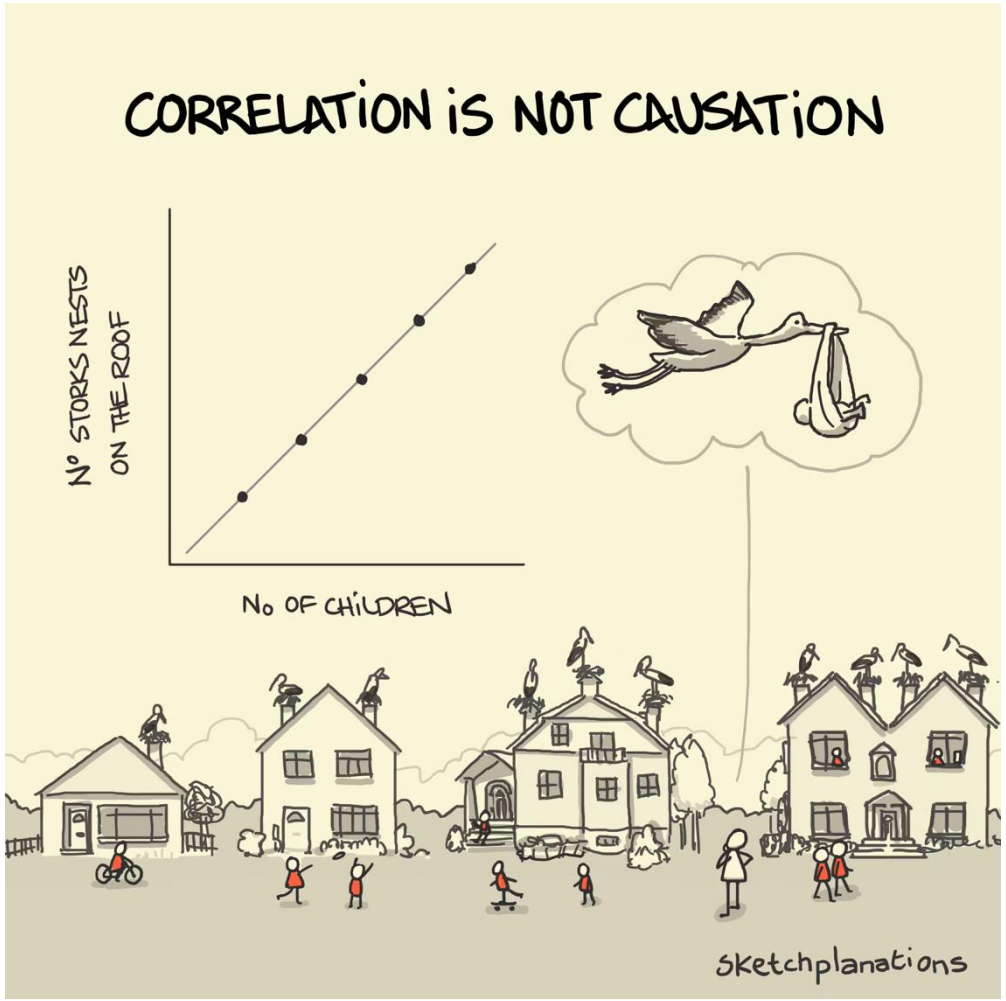
Correlation != Causation

Causation issue

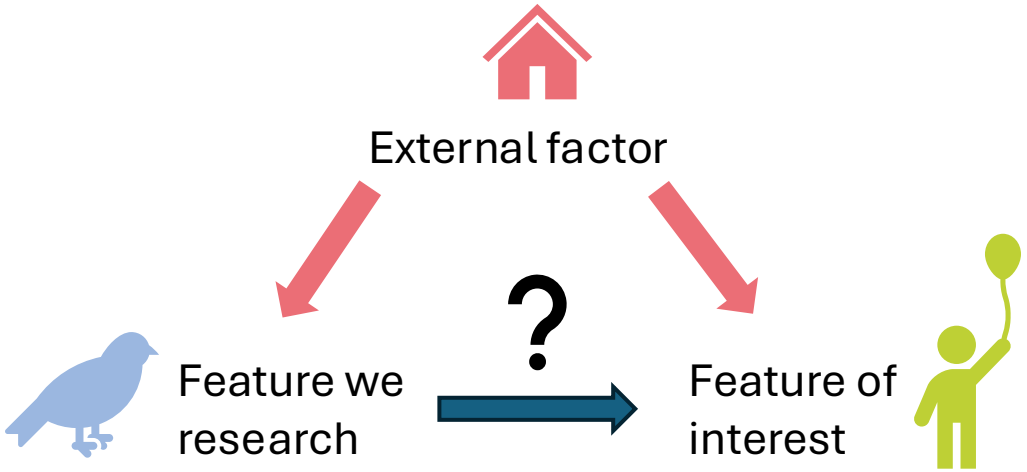


Causation issue





Causation issue



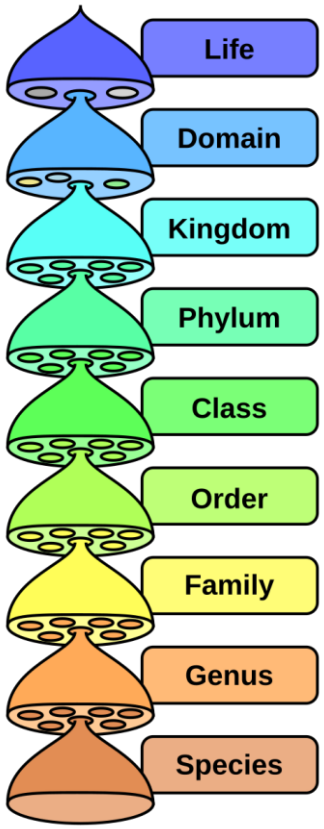
WHAT WE CAN DO ABOUT IT?

1. Even whe causation questionable, correlation may be use as a predictor

Usefull for early diagnosis

2. We can design additional experiments to check causeation

Different level abundance tables



	Tom	Mary
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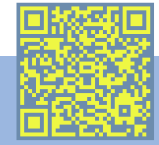
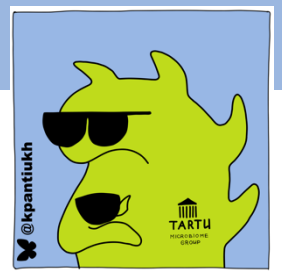
	Tom	Mary
Species 1	0.1	3.7
Species 2	0.0	0.2
Species 3	2.3	0.0

Low dimensionality → fewer multiple-testing problems.

Species & strain level: can link associations to particular functions or pathogenic potential.

BUT: group size metters!





How to decide what taxonomic level to use?

1.Cohort size and statistical power

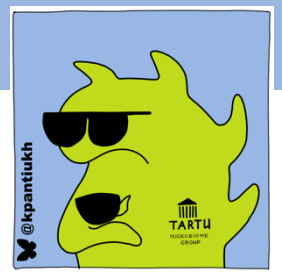
- •species/strain have many rare or zero-count taxa.
- •Smaller cohorts may not provide enough observations per taxon to detect associations reliably.
- •Broader levels (phylum, class, family) aggregate taxa, increasing counts and power but may hide specific effects.

2.Expected prevalence of taxa

- •Rare taxa are often absent in many samples.
- •Presence/absence models can work for very rare taxa, but effect size estimates become unstable.
- •Focus on taxa that occur in a meaningful fraction of samples (e.g., >10–20% prevalence).
- **Tip:** Check prevalence at different levels before deciding; sometimes grouping into higher levels improves coverage.

3.Biological interpretability

- •Broad levels show general trends (e.g., Bacteroidetes increase) but often lack actionable insights.
- •Genus or species level allows linking findings to metabolic pathways, pathogenicity, or prior literature.
- •Strain-level associations are most informative for functional or mechanistic hypotheses but require high-resolution sequencing.



How to decide what taxonomic level to use?

4.Multiple testing burden

- •Species/strain levels increase the number of tests, requiring stricter p-value correction and reducing statistical power.
- •Consider pre-filtering low-abundance taxa or focusing on taxa with prior evidence to reduce false negatives.

5.Sequencing depth and MAGs aviability

- •Low-depth sequencing may not resolve species or strains reliably.
- •High-resolution analysis is only meaningful if the data can support it; otherwise, stick to genus/family.

6.Hierarchical approach

- •Start broad to detect global shifts, then zoom in to finer levels for taxa showing signals.
- •This balances power, interpretability, and control of multiple testing.

How to decide what taxonomic level to use?

It's about finding the right balance and testing many different options before discovering what works

- Small cohort, rare taxa, low sequencing depth → use higher taxonomic levels (family/genus).
- Large cohort, common taxa, high-resolution data → species or strain level can be explored.
- Always consider prevalence, expected group size, and interpretability.

Preparing an abundance table

1. Filter low-abundance and rare taxa

   - Remove taxa that are present in very few samples (<1% prevalence)
   - Remove taxa with extremely low relative abundance (not popular)

   This reduces sparsity, improves statistical power, and decreases the multiple-testing burden.

2. Handle zeros & Compositional transformation

   - Zero counts are common in microbiome data.
   - Perform CLR transformations

Bacterial species
main characteristics

Ecological niche / lifestyle

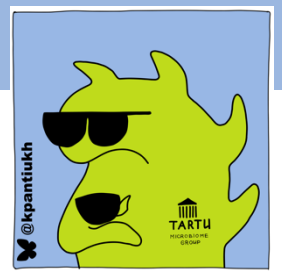
Functional traits / metabolism

Genomic

Morphology and structural features

Interaction with other microbes

Clinical or industrial relevance



Bacterial species
main characteristics

Ecological niche / lifestyle

Functional traits / metabolism

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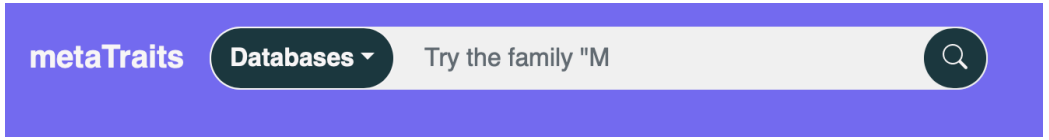
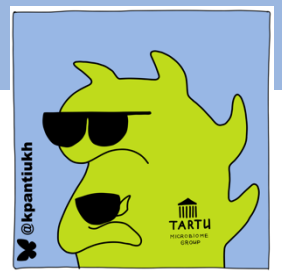
Interaction with other microbes

Clinical or industrial relevance

- **Habitat:** gut, oral cavity, soil, water, skin, etc.
- **Host association:** commensal, symbiont, opportunistic pathogen, obligate pathogen.
- **Temperature preference:** psychrophile, mesophile, thermophile.
- **pH tolerance** and other environmental tolerances.

https://metatraits.embl.de





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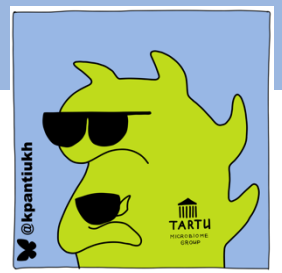
Interaction with other microbes

Clinical or industrial relevance

- **Carbon source utilization:** sugars, proteins, lipids.
- **Energy generation:** respiration, fermentation, photosynthesis, chemolithotrophy.
- **Nitrogen/sulfur cycling capabilities:** nitrate reduction, sulfate reduction, ammonia oxidation.
- **Secondary metabolite production:** antibiotics, bacteriocins, signaling molecules.



Bacterial species
main characteristics

Ecological niche / lifestyle

Functional traits / metabolism

Genomic

Morphology and structural features

Interaction with other microbes

Clinical or industrial relevance

- **Genome size** and GC content.
- **Plasmid presence** or mobile genetic elements.
- **Virulence genes** or toxin production.
- **Antibiotic resistance genes**.

https://gtdb.ecogenomic.org

Bacterial species
main characteristics

Ecological niche / lifestyle

Functional traits / metabolism

Genomic

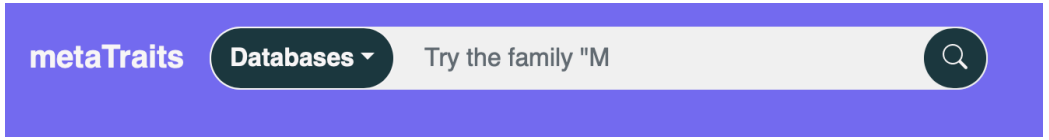
Morphology and structural features

Interaction with other microbes

Clinical or industrial relevance

- **Cell shape:** cocci, rods, spirals.
- **Motility structures:** flagella, pili.
- **Surface structures:** capsule, S-layer, biofilm-forming ability.
- **Sporulation ability.**

https://metatraits.embl.de



Bacterial species
main characteristics

Ecological niche / lifestyle

Functional traits / metabolism

Genomic

Morphology and structural features

Interaction with other microbes

Clinical or industrial relevance

- **Symbiosis or antagonism:** production of inhibitory compounds, mutualistic relationships.
- **Biofilm formation:** ability to form communities on surfaces.
- **Quorum sensing / communication:** signaling mechanisms.

https://pubmed.ncbi.nlm.nih.gov

https://www.biorxiv.org

Bacterial species
main characteristics

Ecological niche / lifestyle

Functional traits / metabolism

Genomic

Morphology and structural features

Interaction with other microbes

Clinical or industrial relevance

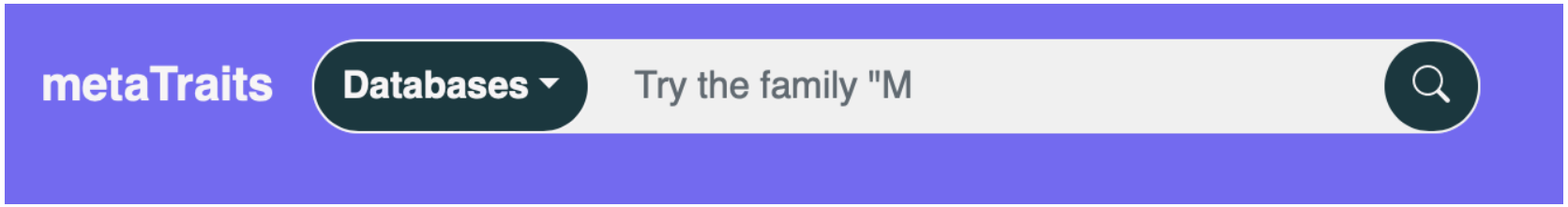
- Pathogenicity to humans, animals, or plants.
- Probiotic potential.
- Industrial applications: fermentation, bioremediation, enzyme production.

https://pubmed.ncbi.nlm.nih.gov

https://www.biorxiv.org

MetaTraits demo

https://metatraits.embl.de



GTDB demo

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