



Metabolic complementarity applied to the screening of microbiota and the identification of key species

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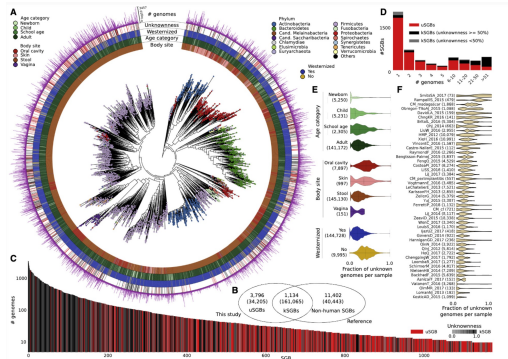


2021 **Metabolic
Pathway
Analysis:
The Frontiers**

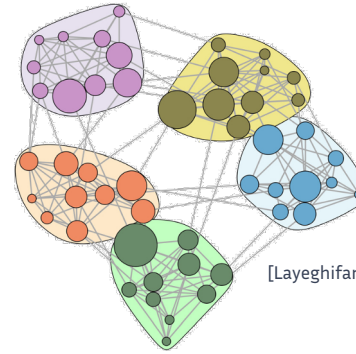
August 2, 2021



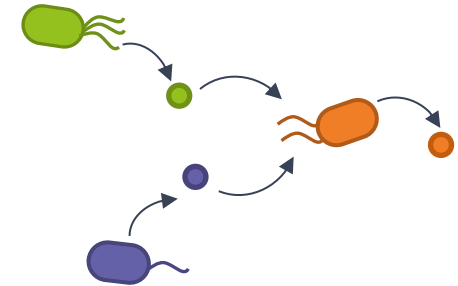
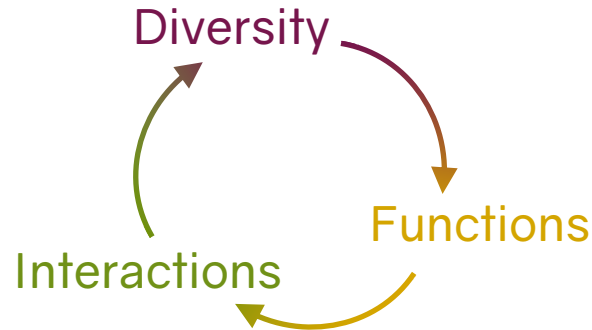
An era of microbiomes



[Pasolli et al 2019]



[Layeghifard et al 2017]

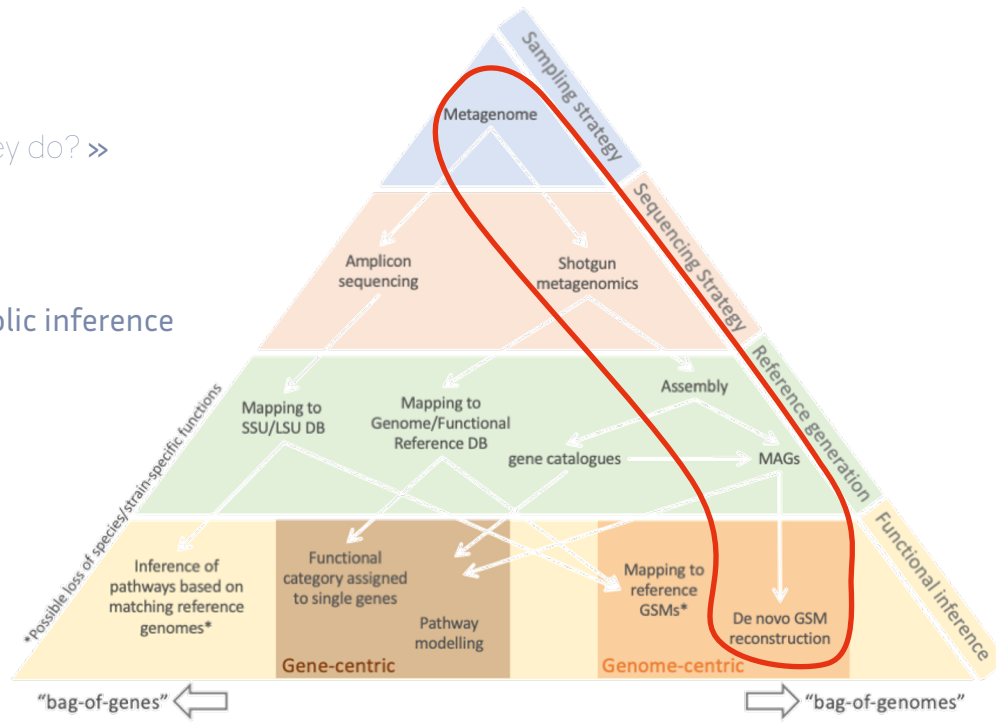


Identification of microbial interactions from metagenomic data is key to better understand complex ecosystems

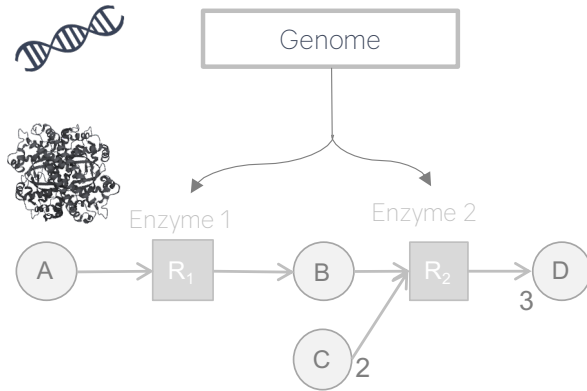
Metagenomics meets metabolism

- From « who is there? » to « what might they do? »
- Inferring functions in metagenomes
- Genome-centric approaches enable metabolic inference

Combine genome reconstruction and metabolic modelling to unravel the functional repertoire and community organisation of uncultivated species



Metabolic networks and communities

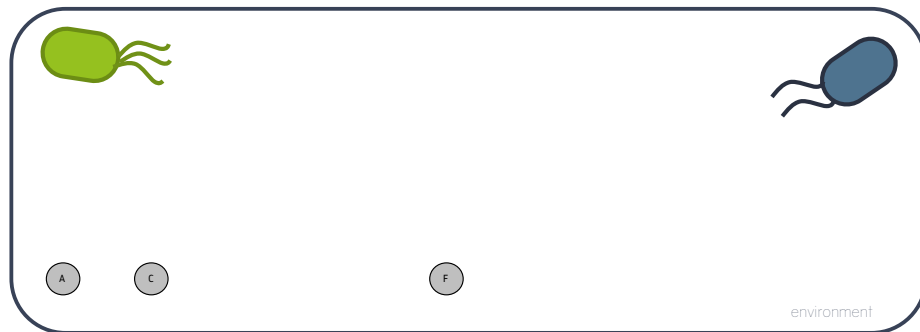
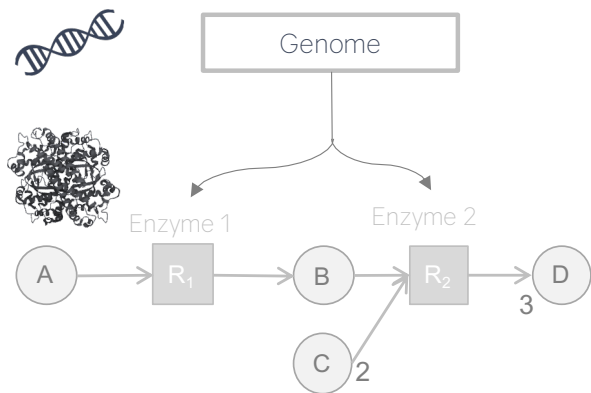


1 genome → 1,000 to 5,000 reactions

Genome-scale metabolic network (GSMN)

Functional repertoire of the cell

Metabolic networks and communities

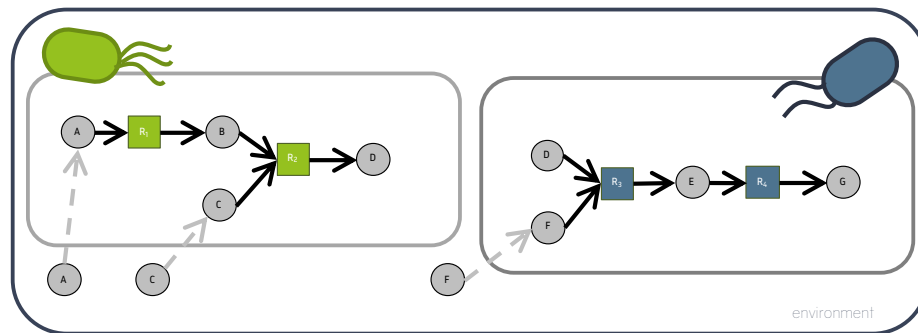
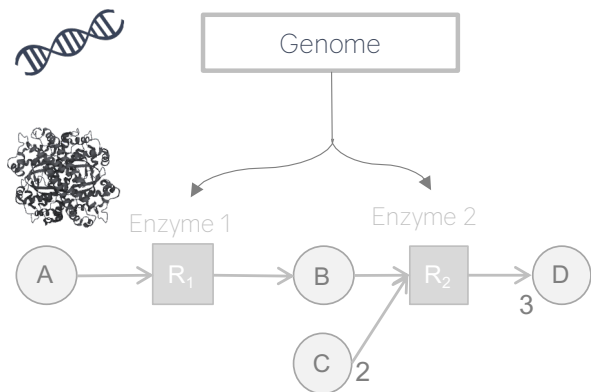


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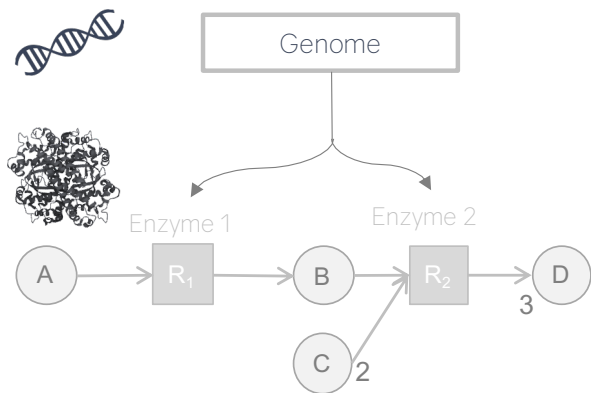
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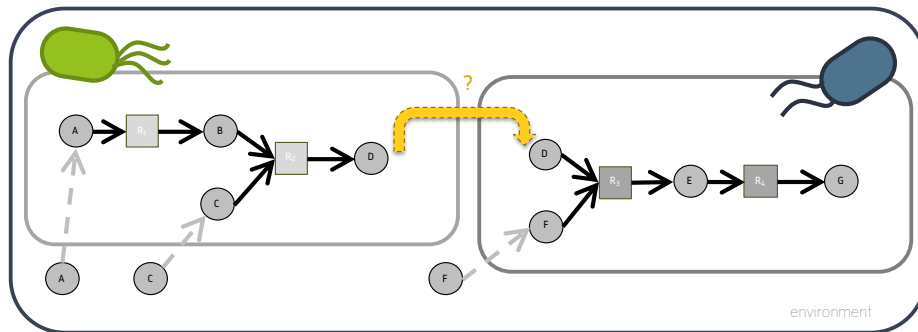
Metabolic networks and communities



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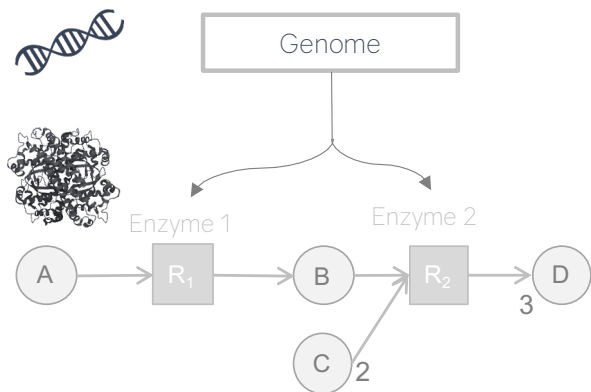
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1. Reconstruct GSMNs for all genomes
2. Assess putative complementarity between them

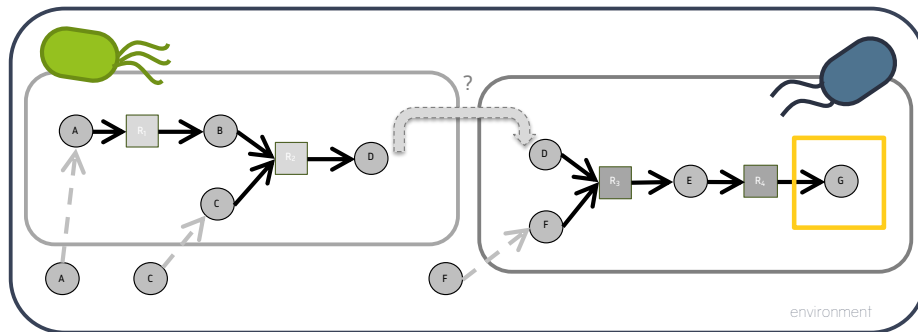
Metabolic networks and communities



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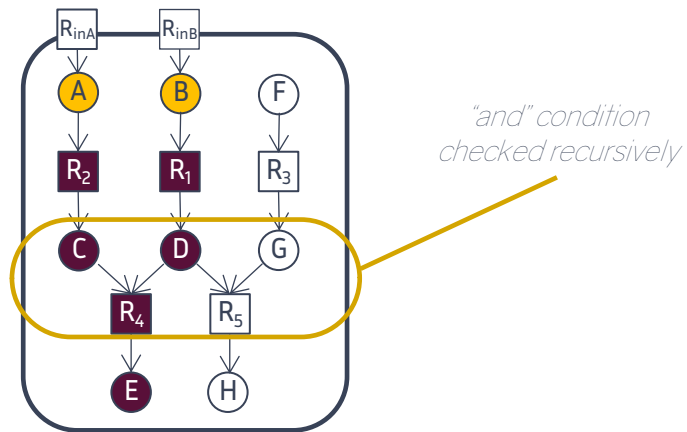
Genome-scale metabolic network (GSMN)

Functional repertoire of the cell



1. Reconstruct GSMNs for all genomes
2. Assess putative complementarity between them
3. Identify key species associated to a function

Metabolic modelling



Network expansion [Ebenhöh et al 2004]

- Scope of seeds
- Qualitative
- Implementation in Answer Set programming

$$\text{scope}(G, S) = \bigcup_i M_i,$$

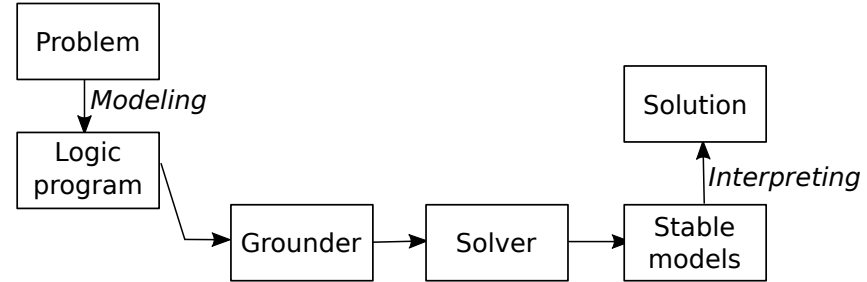
where $M_0 = S$ and
 $M_{i+1} = M_i \cup \text{products}(\{r \in R \mid \text{reactants}(r) \subseteq M_i\})$

Robustness to incomplete data, similarity to prediction from qualitative simulations \rightarrow suitability to a metagenomic context

Answer Set Programming

[Gebser et al 2012]

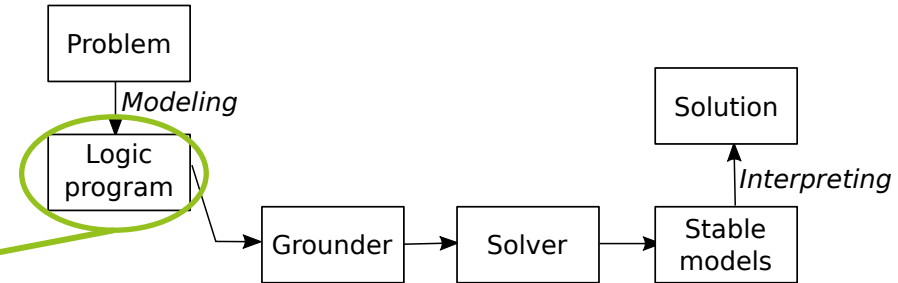
- Logic paradigm
- Knowledge representation & reasoning
- Optimisation



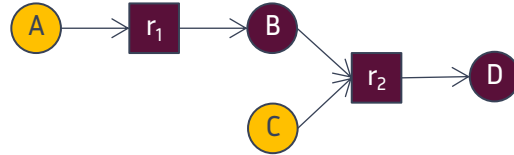
Answer Set Programming

[Gebser et al 2012]

- Logic paradigm
- Knowledge representation & reasoning
- Optimisation



```
reaction("r1").
reactant("A", "r1").
product("B", "r1").
%
reaction("r2").
reactant("B", "r1").
reactant("C", "r1").
product("D", "r1").
%
seed("A").
seed("C").
%
scope(M) :- seed(M).
scope(M) :- product(M,R); reaction(R); scope(N) : reactant(N,R).
```



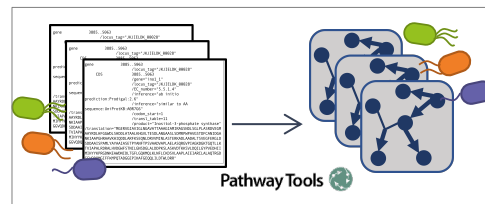
A pipeline for metabolic screening of communities

Microbiota

- MAGs
- Reference genomes



Automatic GSMN reconstruction



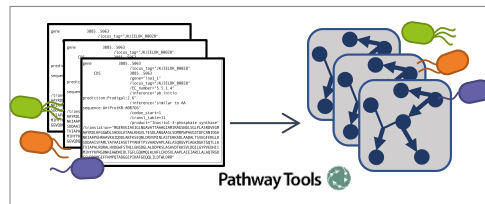
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Microbiota

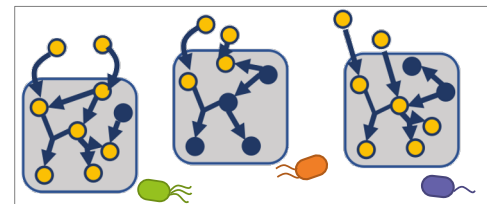
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Automatic GSMN reconstruction



Individual metabolic capabilities

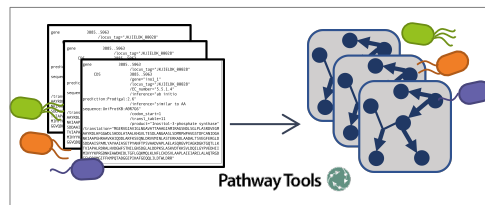


A pipeline for metabolic screening of communities

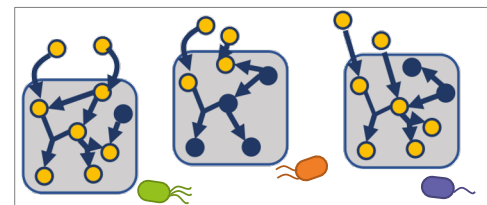
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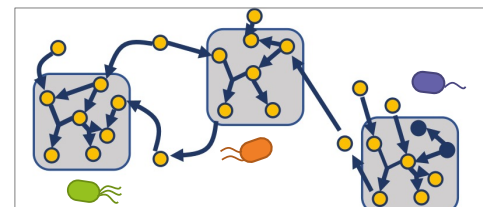
Automatic GSMN reconstruction



Individual metabolic capabilities



Collective metabolic capabilities

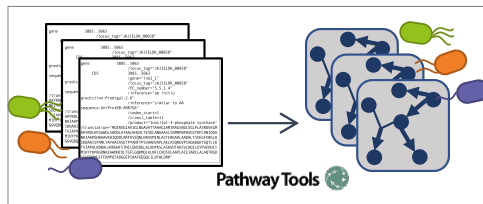


A pipeline for metabolic screening of communities

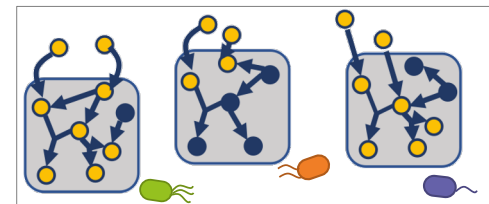
Microbiota

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- Reference genomes

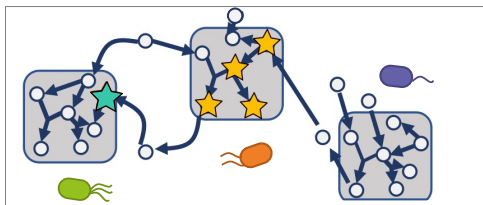
Automatic GSMN reconstruction



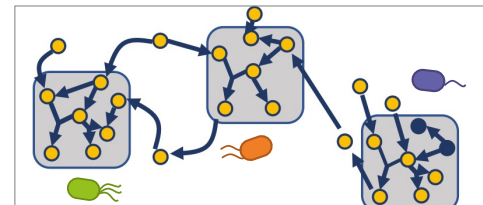
Individual metabolic capabilities



Added value of cross feeding



Collective metabolic capabilities

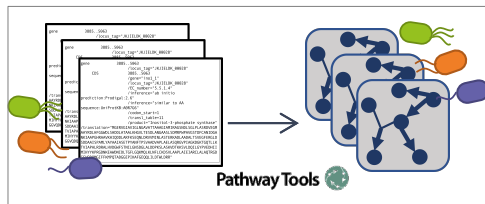


A pipeline for metabolic screening of communities

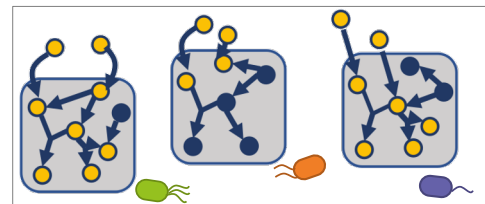
Microbiota

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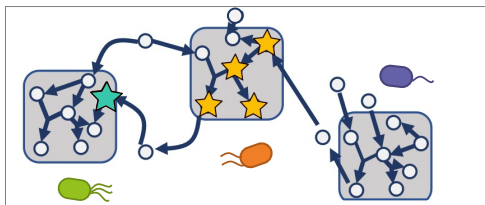
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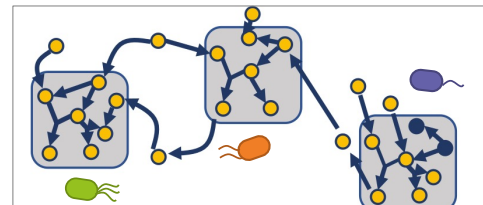
Individual metabolic capabilities



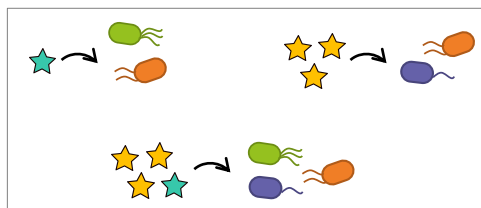
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Collective metabolic capabilities



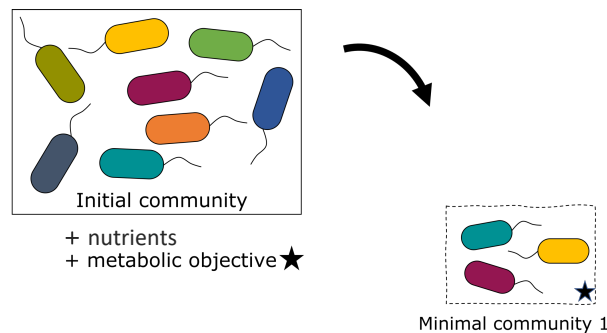
Minimal community selection



Systematic screening of **metabolic potential** and **mutualistic potential** in a microbiota

Key species

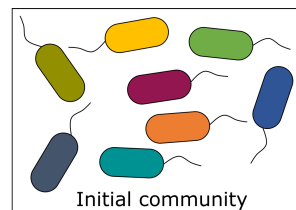
- Minimal community selection:
combinatorial problem solving



The concept of **key species** addresses the **combinatorial** challenge brought by **functional redundancy** in microbiomes

Key species

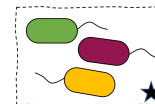
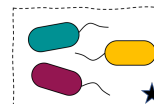
- Minimal community selection:
combinatorial problem solving
- However... 1 solution (= 1 minimal community) among
millions? **Huge combinatorics**



+ nutrients
+ metabolic objective★

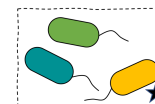
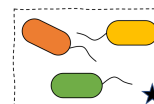


All minimal communities satisfying
the metabolic objective



Minimal community 1

Minimal community 2



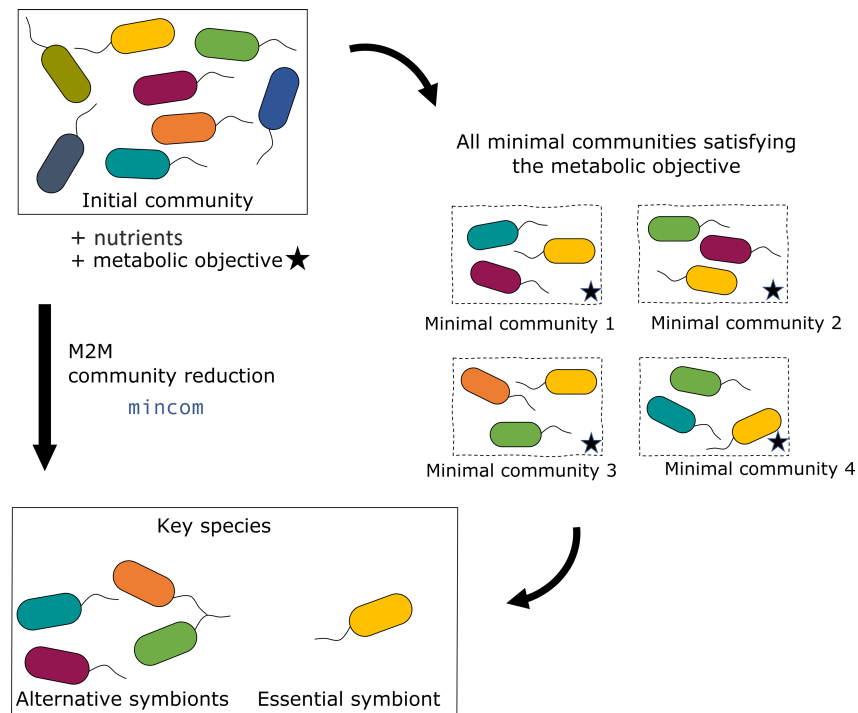
Minimal community 3

Minimal community 4

The concept of **key species** addresses the **combinatorial** challenge brought by **functional redundancy** in microbiomes

Key species

- Minimal community selection:
combinatorial problem solving
- However... 1 solution (= 1 minimal community) among millions? **Huge combinatorics**
- *Key species* = species found in at least one of the predicted minimal communities (MC)
 - > *Key symbionts* : species predicted in **every** MC
 - > *Alternative symbionts* : species predicted in some but not all MC



The concept of **key species** addresses the **combinatorial** challenge brought by **functional redundancy** in microbiomes

Application to 1,520 reference genomes of cultivable species of the human gut microbiota

	Gut dataset
initial data	draft reference genomes
number of genomes	1,520
<i>GSMN reconstruction</i>	
all reactions	3,932
all metabolites	4,001
avg reactions per GSMN	1,144 (\pm 255)
avg metabolites per GSMN	1,366 (\pm 262)
avg genes per mn	596 (\pm 150)
% reactions associated to genes	74.6 (\pm 2.17)
avg pathways per mn	163 (\pm 49)
<i>metabolic potential</i>	
number of seeds	93
avg scope per mn	286 (\pm 70)
union of individual scopes	828

156 metabolites that cannot be produced by individual species

Classification into categories: lipids (28), carbohydrate derivatives (58)...

Key species computation for categories of metabolites + minimal communities enumeration

Analysis and visualisation

Key species by groups of metabolic end-products

		Firm.	Bact.	Acti.	Prot.	Fuso.	total
aminoacids and derivatives (5 targets)	KS	142	52	0	27	6	227
4 bact. per community	ES	0	0	0	0	0	0
120,329 communities	AS	142	52	0	27	6	227
aromatic compounds (11 targets)	KS	52	0	0	20	0	72
5 bact. per community	ES	2	0	0	1	0	3
950 communities	AS	50	0	0	19	0	69
carboxyacids (14 targets)	KS	16	13	0	28	2	59
9 bact. per community	ES	2	0	0	2	0	4
48,412 communities	AS	14	13	0	26	2	55
coA derivatives (10 targets)	KS	106	0	50	17	1	174
5 bact. per community	ES	0	0	0	0	1	1
95,256 communities	AS	106	0	50	17	0	173
lipids (28 targets)	KS	3	140	22	20	0	185
7 bact. per community	ES	3	0	0	1	0	4
58,520 communities	AS	0	140	22	19	0	181
sugar derivatives (58 targets)	KS	11	30	78	23	0	142
11 bact. per community	ES	5	0	0	0	0	5
7,860,528 communities	AS	6	30	78	23	0	137

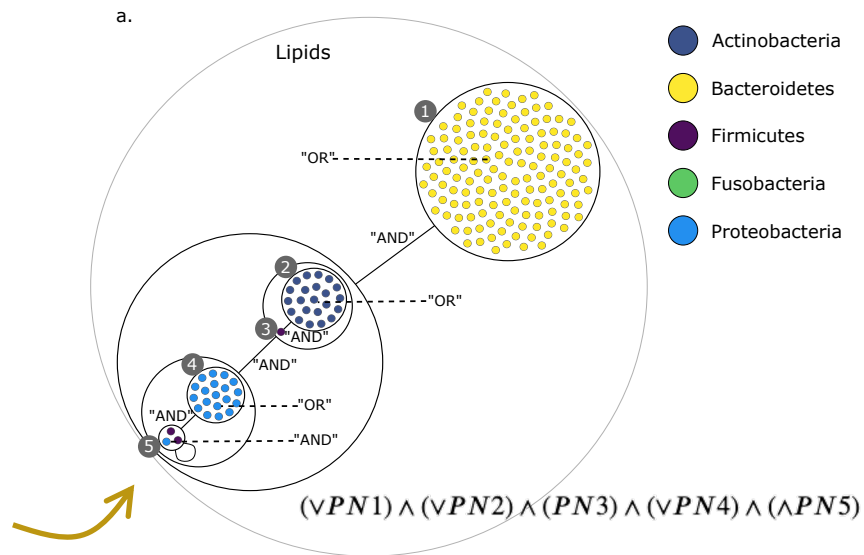
Enumeration of all minimal communities



How do bacteria associate within them?
Any patterns?

Key species by groups of metabolic end-products

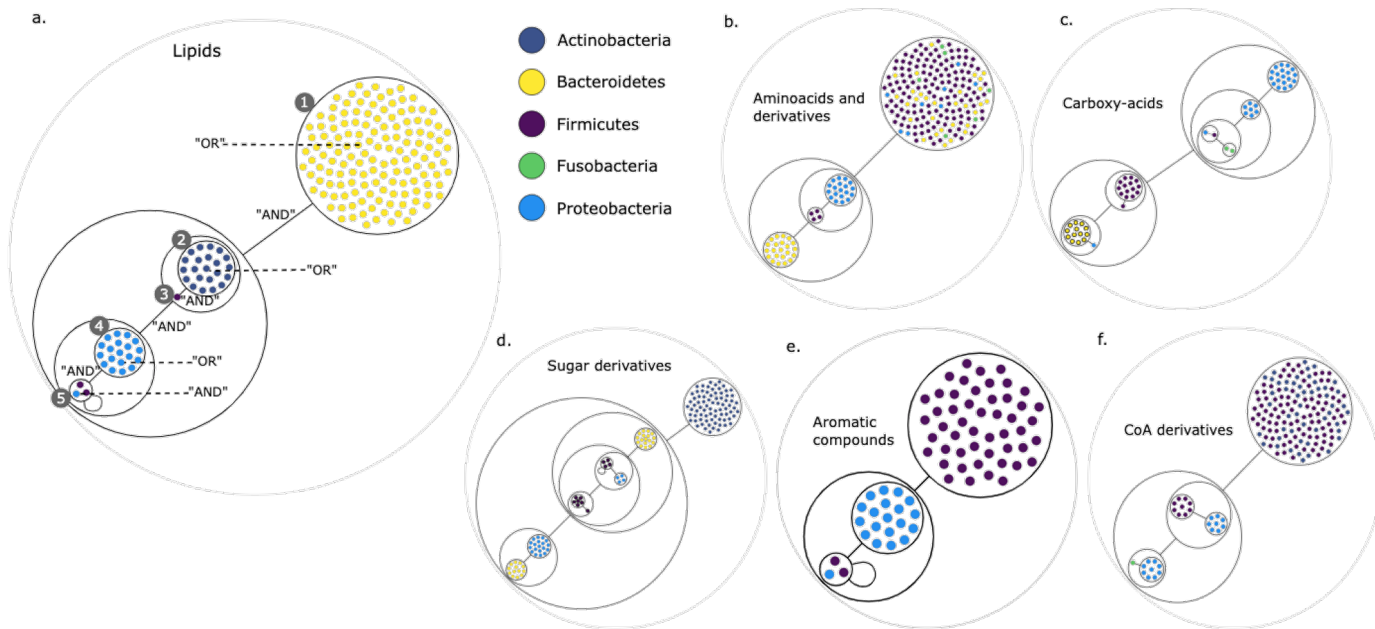
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58,520 minimal communities associated to the producibility of the 28 lipids can be visualised as a compressed association graph.

→ Species with equivalent roles in the communities

Same “reading pattern” for all groups



Essential symbionts (ES) shared between several groups of targets
 → species with unique functions among the 1,520?
 4/5 ES for carbohydrates are studied as probiotics

MAGs vs reference genomes

Metagenome-assembled genomes (MAGs)

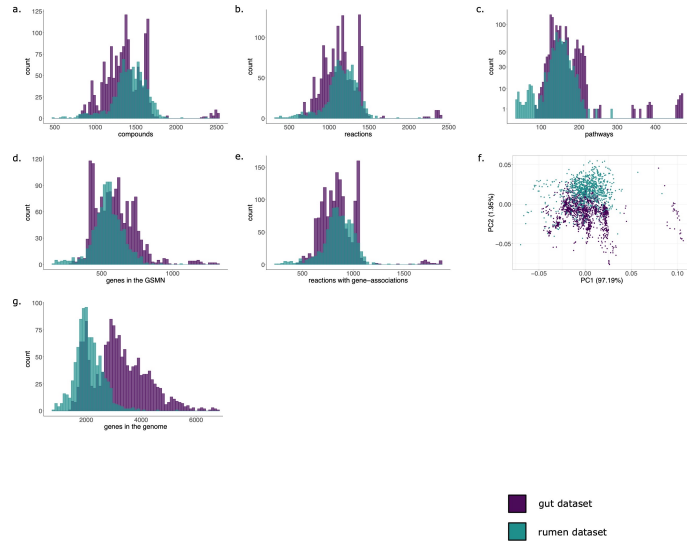
- > Possibly incomplete
- > Possibly contaminated

Experimental setup

- 913 cow rumen MAGs [Stewart et al 2018]
- Random degradation
 - > Removal of 2% genes in all MAGs
 - > Removal of 5% genes in 80% of MAGs
 - > Removal of 5% genes in all MAGs
 - > Removal of 10% genes in 70% of MAGS
- Reconstruct & compare GSMNs, apply M2M screening and community selection

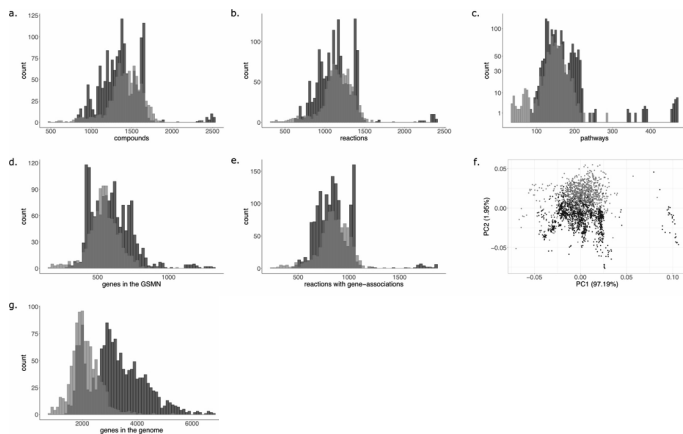
It's ok to use MAGs

GMSN characteristics are similar for reference genomes and MAGs



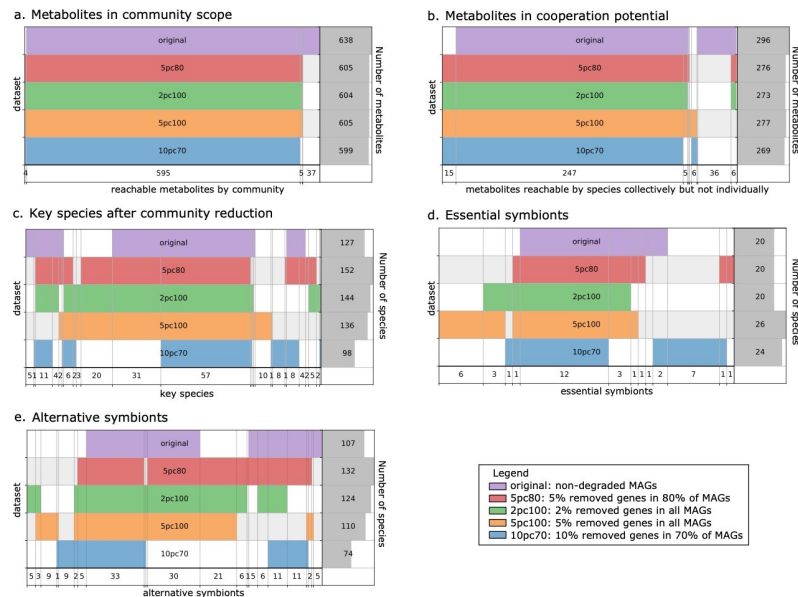
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■ gut dataset
■ rumen dataset

Modelling predictions are stable despite MAG degradation



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¹Univ Rennes, Inria, CNRS, IRISA, F-35000 Rennes, France; ²Inria Bordeaux Sud-Ouest, France; ³Gut Microbes and Health, Quadram Institute, NR4 7GJ, Norwich, United Kingdom; ⁴Digital Biology, Earlham Institute, NR4 7GJ, Norwich, United Kingdom; ⁵INRAE, UMR IGEP, Bioinformatics Platform for Agroecosystems Arthropods (BIPAA), 35042 Rennes, France; ⁶Inria, IRISA, GenOuest Core Facility, 35042 Rennes, France

A flexible and scalable pipeline

Metage2Metabo

- > Global analysis of metabolism in microbiomes: redundancy - complementarity
- > Key species screening: complexity reduction
- > Robust modelling framework that supports data uncompleteness

Use cases:

- > Comparison of metabolic repertoire in genome collections
- > Comparison of cohort samples through their MAGs composition



- Arnaud Belcour
- Méziane Aïte
- Anne Siegel



- Falk Hildebrand
- NBI Computing
Infrastructure for Science



- Pleiade project team
<https://inria.fr/en/pleiade>



- Simon Dittami



- Anthony Bretaudeau



We're hiring!

Postdoc position - 2 years - at Inria Bordeaux
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