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# From strain potential to bacterial community design for targeted functions

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# From strain potential to bacterial community design for targeted functions



STLOpen Days  
19-21 March 2019



Hélène Falentin – MicroBio  
Valérie Gagnaire – MicroBio



## Food quality & safety



Develop safe, healthy, sustainable and hedonic fermented foods



## Food spoilage



Reduce health risks and food losses and waste

# From strain potential to bacterial community design for targeted functions



## Animal health



Offer alternatives to antibiotic treatments (probiotics)

## Health & wellbeing



Develop functional and designer food against modern lifestyle diseases

## Food quality & safety



Develop safe, healthy, sustainable and hedonic fermented foods

## Food spoilage



Reduce health risks and food losses and waste

# How to properly design bacterial communities?



## Animal health



Offer alternatives to antibiotic treatments (probiotics)

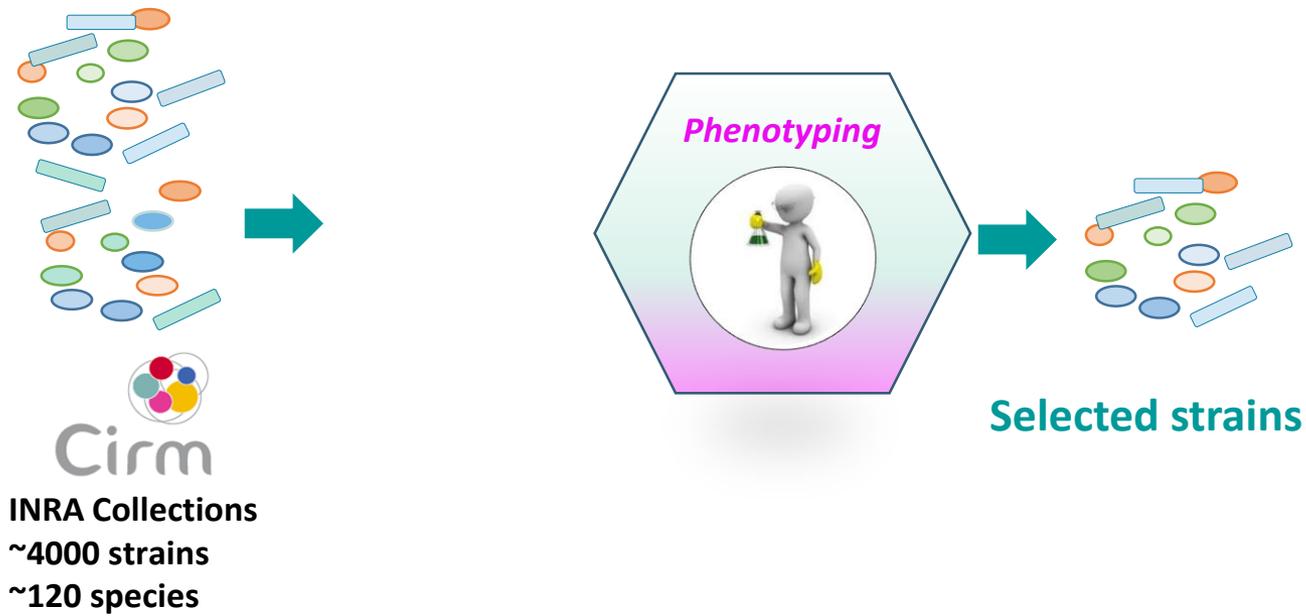
## Health & wellbeing



Develop functional and designer food against modern lifestyle diseases



# How to select bacterial species and strains of interest?



# What is for us phenotyping?

**Phenotype** = expression of the bacteria genotype

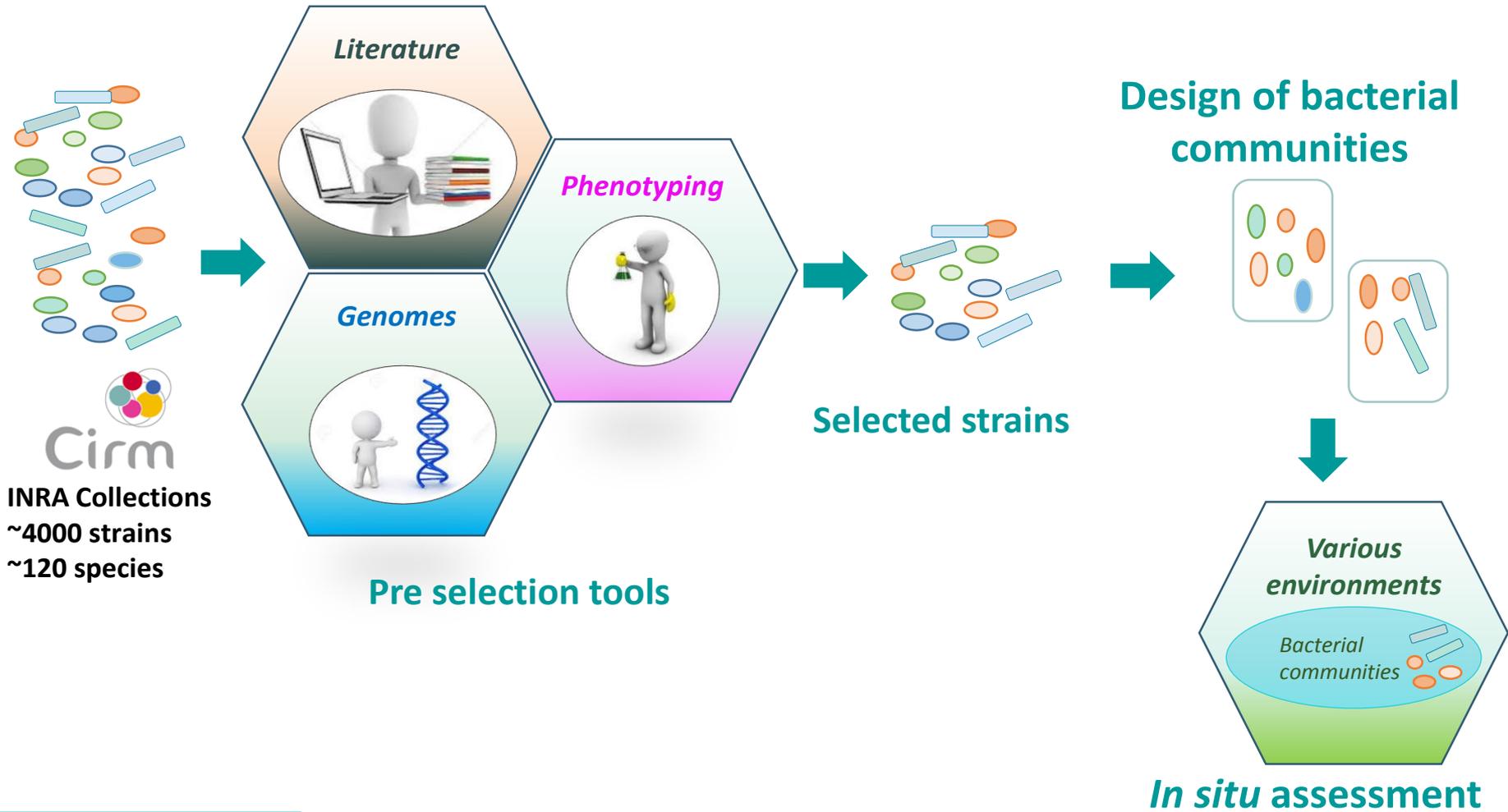


- ✓ morphology or form and structure
- ✓ biochemical and physiological properties
- ✓ metabolites produced

↪ To test observable characteristics = phenotypes of a bacteria in an environment

- Use of media that mimics at best the *in situ* environments : composition, structure and abiotic conditions
- Assess some risk criteria: biogenic amines, antibiotic resistance...

# How to select bacterial species and strains of interest?



## Food quality & safety



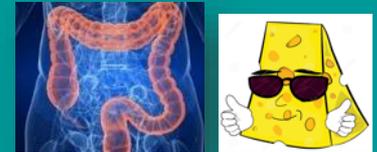
## Food spoilage



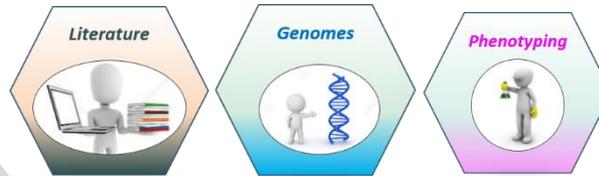
## Animal health



## Health & wellbeing



What information is needed to use the pre selection tools ?



## Food quality & safety



↳ Enzymes from degradation pathways  
↳ synthesis pathways

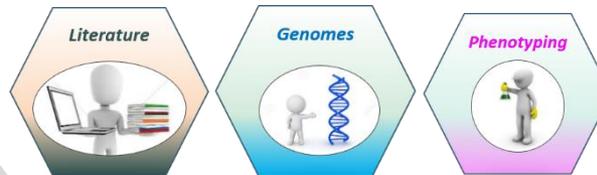
## Food spoilage



↳ Resistance mechanism of spoilage bacteria

## Composition of raw material

### Genes responsible for targeted functions



## Animal health



↳ Virulence regulation  
↳ Colonization

## Health & wellbeing



↳ Anti-inflammatory properties

## Food quality & safety



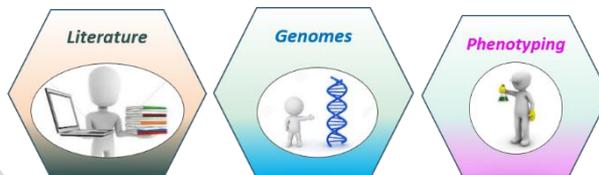
↪ Enzymes from degradation pathways

## Food spoilage



## Composition of raw material

## Genes responsible for targeted functions



## Animal health

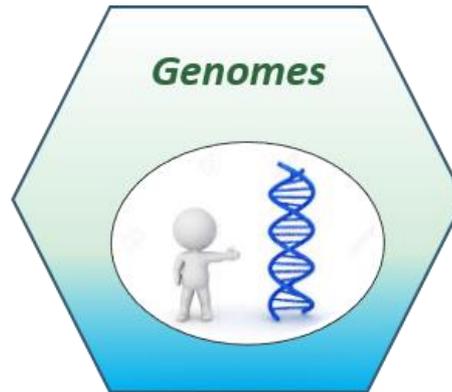


## Health & wellbeing



↪ Anti-inflammatory properties

# Pre-selection tools



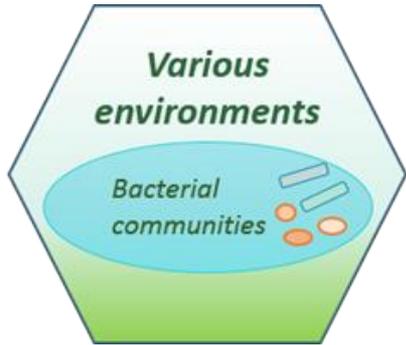
Annotation  
PCR tools



## Limits of the method

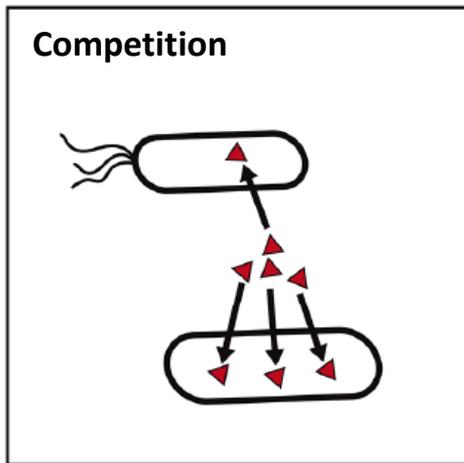
- Some genes are not accurately annotated: cell-wall proteinases ...
- Pathways are not complete
- No direct Links between genome and phenotype:
  - Case of intracellular enzymes released after bacterial cell death
  - Presence of genes cannot predict the observed activity: regulation of gene expression and/or of enzyme production and activity

# What next after the pre-selection of the strains?

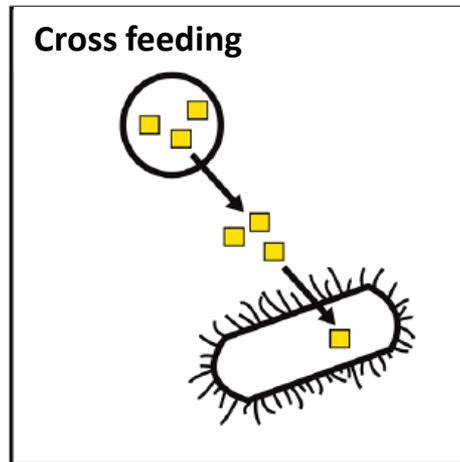


Assembly of the strains for targeting functionalities within bacterial communities

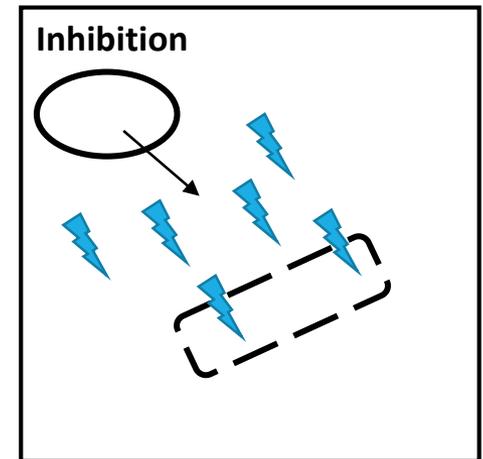
- ↪ *In situ* assessment through various environmental conditions
- ↪ And different kinds of interactions within the communities and with the components of the environment



Iron privation of *S. enteritidis* by egg white (1)



Lactic acid bacteria-Propionibacteria (2)



*L. lactis* / *S. aureus* (3)

Posters  
P15 and  
P16

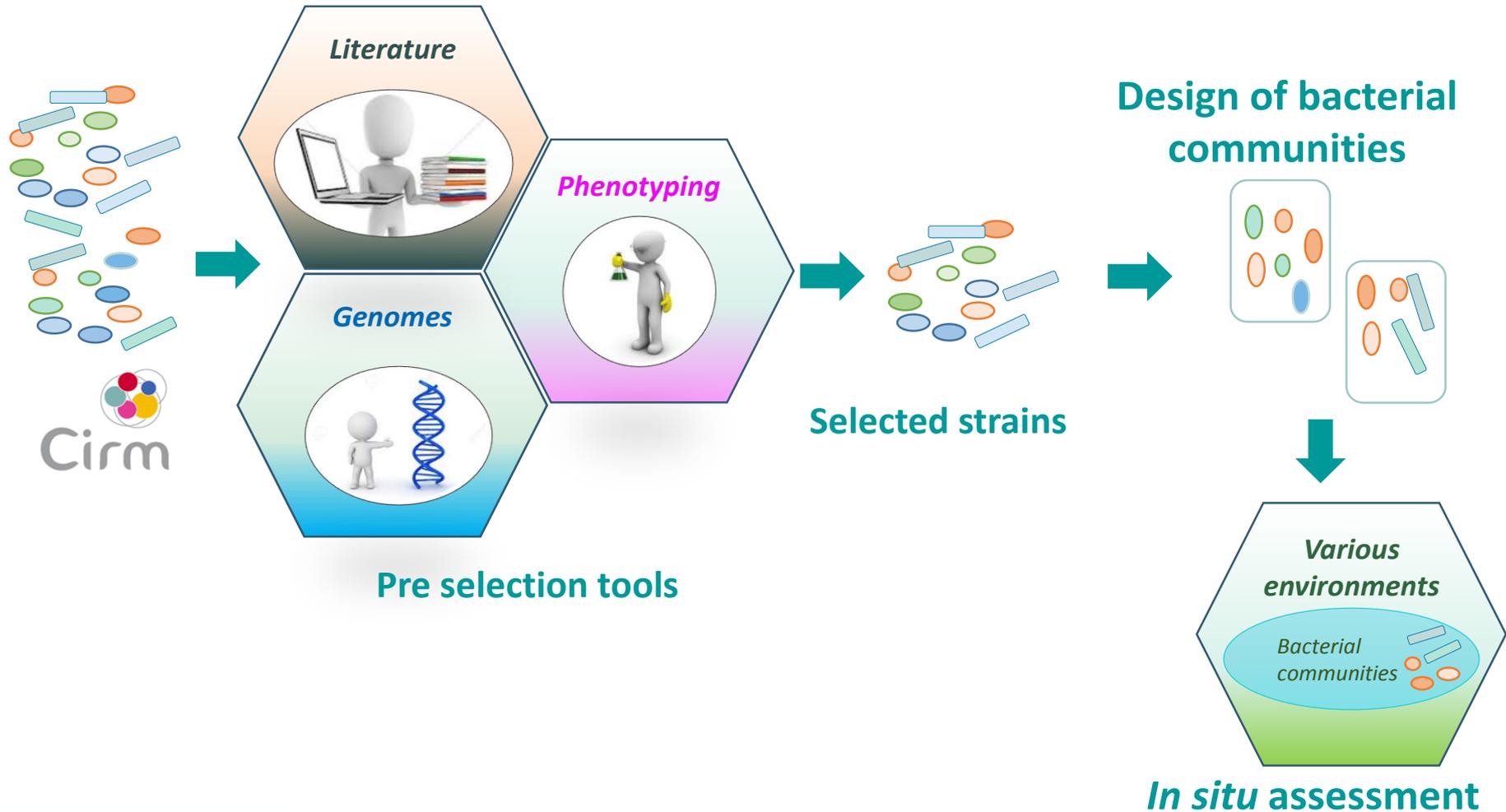
Adapted from Seth and Taga (2017)

- (1) Baron et al. 2017. Front. Microbiol., 8, 1-9
- (2) Thierry et al 2015. Dairy Sci. Technol., 95(6), 895-918
- (3) Cretenet et al. 2011. Environ. Microbiol. Reports, 3(3), 340-351



# Examples of MicroBio results illustrating from the assessment of the strain potential to the design of bacterial communities

# 1- collect data from literature using FLORILEGE database



# Collect data from FLORILEGE : A public database gathering microbial habitats and phenotypes of food interest



**Text mining for automatic extraction of microbial habitats, phenotypes and data gathering**



Exemple :

Meile et al. (2008) reported that *Propionibacterium freudenreichii* is the main species used as a ripening starter in Swiss-style cheeses.

Text-mining  $\Rightarrow$  *Propionibacterium freudenreichii* INHABITS hard-cheese  
USED FOR ripening



# Example of taxons in food habitat

Query in the sheet "Habitat is inhabited by Taxon": **hard cheese AND QPS species**

SOURCE TEXT	HABITAT	RELATION TYPE	TAXON	SOURCE
6168893	Granular	is inhabited by	Bacillus subtilis	OpenMinTeD
21338778	Cheddar	is inhabited by	Bifidobacterium animalis	OpenMinTeD
21338778	Cheddar	is inhabited by	Bifidobacterium longum	OpenMinTeD
12892928	Cheddar	is inhabited by	Debaryomyces hansenii	OpenMinTeD
6394	Cheddar	is inhabited by	Pediococcus pentosaceus	DSMZ
	Beaufort	is inhabited by	Propionibacterium freudenreichii	CIRM
	Comté	is inhabited by	Propionibacterium freudenreichii	CIRM
	Emmental	is inhabited by	Propionibacterium freudenreichii	CIRM
19036465	Cantal	is inhabited by	Kluyveromyces lactis	OpenMinTeD
18218739	hard cheese	is inhabited by	Kluyveromyces lactis	OpenMinTeD
22916882	Caciocavallo	is inhabited by	Lactobacillus casei	OpenMinTeD
11573770, 9812282	Cheddar	is inhabited by	Lactobacillus curvatus	OpenMinTeD
18842314, 17241339, 19754175	hard cheese	is inhabited by	Lactobacillus delbrueckii	OpenMinTeD
15453471	Cheddar	is inhabited by	Lactobacillus delbrueckii	OpenMinTeD
11573770	Cheddar	is inhabited by	Lactobacillus fermentum	OpenMinTeD
18842314, 6434507, 10946835	hard cheese	is inhabited by	Lactobacillus helveticus	OpenMinTeD
10984143, 16987432	Cheddar	is inhabited by	Lactobacillus helveticus	OpenMinTeD
22029927	Emmental	is inhabited by	Lactobacillus helveticus	OpenMinTeD
22916882	Caciocavallo	is inhabited by	Lactobacillus paracasei	OpenMinTeD
16476172	Comté	is inhabited by	Lactobacillus paracasei	OpenMinTeD

1-20 of 34

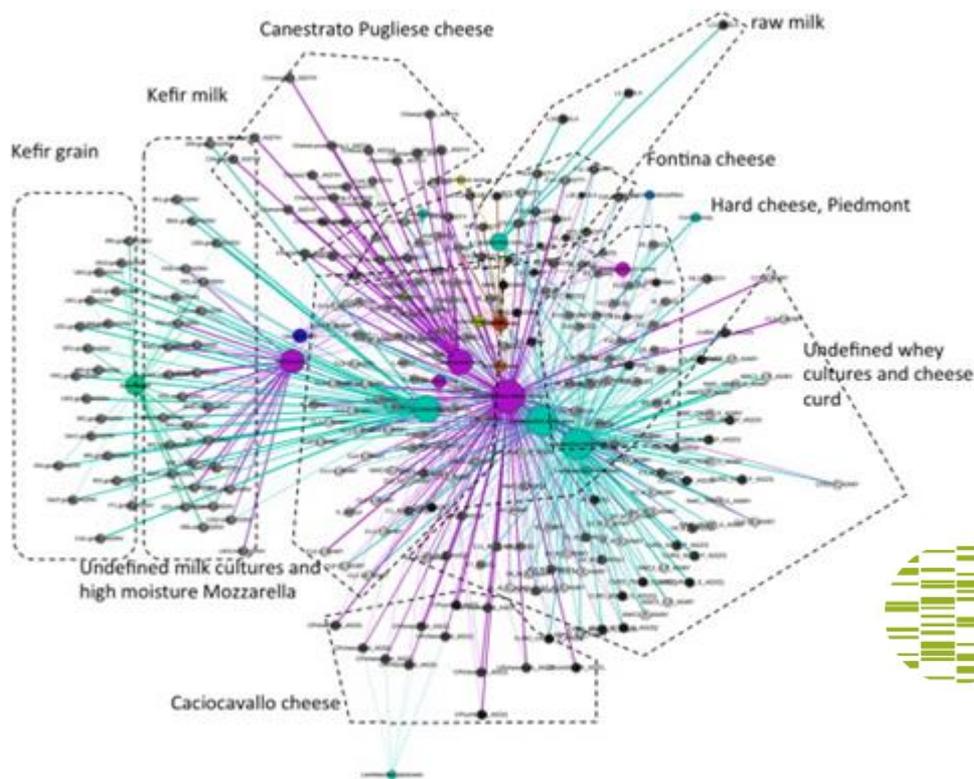


# Example of halotolerance

Query in the sheet “Phenotype is exhibited by Taxon”: halotolerant AND QPS species

➔ 9 species selected

SOURCE TEXT	PHENOTYPE	RELATION TYPE	TAXON
21664643	halotolerant	is exhibited by	Bacillus megaterium
12557391, 21890005, 16847833	halotolerant	is exhibited by	Bacillus subtilis
14643798, 25561404, 18822773	halotolerant	is exhibited by	Bacillus licheniformis
16862599, 9931473, 15756621	halotolerant	is exhibited by	Debaryomyces hansenii
18068256	halotolerant	is exhibited by	Lactococcus lactis
18068256	halotolerant	is exhibited by	Leuconostoc lactis
25039289	halotolerant	is exhibited by	Lactobacillus plantarum
9327565	halotolerant	is exhibited by	Saccharomyces cerevisiae
17897213, 25542205	halotolerant	is exhibited by	Bacillus pumilus



On going improvements to help designing microbial communities by taking into account:

the social network of bacteria according to the food habitat

**E-novFood** in collaboration with



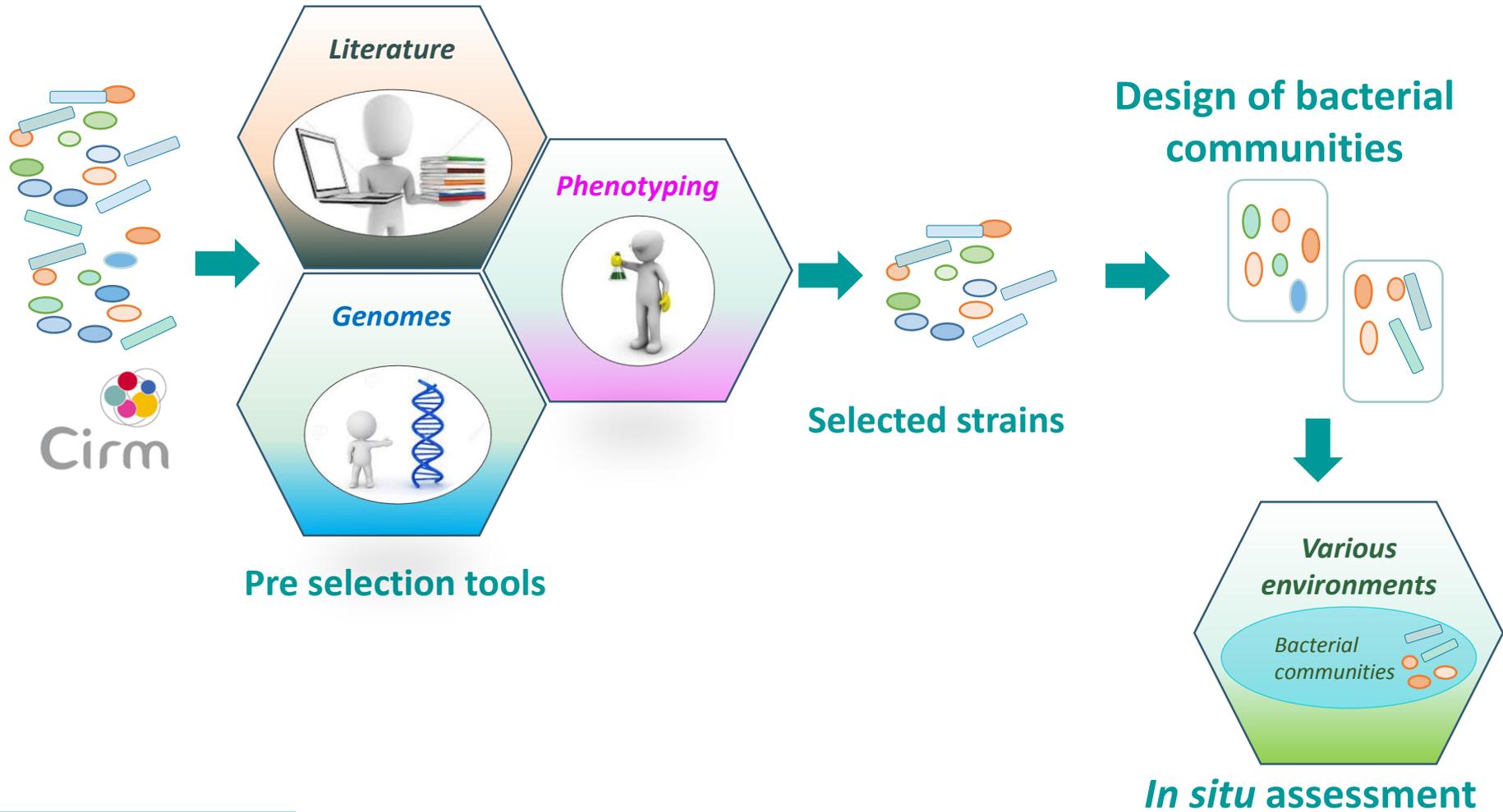
Università degli Studi

Basilicata

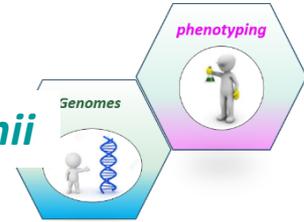
University of Potenza

- Tools to select strains that are co-occurring in a given food habitat
- providing information on the structure of bacterial communities in raw materials, fermented and spoiled foods which can be used for food process development

# 2- links between genome and phenotypes ?

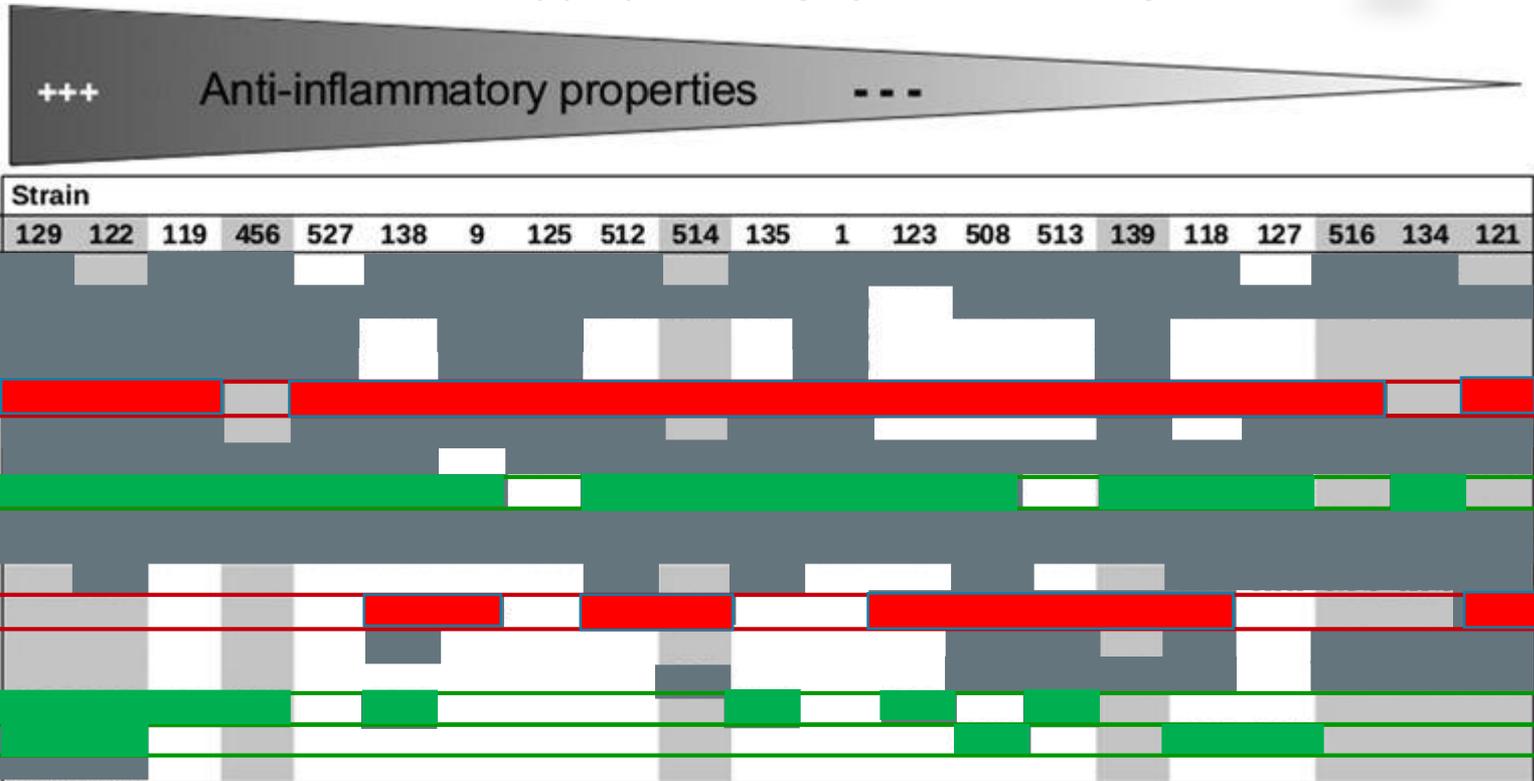


# Select strain with genes of interest (1)



## ➤ Mastering the anti-inflammatory potential of *Propionibacterium freudenreichii*

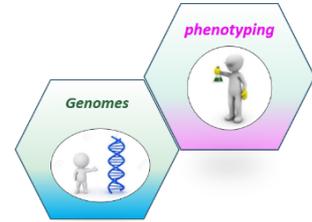
*P freudenreichii* has anti inflammatory properties highly variable among strains



- There was a multi-gene determinism according to the strains
- 2 surface protein *SlpE* et *SlpB* and *hsdM3* genes associated with high anti-inflammatory properties
- 2 genes encoding cytoplasmic proteins associated with antagonist effects

➤ Tools to select strains with the genes of interest to find strains with high anti-inflammatory potential

## Select strain with genes of interest (2)

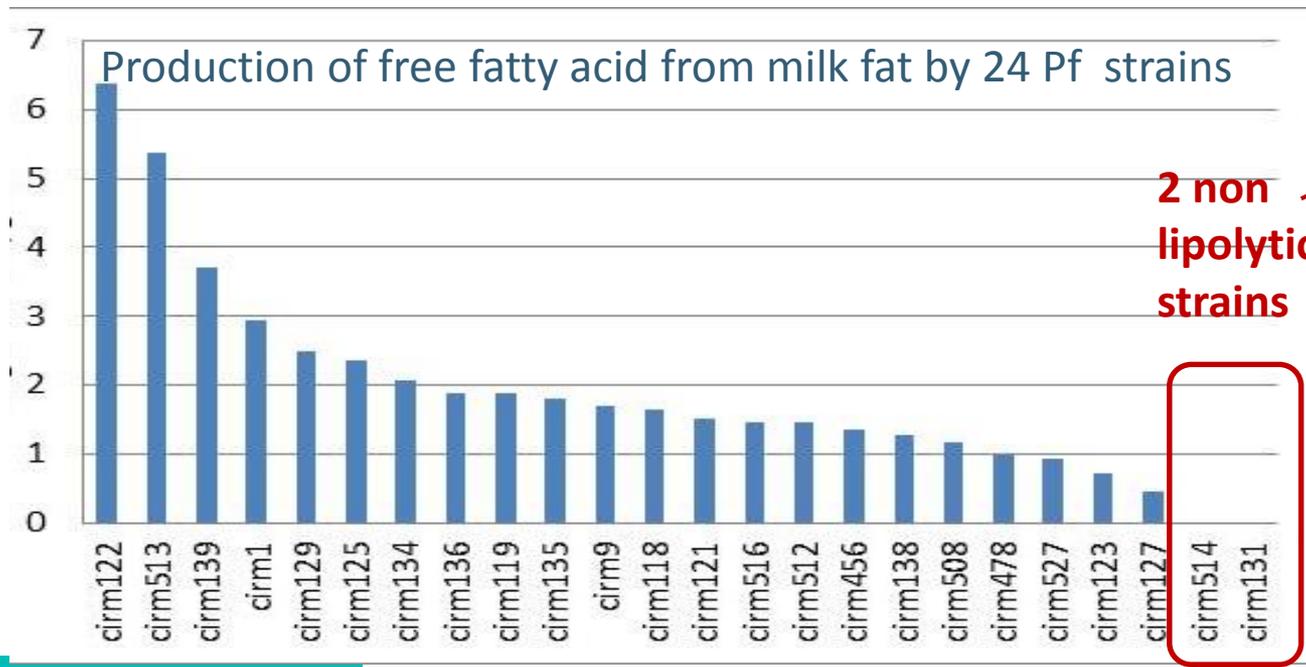


### ➤ Modulating cheese flavor related to lipolysis by *P. freudenreichii*

- A total of 12 putative esterase-coding genes identified in *P. freudenreichii* genome
- Sequence of all esterases are highly conserved
- All esterases were expressed regardless of the presence of milk fat

**But 1 secreted lipolytic esterase with a major role in cheese lipolysis**

- Lipolytic activity markedly strain-dependent:

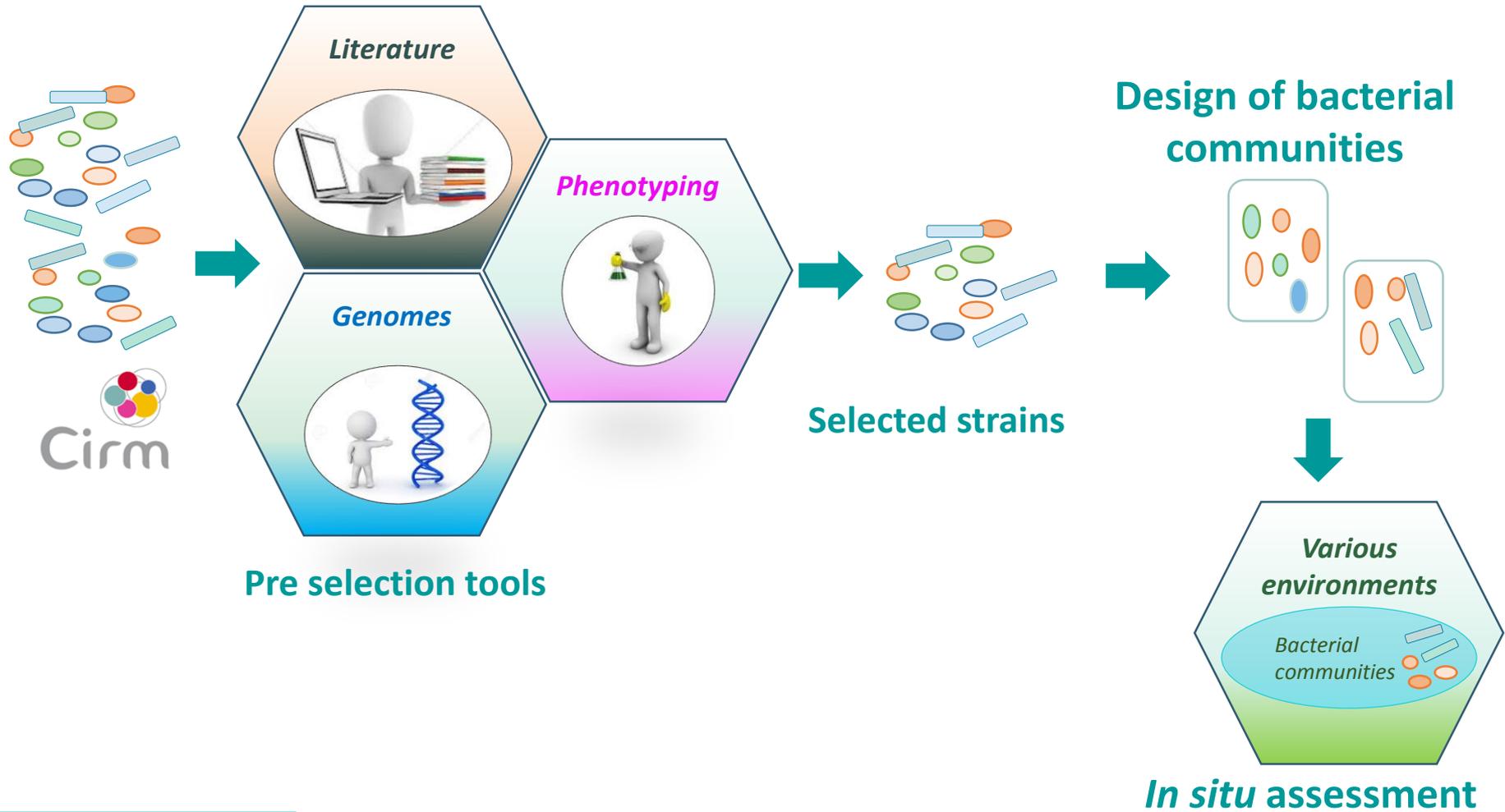


**2 non lipolytic strains**

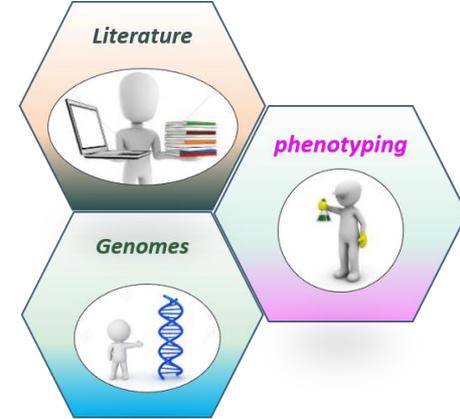
characterized by the same single-nucleotide deletion leading to a premature stop codon in the gene encoding the secreted esterase

➔ Can be used to predict a « null » phenotype

# 3 - Exemple of assembly of strains for functionalizing bacterial communities

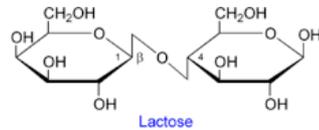


# Knowing the composition of the raw material as a way to select bacteria



➤ Ferment sugars from various resources :

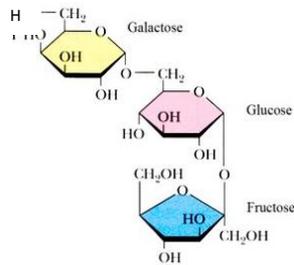
- lactose from milk,



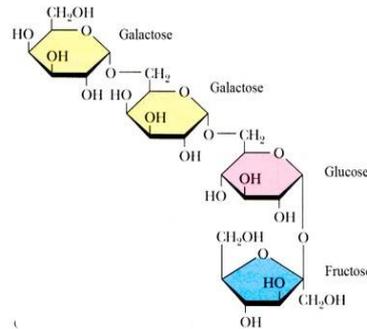
$\beta$ -galactosidase

- raffinose, stachyose, verbascose and sucrose from legume resources

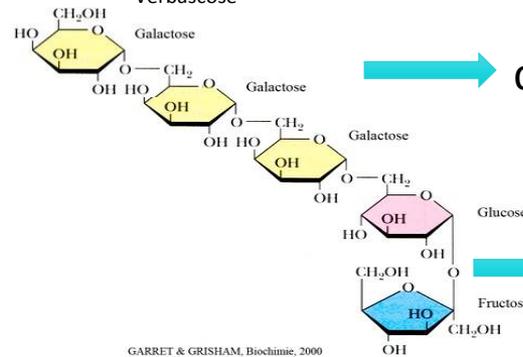
Raffinose



Stachyose



Verbascose



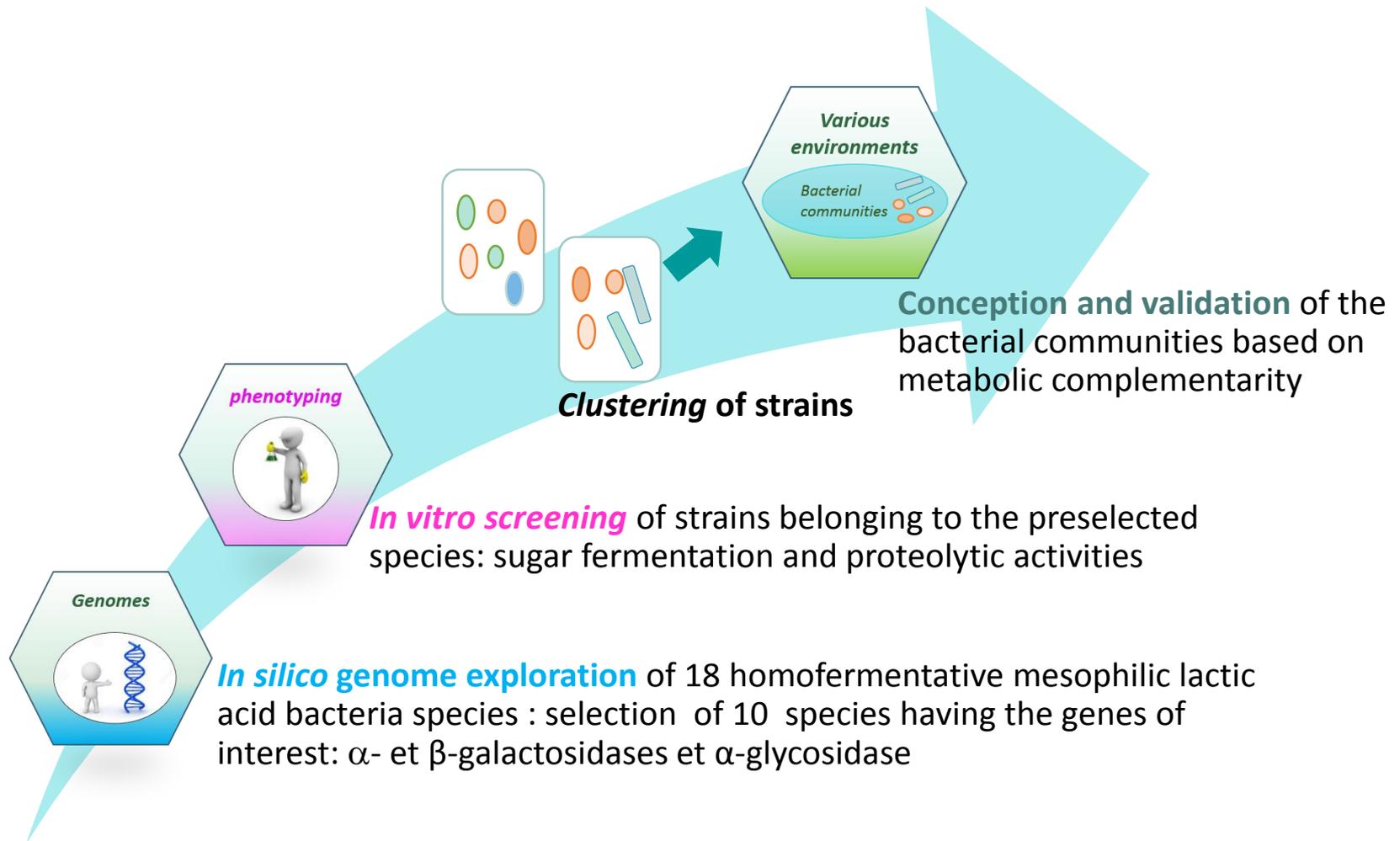
$\alpha$ -galactosidase



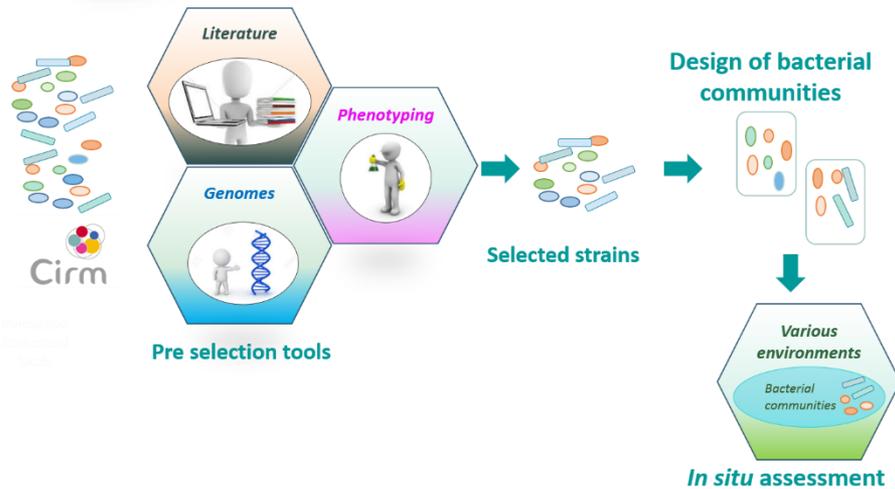
$\alpha$ -glycosidase

GARRET & GRISHAM, Biochimie, 2000

# Design based on metabolic complementarity



Take home messages



We developed key tools and approaches to be able to explore:

- How strains respond to environmental variations
- How to target specific metabolites or functional properties
- How to assemble strains into communities



***MERCI***



***THANK YOU FOR  
YOUR ATTENTION***