



# Bioinformatics tools as a way to select microbial strains for fermented food products

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27 mai 2019



# **1- Context on fermented food products**

# Making fermented food products



Raw material from animal or plant origin

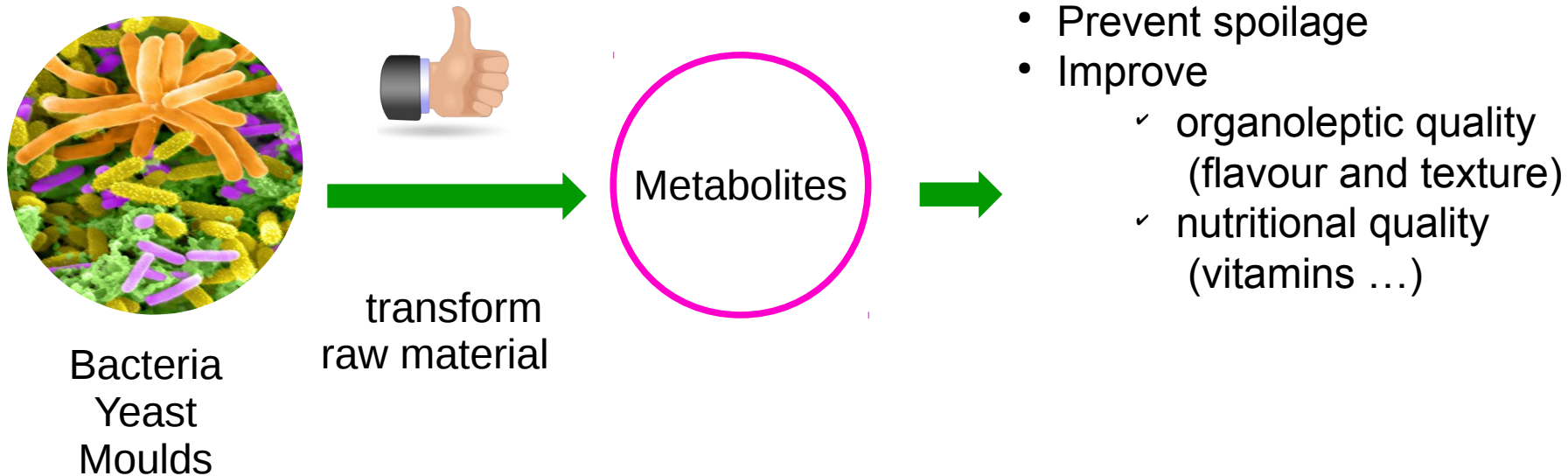


Complex microbial community

Microbial growth during fermentation

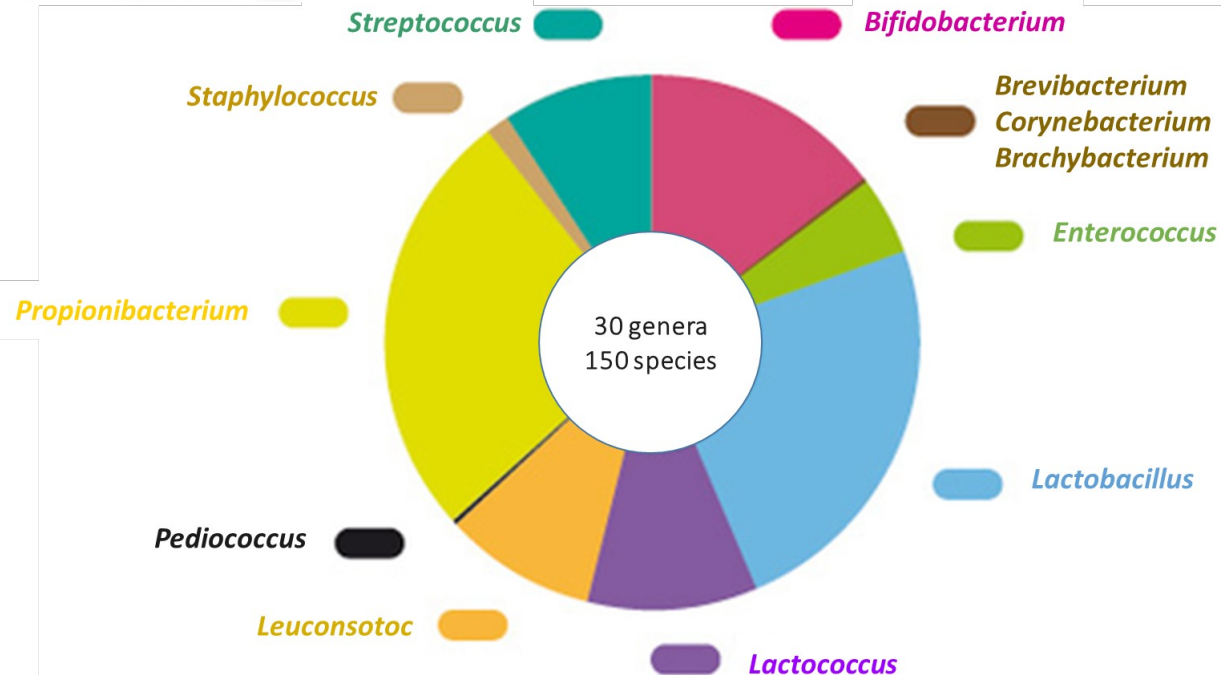


# Role of the food microbial ecosystems during fermentation



# Biological Ressource Center dedicated to species of food interest

- Strains from various habitats (food, environment)
- Taxonomic assignation at the species level
- Many strains per species



4000 strains of food interest in CIRM-BIA Rennes collection

# Development of fermented foods adapted to sustainable production



Using raw materials with huge diverse compositions (milk, legumes and cereal juices)



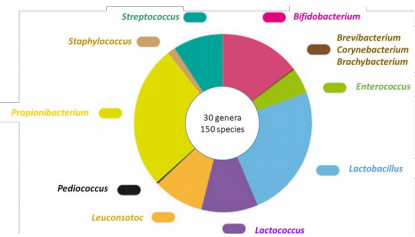
**How to choose advisedly the species and strains to be inoculated ?**



FERMENTATION

## **2- Strategy and bioinformatic tools**

# Used strategies to choose species/strains for fermentation

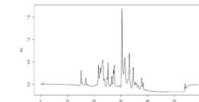


4000 strains



***In vitro***  
**biochemical**  
**approach**

Bacterial growth  
Metabolite  
quantification



Strain  
selection

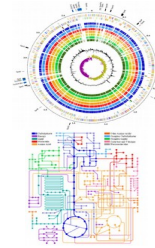


# Used strategies to choose species/strains for fermentation

## Pre-screening step

### *in silico* approach

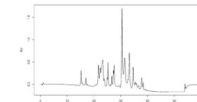
Mining public genome annotation databases  
Search for enzyme sequences



## Screening

### *In vitro* biochemical approach

Bacterial growth  
Metabolite quantification



Strain selection

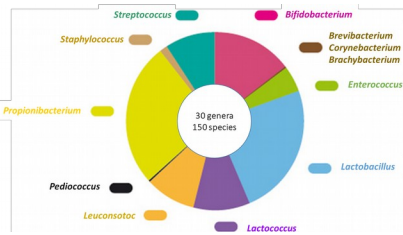
Less than 200 strains

### Bibliographic approach using text-mining

Occurrence of bacterial species in fermented food

Building of the Florilège DB using text-mining algorithms

PubMed



4000 strains



# AlvisIR reads Pubmed articles and extracts Taxonomic, phenotypic and biotope data



period, yeasts (primarily *Debaryomyces hansenii* for semi-soft cheeses and additionally *Geotrichum candidum* for soft cheeses and coagulase-negative staphylococci (*Staphylococcus equorum*) are present [3, 21, 26]. Generally, *D. hansenii* and staphylococci on cheese surface are assumed to originate from the cheese brine which often is not changed or pasteurised between salting of different batches [2, 3, 42]. For *D. hansenii*, Petersen et al. [46] showed that the dominating *D. hansenii* strain on cheeses of the Danish Danbo type did not originate from the added ripening culture, but from the dairy housemicrobiota present in the ripening room. *D. hansenii* is important during cheese ripening as it assimilates lactate and produces alkaline metabolites such as ammonia thereby increasing pH of the cheese surface [23, 46], which enables the growth of the less acid tolerant bacterial microbiota, primarily Gram-positive corneforms (*Brevibacterium* spp., *Cornebacterium* spp.) and *Microbacterium* spp. [4]. In addition, subpopulations of

Annotations Text selection

Id	Annotation Set	Ki	Type	Details
53	[imported] imported from annotation by bdridi in campaign 53		Exhibits	Agent ( Taxon <i>Brevibacterium</i> spp. ) + Property ( Phenotype Gram-positive )
4d443...e7	[imported] imported from annotation by bdridi in campaign 53		Exhibits	Agent ( Taxon <i>Staphylococcus equorum</i> ) + Property ( Phenotype coagulase-negative )

# Florilege DB integrates automatically



## Sampling, isolation biotopes

(named Habitat)

from Genbank, DSMZ, CIRM and litterature

Soy juice  
Sausages  
Cheese

## Taxonomy

from Genbank and litterature

*Streptococcus thermophilus*  
*Lactococcus lactis*  
*Lactobacillus plantarum*



## Phenotypic data

from BacDive (DSMZ)  
and GOLD and litterature

Rod shape  
Halophilic  
Mesophilic

# Florilege queries

'yoghurt from fermented soybean milk'



Tab 'Habitat'



<http://migale.jouy.inra.fr/Florilege/>

[Welcome](#)
[Taxon lives in Habitat](#)
[Habitat is inhabited by Taxon](#)
[Taxon exhibits Phenotype](#)
[Phenotype is exhibited by Taxon](#)

Search relations by habitat:  [TSV Download](#) [Filter Selection](#)

**32 relations for the habitat "yoghurt from fermented soybean milk"**

Source:    
 Taxon: 
 QPS only [Apply](#)

SOURCE TEXT	HABITAT	RELATION TYPE	TAXON	SOURCE
EU855186, EU855184, EU855194	yoghurt from fermented soybean milk	is inhabited by	Bacillus amyloliquefaciens	GenBank
AY422986	yoghurt from fermented soybean milk	is inhabited by	Bacillus flexus	GenBank
EF439666	yoghurt from fermented soybean milk	is inhabited by	Bacillus licheniformis	GenBank
EU855196, EU855214, EU855220	yoghurt from fermented soybean milk	is inhabited by	Bacillus pumilus	GenBank
FJ434652	yoghurt from fermented soybean milk	is inhabited by	Bacillus subtilis	GenBank
EU855223	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus curvatus	GenBank
EU855222	yoghurt from fermented soybean milk	is inhabited by	Pediococcus pentosaceus	GenBank
18084044, 19157616, 22283589	yoghurt from fermented soybean milk	is inhabited by	Bacillus licheniformis	OpenMinTeD
18298737, 18686970, 18541163	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus acidophilus	OpenMinTeD
18387120, 18541163, 24500482	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus casei	OpenMinTeD
16102864	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus sakei	OpenMinTeD
4205191, 25887264, 21115709	yoghurt from fermented soybean milk	is inhabited by	Lactococcus lactis	OpenMinTeD
18230145, 24547696, 17048069	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus fermentum	OpenMinTeD
21801422, 22954488, 25892687	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus helveticus	OpenMinTeD
17292991	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus johnsonii	OpenMinTeD
17292991, 19269578	yoghurt from fermented soybean milk	is inhabited by	Lactobacillus rhamnosus	OpenMinTeD

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**3- Comparison of the 2 different approaches (*in silico* and bibliographic) for the fermentation of legumes juices**

# Fermenting legume juices : soy and lupin projects

## Specifications :

- no gas production = no 'obligatory heterofermentative' species
- mesophilic species = growth at 30 °C
- belonging to *Lactobacillus* (Firmicutes)

# Fermenting legume juices : soy and lupin projects

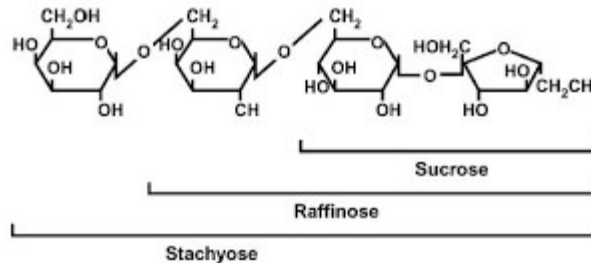
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Both soy and lupin juices contain :

- all amino acids
- sucrose
- non digestible oligosaccharides : raffinose and stachyose



# Fermenting legume juices : soy and lupin projects

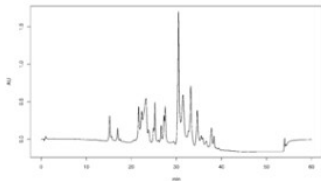
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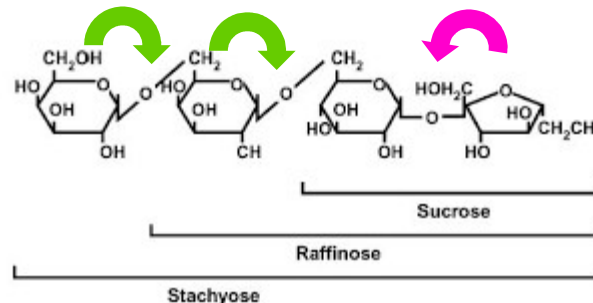


Both juices soy and lupin contain :

- all amino acids
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- non digestible oligosaccharides : raffinose and stachyose



$\alpha$ -galactosidase

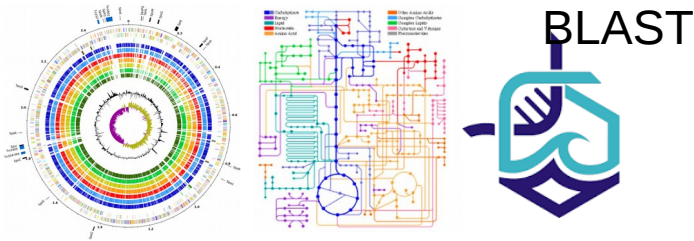


invertase



# Pre-screening for lupin and soy juices fermentation among *Lactobacillus* genus


## Lupin juice *in silico* approach



  $\alpha$ -galactosidase genes  
Invertase genes

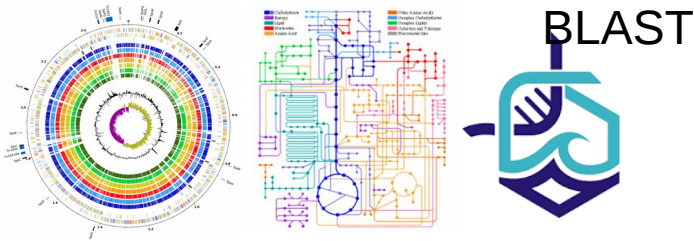
## Soy juice Bibliographic approach



 Species associated with  
'yoghurt from fermented  
soybean milk'

# Pre-screening for lupin and soy juices fermentation among *Lactobacillus* genus

## Lupin juice *in silico* approach




  $\alpha$ -galactosidase genes  
Invertase genes

**9 species** from the  
CIRM-BIA collection  
represented by  
**67 strains**

## Soy juice Bibliographic approach



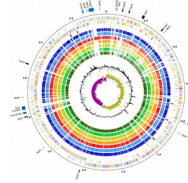
 Species associated with  
'yoghurt from fermented  
soybean milk'

**17 species** from  
CIRM-BIA collection  
represented by  
**130 strains**

# Biochemical validation

In vitro screening on different sugars in synthetic medium

**in silico  
approach**



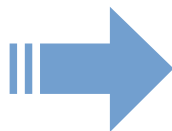
**67 strains**

**Bibliographic  
approach**



**130 strains**

Sucrose +	81 %	80 %
Raffinose +	41 %	39 %
Stachyose +	11 %	4.5 %



- Targeted functionalities are reached
- Same proportion of positive strains with both approaches !

# Take home messages

- Bioinformatic tools are useful to pre-screen strains *in silico* and powerful since huge quantities of annotated genomes are available in public databases
- A biochemical screening of preselected species is still needed but focused on the most promising species
- Bioinformatic tools can facilitate the development of new sustainable fermented food.

# Perspectives



We would like to :

- Enrich Florilege database with new habitats and new phenotypes to expand its use to other ecosystems
- Associate different bacterial strains to develop fermented foods with multiple functionalities

# Thank you for your attention !



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