

METAGENOMIC INSIGHTS INTO THE DYNAMICS AND FUNCTIONALITY OF FOOD MICROBIAL COMMUNITIES

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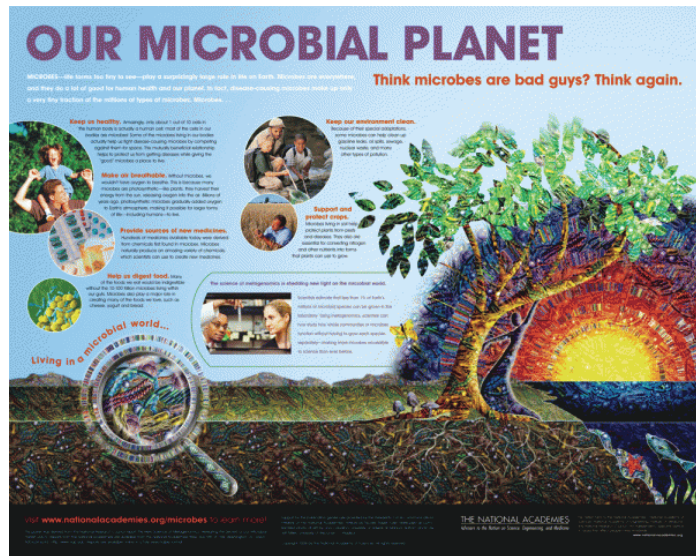
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Summary

- Introduction
- Metagenomics tools
- Diversity characterization
- Functionality understanding
- Future



The food microbial ecosystem

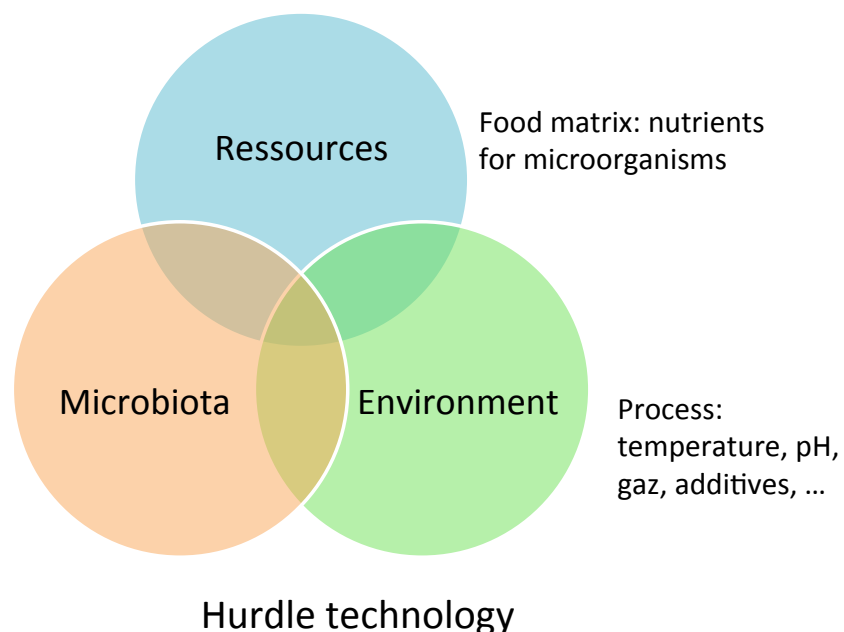
<https://microbialmodus.wordpress.com/tag/fungi/>

INTRODUCTION

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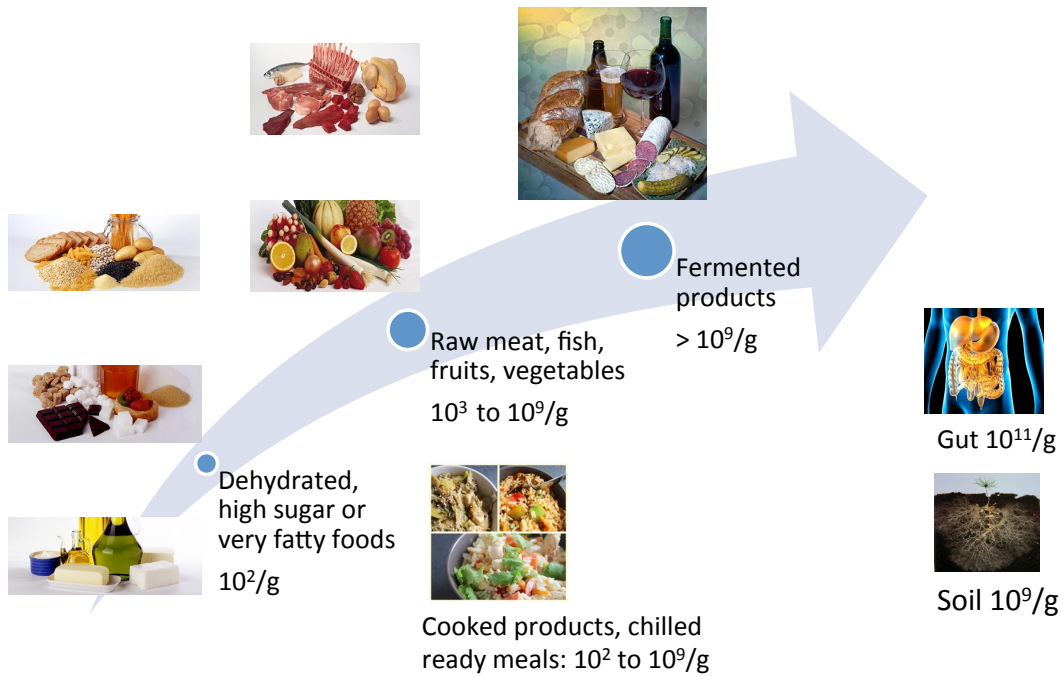
The food ecosystem



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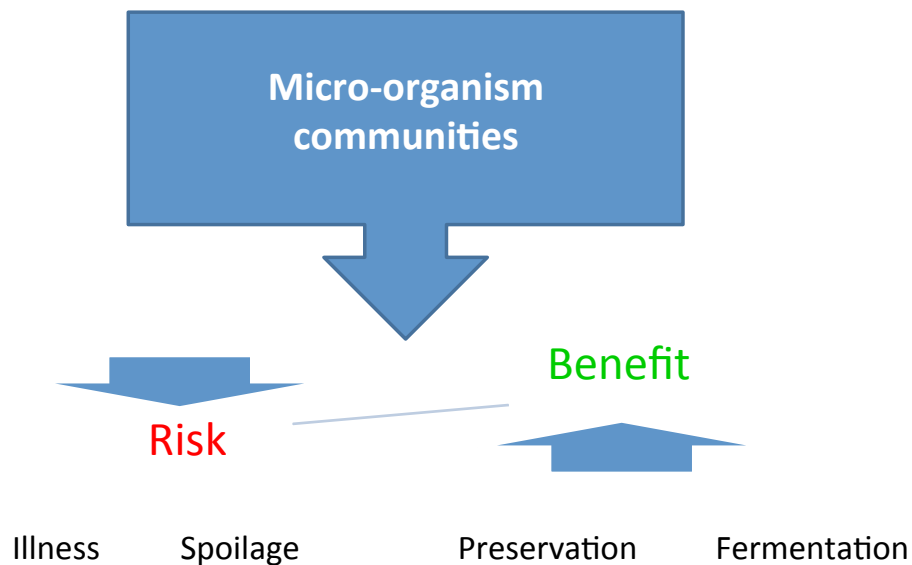
Food microbiota



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Microorganisms in food



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Which challenges?

- Moderate complexity of food ecosystem (regarding gut and soil)
- High stringent environment (fermentation/storage)
- No functional resilience?
- Which functionality to target ?
- Fermentation ? Spoilage? Preservation? safety? Health benefit?

Microbial ecologist has to face to uncultivability of species and to link single approach and community approach

Metagenomics
Culture independent
analysis of genetic
material of microbial
communities

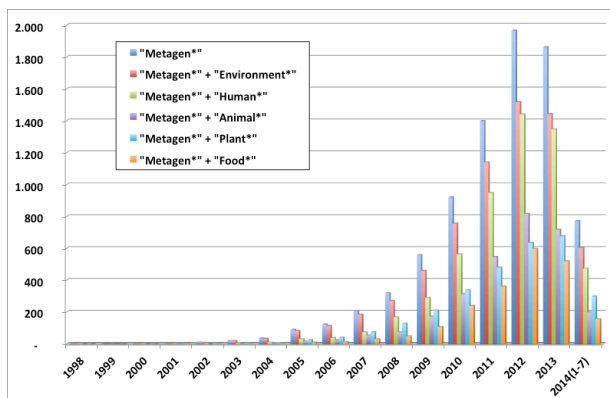


- Characterizing products
- Looking for new functionalities
- Monitoring process
- Selecting strains

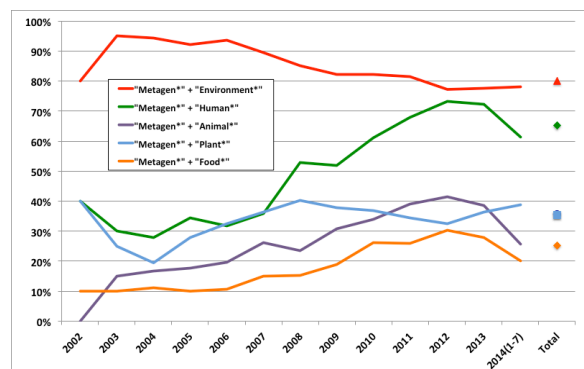
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Increasing reports in metagenomics



number



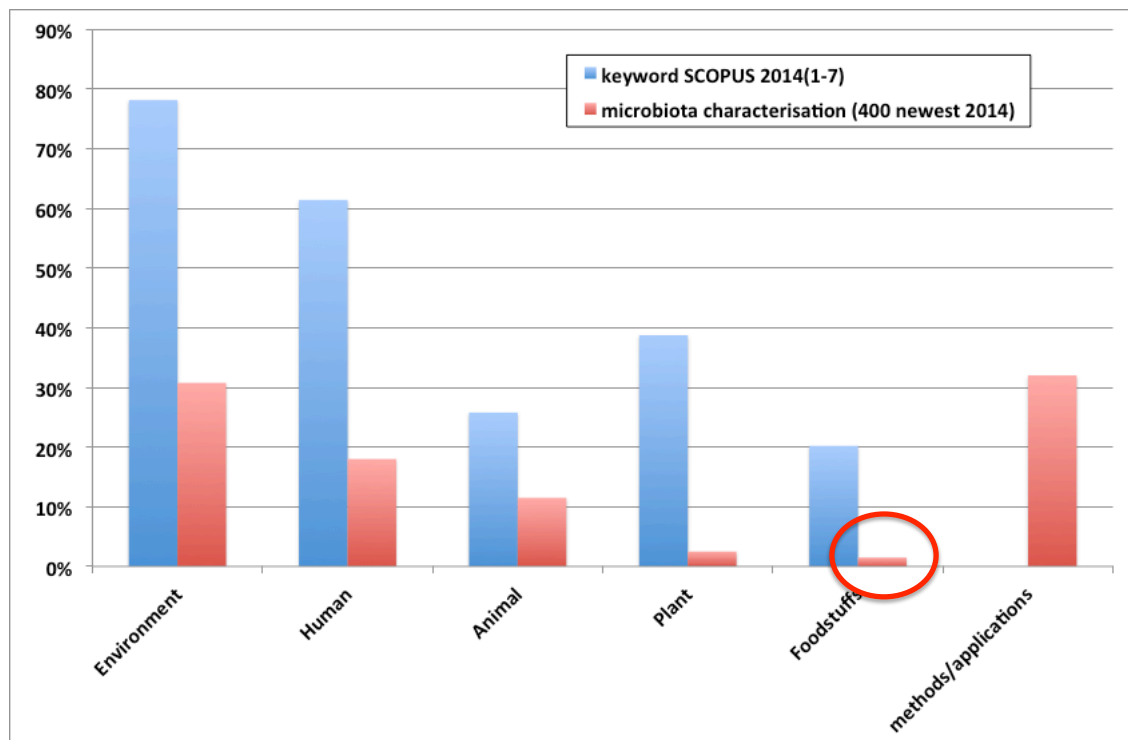
% total

Publications in Scopus

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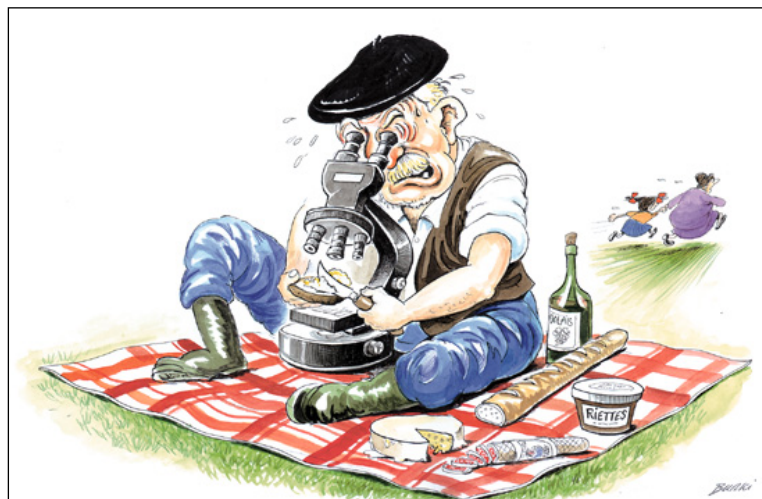
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But few food microbiota available



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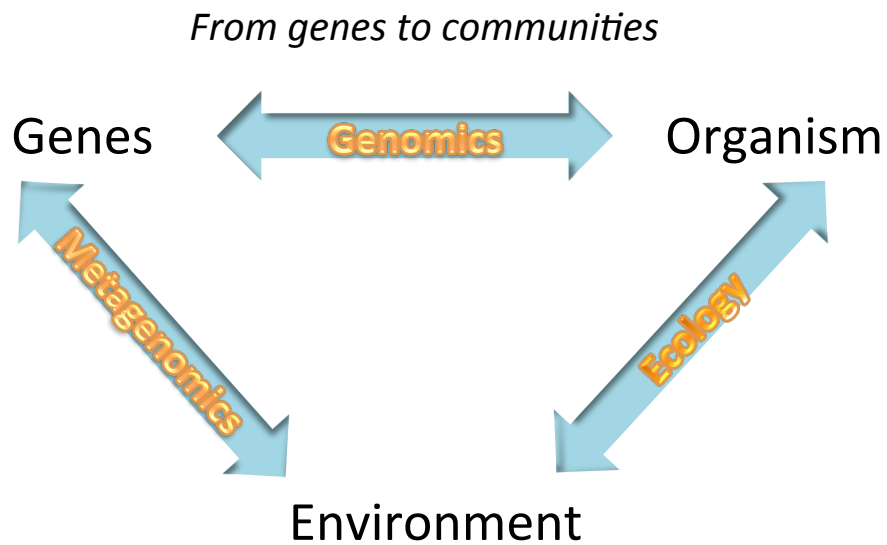
Which tools for which questions?

META(GEN)OMICS APPLICATIONS

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Metagenomics and ecosystem



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Metagenomics applications

- Who? What? How?
- Who is here?
- What do they do?
- Who makes what?

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How to identify microorganisms?

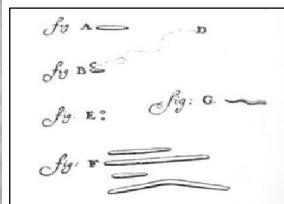
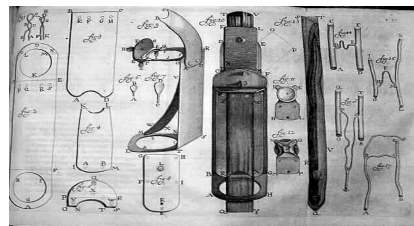
- How do they look like?
- What do they do, eat or produce?
- Who are they: genetic background?
- Alone, single cells
- All together (ecosystem)



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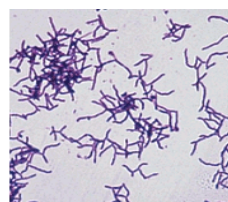
To see



Antonie Van Leeuwenhoek, 1676, first description of « animalcules »



Gram Negative



Gram Positive



Microscopy (Gram staining, 1884)

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To detect, to identify, to count



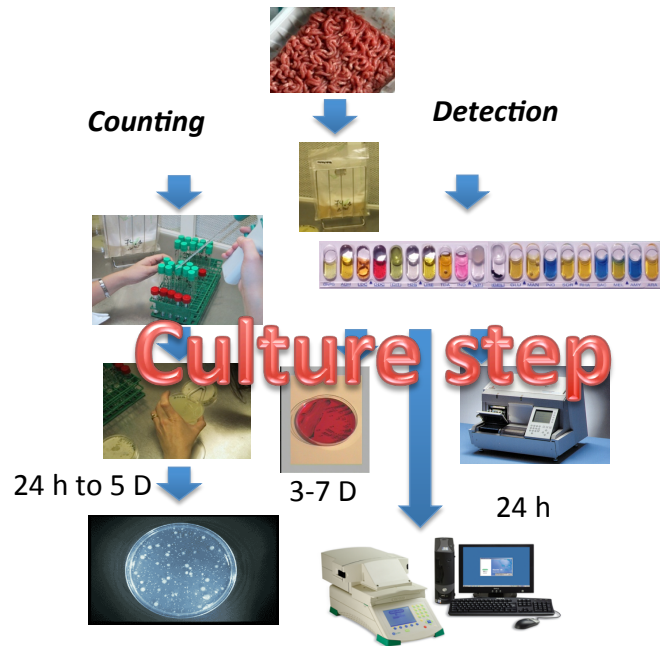
Louis Pasteur,
1822-1895



Robert Koch,
1843-1910



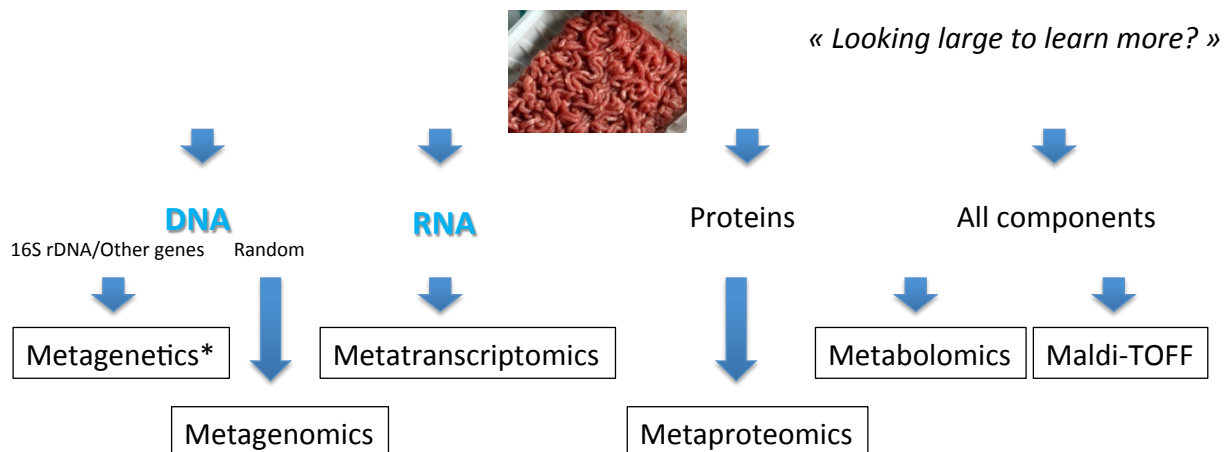
Julius Pétri, 1852-1921



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Culture-independent tools

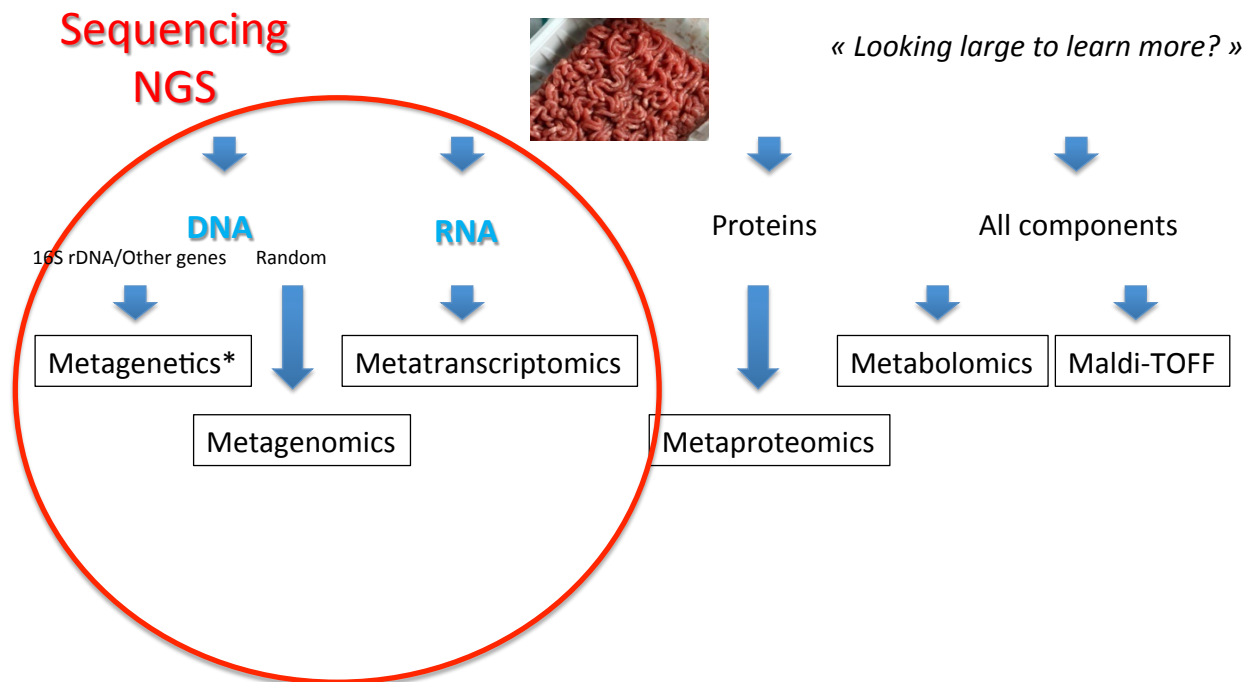


*Esposito and Kirschberg 2014, FEMS microbial lett 351 145-146

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Culture-independent tools

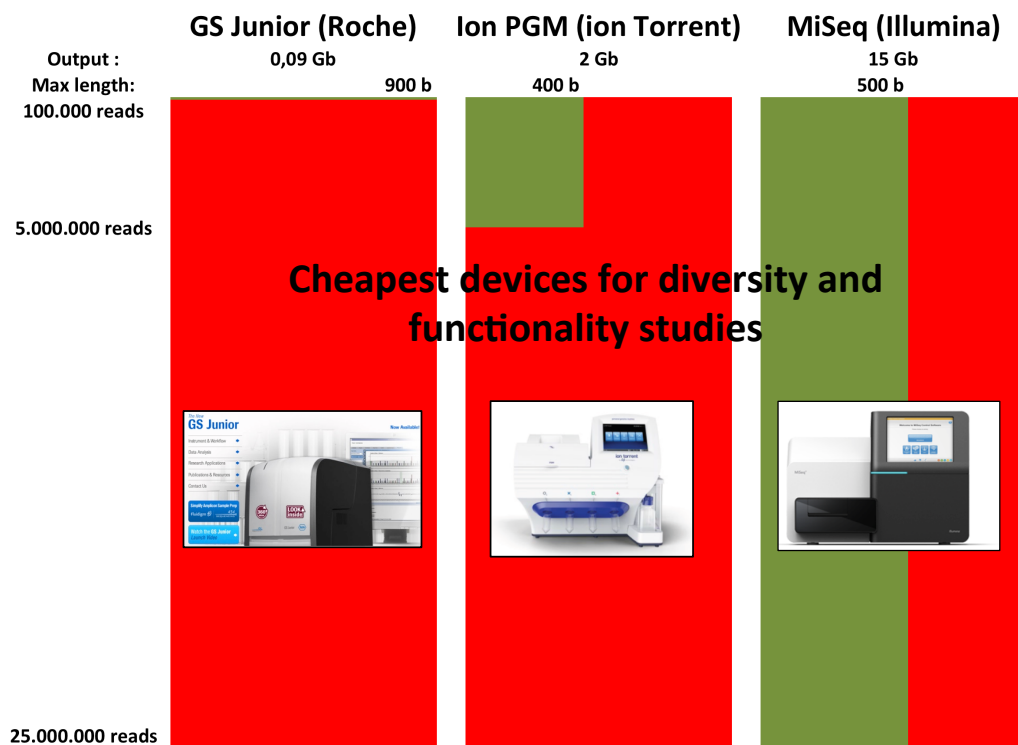


*Esposito and Kirschberg 2014, *fFEMS microbial lett* **351** 145-146

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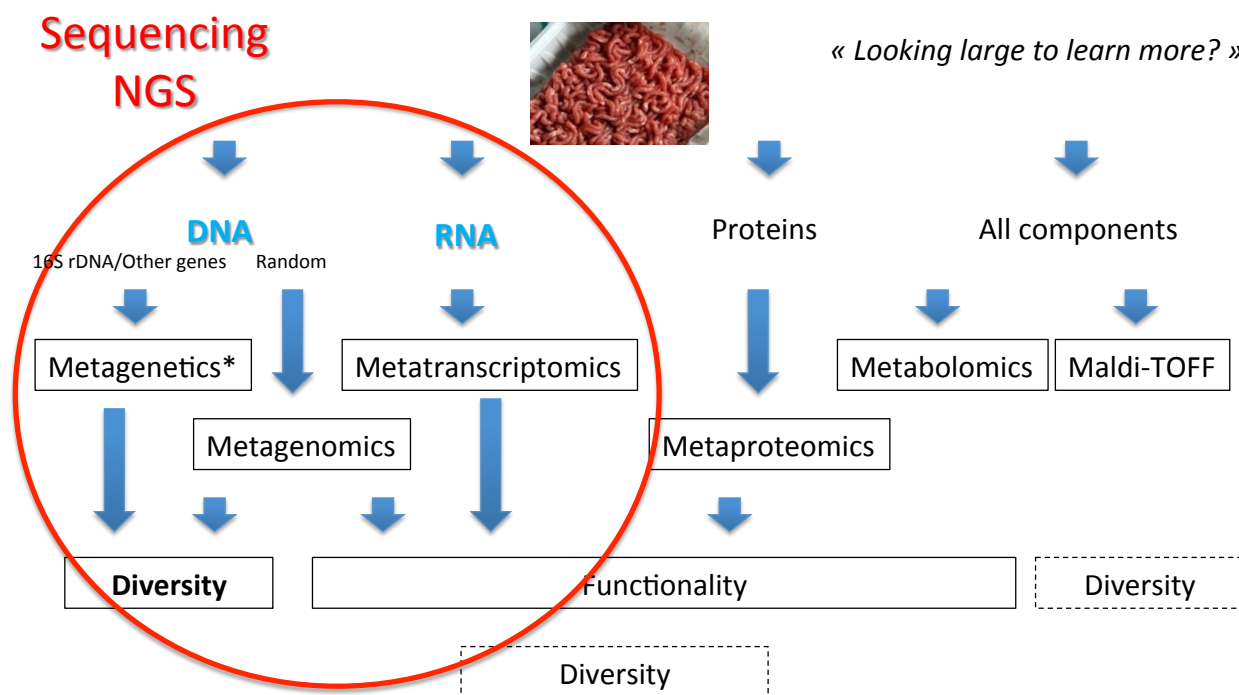
Sequencing: a fast evolving technology



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Culture-independent tools



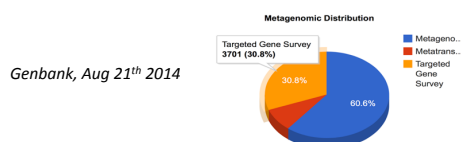
*Esposito and Kirschberg 2014, *fFEMS microbial lett* **351** 145-146

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Main applications of metagenetics, metagenomics, metatranscriptomics in food

Omics	Molecule	Data	Applications in food
Metagenetics	16s rDNA	Bacterial composition Phylogeny Specific target genes	Structure of Food microbiota Strain monitoring
Metagenomics	Genomic DNA	Gene catalogs	Population diversity Functions in food
Metatranscriptomics	mRNA (cDNA)	Gene expression	Functions in food



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Which microbes are present?

CHARACTERIZING DIVERSITY

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What for in food?

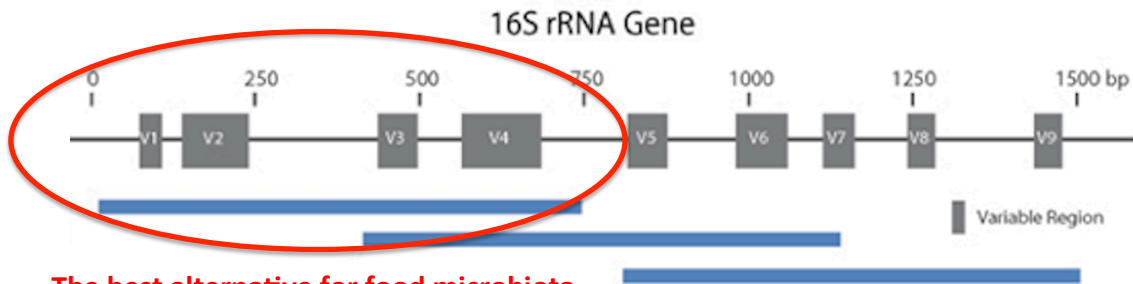
- To know the origin of products
- To identify bacteria responsible for food spoilage
- To follow and control process/storage
- To monitor strains

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16S rDNA metagenetics in food

A universal, highly conserved bacterial genetic marker



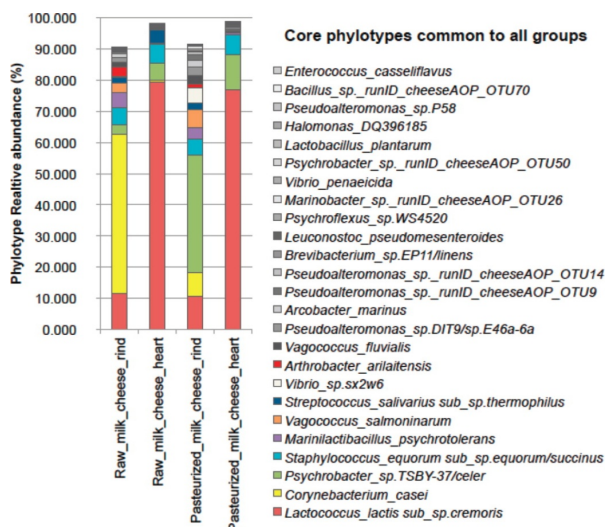
The best alternative for food microbiota characterization

- Many reports
- Microbiota characterization
- Fermented food (Asia), cheese, meat, sourdough, cocoa)
- Unsuspected diversity (species number)
- Unexpected groups (ex *Archaeobacteria*)

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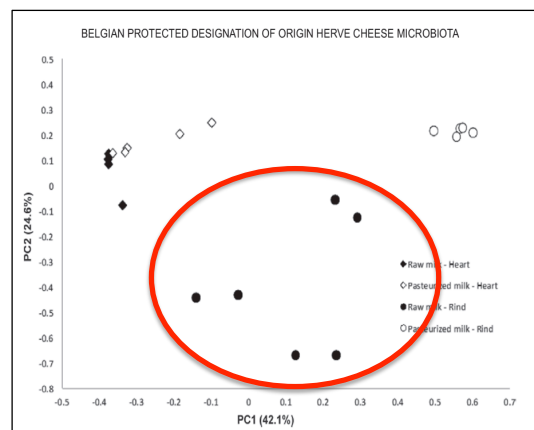
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Characterizing microbiota of a traditional artisanal cheese



Describing microbiota

A common core for raw and pasteurized cheeses



Comparing 22 cheeses

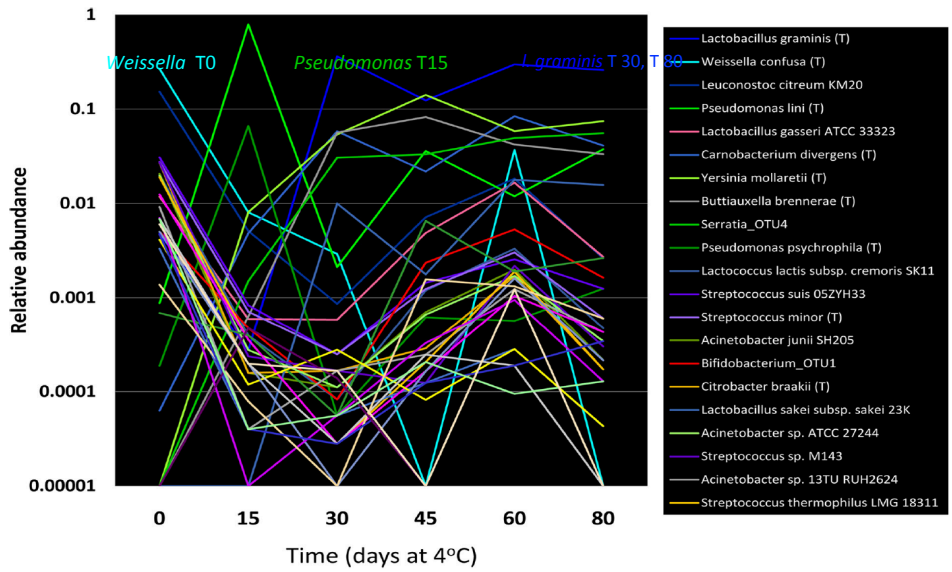
Diversity among raw milk cheese rinds

Delcenserie et al 2014, J Dairy Sci, 97: 1-11

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Following population dynamics in fresh sausage

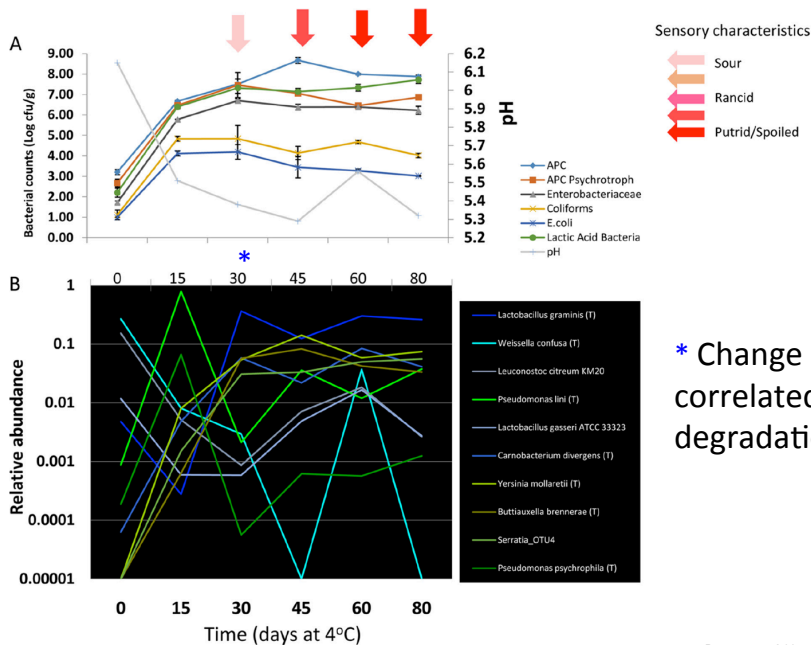


A quite long shelf life, different phases of population changes

Benson AK et al., 2014, Appl Env Microbiol **80**: 5178-94

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The actors of spoilage



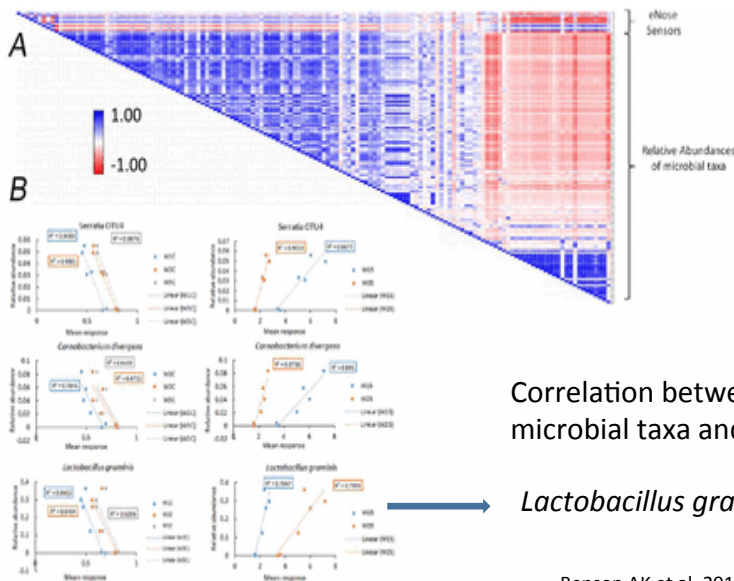
* Change in dominant species correlated with sensory degradation

Fresh sausage

Benson AK et al., 2014, Appl Env Microbiol **80**: 5178-94

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The actors of spoilage



Correlation between abundance of microbial taxa and sensorial analyses

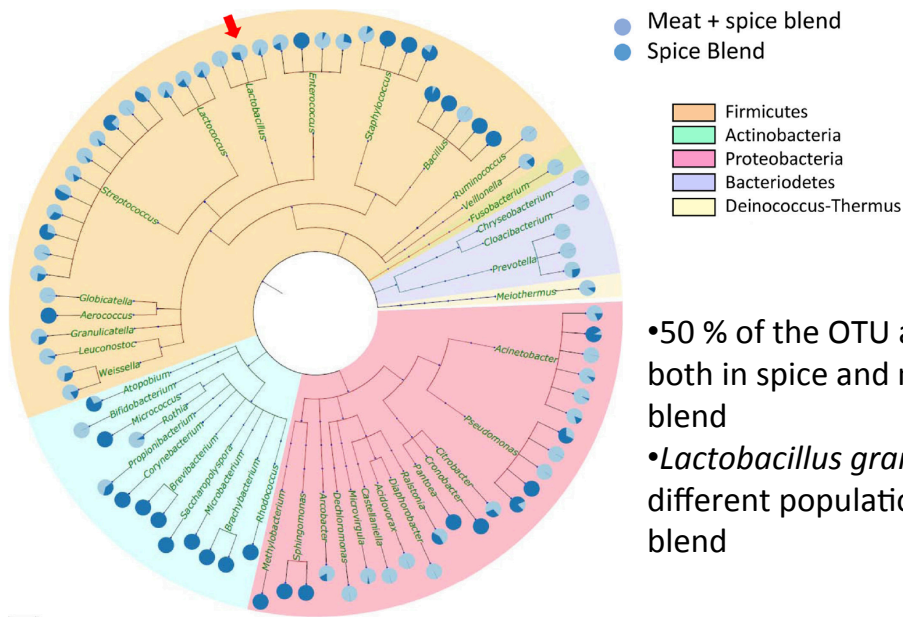
Lactobacillus graminis

Benson AK et al, 2014, Appl Env Microbiol 80: 5178-94

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Origin of microbiota



- 50 % of the OTU are present both in spice and meat + spice blend
- Lactobacillus graminis*: two different populations in spice blend

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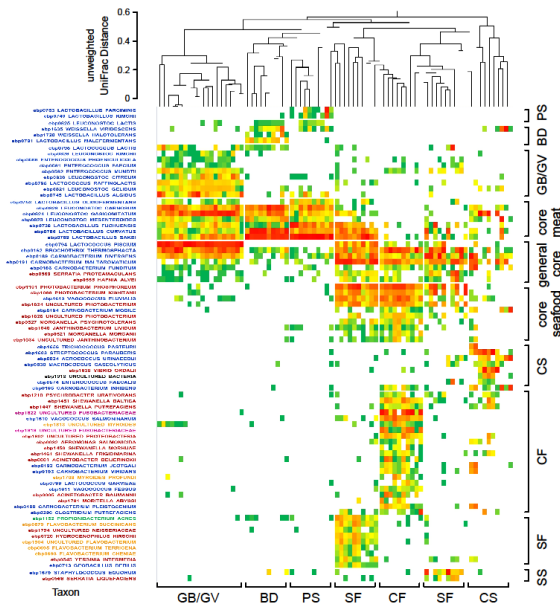
Benson AK et al., 2014, Appl Env Microbiol 80: 5178-94

Spice blend : a source of dominant *Lactobacillus*

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A revisited vision of food spoilage?



- Spoiled animal food products: fish and meat
- 15 to 60 species
- Putative new dominant spoiling species for fish?
- Yet uncultured

Chaillou et al in revision ISME J.

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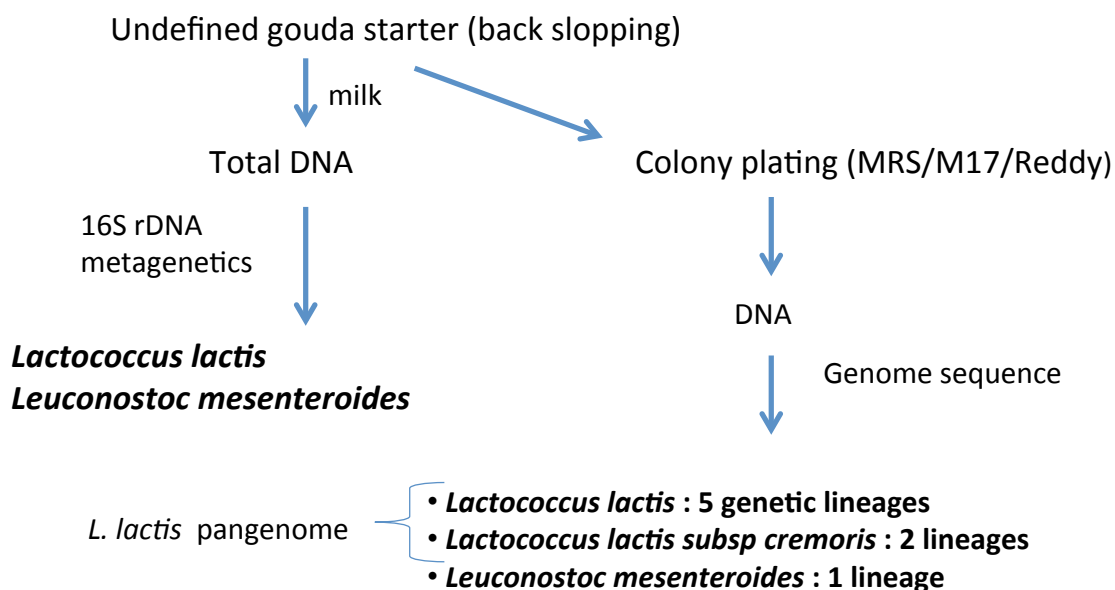
Beyond the species level: towards exploring strain diversity in communities

- 16S rDNA too much conserved to discriminate strains of the same species
- Find other approaches to discriminate strains within communities
- High selective pressure of fermentation process (cheese making)
- Reduced communities
- More robust performance of mixed strain starters
- High degree of genetic heterogeneity at the strain level rather than the species level

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Following mixed starters



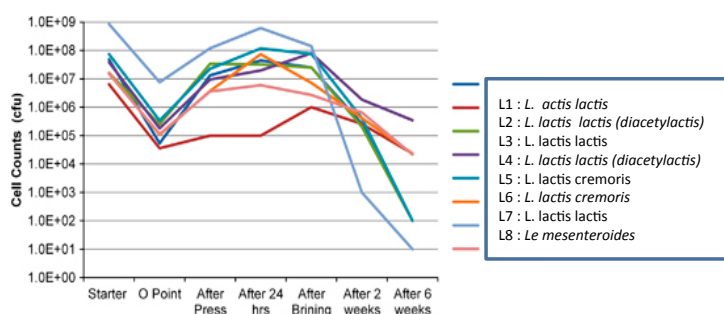
Erkus et al, 2013. ISME 7, 2126-2136

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Following mixed starters

« multifactorial diversity sustains microbial community stability »



Relative abundance of genetic lineages during cheese manufacturing reveals:

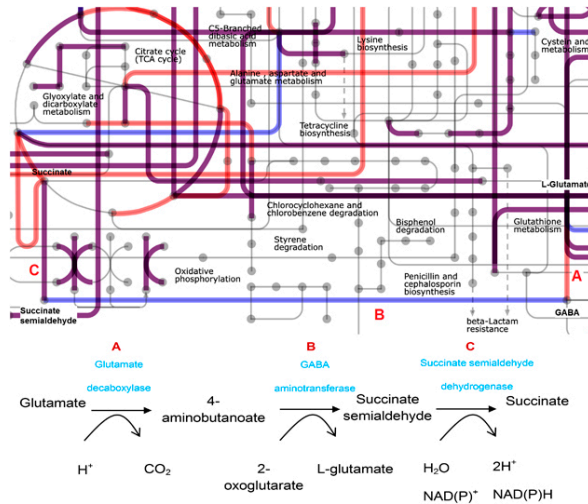
- effect of dynamic of the community on cheese making
- better survival lineage 2 and 4 and 8 (citrate utilizing) enhanced flavor

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Lessons from pangenome

Towards functionality



red: *Lactococcus lactis* pangenome
 blue: *Leuconostoc mesenteroides*
 purple : shared

Complementary reactions between strains in the community to fulfill the function : glutamate degradation

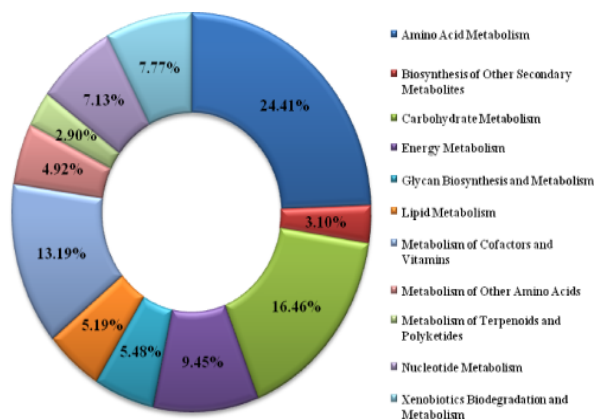
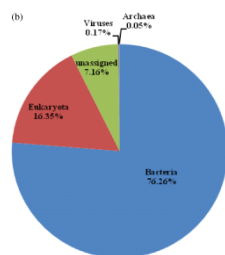
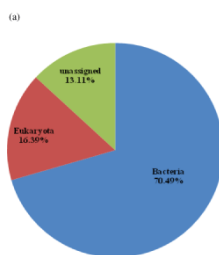
Erkus et al, 2013. ISME 7, 2126-2136

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Metagenomics in food

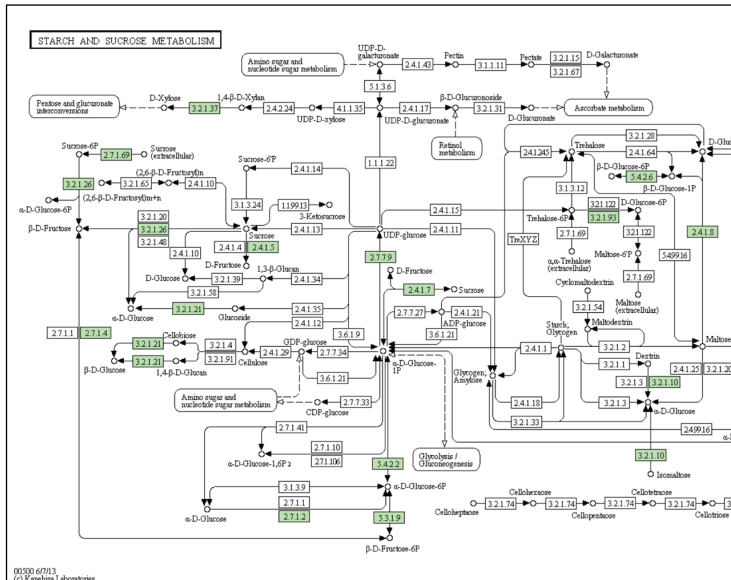
- Few studies
- Fermented Puer tea
- DNA pyrosequencing
- Bacteria and Eucaryota (yeast and moulds)
- Predicted metabolic profiles



Lyu et al 2013, J Sci Food Agri 93:3165-3174

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What do they do?

UNDERSTANDING FUNCTIONALITY

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Fermented food



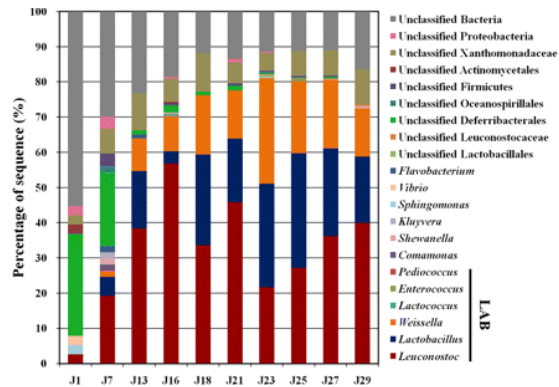
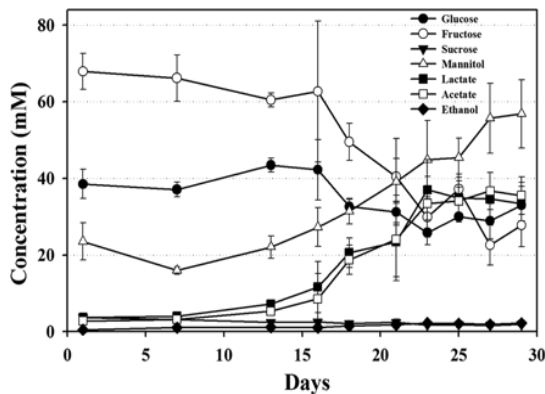
- Safe and sustainable
- Microbial communities moreless complex
- Generally low number of species for fermentation process
- Challenge: knowing and monitoring their interactions
- Starter selection

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Kimchi: a nice case of study

Spontaneous fermentation of vegetables (cabbage /radish)



A community where LAB are prevalent

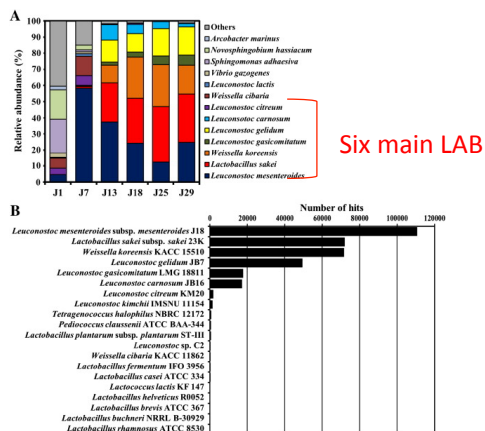
Park et al 2011 Appl. Environ. Microbiol. 77 : 2264-2274

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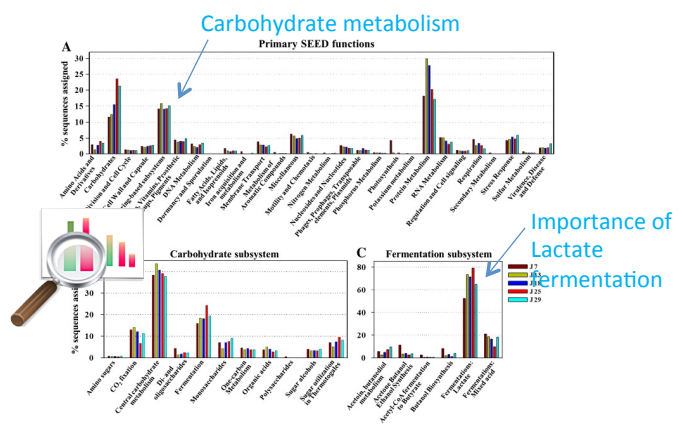
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What do they express?

Is this LAB community described in databases? Six main LAB



What is the metabolic potential expressed by this LAB community?

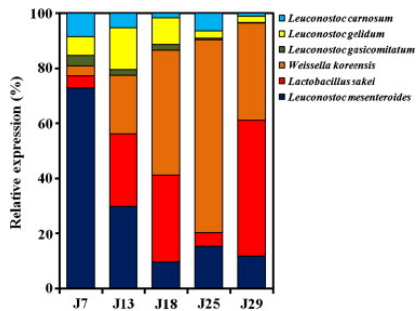


jung et al 2013 Int J food microbiol 163 :171-179

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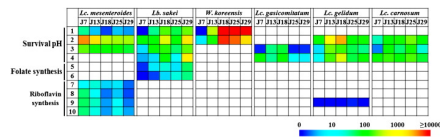
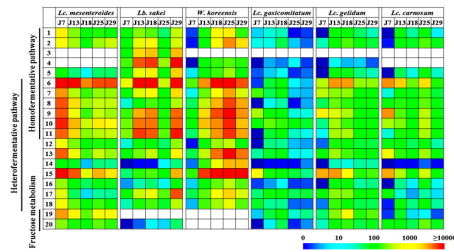
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Which species exprimes what and when?



During fermentation

- decreased expression of *Leuconostoc*
- increased expression of *L. sakei* and *Weissella*



Understanding dynamics of functional role of each species
Understanding dynamics of gene expression

Jung et al 2013 Int J food microbiol **163** :171-179

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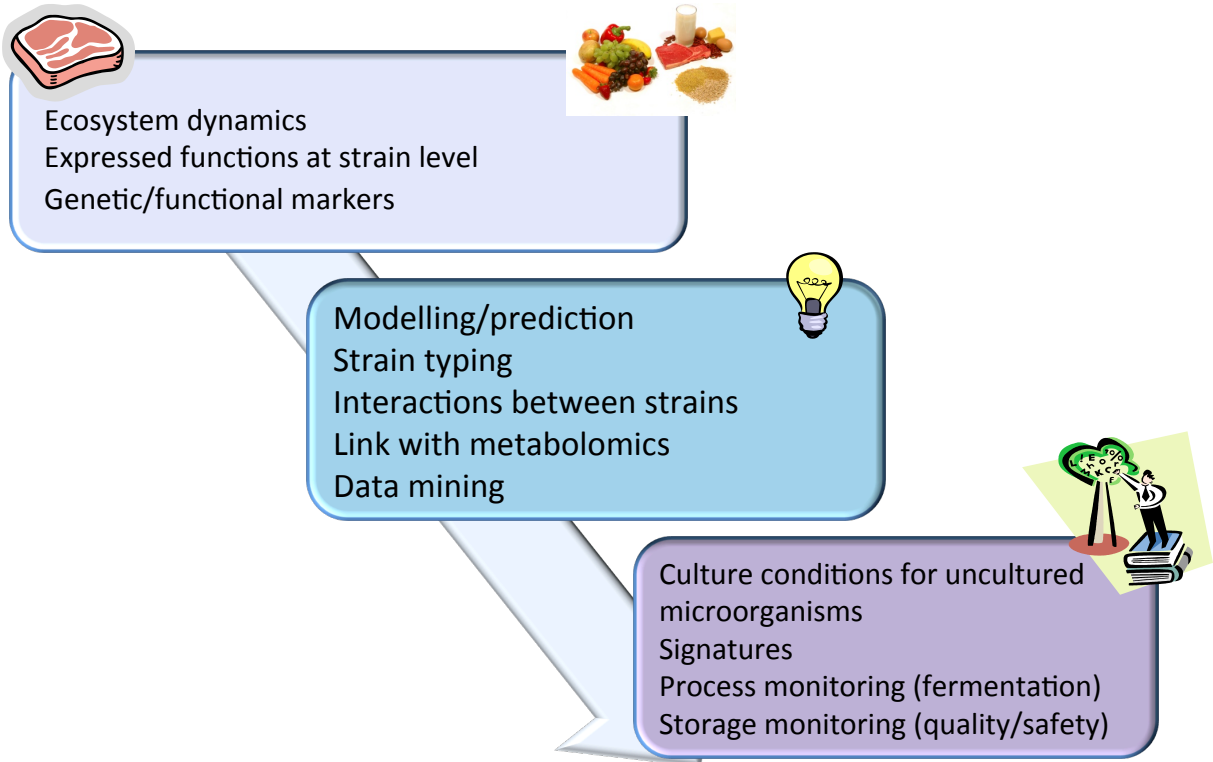
Dreaming

FUTURE

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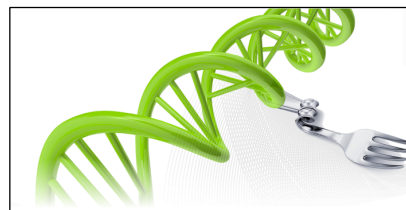
Further exploration of food microbial consortia



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CONCLUSIONS



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Take home message

- A new starting era for food microbiology
- A revisited vision of food ecosystems
- Exciting and promising future tools
- BUT SOME LIMITS

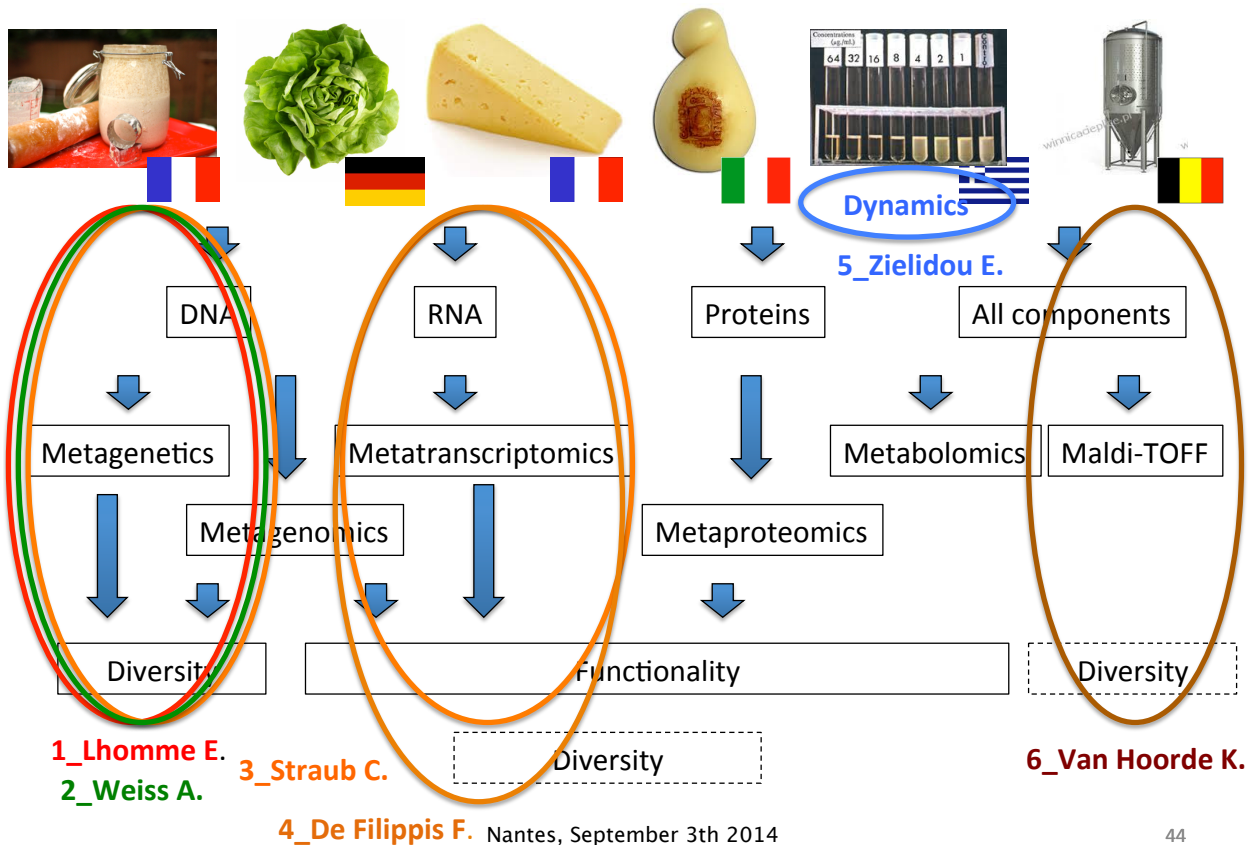
Needs for:

- A lot of microbiota associated with the composition/history of the food product in order to help to the interpretation
- Consolidated/validated databases
- Widespread bioinformatics tools
- Time!

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SESSION 2 – DYNAMICS AND FUNCTIONS OF MICROBIAL CONSORTIA



Thanks for your attention!