

## DESIGN OF BACTERIAL CONSORTIA FOR FERMENTATION OF NOVEL FOOD MIXING MILK AND LUPIN FLOUR

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### Introduction

Lactic acid bacteria (LAB) are widely used to ferment a variety of food resources. To ferment ‘new’ resources developed in the context of sustainable food systems, such as animal and plant mixes, the challenge consists in designing *de novo* specific consortia of strains that bring extra sensory and nutritional benefits to these raw materials.

In our approach, we chose the strains on their capability to use the main components present in animal and legume resources. This was challenging because it needs to bring together bacteria that do not naturally colonize the same habitats. For that purpose, we used different lactobacilli and lactococci strains. In order to design LAB consortia capable to hydrolyze the proteins and the carbohydrates of a mixed food composed of bovine milk and lupine flour, we developed a strategy that consisted in: i) selecting from an *in silico* study several mesophilic homofermentative LAB species, showing a high prevalence of the genes required to catabolize lupine and/or milk carbohydrates; ii) screening *in vitro* a pool of strains of these selected species for their ability to use milk and/or lupine carbohydrate and proteins; and iii) designing consortia of strains exhibiting complementary functional properties (figure 1).

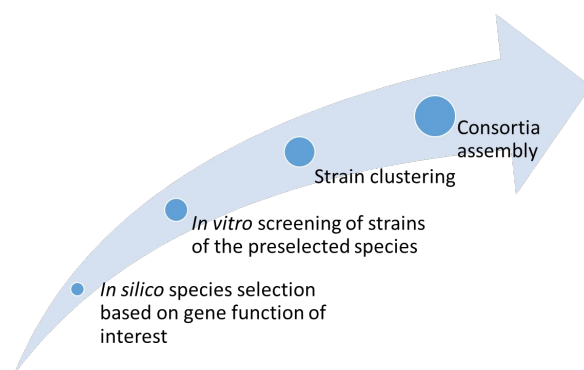
### Material and methods

*In silico* study was performed by using all the genome sequence data available for mesophilic homofermentative LAB strains to search for the genes encoding three carbohydrate-degrading enzymes:  $\alpha$ -galactosidase,  $\beta$ -galactosidase, and  $\alpha$ -glucosidase, for raffinose and/or stachyose, lactose, and sucrose hydrolysis, respectively. *In vitro* screening was performed on strains of all the preselected species on these enzymatic activities and on the proteolytic activities. Clustering was applied to bring together the strains that were considered as ‘metabolically equivalent’ and to design consortia that exhibit complementary abilities of carbohydrate and protein hydrolysis. Milk-lupine mixes were then fermented by LAB consortia consisted of several lactococci and lactobacilli strains and by pure cultures, separately. LAB populations, pH, content in residual carbohydrates, organic acids, volatile compounds, and free amino acids were determined in the fermented mixes.

## Results and discussion

The total LAB populations in consortia-fermented media were higher than the population observed in pure cultures. In agreement, consortia-fermented media had a lower pH and a higher content in lactate, the main acid produced, compared to pure cultures. Moreover, they contained significantly lower amounts of the various carbohydrates present, lactose and the lupine galacto-oligosaccharides, verbascose, stachyose, and raffinose, which can be beneficial to reduce the digestive discomfort. Glucose and sucrose were also totally utilized by the strains as in some consortia. Proteolysis indices markedly varied among the fermented mixes. Some non-proteolytic strains grew better in consortia than in pure culture, suggesting that they used peptides and amino acids released by proteolytic strains. These results suggest commensalism or mutualism between strains. Moreover, a diversity of volatile compounds were identified in the fermented mixes. Among these volatiles compounds, all the consortia were able to decrease the initial content in hexanal, which causes “greeny” off-flavor in legumes, in agreement with other studies (Schindler et al., 2011). Diacetyl or compounds derived from amino acid catabolism were present at a significantly higher amount in some fermented mixes.

These results confirm that the methodology of assembling consortia was successful. They actually illustrate how genomic and phenotyping data can be exploited to design consortia and produce ‘new’ fermented resources with different compositions susceptible to modulate their sensory and nutritional properties.



**Figure 1:** Workflow used to assemble bacteria according to targeted genes and complementarity of functions

## Keywords

Targeted functions, carbohydrates, proteins, consortia, mixed food

## References

Schindler S, Wittig M, Zelena K, Krings U, Bez J, Eisner P, Berger RG (2011). *Food Chemistry* 128:330–337.