Novel Protective Cultures for High Safety and Clean-Label Food Products

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State of the Art

In recent years, great attention has been paid to food safety (Al-Tayyar et al. 2020) highlighting the need to adopt "clean label approaches" as screens against foodborne spoilers and pathogens (Heymich et al. 2021). Bio-preservative strategies, including microorganisms (protective cultures -PCs-) or their metabolites, have been investigated. In the control of foodborne pathogens, several authors (Hammani et al. 2019; Mota-Gutierrez and Cocolin, 2021), reported a great number of protective lactic acid bacteria strains which commonly produce organic acids, peroxides, enzymes and bacteriocins. To date, the antagonistic action of commercial protective cultures is mainly due to the production of bacteriocins. However, the efficiency of the application of bacteriocins as food additives may be limited for various reasons, particularly for the low effectiveness against spoilage and pathogenic bacteria in food environment.

Therefore, it is important to search for other compounds with high antimicrobial activity with higher specificity.

On highlights bases, novel and clean-label approaches in food systems will be developed. Particularly, protective and non-bacteriogenic strains, more effective than those currently available, will be selected and validated in food system.

PhD Thesis Objectives and Milestones

A1) Producer strains isolation and identification

Lab from food products will be isolated and identified at species level by Polymerase Chain Reaction-Denaturing Gradient Gel Electrophoresis (PCR-DGGE) and 16S rRNA gene sequencing.

A2) Selection of protective cultures

Primary screening: antagonistic activity of the producing strains against a wide range of bacteria and moulds will be ascertained. Chromatographic and electrophoretic approaches will be applied to detect one or more metabolites involved in the antimicrobial effects. (A.2.1).

Secondary screening: non-bacteriogenic antagonist strains will be investigated for their effect growth and biofilm formation of spoilers and pathogens. (A.2.2) Additional, functional activity and flavouring properties (A2.3 A2.4)

A3) PCs validation in food system

The effectiveness of PCs as replacers of conventional and unhealthy additives and/or ingredients in innovative food products will be evaluated by PCs challenge tests.

A4) Writing and Editing

PhD thesis, scientific papers and oral and/or poster communications will be prepared.

Isolation identification
- Foods with harsh environmental conditions;
- Specie level identification: PCR-DGGE;
- Sequencing

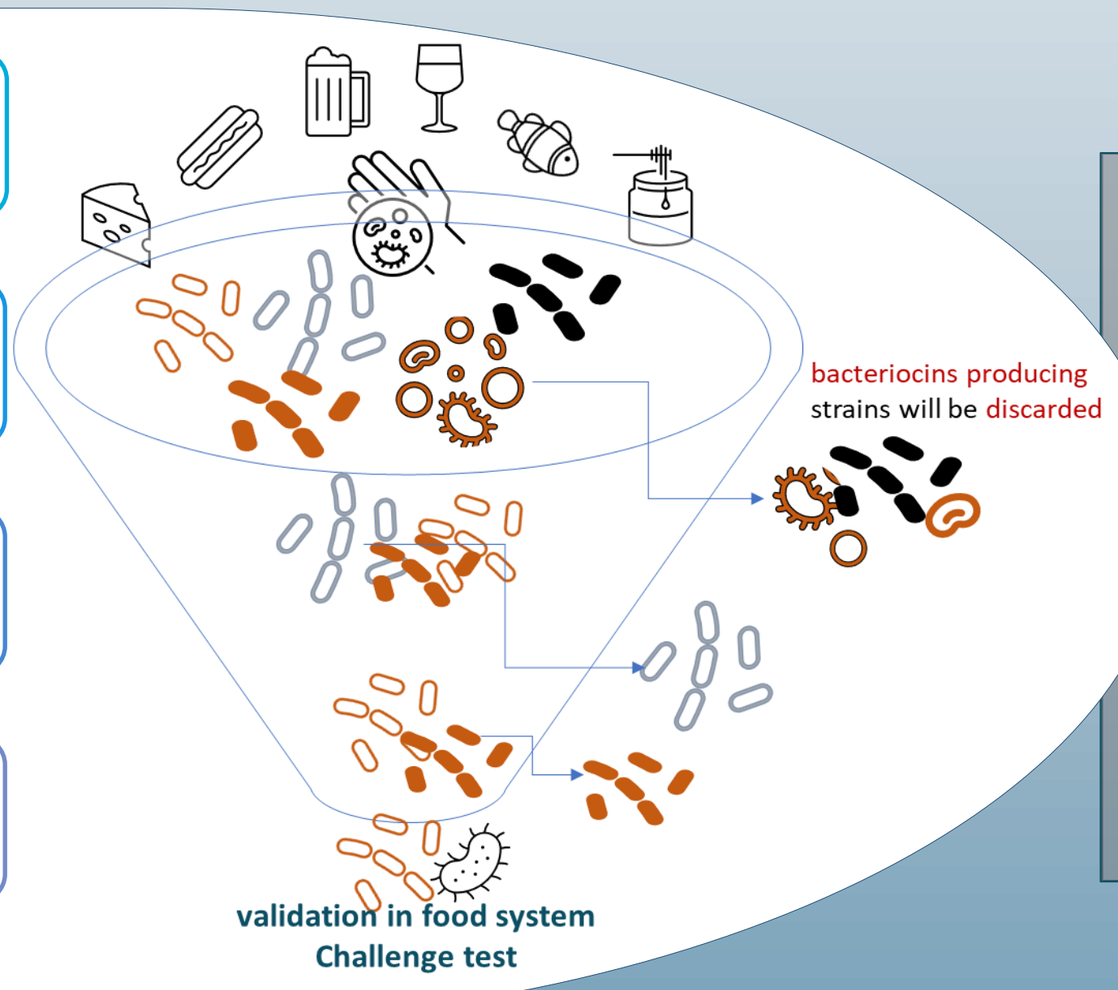
Primary screening

Effect on undesirable microorganisms
- Agar Well Diffusion Assay
- Antimicrobial metabolites (HPLC)
- Mode of action (2ED, PCRs)

Secondary screening

Effect on undesirable microorganisms
- Survival model and polynomial equations biofilm formation (crystal violet assay)

Functional flavouring properties
- **Health promoting activity** (adhesion to intestinal cellular line, resistance to human beta-defensins, short chain organic acids production)
- **Volatilome** characterization in model food



The main goal of the PhD project is the development and validation of biotechnologies to produce healthy food products, based on the use of innovative and multi-functional protective cultures, and the concomitant reduction of additives and anti-health ingredients

Gantt diagram for this PhD thesis project

Activity	Months	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24
A1)	**Producer isolation and identification**	■	■	■	■	■																			
	Isolation	■	■																						
	PCR-DGGE	■	■	■	■																				
	16S rRNA sequencing		■	■	■	■																			
A2)	**Selection of protective cultures**			■	■	■	■	■	■	■	■	■	■	■	■	■	■								
	Primary screening			■	■	■	■	■	■	■	■	■	■												
	Secondary Screening									■	■	■	■	■	■	■	■								
	Additional functional activity													■	■	■	■								
	Additional flavouring properties													■	■	■	■								
A3)	**PCs validation in food system**																	■	■	■	■	■	■	■	■
A4)	**Thesis and Paper Preparation**	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

References

Al-Tayyar, N. A., Youssef, A. M., & Al-Hindi, R. (2020). Antimicrobial food packaging based on sustainable Bio-based materials for reducing foodborne Pathogens: A review. Food chemistry, 310, 125915.

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