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Molecular interactions between *Staphylococcus aureus* and bacteriocin-producing *Lactococcus lactis* in experimental matrices

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This PhD research project aims to characterize the microbial interaction between nisin-producing *Lactococcus lactis* and *Staphylococcus aureus* strains under experimental conditions. In particular, the project will predominantly focus on elucidating the molecular interactions between both microorganisms, in order to assess their transcriptomic and proteomic response profiles when submitted to different culture conditions.

Interazioni molecolari tra *Staphylococcus aureus* e *Lactococcus lactis* produttore di batteriocina in matrici sperimentali

Questo progetto di tesi di dottorato ha lo scopo di chiarire l'interazione microbica tra ceppi di *Lactococcus lactis* produttori di nisina e *Staphylococcus aureus* in condizioni sperimentali. In particolare, questo studio si concentrà prevalentemente sulla caratterizzazione moleculare della interazione tra questi due microrganismi, al fine di valutare i loro profili di risposta trascrittomica e proteomica sotto diverse condizioni di coltura.

1. State of the Art

Lactic acid bacteria (LAB) are predominant constituents of the autochthonous microbiota of various foods, such as milk and dairy products (AXELSSON et al., 2001). Several LAB species are capable of interfering with the development of spoilage and pathogenic microorganisms in foods, mainly by reducing the pH, competing for nutrients or production of antimicrobial substances. Among these, it is notable the role of bacteriocins, extracellular proteinaceous compounds which were proven to have bacteriostatic and/or bactericidal effects against several microorganisms, usually phylogenetically related bacteria (CARR et al. 2002). Bacteriocins are ribosomally synthesized proteins that lead to pore formation in the plasma membrane of the target cell, and for which the producer cell has a specific mechanism of protection. *Lactococcus lactis* subsp. *lactis* is a well-characterized LAB specie capable of producing nisin, a bacteriocin approved for application in foods to control the growth of certain pathogens (COTTER et al. 2005).

Staphylococcus aureus is a pathogen of major concern in foodstuffs, especially in milk and dairy products, due to the ability of certain strains to produce thermoresistant enterotoxins that may lead to gastrointestinal disorders when ingested (LE LOIR et al., 2003). The growth and virulence of *S. aureus* can be strongly affected by the presence of *L. lactis* in a food context. Moreover, it was demonstrated that the inhibitory effect of nisin on *S. aureus* when applied in foods is mostly revealed as a remarkable reduction in pathogen counts and/or lack of staphylococcal enterotoxin production (CHARLIER et al., 2009).

Despite the recognized inhibitory influence that LAB may have over certain food pathogens, the molecular mechanism of these inhibitory effects is still poorly documented. Therefore, the molecular characterization of interactions involving bacteriocinogenic LAB and enterotoxigenic *S. aureus* may significantly lead to novel approaches for the control of this major pathogen in foods.

The general aim of this study is to characterize the microbial interactions occurring between nisin-producing *L. lactis* and enterotoxigenic *S. aureus* under experimental conditions.

2. PhD thesis Objectives and Milestones

Within the overall objective mentioned above, this PhD thesis project could be divided into the following activities according to the Gantt diagram given in Table 1:

A1) Bibliographic Research

A2) Optimization of culture conditions

1) Selection of most suitable strains
2) Assessment of most appropriate culture conditions

A3) Transcriptomic Analysis

1) Preparation of experimental matrices (culture media, reconstituted milk and soft cheese)
2) RNA extraction and cDNA conversion
3) RNA-Seq gene expression analysis

A4) Proteomic Analysis

1) Preparation of experimental matrices (culture media, reconstituted milk and soft cheese)
2) Protein extraction and manipulation
3) Assessment of proteomic profiles by Peptide Mass Fingerprinting

A5) Writing and Editing of the PhD thesis, scientific papers and oral and/or poster communications.

Table 1. Gannt diagram for the present PhD thesis project.

Activity		Months																	
		1	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30	32	34	36
A1	***Bibliographic Research***																			
A2	***Optimization of culture conditions***																			
	Selection of most suitable strains																			
	Assessment of most appropriate culture conditions																			
A3	***Transcriptomic Analysis***																			
	Preparation of experimental matrices																			
	RNA extraction and cDNA conversion																			
	RNA-Seq gene expression analysis																			
A4	***Proteomic Analysis***																			
	Preparation of experimental matrices																			
	Protein extraction and manipulation																			
	Assessment of proteomic profiles by Peptide Mass Fingerprinting																			
A5	***Writing and Editing of PhD thesis***																			

3. Selected References

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