Anti-Quorum Sensing Activity of Probiotics against Foodborne-Spoilage and Pathogenic Bacteria

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Quorum sensing (QS) is a mechanism for cell-to-cell communication between inter- and intra-bacterial species that is employed to regulate the gene expression of virulence factors. Microbial control through QS inhibition is a rising interest of research. However, utilisation of probiotics as a source of QS inhibitors is under-researched, in particular, against food spoilage bacteria. This project aims at the following: 1) to identify the type of QS in isolates of foodborne-Pseudomonas and Campylobacter species. 2) to investigate the anti-QS activity of probiotics against these isolates. 3) to enhance the anti-QS activity through microencapsulation.

**Attività di rilevamento anti-quorum sensing dei probiotici contro i batteri patogeni e il deterioramento di origine alimentare**

Questo progetto di tesi di dottorato mira a mettere a punto un procedimento sperimentale, in batch o a riciclo totale, prima in impianto da banco e poi in impianto pilota, atto ad individuare il modello matematico in grado di simulare il processo di recupero di selezionati biopolimeri di interesse alimentare mediante moduli a membrana di ultrafiltrazione tubolari o a fibre cave, consentendone il dimensionamento ottimale in scala industriale.

# 1. State-of-the-Art

Quorum sensing is a cell-to-cell communication that occurs between intra-and inter-bacterial species and is regulated by signalling molecules called autoinducers (AIs). When the AIs reach a certain threshold of levels, they bind to protein receptors, which then initiates QS circuit. There are three common types of autoinducers, which are produced for initiation of QS. The first is acyl-homoserine lactones (AHLs) that is produced by *luxI* gene in Gram-negative bacteria. The second is autoinducer peptides (AIP) that is produced by *agrD* gene in Gram-positive bacteria. The third one is called AI-2 that is synthesized by *luxS* gene, and this can be present in Gram-negative and Gram-positive bacteria.

Bacterial communication in foods through QS plays a role in the food quality and safety. That is, production of virulence factors by foodborne-pathogenic and spoilage bacteria is regulated by QS mechanism. For example, spoilage of dairy products by biofilm and enzymatic activity (proteolytic, and lipolytic) of *Pseudomonas* species (*P. fluoroscens, P. fragi, P. gassari, P. putida* and *P. lactis*) is regulated by AHL-type QS system (Quintieri et al., 2021. In addition, spoilage in meat and vegetables is also induced by Gram-negatives, where acyl-homoserine lactones (AHL) and AI-2 act as signalling molecules for QS induction. The AHL autoinducers (C4-HSL, C6-HSL and C6-3-oxo-HSL) were detected in chilled-stored meat products and vegetables (broccoli, parsley, carrots, and spinach) spoiled by Enterobacteriaceae and Aeromonas species. In addition, AI-2 activity was also found in meat products contaminated with Pseudomonas fragi and E. coli O157:H7 (Skandamis *et al*., 2012). Milk can be also contaminated with pathogenic Gram-positive bacteria such as Listeria. monocytogenes that uses peptides (i.e., AIP) as QS signalling molecules to trigger gene expression for biofilm formation (Bai *et al*., 2021).

With the widespread use of antibiotics in animal industry, spread of antimicrobial-resistance bacteria in foods is of a major concern for food industry. Microbial control through QS inhibition is an alternative strategy to overcome antimicrobial resistance of bacteria due to the fact that QS disruption would inhibit expression of virulence-related genes without growth inhibition. A wide range of plant-derived compounds (such as phenol acids) and QS signal-degrading enzymes were demonstrated as QS inhibitors (QSIs) (Machado *et al*., 2020). Oregano essential oil, cinnamaldehyde and catechins inhibited the expression of virulence factors by *P. fluorescens* (extracellular protease activity, biofilm formation, swarming and swimming motility) through anti-QS activity (Rossi *et al*., 2018; Ding *et al*., 2019). In addition, in-vitro studies showed that probiotics are a promising source of QS inhibitors against foodborne pathogens (Davares *et al*., 2022). However, in-situ studies investigating anti-QS activity of probiotics is yet to be demonstrated. Furthermore, QS inhibition in foodborne-spoilage bacteria is still lacking. Indeed, QS inhibitors from probiotics can be utilised as a sustainable and safe method of food preservation.

# 2. PhD Thesis Objectives and Milestones

Within the overall objective mentioned above this PhD thesis project can be subdivided into the following activities according to the Gantt diagram given in Table 2:

A1) **Isolation of foodborne pathogenic and spoilage bacterial strains from dairy and meat products** (*Pseudomonas* and *Campylobacter* species), *Pseudomonas* species are main spoilage species in meat and dairy products. *Campylobacter* species are causative of Campylobacteriosis, which is the main foodborne illness in the European union in the last few years. Assessment of virulence factors of the isolates such as enzymatic activity and biofilm formation.

A2) **Identification of the type of QS system** in the isolated *Pseudomonas* and *Campylobacter* strains

A3) **Investigation of the anti-virulence and anti-QS activity** of a wide range of probiotics *lactobacillus* and *Bifidobacterium* strains (as planktonic cells, or their metabolites and cell-free extract) against the isolates to select the most effective probiotic strains.

A4) **Transcriptomic analysis** of QS-related and virulence-related genes to study the change in genes expression after treatment with probiotics (in-vitro and in situ).

A5) **Studying the effect** of microencapsulation on the anti-QS activity of probiotics strains and the expression of genes encoding production of QS inhibitors.

A6) **Writing and Editing** of the PhD thesis, scientific papers and oral and/or poster communications.

***Table 2***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) | ***Isolation of Target Bacteria*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Isolation of *Pseudomonas* and *Campylobacter* species |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Assessment of virulence factors |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2+3) | ***Screening of QS Systems in the Isolates*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Identification of the type of QS system using bioreporter strains |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Investigation of anti-QS activity of probiotics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Transcriptomic Analysis*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Transcriptomic analysis of QS-related genes |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | Transcriptomic analysis of virulence-related genes |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Effect of Microencapsulation on QS*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Effect of microencapsulation of QS-related genes expression in probiotics |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Anti-QS activity of probiotics microcapsules |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

# 3. Selected References

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