Study of the Effects of Plant-Derived MicroRNAs on the Human Gut Microbiota

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This PhD project aims to investigate the stability of plant-derived microRNA (miRNA) throughout the human digestion process, as well as the potential impacts of miRNA on the gut microbiota and their subsequent metabolic responses. In vitro and in-vivo models will be used to evaluate miRNA stability and their impact on the gut microbiota and metabolites. The study has implications for the food and healthcare industries and could contribute to the development of therapeutic treatments.

Studio degli effetti dei microRNA di origine vegetale sul microbiota intestinale umano

Questo progetto di dottorato si propone di valutare la stabilità dei microRNA di origine vegetale assunti dall’uomo con la dieta durante il processo di digestione, così come i suoi potenziali effetti sul microbiota intestinale e la sua risposta metabolica. A tale riguardo, saranno utilizzati modelli in vitro e in vivo. I risultati ottenuti potranno suppportare le industrie alimentari e farmaceutiche nel potenziale sviluppo di nuovi alimenti funzionali e trattamenti terapeutici.

# **1. State-of-the-Art**

The gut microbiota, commonly called our "forgotten organ," with a thriving microbial population of approximately 100 trillion bacteria plays a crucial role in human health and disease. The stability of the intestinal microbiota is directly linked to the well-being and disease of mammals. Indeed, it is responsible for various metabolic processes such as energy generation and storage, digestion and absorption of undigested carbohydrates, and communication with the immune system. Microbiota helps to support immune cell maturation and appropriate immune responses, regulating biological functions through diverse metabolic genes, enzymes, and biochemical pathways (Clemente et al., 2012). Additionally, the production of bioactive substances like vitamins, amino acids, and lipids is heavily dependent on gut bacteria (Hou et al., 2022). Eukaryotic organisms commonly contain miRNAs, a type of non-coding RNA that undergoes processing within the nucleus before acquiring functionality within the cytoplasm. MicroRNAs are frequently present within small, membranous vesicles such as exosomes, microvesicles, and apoptotic bodies, or they can associate with RNA-binding proteins and high-density lipoproteins. These miRNA vesicles possess varying sizes, ranging from 20 to 5000 nm in diameter (Li et al., 2019). miRNAs regulate more than 60% of human protein-coding genes, in which the human genome contains 1881 high-confidence miRNAs (Cui et all., 2016). Endogenous miRNAs are present in human plasma, urine, saliva, and body fluids, and they have been linked to various diseases including obesity, diabetes, and cancer (Li et al., 2019). Moreover, microRNAs (miRNAs) act as regulators of epigenetic mechanisms by influencing the synthesis of proteins that modify gene expression without causing DNA mutations. Epigenetic regulation plays a critical role in developing and maintaining cellular identity, function, and response to environmental stimuli. The reversible nature of epigenetic changes makes them potential targets for therapeutic interventions in various diseases (Yao et al., 2019; Ramzan et al., 2021). The miRNA-epigenetic feedback loop is regulated by DNA methylation, RNA modification, and histone modification (Yao et al., 2019). The diet could modify gene expression through miRNA regulation and its potential impact on disease development has been supported by the recent emergence of a new field called nutrimiRomics. NutrimiRomics combines health, diet, and genetics to understand how dietary elements influence gene expression, with miRNA research. Recent studies suggest that miRNAs present in diet can be absorbed by the host gastrointestinal system and modulate miRNA machinery, similar to minerals, vitamins, and micronutrients, potentially aiding in the maintenance of healthy homeostasis. The stability of miRNAs is controversial. Various studies have revealed that miRNAs are sufficiently stable throughout food processing and digestion whereas other studies widely believed that RNA could be destroyed during boiling. Nevertheless, miRNA networks play a significant role in regulating tumour metastasis-induced gut metabolites. Host intestinal cells secrete miRNAs that can regulate microbial growth and the abundance of intestinal microbiota by exchanging DNA. The gut microbiota can affect host intestinal miRNAs and modulate innate and adaptive intestinal immunities (Fan et al., 2022; Bi et al., 2020). Therefore, the role of miRNAs in regulating gene expression, modulating the gut microbiome, and their potential as a dietary intervention to improve gut health and prevent gastrointestinal diseases is an active area of research. The interaction between miRNAs and gut microbiota is crucial in regulating intestinal homeostasis, and dysbiosis of the gut microbiota is strongly correlated with a variety of intestinal diseases. Understanding the connections between gastrointestinal diseases, miRNA stability, and other variables impacting intestinal health could potentially lead to the development of miRNA-based therapies for digestive disorders (Bi et al., 2020). Thus, this PhD project will address the controversy surrounding miRNA stability and its impact on gut microbiota and their metabolites, specifically focusing on how miRNAs regulate the gene expression of microorganisms. The results of this research will provide valuable insights into the potential of plant-derived miRNAs for gut health.

# **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 1:

A1) **Stability of miRNAs** will be evaluated through both in vitro assessment using commercial enzymes (A1.1) and in vivo trials involving oral digestion (A1.2). This approach relies on the digestion of microRNA-containing purified extracts provided by Mirnagreen and subsequent quantification to determine its effectiveness.

A2) **Capacity of miRNAs to change the functionality of high-potential probiotics** will be testedto assess differences in fermentative profiles, phenotypes, and functionality of probiotics at different conditions using the MicroArray (PM) platform (OmnilogSystem) (A2.1) and genomics analysis (A2.2). Based on that investigation, the capability of microRNAs to enhance the production of neurotransmitters or other compounds that have health-promoting effects will be assessed.

A3) **Microbiota profile at the gut level using SHIME** will be determinedto investigate the stability of microRNAs during gastrointestinal transit and their impact on gut microbial populations and cell activity. The system simulates the human gastrointestinal tract and includes five bioreactors to mimic the stomach, small intestine, and colon. The microRNA-containing extracts will be fed to the system and miRNA survivability will be evaluated through extraction and quantification methods (A3.1). During the treatment, short-chain fatty acids (SCFA), 16S rRNA and volatile compounds (VOCs) will be analyzed (A3.2).

A4) **Health-promoting properties of microRNAs at the intestinal level using Caco-2 cell** will be analyzedto assess the capacity of miRNA extracts to affect the cellular redox state (A4.1), the release of pro-inflammatory mediators, and the permeability of the human colon carcinoma Caco-2 cells (A4.2).

A5) **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) | ***MicroRNA stability*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Static *in-vitro* digestion |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Oral digestion (*in-vivo* trials) |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A2) | ***Effect of miRNAs on the functionality of potential probiotics*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Evaluation of metabolic profile |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Genomic Analysis |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A3) | ***Effect on gut microbiota (SHIME)*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Quantification of miRNAs |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) 16S taxonomy and VOCs, SCFA |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A4) | ***Conducting Caco-2 cells model*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 1) Antiradical properties |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
|  | 2) Anti-inflammatory properties |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| A5) | ***Thesis and Paper Preparation*** |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |

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