Microbiome Mapping in Meat Food Chain from Farm-to-Fork

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This Ph.D. research project develops in collaboration with Dawn Meats Group, a main meat producer and processor in Ireland. The project aims to evaluate the main microbial players in meat spoilage, with a focus on identifying these microbial spoilers as well as their possible routes of contamination throughout the meat production chain (i.e., at the farm, slaughterhouses, processing and packaging facilities, transport, retailer/market, up to the final consumer) using culture-independent shotgun metagenomics. Metagenomics will allow to investigate the presence of microbial genes associated with potential hazards (e.g., antibiotic resistance, virulence factors, and toxin production) and spoilage-related activities (e.g., proteolysis, production of volatile compounds and off-odours, slime, biofilm production). Finally, novel preservation technologies (PAW, plasma activated water) will be tested to improve beef safety and shelf life.

**Mappatura del microbioma lungo la catena di produzione della carne bovina “from farm-to-fork”**

Il progetto di ricerca verrà sviluppato in collaborazione con Dawn Meats Group, uno dei principali produttori di carne in Irlanda. Il progetto mira a valutare i principali agenti microbici responsabili del deterioramento della carne, con particolare attenzione all'identificazione delle loro possibili vie di contaminazione lungo tutta la catena di produzione della carne (ovvero, dall'allevamento, al macello, fino agli impianti di lavorazione e confezionamento, e lungo il trasporto fino al consumatore finale) utilizzando un approccio di metagenomica shotgun, che consentirà di indagare la presenza di geni microbici associati a potenziali pericoli per la salute (ad es. resistenza agli antibiotici, fattori di virulenza e produzione di tossine) e attività correlate al deterioramento (ad es. proteolisi, produzione di composti volatili dall’odore sgradevole, produzione di biofilm). Infine, verrà testato l’utilizzo dell’acqua attivata al plasma per prolungare la sicurezza e la shelf-life della carne.

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

Meat consumption has been steadily increasing worldwide due to its nutritional value and delicious flavours, with an estimated 346 million metric tons consumed in 2021 alone (FAO, 2021). However, meat and meat products are extremely vulnerable to the colonization and development of a wide range of microorganisms (Cauchie *et al.,* 2020). Meat spoilage is a major issue around the world, accounting for up to 20% of total meat production losses (Karwowska *et al.,* 2021). In addition, several pathogens may develop in raw beef, representing a health concern (e.g., *Listeria monocytogenes, Salmonella* spp., pathogenic *Escherichia coli*).

The advent of next-generation sequencing revolutionized the collection of massive amounts of data from microbial ecosystems, overcoming the limitations of culture-based and PCR-based methods (Almeida and De Martinis, 2019).

# **2. PhD Thesis Objectives and Milestones**

The goal of this research project is to map the microbiome of the entire beef chain using metagenomics, providing insights into how to improve microbiome monitoring and management throughout the beef supply chain.

The project can be divided into the following activities according to the Gantt diagram reported in Table 1:

**A1) Literature review on the role of microbiome on fresh beef spoilage:** A literature review will be carried out to identify the main contaminations routes in order to effectively plan the sampling activities.

**A2) Analysis of contamination routes in raw beef chain:** The primary goal of this study is to identify and characterize the microbes involved in meat spoilage, along with identifying potential routes of microbial contamination, such as the processing environment, equipment, or personnel (i.e., from farm to end retailer) in the entire meat production chain. This activity will be in collaboration with the Dawn Meats Group in Ireland, that own 6 different slaughterhouses and processing facilities across Ireland, as well as several farms (Figure 1). We plan to sample 5 different animals in each farm and follow the same animals along the processing chain, The sampling will include environmental swabs taken on surfaces, tools and equipment in the different facilities, as well as raw beef before and after the maturation, after the slicing and packaging and during the shelf life, for a total of around 300 samples. The sampling will be carried out twice, during summer (August-September 2023, when the animals are grazing) and in winter (January-February 2024), when they are in the stalls. Samples will be sequenced using a shotgun metagenomics approach. We will be able to identify the routes of transmission of the microbiome through the meat production chain, from farm to table, and determining its ability to survive in various environmental conditions. In addition, we will figure out how these microbiomes cause meat spoilage, identifying the microbial genes and metabolic pathways that contribute to meat spoilage, such as the production of off-flavors, protein breakdown, meat discoloration, as well as genes related to potentially harmful activities (toxin production, virulence factors).

A3) **Exploring new strategies for meat prevention and preservation** in a second part of the study, we will focus to develop and test novel strategies for controlling meat spoilage. During a secondment at the University of Leon (Spain), the effect of Plasma Activated Water on beef contamination will be evaluated, to define a potential use for carcass washing.

A4) **Thesis and Publications writeup:** We will share our findings with the scientific community as well as the cooperating company. Research findings will be published in peer-reviewed scientific journals.

A5) **Research dissemination:** key findings will be presented at national and international scientific conferences and meetings.

**Table 1**Gantt diagram for this PhD thesis project.

|  |  |  |
| --- | --- | --- |
| **S. No** | **Activities** | **Time Frame (3 Years)** |
| **1st January 2023 – 31st December 2025** |
| **2023** | **2024** | **2025** |
| A1) | **Literature review on the role of microbiome on fresh beef spoilage** | **Q1** | **Q2** | **Q3** | **Q1** | **Q2** | **Q3** | **Q1** | **Q2** | **Q3** |
| A2) | **Analysis of contamination routes in raw beef chain** |  |  |  |  |  |  |  |  |  |
|  | 1) Microbiome Sampling from different locations and in different seasons (2 times) |  |  |  |  |  |  |  |  |  |
|  | 2) DNA extraction and shotgun metagenome sequencing |  |  |  |  |  |  |  |  |  |
|  | 3) Bioinformatics data analysis  |  |  |  |  |  |  |  |  |  |
| A3) | **Exploring new strategies for meat prevention and preservation** |  |  |  |  |  |  |  |  |  |
|  | 1) Evaluating Plasma Activated Water (Secondment at Univ. Leon, Spain) |  |  |  |  |  |  |  |  |  |
| A4) | **Thesis and Publications writeup** |  |  |  |  |  |  |  |  |  |
| A5) | **Research Dissemination** |  |  |  |  |  |  |  |  |  |

**Note: Q:** Quadrimester

**Figure 1.** The sampling plan discussed with the Dawn Meat Group, Ireland, and the different facilities that will be sampled.

**DAWN MEAT IRELAND:**

Flow chart for sampling to investigate identified interest points.

﻿Primary route will be:

1. Farm
2. Slaughter Plant
3. Cutting Plant (could be same site or different)
4. Retail Packing Plant
5. Distribution (lorry & depot)
6. Retail stores

# **3. References:**

1. Almeida, O.G.G.D. and De Martinis, E.C.P., 2019. Relating next-generation sequencing and bioinformatics concepts to routine microbiological testing. *Electron. J. Gen. Med*, ***16***.
2. Cauchie, E., Delhalle, L., Taminiau, B., Tahiri, A., Korsak, N., Burteau, S., Fall, P.A., Farnir, F., Baré, G. and Daube, G., 2020. Assessment of spoilage bacterial communities in food wrap and modified atmospheres-packed minced pork meat samples by 16S rDNA metagenetic analysis. *Frontiers in microbiology*, ***10***, p.3074.
3. Food and Agriculture Organization of the United Nations (FAO), 2021. Meat Market Review: Emerging Trends and Outlook.
4. Karwowska, M., Łaba, S. and Szczepański, K., 2021. Food loss and waste in meat sector—why the consumption stage generates the most losses?. *Sustainability*, ***13***(11), p.6227.