Metagenomic Characterization of Microbial Communities in Different Food Samples

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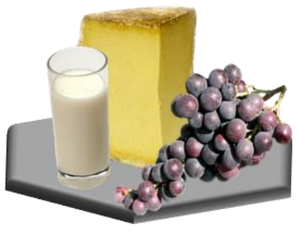

The aim of this PhD project is the characterization of microbial communities in different food samples by using the Illumina high-throughput whole metagenome sequencing technique. This approach will make it possible to determine the microbial populations operating in the samples, to highlight any differences between two or more samples and to assess variations in the presence of microbial populations in the different ripening phases of the same sample.

1. State of the Art

DNA sequencing is an evolving field of molecular biology. For years, most applications of sequencing were based on the use of the Sanger method[1].

Only in recent years, Next Generation Sequencing technologies, with the ability to economically deliver millions of DNA sequences per run, have changed the way we approach genome analysis[2]. The cost and time reductions introduced by these tools have spilled over into various disciplines, such as metagenomics, cancer research, animal research and biological selection. NGS technologies have deeply changed our approach to the study of food microbial communities[3]. The leading technique by far in the second-generation sequencing arena is the Illumina technique, using technology first developed by Solexa. Nowadays, NGS is used to sequence complete genomes from different biological matrices, including food and beverage.

The NGS techniques also allow the detection of nondominant communities that may play an important role in the studied niches. NGS platforms share a common technological feature based on massively parallel sequencing of clonally amplified or single DNA molecules that are spatially separated in a flow cell. As a massively parallel process, NGS generates hundreds of megabases to gigabases of nucleotide sequence output in a single instrument run, depending on the platform[4].

The main aim of this project will be the characterization of the microbial populations operating in different food matrices using the Illumina technique, in order to identify the specific characteristics of food products during and after production processes. Indeed, several investigations suggest the presence of characteristic microbial strains in different food products, which represent one of the key factors for the acquisition of peculiarities that contribute to their quality and distinctiveness[5].

2. PhD Thesis Objectives and Milestones

A1) **Bibliographic research.** DNA extraction methods from complex matrices, NGS techniques;
A2) **Sampling and sample storage.** Grape samples of Cabernet-sauvignon and Aglianico varieties and Pecorino cheese, at various stages of maturity, taken from several farms in different Italian regions and frozen at -20°C;
A3) **Sample preparation for extraction of a sufficient amount of DNA.** Development and/or optimization of a DNA extraction protocol (A3.1) and quantification of DNA (A3.2) to obtain a good amount of genetic material (ng/µl) and a good purity ratio (A260/280);
A4) **Sample preparation for Illumina sequencing;**
A5) **Illumina sequencing;**
A6) **Statistical analysis of microbial genomic sequences.** Statistic tools to highlight similarities and differences in microbial sequences/ populations, their distribution and interactions between the environment, the production area and the product itself.
A7) **Writing and Editing of the PhD thesis, scientific papers, oral and poster communications.**

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)	***Bibliographic research***		■	■	■	■	■	■																		
A2)	***Sampling and sample storage***		■	■	■	■	■	■	■	■		■		■	■	■	■	■	■	■	■		■		■	
A3)	***Sample preparation***		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
	3.1) Development of DNA extraction protocol		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
	3.2) DNA quantification		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	
A4)	***Sample preparation for Illumina sequencing***														■	■	■	■	■	■	■	■	■	■	■	
A5)	***Illumina sequencing***															■	■	■	■	■	■	■	■	■	■	■
A6)	***Statistical analysis***																		■	■	■	■	■	■	■	■
A7)	***Thesis and Paper preparation***		■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■	■

3. References

1. Sanger F, Nicklen S, Coulson AR (1977). DNA sequencing with chain terminating inhibitors. *Proceedings of the National Academy of Sciences USA*; 74(12):5463-5467.
2. Mardis ER (2017). DNA sequencing technologies: 2006-2016. *Nature Protocols*; 12(2):213-218.
3. De Filippis F, Parente E and Ercolini D (2018). Recent Past, Present, and Future of the Food Microbiome. *Annual Review of Food Science and Technology*; 9:589-608.
4. Voelkerding KV, Dames SA and Durtschi JD (2009). Next-Generation Sequencing: From Basic Research to Diagnostics. *Clinical Chemistry*; 55(4):641-658.
5. Castellani F, Vitali A, Bernardi N, Marone E, Palazzo F, Grotta L, Martino G (2017). Dietary supplementation with dried olive pomace in dairy cows modifies the composition of fatty acids and the aromatic profile in milk and related cheese. *Journal of Dairy Science*; 100(11):8658-8669.

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