DOMANDA

Identificativo Flusso: null

null

Struttura: null

ID Domanda	**null**
Accordo Bilaterale	**null**
Bando	**null (null)**
Scadenza Presentazione Domande	**null**
Responsabile Italiano :	**null**
Codice Fiscale Responabile Italiano	**null**

Proposta di Ricerca :

Description of the project\/span>\/b>\/p>
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Interest in packaged salads is steadily increasing throughout Europe and has enormous growth potential. Ready-to-eat \/span>leafy vegetables\/span> (RTE, as "minimally-processed salads")\/span> typically possess a natural
microbiota that changes in composition and abundance throughout the different stages of processing and manipulation of the product from field to table. The preservation and freshness of the product in salad bags can be determined by the initial microbial load and the washing, handling and storage techniques that follow through to the consumer. Sometimes, bacterial species harmful to humans, such as the food pathogens \/span>*Listeria monocytogenes,\/i> \/span>Yersinia enterocolitica\/i> and \/span>Escherichia coli\/i> can proliferate despite controls and accuracy of the food chain (Mir et al., 2018).\/span>\/p>*

The availability of tools and processes that are quick to use, cheap and reliable for studying the microbiome that causes the spoilage of ready-to-eat packed salads is an important objective in many phases of their industrial food chain. However, the analysis of the microbial communities affecting stored leafy vegetables presents some difficulties. Diagnostic applications in areas where microbial communities are rarefied or characterised by high spatial heterogeneity, such as salad leaves, are still scarce and require many verifications to be reliable.\/span>\/p>

The biodiversity of fungi and bacteria and the structure of their communities in natural or artificial environments is the subject of endless studies that have accelerated in recent decades thanks to new techniques. DNA and RNA-based biodiversity studies can overcome the limits of the non-culturability of most bacterial and fungal species (in many environments only 1-5% of \/span>bacterial species can be isolated in vitro). It is now possible to identify and enumerate microorganisms by extracting their DNA from environmental matrices without culturing them (Mira Miralles et al., 2019; \/span>Nilsson et al., 2019\/span>). Our understanding of how microbes colonise and spread in food and how they influence human health is constantly evolving as public databases are created and new techniques based on high-throughput sequencing are developed (\/span>Sekse et al., 2017;

Quijada et al., 2020\/span>).\/span>\/span>\/p>

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This collaboration will allow to: (i) develop an artificial model community containing the species commonly found in salads (ii) use appropriate dilutions to test the sensitivity of total DNA extraction techniques, (iii) test and compare a rapid shotgun metagenomics protocol and an amplicon sequencing protocol based on the nanopore sequencing technique, (iv) investigate whether foodborne microbiota and bacterial pathogens can be detected in amplicon and shotgun metagenomic datasets (v) compare the microbiota of salads with mixed ingredients before and after seven days of storage at 8 °C using the best extraction and sequencing method among those tested.\/span>\/span>\/p>

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The project will be based on the application of Oxford Nanopore sequencing Technologies (ONT), and specifically on the innovative and portable MinIon DNA sequencer.\/span>\/p>

High-throughput sequencing (HTS) is considered a revolutionary approach capable of identifying the microbial communities of a target environment. Different HTS platforms are now available, and the MinION device (Oxford Nanopore Technologies, ONT) is currently considered the most economically affordable one (Buytaers et al., 2021; Zhou et al., 2022\/span>). With this sequencing platform it is possible to apply several protocols and analytical pipelines based on both shotgun sequencing or PCR amplification and sequencing. The crucial point is if the data produced by these two different pipelines are reliable for describing the microbiota present in an environment. To study this important aspect, it is necessary to optimize and verify the methods by assembling an artificial microbial community composed of microorganisms with a known concentration ratio (mock community). Our target mock communities will include microorganisms often occurring in salads before and after packaging. These will comprise different strains of bacteria including pathogens such as Staphylococcus aureus\/i>, Lactobacillus\/i> sp., Escherichia coli\/i>, Salmonella \/i>sp. Pseudomonas aeruginosa\/i> and Listeria monocytogenes\/i> and also potential fungal spoilers represented by strains of the genera Aspergillus\/i>, Penicillium,\/i> Fusarium\/i>, Phoma, Alternaria\/i> and some yeasts.\/span>\/p>

\/span>\/b>\/p>Expected reciprocal benefit of the bilateral cooperation\/span>\/b>\/p>

\/span>\/b>\/p>The partnership in this project shares an interest in studying food microbiology using massive sequencing techniques. Still, it has complementary experience in the systematics and ecology of the groups of microorganisms whose distribution and environmental diversity is to be analyzed. Furthermore, the collaboration is new from the point of view of the institutions involved. In fact, the Institute for Biological System (CNR) has never been able to formalize its cooperation with the Institute of Slovak Academy of Sciences. However, the researchers involved have in the past shared some research in different contexts (cultural heritage microbiology), showing that they constitute an operationally robust and effective team.\/span>\/p>

In the past years, the Slovak team has developed protocols for studying and analysing microbiomes from environmental and food matrices using different sequencing platforms and optimising protocols for microbes' identification based on the use of molecular markers. The Slovak institute is at the forefront of food microbiology. In contrast, the CNR institute has extensive expertise in the field of leafy vegetables food chain. Moreover, the CNR institute is currently involved in a regional project for the application of ONT sequencing systems for the use of metagenomics in the study of soils and rhizospheric environments

in tomato crops. The CNR staff is expert in applied mycology and fungal ecology while the Slovak institute has long proficiency in the study of bacteria, making them complimentary in terms of the expertise needed to complete the project's tasks.\/span>\/p>

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The project will enable the collaborating team to formulate new ideas for shared project proposals with broader research purposes. The aims of the herewith suggested research are, in fact, of potential interest in many food-related industrial fields. Reliable, fast, and affordable protocols for the study of the microbiome of stored food will allow a better understanding of the colonisation dynamics of raw materials and finished products and enable more effective prevention of contamination. More durable and hygienically safer preserved products are the basis of important societal challenges, such as less toxins in food and adequate prevention of food poisoning. Inexpensive and readily available protocols for the analysis of packaged salads will facilitate the acquisition of data. This will create valuable microbial colonisation and development models for designing new preparation and packaging methods and will support the development of safer and more efficient production environments with, ultimately, healthier food.\/span>\/p>
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Role of the Slovak research team\/span>\/b>\/p>
\/span>\/b>\/p>The Slovak team will be involved in the isolation of bacteria from ready-to-eat vegetables in their identification and in the creation of a bacterial mock community with the isolated strains.\/span>\/p>
The group will be responsible for the optimization of the long amplicon sequencing of bacterial and fungal mock communities and the creation of a dedicated reference bacterial database and bioinformatics pipeline analysis system.\/span>\/p>
The optimized long amplicon sequencing approaches (for bacteria and fungi) and the bioinformatics tools will be used for the analysis of microbiota in real ready-to-eat vegetable samples during the second project year. Aliquots of DNA extracted from bacterial assemblages will be sent to the Italian group to apply their shotgun metagenomics approach.\/span>\/p>
\/span>\/b>\/p>Role of the Italian research team\/span>\/b>\/p>
\/span>\/b>\/p>The duties of the Italian research team will focus on the isolation of fungal strains from ready-to-eat vegetables and their identification. Part of the isolated fungi will be utilized for the development of a fungal mock community miming the fungal contaminants in ready-to-eat vegetable. The DNA extracted from the fungal mock community will be sent to Slovakia in order to optimize the long amplicon sequencing method.\/span>\/p>

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The Italian
group will evaluate the shotgun metagenomics sequencing with both the DNA from the fungal mock community and the DNA from the bacterial assemblage prepared by the Slovak group. The shotgun metagenomics approach will be combined also with a new specific fungal database and bioinformatics processing pipeline. The shotgun metagenomics strategy will be applied in the second year of the project to the analysis of the microbiota (bacteria and fungi) present in ready-to-eat vegetables.\/span>\/p>

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Travelling\/span>\/b>\/p>

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In the first year, the exchange of researchers within the framework of the collaboration will be addressed in a planning meeting at the beginning of the project with a trip of the Italian researchers to Bratislava. Then, at the end of the first year, there will be a meeting in Rome with Slovakian researchers travelling to Rome and the organisation of a workshop on the topic of the collaboration and on the sequencing methods that will be organised in Montelibretti for all staff. In this context, there will be an exchange of information and protocols, and shared activity of data analysis will be initiated. In the middle of the second year, two Italian researchers will travel to Bratislava to complete the data analysis on Slovakian platforms, and the team will start writing a collaborative publication. Samples will then be taken from bags of salads in real situations and strategies for comparing microbial communities will be decided. At the end of the project, a concluding seminar will be organised in Rome to present the results.\/span>\/p>