Abstract for the general public

The consumption of fish and seafood brings many benefits, such as the provision of the highest quality protein or polyunsaturated fatty acids with an excellent ratio of Omega-3 to Omega-6 fatty acids, however, the consumption of those can also be associated with risks. The most widely discussed issue is the consumption of large amounts of heavy metals, and in the case of some seafood, the accumulation of toxins, widely.

Pathogens can live in the natural environment in two forms: planktonic, i.e. as free cells, and in complex matrices known as biofilms. A characteristic feature of biofilms is the significantly increased resistance of the bacteria to stress factors, and their ability to form biofilms with the participation of other species, including pathogenic ones.

The project focuses on molluscs because among the raw materials of animal origin, molluscs are one of the main sources of isolation of *Listeria* sp., therefore the selection of this group of animals is justified, and additionally, this topic was not taken up by researchers. The main goal of the project is to characterize the biofilms produced on the shells of molluscs by species of the genus *Listeria*, isolated from the meat and shells of as many as 6 species of molluscs: smooth clams, lagoon cockle, veneridae, oysters, Blue mussels, razor clams and scallops.

The first stage of the study will be to collect DNA from thirty strains belonging to the genus Listeria, which will then be sequenced using the modern NGS technique. This will allow to distinguish groups of strains for the next stages of research, based on the presence of genes responsible for the ability to produce biofilms, virulence, and resistance to technological processes. The next stage of the study is to investigate bacterial compromises during growth and bacterial competition between *Listeria* sp. and other typical pathogens found in fish and seafood (*Vibrio* sp., *Aeromonas* sp. and *Salmonella* sp.) as well as typical fish microbiota (*Pseudomonas* sp., *Hafnia* sp. and *Serratia* sp.) to understand interactions naturally occurring in the environment that form the basis for the analysis of mature multispecies biofilms. Then, the characteristics of the biofilms produced on the shells of the mussels listed above will be performed, it will allow to determine the differences between the biofilms produced on the shells of different species, and also allow to compare the biofilms of *Listeria* strains with different pathogenic potentials. Then, the effectiveness of the processes used in the processing of mussels, pascalisation, ozonation and sous vide cooking will be assessed using flow cytometry, which is currently the most precise tool for examining the viability of bacteria after the processes. The final step will be to analyse the transcriptome of *Listeria* strains from planktonic cultures, from mature biofilms, and from mature biofilms that have survived the physical methods used in processing. This study will be performed using RNA sequencing, which will allow to assess whether the expression of genes responsible for virulence is increased in biofilms produced on chitin shells and after technological processes. This study will be extended to the assessment of pathogenic potential using an in vivo model (on *Galleria mellonella* larvae).

The implementation of the research will allow to understand the functioning of *Listeria* sp. in biofilms on shells of molluscs, focusing the research on increasing the safety of seafood processing.