Summary for the final report
Control of psychrophilic photobacteria in meat spoilage – (Psychrophilic Photobacteria)

First examinations within the scope of the project 'Control of psychrophilic photobacteria in meat spoilage' focused on the development of a suitable cultivation medium for photobacteria. The combination of the pre-mixed medium Marine Broth supplemented with 2% meat extract proved to be suitable for the optimum growth of *Photobacterium* species. Lowering the cultivation temperature from the commonly used 30-37 °C to 15 °C for 3 days proved to be a key implementation for the isolation of photobacteria.

During application of the developed cultivation method the species *P. phosphoreum*, *P. carnosum* and *P. iliopiscarium* (the two former constituting the majority of isolates) were detected on different types of meat, including pork, beef, chicken and turkey. Contaminations were present in raw meat independently of packaging/preserving conditions (vacuum, modified atmospheres, marinated) and of manufacturer and brand. Contamination also affected both, small and local meat processing facilities, as well as large supermarket chains. Increasing numbers of contaminated samples were observed during the summer months, which could be traced back to enhanced activity of the air conditioning in meat processing facilities, suggesting it might be a relevant source of contamination. Suitable methods for reduction of the *Photobacterium* contamination on meats are therefore suggested to include careful cleaning of the air conditioning system.

A selection of other foods tested negative for photobacteria, including several animal-associated products and products related to the sea. Results from other screened products revealed also that presence of photobacteria is limited to raw meat (and fish). In addition, two meat processing plants were screened for contamination. One of said plants turned out to be positively contaminated by photobacteria. However, contamination was only detected on the final product, and not on the processing steps, and therefore the exact location of the source of contamination remained unknown. Since the contamination could only be detected after a few days of incubation and only on one packaged product, low initial cell counts were assumed.

In order to improve detection of photobacteria and better describe distribution on packaged meat and source of contamination on the slaughter plants, the development of a culture-independent and highly sensitive methodology for the detection of *Photobacterium* contaminations in meat processing facilities was required. The application of cultivation methodology is additionally not suitable for quick application in the industry, increasing the need for a DNA-based method. A loop-mediated isothermal amplification (LAMP) based method for the qualitative assessment of presence of *P. phosphoreum*, *P. iliopiscarium* and *P. carnosum* was developed, together with a quick processing protocol for meat-samples. This LAMP assay provides a test result within 2 hours of preparation/incubation and requires only minimal processing, equipment and training. The assay is able to detect photobacteria at initial contamination stages when cell numbers are still low. However, performance of the LAMP-assay could not be transferred into the industry yet, due to the current situation of the pandemic. Available data nevertheless point at a major role of the industry in the process of contamination.

Results from comparative genomics and physiological studies revealed differentes both intra- and inter-species. The three species display high diversity within their genomes and metabolic capabilities, and appear to benefit from frequent events of horizontal gene transfer in order to diversify their adaptive responses. *P. phosphoreum*, comprised of two different subspecies, is able to perform better in growth experiments and it is predicted to be able to cope with stress better than the other two species. It also displays a more diverse antimicrobial activity and production of secondary metabolites than the other two species. Despite taking into account strains from multiple origins, the two subspecies did not show specific genomic and physiological adaptation that proved an environmentally driven division of the species. The species appears mostly tied to marine environments, showing a stronger adaptation to high pressure and increased salt content, and retaining symbiotic-related characteristics such as its bioluminescence. The species *P. carnosum*, although slower in growth than *P. phosphoreum*, appears as a conserved unique clade that has focused its adaptative strategies to the diversification of carbohydrates as carbon sources and its ability to potentially colonize not only meat and fish, but also plant-based niches. *P. iliopiscarium*, the least abundant of the three species, is the only one displaying source-related environmental adaptations. The species also grows slower than *P. carnosum*, shares with it utilization of some carbohydrates and displays stress coping mechanisms somehow in a middle point between the other two species. Meat-isolated strains of the species show wider carbon utilization as *P. carnosum*, while marine-related strains retain use of marine-related compounds and motility. Additionally, all detected *Photobacterium* species were sensitive to increased temperature and dehydration. The three species were predicted to produce more than one type of biogenic amines among which putrescine, cadaverine and tyramine are found. Production of histamine was predicted for a few isolates of *P. phosphoreum* and *P. carnosum* via an alternative histidine decarboxylase from the commonly described in literature.

Since photobacteria are found in all types of screened packaging atmospheres, the influence of the gases used in the industry to increase the shelf-life of raw meat was tested. Growth under air, only nitrogen, MAP with high oxygen and MAP without oxygen of *P. carnosum* and *P. phosphoreum* was monitored to determine growth parameters of the bacteria and comparative proteomics was used to establish the mechanisms behind their behaviour. Results revealed that the bacteria do respond to a changing gas atmosphere and that oxygen concentration and presence/absence of oxygen and carbon dioxide can shape their growth dynamic. Air remains the optimum gas atmosphere that allows a fastest and highest growth, with improved energetic metabolism derived from pyruvate metabolism and aerobic respiration, increase of its concentration mainly increases the oxidative stress of the bacteria and limits them. Complete absence of oxygen does force photobacteria to switch to anaerobic respiration and fermentative pathways, especially in the case of *P. carnosum*. The effect of carbon dioxide is not able to inhibit photo bacteria by itself, and although a reduction in growth was observed, the indirect nature of its effects on bacteria makes it hard to understand the mechanism behind it. However, it is when mixing high oxygen and carbon dioxide that the strongest effects are observed. Growth of *P. carnosum* is fully inhibited and that of *P. phosphoreum* is severely hindered and reduced to a minimum. Proteomics regulation revealed in the case of *P. phosphoreum* an upregulation of most metabolic pathways and enzymes, regardless of their utility in the growing conditions. This result

suggests an extreme response to stress conditions that override the regulatory tools of the bacteria. The overexpression of so many proteins might unbalance the energetic yield and place the cells in a survival state rather than growth.
Results show that photobacteria do not grow on modified atmospheres with high oxygen. However, they are found abundantly on meat packaged under said gas combination. In order to understand possible interactions with other meat spoilers or concomitant microbiota, studies on competition experiments on meat followed by a transcriptomic analysis were performed with photobacteria. The aforementioned suggested adaptation of *P. carnosum* to more diverse environments (among which meat is included) is also reflected in the positive influence that the presence of other meat spoilers had on the growth of this species in a protective gas atmosphere, that otherwise, when being alone, showed much poorer or no growth. Under the same circumstances in which other species were present, *P. phosphoreum* tended to grow poorly, possibly due to nutrient competition. These conclusions were first suggested from the competition experiments, and later confirmed by the examined transcriptomics data. Protective gas atmosphere showed little effect on the growth of photobacteria on meat, provided that only carbon dioxide was used as the active gas, and therefore supporting the idea that carbon dioxide alone is not enough to inhibit the presence of photo bacteria on raw meat. In contrast, the combination of carbon dioxide and oxygen in high concentration led to a significant reduction in growth under laboratory conditions compared to growth in air. Based on the investigated transcriptome data, this can possibly be attributed to increased oxidative stress caused by oxygen, accompanied by reduced respiratory activity, and various cellular inhibitory activities caused by carbon dioxide.
Overall, it was shown that contamination by photobacteria can develop into a problem in meat processing, primarily through the formation of various biogenic amines and other spoilage products responsible for changes in the organoleptic characteristics of meat, through reaching spoilage-relevant cell counts and through a comparatively high tolerance to protective gas packaging. Suitable countermeasures can include careful cleaning of the operations with tap water, temperature increase and tracking of contamination chains using DNA-based detection.

IVLV members can download the complete final project report from our homepage. All you need is to register in the section “My IVLV”. Non-members can request the final report from the IVLV office at office@ivlv.org.

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