25th International ICFMH Conference - FoodMicro 2016
July 19th - 22nd 2016 – University College Dublin, Ireland

FLORPRO project: Selection of beneficial bacteria from chilled food products to protect them from bacterial spoilage and increase their shelf life

Gilles Kergourlay[1], Sabrina Labrozzo[1], Julien de Barsy[1], Marie Vassen[1], Bernard Taminiau[1] and Georges Daube[1]

[1]University of Liege, Faculty of Veterinary Medicine, Department of Food Science & FARAH, Liège, Belgium

*gilles.kergourlay@ulg.ac.be

INTRODUCTION

Food industry players are subject to a high competition. In this way, their food products have to meet the requirements in terms of quality and safety coming from regulation, food sector, and consumers. To do that, it could be interesting to add to food products a logistical flexibility or an ecological added value (Clean Label). Meanwhile, microbial food spoilage has become a major issue in the food industry. It has been estimated to be responsible for 25 % of the world's food supply losses. In this context, the need for innovative and natural solutions to protect food products have become stronger and microbial biopreservation prove to be a promising alternative.

OBJECTIVE

The main goal of FLORPRO project is to enable industrial partners to provide on the market products with a perfect sensory and microbiological control. In order to do so, we intend to act at several levels, either in the recipe of the product, or in its packaging, but especially in its microbiological composition. Several chilled food products were selected from different sectors of the food industry. Thus, thorough studies concerning these food products and their changes during aging were realized. Through this knowledge, we were able to select part of their microbiota with no organoleptic impact in order to protect them from bacterial spoilage and extend their shelf life.

MATERIALS & METHODS

Food products characterisation

Microbiology: *Pseudomonas*, yeast & mould, total mesophilic bacteria count, *Brochothrix thermosphac*ta, lactic acid bacteria, *Enterobacteriaceae*.
Metagenetics: Day 0 & end of the shelf life
Physico-chemistry: pH, gaz, lipid & protein alteration (biogenic amine, aldehyde, etc.)

Methodology development

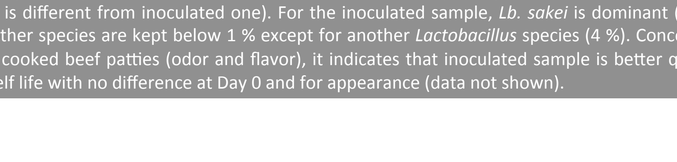
- Isolation of dominant bacteria
- Characterisation of bacteria of interest
  - ✓ Bioscreen™
  - ✓ RAPD
- Inoculation method of the selected bacteria
- Monitoring of the inoculated bacteria
  - ✓ qPCR
  - ✓ Metagenetics

Process validation in pilot plant

- Inoculation of bioprotective strains
  - ✓ Alone or in mix
  - ✓ Different concentrations
- Modified atmosphere packaging
  - ✓ Ratio CO_2 / 0_2 / N_2
  - ✓ Ratio weigh / volume / gaz
- Aging
- Microbiological, physico-chemical, metagenetic and sensory analysis at Day 0 and at the end of the shelf-life

Industry trial

- Implementation of inoculation methods in practical conditions of use
  - ✓ Beef patties: into the food matrix
  - ✓ Precooked passta (w/o egg): into the surface-added oil
  - ✓ White pudding: spraying on the surface
- Real packaging
- Aging



- Shelf-life analysis
  - ✓ 16S rDNA metagenetics → Technology: Illumina (MiSeq) ; Processing data: Mothur
  - ✓ Sensory analysis → Untrained panel (6 to 8 members) ; Raw samples and/or cooked samples ; 3 attributes (appearance, odor, and flavor) ; Scoring from 1(= dislike) to 5 (= like) ; ANOVA with post-hoc Tukey HSD

RESULTS

White pudding

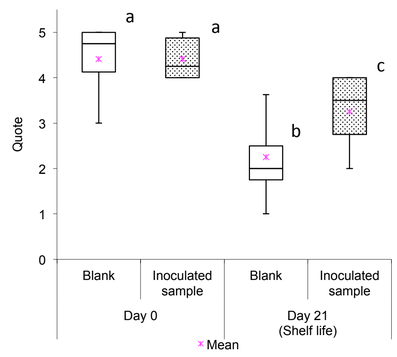


Fig. 1 Sensory analysis of raw white pudding (odor)

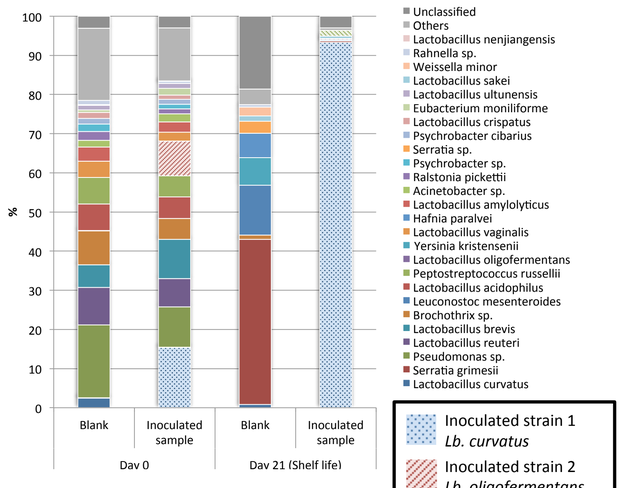


Fig. 2 16S rDNA metagenetic analysis of white pudding

Metagenetic analysis (Fig. 2) show that at Day 0, white puddings are contaminated with different dominant species such as several *Lactobacillus* species, *Brochothrix* sp., and *Pseudomonas* sp.. While for the inoculated sample, the 3 inoculated strains are found: *Lb. curvatus* (15 %) ; *Lb. oligofermentans* (9 %) and *Lb. nenjiangensis* (1 %). At the end of the shelf life, the blank is dominated by *Serratia grimessi* up to around 40 % of the total microbiota but also with other species known to be potentially involved in spoilage such as *Leuconostoc mesenteroides*, *Hafnia paralvei* and *Serratia* sp. For the inoculated sample, *Lb. curvatus* is dominant (93 %) and all the other species are kept below 1 % except for *Lb. nenjiangensis* (1.5 %). Concerning the sensory analysis (Fig. 1) of raw white pudding (odor), it indicates that inoculated sample is better quoted ($p<0.05$) than blank at the end of the shelf life with no difference at Day 0 and for appearance (data not shown).

Beef patties

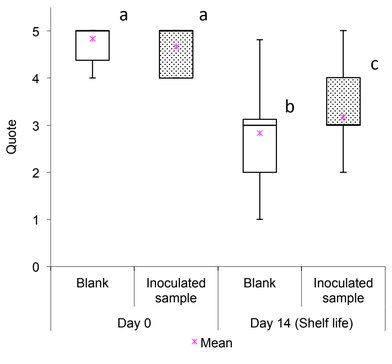


Fig. 5 Sensory analysis of raw and cooked beef patties (odor and flavor)

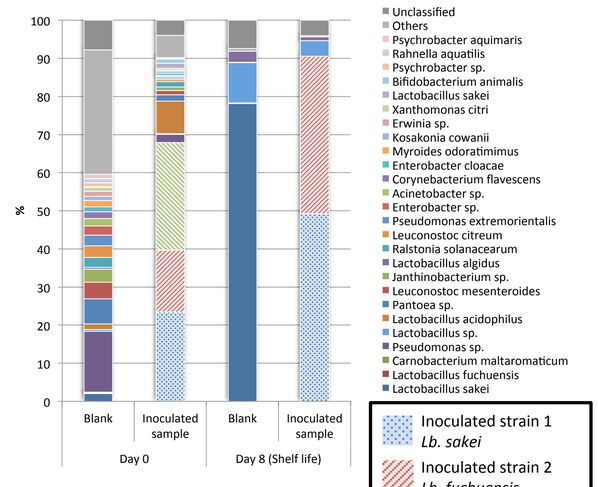


Fig. 6 16S rDNA metagenetic analysis of beef patties

Metagenetic analysis (Fig. 6) show that at Day 0, beef patties are contaminated with different species such as *Pseudomonas* sp., *Pantoea* sp., and *Leuconostoc mesenteroides*. While for the inoculated sample, the 3 inoculated strains are found to be dominant (about 70 % of the microbiota) : *Lb. sakei* (23 %) ; *Lb. fuchuensis* (16 %) and *C. maltaromaticum* (28 %). At the end of the shelf life, the blank is dominated by *Lactobacillus* species up to around 90 % of the total microbiota, especially *L. sakei* (80 %, without the ability to determine whether this strain is different from inoculated one). For the inoculated sample, *Lb. sakei* is dominant (49 %) with *Lb. fuchuensis* (41 %) and all the other species are kept below 1 % except for another *Lactobacillus* species (4 %). Concerning the sensory analysis (Fig. 5) of raw and cooked beef patties (odor and flavor), it indicates that inoculated sample is better quoted ($p<0.05$) than blank at the end of the shelf life with no difference at Day 0 and for appearance (data not shown).

Precooked pasta

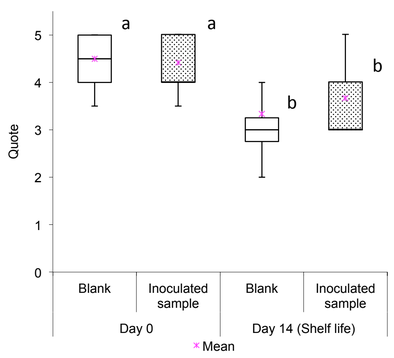


Fig. 3 Sensory analysis of precooked pasta (odor)

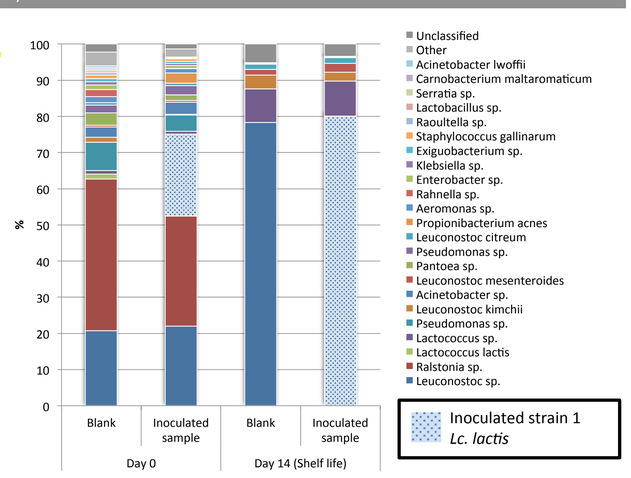


Fig. 4 16S rDNA metagenetic analysis of precooked pasta

Metagenetic analysis (Fig. 4) show that at Day 0, precooked pasta are contaminated with different species such as *Leuconostoc* sp., *Ralstonia* sp., and *Pseudomonas* sp. While for the inoculated sample, the inoculated strain is found: *Lc. lactis* (23 %). At the end of the shelf life, the blank is dominated by several species of *Leuconostoc* spp. up to around 85 % of the total microbiota, this genus is known to be potentially involved in spoilage. For the inoculated sample, *Lc. lactis* is dominant (80 %) and *Leuconostoc* spp. is kept below 5 %. Concerning the sensory analysis (Fig. 3) of raw precooked pasta (odor), it indicates that inoculated sample is better quoted than blank at the end of the shelf life with no difference at Day 0 and for appearance (data not shown).

CONCLUSION

To conclude, these results show that the strategy developed in the FLORPRO project, which involves the selection of bacteria naturally present in the product for a biopreservative purpose, seems to be a very promising way to enhance food products. Thus, the selected cocktails have partially inhibited the development of potentially spoilage bacteria such as *Leuconostoc* sp. and *Serratia* sp.. These cocktails have also protected the tray from swelling (white pudding) and stabilised the sensory evaluation after the end of the shelf life (Data not shown). Currently, the assessment of the bioprotective effect of this cocktail against pathogens is being undertaken. Moreover, this strategy is being carried out on other food products.