INFLUENCE OF DIFFERENT PHYSICO-CHEMICAL TREATMENTS ON SURVIVAL OF *LISTERIA MONOCYTOGENES*, *PSEUDOMONAS FLUORESCENS* AND *PSEUDOMONAS FRAGI* STRAINS.

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KEYWORDS: *Listeria monocytogenes*, *Pseudomonas* species, physico-chemical treatments, decontamination.

BACKGROUND

Listeria monocytogenes is present along the food processing lines and can be detected in a variety of foodstuffs such as meat, milk and their by-products. This bacterium has become one of the most important foodborne pathogen and is of major public health concern for food industries since it is occasionally responsible for outbreaks of listeriosis (Palumbo and Williams, 1994). In industrial plants, *L. monocytogenes* can be isolated with *Pseudomonas* species such as *Pseudomonas fluorescens* and *Pseudomonas fragi* which are responsible for meat spoilage (Delaquis and Mc Curdy, 1990). Inhibiting the growth or eliminating these both organisms at the food chain level is consequently an important task of hygienists.
For these reasons, drastic decontamination procedures are generally used to eliminate them.
Additionally, in industrial plants, various stressing agents are applied during food processing and during cleaning and disinfecting procedures. It is then interesting to evaluate the bacteriostatic or bactericidal effects of some treatments on these micro-organisms. Moreover, understanding their adaptative responses to overcome these treatments would help to better control them in food products and plants.

OBJECTIVES

This present work aimed at studying survival of *L. monocytogenes* and two *Pseudomonas* strains to different physico-chemical treatments. This study was undertaken in directed microbial ecology prospects which consisted in the selection of appropriate parameters and combination of physico-chemical treatments, in order to focus an efficient and selective decontamination procedure. Thus, they should have lethal impacts on pathogen and spoilage (negative) flora but limited influence on neutral or technological positive micro-organisms.

METHODS

L. monocytogenes and *P. fluorescens*, *P. fragi* strains were respectively isolated from industrial plants and from a slaughterhouse.
Bacteria were grown at 20°C in a meat broth medium up to the early stationary phase (20 h culture). Then, cells (about 10^7 cells. ml^{-1}, final concentration) were exposed to the selected shock solutions at 20°C or to thermal shocks. A shock was considered as a stress applied for a 30 min period. Shock solutions were prepared by adjusting the culture medium to the concentrations or pH studied. In the first experiments, individual and combined shocks were applied. For the individual shocks, the medium was adjusted to pH 5.4 (acetic acid), pH 10.5 (sodium hydroxyde (NaOH)), or transferred from 20°C to 45°C or 55°C for 30 min. When the acid and alkaline shocks were combined, bacteria were exposed to the first shock solution : pH 5.4 or pH 10.5 then the cultures were pelleted and resuspended in the second shock solution : pH 10.5 or pH 5.4 respectively.

In a second set of experiments, the studied treatments were pH 5.4 and pH 10.5 with or without 10 % NaCl and with or without biocides (A, B, C). When pH, NaCl and biocide were combined, the first shock consisted of a pH / NaCl treatment followed by the biocide one. Experiments were performed using a 16 assays (2^4) experimental design.

Initial and final populations (Colony Forming Unit. ml^{-1}) after shocks were evaluated by serial dilutions in tryptone saline solutions and spread plating onto Trypticase Soja Agar (*L. monocytogenes*) or Plate Count Agar (*Pseudomonas* spp.). The population was enumerated after a two days incubation period either at 37°C for *L. monocytogenes* or at 24°C for *Pseudomonas* strains.

RESULTS AND DISCUSSION

Results of the first experiments are indicated in Figure 1. The two *Pseudomonas* strains showed similar behaviours.

The heat shocks led to a low reduction of the *L. monocytogenes* population at 45°C and to its complete destruction at 55°C. On the opposite, for *Pseudomonas* spp., a 2 log reduction was observed at 45°C and a 5 log reduction at 55°C. The treatment at 55°C appeared more bactericidal for *L. monocytogenes*. Temperature, itself is an important parameter.

For the individual pH shocks, both acid and alkaline shocks studied had no efficiency against *L. monocytogenes*. The acid shock was inefficient against *Pseudomonas* spp. but the alkaline shock allowed a 4 log population reduction.

When these shocks were combined, the treatment pH 5.4 + pH 10.5 had no efficiency against *L. monocytogenes* whereas the combination pH 10.5 + pH 5.4 led to a population reduction of 3 log. *Pseudomonas* strains were more sensitive and population reductions of 5 log and 8 log (total destruction) were respectively observed.
When the first shock was acid, the cells appeared to be able to resist to a second alkaline shock. Consequently, the application order really influenced the efficiency of the treatment since a combination with a first alkaline shock was more lethal than a first acid shock one.

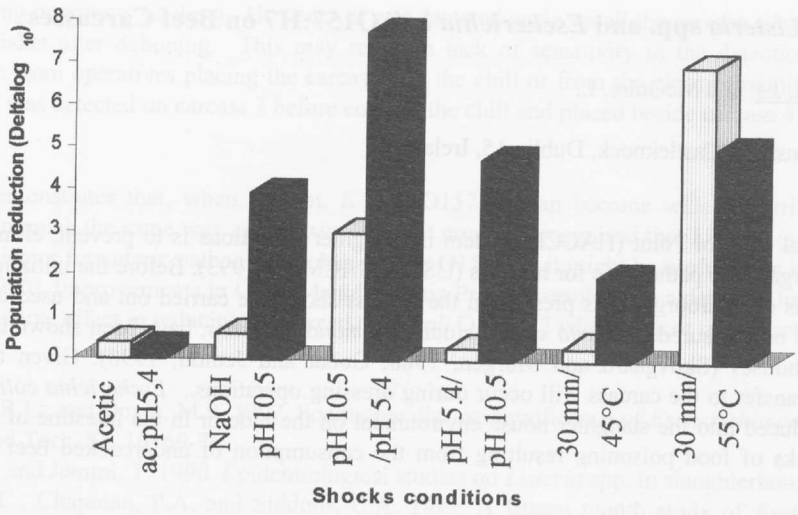

Figure 1: Population reduction of *L. monocytogenes* (▒) and *Pseudomonas* spp. (■) depending on pH or heat shocks.

In the second part of the study, experiments were carried out with the same pH parameters but with or without NaCl in the first shock and plus or no biocides in the second shock (Figure 2). For the biocides A and B, both *Pseudomonas* strains were studied and showed similar results (*Pseudomonas* spp.) whereas the biocide C was only tested on *P. fluorescens*.

Most of the combinations studied at pH 5.4 (organic acid) were inefficient to reduce *L. monocytogenes* or *Pseudomonas* spp. (<1 log). However, the combination pH 5.4/ 10%NaCl + biocide C led to a 2 log reduction of the *P. fluorescens* population.

On the contrary, most of the alkaline (pH 10.5) combinations studied allowed a reduction of the flora, particularly for the *Pseudomonas* spp. In the absence of NaCl in the first shock solution, a second (biocide) shock synergistically increased the combination efficiency noticeably against *Pseudomonas* spp.

When three stressing agents were combined, the treatments allowed high levels of population reduction. The combination pH10.5/ 10% NaCl + biocide (A, B, C) showed reductions of 5 to 8 log for both flora. The treatment with biocide A was the most bactericidal.

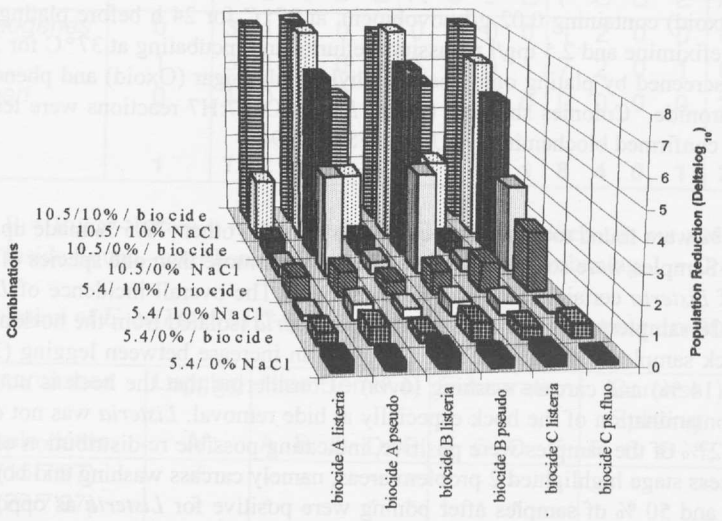

Figure 2: Population reduction depending on the microorganisms and combinations studied.

CONCLUSIONS

This study highlights some combinations of shocks which could decrease or eliminate *L. monocytogenes* and *Pseudomonas* spp. The most efficient appear to be combinations of alkaline, osmotic and biocide shocks. The next step of the work will be to study the effects of the most efficient treatments on micro-organisms present on surfaces of production lines or in food products (technological flora) in order to develop selective decontamination procedures. It would be also interesting to study the consequences of these treatments at a molecular level by proteomic analysis in order to understand why some bacteria are able to overcome some treatments.

REFERENCES

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