Effects of Hydrogen Peroxide on persistent and non-persistent strains of *Listeria monocytogenes* recovered from cheese processing plants

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Introduction

Listeriosis, a food-borne disease caused by consumption of food contaminated by *Listeria monocytogenes*, can produce infections in susceptible human populations, such as immuno-compromised persons, the elderly, pregnant women, their fetuses and new-borns (1). A wide variety of foods have been found to be contaminated with *L. monocytogenes,* namely: soft cheeses, dairy products, pâtés, sausages, smoked fish, salads, infant cereals, cakes, cream, butter and, in general, refrigerated ready-to-eat commercially produced products, consumed without further cooking or reheating (2).

L. monocytogenes is particularly difficult to control, since it is widespread in nature, and because it possesses physiological characteristics that allow it to grow under conditions that are usually adverse for most human pathogenic bacteria. Persistence of *L. monocytogenes* in food processing plants (i.e. long term survival in a complex environment) has been reported, for years or even decades (3, 4), being an important factor in the transmission of this food-borne pathogen. Despite research showing the lethality of sanitizers to *L. monocytogenes*, the pathogen is occasionally isolated from food-processing environments, even following cleaning and sanitizing of equipment surfaces (5, 6).

Several authors have tested the susceptibility of *L. monocytogenes* to different industrial disinfectants, such as quaternary ammonium compounds, alcohols, chlorinated compounds, and other oxidizing agents such as peracetic acid, ozone and peroxide derivatives (7, 8, 9). The use of hydrogen peroxide (H_2O_2) has been proposed as an alternative for decontaminating fruit and vegetables due to its low toxicity and safe decomposition products. It is effective against a wide spectrum of bacteria, yeasts, moulds, viruses and spore-forming bacteria (10). H_2O_2 rapidly degrades into oxygen and water (nontoxic products) upon contacting organic material, thus having no long-term residual activity.

This study was performed to evaluate the ability of 31 persistent and 18 non-persistent strains of *L. monocytogenes,* isolated from cheese processing environments, to survive different concentrations of H_2O_2(i.e. 1.5 and 0.75 % v/v) at different exposure times (5 and 20 minutes), at 22 ºC.

Methods

✓ **Inoculum preparation**

1) Persistent and non-Persistent isolates were cultured in 10 ml of TSB-YE, overnight, at 37ºC (to achieve a cell concentration of c.a. 10^9 CFU/ml)

2) Cultures were centrifuged at 6000 rpm x 5 min

3) Pellets were re-suspended in 10 ml of phosphate-buffer solution (PBS)

✓ **Suspension Test**

1) 500 µl of microbial suspension were added to 4.5 ml of prepared sanitizer solution at different concentrations (H_2O_2 1.5 and 0.75 % v/v); for controls PBS was used in place of sanitizer

2) Tubes were incubated at 22 ºC for 5 min and 20 minutes

3) One ml of each tube was transferred to another tube to neutralize with catalase solution (0.2 mg/ml) for 10 minutes at 22 ºC

4) Serial dilutions (1:10) were prepared and plated in TSA-YE plates

5) Plates were incubated at 37 ºC for 24 hours

6) Colonies were counted to obtain a result for CFU/ml

✓ **Calculation of antimicrobial activity**

The difference between Log CFU count after treatment with PBS and with sanitizer was used to define antimicrobial activity.
The value represents how many log units the sanitizer reduced the microbial population during the time of exposure. The sanitizer was considered efficient if a 5 log units reduction was observed.

✓ **Statistical analysis**

An analysis of variance (one-way ANOVA) was performed in order to test the significant differences ($p<0.05$) between (i) exposure time, (ii) sanitizer concentration, (iii) persistent and non-persistent strain, and (iv) serogroup. All calculations were carried out using the software KaleidaGraph (version 4.04, Synergy Software, Reading, USA).

Results

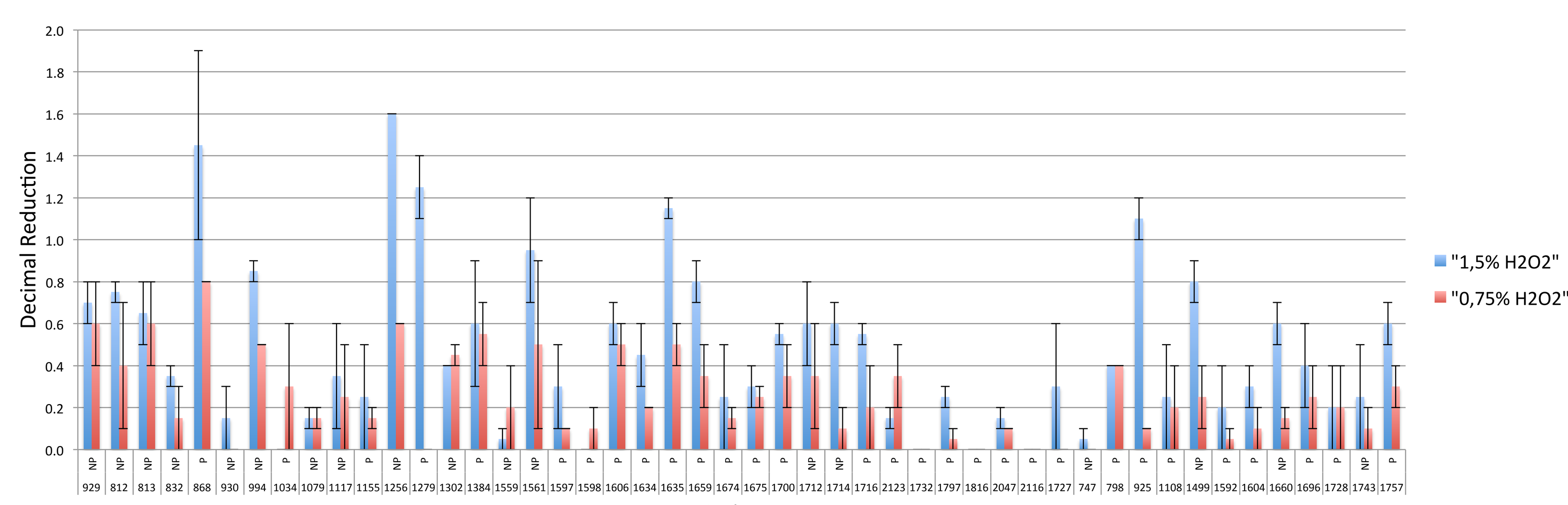

Figure 1: Decimal reduction for the persistent and non-persistent strains in 5 minutes of exposure at different concentrations of H_2O_2

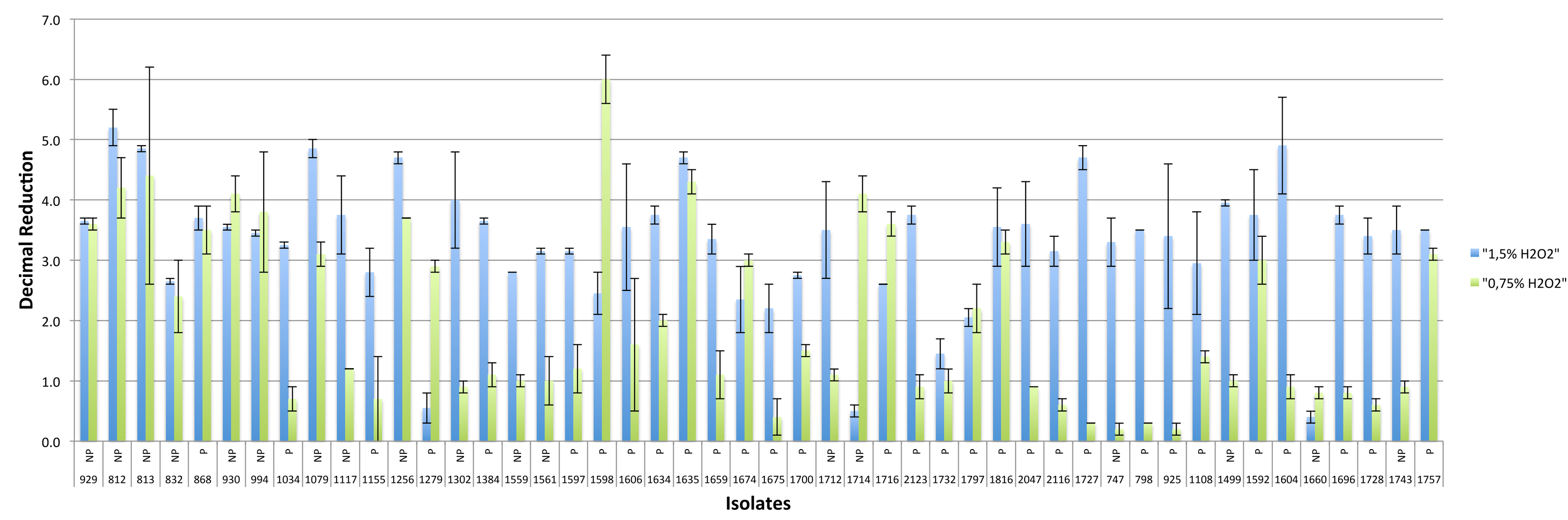

Figure 2: Decimal reduction for the persistent and non-persistent strains in 20 minutes of exposure at different concentrations of H_2O_2

Conclusions

Persistent strains have been isolated from food-processing environments after cleaning and disinfection. While this may simply indicate presence of these strains in locations that cannot be reached by sanitizers ("niches"), it has also been proposed that resistance to cleaning and disinfection agents could explain the persistence of specific *L. monocytogenes* strains in these environments. The present study aimed to evaluate the ability of persistent and non-persistent strains to survive at different concentrations of H2O2. The maximum of log reduction observed was 1.6 and 5.2 log for 1.5% v/v of H_2O_2 and 0.8 and 6 for 0.75 % v/v of H_2O_2, after 5 and 20 minutes of exposition, respectively. No significant differences in the resistance between persistent and non-persistent strains was observed for the conditions tested ($p<0.05$). Other authors found no relation between persistence and increased tolerance to disinfectants when comparing susceptibility of persistent and sporadic isolates to commonly used commercial disinfectants (7, 8, 9). As cells organized in a biofilm are generally less susceptible to disinfectants than when in their planktonic state, and consequently, shielded against cleaning and disinfection procedures, It could be important to compare the results obtained with susceptibility of these strains in a biofilm condition.

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