Effect of osmotic, alkaline, acidic and thermal stresses on the growthand inhibition of *Listeria monocytogenes.*

C. Vasseur, L. Baverel, M. Hebraud, J. Labadie. SRV.
INRA de Theix. 63122. St Genes Champanelle. France., Fax: 33. (0)4 . 73. 62. 42. 68. E-Mail: Jean.Labadie(~clermont.inra.fr

INTRODUCTION

Listeria monocytogenes is a gram-positive, aerobic to facultatively anaerobic bacterium that is pathogenic to man and animals. This pathogen is an agent of foodborne disease. It is isolated from a variety of foods and meats particularly. To better assess the potential means of controlling this bacterium, it is important to evaluate the effects of various physicochemical decontaminating agents on the survival of selected *L. monocytogenes* which are representative of the populations growing in foods.

1VIATERIAL AND METHODS

Bacterial strains and growth conditions

Five isolates of *L. monocytogenes* selected among 1500 strains were used. Strains were grown in tubes on TSA incubated 8 h at 37°C. Cultures were realised at 20°C, in 100 ml of BTVs, Begot *et al* (1996) buffered with K2HPO4-KH2PO4 and ajusted at pH 7.

Preparation of the shock solut~ons

Shock solutions (4 times concentrated) were prepared by adding NaCl, NaOH, HC1, Acetic acid and Lactic acid to BTV5 in order to obtain the following final values: 4%; 6.25% and 8%NaCl ; pH 9.5; 10; 10.5; 11 end pH 5.4; 5.6 and 5.8.

Effects of osmotic, alkaline and acidic shocks.

Shocks were performed on the early exponential-phase cells (BTV,, 20°C) by adding 7,5 ml of bacterial cultures to 2,5 ml of 4 times concentrated shocks solutions. Shocked cultures and controls where dispensed into microplates and placed in a Bioscreen C (20°C), an automated optical density monitoring system (Labsystems (UK)). The absorbance of each microwell was measured at 600 nm.

Effect of thermal shocks

Early exponential-phase cells were transferred either in ice (cold shock) or in waterbaths regulated to 55°C and 63°C (heat shocks). After 30 min. shocked cultures were dispensed into microplates and growth was followed at 20°C in the Bioscreen.

Bioscreen data

Calculations of log optical density were performed prior to determine the growth parameters (growth rate, lag time) using a non linear regression model, Begot *et al (1996).*

Analysis of Principal Composantes (A.P.C.)

This analysis was performed using the software Statbox (Microsoft). It was realised on the following parameters: latency (L), maximal population (A), growth rate (~). Values obtained after alkaline and acids shocks (pH) for the 5 strains were taken in account.

RESULTS

Effect of the NaCI concentration

Increasing the NaCl concentration in the media reduced the growth and increased the lag phase (Fig. 1). Lags varied from a range of 1.27h - 1.58h (A and B strains respectively at 4% to 1.51h (A) - 4.73h (E) at 6.25% and 2.12h (D) - 5.32h (E) at 8% NaCl. The generation time was doubled from 4% to 8% NaCl in the medium. E was the most sensitive strain to the highest NaCl concentration tested (8%).

Effect of the pH

Alkaline shocks

When the pH was adjusted to pH 9.5, the lag times varied from lh (D) to 3.83h for the slowest one (A). Increasing the pH increased the lag phases for all strains (Fig. 1). The A strain was particularly inhibited at pH 10 and 10.5.

Acid shocks

The strains D and E, were the most rapid (generation time of 14.4 h and 19.29 h respectively at pH 5.4) and the most tolerant to acetic acid. A, B and C were more sensitive with generation times of 20 h (A) to 26 h (C). Lactic acid has similar effects although less marked than the acetic acid ones. The generation time varied from 2.53 h at pH 5.8 to 3 h at pH 5.4 (strain D) whereas the lag time varied from 1.12 h to 1.54 h at the same pH. A, B and C were the most sensitive to lactic acid compared to D and E. An increasing lag phase and a growth rate decrease was observed in media acidified to pH 5.4, 5.6 and 5.8 with HC1. For instance, lag of the strain D varied only from 0.88 h at pH 5.8 to 1.07 h to pH 5.4. HC1 had the least deleterious effect on the initiation of growth of the strains.

Effect of the thermal shocks

The cold shock did not influence the growth of the strains. The lag did not exceed 1.35 h (strain C). But after heat shocks, lag times varied from 17.7 h at 55°C to 30.5 h at 63°C i.e an increase of 8°C did nearly doubled the latency.

Analysis of Principal Composantes (Not shown)

The A.P.C. realised according to pH shocks results highlighted three groups of strains: A, B-C, and E-D. However, the strains D and E appear to be the more resistant of the 5 strains tested by revealing short lag time and high bacterial population.

DISCUSSION

Effect of NaCI

The different NaCl concentrations added to the culture media led to a slowing down of the growth (start) and to a slight variation of the lag phases depending on these concentrations and on the strains studied. Different authors investigated the mechanisms of osmotic stress adaptation of *Listeria monocytogenes.* Patchett *et al. (1992)* described that *Listeria monocytogenes* cells grown in the presence of 7.5% NaCl contained higher concentrations of K+, betaine, glycine, alanine and proline than cells grown in the absence of NaCl. Also, Ko *et al.* (1994) showed that *Listeria monocytogenes* accumulates glycine betaine intracellularly when grown under osmotic which resulted in enhanced growth rate of stressed cells.

Effect of pH

Alkaline shock

The alkaline shocks realised led to an icrease of the log phase, particularly at pH 11. Alkaline solutions such as NaOH are used in the formulation of detergents in order to eliminate carbonized settlings, oils or grease. They allow proteins denaturation, fats saponification and have a bactericidal activity.

Acid shock

The experiments performed in this study showed that the acetic acid anti-listerial activity was highest than the lactic acid and

hydrochloric ones, for the five strains. These results were in agreements with those of Ahamad and Marth (1989), Sorrells *et al.* (1989), Elshenawy and Marth (1989). On an equal weight basis, all these studies indicated that the highest relative bactericidal activity against *Listeria monocytogenes* was acetic acid followed by lactic acid and citric acid (non tested in our study). A number of investigators have reported the inhibitory effects of low pH and organic acids on *L.monocytogenes.* Two mechanisms are generally proposed regarding to this inhibition: in one hand, an intracellular acidification (lost of homeostasis) and on the other hand, a specific effect of the acid non dissociated form on the metabolic activities.

According to Ita and Hutkins (1991), the efficency of the treatments using organic acids would be due to the non dissociated fraction rather than to the protons toxicity.

Effect of temperature

As shown on the Fig 1, cold shocks have no inhibiting effects on the growth of *L. monocytogenes.* On the opposite, heat shocks, 55°C or 63°C (30 min) induced increased latencies prior growth, particularly at 63°C. The possible heat resistance of *Listeria monocyfogenes is* a conflicting subject. Beams and Girard (1958) were the first to describe this strain as a microorganism able to survive to the pasteurization process of 61.7°C, 35 min. They concluded that *Listeriamonocytogenes* was more thermotolerant than most nonsporeforming bacterial pathogens. On the contrary, Donnelly *et al.* (1987) claimed that this organism does not survive the lowest legal pasteurization temperature. They reported the importance of the methodology employed to determine the thermal inactivation (sealed, immerged, preheated test tubes or not). According to these authors, Beams and Girard (1958) would have overestimated the thermoresistance of the organism.

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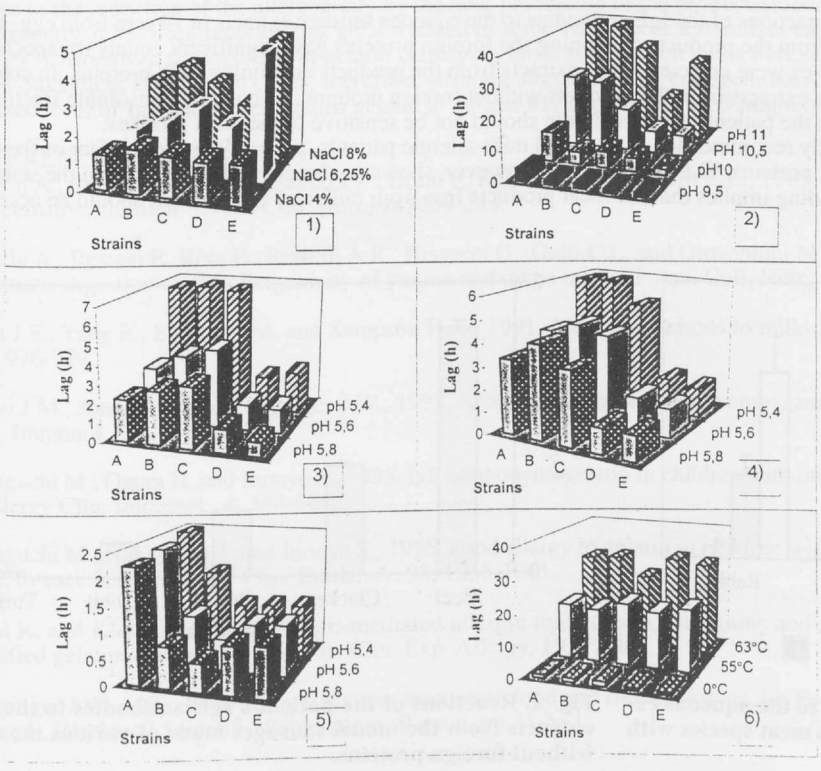

Fig 1. Lag times induced by the different treatment on Listeria monocytogenes. ~ I) NaCL, 2) NaOH, 3) Acetic acid, 4) Lactic acid, 5) HCI, 6) Temperature.