Food Microbiology 28 (2011) 702–711

Contents lists available at ScienceDirect

Food Microbiology

journal homepage: www.elsevier.com/locate/fm

Towards the quantification of the effect of acid treatment on the heat tolerance of *Escherichia coli* K12 at lethal temperatures

E.G. Velliou [a,b,1], E. Van Derlinden [a,b,1], A.M. Cappuyns [a,b,1], E. Nikolaidou [b], A.H. Geeraerd [a,c,1], F. Devlieghere [a,d,1], J.F. Van Impe [a,b,*,1]

[a] *CPMF2-Flemish Cluster Predictive Microbiology in Foods*
[b] *BioTeC-Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium*
[c] *Division of Mechatronics, Biostatistics and Sensors, Department of Biosystems, Katholieke Universiteit Leuven, W. de Croylaan 42, B-3001, Leuven, Belgium*
[d] *Laboratory of Food Microbiology and Food Preservation, Department of Food Technology and Nutrition, Ghent University, FOOD2KNOW, Coupure Links 653, B-9000 Ghent, Belgium*

ARTICLE INFO

Article history:
Received 10 March 2010
Received in revised form
18 June 2010
Accepted 23 June 2010
Available online 7 July 2010

Keywords:
E. coli K12 MG1655
Heat resistance
Stress
Stress adaptation
Acid treatment
Thermotolerance
Inactivation

ABSTRACT

The aim of this work is to investigate the effect of acid treatment -before and during heat inactivation- on the heat resistance of *Escherichia coli* K12 MG1655 cells at lethal temperatures. *E. coli* cells were grown in Brain Heart Infusion broth until they reached the stationary phase ($\approx 10^9$ cfu/mL). Approximately 30 min before thermal inactivation the early stationary phase cells were added in Brain Heart Infusion broth with a specific pH value, achieved with addition of either acetic (50% (v/v)), lactic (50% (v/v)) or hydrochloric acid (30% (v/v)), and inactivation experiments took place at 54 °C and 58 °C. The inactivation dynamics are analysed using the inactivation model of Geeraerd et al. (2000). This enables to define the induced thermotolerance of *E. coli* as a prolongation of the shoulder and/or a reduction of the inactivation rate. Generally, addition of acids increased the heat resistance of *E. coli*. The induced resistance depends on the type of acid and on the quantity added, i.e. different levels of acidification lead to a different level of heat resistance.

This work provides additional knowledge on the reaction of bacterial cultures to heat after acid treatment -before and during heat treatment- and, therefore, it contributes to an improved understanding of the effect of acid exposure on the bacterial heat resistance.

1. Introduction

Recent food processing procedures tend towards less aggressive techniques, i.e., high hydrostatic pressure, irradiation and mild heat treatment. In contrast to the more traditional processes like pasteurization and sterilization, these new processing techniques maintain the textual and sensory characteristics of fresh food products. However, as the applied conditions are less harsh, microbial survival and even stress adaptation might not be prevented.

Microorganisms reveal stress adaptation, which is actually the increase of a microorganism's resistance to environmental conditions, i.e., temperature, acidity, increased concentrations of salt, presence of (toxic) chemical agents, etc., that would normally be lethal and this by pre-exposure to a similar stress factor (Cebrian et al., 2009; Hassani et al., 2005, 2006; Juneja and Marks, 2003; Valdramidis et al., 2006, 2007) or a different (Leyer and Johnson 1993; Juneja and Novak, 2003; Skandamis et al., 2008, 2009) kind of stress, i.e., another stress factor than that responsible for the initial microbial stress response.

For example, exposure to a temperature shock may lead to bacterial resistance to another type of environmental stress. Other studies have proven that heat (or acid) shocking may increase the resistance of bacteria to (heat), acids, ethanol and/or NaCl (see as examples Leyer and Johnson, 1993; Tetteth and Beuchat, 2003). The ability of a stress adapted microorganism to resist when it is exposed to another kind of environmental stress is known as *cross protection* (Juneja and Novak, 2003).

When the bacterial cells are exposed to an environmental stress, they respond in several ways. Most likely they produce proteins which are known as shock proteins- e.g. GroEL, DnaK, DnaJ, etc.- and their main function is to repair the damages caused by the stress factor or eliminate the stress agent (Ohtsuka et al., 2007; Yousef and Courtney, 2003).

* Corresponding author. BioTeC-Chemical and Biochemical Process Technology and Control, Department of Chemical Engineering, Katholieke Universiteit Leuven, W. de Croylaan 46, B-3001 Leuven, Belgium. Fax: +32 16 32 2991.
E-mail address: jan.vanimpe@cit.kuleuven.be (J.F. Van Impe).
[1] http://www.cpmf2.be/

0740-0020/$ – see front matter © 2010 Published by Elsevier Ltd.
doi:10.1016/j.fm.2010.06.007

Another way the bacteria protect themselves from an environmental stress factor is via the alternation of the ratio of the fatty acids of the cellular membrane. When the concentration of saturated fatty acids in the membrane increases and is higher than that of the unsaturated fatty acids the membrane fluidity decreases and, therefore, the bacterial cells become more resistant to stress factors such as heat. Brown et al. (1997) observed for five strains of *E. coli*, i.e., MJR, M23, R172, R91 & O157:H-, that when the bacteria were acid shocked (exposed to a low pH of 3) the non saturated fatty acids were replaced by saturated ones and the bacterial acid resistance increased, respectively. Similar phenomena were observed by Alvarez-Ordonez et al. (2008, 2009) for *Salmonella typhimurium* and *Salmonella sentfenberg*, respectively.

Acid stress can be defined as the combined biological effect of low pH and weak (organic) acids present in the bacterial cellular environment. At low pH weak (organic) acids are mostly uncharged and they diffuse via the cellular outer membrane to the inner part of the cell, where they dissociate, leading to a decrease of the internal pH of the cell. The lower the external pH is, the higher the influx of organic acids in the cell. Strong acids, like HCl, lead to trafficking of the dissociated [H^+] in the cell via the membrane leading to an increase of the internal pH of the cell. Generally, the constant influx of protons in the cell leads to cellular death due to energy depletion (Foster, 2004; Bearson et al., 1997).

The level of the induced tolerance is dependent –among other factors, i.e., the duration of the exposure, the rate, etc., on the growth phase of the cells. Small et al. (1994) stated that, under acid stress conditions, when *E. coli* K12 enters the stationary phase slowing down and finally ceasing growth, morphological and genetic alternations lead to an increased resistance to a variety of stress conditions. According to Buchanan and Doyle (1997) non-pathogenic *E. coli* culture is more acid resistant during the stationary phase compared to the log-phase growth phase.

Differences at the level and the extent of acid resistance are observed between strains of a specific microorganism. For example, Gorden and Small (1993) concluded that the pathogenic *E. coli* strains, i.e., enterohaemorrhagic, enteroinvasive and enteropathogenic, have a higher acid resistance compared to the non-pathogenic *E. coli* K12. In the present study *E. coli* K12 (Jensen, 1993) has been chosen as a surrogate for the food-borne pathogen strain *E .coli* O157:H7. The K12 strain differs from the pathogenic O157:H7 by the absence of a number of features, i.e., plasmids and phage that encode specific virulence factors (Kaper et al., 2004). Nevertheless, the systematic study of the (non-pathogenic) *E. coli* K12 strain is informative for the understanding of the bacterial stress adaptation.

Understanding the mechanisms and the relation between acid stress and heat adaptation is of great importance for food safety issues, since during a food process or in a food product microorganisms are exposed to a variety of factors that may lead to stress and further adaptation. For instance, acids are used as preservatives in food products or they might be produced due to a fermented vegetation. Moreover, mild heat treatments or innovative techniques, like high hydrostatic pressure may lead to a progressive heat adaptation of the -possibly present- microorganisms (see as examples Leyer et al., 1995; Yousef and Courtney, 2003; Skandamis et al., 2008).

This work aims at providing additional knowledge on the reaction of bacterial cultures to heat after exposure to acids shortly before and during heat inactivation and, therefore, it contributes to an improved understanding of the effect of acid exposure on the bacterial heat resistance.

More specifically, the heat resistance of *E. coli* K12 at 54 °C and 58 °C is studied, when exposed to different kinds of acids. Early stationary phase cells are brought in an acidified BHI solution approximately 30 min before heat inactivation. The acidified BHI -of specific lower pH than the unmodified BHI- was gained by addition of acetic (50% (v/v)), lactic (50% (v/v)) or hydrochloric (30% (v/v)) acid.

2. Materials and methods

2.1. Inoculum preparation

E. coli K12 MG1655 stock culture was stored at −80 °C in Brain Heart Infusion (BHI) broth (Oxoid Limited, Basingstoke, UK) with 25% (v/v) glycerol (Acros Organics, NJ, USA). For the preparation of the inoculum a loopful of the stock culture was transferred in 20 mL of BHI broth and was incubated at 37 °C on a rotary shaker (175 rpm) for 9.5 h. 20 μL of the cell suspension was transferred to 20 mL of fresh, i.e. unmodified, BHI broth and was incubated at 37 °C for 15 h. Early stationary phase cultures were harvested by centrifugation (1699 g, 2 min, 20 °C) and portions of the cell suspensions were washed in acidified BHI.

2.2. Preparation of the inoculated heating medium

The pH value of the unmodified BHI broth is approximately 7.5. It should be mentioned that during growth the pH of the medium decreases to approximately 6.5 due to the production of acetic acid. Afterwards, the pH increases again to approximately 7.8 when cells reach the stationary phase. This pH fluctuation could lead to a specific acid adaptation during growth. However, this possible adaptation is equal and present in all conditions under study, enabling the comparison between treatments.

Acidified BHI of pH 5.0, 5.5 and 6.0 was prepared with addition of different acids each time, in fresh BHI broth. The acids added were hydrochloric acid 30% (v/v) (Acros Organics, NJ, USA), acetic acid 50% (v/v) (Acros Organics, NJ, USA) and lactic acid 50% (v/v) (Acros Organics, NJ, USA). For acetic acid, one additional experiment at pH 4.0 was performed. The harvested cells prepared from the step described in paragraph 2.1, remained in the acidified BHI for approximately 30 min at room temperature.

2.3. Thermal inactivation of E. coli at static temperatures

Static inactivation experiments took place in sterile glass capillary tubes in which a volume of 60 μL cell suspension (prepared as described in paragraph 2.2) was pipetted. Tubes were then sealed by a gas flame and immersed in a water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK), at static temperatures of 54 °C and 58 °C. At regular times two capillaries were removed from the water bath, placed in an ice-water bath and analysed within approximately 45 min. Decimal serial dilutions of the samples were prepared in a BHI solution and surface plated on BHI agar (1.2% (w/v)) using a Spiral Plater (Eddy Jet IUL Instruments, Barcelona, Spain). The volume plated was 49.2 μL. Plates were incubated for 24 h at 37 °C and colony forming units were enumerated. The detection limit was 3log cfu/mL. Each experiment was repeated in duplicate.

2.4. Mathematical modelling

The experimental data (cell density data) were *log*-transformed and plotted as a function of time. The inactivation model of Geeraerd et al. (2000) (Geeraerd et al., 2000; Valdramidis et al., 2005) was fitted to the data (Equation (1)).