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Heat adaptation of *Escherichia coli* K12: effect of acid and glucose

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Abstract

The objective of this work is to investigate the effect of the (possible) acid adaptation during growth in a glucose rich environment on the heat resistance of *Escherichia coli* K12 MG1655. *E. coli* cells were grown in TSB and/or TSB dextrose free broth until they reached the stationary phase. Afterwards, the stationary phase cells were added in TSB and/or TSB dextrose free broth and inactivation took place at 54°C and 58°C. It was observed that growth in a glucose rich environment leads to an increased heat resistance, most likely due to a certain level of acid and further heat adaptation via cross protection.

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Keywords: *E. coli*; acid adaptation; heat resistance; cross protection; glucose

1. Introduction

Nowadays, consumers demand food products of high quality sensory characteristics, i.e., flavour, taste and texture. Therefore, recent food processing procedures tend towards less aggressive techniques, e.g., mild heat treatment, high hydrostatic pressure etc. In contrast to more conventional processes like sterilization and pasteurization, these new processing techniques maintain the textural and sensory characteristics of fresh food products. However, as the applied conditions are less harsh, microbial

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survival and even stress adaptation might not be prevented. Therefore, a more profound understanding of the mechanisms of microbial stress adaptation and resistance is of significant importance for food safety issues.

Generally, microorganisms reveal stress adaptation, i.e., the increase of a microorganism's tolerance to environmental conditions which would normally be lethal, e.g., heat, acidity, increased salt concentration, metabolic products and presence of (toxic) chemical components. The stress adaptation usually results from pre-exposure to a similar stress factor [1-6] or a different kind of stress, i.e., another stress factor than that responsible for the initial microbial stress response [7-10]. For example, exposure to a sub-optimal temperature may lead to resistance when exposed to sub-optimal levels of another environmental factor. Studies have proven that heat (or acid) stress exposure may increase the resistance of bacteria to (heat), acids, ethanol and/or NaCl (see as examples [7] and [11]). The ability of a stress adapted microorganism to resist when it is exposed to another kind of environmental stress is known as *cross protection* [8].

For example, exposure to a sub-optimal temperature may lead to bacterial resistance when exposed to sub-optimal levels of another environmental factor. Other studies have proven that heat (or acid) stressing may increase the resistance of bacteria to (heat), acids, ethanol and/or NaCl (see as examples [7] and [11]). The ability of a stress adapted microorganism to resist when it is exposed to another kind of environmental stress is known as *cross protection* [8].

When microbial cells are exposed to an environmental stress, they can respond in several ways. Most likely, specific genes, related to the cellular stress response are expressed and/or over-expressed, finally leading to the production of proteins which are known as *shock proteins*. The main function of these proteins is to repair the damages caused by the stress factor or to eliminate the stress agent [8, 12, 13]. Another way bacteria respond to a stressing factor and further protect themselves is via the alternation of the ratio of the fatty acids of the cellular membrane, i.e. increase of the saturated compared to the un-saturated fatty acids of the membrane that decreases the membrane fluidity, which leads to increased acid resistance [14-16].

Understanding the mechanisms and the relation between acid and further heat adaptation is of significant importance for food safety, since in a food product or during certain (food) industrial processes microorganisms are exposed to a variety of environmental stressing factors that could lead to a further microbial adaptation and further survival in a food system. For instance, acids are used as preservatives in food products or they might be produced due to 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 [9, 13, 17, 18].

This research studies the reaction of bacterial cultures to heat when they are pre-acid adapted during growth in a glucose rich environment. Hereto, E. coli K12 is either grown in TSB or TSB glucose free before inactivation qt 54C and 58C, in one of these two media.

2. Materials and Methods

2.1. Inoculum preparation

E. coli K12 MG1655 stock culture was stored at -80^{o}C in Tryptic Soy Broth (TSB) (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 TSB or TSB dextrose free (TSBdf) broth and was incubated at 37^{o}C on a rotary shaker (175 rpm) for 9.5 h. Next, 20 μL of the cell suspension was transferred to 20 mL of TSB or TSBdf broth and incubated at 37^{o}C for 15 h. Early stationary phase cultures were harvested by centrifugation (1699 g, 2 min, 20^{o}C) and portions of the cell suspensions were washed in TSB or TSB df.

The different combinations of growth (at 37°C) and thermal inactivation (at 54°C and/or at 58°C) media, i.e., different combinations of TSB and TSBdf, under study are the following:

- Growth in TSB broth and inactivation in TSB broth.
- Growth in TSBdf broth and inactivation in TSBdf broth.
- Growth in TSB broth and inactivation in TSBdf broth.
- Growth in TSBdf broth and inactivation in TSB broth.

2.2. 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 above) was pipetted. Tubes were then sealed by a gas flame and immersed in a water bath (GR150-S12, Grant Instruments Ltd, Shepreth, UK), at 54°C and/or 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 TSB solution and surface plated on TSB 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 3 log CFU/mL. Each experiment was repeated in duplicate.

2.3. 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) ([19], [20]) was fitted to the data (Equation 1).

$$N(t) = \left(N(0) - N_{res}\right) \cdot \exp(-k_{\max} \cdot t) \cdot \left(\frac{\exp(k_{\max} \cdot S\imath)}{1 + \left(\exp\left(k_{\max} \cdot S\imath\right) - 1\right) \cdot \exp\left(-k_{\max} \cdot t\right)} \right) + N_{res} \qquad (1)$$

with N [CFU/mL] the cell population, $N(0)$ [CFU/mL] the initial cell population, N_{res} [CFU/mL] the stress resistant subpopulation, k_{max} [1/min] the maximum specific inactivation rate, Sι [min] the shoulder period and t [min] the time.

2.4. Data analysis

Modelling of the data was performed with GinaFiT (Version 1.5), a freeware add-in for Microsoft® Excel [21]. Graphical illustrations were generated in MatLab® Version 7.4 (The Mathworks, Inc., Natick, USA).

3. Results & Discussion

The heat resistance of *E. coli* K12 at 54°C and 58°C is studied, when grown and (thermally) inactivated in TSB, TSBdf and in combinations of them, i.e., growth in TSB and inactivation in TSBdf or growth in TSBdf and inactivation in TSB.

The results of the inactivation experiments are summarized in Figures 1 and 2. Microbial inactivation was followed approximately until 3 log CFU/mL (i.e., the detection limit). Generally, duplicate experiments revealed rather similar behaviour. A typical microbial curve of the present study includes, in most cases, a shoulder phase followed by a linear part (Figures 1 & 2). Experimental data were described after parameter identification using the inactivation model of Geeraerd et al. (2000), as shown in Figures

1 & 2. The resulting estimates for the inactivation rate, k_{max}, and the corresponding standard errors are shown on Table 1. In this study, the change in the heat resistance is defined by the duration of the shoulder phase and/or a change in the inactivation rate, k_{max}.

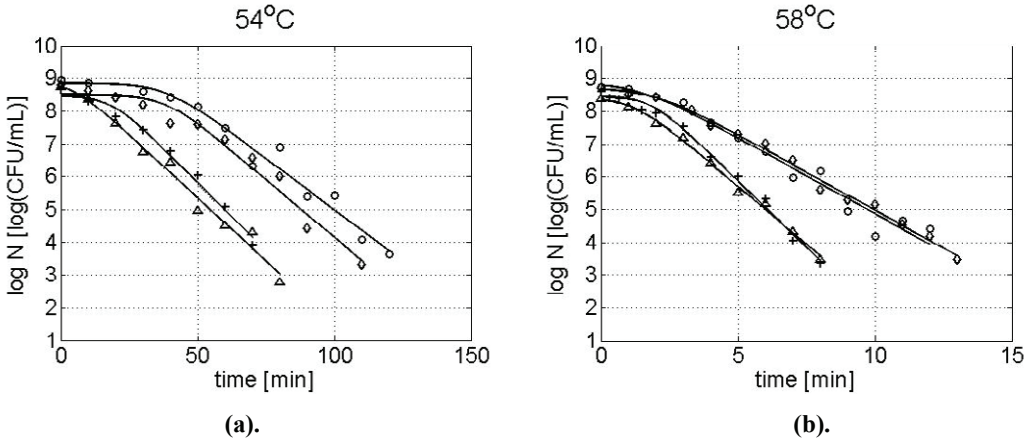

Fig. 1. Fits of the Geeraerd et al. (2000) model on the experimental data at (a) 54 °C and (b) 58 °C : (+) & (Δ) Growth and inactivation in TSBdf broth, and (o) & (◊) Growth and inactivation in TSB broth.

When *E. coli* is grown and heat inactivated in TSBdf, inactivation starts rapidly without any preceding shoulder phase at 54°C as well as at 58°C (Figure 1(a) and 1(b) respectively). When grown and inactivated in TSB with glucose a clear shoulder precedes the linear inactivation at 54°C. At 58°C a shoulder phase is present but less pronounced (Figure 1). Generally, the shoulder represents a time period during which bacterial cells are capable to re-synthesize a vital component. Death ensues only when the rate of destruction exceeds the rate of synthesis [19]. The presence of the shoulder period is an indication of increased heat resistance. When examining the inactivation rates of the log-linear part of the inactivation (Table 1) it is observed that in the case of TSB medium the values are lower, i.e., slower inactivation, compared to TSBdf at both studied temperatures, indicating an increased thermotolerance in the case of growth in TSB broth. The presence of glucose in the environment during growth leads to the production of acetic acid which lowers the pH. The concentration of acetate during growth of *E. coli* at 37°C in presence of glucose increased from 0 to $1{,}6 \times 10^{-3}$ g/L after 6 hours (data not shown). This acidic environment during growth is a –shelf induced- acid stress for the bacteria, possibly leading to a certain level of acid adaptation and further heat resistance –via *cross protection*- [8].

In order to investigate whether the observed bacterial heat resistance is a result of the presence of glucose during growth and/or during heat inactivation, inactivation experiments were performed at 54°C in TSB and TSBdf medium after growth in TSBdf and TSB medium, respectively (Figure 2). Bacterial inactivation at 54°C is similar after growth in TSBdf medium independently of the inactivation medium, i.e. TSB or TSBdf, as can be seen in Figure 2(b) and on Table 1. When *E. coli* is grown in a glucose rich environment, i.e. in TSB broth, and further inactivated at 54°C in TSBdf broth the induced resistance is equal to the one observed for inactivation in TSB broth (after growth in TSB) by means of similar prolongation of the shoulder and approximately equal values of the inactivation rate (Figure 2(b), Table 1). These findings indicate that the *presence of glucose during growth* is critical regarding the induction of heat resistance of *E. coli* at lethal temperatures. However, the presence or absence of glucose in the inactivation medium seems not to affect the inactivation kinetics.

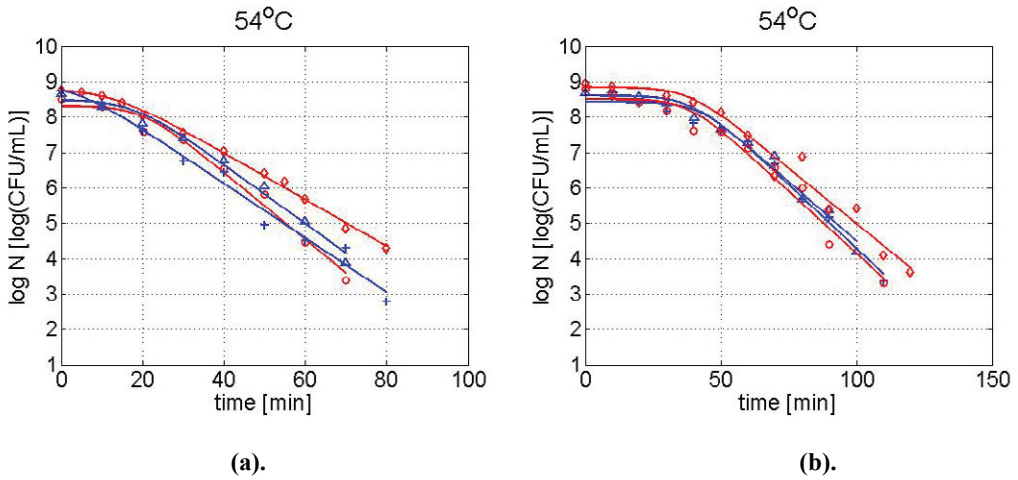

Fig. 2. Fits of the Geeraerd et al. (2000) model on the experimental data at 54 °C: (a). (+) & (Δ) Growth and inactivation in TSBdf (o) & (◊) Growth in TSBdf and inactivation in TSB, (b). (+) & (Δ) Growth in TSB and inactivation in TSBdf (o) & (◊) Growth and inactivation in TSB. Different symbols represent replicate experiments.

Generally, in the present research it was observed that growth in a glucose rich environment leads to a certain level of acid adaptation and further heat resistance of *E. coli* at lethal temperatures, most likely via cross protection. Several other researchers as well, have proven a relation between acid adaptation and heat resistance. For example, Leyer et al. [7] showed that there is a relationship between acid adaptation and –increased- tolerance to other environmental stresses, e.g., heat and salt, for *Salmonella* Typhimurium. Tetteh et al. (2003) [11] have proven that heat and/or acid stress leads to an increase of the concentration of heat shock proteins and, therefore a further increased acid tolerance of *Shigella flexneri*. Jorgensen et al. [22] observed that the presence of lactic acid has a specific positive effect on the response of *Listeria monoytogenes* to heat. Velliou et al. [18] observed an increased thermotolerance of *E. coli* at 54 and 58°C in presence of different types of acids, i.e., HCl, acetic acid and lactic acid. The level of the induced heat resistance was dependent on the acid type and the level of acidification, i.e., pH value. Skandamis et al. [9] showed that there is an increased heat resistance of stationary phase cultures of *Listeria monocytogenes* at 52, 57 and 63°C, following exposure to an acidic environment.

The present research aims at giving more light on the (possible) changes in the bacterial thermotolerance when they are pre-acid adapted due to growth in a glucose rich environment. Therefore, it contributes to an improved understanding of the level of the induced heat resistance when pre-exposed to a different environmental stressing factor during growth.

Table 1. Values of the inactivation rate, k_{max}, of the model of Geeraerd et al. (2000) for all conditions under study.

Medium/ Tempeture (°C)	Replicate	k_{max} (SE) [1/min]
Growth and inactivation in TSB at 54 °C	1	$1{,}44*10^{-1}$ ($1{,}21*10^{-2}$)
	2	$1{,}62*10^{-1}$ ($1{,}58*10^{-2}$)
Growth and inactivation in TSB at 58°C	1	$1{,}07*10^{0}$ ($3{,}65*10^{-2}$)
	2	$1{,}06*10^{0}$ ($8{,}94*10^{-2}$)
Growth and inactivation in TSBdf at 54 °C	1	$1{,}77*10^{-1}$ ($1{,}31*10^{-2}$)
	2	$1{,}92*10^{-1}$ ($1{,}58*10^{-2}$)
Growth and inactivation in TSBdf at 58 °C	1	$1{,}86*10^{0}$ ($9{,}17*10^{-2}$)
	2	$1{,}62*10^{0}$ ($6{,}15*10^{-3}$)
Growth in TSB and inactivation in TSBdf at 54 °C	1	$1{,}54*10^{-1}$ ($1{,}28*10^{-2}$)
	2	$1{,}66*10^{-1}$ ($1{,}21*10^{-2}$)
Growth in TSBdf and inactivation in TSB at 54 °C	1	$1{,}52*10^{-1}$ ($4{,}83*10^{-3}$)
	2	$2{,}18*10^{-1}$ ($1{,}97*10^{-2}$)

4. Conclusion

Increased heat resistance of *E. coli* has been observed after growth in a glucose rich environment (TSB), compared to growth in absence of glucose (TSB dextrose free). In a glucose rich environment, the main cellular metabolic product is acetic acid. The presence of acetic acid lowers the pH of the medium leading to acid stress and, as a reaction, (self-induced) acid adaptation. This acid adaptation leads to an increased heat resistance of *E. coli* at lethal temperatures. However, the presence or absence of glucose in the inactivation medium has no clear effect of the bacterial thermotolerance.

This work provides additional knowledge on the reaction of bacterial cultures to heat when they are acid adapted. Additionally, it contributes to an improved understanding of the role of glucose during growth and/or heat inactivation.

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