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Effect of salt concentrations on the growth of heat-stressed and unstressed *Escherichia coli*

S. M. Abdulkarim [1] *, A. B. Fatimah [1] and J. G. Anderson [2]

[1] *Department of Food Science, Faculty of Food Science and Technology, Universiti Putra Malaysia, 43400 UPM Serdang, Selangor, Malaysia.* [2] *Strathclyde Institute of Pharmacy and Biomedical Sciences University of Strathclyde, Royal College, 204 George Street, Glasgow G1 1XW Scotland. *e-mail: ak@food.upm.edu.my*

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Abstract

The effect sodium chloride (NaCl) and potassium chloride (KCl) concentration on the growth of *Escherichia coli* cells cultivated at 37 and 44°C was studied in an effort to understand the importance of the salts and glucose in medium to the growth of *E. coli*. A turbidimetric method was used to measure the growth of *E. coli* after a 24 hours incubation period. The turbidimetric method used gave a high correlation (R^2 = 0.9606) with the traditional surface colony count method. Four sets of salt concentrations, 0, 0.5, 1.0 and 1.5% (w/v), were employed throughout this study. Absence of NaCl in the medium was found to slightly decrease the growth of *E. coli* at 37°C. *E. coli* grew optimally at 0.5% (w/v) NaCl concentration. Addition of 0.5% KCl was found to have less beneficial effect on the growth of *E. coli* at 37°C compared to cells grown in medium with 0.5% NaCl. Increase in the concentrations of both salts above 0.5% decreased growth at 37°C. The extent to which growth was suppressed was directly proportional to the concentration of salts. At zero concentration of both salts, growth of *E. coli* was very low at 44°C. Increase in the concentrations of both NaCl and KCl from 0.5% to 1.5% resulted in growth enhancement. Glucose affected significantly the growth of *E. coli* at 37°C. Addition of 140 mM (w/v) of glucose to the medium increased the growth of *E.* coli at 37°C to a greater extent than was obtained by salt addition. However, the addition of the same concentration of glucose was found to have only a very slight effect on growth at 44°C.

Key words: Heat-stressed *Escherichia coli,* salt concentration, growth.

Introduction

Enrichment media have long been and continue to be extremely important for the detection and isolation of indicator and pathogenic bacteria. Optimization of growth in these media to shorten the analysis time is now even more important when these media are used in conjunction with rapid PCR or immunodiagnostic techniques. Great attention is usually paid to the nature of C and N sources and selective inhibitors in enrichment media, but there is less understanding of the importance of ionic composition. This can be particularly important when low numbers of stressed cells must be recovered. Microorganisms require variable amounts of salt for growth and metabolism. In general, the requirement for salt is not an exclusive need for sodium chloride (NaCl) because many halophiles require low levels of K^+ (potassium ions), Mg^{++} (magnesium ions), and other cations and anions in addition to NaCl [1]. Furthermore, for some bacteria, the apparent requirement for NaCl is not specific and other salts and sugars can be substituted.

Microbes are able within limits to adapt to stress conditions such as heat or acidity. The mechanism of adaptation is by signal transduction system, which controls the coordinated expression of genes involved in cellular defense mechanisms. Inclusion of preservative is one of the factors that affect microbial growth. Sodium chloride has been used as an additive and preservative in food. As antimicrobial agent, NaCl has often been incorporated as an ingredient in meat and meat product [2, 3].

Generally, bacteria belonging to the family Enterobacteriaceae, such as *E. coli* and *Salmonella,* do not tolerate high salt levels [4]. However, certain strains of *E. coli* are halo-tolerant and are able to survive and grow in high salt concentrations. This high osmotic strength seems to be due to the production of proline in the cells [5]. When enteric and pathogenic bacteria are released from their hosts into natural environments [6, 7], they are often challenged by various environmental stresses, such as nutrient starvation, osmotic shock, temperature variation, oxidative stress, etc. [8]. Greater understanding of the effects of environmental stresses on *E. coli* is urgently required. Transmission to a new host often involves a period of exposure to a hostile external environment. How *E. coli* cells cope with such exposure and the possible role of the new information concerning the influence of environmental factors on the physiology of *E. coli* cells should help increase our understanding of how these organisms survive and retain infectivity in natural environments and may also help in the development of improved methods for the resuscitation and recovery of environmentally stressed cells. A number of environmental factors has been examined previously. In this study the responses of heat-stressed and unstressed *E. coli* cells to exposure to media having different salt (sodium and potassium chloride) concentrations was examined.

Materials and Methods

***Media and chemicals*:** All media used in this work were from Oxoid. Other chemicals were from BDH and Oxoid Laboratories UK. The media used were commercial grades from Oxoid. Preparations of these media were done according to the manufacturer's instruction manual. A modified medium was also prepared and used. The media used and their constituents are as follows: (a) MN Broth (Modified Nutrient Broth), which basically has the same composition as nutrient broth No. 2, from Oxoid but no sodium chloride was added during preparation. Other media used were: (b) MacConkey agar No. 3., (c) Yeast Extract Agar and (d) Brilliant Green Bile (2%) broth.

Media modifications: The compositions of media were modified by addition of sodium chloride, potassium chloride and glucose in different concentrations as follows:

1. Modified Nutrient Broth (MNB) + 0.5% NaCl
2. MNB + 1.0% NaCl
3. MNB + 1.5% NaCl
4. MNB + 0.5% KCl
5. MNB + 1.0% KCl
6. MNB + 1.5% KCl
7. MNB + 70 mM NaCl
8. MNB + 70 mM KCl
9. MNB + 140 mM glucose

Dilution solvents: Serial dilutions for surface colony counts were made using a quarter strength Ringer solution (Oxoid), preparation of which was according to the manufacturers' instruction manual. Where necessary, dilutions were made with blank MNB medium. All dilutions carried out were in ten fold. For the most accurate colony count, dilutions were selected so that the total number of colonies will be small.

Bacterial strain: *Escherichia coli* was used throughout this work. The bacterial strain was supplied from the culture collection of the Department of Bioscience & Biotechnology, University of Strathclyde, Scotland. A pure culture was supplied. All identification procedures were carried out at the Bacteriology Section of the Department of Bioscience and Biotechnology of the University of Strathclyde as recommended for *E. coli* [9].

Maintenance and purity check of cultures: Pure cultures of the *E. coli* strain obtained were grown on nutrient agar slope, incubated at 37°C for 24 hours. The culture growth was then washed with 10 ml of nutrient broth into a 1 oz. screw-capped bottle previously sterilized and fully labeled. The culture was then stored at 4°C until needed. The purity of the culture was regularly checked by plating on agar.

Inoculation of media: Ten ml each of media was dispensed into 10 screw capped 1oz. bottles, containing inverted Durham tubes. The bottles were labeled and sterilized by autoclaving at 121°C for 15 minutes. A loop full of pure *E. coli* culture from the stock culture was inoculated into each of the 10 bottles. Of these 10 bottles 5 were incubated at 44°C and 37°C in a thermostatically controlled water bath for 24 hours. At the end of incubation, the bottles were examined for presence of gas and/or change in turbidity. Procedure above was repeated on each of the modified media with different salt and glucose concentration and on a medium with no added salt or glucose.

Counting of viable cells: Surface colony count method was employed. A drop (0.05 ml) of serial dilution was dispensed onto the surface of poured agar plates, and the colonies which developed upon incubation of the plates at 37°C were counted using surface plate count method. Of 10-fold serially diluted culture grown in media with different salt and glucose concentrations at temperatures of 37 and 44°C, 0.05 ml was dispensed onto the surface of yeast extract and MacConkey agar plates. Using a sterile glass rod the cultures were evenly spread and the plates incubated at 37°C for 24 hours. At the end of 24 hours, plates were examined for the development of colonies. Plates showing development of colonies were selected and the colonies counted using a colony counter and with the aid of a low power magnification lens.

Turbidimetric measurements: The media used for growing *E. coli* was first scanned using a spectrophotometer. The scanning helps detect wavelengths at which absorption due to the color of media occur. Once the range of potentially interfering wavelength was found, a new wavelength is set to read the absorbance due to the turbidity as a result of bacterial growth. The absorbance of the culture growth was determined by using visible light at a wavelength of 650 nm. One ml of the culture growth was placed in micro cuvette and slotted into the spectrophotometer and the absorbance reading recorded.

Preparation of standard curve: Media with different salt compositions were inoculated with a loop full of *E. coli* culture, and incubated at 37°C. Two samples were obtained from each culture growth at every hour. One sample was serially diluted and plated on agar and the second sample was used to obtain absorbance readings due to turbidity caused by growth. The absorbance was plotted against the number of colonies (log CFU) obtained from colony counts (Fig. 1).

Effect of NaCl and KCl salts on growth of E. coli at 37 and 44°C: Thirty bottles containing 10 ml each of medium were used. Out of the 30 bottles 15 contained NaCl, 5 each with 0.5, 1.0 and 1.5% (w/v) NaCl and the other 15 bottles contained KCl, 5 each with the same concentration of KCl as in NaCl. The bottles were inoculated with a loop full of *E. coli* culture and incubated at 37°C for 24 hours. The same procedure was repeated and the incubation

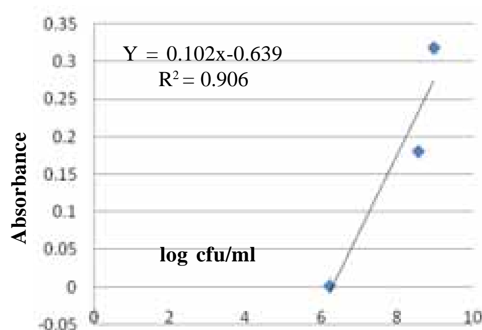

Figure 1. Standard curve for the growth of *E. coli* at 37°C.

was done at 44°C for 24 hours. The growth in each bottle was measured by APC.

Results and Discussion

Effect of NaCl and KCl salts on growth of E. coli incubated at 37°C: Table 1 shows results for NaCl and KCl. Cultures grown at 37°C showed a significant ($P < 0.05$) increase in growth with increase in both salt concentrations from 0% (No salt) to 0.5%. The 0.5% salt represents the highest growth obtained (9.20×10^7 CFU/ml) and (5.56×10^7 CFU/ml), respectively, for NaCl and KCl. With further increase in the NaCl concentration to 1.0 and 1.5% growth was found to steadily decrease accordingly to 8.20×10^7 CFU/ml and 4.04×10^7 CFU/ml. Growth sharply decreased in the case of KCl as the concentration increased to 1 and 1.5%. Figs 2 and 3, shows the relationship between increases in NaCl and KCl concentration, respectively, to total cell growth at 37°C. The optimum concentration for *E. coli* growing at 37°C was 0.5%. This may explain the reason why most of the media formulations contain 0.5% (w/v) NaCl. The decrease in total growth due to increase in the salt concentration was probably as a result of hyper osmotic effect.

Culture cultivated with 0.5% KCl in medium showed very little increase in total growth compared to culture cultivated without salt. Growth was, however, generally affected in the same manner as with NaCl for the different KCl concentrations, only that growth at 1.0 and 1.5% KCl are much lower compared to growth with NaCl at the same concentration.

Effect of NaCl and KCl salts on growth of E. coli incubated at 44°C: Table 1 shows growth at 44°C with NaCl and KCl at various concentrations. At this temperature increase in the concentrations of both salts significantly ($P<0.05$) influenced the total growth of *E. coli* up to a certain limit (not fully determined in the experiment). The total cell growth was found to increase with increase in NaCl concentration from 0 to 1.5% (w/w). With increase in the temperature from 37°C to 44°C, increase in both NaCl and KCl concentration showed marked effect. Figs 4 and 5 show the relationship between the increase in NaCl and KCl concentration, respectively, and total growth of *E. coli* cells at 44°C. It has already been established that high salt concentration, which increases osmolarity of the medium, not only overcome temperature sensitive mutations but also increase the high temperature limit for the growth of many bacteria [10-12]. There are several studies that have shown the combined effect of salt and heat treatment on bacterial growth. Adding salt or other ingredients increases the heat resistance of pathogenic bacteria including *E. coli* O157:H7

Table 1. Effect of salt and glucose content of media on the growth of heat-stressed and unstressed *E. coli.*

	Conc. (%w/w)	Growth (10^7 CFU/ml) 37°C	44°C
Control	0	4.29 ± 0.79	0.28 ± 0.03
	0.5	9.20 ± 0.51	2.62 ± 0.43
NaCl	1.0	8.20 ± 1.07	5.80 ± 1.51
	1.5	4.04 ± 0.93	6.08 ± 1.08
	0.5	5.56 ± 0.79	1.58 ± 0.17
KCl	1.0	0.79 ± 0.02	8.34 ± 1.05
	1.5	0.54 ± 0.06	8.00 ± 0.79
Glucose	140 mM	++	0.42 ± 0.03

++ Bacterial counts > 10^8 CFU/mL

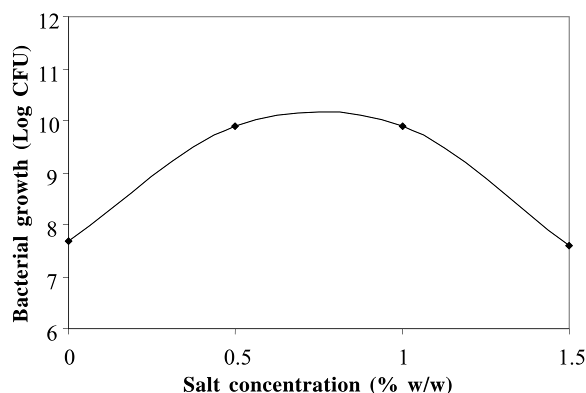

Figure 2. Bacterial growth at varying NaCl concentrations at 37°C and 24 h incubation.

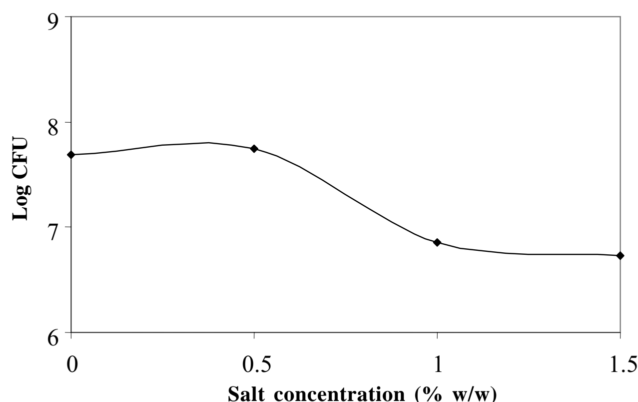

Figure 3. Bacterial growth at varying KCl concentrations at 37°C and 24 h incubation.

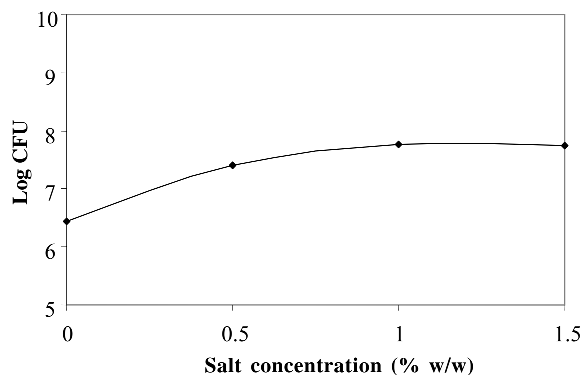

Figure 4. Bacterial growth at varying NaCl concentration at 44°C and 24 h incubation.

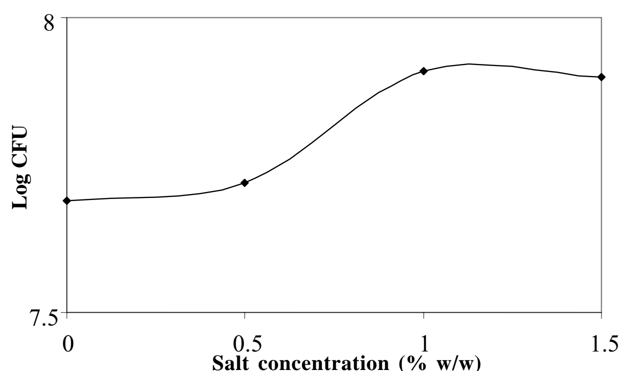

Figure 5. Bacterial growth at varying KCl concentrations at 44°C and 24 h incubation.

because they decrease water activity (a_w) of samples. Blackburn *et al.*[13] reported that heat resistance of *E. coli* O157:H7 increased with increasing NaCl up to 8.5% (w/w) and the resultant model predicted maximum D-values at about 5-7% (w/w) NaCl. A similar effect has also been seen for *Listeria monocytogenes* at NaCl concentrations up to 4.5% [14].

Effects of glucose addition to medium on growth of E. coli at 37 and 44° C: In this experiment the effect of glucose addition to medium on the total cell growth was determined. Nutrient broth medium prepared without salt was supplemented with 140 mM glucose. Results obtained (Table 1) showed that glucose supplementation of medium greatly enhanced growth at 37°C. The total cell growth (>10^8 CFU/ml) was higher than the total cell growth for any of the salts used at this temperature. However, at 44°C the addition of glucose does not influence the cells in overcoming the growth inhibitory effect of the temperature. Total cell growth (0.42 x 10^7 CFU/ml) was only slightly higher than cells grown at 44°C without salt or glucose.

Addition of glucose to medium at 37°C was more important to the growth enhancement than the salts. Glucose provides a carbon source for the bacterial cells for growth, metabolism and other energy requiring processes. At the higher temperature of 44°C, however, the high salt concentration was more important than glucose to help overcome the growth inhibitory effect of this temperature and therefore stimulated growth.

Conclusions

Higher salt concentrations of the medium increase its osmolarity, which is likely to have resulted in hyper osmotic shock to *E. coli* cells causing growth suppression. Consequently, the importance of increasing the salt concentration of medium on the growth of *E. coli* became more apparent when the temperature was raised from 37°C (optimum temperature for growth of *E. coli*) to 44°C. It was concluded that increasing the salt concentration in the medium partially overcame the inhibition of growth of *E. coli* at high temperature.

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