



Effect of Varying NaCl Concentrations on the Growth Curve of *Escherichia coli* and *Staphylococcus aureus*

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To cite this article:

Omotoyinbo O. V., Omotoyinbo B. I. Effect of Varying NaCl Concentrations on the Growth Curve of *Escherichia coli* and *Staphylococcus aureus*. *Cell Biology*. Vol. 4, No. 5, 2016, pp. 31-34. doi: 10.11648/j.cb.20160405.11

Received: January 18, 2017; **Accepted:** March 3, 2017; **Published:** March 18, 2017

Abstract: The effect of NaCl concentration on the growth of gram negative *Escherichia coli* and gram positive *Staphylococcus aureus* cells cultivated at 37°C was studied in an effort to understand the importance of NaCl to the growth of these bacteria. The bacteria were grown on standard nutrient agar in three groups containing NaCl concentrations; 0, 1.0 and 3.0% (w/v) with turbidimetric readings of absorbance taken at hourly intervals to obtain their growth curve. NaCl had an effect of shortening the latent period of growth in *S. aureus* previous to rapid growth, producing a characteristic lag phase which appears to have a somewhat greater accelerating effect than that obtainable in *E. coli* which is characteristic of most standard growth curves. At 0% (w/v) NaCl concentration, optimal growth of both *E. coli* and *S. aureus* was observed (0.557 and 0.583 respectively). However, an increase in the concentrations of NaCl above 0% to 1.0 and 3.0% decreased growth at 37°C. Hence, it was observed that the extent to which growth was suppressed was directly proportional to the increasing concentration of NaCl. Therefore NaCl was observed to inhibit growth of both bacteria at 37°C.

Keywords: NaCl Concentration, Growth Curve, Turbidity, *Escherichia coli*, *Staphylococcus aureus*

1. Introduction

For all living organisms to survive, they must be able to sense environmental parameters that define their habitats and react accordingly to the changes that come with various adaptive mechanisms [17]. Generally, bacteria belonging to the Enterobacteriaceae family, such as *Escherichia coli* and *Salmonella*, do not tolerate high salt levels, but some species, such as *Serratia rubidea*, are very salt-tolerant (up to 10% NaCl) [1]. Halotolerant strains of *E. coli* are able to survive and grow even at very high salt concentrations. It seems that this high osmotic strength is due to the production of proline in the cells [2, 3]. However, tolerance of *Staphylococcus aureus* to high concentrations of NaCl in liquid medium has been reported, with no damage or shrinkage observed in its cellular structure, while in the case of *E. coli*, cell injury occurred. NaCl was therefore reported to affect the morphology of *E. coli* and *S. aureus*, while having a milder effect with respect to cell damage, especially on *S. aureus*

[4]. A low salinity leads to an immediate influx of small solutes thus relieving physical stress, in contrast, high salinity leads to water efflux which is counterbalanced by an increase of compatible solutes such as proline, glutamate, glycine betaine, ectoine and trehalose [16]. This study aims at investigating the effect of the varying NaCl concentrations on the growth of *E. coli* and *S. aureus* at the various growth phases.

2. Materials and Methods

2.1. Media and Chemicals

All media used in this work were from Biomark Laboratories, Pune- India. Other chemicals were from Sigma. Preparations of these media were done according to the manufacturer's instruction manual. Media modifications were made by addition of Sodium chloride (NaCl) in different concentrations to standard media as follows: Media + 0% NaCl (Control), Media + 1.0% NaCl, Media + 3.0% NaCl.

2.2. Bacterial Isolates Identification

Clinical isolates of *E. coli* and *S. aureus* were used throughout this work. The bacterial strain was supplied from the culture collection of the Department of Microbiology, Federal University of Technology, Akure, Nigeria. A pure culture was supplied and all identification procedures were carried out at the Bacteriology Section of the same department as recommended for *E. coli* and *S. aureus*. Pure cultures of the bacteria strain obtained were grown on nutrient agar slants, incubated at 37°C for 18 hours. The culture growth was then stored at 4°C until needed. The purity of the culture was regularly checked by plating on fresh nutrient agar [5].

2.3. Inoculation of Media

Nutrient broth was produced according to manufacturer's instruction manual. Fifty (50) ml each of the three media NaCl concentration was dispensed into two conical flasks per category of the same salt enrichment and corked tightly. The flasks were labeled and sterilized by autoclaving at 121°C for 15 minutes. A loop of pure *E. coli* and *S. aureus* culture from the stock culture was inoculated into three of the flasks of different NaCl concentration respectively. The inoculated nutrient broth was then incubated at 37°C for 12 hours. At the end of the incubation, the flasks were examined for presence of change in turbidity.

2.4. Measurements of Growth Pattern

The absorbance of the culture was determined at a wavelength of 660nm using spectrophotometer. The absorbance of each growth cultures with different NaCl concentration were obtained every hour for eleven hours. The mean of duplicate readings were plotted against the hour intervals to obtain the standard growth curve at the different NaCl conditions.

3. Results and Discussion

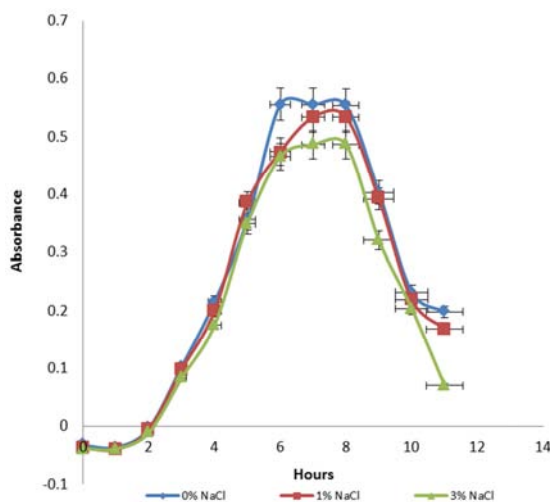


Figure 1. Growth curve of *E. coli* grown in medium of varying NaCl concentration.

Figure 1 shows results for *E. coli* grown in cultures of varying NaCl concentration at 37°C. A significant difference was observed in growth with decreasing turbidity from 0% (No salt enhancement- control) to 1.0% NaCl concentration. The observed steady decrease in growth of *E. coli* as the NaCl concentration increases have also been reported by Abdulkarim *et al.* [6], especially at the lag and early log phase. This may explain the reason why most of the media formulations contain 0.5% (w/v) NaCl (equivalent to 5.0 g/L NaCl) to obtain optimum growth. It also appears that the NaCl has little shortening effect upon the latent period previous to rapid growth [7]. The effect of NaCl upon the period of lag was therefore extended in the case of *E. coli* in the varying NaCl conditions experimented as shown in Figure 1.

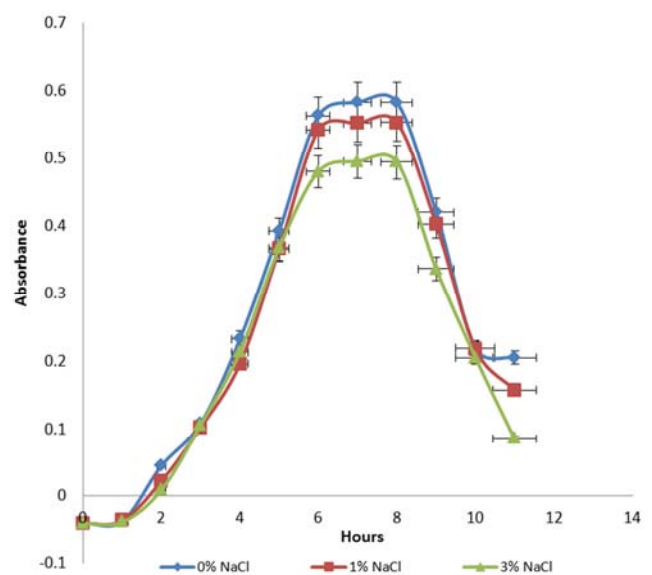


Figure 2. Growth curve of *S. aureus* grown in medium of varying NaCl concentration.

A characteristic lag growth phase for *S. aureus* as shown in Figure 2, was quite different from those observed in Figure 1 where *E. coli* was grown under same condition. The NaCl had an effect of shortening the latent period previous to rapid growth, producing a characteristic lag phase which appears to have a somewhat greater accelerating effect than that obtainable in *E. coli*. It is notable since *E. coli* is a non-halophile while *S. aureus* is halotolerant and can grow in the presence of high NaCl concentrations [8], such as on skin surfaces which often have high NaCl concentration (10% NaCl) [9]. This may suggest that *S. aureus* may exhibit a better ability to overcome the osmotic shock due to NaCl, compared to *E. coli*, hence, the former has a characteristic increase in the velocity of growth and shortened lag period as compared to the latter.

Generally, both bacteria at all NaCl conditions considered showed similar accelerating action and a shortened period of logarithmic growth when compared to the total incubation period during the log phase as also observed by Banwart [7]. Since the number of bacteria present in the culture at the

breaking point of the growth curve for both bacteria which was approximately the same in all cases, hence, there was no significant difference in the velocity of growth during the log phases at the different NaCl conditions. However, the higher the NaCl concentration, the faster the onset of the stationary phase in both bacteria.

It was observed that the optimum growth recorded was in a decreasing order for both *E. coli* (0.556, 0.534 and 0.486) and *S. aureus* (0.583, 0.553 and 0.495) at NaCl levels 0%, 1% and 3% respectively. These shows that the higher the NaCl concentration the lesser both bacteria will reach their optimal growth. These decreasing optimal growths as a result of increasing salt concentration, was also reported by Hajmeer [10] and Abdulkarim *et al.* [6], and they further suggested that it may be due to hyper osmotic effect on the bacteria. Explaining that, the osmotic shock on the organisms, may have led to the growth suppression. Also, *S. aureus* showed a higher optimal growth, seen at its exponential (log) phase than *E. coli* at all the NaCl concentrations; this also confirmed the adaptive ability of *S. aureus* to perform better in a NaCl stressed environment. The exponential phase of growth which shows a pattern of balanced growth wherein all the cells are dividing regularly by binary fission, and growing by geometric progression [11], allows the proper account of the generation time at this phase.

From the work of Friedrich [12], the mathematical development of the growth equation, generation time (μ) has been derived at:

$$\mu = \frac{2.303 (\log OD_2 - OD_1)}{(t_2 - t_1)} \quad (1)$$

where μ is determined from two data points, OD_1 and OD_2 ; points that are chosen from the exponential phase which mathematically are on the best-fitted line, while t_2 and t_1 are their corresponding difference in time values. While generation time (t_d) is obtained from the formula:

$$\mu = \frac{\ln 2}{t_d} \quad (2)$$

Therefore, the relationship between μ and t_d as shown in Table 1 for the growth of *E. coli* and *S. aureus* showed that the NaCl conditions considered allows the growth of these bacteria at different rates during the exponential phase.

The results presented in Table 1 showed an increase in the specific growth rate of *E. coli* as NaCl concentration increased with a corresponding decrease in doubling time, without a characteristic pattern, while, in the case of *S. aureus*, though specific growth rate increased with increasing NaCl concentration, there was nevertheless no effect of this two factors on the doubling time in having a characteristic growth pattern.

Therefore, it has been suggested that, a considerable variability exists in the generation times of individual bacteria in a population [13], with the nature of the distribution of these generation times being the subject of a number of studies [14, 15]. These workers observed different

organisms directly and for example *E. coli*, and unequivocally concluded that a particular pattern of the generation time distribution could not be ascertained. This study also supports those claims of lack of pattern in the distribution of generation times, which may be due to the difficulty in maintaining a uniform environment around cells during microscopic observation as suggested by Harvey [15] and Plank and Harvey [13].

Table 1. Effect of NaCl on growth of bacteria.

Effect of NaCl concentration on the growth of <i>E. coli</i>		
NaCl concentration of Growth medium	Specific growth rate μ (h^{-1})	Generation/Doubling time t_d (h)
0%	0.009	77.0
1%	0.015	46.2
3%	0.047	14.7
Effect of NaCl concentration on the growth of <i>S. aureus</i>		
0%	0.009	40.8
1%	0.010	69.3
3%	0.013	53.3

4. Conclusion

NaCl had no effect on the velocity of log phase. The growth curves obtained showed *S. aureus* had a better optimal growth performance as compared to *E. coli* at control and other levels of NaCl inclusions. However, the higher the NaCl concentrations of the growth environment, the lower the optimal levels of growth being reached by both bacteria.

Acknowledgments

We wish to appreciate the laboratory staffs in the department of Microbiology and Biochemistry for their kind assistance.

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