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Influence of Temperature on *Escherichia coli* Growth in Different Culture Media

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The cellular response against high temperatures and nutrient depletion might be essential regulatory feature among all organisms. Present study was designed to examine the growth and physiology of *Escherichia coli* at different temperatures in our laboratory condition. Patching and spot tests were carried out and the effect of different temperatures (25 °C, 30 °C, 37 °C, and 45 °C) on the growth of *E. coli* in different media varying in nutrients was studied. The results showed that growth of *E. coli* was inhibited at 45 °C and the normal phenotypic colony characteristics were disabled. Also, it was observed that, in comparison to the nutrient agar media, Luria-Bertani (LB) agar media and the minimal agar (MM) media suppressed the bacterial growth. Growths in the liquid media were in consistence. Present study revealed that the growth of laboratory strain of *E. coli* declined with the increase in temperature. Such an inhibition might an impact on the isolates to be viable but nonculturable (VBNC).

Key words: *Escherichia coli*; heat stress; growth; magnesium chloride**,** outer-membrane porin**.**

The temperature variation is one of the most important stress factors for the existing microorganisms in the environment[1]. For instance, a temperature increase induces the bacterial heat shock response which allows cells to adapt and survive against the thermal stress conditions[2-4]. Now-a-days the heat shock response is of importance for many scientific and industrial applications, for example, in processes where temperature-induced heterogonous protein production takes place[5]. All living organisms have developed sophisticated strategies to respond against several environmental stresses including the variations in osmolarity, pH, and also due to the nutrition depletion.

Bacteria in natural environments are constantly challenged by the need to adapt to changes in nutrient availability and stress condition. A range of bacteria, including *Escherichia coli*[1, 6-11], *Salmonella* spp.[12] *Pseudomonas* spp[13] and *Vibrio* spp.[14] have now been shown to elicit sophisticated intracellular reorganization programmes in response to such changes. Typically, these programmes are characterized by a series of physiological and genetic changes that facilitate the development of multi-stress resistant cells capable of long-term survival as well as immediate recovery and outgrowth[14-15].

Previously it has been demonstrated that *E. coli* W3110 strain showed inhibited growth at 45 °C in the Luria-Bertani media[7]. Based on this finding, current study was designed to detect the

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effect of the same variable on our laboratory strain of *E. coli*, and also to observe the influence of media composition on the growth at different temperatures. Thus, the present study was designed to distinguish among the growth patterns of our laboratory *E. coli* strain at room temperature (25 °C) followed by at 30 °C, the optimum temperature (37 °C), and finally at higher (45 °C) temperatures in three types of media (nutrient agar, Luria-Bertani agar and minimal agar) in order to extensively compare their growth characteristics.

MATERIALS AND METHODS

Bacterial culture and media

The bacterial strain used in this experiment was the laboratory strain of *E. coli* in the Dept. of Microbiology, Stamford University Bangladesh. The medium used for the growth and subsequent subcultures of the bacteria were Eosin-Methylene Blue (EMB) agar, MacConkey Agar and Nutrient Agar (NA) plates.

Microscopy

A colony from individual culture was incubated at 25 °C, 30 °C, 37 °C, and 45 °C. Gram staining was performed, and the shape, size, arrangement and gram reaction of the culture were observed in a microscopic field using bright field microscope (Humascope) under 1000× magnification.

Patching

A single colony of a freshly grown *E. coli* culture on nutrient agar plate was picked by a sterile toothpick and then was patched on nutrient agar, Luria-Bertani (LB) agar, and minimal agar media (MM) plates. Patching was done in a replica fashion maintaining the serial of patching site in each plate. Plates were incubated at the temperatures mentioned above.

Spot test

After 6 hours of growth of freshly grown *E. coli* culture (pre-culture) in nutrient broth, LB broth, and MM broth, the OD_{600} of respective culture broth was adjusted to 0.1. The bacterial suspension was serially diluted in 0.85% normal saline up to 10^{-3}. Then from each of the dilution, 10 µL of the bacterial suspension was spotted on nutrient agar, Luria-Bertani agar, and minimal media agar plates. After the spots dried off, plates were incubated at 25 °C, 30 °C, 37 °C and 48 °C for 24 to 48 hours

Determination of growth in liquid media

As stated above, the OD_{600} of respective pre-culture was adjusted to 0.1, and 30 µl of the pre-cultures were introduced into 4 different sets of 30 mL of nutrient broth, Luria-Bertani (LB) broth, and minimal media (MM) broths. Incubation was carried out at the temperatures mentioned above. At the time interval of 12, 24, 36 and 48 hours, the respective OD at 600 nm were measured and recorded. Besides, the respective colony forming units per mL (cfu/ mL) were enumerated.

RESULTS AND DISCUSSION

As stated earlier, responses of *Escherichia coli* W3110 strain against high temperatures have been extensively investigated[1, 7]. The present study was performed

Table 1. Observation of patching morphology at different temperatures

Temperature (°C)	Growth on different media		
	Nutrient Agar	Luria-bertani Agar	Minimal Agar
45	+	-	-
37	+++	+++	+++
30	++	++	++
25	++	++	++

+++ Rapid Growth
++ Moderate Growth
+ Indicates Slow Growth
- No Growth

Table 2. Influence of temperature on *E. coli* growth observed through spot tests

Temperature (°C)	Growth on different media		
	Nutrient Agar	Luria-bertani Agar	Minimal Agar
45	+	-	-
37	+++	+++	+++
30	++	++	+
25	++	++	+

+++ Rapid Growth
++ Moderate Growth
+ Indicates Slow Growth
- No Growth

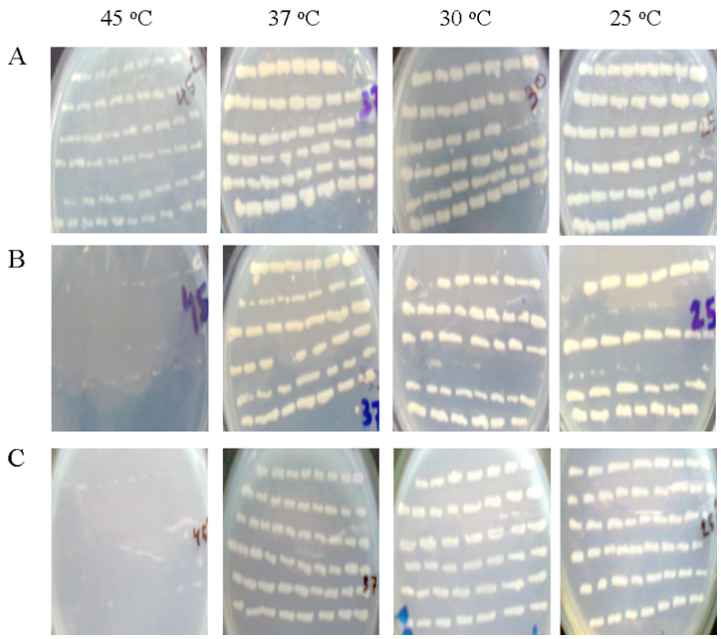

Fig. 1. Observation of growth after patching in Nutrient Agar media (A), Luria-Bertani Agar media (B) and Minimal Agar media (C) at 25 ºC, 30 ºC, 37 ºC , and 45 ºC. Considerable growth was found in all the three media at 25 ºC, 30 ºC & 37 ºC. No growth was observed at 45 ºC in LB agar & minimal agar media, a relative slow growth was observed at 45 ºC in nutrient agar media

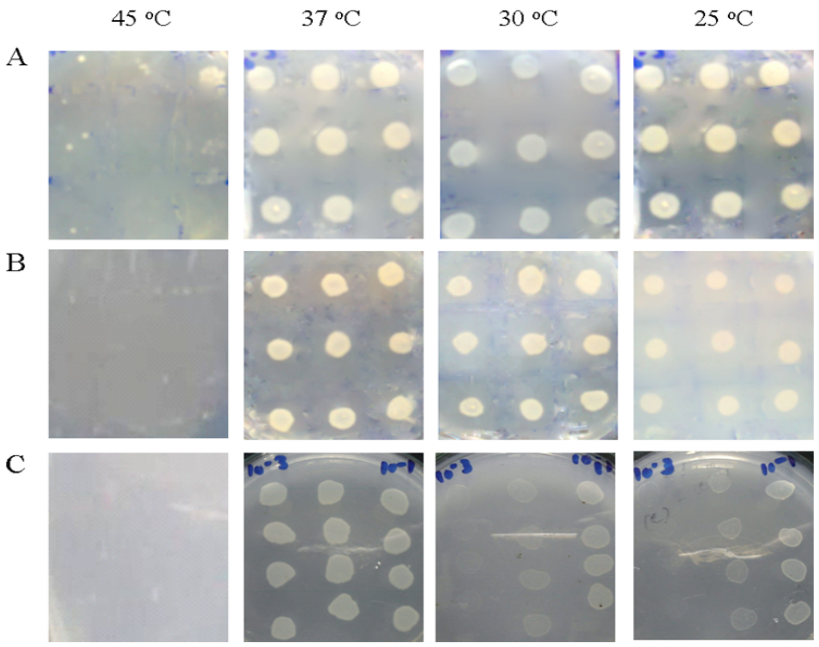

Fig. 2. Observation of growth after spot tests in Nutrient Agar media (A), Luria-Bertani Agar media (B) and Minimal Agar media (C) at 25 ºC, 30 ºC, 37 ºC , and 45 ºC. Considerable growth was found in all the three media after 24 hours of incubation at 25 ºC, 30 ºC & 37 ºC. No growth was observed at 45 °C in Luria-Bertani agar & minimal agar media, but a slow growth was observed at 45 ºC in nutrient agar media after 48 hours

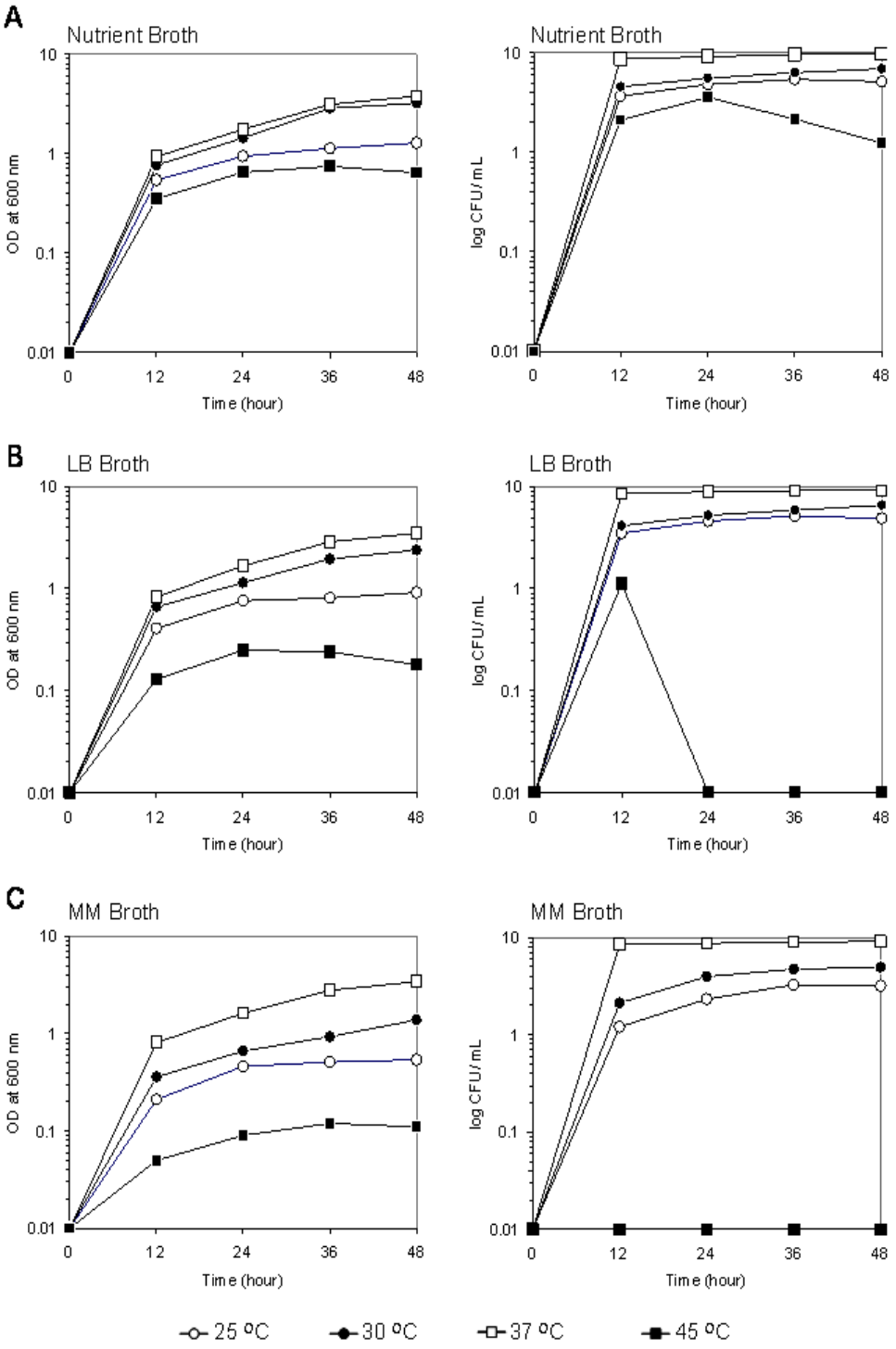

Fig. 3. Observation of growth in liquid media including Nutrient Broth (A), Luria-Bertani (LB) Broth (B) and Minimal Medium (MM) Broth (C) at 25 °C (indicated by open circles), 30 °C (filled circles), 37 °C (open squares), and 45 °C (filled squares)

as a consequence of this study with the laboratory strain of *E. coli* to observe the growth patterns of *E. coli* on different culture media at different temperatures. The growth characteristics of *E. coli* cells were determined through patching and spot tests.

Effect of high temperature on cell morphology

Under microscope, normal morphological characteristics of *E. coli* cells were observed at 30 °C and 37 °C while at 25 °C, cell sizes were found a bit smaller than those at 30 °C and 37 °C. At 45 °C, to be smaller and aggregated form of cell structures was observed (data not shown). Therefore, it was notified in consistence to our previous data [7] that as the temperature increased, the growth of *E. coli* ceased. However, to confirm this finding, patching and spot tests were performed at 25 °C - 45 °C) in different media.

Effect of high temperature on cell growth in different media

At 25 °C and 30 °C, normal growth was observed after 24 hours whereas at 37 °C, growth was maximal and confluent in all three media (Table 1, figure 1). However, growth was not observed in LB agar and MM agar at 45 °C (Table 1, figure 1). Next, in order to confirm such a phenotype, we investigated the influence of temperatures in different media through the spot tests. As was in the patching experiments, at 25 °C and 30 °C, normal growth was observed. At 37 °C, growth was maximal and confluent in all media. No growth was observed in LB agar and MM agar at 45 °C. In addition, a slow growth was observed at nutrient agar media which supports the results obtained through patching experiments (Table 2 and Fig. 2).

In consistent with these observations, growth in the all three liquid media exhibited that at 45 °C, the growth of *E. coli* was suppressed at the lower temperatures (both at 25 °C and 30 °C) and drastically at 45 °C (Figure 3). Interestingly, nutrient broth exhibited to be a better growth medium for our laboratory strain than those of LB and MM media. An apparently constant level of colony forming units at 37 °C in all three media might be assumptive of the existence of viable but nonculturable (VBNC) cells as previously indicated in case of *E. coli* W3110 strain by Noor *et al* (2009a)[7]. Suppression of growth as indicated by a lower OD_{600} and colony numbers at 45 °C might be explained by the generation of reactive oxygen species (ROS) as discussed previously[8]. However, the findings of this very study is not only confirmative of our previous studies[7-8] but also is unique in perspective of the new strain of *E. coli* used in our laboratory.

CONCLUSION

In summary, we demonstrated a phenotypic comparison between our laboratory strains of *E. coli* with the reference strain (*E. coli* W3110). This study also give an idea about nutritional composition of different culture media and their effect on the growth of *E. coli* at variable heat stress conditions. This finding, to our knowledge, has been revealed for the first time in the research perspective with *E. coli* in our country. Overall, the present study clearly depicts that with the increase in temperature, the *E. coli* cells lose its cultivability. It may note the revelation of a new property of our laboratory strain which is generally termed as the viable but non-culturable (VBNC) state[6-8]. The outcome of such speculated VBNC state may be further investigated for our laboratory *E. coli* strain.

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