Bangladesh J Microbiol, Volume 32, Number 1&2, June-December 2015, pp 39-44

Original Article

Impact of Different Carbon Sources on the *in vitro* Growth and Viability of *Escherichia coli* (SUBE01) and *Salmonella* spp. (SUBS01) Cells

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With a previous observation of *Escherichia coli* growth cessation along with temperature variation within three different bacteriological culture media (nutrient agar, Luria-Bertani agar and minimal agar), current investigation further depicted on the possible growth dynamics of *Escherichia coli* (SUBE01) and *Salmonella* (SUBS01) growth and viability upon supplementation of different carbon sources (dextrose, sucrose, lactose, glycerol and tween 20) at 37°C under the aeration of 100 rpm. Viability of the tested bacterial species was assessed through the enumeration of the colony forming unit (cfu) appeared upon prescribed incubation for 12-24 hours on different agar plates consisting of the above mentioned carbon sources. Besides, to inspect the cellular phenotypic changes, morphological observations were conducted under the light microscope. Variations in bacterial growth (either growth acceleration or cessation) were further noticed through the spot tests on the agar plates. Considerable shortfalls in the culturable cells of *E. coli* and *Salmonella* spp. were noted in the minimal media separately consisting of sucrose, lactose, glycerol or tween 20 while an opposite impact of accelerated growth was noticed in the media supplied with dextrose. The data revealed a hierarchy of consequence of carbon sources as nutrient generators whereby the favourable bacterial growth and survival order of the carbon sources was estimated as dextrose > glycerol > lactose > tween 20 > sucrose.

Keywords: Carbon sources; Colony forming unit (cfu), *Escherichia coli*, Nutrient diversity, *Salmonell*a spp.

Introduction

Bacterial survival in natural environments are usually challenged by brusque changes in the nutrient exhaustion and by an array of physicochemical stimuli including temperature, pH, sugar/ salt concentrations, the redox state, toxic compounds, etc[1-9]. In natural habitats, bacterial cells must need to meet their nutritional requirements (including the carbon sources, major elements and minor elements) for cellular biosynthesis and metabolic functions[10]. Thus, apart from the natural habitats, for the *in vitro* cultivation of bacteria in laboratory culture media, bacteria cells are to be provided with a suitable source of carbon in simpler form (regarding the ease of uptake) for cellular biosynthesis primarily through carbon catabolic repression[10-**14**]. In consistent to this concept, the earlier studies indeed reported that abrupt changes in the source of carbon stimulated metabolic potential in *E. coli* cell through the elicitation of specific catabolic operons[10,15-17].

Through physiological and genetic analysis on *E. coli*, our former studies unravelled the impact of the *in vitro* nutrient composition accompanied with the temperature up-shift with the generation of oxidative stress retarding the cell viability of the bacterium[6,8,18-19]. In a separate study the influence on cell viability and culturability in response to the external and internal oxidative stresses[9,18-21] in *E. coli* (SUBE01), *Pseudomonas* spp. (SUBP01), *Bacillus* spp. (SUBB01) and *Salmonella* spp. (SUBS01) has been inquired well and apparently appended the new information on the defense strategy of these bacteria especially those belonging to *Salmonella* (SUBS01)[9,21]. Along these lines of phenotypic observations on the cellular behaviour against the heat shock and oxidative stress, the current investigation further emphasized (1) to distinguish among the growth patterns of *E. coli* (SUBE01) and *Salmonella* spp. (SUBS01) on culture media with variations in carbon sources (dextrose, glycerol, lactose, tween 20 and sucrose), (2) to observe the retention within the cell viability in response to the diversity within carbon sources as major nutrients for *in vitro* cultivation.

Methods and Materials

Demonstration of culturable cells upon variation in carbon sources

Laboratory stock culture of *Escherichia coli* (SUBE01) and *Salmonella* spp. (SUBS01) were used in this study. Experiments demonstrating the bacterial growth in terms of cell turbidity (optical density at 600 nm; *i.e.,* OD_{600}) and colony forming units (CFUs) were conducted as described earlier by Nur *et al*.[21]. Minimal media (dextrose 1.0 g/l, dipotassium phosphate 7.0 g/l, monopotassium phosphate 2.0 g/l, sodium citrate 0.5 g/l, magnesium sulfate 0.1 g/l and ammonium sulfate 1.0 g/l) for both agar (MA) and broth (MB) were used for the assay of the bacterial culturability[8].

D-Sucrose, D-lactose, glycerol and tween 20 (Sigma-Aldrich Corporation, USA) were used separately as carbon source, which were included in minimal media (both agar and broth) instead of dextrose (D-glucose) (Sigma-Aldrich Corporation, USA). After 24 hour incubation on MA plates at 37°C, one loopful of the bacterial culture was introduced into 5 ml MB followed by incubation at

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37°C for 4-6 hours at 100 rpm to form the pre-culture[8]. After adjusting the optical density of the pre-culture at OD_{600} to 0.1, 30 µl each was introduced into 5 different sets of 30 ml of MB and incubated at 37°C at shaking condition (100 rpm). At the time points of 12, 36 and 72 hours, the cell growth was monitored by measuring OD_{600} and by counting the CFUs on agar plates[8-9,18,20-21].

Manifestation of culturable cells was further confirmed by the supportive spot tests[8-9,18,20-21]. As described previously, each the culture suspension was serially diluted in 9 ml minimal broth to obtain up to 10^{-4} fold dilution. From each dilution, an aliquot of 5 µl was dropped on to the minimal agar, dried off for 15 minutes, and finally the plates were incubated at 37°C for 24 hours. Spotting on the agar was accomplished at 24 hours of growth. Results were statistically analyzed by determining the *p* value through *t* test. Standard deviations were also measured through statistical hypothesis testing[21]. All the data found in this study were estimated as significant ($p = 0.05$).

Demonstration of morphological changes

Simple staining (Crystal violet, Hucker's solution) was conducted to assess the cellular morphology as was done previously[9,20-22]. An aliquot of 10 µl from the bacterial culture suspension was removed at 24 hours of growth, and the cellular morphology, shape and organization were observed under the light microscope (Optima Biological Microscope G206, Taiwan) at 1,000x magnification[21].

Results and Discussions

Growth retardation of E. coli (SUBE01) and Salmonella spp. (SUBS01) upon carbon source variation

Numerous studies so far reported that in the natural environments bacterial diversity is found to be excessively higher than those estimated through the conventional plating techniques; indeed less 1% of total bacteria are culturable in laboratory[23-28]. Many of them have been isolated by employing different laboratory techniques; nevertheless, no stringent method so far seems to fascinate all microorganisms for *in vitro* growth collected from their natural habitats[29]. Hence, the present study was an introductory attempt apparently in small scale to investigate bacterial growth dynamics in response to a variety of carbon sources.

In this investigation, when *E. coli* (SUBE01) and *Salmonella* spp. (SUBS01) cells were grown separately in sucrose, lactose, glycerol and tween 20 within minimal media (both broth and agar), a relatively minor variation both in the cell turbidity and in the colony forming units (CFUs) were observed, which was quite comparable to that grown in dextrose comprising minimal media (Figure 1). Notably, at the mid stationary phase both *E. coli* and *Salmonella* spp. exhibited their highest growth rate, *i.e.*, 10^7 cfu/ml and 10^5 cfu/ml in dextrose within minimal media, respectively, which was comparable to that grown in sucrose, lactose, glycerol and tween 20 within minimal media (Table 1). However, no significant changes in their morphology were noticed during stasis up to 72 hours (Figure 2).

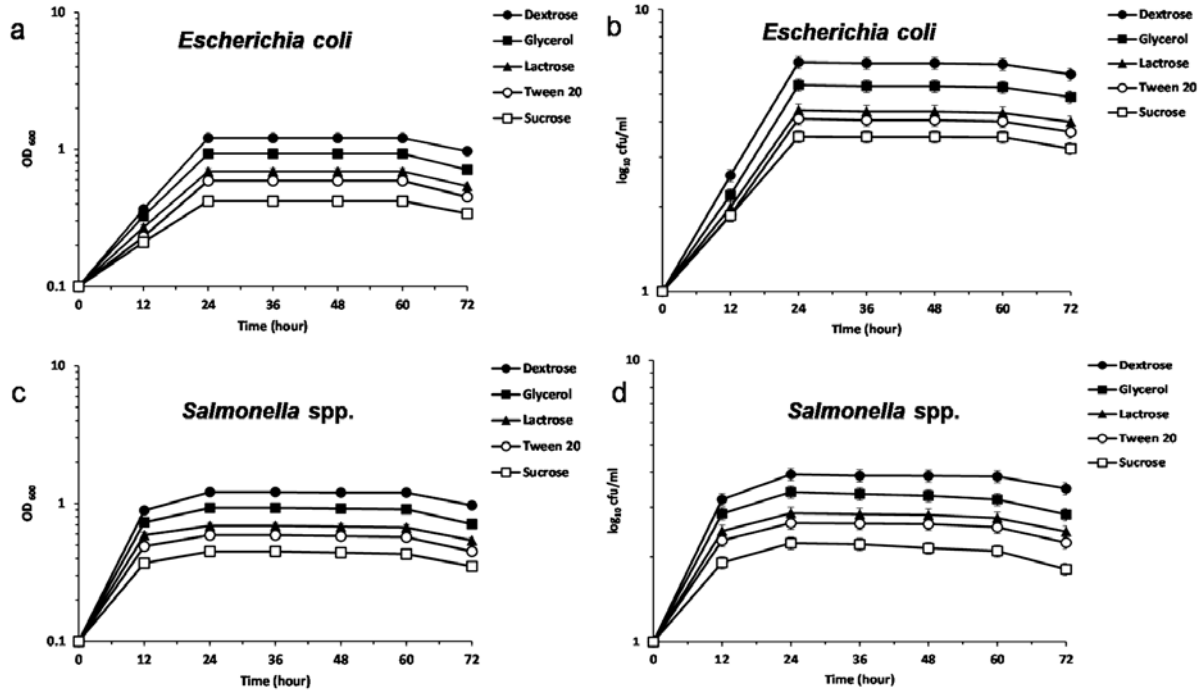

Figure 1. *Assessment of cell culturability through the examination of growth of E. coli (a, b) and Salmonella spp. (c, d) on different carbon sources (sucrose, lactose, glycerol and tween 20) comprising minimal media (both agar and broth). However, slight variation of growth was observed for both bacterial species, wherein E. coli exhibits 7 log cfu/ml in dextrose, 5 log cfu/ml in glycerol 4 log cfu/ml in D-lactose, 4 log cfu/ml in tween 20, 3 log cfu/ml in D-sucrose as well as Salmonella spp. exhibits 5 log cfu/ml in dextrose, 4 log cfu/ml in glycerol 3 log cfu/ml in D-lactose, 3 log cfu/ml in tween 20, 2 log cfu/ml in D-sucrose after 24 hours of incubation periods. Standard deviations for all data have been indicated by error bars.*

Table 1. *A quantitative estimation of highest load of Escherichia coli and Salmonella spp. in different carbon sources*

Substrate	Enumeration of microorganisms (cfu/ml) (at mid-stationary phase, after 24 hours of incubation)	
	Escherichia coli	*Salmonella* spp.
Dextrose (1.0 g/l)	10^7	10^5
Glycerol (5.0 ml/l)	10^5	10^4
D-Lactose (10.0 g/l)	10^4	10^3
Tween 20 (0.1 ml/l)	10^4	10^3
D-sucrose (5.0 g/l)	10^3	10^2

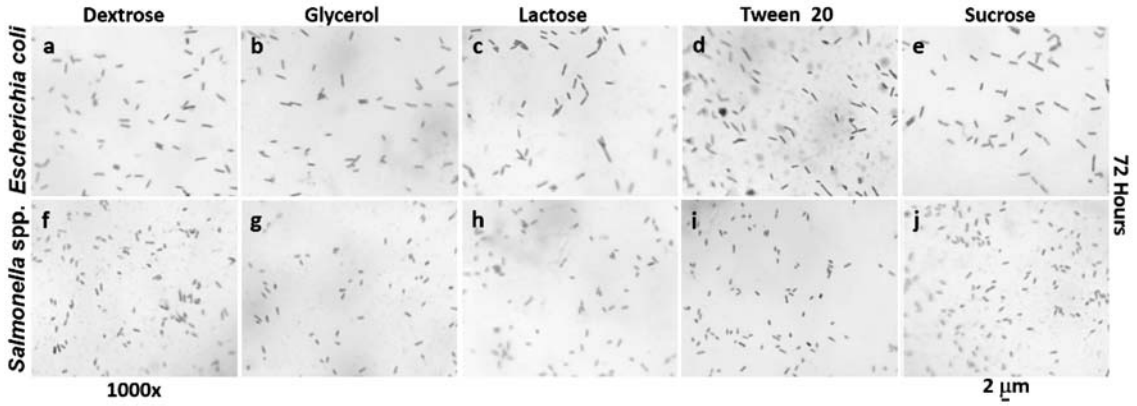

Figure 2. *Observation of the morphological changes of Escherichia coli (a-e) and Salmonella spp. upon nutrient variation (f-j). Cells were grown on different carbon source (dextrose 1.0 g/l, sucrose 5.0 g/l, lactose 10.0 g/l, glycerol 5.0 ml/l and tween 0.1 ml/l) within the minimal media (both in agar and broth forms). Cellular morphology and arrangements were observed after 72 hours, wherein no significant morphological changes were observed.*

Confirmative demonstration of loss of culturability of Escherichia coli (SUBE01) and Salmonella (SUBS01) cells

When *E. coli* (SUBE01) and *Salmonella* spp. (SUBS01) cells were grown separately in different carbon sources within the minimal media, a quantitative estimation of highest load for both species was recorded as shown in Table 1. Indeed, a dramatic decline in the culturable fraction of cell population (Figure 1) together with an apparently uninfluenced morphology of *E. coli* (SUBE01) and *Salmonella* (SUBS01) cells (Figure 2) led us further to cross-check of the culturability of the cells through the spot tests as has been done in the earlier studies[8-9,20-21]. Reproducible to the growth study results (Figure 1), a substantial growth variation was observed when cells were grown separately in sucrose, lactose, glycerol, tween 20 and dextrose within the minimal media (Figure 3).

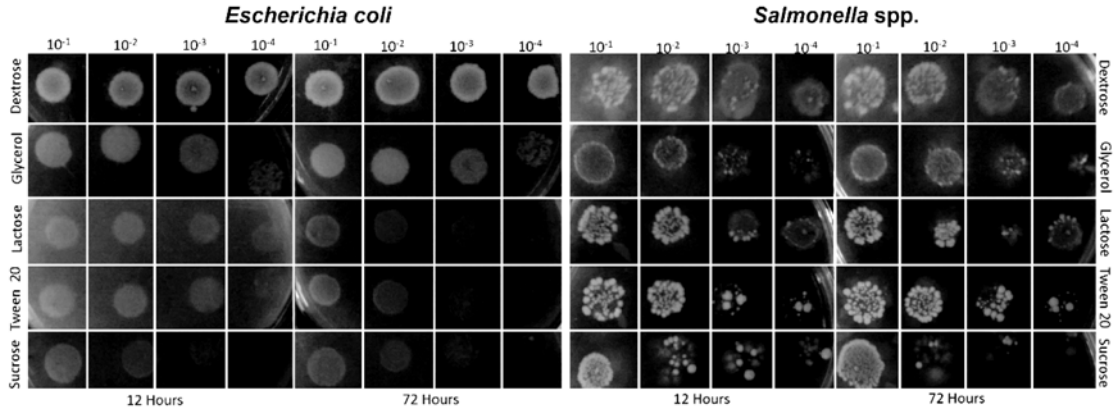

Figure 3. *Confirmative demonstration of culturability and survival potential of Escherichia coli and Salmonella spp., cells by spot test on different carbon source (dextrose 1.0 g/l, sucrose 5.0 g/l, lactose 10.0 g/l, glycerol 5.0 ml/l and tween 0.1 ml/l) within the minimal agar after 12 and 72 hours of incubation periods. Notably, from the dilutions up to 10^{-4}, both bacterial species showed a growth variation on different carbon source within the minimal agar.*

Metabolic properties of *E. coli* using acetate, glucose, lactose and sucrose as carbon energy source has been inquired well and evidently brought the new information[10,13,30-35]. Concisely, *E. coli* is very well known to uptake glucose using the phospho-transferase transport process coupled to the conversion of glucose into glucose-6-phosphate[10,36]. This transport system is not specific to glucose only but also for other sugars as well (mannose, fructose and other hexoses) transport system[10,33,37-38]. Notably, in *E. coli* the adenylate cyclase is activated in the absence of glucose resulting in an elevated amount in cyclic adenosine mono phosphate (cAMP) that activates the expression of a large set of catabolite derepression genes through binding to the cAMP receptor protein[13,31,33,38-41]. Nevertheless, the cAMP-receptor protein (CRP) regulated carbon utilization process are nearly similar in *E. coli* and *Salmonella* spp.[37,42-43]. Hence, in the current study both species exhibit similar results, which is in supportive with the previous suggestive reports[13,31,33,38-42]. Moreover, besides the current study findings, the uptake and subsequent utilization of sucrose and glycerol is also well explained by the previous studies[44-45]. Interestingly, in the current study, tween 20 was found to be well utilized by both *E. coli* and *Salmonella* spp. Possibly, tween 20 is first hydrolyzed into oleoic acid followed by the subsequent conversion into oleaolyl CoA, which in turn enters into the cell through the b-oxidation pathway. However, in a very early study *E. coli* K12 strain was found to lose the culturability in the tween 80-supplemented minimal medium[46]. A simplified model showing possible uptake mechanisms of various substrates employed by *E. coli* has been plotted in Figure 4.

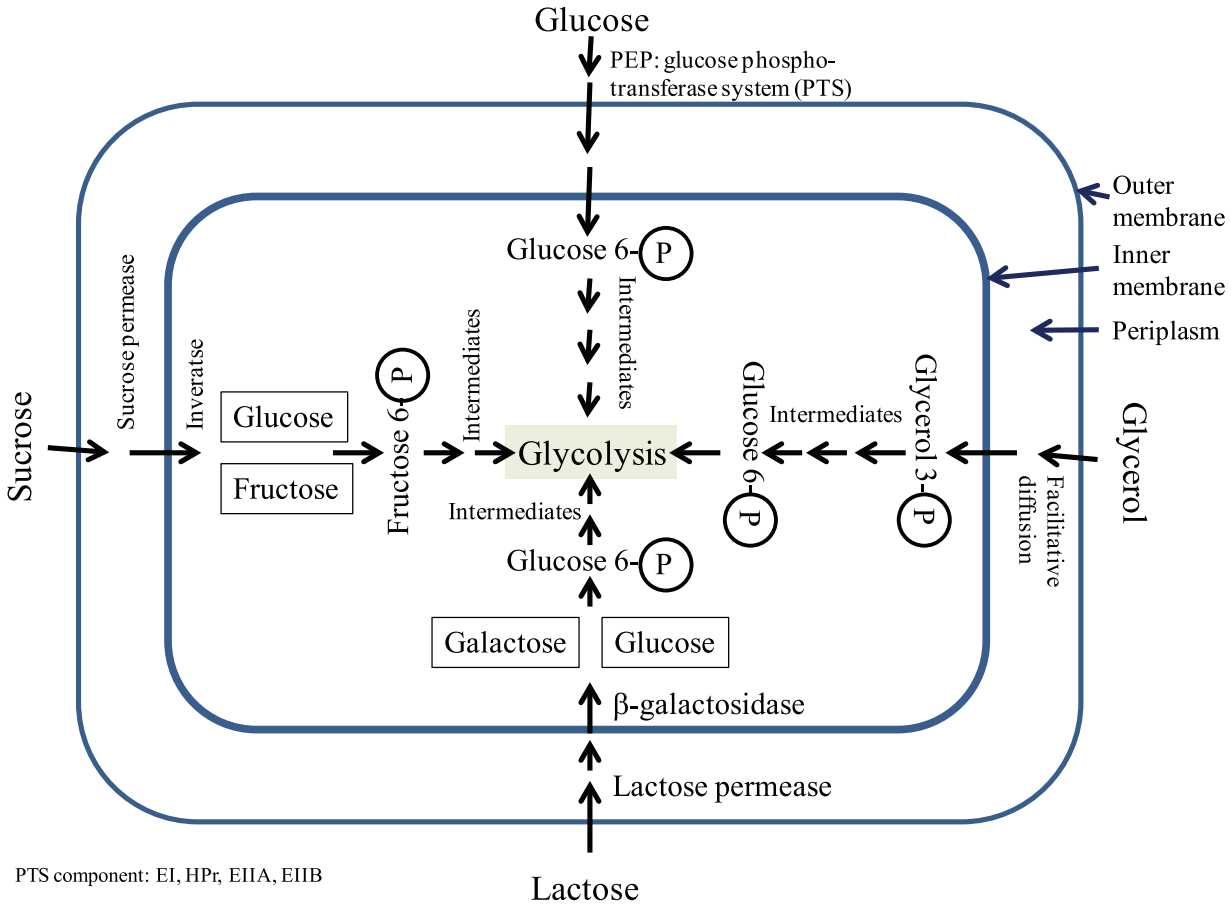

Figure 4. *A simplified model showing the scheme of utilization of glucose, sucrose, lactose and glycerol in Escherichia coli. The bacterium consists of PEP phosphotranferase system, responsible for the uptake of glucose and sucrose. As has been shown in the model, glucose and fructose eventually convert into glucose-6-phosphate and enters the series of conversions in glycolysis. Lactose crosses the membrane through the action of the permease enzyme which is an energy dependent process, and converted into glucose and galactose in the cytoplasm, which in turn enters the glycolytic catabolism. Glycerol is converted into glycerol-3-phosphate which afterwards assembles into the glucose-6-phosphate to be catabolized through glycolysis.*

Conclusion

With a consecution of those works, the presented study revealed the prelusive nutritional diversity and proposed that there is a hierarchy that abide by the carbon source consequence (dextrose > glycerol > lactose > tween 20 > sucrose) and it would be useful during the enrichment techniques. Furthermore, tween 20, even being into the category of detergent with a possible role in despairing the cell membrane; yet the molecule has been found to be better utilized compared to that of sucrose. Further studies on tween molecule degradation by bacteria would be of greater interest to understand the overall bacterial catabolic strategies for diversified substrate utilization.

Acknowledgement

We thank the Laboratory of Microbiology, Stamford University Bangladesh for the logistic and financial supports to conduct the experiments. We also thank Saurab Kishore Munshi, a faculty member of the similar discipline for helping to construct the metabolic model presented in the paper (Figure 4).

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