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Full Length Article

The Effects of some Physicochemical Stresses on *Escherichia coli* O157:H7 as Clinical Pathogenic Bacteria

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Abstract

Escherichia coli O157:H7 is a member of *Enterobacteriaceae cocobacilla*, which is gram negative and it causes gastrointestinal disease such as Diarrhea, Hemorrhagic colitis, and Hemolytic uremic. The first aim of this study was to examine *E. coli* O157:H7 in planktonic and biofilm state under stressed conditions of NaCl, pH, temperature and ORS (Oral Rehydration Salt) powder and then make comparisons. The second aim was to examine the shape of the bacterium in microscopic (bacteria shape) and macroscopic (bacterial colonies in Nutrient Agar culture media). Bacterial strains of *E. coli* O157:H7 were purchased (No. (NCTC) 12900). The bacteria were placed in liquid Nutrient Broth culture media and then transferred to Nutrient Agar culture media into plates to examine colonial shape. Microtitration plate was used in order to determine the biofilm formation under stressed conditions. Under these conditions, colonial morphology and bacterial shapes were significantly changed and their shapes tended to Cocci and Bacilli in order to survive more. Bacteria survival under stressed conditions was more in biofilm state rather than in Plankton state. This research demonstrated that *E. coli* O157:H7 had a different kind of effects in physicochemical stresses, while the optimal conditions were found in NaCl, pH, Tm and ORS stresses.

Keywords: Stress; Morphotype; Biofilm; *Escherichia coli* O157:H7

Introduction

Escherichia coli belong to a family Enterobacteriaceae. They are gram-negative, short rod-shaped bacteria and move with peritrichous flagella, owning fimbriae and are facultative anaerobes that are capable of fermentative and breathing metabolism. Enterohemorrhagic *E. coli* (EHEC) were documented as significant human pathogens in the US in 1982 (Rogerie *et al.*, 2001; Bhekisisa, 2008; Mirnejad *et al.*, 2010; Jamshidi *et al.*, 2015; ÖZtÜRk and Halkman, 2015). Many strains of *E. coli* O157:H7 showed higher acceptance to hostile ecological situations than those of other pathogenic and nonpathogenic bacteria (Chen *et al.*, 2004).

Typical habitat of *E. coli'* is the lower portion of the most warm-blooded animal intestine. Some strains of *E. coli* are harmless commensal members of the intestinal flora of mammals in which some strains adhere to the intestinal mucosa while others are only impermanent transients in the lumen of the colon. *E. coli* can reply to ecological signals such as chemicals, pH, Tm, and osmolality in a number of very extraordinary ways since it is a single-celled bacterium. *E. coli* O157:H7 is one of emerging food-borne infections. It is responsible for 14000 cases reported in the United States in 1994, 1600 cases in Canada in 1992 and additional sporadic cases reported worldwide have raised *E. coli* O157:H7 to a food-borne pathogen of universal importance. It causes severe illnesses such as HUS, which is more common in young children and TTP which mainly affects the elderly. The pathogen is exceptional in its severe consequences of infection, low infection dose and acid tolerance (Bhekisisa, 2008; Marouani-Gadri *et al.*, 2009).

Biofilm is demarcated as a microbial population in a supporter surrounding substance. In the environment, bacteria typically fasten to compacted sides (specifically to fluid–hard edges). After add-on, they form microcolonies, frequently it makes extracellular polymeric substances (EPS), catch debris and other species of cells, and produce biofilms. In therapeutic and industrial situations, bacteria are found mostly as biofilm cells and not as planktonic cells (Kawarai *et al.*, 2009).

In normal situations, bacteria are as biofilms in most of the times, sessile collections of bacterial cells, attached to abiotic sides. Conversion is a complicated procedure from planktonic cells in liquid to biofilms in surfaces that it can

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be divided into four phases: a first connection to abiotic sides, microcolony construction, and preservation of a matured biofilm. Throughout biofilm development, many features are also involved. For example, nutrient accessibility, surface properties of abiotic, and cell sites may be involved in the first connection to abiotic surfaces (Oh *et al*., 2007; Uhlich *et al.*, 2014). In other studies, role stress were investigated in mental health (Tehrani *et al.*, 2013), mood state of veterans with post-traumatic stress disorder (Omidi *et al.*, 2013), effectiveness of stress management skill training on the depression (Habibi *et al.*, 2013), and Stress in Women With Multiple Sclerosis (Kolahkaj and Zargar, 2015). In the paper for first was investigated role physicochemical stresses on *E. coli* O157:H. The purpose of research was the survey *E. coli* O157:H7 in plankton and biofilm under some physicochemical stresses. The main goal of this study was the experimental *E. coli* O157:H7 in plankton and biofilm under some physicochemical stresses and then the shape of this bacterium was identified in microscopic and macroscopic manners.

Materials and Methods

Growth Conditions

The culture of *E. coli O157* (NCTC) 12900 (Laboratory Razi Vaccine and Serum Research Institute, Karaj, Iran) was grown up in nutrient broth culture (NB) for 24 h at 37°C to determine pH, NaCl, ORS and temperature stress factors on bacterial growth. The bacteria were experimented in different conditions including; pH (4 to10), NaCl (0.5 to 5 g), ORS (1 to 8 g) and temperature (4 to 55°C) in Ben Murray, Incubator, Electrode pH meter and Refrigerator (Dewanti and Wong, 1995; Gabriel and Nakano, 2010a).

Preceding each experimentation, *E. coli* O157:H7 was streaked on a nutrient agar (BHI, Difco, Detroit, MI, USA), incubated overnight at 37°C and transported to a 50 mL flask having 20 mL of medium. The flask was disturbed in an orbital shaker (150 rpm) for 24 h at 20°C and then transported to 100 mL of the similar medium which was cultivated in similar circumstances. (Trémoulet *et al.*, 2002).

Bacteria were cultured under stressed situations by poor plate method and dilute preparation. The bacterial growths were measured by turbidity measurement (OD 650 nm) in nutrient broth culture and also growth was evaluated as OD (650 nm) and CFU. All of these performances have been done in two fixed time (12 and 24 h) (Chen *et al*., 2004; Gabriel and Nakano, 2010b).

Planktonic and Biofilm Cultures

A 100 mL of medium were accustomed to an OD at 600 nm of 0.1 for planktonic culture. They were grown-up in a rotary shaker (150 rpm) at 20°C for 7 days. For producing biofilms, Castonguay's method was done (Castonguay *et al*., 2006). Developed biofilms and planktonic bacteria with similar age were diluted (1/10, w/v) in saline and tolerated during 5 min and Trémoulet's method was followed (Trémoulet *et al.*, 2002).

Observation of Biofilm Formation Cells

Totally grown *E. coli* cells were relocated into 50 mL of a 1 M medium. Methods of Hiraga and Kawarai were followed for this test. In the end, slides were washed 3 times and dried up at room temperature for 30 min. Then, Biofilms were Gram-stained (Hiraga *et al.*, 1989; Kawarai *et al.*, 2009).

Statistical Analyses

Statistical significance was assessed by using the least significant differences – LSD (T-test). P-value was <0.05. Results were expressed as mean ± S.D. The statistical analysis was done by SPSS software (version – 10).

Results

Effects of Temperature on the Bacterial Growth and Formation

This bacterium was experimented in seven temperatures. The results of this study have been shown in Fig. 1, which indicated that the best condition for bacterial growth was at 37ºC in both formations (biofilm and planktonic). But, the percentage of bacterial growth in biofilm formation approximately was completed at 25, 30 and 37ºC. It could be understood that bacterial biofilm formation could grow wider than bacterial planktonic formation in different ranges of temperature.

The number of bacteria (CFU/mL) in this stress factor approximately was 1.5×10^8 mL at 37ºC in Table 1 which shows that the highest number of bacteria was at 37ºC as an optimal temperature. The number of bacteria in other temperatures was lower than optimal temperature (37ºC) and also, the best temperature for this bacterium was at 37ºC because both formations could grow efficiently. Other temperatures had negative effects on bacterial growth which both method (CFU and OD) proved this subject that *E. coli* O157:H7 could have a perfect growth at 37ºC (Fig. 1). The colony formation was acerate, bright white, wet, round and raised formation at 4 and 55ºC in Table 2. But, the colony formation was changed to small and big at 30 and 37ºC, respectively. All experiments were done during two times (12 and 24 h).

Effects of NaCl on the Bacterial Growth and Formation

In this step, we used eight concentrations (0.5, 1, 1.5, 2, 2.5, 3, 3.5 and 5 g) for releasing the effects of NaCl on bacterial growth, which has been presented in Fig. 1. This bacterium could grow perfectly in 0.5 to 3.5 g of NaCl whose

Table 1: The number of bacteria (CFU/ml) in physicochemical stresses (Tm, pH, NaCl and ORS)

ORS (g)	CFU/mL		NaCl(gr)	CFU/mL		pH	CFU/mL		Temp (°C)	CFU/mL	
	12 hr	24 hr		12 hr	24 hr		12 hr	24 hr		12 hr	24 hr
1	12×10^6	12.3×10^7	0.5	13.5×10^7	12×10^7	4	7.5×10^6	3×10^6	4	7.5×10^6	3×10^6
2	12×10^7	11.8×10^7	1	12.7×10^7	10×10^7	5	6×10^7	4.5×10^7	25	7.5×10^7	6×10^7
3	11.7×10^7	11.4×10^7	1.5	12.1×10^7	9.7×10^7	6	10.5×10^7	9.5×10^7	30	10.5×10^7	6×10^7
3.5	6.45×10^7	6×10^7	2	10.8×10^7	9×10^7	7	10.5×10^8	10.5×10^8	37	1.5×10^8	1.5×10^8
5	5.7×10^7	3.75×10^7	2.5	9.6×10^7	7.5×10^7	8	6.1×10^7	4.5×10^7	40	9×10^6	8.2×10^7
5.5	3×10^7	2.25×10^7	3	6×10^7	4.8×10^7	9	2.1×10^7	4.5×10^6	50	4.5×10^7	3.1×10^7
7	2.25×10^7	1.8×10^7	3.5	3.3×10^7	1.5×10^7	10	0	0	55	1.5×10^7	6×10^6
8	3×10^6	1.5×10^6	5	4.5×10^6	1.5×10^6	-	-	-	-	-	-

*The initial number of bacteria is 1.5×10^8; The data in each column represents three independent experiments ($p<0.05$)

Table 2: The effects of physicochemical stresses on colony formation

Physicochemical stresses	Ranges	Morphological characteristics of the colonies
Temperature (°C)	4	Acerate, bright (white), wet, round and raised formation
	25 and 30	Small, bright (white), wet, round and raised formation
	37	Big, bright (white), wet, round and raised formation
	40 and 50	Small, bright (white), wet, round and raised formation
	55	Acerate, bright (white), wet, round and raised formation
pH	4, 5 and 6	Small, bright (white), wet, round and raised formation
	7	Big, bright (white), wet, round and raised formation
	8, 9 and 10	Acerate, bright (white), wet, round and raised formation
NaCl (%)	0.5	Small, white, dry, round and flatted formation
	1, 1.5, 2, 2.5, 3, 3.5 and 5	Acerate, white, dry, round and raised formation
ORS (%)	1	Big, white, wet sticky, round and flatted formation
	2, 3 and 3.5	Average, opalescent, wet sticky, round and flatted formation
	5, 5.5, 7 and 8	Small, opalescent, wet sticky, round and flatted formation

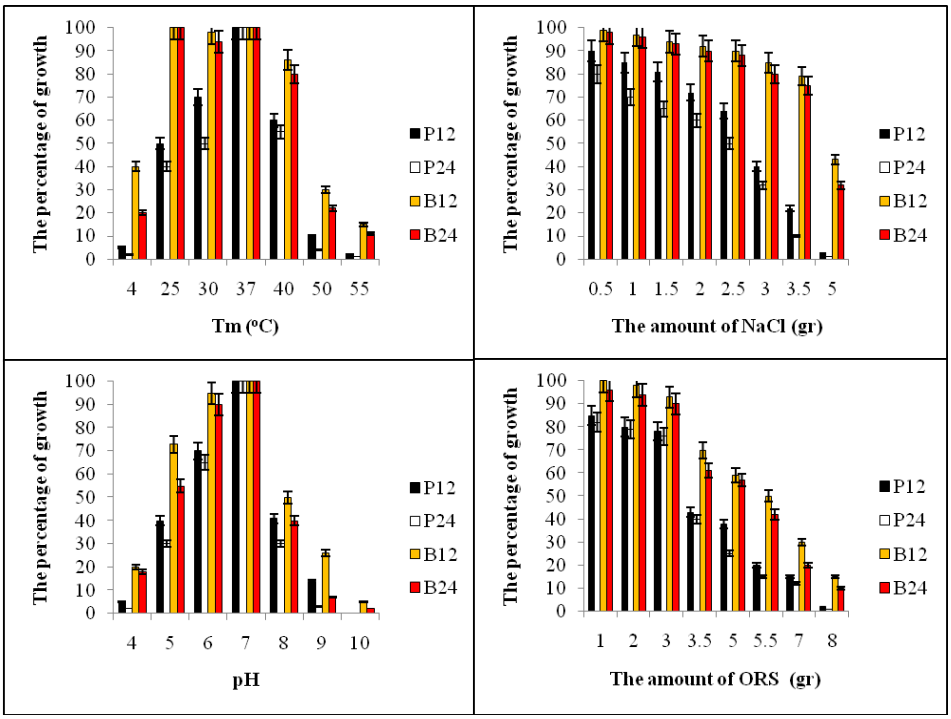

Fig. 1: The percentage of effects under different stresses on bacterial growth (Tm, pH, ORS and NaCl)
Legend: P12: The bacterial growth in planktonic formation during 12 h; P24: The bacterial growth in planktonic formation during 24 h; B12: The bacterial growth in biofilm formation during 12 h, B24: The bacterial growth in biofilm formation during 24 h

percentage was around 90% in during two times (12 and 24 h) but the bacterial growth was reduced in 5 g of NaCl and also, the best condition for growing was in 0.5 g of NaCl because both bacterial forming could grow in this concentration. On the other side, the percentage of bacterial growth was getting fail with increasing the amount of NaCl.

The percentage of bacterial growth was in biofilm formation more than bacterial growth in planktonic formation in all NaCl concentrations which it indicated that biofilm formation overcame and also this formation could protect bacteria from this factor stress. The number of bacteria (CFU/mL) and O.D presented that the bacterial growth was decreased with increasing the amount of concentration while this data were presented in Table 1 and Fig. 1, which confirmed the best concentration for growing was 0.5 gr of NaCl. The colony formation was Small, white, dry, round and flatten formation in 0.5 g of NaCl but colony formation was changed to acerate, white, dry, round and raised formation in the rest of concentrations (1, 1.5, 2, 2.5, 3, 3.5 and 5 g).

Effects of pH on the Bacterial Growth and Formation

The number of bacteria (CFU/mL) were changed in different pH levels which it was presented in Table 1. The heights number of bacteria belonged to pH 6 and 7 which were 10.5×10^7 and 10.5×10^8 during 12 h and 9.5×10^7 and 10.5×10^8 during 24 h; respectively and also the lowest number of bacteria related to pH 10. The colony formation was changed at three levels of pH such as; the formation was small, bright (white), wet, round and raised formation in pH 4, 5 and 6 but it was changed to big formation in pH 7 and acerate formation in pH 8, 9 and 10, which all data were presented in Table 2. On the other hand, the percentage of bacterial growth which was presented in Fig. 1 proved this subject that pH 6 and 7 were optimal pH for *E. coli O157* and also, the percentage of bacterial growth in biofilm formation was more than planktonic formation but the percentage of growth was the same in pH 7 in both formations. All experiments were done during two times which were 12 and 24 h.

Effects of ORS on the Bacterial Growth and Formation

The percentage of bacterial growth was decreased with increasing ORS concentration while the best growth belonged to 1, 2 and 3 g ORS and also the percentage of bacterial growth in biofilm formation was more than planktonic formation in all ORS concentrations during two times (12 and 24 h). The results were presented in Table 1 and Fig. 1. The colony formation was big, white, wet sticky, round and flatten formation in 1 g ORS, which data were presented in Table 2. But it had been changed to average and small with increasing ORS concentration.

Discussion

Escherichia coli O157:H7 is a pathogen often contracted by intake of contaminated water or food. Infection with this agent is associated with a broad spectrum of illness ranging from mild diarrhea and hemorrhagic colitis to the potentially fatal hemolytic uremic syndrome (HUS). The influences of prior exposures to common physicochemical stresses encountered by microorganisms in food and food processing ecologies such as acidity, desiccation and their combinations, on their subsequent susceptibility towards UV-C treatment in coconut liquid endosperm beverage (Glass *et al.*, 1992; Bjornsdottir *et al.*, 2006; Gabriel *et al.*, 2015).

Glass *et al.* (1992) exposed *E.coli* O157:H7 was incapable to grow in TSB having more than 6.5% NaCl (Glass *et al*., 1992). Conner *et al.* (1992) described that *E. coli* O157:H7 growth was reserved by 6% NaCl in TSB (Conner, 1992). Glass *et al*. (1992) discovered *E. coli* 0157:H7 could grow in NaCl meditations (6.5%) (Glass *et al.*, 1992). Results of this study were similar to other studies which have been done by other researchers and also, we found that the bacterial growth was decreased with increasing NaCl concentration which this subject was informed by two methods (CFU and OD) and these results showed that the colony formation was changed in deferent concentrations and the best condition for this bacterium was in 0.5 g of NaCl.

Bjornsdottir *et al.* (2006) showed the first report of the protective effect of organic acids on the survival of *E. coli* O15:H7 under low-pH conditions (Bjornsdottir *et al.*, 2006). Gabriel and Nakano (2010a) contribute in further understanding the behavior of the test organism after exposure to combinations of stresses commonly encountered in food and food processing ecologies (Gabriel *et al.*, 2010). Gabriel *et al*. (2015), stresses were found significantly greatest, making the organism and physiological state an appropriate reference organism for the establishment of UV-C pasteurization process for the beverage (Gabriel *et al.*, 2015). In our research, the best pH for bacterial growth was pH 7. The bacterial growth was dropped in other pH and also the number of bacteria and colony formation were affected by other condition stress and our results are similar to other researchers. The best pH for this bacterium is 6 and 7 in two times (12 and 24 h). Uhlich *et al*. (2001) confirmed cooperative biofilm creation counting an *E. coli* O157:H7 strain (occupied in outbreaks from spinach and lettuce, respectively) (Uhlich *et al.*, 2001; Uhlich *et al.*, 2008). In this study, we found that the best formation was biofilm formation because the percentage of bacterial growth in biofilm formation was more than in planktonic formation in all stressed conditions. This bacterium can be pathogenic in optimal conditions in biofilm and planktonic formation.

Gabriel and Nakano (2010b) suggested that disclosure of cells to high temperature and desiccation damages the cell walls of bacteria that lead to losses in cellular constituents (Gabriel and Nakano, 2010a). There are few studies about effects of temperature on *E. coli* 0157:H7 but in this study, we found that optimum temperature for this bacterium was at 37^{o}C and bacterial growth was reduced at

other temperatures. On the other hand, the colony formation was affected by various temperatures. Glass *et al.* (1992) exposed *E. coli* 0157:H7 raised at all alkaline pH values (to pH 9.0) tested (Glass *et al.*, 1992).

This research demonstrated that *E. coli* O157:H7 could be pathogenic in some conditions and its growth could be affected by some stresses (NaCl, pH, Tm and ORS). Our results were similar to other researchers but in this study, we tried to focus on four stresses, which were important. We found that biofilm formation could have growth in some condition stresses, which planktonic formation could not have the same growth and could be understood that this bacterium could survive more in biofilm formation. On the other hand, the optimal conditions were found for *E. coli* O157:H7 in NaCl, pH, Tm and ORS stresses.

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