Recovery of Cold Impacted Escherichia Coli O157:H7 Bacteria in Ground Beef

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Abstract

Escherichia coli in fresh minced meat was injured by cooling at 4 ºC. A bacterial population has three different physiology which are uninjured or normal cells, sublethally injured cells (or injured cells), and lethally injured cells (or dead cells). Cell injury is defined as any damage to the components of cells themselves by any stresses which weaken the ability of cells to survive or multiply. This will increase the sensitivity of cells to any harmful factors. The cells can repair their injury which can be extended 48hour depending on the nature of stress and degree of injury. The purpose of this study was to: supplemented some cultural media and preparation new cultural media to isolated E.coli with compounds that supplemented the bacterial growth such as yeast extract, sodium pyruvate, n-propyl gallate, catalase, and Tween. Various concentrations of the compound were tested minced beef meat with mixed it and compared with traditional media. The rest of the compound had variable effects on the recovery of cold stressed cells but they weren’t as efficient as needed. It is, therefore recommended that 0.5% of both catalase and tween 80 be used to supplement tryptic soy agar (TSA) in the repair detection procedure.

Keywords: Cold, Beef, Bacteria, Microbial.

1. Introduction

Microbial contamination of food is a major concern for the food industry and consumers. An estimated 76 million people face food-borne illness each year in the United States. The estimated costs related to food-borne diseases in the United States are between $10 and $83 billion annually [1]. Methods such as heating, cooling, freezing, drying, freeze-drying, irradiation, fermentation, or the addition of antimicrobials and chemicals are commonly used to control bacterial contamination and pathogens [2]. After these treatments, one population of microorganisms may be killed, while another may survive (non-injured), and a third population may be sublethally injured [3]. Injured organisms are potentially as important as their normal counterparts because they can recover and become functionally normal in a favorable environment. Since injured cells may not grow well on selective detection media, a recovery step or repair of injured cells on non-selective media is necessarily incorporated with selective enumeration [4]. Determining the presence of injured microorganisms is important in many areas, such as the preservation and spoilage of foods, consumer protection, and the manufacturing of safe foods [5]. While developing rapid detection methods (such as modified culturing and biochemical assays, immunoassays, molecular techniques, and biosensors). It is important to detect both injured and non-injured microorganisms, to distinguish between live and dead cells to prevent false-negative results. It is showed that supplementing the restricted medium with specific nutrients allowed the injured cells to regain the ability to multiply. Additionally, injured cells may show an extended lag phase, compared with non-injured cells, to repair damage and synthesize the proteins and nucleic acids needed for growth. It should be noted that an injured cell can repair the cellular damage (resuscitation) and regain its ability to form a colony in the presence of the selective agent; however, the dead cell cannot form a colony under any condition [6]. A population of surviving microorganisms, after a cold impact treatment, includes dead cells (lethally or irreversibly injured), uninjured cells (normal cells), and injured cells [7,8]. Pathogens and spoilage organisms in meat products can become injured. Injury of microorganisms may result from meat processing and handling procedures, such as cooling, refrigeration, freezing, drying, and irradiation, from exposure to preservatives, acidity, and low water activity, or from being starved [9,10]. Therefore, determining the presence of injured microorganisms is critical to the quality and safety of final meat products. The Gram-negative bacterium Escherichia coli becomes susceptible to the narrow-spectrum antibiotic vancomycin during growth at low temperatures. The outer membrane of Gram-negative bacteria is an effective barrier against extracellular solutes, providing intrinsic resistance to useful antibiotics [11]. Escherichia coli O157:H7 is an important foodborne pathogen associated with cases and outbreaks of food poisoning. Foods of bovine origin, in particular ground beef, are major vehicles of infection [12]. Sensitive and improved methods for detection of this organism are needed by the food laboratories to prevent foods contaminated with E. coli O157:H7 and from reaching the consumer. The objectives of this study were to
evaluate the sensitivity of various cultural methods using three different enrichment media and four selective plating media for recovery and detection of cold stressed \textit{E. coli} O157:H7 inoculated at low concentrations into ground beef samples.

## 2. Materials and Methods

\textit{E. coli} isolate was obtained from the Biology Department/ College of Science /Baghdad University. Bacteria were grown on tryptic soy agar (TSA) slant and stored at 4C. Bacteria were activated in nonselective tryptic soy broth containing 0.3\% yeast extract and incubated at 37C for 24 hours. Grown cells were transferred to 90 ml of tryptic soy broth (TSB) and incubated at 37C for 12 h. The absorbance at 1.0 for the exponential phase culture of \textit{E.coli} grown in TSB was fixed using a Pye unicum spectrophotometer at 650 nm [13]. The cells were centrifuged in a refrigerated centrifuge at 7000 x g for 12 minutes and suspended in sterile distilled water to an optical density at 650 nm of 1. 25 grams of fresh minced beef meat were contaminated with 225 ml of \textit{E. coli} culture grown in TSB to fix a concentration of $10^7$ cfu/ml of fresh chicken meat. Treated meat samples were subjected to stress by refrigeration at 4C for 1,3,5 and 5days. Cells were enumerated before and after being stressed by pour plating with tryptic soy agar supplemented with various concentrations of compounds that either has an antioxidant function or degrade hydrogen peroxide (Table 1).

### Table 1. Source, type, concentration, and function of supplements.

| Supplement to TSB | Concentration(\%) | Function | Time added relative to sterilization | Source |
|-------------------|-------------------|----------|-------------------------------------|--------|
| Yeast extract     | 0.25, 0.5         | Support TSB | Before                              | Oxide ltd |
| Sodium pyruvate   | 0.10, 0.3, 0.05   | Degrade H$_2$O$_2$ | Before                              | Sigma chemical co |
| N.Propyl gallate  | 0.05, 0.1, 0.3    | Anti oxidize Gurtler and Kornacki ,2009 (5) | Before | Sigma chemical co |
| Catalase          | 0.1, 0.2, 0.5     | Blocks formation of H$_2$O$_2$ | After | Sigma chemical co |
| Tween 80          | 0.1, 0.3, 0.5 %   | Degrade H$_2$O$_2$ Gurtler and Kornacki ,2009 (5) | After | Baker Chemical co |

### 2.1. Cells enumeration

In tryptic soy agar medium, a pour plate method was enumerated \textit{E. coli} cells before and after cold stress after serial dilution in peptone water and incubated at 37 ° C for 24 hours.

### 2.2. Microbiological analysis

The percentage of increase in the number of living cells before and after supplementation were calculated according to the following equation

\[
\text{Percentage of increase} = \left( \frac{\text{the number of living cells in supplement media} - \text{the number of living cells in unsupplemented media}}{\text{the number of living cells in unsupplemented media}} \right)\times 100
\]

### 3. Results and Discussion

The phenomenon of outbreaks of foodborne diseases has increased in the past decade, but methods of detection and isolation are still old, so it was necessary to find new media and ways to detect these bacteria. The process of using refrigeration is one of the most important factors in reducing the number of living cells, as a breakdown of some components in the cell wall, cytoplasmic membrane, and damage to the ribosomes. And may occur a breakdown in DNA. Therefore there is a decrease in the number of living cells that culturing in TSA medium for different periods (1,3,5 and 7)days. It was 0day as control when incubated in cooling. The bacterial numbering was increased when the bacteria were incubated without cooling, as illustrated in fig1.
Figure 1. Effect of cooling at 4C for 1,3,5 and 7 days on the number of Escherichia coli bacteria in minced meat on tryptic soy agar medium and compare it with control(without cooling).

The results showed that there were no significant differences for the effect of recovery and recovery of damaged cells when using each TSA medium with the addition of (0.1, 0.25, and 0.5)% yeast extract (Fig. 2) despite the fact that yeast extract is a good nitrogen source, which combines a number of basic factors to restore cell wall of cold-stressed bacteria in addition to supporting components of the used medium.

Figure 2. The effect of using yeast extract on the numbers of Escherichia coli bacteria in fresh minced meat for different periods at 4C.

The effect of sodium pyruvate in different concentrations (0.05, 0.1, 0.3)% on the recovery effect of the damaged cells was clear in (Figure 3) and this maybe comes from its action in destroying hydrogen peroxide and this was confirmed by researchers [14], as the use of both yeast extract and sodium pyruvate at rates of 0.3% for each of them are the best results obtained, as the addition of sodium pyruvate to the medium is one of the most important factors that led to an increase in the number of restored injured cells when using TSA medium.
The results of Figure (4) show that N-Propyl gallate is ineffective when used in low concentrations (0.1 and 0.05)%%, but when used at a concentration of 0.3%, it was the best concentration ratio as it acted as a good antioxidant, which gave more cells recovered.

While the results of supplementing the medium with different concentrations of catalase (Fig. 5) showed more effectiveness in recovery cells to their vitality, especially by using a concentration of 0.5% and in all stages of cryopreservation, which means stopping the formation of hydrogen peroxide.
Figure 5. The effect of using catalase on the numbers of *Escherichia coli* bacteria in fresh minced meat for different periods of time at 4°C.

Figure (6) showed the results of supporting the medium by adding Tween 80 at different concentrations (0.1, 0.3, 0.5)%, respectively. The results showed an increase in the restoration of cells to their vitality at all concentrations and gradually, as the highest percentage of cells recovery was at a concentration of 0.5% and may return because the cold-stressed cells decreased the number of developing cells, as some components of the cell wall were destroyed. When Tween 80 was added, the damage to the cell was repaired (resuscitation) and its ability to form a colony was restored.

Figure 6. Effect of using Tween 80 on numbers of *Escherichia coli* bacteria in fresh minced meat for different periods of time at 4°C.

Figure (7) shows the results of supporting the medium by adding Tween 80 at different concentrations (0.3, 0.5)%, respectively, with different concentrations of catalase (0.3, 0.5)%. The rate of recovery of cells was at a concentration of 0.5% for each, and this may be due to the fact that the cold-stressed cells had repaired the damage caused to the cell (resuscitation) and restored its ability to form a colony significantly.
Figure 7. Effect of using different concentrations of Catalase and Tween 80 on the numbers of *Escherichia coli* in fresh minced meat for different periods at 4°C.

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