Full Length Research Paper

Isolation and identification of *Listeria monocytogenes* from local cheese and evaluation of bacteria growth and proliferation in Hela cell culture

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*Listeria monocytogenes* is Gram-positive, spore-free and facultative anaerobic frequently transmitted to humans via water, meat and its products as well as raw vegetables. It is able to grow both intra and extra-cellular. The main way of bacteria transmission to human is through food material. The aim of this study is to isolate the bacteria from cheese using cold-enrichment and detection of it in Hela cell culture by light microscopy. Thus fresh local cheeses were sampled. Samples were moved to Trypton Soya Yeast Extract (TSYE) medium and were kept at 4°C for 1 to 3 weeks for enrichment. Then, they were cultured in Palcam and *Listeria* selective agar over given intervals. In order to detect colonies, first Gram staining and then biochemical tests were applied. Bacteria were inoculated in flasks containing Hella cell so as to detect bacteria entrance to the cell. Afterward, this cell culture medium was sampled on lam regularly. Samples were stained with Giemsa and then they were examined using light microscope. This investigation indicated that isolation of *L. monocytogenes* from diaries is possible using cold-enrichment without need for inhibitors antibiotics. In addition, it was found that this bacterium is able to enter into the cell and grow and proliferate in laboratory conditions. It lyses host cell after 48 h. The results of this study imply that *L. monocytogenes* exist in some contaminated local cheese. It also was found that it is able to grow and proliferate in cellular culture mediums and in laboratory conditions. Regarding the fact that the bacteria are omnipresent, more control over food material production and distribution cycle is needed so that it is prevented.

**Key words:** *Listeria monocytogenes*, cheese, intracellular bacteria, Hela cell cultures.

INTRODUCTION

*Listeria monocytogenes* are Gram-positive, spore-free and facultative anaerobic bacteria which are able to grow inter and extracellular. It can be transmitted via contaminated foods (Lyytikainen et al., 2000), cheese (Larson et al., 1999), pasteurized milk (Ryser and Marth, 1999; Teo and Knabel, 2000), raw vegetables (Ryser and Marth, 1999), meat and its products (Carroll et al., 2000; Kawasaki et al., 2009), and seafood (Weagant et al., 1988) to human. Several cases have been reported on listeriosis outbreak caused by contamination of foods like meat or diaries and other processed foods which have been consumed without enough heating or pasteurization. This indicates ability of the bacteria in growth and proliferation in refrigerator temperature. *L. monocytogenes* is one of the few bacteria which can pass through placenta. In pregnant women with listeriosis, it can contaminate fetus and thus lead to miscarriage, premature delivery or delivery of live infants...
with systemic infections by this bacterium (Jawetz et al., 2001). Invasive listeriosis clinical symptoms are usually severe and appear as miscarriage, sepsis, and meningoencephalitis (Vazques- Boland et al., 2001).

Erdogan et al. (2002) studied isolation of L. monocytogenes from fecal samples using cold enrichment (4°C) for 14 weeks and cultured on enriched selective medium at 30°C. They reported when sample is kept at refrigerator temperature in physiologic serum lacking culture, L. monocytogenes is isolated in third week in 94% cases. If agar selective medium is combined with cold enrichment method, it can be isolated in first week in 100% cases (Erdogan et al., 2002; Erdogan, 2010).

L. monocytogenes enters human body through gastrointestinal tracts following eating contaminated foods such as cheese. It involves a bacterial protein called interalin which reacts with gastrointestinal epithelium known as E cadherin and then it enters epithelial cells (Goldberg, 2001; Ryser and Marth, 1999).

The aim of this study is to present a simple practical method considering available resources (using fewer materials) for isolation of this bacterium from fresh local cheese, and detection of it in Hela cell culture by light microscopy.

MATERIALS AND METHODS

In this experimental study, a sample of fresh local cheese was selected. In order to carry out the experiment, 25 g local cheese was transferred to 225 ml Trypton Soya Yeast Extract (TSYE) medium already heated up to 37°C. Then sample was stirred up so as for its constituents to be scattered. It was kept in prepared medium for one to three weeks at 4°C. One week later this medium was sampled every two days and the sample was diluted with potassium hydroxide 0.5%. Resulting dilution was at 10.1. In this experiment, 25 g local cheese prepared by Razi Vaccine and Serum Research Institute, Hesarak, Karaj (4524 of testes well that; this experiment, 25 g local cheese nolayer was formed on cover slips, in this prepared by Razi Vaccine and Serum Research Institute, Hesarak, Karaj (4524 of testes well that; 25 g local cheese nolayer was formed on cover slips, in this

After isolation, 25 g local cheese was frozen and was kept at 37°C to lighten tubes and two additional tubes without addition of bacteria were considered as controls. Lighten tubes containing slip cover were kept at 37°C and one sample of each tube was taken after 12, 24, 36, and 48 h and it was fixed for half an hour by Carnoy fixator. Subsequently, Carnoy was emptied and phosphate-buffered saline (PBS) was added. Following this stage, cover slips were prepared for Giemsa staining. Prepared cover slips were observed and photographed by Olympus BH-2 light microscope (Takano et al., 1999).

RESULTS

In this method L. monocytogenes was obtained without using mediums such as Oxford agar, nalidixic Acid, cycloheximide, and acriflavine. Following appearance of susceptible colonies and performing glucose tests and other biochemical tests, bacteria were identified according to Australian/New Zealand Standard (1998). Results indicated that L. monocytogenes bacteria are available in 9% of fresh local cheese samples. L. monocytogenes was cultured in virology sector of Razi Vaccine and Serum Research Institute. In this research, various dilutions of L. monocytogenes bacteria were inoculated in Hela cell culture. Cellular culture was used in order to show the ability of this bacterium to enter into cell. Investigation of photographs showed that this bacterium was able to enter Hela cells in concentrations more than 5 \times 10^3. As given in Figure 1, abundant bacteria can be seen around and few of them can be seen in the cell. This figure demonstrates well that L. monocytogenes entered the host cell after 12 h and it could proliferate and grow in intracellular medium and finally after 48 h lysed the host cells (Figure 2).

DISCUSSION AND CONCLUSION

Isolation of L. monocytogenes is often difficult and time consuming. In this research, it was attempted to develop the simplest method for its isolation using works by other researchers and concerning available resources. Basic method for this research were Australian/New Zealand Standard (1998) and Standard 4524 of Institute of Standards and Industrial Research of Iran (1999). In Australian/New Zealand Standard, after addition of the sample to enrichment medium, culture medium was kept at 30 and 4°C, temperature of the refrigerator, wasn’t used. In addition, antibiotics and bacteriostatic substances such as nalidixic acid, cycloheximide, and acriflavine hydrochloride were added to enrichment culture medium so that growth and proliferation of pesky bacteria and fungi is prevented. In this method, refrigerator temperature was used (cold enrichment) instead of using antibiotics and bacteriostatic substances.
Figure 1. 12 h after bacteria inoculation in cellular culture, abundant bacteria can be seen around and few of them can be seen in the cell.

Figure 2. 48 h after bacteria inoculation; contaminated cell is lysed due to bacteria growth and proliferation.
Erdogan et al. (2002) recommended cold enrichment for isolation of this bacterium, which is consistent with our findings.

Due to importance of *L. monocytogenes* in food products health and public health, many studies have been done in various countries including Iran on this bacterium. For instance, 3 cases of listeriosis were observed during 2 weeks in North Carolina in 2001, reason of which was using home-made Mexican style cheese (Anonymous, 2000; MacDonald et al., 2005). In Brazil, 11 (10.6%) cheese samples were contaminated by *L. monocytogenes* out of 103 cheese samples (da Silva et al., 1998). In our country, 3 samples out of 28 samples of sausage and salami (10.71%) and 3 samples (9.09%) out of 22 dairy samples were contaminated by *L. monocytogenes* (Noruzi et al., 2002) which is somehow in consistency (9%) with our findings.

Since prevalence of listeriosis may bring unpleasant and irreversible effects to the elderly, immunocompromised patients and pregnant women, it is necessary to use accurate and reliable methods in order to obtain and detect this bacterium in health centers. On the other hand, one of the food-related listeriosis prevalence causes in different communities including Iran is contaminated cheese, especially soft cheeses. So, regarding high consumption of traditional cheeses in our country, it is necessary to have specific surveillance over the production and distribution of dairy products by small traditional producers and factories and avoid consuming dairies which are offered traditionally in non-healthy manner.

Concerning the fact that *L. monocytogenes* is able to grow and proliferate on extracellular mediums and synthesized culture mediums and it is possible to identify appropriate medicine for fighting against bacteria using antibiogram test, however, recurrence of infection with Listeria (30%) implies that this bacterium in intracellular and using antibiotics is useful just for annihilation of extracellular bacteria and intracellular bacteria are secured from fatality of antibiotics (Molder, 1985).

Findings of this research suggest that *L. monocytogenes* is able to enter host cell and lyse it. Regarding identified concentrations (more than $5 \times 10^5$) and intervals (12 to 48 h) in this research, in which bacteria enter cell and lyse it, it is recommended to use these findings in future works in plans for developing vaccine.

REFERENCES


