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Study of Storage Periods of Culture Suspension of *Escherichia coli*

Kundan Kumar*, I. N. Mishra, Sandhya Jha

University Department of Biotechnology, L. N. Mithila University, Darbhanga-846 004, Bihar, India

ABSTRACT

Escherichia coli strain ATCC 8739 is an important strain of pharmaceutical and biotechnological industries. We studied the viability of culture suspension of *Escherichia coli* strain ATCC 8739 at 2 to 8°C up to 360 days in 0.9% w/v NaCl. The culture suspension containing 10000 cfu/ml used in the study was stored at 2 to 8°C for 360 days in 0.9% w/v NaCl. Using 10µl of above culture suspension, the viable count was made by the pour plate technique using Soyabean Casein Digest Agar medium in fixed interval of time during the 360 days storage period. During the storage period, population of *Escherichia coli* strain ATCC 8739 decreased from 10000 cfu/ml to 9900 cfu/ml during the first 30 days, whereas the population decreased to 0 cfu/ml in 360 days. Findings emanate from the study indicates that 30 days storage period of *Escherichia coli* strain ATCC 8739 at 2 to 8°C in 0.9% w/v NaCl is suitable for laboratories testing purposes on account of fact that in 30 days storage period, population of *Escherichia coli* strain ATCC 8739 decreased from 10000 cfu/ml to 9900 cfu/ml which is very low.

Keywords: Microbial limit test, Non sterile products, Sterility test, Sterile products, Viability.

INTRODUCTION

Escherichia coli is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E. coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls due to food contamination. [1] The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2, [2] and by preventing the establishment of pathogenic bacteria within the intestine. [3-4] Virulent strains of *E. coli* can cause gastroenteritis, urinary tract infections, and neonatal meningitis. In rare cases, virulent strains are also responsible for hemolytic-uremic syndrome, peritonitis, mastitis, septicemia and Gram-negative pneumonia.

In pharmaceutical and biotechnological industries, it is recommended by the Indian Pharmacopoeia-2010 [5], that the presence of *Escherichia coli* in products is strictly not allowed. It is therefore pertinent to assure the absence of *Escherichia coli* by microbial limit test for non sterile products and the sterility test for sterile products, time to time in each case. The requirement of culture suspension of known cfu/ml of Escherichia coli ATCC 8739 is essential to conduct microbial limit test and sterility test as growth promotion test of culture media, used in microbial limit test and sterility test.

The preparation of culture suspension of known cfu/ml is a complicated process, along with this every day preparation of culture suspension is time consuming and costly business for the industries. Now taking into consideration above mentioned factors, there was requirement to study the storage period of prepared culture suspension of known cfu/ml of *Escherichia coli* ATCC 8739 at a particular temperature for the growth promotion test of culture media used for microbial limit test and sterility test in the industries. [6]

***Corresponding author: Mr. Kundan Kumar,**
PhD Student, University Department of Biotechnology, L. N. Mithila University, Darbhanga-846004, Bihar, India;
Email: kk_bt82@rediffmail.com

MATERIAL AND METHODS

Preparation of culture media

Culture media of HiMedia Laboratories Pvt Ltd was used in the study. Growth promotion test of culture media was checked by *Escherichia coli* ATCC 8739. Required quantity of Soyabean Casein Digest Agar and 0.9% w/v NaCl [7] were prepared and sterilized in an autoclave at 121°C for not less than 20 minutes at 15 lbs pressure. The pH of media was to be maintained before and after sterilization. [8-9]

Preparation of culture suspension of *Escherichia coli* ATCC 8739

Required numbers of Soyabean Casein Digest Agar slants and tubes containing 0.9% w/v NaCl [7] were prepared. After solidification, the media slants were transferred to incubator for pre-incubation at 35±2.5°C for 48 hours for checking any contamination. Working culture of *Escherichia coli* ATCC 8739 was added over the surface of the media slant by streaking method. These streaked media slants were placed in incubators at 35±2.5°C for 48 hours. After completion of

incubation period, the Soyabean Casein Digest Agar slants and 0.9% w/v NaCl tubes were transferred for serial dilution to Laminar Air Flow. 02 ml of 0.9% w/v NaCl solution was added over the surface of freshly prepared slants of *Escherichia coli ATCC 8739* after which surface of slants were scraped by using sterile inoculating loop. Serial dilution was done and 10 µl of culture suspension was transferred into separate sterile petriplate in duplicate from the five dilutions *i.e.* 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} and 10^{-8}. 15 ml of Soyabean casein digest agar was added aseptically into each petriplate along with negative control and incubated at 35±2.5°C for 48 hours. The number of colonies were observed and counted. The dilution 10^{-6} containing 10000 cfu/ml was selected for study and preserved at 2 to 8°C. [10-11]

Experimental details

All experiments were conducted under Laminar Air Flow and media plates were incubated in incubators. The test suspension of *Escherichia coli* ATCC 8739 containing 10000 cfu/ml was used for study. 10µl of culture suspension was transferred into separate sterile petri plate in duplicate from the test suspension. 15 ml of Soyabean Casein Digest Agar media was added aseptically into the each petri plate along with negative control and incubated at 35±2.5°C for 48 hours in incubator. The number of viable colonies were observed and counted in fixed interval of time by the same process during the whole storage period of 360 days at 2 to 8°C. (Table 1 & Fig. 1)

Table 1: Observed cfu/ml throughout the storage period of 360 days of *Escherichia coli* ATCC 8739

STORAGE PERIOD IN DAYS	OBSERVED CFU/ML
0	10000
30	9900
60	8800
90	7200
120	5800
150	4200
180	3300
210	2100
240	1400
270	800
300	500
330	100
360	0

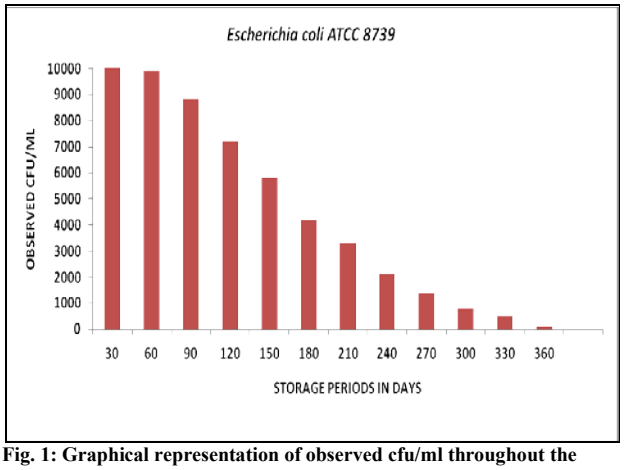

Fig. 1: Graphical representation of observed cfu/ml throughout the storage period of 360 days of *Escherichia coli* ATCC 8739 stored at 2 to 8°C

RESULTS

Study indicates that during the first 30 days storage period of *Escherichia coli* ATCC 8739 at 2 to 8°C in 0.9% w/v NaCl, population decreased from 10000 cfu/ml to 9900 cfu/ml, while the population decreased to 3300 cfu/ml in 180 days and to 0 cfu/ml in 360 days of storage period. During the first 30 days storage period, population of *Escherichia coli* ATCC 8739 decreased from 10000 cfu/ml to 9900 cfu/ml, which is very low reduction in population.

DISCUSSION

The preparation of culture suspension of known cfu/ml is a complicated process, along with this every day preparation of culture suspension is time consuming and costly business for the industries. Now taking into consideration above mentioned factors, there was requirement to study the storage period of prepared culture suspension of known cfu/ml of *Escherichia coli* ATCC 8739 at a particular temperature for the growth promotion test of culture media used for microbial limit test and sterility test in the industries. [6]

This research study will lessen the manufacturing cost and testing time of pharmaceutical and biotechnological products in the industries. The results obtained shows that the 30 days storage period of culture suspension of *Escherichia coli* ATCC 8739 stored at 2 to 8°C in 0.9% w/v NaCl is suitable for growth promotion test of culture media in pharmaceutical and biotechnological industries.

REFERENCES

1. Vogt RL, Dippold L. *Escherichia coli* O157:H7 outbreak associated with consumption of ground beef. Public Health Rep. 2005; 120 (2): 174-178.
2. Bentley R, Meganathan R. Biosynthesis of vitamin K (menaquinone) in bacteria. Microbiol. Rev. 1982; 46(3): 241-280.
3. Hudault S, Guignot J, Servin AL. *Escherichia coli* strains colonizing the gastrointestinal tract protects germ-free mice against *Salmonella typhimurium* infection. Gut 2001; 49(1): 47-55.
4. Reid G, Howard J, Gan BS. Can bacterial interference prevent infection? Trends Microbiol. 2001; 9(9): 424-428.
5. Indian Pharmacopoeia, Controller of Publication, Delhi, MHRD, Govt of India, 2010, Vol 1, pp 35-40.
6. Davis BD, Mingioli ES. Mutants of *Escherichia coli* requiring methionine or vitamin B_{12}. J. Bacteriol. 1950; 60: 17-28.
7. Kropinski, AM. Stability of bacterial mutants in saline. Applied Microbiology 1975; 29(4): 448-450.
8. DeVay JE, Schnathorst WC. Single-cell isolation and preservation of bacterial cultures. Nature (London) 1963; 199: 775-777.
9. Lennox ES. Transduction of linked genetic characters of the host by bacteriophage P1. Virology1955; 1: 190-206.
10. Grivell AR. Ganesan AK, Hanawalt PC. Preservation of *Escherichia coli* on silica gel. Microb. Genet. Bull.1972; 34: 15.
11. Howard DH. The preservation of bacteria by freezing in glycerol broth. J. Bacteriol.1956; 71**:** 625.