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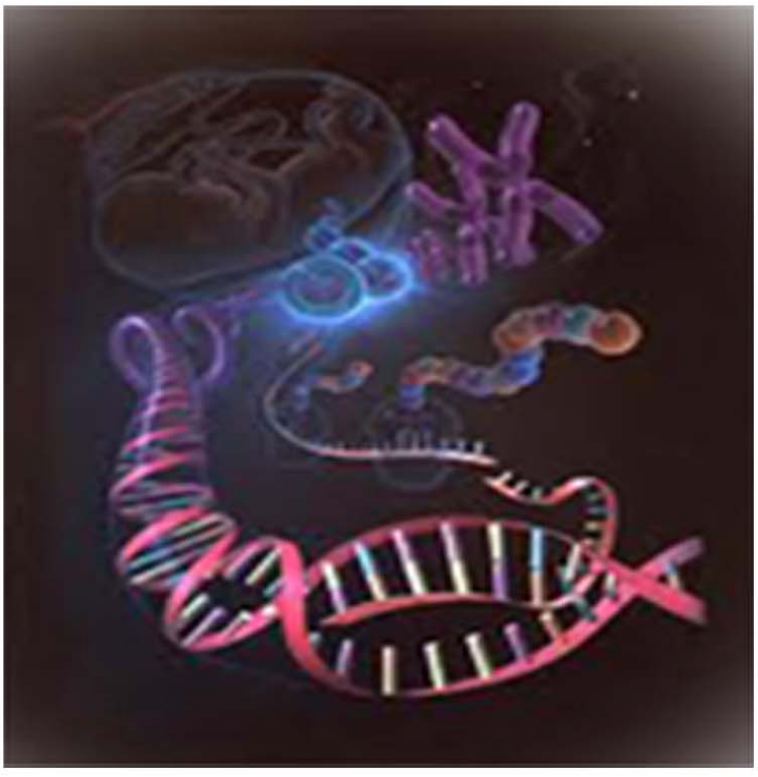

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Research Paper

STUDY OF VIABILITY PERIOD OF CULTURE SUSPENSION OF *SALMONELLA ENTERICA*

Kundan Kumar[1]* and Sandhya Jha[1]

*Corresponding Author: **Kundan Kumar,** ✉kk_bt82@rediffmail.com

Salmonella enterica ATCC 14028 is an important strain of pharmaceutical and biotechnological industries. The present study indicates the viability of culture suspension of *Salmonella enterica* strain ATCC 14028 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 10 µl 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 of storage period. During the storage period, population of *Salmonella enterica* ATCC 14028 decreased from 10000 cfu/ml to 9900 cfu/ml during the first 30 days, whereas the population decreased to 100 cfu/ml in 360 days. Findings emanate from the study indicates that 30 days storage period of *Salmonella enterica* ATCC 14028 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 *Salmonella enterica* ATCC 14028 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

Salmonella is a genus of rod-shaped, Gram-negative, non-spore-forming, predominantly motile enterobacteria with diameters around 0.7 to 1.5 µm, lengths from 2 to 5 µm, and flagella that grade in all directions (i.e., peritrichous). They are chemoorganotrophs, obtaining their energy from oxidation and reduction reactions using organic sources, and are facultative anaerobes. Most species produce hydrogen sulfide (Clark, 1987) , which can readily be detected by growing them on media containing ferrous sulfate, such asTSI. Most isolates exist in two phases: a motile phase I and a nonmotile phase II. Cultures that are nonmotile upon primary culture may be switched to the motile phase using a Cragie tube. *Salmonella* is closely related to the *Escherichia* genus and are found worldwide in cold- and warm-blooded animals (including humans), and in the environment. They cause illnesses such as typhoid fever, paratyphoid fever, and foodborne illness (Ryan,2004). *Salmonella* species are facultative intracellular pathogens (Jantsch, 2011) that enter cells via macropinosomes (Kerr, 2010). Enteritis *Salmonella* (e.g., *Salmonella enterica* subsp. *enterica serovar* Enteritidis) can cause

* University Department of Bio-Technology, L. N. Mithila University, Darbhanga-846004, Bihar.

diarrhoea, which usually does not require antibiotic treatment. However, in people at risk such as infants, small children, the elderly, *Salmonella* infections can become very serious, leading to complications. If these are not treated, HIV patients and those with suppressed immunity can become seriously ill. Children with sickle cell anaemia who are infected with *Salmonella* may develop osteomyelitis.

In pharmaceutical and biotechnological industries, it is recommended by the Indian Pharmacopoeia-2010 (Indian Pharmacopoeia, 2010), that the presence of *Salmonella enterica* in products is strictly not allowed. It is therefore pertinent to assure the absence of *Salmonella enterica* 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 *Salmonella enterica* ATCC 14028 is essential to conduct microbial limit test and sterility test (Indian Pharmacopoeia, 2010) 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 *Salmonella enterica* ATCC 14028 at a particular temperature for the growth promotion test of culture media used for microbial limit test and sterility test in the industries (Davis and Mingioli, 1950).

MATERIALS 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 *Salmonella enterica* ATCC 14028. Required quantity of Soyabean Casein Digest Agar and 0.9% w/v NaCl (Kropinski, 1975) were prepared and sterilized in an autoclave at 121°C for not less than 20 min at 15 lbs pressure. The pH of media was to be maintained before and after sterilization (DeVay, 1963; Lennox, 1955; Miller, 1972; Howard, 1956).

Preparation of Culture Suspension of *Salmonella enterica ATCC 14028*

Required numbers of Soyabean Casein Digest Agar slants and tubes containing 0.9% w/v NaCl (Kropinski, 1975) were prepared. After solidification, the media slants were transferred to incubator for pre-incubation at 35±2.5°C for 48 h for checking any contamination. Working culture of *Salmonella enterica* ATCC 14028 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 h. 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 *Salmonella enterica* ATCC 14028 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 SCDA was added aseptically into each petriplate along with negative control and incubated at 35±2.5°C for 48 h. The number of

colonies were observed and counted. The dilution containing 10000 cfu/ml was selected for study and preserved at 2 to 8°C (Grivell, 1973; Lapage, 1970; Thomas, 1978).

Experimental Details

All experiments were conducted under laminar air flow and media plates were incubated in incubators. The test suspension of *Salmonella enterica* ATCC 14028 containing 10000 cfu/ml was used for study. 10 µl 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 h 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.

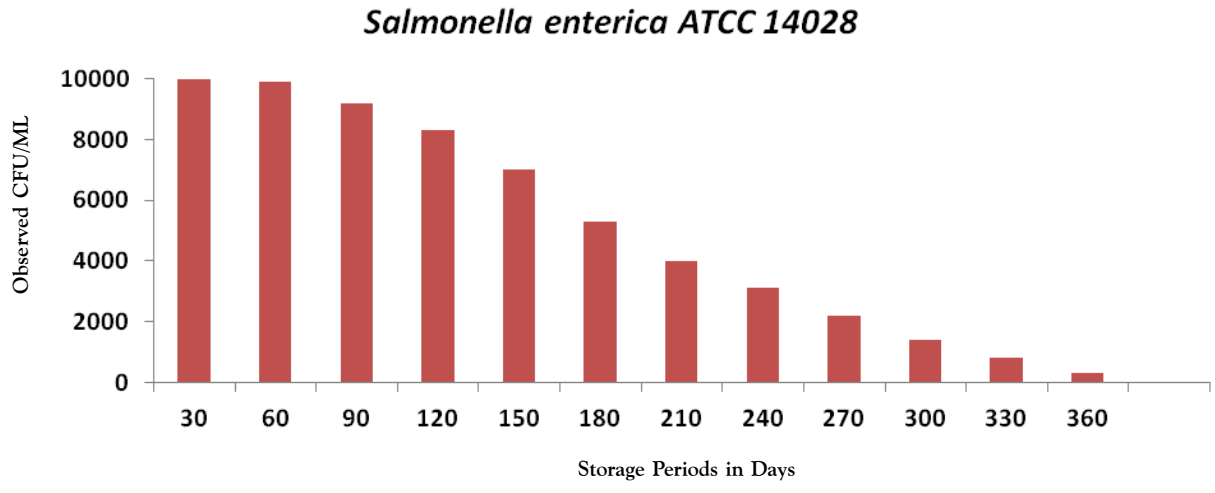
RESULTS AND DISCUSSION

Study indicates that during the first 30 days storage period of *Salmonella enterica* ATCC 14028 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 4100 cfu/ml in 180 days and to 100 cfu/ml in 360 days of storage period.

Table 1: Observed cfu/ml Throughout the Storage Period of 360 days of *Salmonella enterica ATCC 14028*

Storage Period in Days	Observed Cfu/Ml
0	10000
30	9900
60	9200
90	8300
120	7000
150	5300
180	4000
210	3100
240	2200
270	1400
300	800
330	300
360	100

Graph 1: Graphical Representation of Observed cfu/ml Throughout the Storage Period of 360 days of *Salmonella enterica* ATCC 14028 Stored at 2 to 8°C



In the present study, determination of storage periods of prepared culture suspension of known cfu/ml of *Salmonella enterica strain ATCC 14028*, at a particular temperature for the growth promotion test of culture media used for microbial limit test and sterility test in the industries was undertaken. This will lessen the manufacturing cost and testing time of pharmaceutical and biotechnological products.

Results were presented in form of Table 1 and Graph 1.

CONCLUSION

The results obtained shows that the 30 days storage period of culture suspension of *Salmonella enterica ATCC 14028* stored *at* 2 to 8°C in 0.9% w/v NaCl was suitable for growth promotion test of culture media in pharmaceutical and biotechnological industries. This research study will lessen the manufacturing cost and testing time of pharmaceutical and biotechnological products in the industries.

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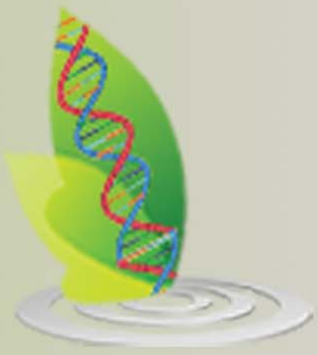

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