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STUDY OF STORAGE PERIODS OF CULTURE SUSPENSION OF *STAPHYLOCOCCUS AUREUS*

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ABSTRACT

Staphylococcus aureus ATCC 6538 is an important strain of pharmaceutical and biotechnological industries. We studied the viability of culture suspension of *Staphylococcus aureus* strain ATCC 6538 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 storage period. During the storage period, population of *Staphylococcus aureus* ATCC 6538 strain 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 *Staphylococcus aureus* strain ATCC 6538 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 *Staphylococcus aureus* ATCC 6538 decreased from 10000 cfu/ml to 9900 cfu/ml, which is very low.

INTRODUCTION: *S. aureus* meaning the "golden grape-cluster berry," and also known as "golden staph" and Oro staphira, is a facultative anaerobic Gram-positive coccal bacterium. It is frequently part of the skin flora found in the nose and on skin, and in this manner about 20% of the human population are long-term carriers of *S. aureus.*

S. aureus is the most common species of staphylococci to cause Staph infections. One of the reasons for this is a carotenoid pigment staphyloxanthin that is responsible for the characteristic golden colour of *S. aureus* colonies. This pigment acts as a virulence factor, with an antioxidant action that helps the microbe evade death by reactive oxygen species used by the host immune system. *S. aureus* can cause a range of illnesses from minor skin infections, such as pimples, impetigo, boils (furuncles), cellulitis folliculitis, carbuncles, scalded skin syndrome, and abscesses, to life-threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia, and sepsis. Its incidence is from skin, soft tissue, respiratory, bone, joint, endovascular to wound infections. It is still one of the five most common causes of nosocomial infections, often causing postsurgical wound infections.

In pharmaceutical and biotechnological industries, it is recommended by the Indian Pharmacopoeia-2010 [1], that the presence of *S. aureus* in products is strictly not allowed. It is therefore pertinent to assure the absence of *S. aureus* 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 *S. aureus* strain ATCC 6538 is essential to conduct microbial limit test and sterility test [1] 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 *S. aureus* strain ATCC 6538 at a particular temperature [2] for the growth promotion test of culture media used for microbial limit test and sterility test in the industries [3].

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 *S. aureus* strain ATCC 6538. Required quantity of Soyabean Casein Digest Agar and 0.9% w/v NaCl [4] 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 [5].

Preparation of culture suspension of *Staphylococcus aureus* strain ATCC 6538: Required numbers of Soyabean Casein Digest Agar slants and tubes containing 0.9% w/v NaCl [6] 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 [7] of *S. aureus strain* ATCC 6538 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 *S. aureus* strain ATCC 6538 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 hours.

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 [8].

Experimental details: All experiments were conducted under Laminar Air Flow and media plates were incubated in incubators. The test suspension of *S. aureus* strain ATCC 6538 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 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 and Fig. 1**).

RESULTS AND DISCUSSION: Study indicates that during the first 30 days storage period of *S. aureus* strain ATCC 6538 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 4200 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 *S. aureus* strain ATCC 6538 decreased from 10000 cfu/ml to 9900 cfu/ml, which is very low reduction in population.

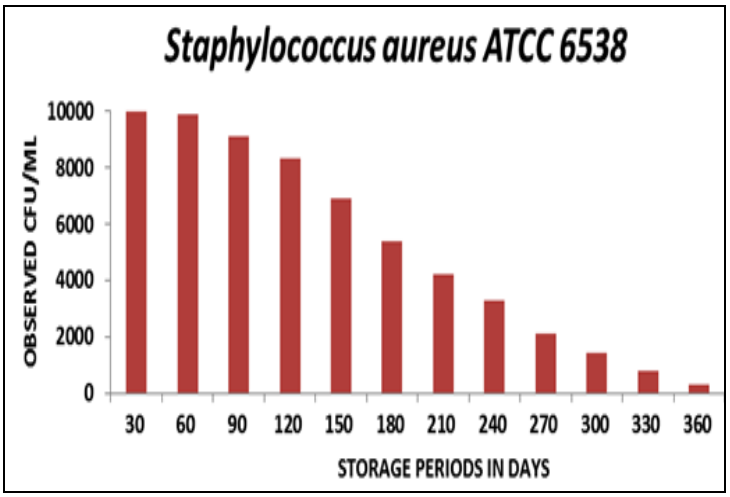

FIG. 1: GRAPHICAL REPRESENTATION OF OBSERVED CFU/ML THROUGHOUT THE STORAGE PERIOD OF 360 DAYS OF *STAPHYLOCOCCUS AUREUS* STRAIN ATCC 6538 *STORED AT 2 TO 8˚C*

TABLE 1: OBSERVED CFU/ML THROUGHOUT THE STORAGE PERIOD OF 360 DAYS OF *STAPHYLOCOCCUS AUREUS ATCC 6538*

STORAGE PERIOD IN DAYS	OBSERVED CFU/ML
0	10000
30	9900
60	9100
90	8300
120	6900
150	5400
180	4200
210	3300
240	2100
270	1400
300	800
330	300
360	0

CONCLUSION: The results obtained shows that the 30 days storage period of culture suspension of *S. aureus* strain ATCC 6538 at 2 to 8°C in 0.9% w/v NaCl is suitable for laboratories testing purposes for pharmaceutical and biotechnological industries.

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