

Preservation of Thermophilic Bacterial Spores Using Filter Paper Disc Techniques

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Abstract

Any microbial process requires a reliable source of culture stock that is reproducible, viable and taxonomically determined and also preserved without changes in genetic and phenotypic traits.

Here we have described most effective and reproducible method for preservation of bacterial spores on filter disc at -20°C, 4°C and RT for use in laboratory for microbial process.

The filter disc is used as base to support the spores inside the tiny holes. The filter paper discs are dipped into the medium after the spore phase growth of microorganisms. The whatman filter wet discs are dried at 55-60°C till they are completely dry. The viability of discs stored at room temperature and -20°C were checked at 3, 4, 6 and 2 month intervals. This is cost effective technique. In absence of preservative, the viability of the cultures were checked on liquid medium and monitored for the production of the biomolecules such as cellulase. These techniques could be used for other bacterial spore species.

Keywords: Preservation; Drying techniques; Thermophilic bacteria; *Streptomyces*; Filter disc

Introduction

The role of culture collection is to preserve and maintain microbial cultures without alteration of genetic or phenotypic characteristic of strain as source of microbial cell for various biotechnological processes.

There are various preservation methods described and is used for microbial cultures for short and long term preservation. Method for preservation of fungal culture using modified filter paper technique for long term preservation was described by Fong et al. [1].

Some preservation methods have limitations such as time consuming, cost effective and genetic and physiological variations during long term maintenance [2].

Increased interest in culturable diversity has made microbiologists to think seriously about microbial preservation [3]. Microbes are the backbone of modern biotech industries and are utilized for generation of bioenergy and biofuels [4,5] and they are natural source of novel therapeutics.

Cultivation and characterization of microorganisms alone is not adequate without preservation techniques that do not alter the morphology, physiology or genetics of pure strains. Careful preservation is important for future research, teaching and industrial applications [6,7]. Current paper highlights the new preservation techniques for bacterial spore, using filter disc techniques which showed the reported biomolecules like cellulase produced after preservation for more than one year.

To overcome these limitations other methods of storages have been developed to preserve bacterial and fungal strain. There are various methods of preservation of the bacterial cultures described. These are preservation through glycerol stock, sub-culturing of the vials or slants, lyophilization, cryopreservation, preservation in liquid nitrogen etc. Here author prepared simple, low cost technique for preservation of spore sample for long term preservation. The existing techniques need preservatives. The preservation of cultures for long and short term storage use glycerol stock, lyophilization, liquid nitrogen, etc.

The developed techniques used only dried filter paper disc and stored at room temperature, 4°C and -20°C. The storage conditions showed viability of stocks and further long term viability check is in progress.

Materials and Methods

Bacterial growth medium such as glucose, yeast extract, peptone, were purchased from SRL laboratory, whatman filter paper was purchased from Millipore.

Bacterial growth and harvesting

The glycerol spore suspension was inoculated into the glucose yeast extract and peptone medium pH 8.0. The flask was incubated at 55°C for 130 rpm, 20-22 hrs. The mycelium was turned into the sporulation phase observed under the microscope. The filter discs were added into the medium for soaking and incubated further 2-3 hrs. The remaining supernatant was discarded and then complete drying of wet filter paper disc was carried out for 2-3 days at 55°C.

The dried filter discs were added into the screw cap bottle and incubated at R.T, 4°C and -20°C. The filter disc containing bottles were kept at respective temperatures to check viability.

Simple preparation of filter discs

The whatman filter paper punched using the punching machine the uniform 6 mm in diameter. The filter discs are presterilized into the screw cap bottles and used for the drying of the spore suspension of broth.

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Growth curve and harvesting

Filter discs were added at particular spore formation phase and incubated further for 24 hrs and extracted and dried at 55°C. The culture was observed for its viability in terms of turbidity and microscopic examination.

Drying of the filter discs

Photograph of dried filter disc containing bacterial spore is preserved in form are shown in Figures 1 and 2. The dried filter disc is ready for inoculation for the generation of seed culture. The viability of the spore stock has been checked in liquid medium.

Whatman filter (3 mm in diameter) punched using punching machine and autoclaved in screw cap bottle for 20 min at 121°C. After autoclaving the filter paper disc is soaked soaked in the spore containing broth and remaining broth is drained out. The soaked filter disc are then dried at 55°C using dry heat. The thermophilic spore specially withstands this temperature. The spore with broth was incubated further for colonization or to adhere into tiny holes. After 24-48 hrs the complete drying of paper disc at 55°C was carried out and stored in sterile screw cap tubes at three different temperature storage was performed as -20°C, 4°C and -20 °C.

Storage and preservation of filter discs

The glass screwed cap bottle containing the pieces of filter paper disc were kept at R.T., 4°C and -20 °C.

Viability evaluation

The viability of the cultures are evaluated by microscopic observation of growth medium after inoculation and plating on appropriate medium as shown in Figures 3a and 3b the supernatant after growth was used to detect enzyme activity.

Results and Discussion

Spore forming microbial cultures were preserved for more than a year and checked for viability. Growth medium was inoculated with 2-3 pieces of filter discs. Growth was monitored by turbidometric and microscopic methods like gram staining to observe the growth turbidity



Figure 1: Paper disc of preserved spore stock of culture 'A'.



Figure 2: Paper disc of preserved stock of two cultures A and B.

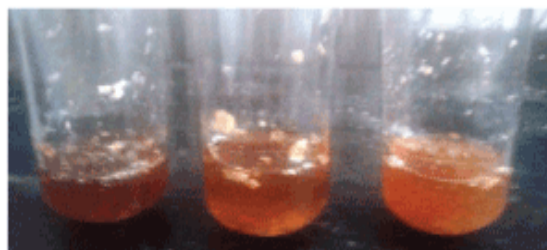


Figure 3A: Viability test of preserved dried filter disc in liquid medium viability of the dried filter disc spore stock preserved 1-A, 2-B, 3-C in liquid medium.

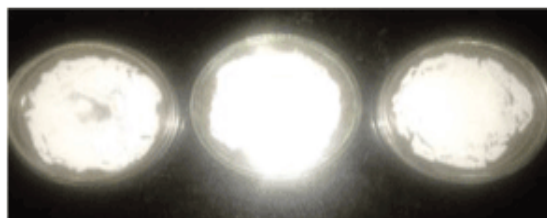


Figure 3B: Viability of the culture broth plated on the medium for growth, 1-A, 2-B, 3-C cultures on plates.

and microscopic, Gram staining was performed to observe the growth. The crude enzyme was extracted from supernatant broth for the enzyme activity and stability.

There are many preservation techniques described, such as cryopreservation of microorganisms [8,9]. Repeated transfer of culture to respective medium or slant (sub-culturing), preservation on agar beads [10], use of silica gel and other sterile supports [11,12], cryopreservation [13] and lyophilization [14]. During cryopreservation, cryovials can be stored immersed in liquid nitrogen (at 196°C) or in its vapour phase (-135 to -150°C). Among these, cryopreservation and lyophilization have been used by various researchers. Among these techniques this paper disc method does not use cryopreservative or lyophilizer cryopreservative and lyophilizer etc. therefore this method is reliable and cost effective technique for preserving spore forming microorganisms. This method is reliable and cost effective technique to preserve the spore forming microorganisms.

This method offers scope for modification of the preservation techniques for respective different species according to their physiological parameter for the growth cycle of microorganism. Here we have developed Method for the preservation of spores of spores of thermophilic *Streptomyces sp.*

Compared to existing techniques, this technique is cost effective and reliable in terms of viability of bacterial cultures, as we have observed viability after one and half years of storage. The viability test is still ongoing at room temperature, 4°C and -20°C

Conclusion

The bacterial spores are preserved using filter disc technique and viability evaluation is carried out.

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