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Article

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Experimental comparison: Methods for the preservation of fungal cultures

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Abstract

Fungal preservation is of utmost importance both with respect to commercial as well as research purpose; however its long term maintenance is troublesome. Frequent sub-culturing and re-stocking of the preserved culture often result in mutations. Most preservation techniques like cryopreservation, lyophilisation and silica gel are widely used but are researcher unfriendly for regular recovery and longstanding research. In this study different fungal species were preserved using the slant method, stab method and glycerol-slice method to elucidate the most effective technique feasible for both regular use and long term fugal preservation. Dataset revealed that stoage in glycerol-slice method lasts longer than the other methods and is recommended for laboratories where specialized conservation facilities are unavailable.

Key words – Cryopreservation – culture maintenance – fungal slants – fungal stabs – glycerol slice

Introduction

Microbes are the foundation of modern biotechnology based industries and are exploited for the generation of bio-energy and bio-fuels (Stahl & Wagner 2006, Bhardwaj & Garg 2012, Prakash et al. 2013). They are an innate source of novel medicines, also used as biopesticides, biofertilizers, and for maintaining the sustainability of the environment (Zaidi et al. 2009, Hayat et al. 2010). Along with the isolation and cultivation of pure strains, suitable preservation facilities for conserving their morphological, physiological and genetic traits are of utmost priority (Prakash et al. 2013). The need to store cultures is essentially required and the solution of most optimal preservation technology is the necessity of the present era that is convenient, efficient and affordable for technological advancement. The presently practiced processes are comprehensive, need expertise, resources, time and money that can be saved by carefully weighing the real purposes behind preserving cultures with the available financial and physical resources for any specific storage technique. The requirement of space, equipments, record-keeping and revival is much lower in case of researchers/scientists maintaining a small number of cultures for current research; teaching laboratories maintaining small archival collections at institution level and/or general service culture collections for active acquiring, storage and distribution of large numbers of cultures (Lacey 1997).

The task of preserving this vast number of fungi having heterogeneous nature and diverse

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growing habits is tedious. Freeze-drying (lyophilization) is used as one of the preservation method however, it cannot be universally applied to all the fungi as the mycelium does not survive the process of lyophilization and also the spores of certain strains are lethally damaged by this process. Preservation of specimens in liquid nitrogen maintains the original properties of the organism and is relatively simpler. It is, nowadays, an ideal system for preserving living cultures (Hwang 1966, Mueller et al. 2004).

However, it is necessary to triumph over the limitations of long term preservation of fungus cultures for routine captivity and researcher friendly (Paul et al. 2015). In the current study, an attempt was made to develop an effective system for long-term preservation of fungi which would meet the needs of researchers globally. The present study aims to investigate and deliver a methodology for preservation of fungus for a long duration to solve the problems that arrive with the routine maintenance of the culture.

Materials & Methods

Sample collection and isolation of different fungi

Various fruit samples (peel and pulp discarded as waste) were acquired from the fruit juice shop at Banasthali Vidyapith, Banasthali, Rajasthan during the year 2018-19. Samples were collected and transported to the laboratory in sterile zip-lock poly bags and further analyzed. Isolation of the fungi from samples was carried out on potato dextrose agar (PDA) plates. Samples were kept at three equi-distance position and the plates were incubated for 7 days at 29±2 °C. The media was supplemented with kanamycin to prevent bacterial growth. The isolated fungus was cautiously sub cultured on separate PDA plates and slants (Alexopoulos 1952). The fungal cultures were examined culturally and microscopically by Lactophenol cotton blue (LPCB) mount followed by identification (Fig. 1).

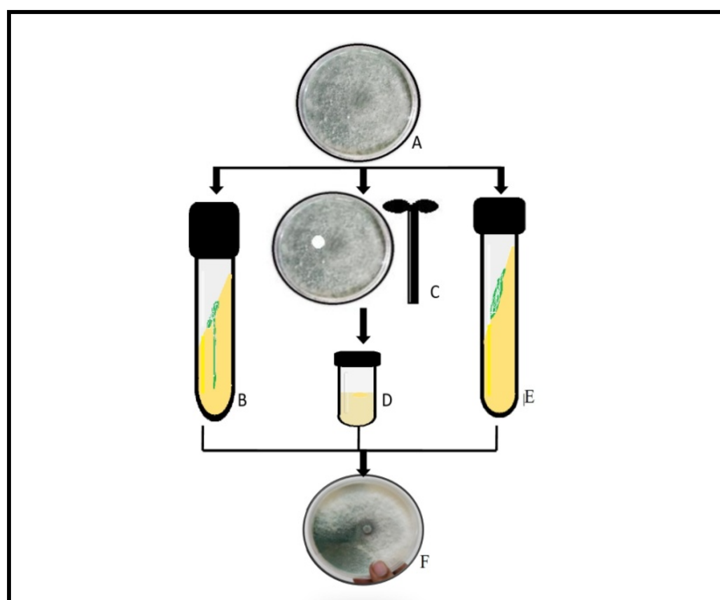

Fig. 1 – Pictorial representation of different methods used for the fungal culture maintenance A 7 days old fungal culture. B Agar stab method. C Fungal culture with corkborer. D Glycerol slice method. E Agar slant method. F Revival of fungal culture.

Preservation of isolated fungi

Preservation of the isolated samples was done in three alternative approaches to get the most efficient way of preserving the fungal cultures for a long duration. First approach involves the preparation of slants with PDA and kept at 4°C (Sasi et al. 2010, Mukunda et al. 2012). Second approach involves stabbing of fungal culture in 1ml PDA in eppendorf and overlaying with

glycerol and keeping them at -20°C. Thirdly, glycerol stock were prepared by placing fungal disc of equal diameters cut from the secondary cultured plates using a sterile cork borer in 50% sterile glycerol. These eppendorf were tagged and kept at -20°C until recovery (Fig. 1).

Revival and comparative microbial analysis

After 6 months, freshly prepared PDA plates were re-inoculated with the fungal disc preserved in glycerol and the slants. These plates were incubated for 7 days at 29±2°C. The fungal isolates were identified and analysed, based on cultural/macroscopic and microscopic characteristics using LPCB mount method (Sasi et al. 2010, Mukunda et al. 2012).

Results

Data indicated that during primary screening *Fusarium* sp., *Trichoderma* sp., *Cladosporium* sp. and *Penicillium* sp. fungi were recorded from different fruit samples, followed by the identification by LPCB mount based on morphological and microbiological characteristics (Fig. 2A-D).

Out of the three methods used as revival from slants, stabs, and glycerol slice, the percent survival was significantly optimal with glycerol slice method. This method indicated clear and pure growth of the test fungi, whereas in slants and stabs the media was found to be exhausted and needed to be sub-cultured before retrieval and pure culture isolation respectively. In some cases the plates were encountered with non-specific growth that was not detected in case of glycerol slice method.

The slants need to be refreshed after short regular interval of times due to depletion of media. On the other hand, repeated opening the slant increases the chances of contamination. This method is feasible for limited number of isolates for a short span of time (Fig. 2E).

The stab prepared in eppendorf requires limited space but each stab is consumed at a time of use hence several sets have to be prepared each time. Since these stabs are kept at a very low temperature, they do not survive for a very long time due to quick media exhaustion. Their timely revival is another challenge that was often encountered (Fig. 2F).

For obtaining active culture, single fungal slice can be used for its revival. This method turned out to be user-friendly and is recommended for various laboratories for successful storage and maintenance of fungal cultures (Fig. 2G). Resumption of growth was observed without loss of morphological characteristics from thawed specimens of all the test organisms.

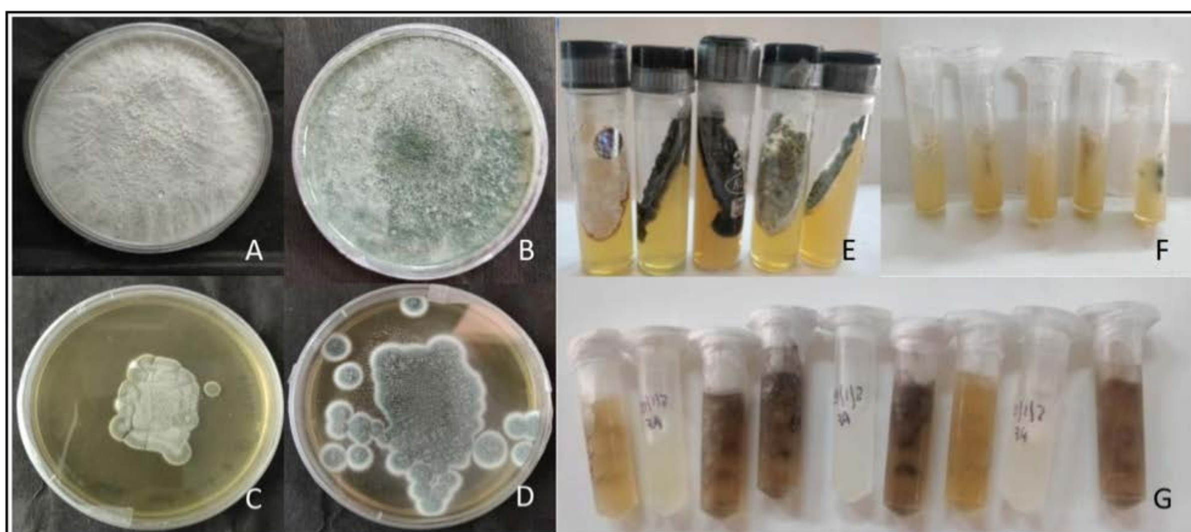

Fig. 2 – Photoplate showing different methods used for the fungal culture maintenance A *Fusarium* sp. B *Trichoderma* sp. C *Cladosporium* sp. D *Penicillium* sp. E Maintenance by Agar slant method. F Maintenance by Agar stab method. G Maintenance by Glycerol slice method.

Discussion

Present investigation suggests various methods for culture maintenance and preservation. Out of the methods used glycerol slice method revealed best results. Similar observation were suggested by Smith & Thomas (1997) and Lalaymia et al. (2014) for using glycerol that acts as a cryoprotectant. Another study suggested observations of isolated fungal strains frozen and revived justifies that neither freezing nor ultra-low temperature preservation results in apparent alteration of the cultures subjected to the treatment in presence of a cryoprotectant (Espinel-Ingroff et al. 2004).

On the contrary, Iqbal et al. (2017) reported that slants are the most frequently used way of preserving microbial isolates as they are simple and inexpensive but time consuming and labor-intensive at the same time. Similarly Lopez-Martinez et al. (1999) demonstrated that however not all the isolates conserve their typical morphology by PDA slants method. The glycerol effendorf disc/slice is also consumed one at a time hence several copies need to be made but they need limited space and remain same for a longer period of time without getting altered, morphologically as well as physiologically.

Conclusion

Preservation and maintenance of fungal species has been going on for decades and scientist are still working for more researcher friendly, cost-effective and long lasting techniques both for research as well as educational purpose. Multiple techniques are used for this purpose but the present work for the first time comparison between most frequently used techniques and glycerol-slice method, in which glycerol acts as a cryoprotectant. One cork-borer slice solves the contamination problem that is caused due to multiple usage of same slant. Storage at -20ºC increases the longevity of the process. It solves the majot issues of economy, need for revival of the stored samples; is user-friendly on a routine basis and also occupies less space.

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Conflict of interest

Disclosure of potential conflicts of interest indicates that there is no conflict of interest during the study conduct in any of the authors. The study is conducted in compliance with Ethical Standards.

References

Alexopoulos CJ. 1952 – Introductory Mycology. John Wiley & Sons, New York, 1-613, ISBN-100471022136.

Bhardwaj V, Garg N. 2012 – Importance of exploration of microbial biodiversity. ISCA Journal of Biological Sciences 1, 78–83.

Espinel-Ingroff A, Montero D, Martin-Mazuelos E. 2004 – Long Term Preservation of Fungal Isolates In Commercially Prepared Cryogenic Microbank Vials. Journal of Clinical Microbiology 42, 1257–1259.

Hayat R, Ali S, Amara U, Khalid R, Ahmed I. 2010 – Soil beneficial bacteria and their role in plant growth promotion: A Review. Annals of Microbiology 60, 579–598.

Hwang SW. 1966 – Long-term preservation of fungal cultures with liquid nitrogen refrigeration. Applied Microbiology 14, 784–788.

Iqbal S, Ashfaq M, Malik AH, Inam-ul-haq, Khan KS, Mathews P. 2017 – Isolation, preservation and revival of Trichoderma Viride in culture media. Journal of Entomology and Zoology Studies 5, 1640–1646.

Lacey LA. 1997 – Fungi: Preservation of cultures. Manual of techniques in insect pathology. Elsevier Academic Press, Kent, UK, 153–185, ISBN 0-12-4325556,

Lalaymia I, Cranenbrouck S, Declerck S. 2014 – Maintenance and preservation of ectomycorrhizal and arbuscular mycorrhizal fungi. Mycorrhiza 24, 323–337.

Lopez-Martinez R, Hernandez-Hernandez F, Bazan-Moraand E, Castanon-Olivares LR. 1999 – Comparative study of two culture conservation methods in medical mycology. World Journal of Microbiology and Biotechnology 15, 471–474

Mueller G, Bills G, Foster M. 2004 – Biodiversity of fungi Inventory and monitoring methods. Elsevier Academic Press, Burlington, MA, 37–47, ISBN 0125095511.

Mukunda S, Onkarappa R, Prashith KTR. 2012 – Isolation and screening of industrially important fungi from the soils of Western ghats of Agumbe and Koppa, Karnataka, India. Science, Technology and Arts Research Journal 1, 27–32.

Paul JS, Tiwari KL, Jadhav SK. 2015 – Long Term Preservation of commercial Important Fungi in Glycerol at 4ºC. International Journal of Biological Chemistry 9, 79–85.

Prakash O, Nimonkar Y, Shouche YS. 2013 – Practice and prospects of microbial preservation. FEMS Microbiology Letters 339, 1–9.

Sasi A, Kani M, Panneerselvam A, Jegadeesh G et al. 2010 – Optimizing the conditions of amylase by an Esturian strain of Aspergillus spp. African Journal of Microbiology Research 4, 581–586.

Smith D, Thomas VE. 1997 – Cryogenic light microscopy and the development of cooling photocols for the cryopreservation of filamentous fungi. World Journal of Microbiology and Biotechnology 14, 49–57

Stahl DA, Wagner M. 2006 – The knowledge explosion in environmental microbiology offers new opportunities in biotechnology. Current Opinion in Biotechnology 17, 227–228.

Zaidi A, Khan MS, Ahemad M, Oves M. 2009 – Plant growth promotion by phosphate solubilizing bacteria. Acta Microbiologica et Immunologica Hungarica 56, 263–284.