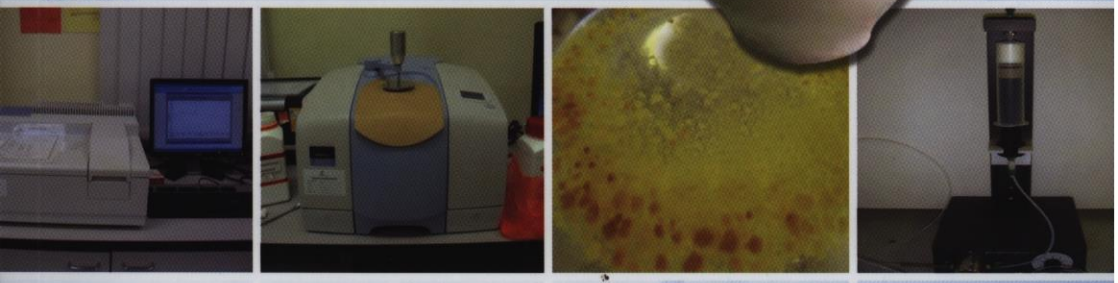
EXPERIMENTAL METHODS in MODERN BIOTECHNOLOGY

Volume 2



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Gombak • 2016

First Edition, 2016

IIUM Press is a member of the Majlis Penerbitan Ilmiah Malaysia – MAPIM
(Malaysian Scholarly Publishing Council)

Perpustakaan Negara Malaysia Cataloguing-in-Publication Data

Parveen Jamal
Experimental Methods in Modern Biotechnology.
Volume 2 / Prof. Dr. Parveen Jamal.
ISBN 978-967-418-384-4
1. Biotechnology. I. Title.
660.6

Published by:
IIUM Press
International Islamic University Malaysia
P.O. Box 10, 50728 Kuala Lumpur, Malaysia
Tel: +603-6196 5014; Fax: +603-6196 4862/6298

Printed in Malaysia by:
REKA CETAK SDN. BHD.
12 & 14, Jalan Jemuju Empat 16/13D
Seksyen 16
40200 Shah Alam, Selangor, Malaysia

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Chapter 12

Bacterial Preservation for Short and Long-term Storage

Raha Ahmad Raus

Introduction

Each lab that uses bacteria for their studies will accumulate numerous bacterial isolates, mutants, genetically engineered variants and purchased bacteria from commercial companies. Without proper preservation of these cultures, future studies could not be carried out and money will be wasted on purchasing the new bacterial strains. Thus, appropriate preservation methods should be adopted by the lab personnel to ensure viability and genetic stability when the preserved cultures are revisited.

In general, the bacterial cultures could be preserved at low temperature. Consistently, it has been shown that the viable storage period of bacteria increases as the storage temperature decreases (Mazur, 1977). However, preservation at temperature below the freezing point requires cryoprotectants as they reduce cell damage caused by the freezing process (Meryman, 1971). Cell damage and cell death are inevitable during storage and it should be minimised as much as possible. Fortunately, most bacterial strains will remain viable in a given storage condition except for a few strains. For bacterial cultures that are used daily or weekly, it is better to plate it on the agar plates or store it as stab cultures in normal refrigerator at 4°C (short term storage). If cultures are not intended to be used for more than a few weeks, more long-term storage methods should be considered for maximum bacterial viability (Table 1).

Table 1. Approximate time bacterial cultures remain viable in different storage conditions

Method	Temperature (°C)	Time
Agar plates	4	4 weeks
Stab cultures	4	3 weeks-1 year
Standard freezer	-20	1-2 years
Super-cooled freezer	-80	1-4 years
Freeze dried	≤4	15 years+

In this chapter, all the methods in Table 1 will be elaborated except for freeze dried method. Agar plates and stab cultures are categorised under short-term storage and; standard freezer and super-cooled freezer are categorised under long-term storage.

Short-term Storage

There is a need to keep bacteria at 4°C although it can survive for a short period. For cultures that are intensively used in a particular short period of time, it is common to use agar plate method where bacteria cultures are streaked on agar and kept at 4°C. This method is preferred over stab culture because if there are mix culture colonies and contaminants on the plate, they can be easily identified and visualised easily. The most common contaminant is *Bacillus sp.* and it is observed as spreading, dull, milky of chalky-opaque colonies with irregular shape. If the plate is heavily contaminated and difficult to pick area or colonies of desired bacterial strain, it should be discarded. However, if there is a small area or colonies of interest that are not disturbed by other bacterial strains or contaminants, it could be transferred to a fresh plate to grow.

The advantage of having stab culture is that the agar is prepared in a tube with a screw cap. This prevents the agar from drying out as what commonly happened to agar plates. For the latter, a parafilm can be used to seal the plate to reduce the drying of agar.