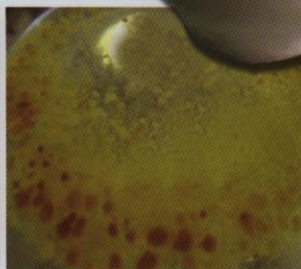


EXPERIMENTAL METHODS in MODERN BIOTECHNOLOGY

Volume 2



Editors

Parveen Jamal

Ibrahim Ali Noorbacha

Azlin Suhaida Azmi



IIUM
Press

IIUM Press Series of Textbook from the Islamic Perspectives

The IIUM Press Series of Textbook from the Islamic Perspectives provide students with the required scientific basics of Islamic perspective in the context of every subject. The series also enable lecturers and students to explore the core principles of knowledge, to apply these throughout their training, and to foster critical thinking at all times. Titles published in the series are fully peer reviewed, to ensure the completeness and clarity of the content, and also are inline with the global and Islamic perspective. Some of the titles published in this series are currently used as textbooks at public universities in the country. The prestigious series provide students with both scientific and Islamic information and appropriate content. It is hoped that each title published in the series will be an ultimate student's companion.

Science, Technology & Medicine

- Akram M. Z. M. Khedher. 2015. *Internet Applications*. Gombak: IIUM Press. ISBN 978-967-418-313-4.
- Akram M. Z. M. Khedher. 2015. *Multimedia and Its Applications*. Gombak: IIUM Press. ISBN 978-967-418-312-7.
- Faiz Elfaki and Zaharah Wahid. 2015. *Computational Methods and Statistics*. Gombak: IIUM Press. ISBN 978-967-418-318-9.
- Kausar Ahmad. 2015. *Manufacturing of Halal Pharmaceuticals*. Gombak: IIUM Press. ISBN 978-967-418-316-5.
- Lukman Hakim Mahamod and Azila Ahmad Sarkawi. 2015. *Islamic Urbanism*. Gombak: IIUM Press. ISBN 978-967-418-302-8.
- Naznin Muhammad. 2014. *Multiple True or False Questions in Haematology and Pathology for Health Sciences Undergraduates*. Gombak: IIUM Press. ISBN 978-967-418-310-3.
- Noor Hasrina Bakar, Noor Azimah Hassan, Mohd Norhaedir Idris and Mahmoud Al-Shawabkeh. 2014. *Programming in C for Foundation*. Gombak: IIUM Press. ISBN 978-967-418-294-6.

EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY

Volume 2

Editors

Parveen Jamal

Ibrahim Ali Noorbatchesa

Azlin Suhaida Azmi



**IIUM
Press**

Gombak • 2016

First Edition, 2016
©IIUM Press, IIUM

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

All rights reserved. No part of this publication may be reproduced,
stored in a retrieval system, or transmitted, in any form or by any means,
electronic, mechanical, photocopying, recording, or otherwise,
without any prior written permission of the publisher.

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

Contents

<i>Preface</i>	ix
Chapter 1: Simple Cell Attachment and Viability Assay for Screening Anticancer Properties of Natural Products <i>Yumi Zuhanis Has-Yun Hashim</i> <i>Chris Gill</i> 3774 / 50933	1
Chapter 2: A Simple and Easy Method for Preparing Solid and Liquid Media for Plant Culture <i>Noor Illi Mohamad Puad</i> <i>Tang Chi Wai</i>	9
Chapter 3: Initiation of Plant Cell Suspension Cultures from Seeds <i>Noor Illi Mohamad Puad</i> <i>Ferda Mavituna</i>	16
Chapter 4: Extraction and Isolation of Xanthine Oxidase Inhibitors from Carica Papaya Leave Extract using Chromatographic Techniques <i>Parveen Jamal</i> <i>Saiful Mohammad Nizam Azmi</i> <i>Azura Amid</i>	25
Chapter 5: Application of HPTLC and HPLC for Purification of Bioactive Compounds from Plant Extract for Gout Remedy <i>Parveen Jamal</i> <i>Saiful Mohammad Nizam Azmi</i> <i>Azura Amid</i>	36
Chapter 6: Determination and Quantification of Ethanol and Byproducts using HPLC (Brand: WATERS) <i>Azlin Suhaida Azmi</i>	47

Chapter 7: Enzymology Method to Determine the Anti-Inflammatory Potentials: Hyaluronidase Inhibitory Activity Bioassay	53
<hr/>	
<i>Nor Hayati Abdullah</i> <i>Ibrahim Ali Noorbatcha</i>	
Chapter 8: Bromelain Enzyme Assay using Casein as Substrate	62
<i>Azura Amid</i> <i>Mohd Jamil Aizat Jamaluddin</i>	
Chapter 9: Isolation and Characterisation of Thermophilic Bacterial Isolates Producing L-asparaginase	72
<i>Dzun Noraini Jimat</i> <i>Intan Baizura Firda Mohammed</i>	
Chapter 10: Estimation of Fungal Biomass in Bioprocess Engineering Experiments	81
<i>Mohamed Ismail Abdul Karim</i>	
Chapter 11: Extraction of Oil from Waste Source	94
<i>Sarina Sulaiman</i>	
Chapter 12: Bacterial Preservation for Short and Long-Term Storage	102
<i>Raha Ahmad Raus</i>	
 <i>Index</i>	 111

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.