# EXPERIMENTAL METHODS IN MODERN BIOTECHNOLOGY


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## Preface

The rapid growth of biotechnology as the interdisciplinary field involving scientists and engineers has given rise to new challenges in carrying out experiments in diverse areas ranging from examining the biological systems to the purification and production of products of commercial importance. The idea of writing a book on Modern Experimental Methods in Biotechnology in such a diverse area is a very daunting task. However, there is a need for such a reference book for senior undergraduate students or beginning researchers in biotechnology, providing an integrated view of the experimental techniques along with the underlying principles, exemplified with case studies or sample results, and pointing out the advantages and limitations of the methods. A modest beginning towards this direction has been attempted in this book by combining the expertise and the experience of the researchers at the Department of Biotechnology Engineering, International Islamic University Malaysia and their collaborators who have been doing or teaching these experiments for the past one decade. For example, a selected set of experiments ranging from straight forward liquid-liquid separation process to the more latest direct nucleation control approach for the control of crystal size distribution in crystallization processes have been described in detail. In addition to extraction and purification of some selected compounds, a set of experiments to screen for metabolic disorders and anti-cancer activities are described with suitable examples. Any biotechnology experimental methods books will be incomplete without the inclusion enzyme assay methods, which has been described in this book as a set of three simple experiments. A flavor for the use of computers in biotechnology is provided in a chapter on homology modeling. Even though the list of experiments included in this book is not exhaustive, the reader will find that the experiments covered are fully self contained with good explanation of the theory behind the experiments and details about how to carry out these experiments. A more exhaustive coverage of a wide variety of other experiments in biotechnology will be included in the later editions.

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CHAPTER 14

# Indicator Microorganisms – Detection of Coliform and *Escherichia Coli*

**Mohamed Ismail Abdul Karim**

## 1. Introduction

Sometime ago in 1885, a German pediatrician known as Theodor Escherich (Escherich, 1885; Neill *et al.*, 1994) identified a bacteria *Escherichia coli* which belong to the original bacteria group known as *Bacterium coli* which was known to be widely distributed in the intestine of many warm-blooded animals and in humans too. This was the predominant facultative anaerobic bacteria found in the bowel and intestinal flora of many healthy animal host (Conway, 1995; Neill *et al.*, 1994) .The bacteria *E. coli* belong to the family of *Enterobacteriaceae* (Ewing, 1986) which include many microbial genera, including the pathogens such as *Yersinia, Shigella* and *Salmonella*. Intestinal contamination of fecal origin is important and the presence of pathogen is responsible for intestinal infections, such as bacillary dysentery, typhoid fever, cholera and paratyphoid dever. Generally many strains of *E. coli* are not pathogens but their presence may be conducive for infectious pathogens to breed and invade the hosts. Pathogenic strains of *E. coli* when ingested, can cause gastrointestinal illness and disorders and may pose a serious threat to healthy humans and babies in general. Their presence can be a nuisance and pose a danger when come into contact on humans and other hosts animals.

Recently *E.coli* is often known to be as an indicator of fecal contamination whereby it is found abundant in human and animal feces and not found in other sources. *E. coli* could also be easily detected by its ability to ferment glucose and lactose and isolated as gastrointestinal pathogens. Their presence in food or water can be accepted as an indicator of recent fecal contamination and likelihood for pathogens to exist and grow. Besides, *E. coli* there are the presence of other enteric bacteria like *Citrobacter, Klebsiella* and *Enterobacter* which can also ferment lactose and are similar to *E. coli* in phenotypic characteristics and are not easily distinguished from *E.coli*. As a result, the term "coliform" was coined to describe this group of enteric bacteria as a group of Gram-negative, facultative anaerobic rod-shaped bacteria that can ferment lactose to produce acid and gas within 48 h at a temperature of 35°C. The U.S. Public Health Service then adopted the enumeration of coliforms as a more convenient standard of sanitary health significance where food found contaminated are implicated in it.

However, since some coliforms are found in the natural environment (Caplenas and Kanarek, 1984) the fecal coliforms usually *E. coli* is often associated with fecal contamination and often designated as an indicator of contamination indicating unsanitary condition might have occurred. Through the work of Eijkman (Eijkman, 1904) it is known the fecal coliforms

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