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DETERMINATION OF OPTIMAL GROWTH PHASE AND INOCULUM SIZE OF *Escherichia coli* (ATCC 25922) FOR LONG TERM STORAGE (STOCK CULTURE)

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DECLARATION

I declare that the work in this thesis/dissertation was carried out in accordance with the regulations of Universiti Teknologi MARA. It is original and is the result of my own work, unless otherwise indicated or acknowledged as referenced work. This thesis has not been submitted to any other academic institution or non-academic institution for any degree or qualification.

I, hereby, acknowledge that I have been supplied with the Academic Rules and Regulations of Universiti Teknologi MARA, regulating the conduct of my study and research.

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TABLE OF CONTENTS

ABSTRACT

Bacterial stock culture for long term storage is necessary for research, diagnostics, teaching and learning purpose. Previously, the Centre of Medical Laboratory Technology, UiTM Puncak Alam was annually purchasing bacterial stock from the American Type Culture Collection (ATCC) which is costly. It was also a problem to maintain a pure stock culture for long term storage. Hence, this study aims to prepare our own bacterial stock culture and provide a standardize method to maintain a pure stock culture for long term storage. This will be achieved by determining the ideal growth phase to harvest cells for stock culture and the optimal inoculum size of *Escherichia coli* (ATCC 25922) required to maintain a long-term storage of pure stock culture. *E. coli* (ATCC 25922) is a gram-negative and commonly isolated in gastroenteritis, urinary tract infections (UTI) and wound infections. The initial stock of *E. coli* (ATCC 25922) was obtained from the stock culture in Microbiology Laboratory, Centre of Medical Laboratory Technology, UiTM Puncak Alam. *E. coli* (ATCC 25922) appear as large and shiny on 5% sheep blood agar, lactose fermenter on MacConkey agar and give positive reaction on indole, motility and methyl red biochemical tests incubated at 37^{o}C. Absorbance (OD 600nm) and colony forming unit (CFU/ml) counts are plotted against incubation time (hours) to analyze the growth curve of *E. coli* (ATCC 25922) in trypticase soy broth (TSB) incubated at 35^{o}C. The bacterial cells were harvested at mid exponential phase at OD 600nm 2.151 with optimal inoculum size between 56.7 x 10^{8} (CFU/ml) to 93.7 x 10^{8} (CFU/ml). Stock cultures were stored in 20% glycerol (4^{o}C, -20^{o}C and -80^{o}C) and microbeads (-20^{o}C and -80^{o}C) and recovered after 1 month storage. To check the purity and viability, recovered organisms were cultured on 5% sheep blood agar and MacConkey agar and biochemical tests were performed. Based on the results, all stock cultures were successfully recovered with pure growth of *E. coli* (ATCC 25922) without any contamination. From this study, it can be concluded that long term storage of bacterial stock culture can be achieved by determining the ideal growth phase of bacteria (exponential phase) and optimal inoculum size of bacteria to harvest cells for stock culture in 20% glycerol (4^{o}C, -20^{o}C and -80^{o}C) and microbeads (-20^{o}C and -80^{o}C). This method can be practiced by laboratory staff and students to perform a pure stock culture of *Escherichia coli* (ATCC 25922) in Microbiology Laboratory, Centre of Medical Laboratory Technology, UiTM Puncak Alam.