UNIVERSITI TEKNOLOGI MARA

DETERMINATION OF OPTIMAL GROWTH PHASE AND INOCULUM SIZE OF *PSEUDOMONAS AERUGINOSA* (ATCC 10145) FOR LONG TERM STORAGE (STOCK CULTURE)

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Thesis is submitted in partial fulfillment of the requirements for the degree of

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AUTHOR'S DECLARATION

I declare that the work in this thesis or dissertation was conducted in accordance with the regulations of University Teknologi MARA (UiTM). It is original and is the result of my own work, unless otherwise indicated for acknowledged as referenced work. This thesis or dissertation 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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ABSTRACT

Pseudomonas aeruginosa is an aerobic Gram negative bacilli that is widely used for research and learning purposes because of its unique characteristics in presenting various significance virulence factors that contribute to broad antimicrobial resistance and causing severe infections in human includes cystic fibrosis, urinary tract infection and dermatitis.. The Microbiology Laboratory in Center of Medical Laboratory Technology, Universiti Teknologi MARA Puncak Alam Campus faces problem in maintaining and storage of pure stock culture due to required high cost by annual repurchasing of American Type Culture Collection (ATCC) strain stock culture from United State of America (USA). This study was conducted to determine the optimal growth condition and inoculum enumeration for long – term storage stock culture. The methodology was initiated with confirmation testing of *P. aeruginosa* ATCC 10145 by performing Gram stain, and biochemical tests including triple sugar iron (TSI), citrate, motility and oxidase. Next, determination of growth curve by turbidimetric assay in trypticase soy broth (TSB) at OD 600 nm and colony counts (CFU/mL) on tryptic soy agar (TSA) plate. The growth curve of *P. aeruginosa* ATCC 10145 was plotted to determine the exponential phase that used for maintain cell viability. Then, the stock culture was prepared in 20% glycerol and Cryobeads followed by storage at 4°C for glycerol stock while in freezer at – 20°C and – 80°C for both glycerol and Cryobeads for one month. The ability to recover after the storage period was checked by performing Gram stain, cultivation on 5% sheep blood agar and MacConkey agar, and biochemical tests on the recovered organism after 1 month storage. The initial stock culture of *P.aeruginosa* ATCC 10145 was a Gram negative rod, the colony on 5% sheep blood agar was greyish, large and ß- hemolytic activity and non – lactose fermenter on MacConkey as the colonies were clean. The biochemical results for *P. aeruginosa* ATCC 10145 were positive for citrate, motility and oxidase while for TSI was alkaline in slant and butt with no gas production (K/K/-). The exponential phase for *P. aeruginosa* ATCC 10145 was achieved after 3 hours incubation at 35°C in TSB with OD 600nm was 1.423 and corresponding with colony count, 2.39 x 10^8 CFU/mL which was harvested for storage. The pure stock culture of *P. aeruginosa* ATCC 10145 was successfully recovered after 1 month storage. In conclusion, stock pure culture of *P. Aeruginosa* ATCC 10145 was prepared for long term storage by determination of optimal growth phase in TSB at 35°C and inoculum size by colony count in TSA.

Keywords: *Pseudomonas aeruginosa*, ATCC 10145, storage, Cryobeads, glycerol