UNIVERSITI TEKNOLOGI MARA

DETERMINATION OF OPTIMAL GROWTH PHASE AND INOCULUM SIZE OF *Proteus vulgaris* (ATCC 6380) FOR LONG TERM STORAGE (STOCK CULTURE)

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DECLARATION

I declare that the work in this thesis/dissertion 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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ABSTRACT

Pure culture is essential to study about bacterial strain's characteristics to diagnose disease associated with *Proteus vulgaris* infection such as nosocomial and human urinary tract infection. The purity status of bacteria is problematic in the Microbiology laboratory as it is often contaminated. Every year, the Centre of Medical Laboratory Technology in Universiti Teknologi MARA Puncak Alam purchases stock culture of *P. vulgaris* (ATCC 6380) from American Type Cell Culture (ATCC) in the United State which is expensive. The purpose of this study is to prepare pure stock culture of *Proteus vulgaris (*ATCC 6380) as a preservation method to maintain the viability of microorganisms for long term storage and use. The initial stock of *P. vulgaris* (ATCC 6380) was obtained from available *P. vulgaris* (ATCC 6380) stock in microbiology laboratory at FSK, UiTM Puncak Alam. Subculture of *P. vulgaris* (ATCC 6380) on 5% sheep blood agar and MacConkey agar was performed followed by identification and confirmation test. *P. vulgaris* (ATCC 6380) maintained as non-lactose fermenter, and give positive reaction in indole, motility, methyl red, phenylalanine deaminase and urea reactions. Preparation of stock culture was done through determination of optimal growth phase and inoculum size of *Proteus vulgaris (*ATCC 6380) to harvest cell for storage in glycerol stock and microbeads at 4^0C, -20^0C and -80^0C. Bacterial growth curve of absorbance (OD 600nm) against incubation time (hours) in fresh TSB was performed to harvest cell at exponential phase. Bacterial colony count was performed to determine the suitable inoculum size of *Proteus vulgaris (*ATCC 6380) for storage. Stock culture of *P. vulgaris (*ATCC 6380) was made from cells harvested at OD 600nm of 1.645 with optimum inoculum size of $24.0X10^8$ cfu/ml. The recovery potential of *Proteus vulgaris (*ATCC 6380) was determined by culturing on 5% sheep blood agar plate and performing biochemical tests to confirm the purity of colony. After one-month storage, stock cultures of *P. vulgaris (*ATCC 6380) in glycerol were successfully recovered at all temperature and in microbeads at -80°C. However, *P. vulgaris* from microbeads stored at -20^0C cannot recovered as pure colony due to contamination of *S. aureus.* In conclusion, stock culture at all temperature can be recovered as pure colony except for microbeads at -20^0C due to contamination caused by improper technique during recovery of *P. vulgaris (*ATCC 6380) stock cultures. The method used to perform pure and viable stock culture was effective but contamination being the only problem.