ABSTRACT

ROFIQ SUNARYANTO. Isolation, Purification, Identification, and Fermentation Medium Optimization of Antibiotic Produced by Marine Actinomycetes. Under direction of TUN TEDJA IRAWADI, ZAINAL ALIM MAS'UD, LIESBETINI HARTOTO, BAMBANG MARWOTO.

Isolation and purification of active compounds produced by marine actinomycetes has been carried out. Marine sediment samples were obtained from 3 different places in Banten West Coast, Cirebon North Coast, and Yogyakarta South Coasts. A total of 40 actinomycetes isolates were obtained 4 isolates were active against *Escherichia coli* ATCC 25922, 5 isolates were active against *Staphylococcus aureus* ATCC25923, 4 isolates were active against *Bacillus subtilis* ATCC 66923, 4 isolates were active against *Pseudomonas aeroginosa* ATCC27853, 4 isolates were active against *Candida albican* BIOMCC00122, and 4 isolates were active against *Aspergillus niger* BIOMCC00134. A11 isolate showed the most active to Gram-positive and Gram-negative bacteria. Species identification using 16S rRNA gene sequencing showed that A11 isolate is *Streptomyces* sp.

Elucidation of its molecular formula and structure using LC-MS, ^{1}H NMR, ^{13}C NMR, and ^{13}C DEPT NMR showed the antibiotic was cyclo(tyrosyl-prolyl), molecule formula was $C_{14}H_{16}N_2O_3$ which has a melting point of 140 °C. Minimum Inhibitory Concentration (MIC) of the antibiotic was determined against 4 bacterial test strains, namely *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 25923, and *Bacillus subtilis* ATCC 66923, which were inhibited at 27, 69, 80, and 74 μg mL^{-1}, respectively.

Fermentation profile of *Streptomyces* sp. A11 showed a lag phase which occurred until 8 hours, a log phase from 9 until 48 hours and a stationary phase from 48 until 144 hours. The growth phase showed maximum specific growth rate (μ_{max}) of 0.04 hour^{-1} and the rate of substrate conversion into biomass ($Y_{x/s}$) of 0.6 gram biomass per gram substrate. The optimum temperature and pH of cyclo(tyrosyl-prolyl) fermentation were 30 °C and 6.5-7.5, respectively.

Optimum composition of fermentation medium was determined with three independent variables: dextrin as a carbon source, peptone as nitrogen source, and a mixture of mineral salts using Response Surface Methodology. The results showed that the three variables significantly affected the activity of cyclo(tyrosyl-prolyl). Peptone gave the strongest effect compared to dextrin and mineral salts. Interaction was found between dextrin and peptone. On the contrary, no interaction was observed between peptone and mineral salts, and between dextrin and mineral salts. Using a mathematical model, the most optimum composition of the medium were found to be dextrin (32.55 g L^{-1}), peptone (11.22 g L^{-1}), and mineral salt (8.65 mL), in which 51.54 g L^{-1} cyclo(tyrosyl-prolyl) was produced. Verification of the model in laboratory showed the cyclo(tyrosyl-prolyl) activity to be 50.04 mg L^{-1}. Thus, the difference between the result of the experiment and the expected response value was 2.9%.

Keywords: marine actinomycetes, antibiotic, *Streptomyces*, cyclo(tyrosyl-prolyl).