

## RINGKASAN

Penelitian untuk mendapatkan enzim protease termostabil dari isolat sumber air panas Gedong Songo telah dilakukan. Isolasi bakteri dilakukan dengan metode goresan di medium Nutrient Agar pada temperatur 50 °C selama 24 jam. Adanya enzim protease diuji dengan menggunakan medium gelatin pada temperatur 50 °C selama 24 jam. Isolat bakteri ditumbuhkan di medium Nutrient Agar pada temperatur 50 °C selama 24 jam. Enzim protease diproduksi di medium *Skim Milk Broth* pada temperatur 50 °C selama 6 jam. Isolasi enzim protease ekstraseluler dilakukan dengan metode sentrifuge dan difraksinasi bertingkat menggunakan amonium sulfat dengan tingkat kejenuhan yaitu F1 (0 - 20 %), F2 (20 - 40 %), F3 (40 - 60 %), F4 (60 - 80 %), F5 (80 - 100 %) dan dilanjutkan dialisis dalam buffer fosfat. Parameter yang diamati adalah aktivitas enzim, aktivitas spesifik enzim dan kadar protein. Fraksi yang diperoleh diuji dengan substrat kasein, menggunakan spektrofotometer UV – Vis pada panjang gelombang optimum kasein dan hasilnya diektrapolasikan terhadap kurva standar kasein untuk mendapatkan nilai aktivitas enzim protease. Kadar protein diuji dengan metode *Lowry*. Karakterisasi yang dilakukan meliputi penentuan temperatur, pH, dan waktu inkubasi.

Hasil penelitian menunjukkan bahwa isolat bakteri yang diperoleh dari sumber air panas Gedong Songo memiliki sifat *Gram* positif, bentuk batang dan aerob. Fraksi yang memiliki aktivitas spesifik tertinggi adalah Fraksi 3 (1716,853 Unit/mg protein). Temperatur optimum 50 °C, pH = 7,5, dan waktu inkubasi 20 menit.

## SUMMARY

The isolation of thermostable protease from thermophilic bacteria from Gedong Songo hot spring has been done. Bacteria isolation has been carried out by streak method using nutrient agar medium at the temperature of 50 °C during 24 hours. Screening on the ability of protease production has been done using gelatine medium at the temperature 50 °C during 24 hours. Bacteria isolate was grown on nutrient agar medium. Enzyme was produced on *skim milk broth* medium at the temperature of 50 °C during 6 hours. The extracellular enzyme isolation has been carried out by centrifuge, fractionating phase has been carried out using ammonium sulphate saturated solution in the concentration of F1 (0 - 20 %), F2 (20 - 40 %), F3 (40 - 60 %), F4 (60 - 80 %), F5 (80 - 100 %) followed by dialysis in phosphate buffer. The parameters observed were enzyme activity, specific activity and protein content. Fractions obtained were then analyzed using casein substrate by UV – Vis spectrophotometer in casein optimum wavelength. The result were then extrapolized on casein standar curve to obtain protease enzyme activity value. Protein content were analyzed using *Lowry* method. The enzyme characterization was done by determined the optimum temperature, pH, and incubation time.

Results showed that the thermophilic bacteria isolate from Gedong Songo hot spring was *Gram* positif, rod shape and aerobic. Fraction 3 (1716.853 Unit/mg protein) has highest specific activity, while the optimal temperature was 50 °C, pH = 7.5, and the incubation time was 20 minutes.

