



## **DETERMINATION OF OPTIMAL PENICILLIN CONCENTRATION FOR PROTOPLASTS AND SPHEROPLAST FORMATION**

**Student:** Arief Nugroho

Degree Program In Microbiology, School of Life Sciences and Technology-ITB,

email: nugroho.arief@gmail.com

**Advisors:** Dr. Pingkan Aditiawati

School of Life Sciences and Technology ITB, email: pingkan@sith.itb.ac.id

**Degree:** Science Degree (S.Si), Conferred October 2010

### **ABSTRACT**

The complete removal of the cell wall results generation of protoplasts, while incomplete removal generates spherplast. Cell wall removal provide basic permeabilized system for ultrastructural, genetic, and physiological studies. Peptidoglycan-targeting penicillin is commonly applied in protoplasts formation. The efficacy of penicillin is highly dependent on the bacterial species or strain being used. This research focused on the optimization of penicillin concentration used for protoplasts/spheroplasts formation. The optimization is carried out using bacterial culture cultivated in presence of different penicillin concentration. Cultures used were *Escherichia coli* (6h,  $4.13 \times 10^8$  cells per mL) and *Bacillus megaterium* (6h,  $6.81 \times 10^7$  cells per mL). The treatment was prepared as follows: 2 mL of bacterial culture, 1 mL of distilled water, 2 mL of 2M sucrose and 0.9 mL 0.1M  $MgSO_4 \cdot 7H_2O$  added to 4 mL of double strength NB medium with the addition of 0.1 mL of penicillin treatment concentration of 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL and 35 mg/mL. Treatment performed for 2 hours at a temperature of  $25^{\circ}C$ . Samples from treatment was observed morphologically with and without staining. Gram staining method was used. The results showed the use of penicillin became effective from the concentration of 30 mg/mL for *Bacillus megaterium* and 35 mg/mL for *Escherichia coli*. Number of *E. coli* cell showed reduction with the average value of  $2.7 \times 10^7$  while the number of *B. megaterium* cell showed growth with an average value of  $2.7 \times 10^7$ .

**Key words:** protoplast, spheroplast, cell wall, penicillin

## **PENENTUAN KADAR PENISILIN OPTIMUM UNTUK PEMBENTUKAN PROTOPLAS DAN SFEROPLAS**

**Mahasiswa:** Arief Nugroho

Program Studi Sarjana Mikrobiologi SITH, email: nugroho.arief@gmail.com

**Pembimbing:** Dr. Pingkan Aditiawati

SITH-ITB, email: pingkan@sith.itb.ac.id

**Gelar:** Sarjana Sains (S.Si), Wisuda Oktober 2010

### **ABSTRAK**

Pelepasan sempurna dinding sel bakteri menghasilkan protoplas, sementara pelepasan sebagian menghasilkan sferoplas. Pelepasan dinding sel ini menyediakan sistem dasar terpermeabilisasi untuk penelitian ultrastruktural, genetika dan fisiologi sel. Penisilin yang mentarget peptidoglikan dinding sel umum diaplikasikan dalam produksi protoplas. Efektifitas penggunaan penisilin untuk pembentukan protoplas sangat tergantung pada jenis atau strain bakteri. Pada penelitian ini dilakukan optimasi kadar penisilin untuk pembentukan protoplas/sferoplas. Penelitian dilakukan dengan metode kultivasi disertai perlakuan variasi konsentrasi penisilin. Kultur yang digunakan adalah *Escherichia coli* umur 6 jam dengan jumlah sel  $4.13 \times 10^8$  per mL serta *Bacillus megaterium* umur 6 jam dengan jumlah sel  $6.81 \times 10^7$  per mL. Perlakuan disiapkan sebagaimana berikut: 2 mL kultur bakteri, 1 mL akuades, 2 mL sukrosa 2M dan 0.9 mL  $MgSO_4 \cdot 7H_2O$  0.1M ditambahkan pada 4 mL medium NB *double strength* dengan perlakuan penambahan 0.1 mL penisilin konsentrasi 15 mg/mL, 20 mg/mL, 25 mg/mL, 30 mg/mL dan 35 mg/mL. Perlakuan dilakukan selama 2 jam pada temperatur  $25^0$  C. Sampel dari perlakuan kemudian diamati morfologinya dengan dan tanpa pewarnaan. Pewarnaan dilakukan dengan metode Gram. Hasil pengamatan menunjukkan penggunaan penisilin mulai efektif dari konsentrasi 30 mg/mL untuk *Bacillus megaterium* dan 35 mg/mL untuk *Escherichia coli*. Jumlah sel *E. coli* menunjukkan pengurangan dengan nilai rata-rata  $2.7 \times 10^7$  sementara jumlah sel *B. megaterium* menunjukkan pertumbuhan dengan nilai rata-rata  $2.7 \times 10^7$ .

**Kata kunci:** protoplas, sferoplas, dinding sel, penisilin