

**Nafisah, Zahrotun, 2019, Konstruksi Pustaka Metagenomik Prokariot dari Permukaan *Eucheuma cottonii* untuk Mencari Gen Penyandi  $\kappa$ -Karaginase. Skripsi di bawah bimbingan Prof. Dr. Afaf Baktir, MS. Apt. dan Dr. Purkan, M.Si., Departemen Kimia, Fakultas Sains dan Teknologi, Universitas Airlangga**

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### ABSTRAK

$\kappa$ -Karaginan adalah koloid hidrofilik yang diekstrak dari makroalga *Eucheuma cottonii*.  $\kappa$ -karaginan mempunyai berat molekul yang sangat besar sehingga pemanfaatannya terbatas. Namun, dalam bentuk *Carrageenan Oligosaccharide* (COs),  $\kappa$ -karaginan mempunyai aktivitas fisik dan biologi yang bervariasi, termasuk anti tumor, anti oksidan dan anti angiogenik. Enzim  $\kappa$ -karaginase berperan memutus ikatan  $\beta$ -(1,4) pada  $\kappa$ -karaginan untuk menghasilkan COs. Ada berbagai macam bakteri yang hidup di permukaan *Eucheuma cottonii*, namun hanya ada 1% bakteri yang dapat dikultur. Metode alternatif untuk eksplorasi bakteri di alam adalah metode metagenomik. Dalam penelitian ini dilakukan eksplorasi gen  $\kappa$ -karaginase dari DNA metagenom prokariot yang menempel di permukaan *Eucheuma cottonii*. Konstruksi pustaka metagenom prokariot dilakukan terhadap kumpulan fragmen DNA hasil digesti dengan enzim restriksi *Sfi*I. Kemudian fragmen DNA diligasi dengan fag  $\lambda$ TriplEx2 dan dilakukan penentuan *titer*. Titer tertinggi didapatkan dari campuran ligasi dengan perbandingan DNA dan fag yaitu 3:2 sebesar  $10,3 \times 10^7$ . Kemudian plak positif diekspresikan melalui *E.coli* BM25,8 untuk mencari gen penyandi enzim  $\kappa$ -karaginase. Aktivitas positif enzim  $\kappa$ -karaginase ditandai dengan terbentuknya daerah *halo*. Kemudian klon rekombinan yang membentuk *halo* dikultivasi untuk produksi enzim  $\kappa$ -karaginase, dan diuji aktivitasnya untuk menentukan suhu dan pH optimum. Berdasarkan uji aktivitas ekstrak  $\kappa$ -karaginase terhadap suhu dan pH didapatkan 4 profil suhu dan pH optimum yang berbeda, dengan demikian telah didapatkan 4 macam gen penyandi enzim  $\kappa$ -karaginase.

**Kata Kunci :  $\kappa$ -karaginase, *Eucheuma cottonii*, metagenomik**

**Nafisah, Zahrotun, 2019, Metagenomic Library Construction of Prokaryotic from the Surface of *Eucheuma Cottonii* to Look for Genes Encoding  $\kappa$ -Carrageenases. The script was under guidance of Prof. Dr. Afaf Baktir, MS. Apt. and Dr. Purkan, M.Si., Departement of Chemistry, Fakultas Sains dan Teknologi, Universitas Airlangga**

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### ABSTRACT

$\kappa$ -Carrageenan was a hydrophilic colloid extracted from macroalgae *Eucheuma cottonii*. The high molecular weight of  $\kappa$ -carrageenan have greatly limited their further application. However, in the form of Carrageenan Oligosaccharide (COs),  $\kappa$ -carrageenan has various physical and biological activities, including anti-tumor, anti-oxidant and anti-angiogenic. The  $\kappa$ -Carrageenase enzyme break  $\beta$ -(1,4) bond in  $\kappa$ -carrageenan to produce COs. There were various kinds of bacteria that live on the surface of *Eucheuma cottonii*, but there were only 1% of bacteria that could be cultured. An alternative method for bacterial exploration in nature was metagenomic method. In this study,  $\kappa$ -carrageenase gene exploration was carried out from prokaryotic metagenom DNA that grew on the surface of *Eucheuma cottonii*. Construction of a prokaryotic metagenom library was carried out against a collection of DNA fragments obtained from digestion with *Sfi*I restriction enzymes. Then DNA fragment ligated with phage  $\lambda$ TriplEx2 and titer was determined. The highest titer,  $10.3 \times 10^7$ , were obtained from the ligation mixture with DNA and phage ratio 3: 2. Then positive plaques were expressed through *E.coli* BM25.8 to look for genes encoding  $\kappa$ -carrageenase enzymes. The positive activity of the  $\kappa$ -carrageenase enzyme was identified by formation of halo region. The recombinant clones that form halo region were cultivated to produce  $\kappa$ -carrageenase enzymes, and their activity based on pH and temperature was measured. Based on these activity, 4 different profiles of optimum temperature and pH were obtained, thus 4 types of genes encoding  $\kappa$ -karaginase enzymes were obtained.

**Keyword :  $\kappa$ -carrageenase, *Eucheuma cottonii*, metagenomics**