IDENTIFIKASI MOLEKULER DAN PRODUKSI BIOSURFAKTAN ISOLAT BAKTERI HIDROKARBONOKLASTIK DARI SUNGAI TERCEMAR DESA WONOCOLO, BOJONEGORO JAWA TIMUR

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INTISARI

Biosurfaktan merupakan senyawa *amphiphilic* yang diekskresikan oleh mikroorganisme khususnya bakteri sebagai metabolit sekunder. Biosurfaktan memiliki banyak manfaat dibidang industri seperti petroleum, bioremediasi, makanan, kosmetik, obat dan nanoteknologi. Sifat biosurfaktan yang lebih ramah lingkungan daripada surfaktan sintetik memberi peluang dalam pengembangan sumber daya alternatif dalam produksi surfaktan. Bakteri karbonoklastik BF 1 dan BF 2 telah ditemukan dan diindikasikan dapat mendegradasi senyawa hidrokarbon (*crude oil*). Hal ini berpeluang bagi isolat bakteri tersebut sebagai agen hayati pemproduksi biosurfaktan. Identifikasi isolat bakteri, serta karakterisasi proses produksi biosurfaktan perlu dilakukan. Identifikasi isolat bakteri BF 1 dan BF 2 dilakukan dengan marker 16S rRNA yang diamplifikasi menggunakan primer 27F dan 1492R. Skrining biosurfaktan dilakukan dengan 3 uji yaitu *hemolysis test, drop collapse assay,* dan *oil spreading test.* Medium produksi biosurfaktan dilakukan modifikasi pada komposisi karbon (C) dan nitrogen (N). Modifikasi sumber karbon (C) yang digunakan adalah *crude oil* dan glukosa sedangkan nitrogen (N) menggunakan yeast ekstrak, urea dan NH_4NO_3. Karakterisasi senyawa biosurfaktan dianalisi dengan teknik KLT. Isolat bakteri BF 1 dan BF 2 berhasil diidentifikasi dengan gen *16S rRNA* dan tertogolong dalam kelas *Gammaproteobacteria*. Isolat BF 1 tergolong dalam genus *Klebsiella* dengan nilai similaritas 99%, sedangkan isolat BF 2 tergolong genus *Stenotrophomonas* dengan similaritas 99%. Kedua isolat bakteri ini positif memproduksi biosurfaktan dan aktifitas emulsi optimal isolat BF 1 pada perlakukan kombinasi medium urea dengan CPO sebesar 66%, sedangkan isolat BF 2 optimal pada perlakuan yeast dengan CPO sebesar 58%. Biosurfaktan yang dihasilkan kedua isolate tergolong dalam fosfolipid, namun diduga tersusun atas komponen yang berbeda karena pola fraksi hasil KLT yang temukan berbeda.

Kata kunci : Identifikasi molekuler, 16S rRNA, Bakteri hidrokarbonoklastik, Biosurfaktan, KLT.

MOLECULAR IDENTIFICATION AND PRODUCTION OF BIOSURFACTANTS BY ISOLATES OF HYDROCARBONOCLASTIC BACTERIA FROM CONTAMINATED RIVER IN WONOCOLO VILLAGE, BOJONEGORO EAST JAVA

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ABSTRACT

Biosurfactant as secondary metabolites is an amphiphilic compound whose is excreted by microorganisms especially bacteria. Biosurfactants have many benefits in the industries such as petroleum, bioremediation, food, cosmetics, medicine and nanotechnology. Eco-friendly of biosurfactant provides an opportunity in developing alternative resources in surfactant production. BF 1 and BF 2 have been found and are indicated to degrade hydrocarbons (crude oil). This ability has the opportunity for those bacteria as biological agents for biosurfactant production. Hence, identification of bacterial isolates and characterization of the biosurfactant production process needs to be studied. Identification of BF 1 and BF 2 was carried out using 16S rRNA markers with 27F and 1492R as primers. Biosurfactant screening was carried out using three methods namely hemolysis test, drop collapse assay and oil spreading test. The biosurfactant production medium was modified to the composition of carbon (C) and nitrogen (N). Modification of carbon source (C) used is crude oil and glucose while nitrogen (N) uses yeast extract, urea and NH4NO3. Characterization of biosurfactant compounds was analyzed by TLC technique. Base on *16S rRNA* gene BF 1 and BF 2 were identified as Gammaproteobacteria class. BF 1 belong to the *Klebsiella* genus with 99% similarity value, while BF 2 belong to the *Stenotrophomonas* genus with 99% similarity. These bacteria are positive for biosurfactant production. Optimal emulsion activity of isolate BF 1 in the treatment of the combination of urea medium with CPO was 66%, while isolate BF 2 was optimal in the treatment of yeast with CPO by 58%. The biosurfactant produced by the two isolates was classified as a phospholipid, but it was thought to be composed of different components because the TLC fraction pattern found was different.

Keywords: Molecular identification, 16S rRNA, hydrocarbonoclastic bacteria, biosurfactant, TLC.