ABSTRACT

Plastic pollution remains a significant environmental challenge without an effective solution to date. The excessive production of plastic, coupled with insufficient management strategies, has resulted in additional issues, including the widespread contamination of microplastics. This study aims to investigate the ability and mechanism of three bacterial isolates, isolated from the mangrove sediment of Segara Anakan, Cilacap, in the biodegradation process of low-density polyethylene (LDPE) microplastics, assessed from substrate changes, quantification of enzyme-encoding functional genes, and enzyme profiling. Changes in the substrates due to oxidation reactions were analyzed in three types of microplastic samples, such as LDPE powder, clear-colored LDPE film, and black-colored LDPE film, by Fourier Transform Infrared Spectroscopy (FTIR). Internal double bond index (IDBI) analysis was then performed on the FTIR spectrum to validate the oxidation reactions and results. Furthermore, quantification of three functional genes, namely AlkB (*alkane monooxygenase*), CYP153 (*cytochrome* P450), and GPx (*glutathione peroxidase*) for isolate no. 8 was carried out by qPCR. Finally, the enzyme profile produced by isolate no. 8 in the biodegradation process was analyzed using *liquid chromatography-high resolution mass spectrometry* (LC-HRMS) proteomics. The results showed that the isolate no. 8 and the isolates belonging to *Lysinibacillus* sp. (98.63%) and *Bacillus cereus* (99.26%) for isolate number 12.1, and 13.3, respectively, had the ability to oxidize LDPE microplastics, assessed by the detection of the C=C double bond functional group and IDBI analysis, but interestingly, the three functional genes (AlkB, CYP153, and GPx) were only detected in isolate no. 8. The results of the LC-HRMS analysis also confirmed the production of GPx enzymes by isolate no. 8 indicated by the detection of thiol-peroxidase and thioredoxin peroxidase enzymes, which are associated with glutathione peroxidase-like enzymes in bacteria. In addition, isolate no. 8 was also suspected to produce lipase due to the detection of the lipase encoding gene (lipA948) through conventional PCR.

Keyword: enzymatic biodegradation, mangrove sediment isolates, LDPE, oxidation

INTISARI

Cemaran plastik di lingkungan menjadi salah satu permasalahan yang belum menemukan solusi efektif hingga saat ini. Tingginya produksi plastik tanpa adanya penanggulangan yang efisien dapat menimbulkan permasalahan baru, salah satunya cemaran mikroplastik. Penelitian ini bertujuan untuk menginvestigasi kemampuan dan mekanisme 3 isolat bakteri dari sedimen *mangrove* Segara Anakan, Cilacap dalam proses biodegradasi mikroplastik jenis *low-density polyethylene* (LDPE) ditinjau dari adanya perubahan substrat, kuantifikasi gen fungsional penyandi enzim yang diduga berperan dalam proses biodegradasi, serta analisis profil enzim. Perubahan susbtrat akibat adanya reaksi oksidasi dianalisis pada sampel 3 jenis mikroplastik yaitu LDPE *powder,* plastik film LDPE bening, dan plastik film LDPE hitam, dengan uji *Fourier Tranform Infrared Spectroscopy* (FTIR). Analisis *internal double bond index* (IDBI) kemudian dilakukan pada spektra FTIR untuk memvalidasi terjadinya reaksi oksidasi. Kuantifikasi 3 gen fungsional yaitu AlkB (*alkane monooxygenase*), CYP153 (*cytochrome* P450), dan GPx (*glutathione peroxidase*) pada isolat no. 8 dilakukan dengan *quantitative* PCR (qPCR). Profil enzim yang dihasilkan oleh isolat no. 8 dalam proses biodegradasi kemudian dianalisis dengan menggunakan *liquid chromatography-high resolution mass spectrometry* (LC-HRMS) proteomik. Hasil penelitian menunjukkan bahwa ketiga isolat (8, 12.1, dan 13.3) memiliki kemampuan dalam mengoksidasi mikroplastik LDPE melalui deteksi gugus fungsi C=C *double bond* dan analisis IDBI, serta diketahui bahwa isolat no. 12.1 memiliki kekerabatan terdekat dengan *Lysinibacillus* sp. (98.63%), dan isolat no. 13.3 memiliki kekerabatan terdekat dengan *Bacillus cereus* (99.26%). Menariknya, hanya isolat no. 8 yang terdeteksi memiliki gen fungsional AlkB, CYP153, dan GPx. Hasil analisis LC-HRMS juga mengkonfirmasi diproduksinya enzim GPx oleh isolat no. 8 dengan terdeteksinya enzim *thiol-peroxidase* dan *thioredoxin peroxidase* yang terkait dengan *glutathione peroxidase-like* pada bakteri. Selain itu, isolat no. 8 juga diduga mampu menghasilkan lipase dikarenakan adanya deteksi gen lipA948 pengkode lipase melalui PCR konvensional.

Kata Kunci: biodegradasi enzimatis, isolat sedimen *mangrove,* LDPE, oksidasi.