

INTISARI

Penggunaan uranium (U) sebagai bahan bakar alternatif penghasil energi menyebabkan kontaminasi U yang bersifat toksik dan radioaktif. Biomineralisasi U menggunakan fosfat inorganik (Pi) merupakan salah satu metode remediasi kontaminan U yang efektif. Biomineralisasi melibatkan metabolisme polyP yang dikontrol oleh gen *ppk* dan *ppx*. Gen *ppx* mengode enzim *exopolyphosphatase* yang berperan dalam hidrolisis polyP menjadi Pi bebas. Penelitian ini bertujuan untuk mengisolasi dan mengetahui karakter gen *ppx* pada mikrobia toleran uranium di Indonesia.

Penelitian ini dimulai dengan kegiatan skrining isolat bakteri toleran 0,4mM (U) yang diisolasi dari limbah radioaktif cair aktivitas rendah Pusat Sains dan Teknologi Akselerator (PSTA) BATAN Yogyakarta secara kualitatif berdasarkan kemampuan tumbuh pada medium bebas-fosfat dan kuantitatif berdasarkan kemampuan akumulasi polyP. Isolat terpilih selanjutnya diidentifikasi secara molekuler dengan metode amplifikasi gen 16S rRNA; diuji kemampuan degradasi polyP, biomineralisasi logam berat Cu^{2+} dan Cd^{2+} ; dan diamplifikasi dan dikarakterisasi gen *ppx*-nya.

Hasil skrining didapatkan isolat potensial polyP yaitu isolat A67I. Berdasarkan hasil analisa gen 16S rRNA isolat A67 I termasuk dalam genus *Acinetobacter*. Isolat A67I diketahui mampu melepas Pi sebesar 50,70% pada jam ke-10 setelah inkubasi. Isolat A67I terbukti mampu mengurangi konsentrasi logam berat Cu^{2+} dan Cd^{2+} sekitar 3,73% untuk Cu^{2+} dan 2,34% untuk logam Cd^{2+} setelah 24 jam inkubasi. Gen *ppx* dari isolat A67I berhasil diisolasi dengan metode PCR menggunakan primer F1-R1 dan F2-R2 berupa “single band” DNA dengan ukuran ± 1500 bp. Penelitian ini berhasil membuktikan bahwa mikroba toleran uranium di Indonesia memiliki gen *ppx* yang menyandi enzim *exopolyphosphatase* yang penting dalam proses mendegradasi polyP menjadi Pi bebas.

Kata kunci: Uranium, *polyP*, Amplifikasi, Karakterisasi, gen *ppx*

ABSTRACT

The use of uranium as an alternative fuel causes radioactive waste. Biomineralisation is one of the bioremediation methods used for removing u contaminant from environment. Biomineralisation is a process of chelating metal using microbial metabolites such as phosphate. polyP metabolism is an essential process for this case. It is known that polyP metabolism is controlled by *ppk* and *ppx* genes. The *ppx* gene encodes an *exopolyphosphatase* enzyme which plays a key role in polyP hydrolysis to excess Pi, while *ppk* gene encodes a polyphosphate kinase enzyme which controlled a polyP synthesis.

The aims of this study was to characterise *ppx* gene present in from uranium tolerant's microbes in Indonesia. The study was conducted by qualitative and quantitative screening of the 0,4 mM uranium-tolerant's bacteria isolated from low-level liquid radioactive waste in *Pusat Sains dan Teknologi Akselerator (PSTA) BATAN*, Yogyakarta. Qualitative screening was based on the isolate ability to grow in the phosphate-free media, while quantitative screening relied on the isolates ability to uptake and accumulate polyP. The polyP metabolism was also analysed by assaying the polyP degradation and metal removal. Finally, the *ppx* gene was amplified and sequenced.

A potential isolate designated as A67I, was identified belongs to *Acinetobacter* genus. A67 I isolate released about 50.70% Pi after 10 hour of incubation and it removed Cu²⁺ and Cd²⁺ ions from the media after 24 hour of incubation. Cu²⁺ and Cd²⁺ ions decreased 3.73% and 2.34%, respectively, following the incubation of the isolate in the media. The amplification resulted a single band of DNA of approximately 1.500 bp in length. The amplified *ppx* gene was found to contain only a partial sequence of exopolyphosphatase gene sequence. However, the blastN analysis showed that it showed similarities with *ppx* gene of *Acinetobacter radioresitens* NBRC's strain and other species. The result of this study truly suggest that the uranium-tolerant bacterial isolate harbors *ppx* gene encoding exopolyphosphatase enzyme.

Key word: Uranium, polyP, Amplification, Characterization, *ppx* gene