Isolasi bakteri dan karakterisasi parsial enzim oksidoreduktase dari Situ Agathis, Kampus Universitas Indonesia, Depok

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Abstrak

Oxydoreductases are enzymes which catalyze oxidation-reduction reaction of their corresponding substrates. Oxydoreductase enzymes from many microorganisms had become major focus of research during last decades. This reaction had been utilized in biosensor (Yuhashi et al. 2005), biotransformation and biofuel (Zu et al. 2006). In the field of biosensor, glucose dehydrogenase application as self-blood glucose monitoring had evolved through several generation to enhance its sensitivity and specificity (Witarto et al. 1997).

Oxydoreductase involve cofactor in their active sites. According to Anthony (1996) among several known cofactors such nicotinamide, flavonoid, and quinone, Pyrollo Quinoline Qinone (PQQ) as the member group of quinon is one of the latest known-cofactors. PQQ differs from other cofactor since it is not covalently bond to its enzyme (Oubrie et al. 1999). PQQ ubiquitously found in all organisms from prokaryote to eukaryote (Bishop et al. 1998). Bacteria is the largest group of PQQ-oxydoreductase producing microorganisms. They successfully isolated from many habitats such: soil, water (Toyama et al. 1995), fruits (Adachi et al. 2003), plants, and in human mouth (Anesti et al. 2005). However, study on PQQ-oxydoreductase producing bacteria isolation had never been reported in Indonesia.

PQQ-Oxydoreductase bacteria are able to utilize organic substrates such glucose, ethanol, methanol, up to polyvinyl alcohol (Ameyama et al. 1985). One of the habitats which provides such organic substrates is Situ Agathis located in University of Indonesia Depok. Situ Agathis contain humic substances that could be degraded in to glucose, ethanol, methanol, also quinone.

In this study, isolation of oxydoreductase-producing bacteria from Situ Agathis University of Indonesia, Depok and characterization of oxydroreductases of selected isolates were performed. The objectives of this research are: to investigate the presence of oxydoreductase-producing bacteria, to isolate the oxydoreductases -producing bacteria, and to partially characterize oxydoreductases from Situ Agathis University of Indonesia Depok. This is the first study on bacteria isolation performed in Situ Agathis UI, Depok. Hence, this study can provide information about the oxydoreductases- producing bacteria from Situ Agathis, which located in UI, Depok. The study consists of two part: first part describe the isolation of oxydoreductase-producing bacteria from Situ Agathis. Second part describe the partial characterization of oxydoreductases which covers enzyme activity, molecular weight, and PQQ effects on the enzymes activity.

The research was carried out at the Protein Engineering Laboratory, Biotechnology Research Centre, Indonesian Institute of Science, Cibinong and the Laboratory of Microbiology, Department of Biology, University of Indonesia, Depok during February ? September 2007. The isolation of bacteria was conducted in three methods i.e : dilution, filtration using filter paper Milipore membran (0.2 m) based on Cappucino and Sherman (2002). Isolation of oxydoreductase-producing bacteria was carried out by using selective media based on Toyama et al. (1995). The assay of oxydoreductases was performed by using Native-PAGE

based on Khodijah (2002).

The result showed that 83 isolates were obtained from Situ Agathis which we assumed could produce oxydoreductase enzymes. Among those isolates, 15 isolates were randomly selected for further study e.g : five isolates which could grow in glucose as sole carbon sources by producing glucose dehdyrogenase, six isolates which could grow on ethanol as sole carbon sources by producing ethanol dehydrogenase and four isolates which could grow on methanol as sole carbon sources by producing methanol dehydrogenase. The selected isolates showed various morphotypes indicating no specific morphological character in oxydoreductase-producing bacteria.

Two oxydoreductases from selected isolates were selected to be analyzed further in second part this thesis. Those enzymes were examined for their possibility to have intracellular PQQ cofactor. Those enzymes were obtained from isolate G1H1D30 (glucose dehydrogenase) and isolate A1H2D60 (ethanol dehydrogenase). Native-PAGE result confirmed that crude extract fraction, dialyzed fraction and elution of open column chromatography of isolate G1H1D30 can produce glucose dehydrogenase and isolate A1H2D60 can produce ethanol dehdyrogenase. The molecular weight of glucose dehydrogenase subunit is about 46 kDa using SDS-PAGE.

SDS-PAGE of ethanol dehydrogenase did not show any protein band in acrylamide gel. We assumed that the amount of protein extracted from cell cytoplasm was not sufficient enough to be detected in SDS-PAGE. Cell of isolate A1H2D60 should be treated by other destruction method such as French pressure or ultrasonicator since this isolate is Gram positive bacteria which had thicker peptydoglycan layer than isolate G1H1D30 which is Gram negative bacteria.

Other characterization performed was addition of PQQ as the cofactor to investigate its effect on enzymes activity. Glucose dehydrogenase from isolate G1H1D30 was known to be PQQ dependent enzymes from its activity increased after addition of PQQ. The addition of PQQ raised the indicating no specific morphological character in oxydoreductase-producing bacteria.

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Other characterization performed was addition of PQQ as the cofactor to investigate its effect on enzymes activity. Glucose dehydrogenase from isolate G1H1D30 was known to be PQQ dependent enzymes from its activity increased after addition of PQQ. The addition of PQQ raised theenzyme activity to eight fold from 0.102 U/mL to 0.94 U/mL of crude enzyme extract. In contrast, addition of PQQ did not give significant effect to EDH enzyme activity (activity of crude enzyme remain 0.082 U/mL in the presence and absence of PQQ). However, further study should be performed to analyze the real cofactor of EDH from isolate A1H2D60. EDH differs from GDH since it had disulphide ring which stabilize PQQ bound to its enzyme. Hence, PQQ could remain bound to EDH as purification procedure performed. PQQ-GDH do not have any disulphide ring which could stabilize PQQ bound. This fact implicated unstable PQQ bound to GDH while isolation and purification performed.