Optimasi Ekspresi Gen Sintetik Penyandi Enzim Pendegradasi Plastik Polietilena Tereftalat (PETase) pada Escherichia coli Arctic Express (DE3) = Optimization of Polyethylene Terephthalate Plastic Degrading Enzyme (PETase) Synthetic Coding Gene Expression in Escherichia coli Arctic Express (DE3)

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Abstrak

Masalah limbah plastik polietilena tereftalat (PET) di Indonesia menjadi perhatian yang serius karena penguraiannya lambat dan berpotensi merusak lingkungan. Solusi yang menjanjikan adalah Ideonella sakaiensis polyethylene terephthalate hydrolase (IsPETase) yang dapat mendegradasi plastik dengan lebih cepat. IsPETase sebelumnya telah diekspresikan di Escherichia coli BL21 (DE3). Namun, IsPETase masih terekspresikan secara insoluble sehingga IsPETase perlu diamati ekspresi gen dan optimasi kondisi ekspresi pada E. coli Arctic Express (DE3). Penelitian bertujuan untuk mengamati ekspresi gen PETase pada E. coli Arctic Express (DE3) dan menentukan kondisi optimal untuk ekspresi. Penelitian ini melibatkan berbagai tahapan seperti peremajaan kultur rekombinan, produksi protein, dan pemanenan, yang dioptimalkan untuk kondisi ekspresi. Ekspresi gen PETase diamati pada fraksi ekstraseluler (dipekatkan), periplasmik (dipekatkan), dan sitoplasmik. Fraksi ekstraseluler belum terekspresikan secara optimal sehingga optimasi kondisi ekspresi dilanjutkan pada fraksi sitoplasmik (soluble) dengan media pertumbuhan Luria Bertani (LB), induksi IPTG 1,0 mM selama 8 jam, dan waktu sonikasi selama 10 menit menghasilkan aktivitas spesifik PETase 0,07 U/mg. Namun, pemurnian protein dan ekspresi perlu dilakukan dengan sel inang Bacillus.

.....The problem of polyethylene terephthalate (PET) plastic waste in Indonesia has become a serious concern due to its slow degradation and potential environmental damage. A promising solution is Ideonella sakaiensis polyethylene terephthalate hydrolase (IsPETase), which can degrade plastic faster. IsPETase has been expressed in Escherichia coli BL21 (DE3), but it is still expressed insolubly, so the expression of the gene and the optimization of the expression conditions in Escherichia coli Arctic Express (DE3) need to be observed. This study aims to observe the PETase gene expression in E. coli Arctic Express (DE3) and determine the optimal conditions for expression. This study involves various stages, such as refreshing culture, protein production, and harvesting, which are optimized for expression conditions. PETase gene expression was observed in the extracellular (concentrated), periplasmic (concentrated), and cytoplasmic soluble fractions. The extracellular fraction has not been optimally expressed, so expression optimization continued in the cytoplasmic fraction with Luria Bertani (LB) growth medium, IPTG induction of 1.0 mM for 8 hours, and sonication time for 10 minutes, resulting in specific activity of 0.07 U/mg. However, protein purification is required and expression is performed with Bacillus host cells.