https://doi.org/10.30970/apmicro.2026.34

Zakalskiy A., Stasyuk N., Zakalska O., Gonchar M.

OVEREXPRESSION OF PENTAERYTHRITOL TETRANITRATE REDUCTASE FROM *ENTEROBACTER CLOACAE PB2* IN *ESCHERICHIA COLI* CELLS

Institute of Cell Biology of the National Academy of Sciences of Ukraine,
14/16 Drahomanov St., Lviv, 79005, Ukraine
e-mail: andriy.zakalskiy@yahoo.com

Abstract. The recombinant strain of *Escherichia coli* overproducing $(His)_6$-tagged pentaerythritol tetranitrate reductase (PETNR) from *Enterobacter cloacae PB2* has been constructed. This strain can be used to develop a cost-efficient large-scale procedure for isolation and one step purification by metal-affinity chromatography of a highly specific PETNR, necessary for the construction of enzymatic kits and biosensors for assay of PETN.
Keywords: pentaerythritol tetranitrate; pentaerythritol tetranitrate reductase; gene cloning.

Pentaerythritol tetranitrate (PETN) is one of the most widely used nitrate ester explosives in military, mining, and civil engineering applications owing to its high detonation performance and chemical stability (Manner et al. 2022). At the same time, the extensive use of PETN has increased the risk of environmental contamination, accidental release, and its misuse in criminal and terrorist activities (Li et al. 2014). Therefore, the development of rapid, sensitive, and portable analytical methods for PETN determination has become an important challenge in environmental monitoring, forensic science, homeland security, and remediation technologies.

Among emerging analytical approaches, biosensors have attracted considerable attention because they combine the high selectivity of biological recognition elements with rapid signal transduction (Naresh et al. 2021). Enzyme-based biosensors are particularly attractive owing to their exceptional catalytic efficiency and substrate specificity (Alvarado-Ramirez et al. 2021).

In current report, we describe a construction of the *Escherichia coli* recombinant strain overproducing $(His)_6$-tagged pentaerythritol tetranitrate reductase (PETNR) from *Enterobacter cloacae PB2*. A codon optimized PETNR gene (GeneArt, Life Technologies) was synthesized and cloned into the pET21a expression vector. The resulting plasmid was introduced into *Escherichia coli* BL21(DE3). The recombinant strain of *E. coli* that overproduces soluble $(His)_6$-tagged PETNR has been constructed. The enzyme preparations are sufficiently stable and possess activity of 185 U/mg. The yield of the PETNR is approximately 120 mg from 1 L culture. The most prominent advantages of the obtained preparation are a high yield and simple isolation of the enzyme. These findings can be used to develop a cost-efficient large-scale procedure for isolation and one step purification by metal-affinity chromatography of a highly specific PETNR, necessary for the construction of enzymatic kits and biosensors for assay of PETN.

References

1. Manner et al. (2022). Chemical Evaluation and Performance Characterization of Pentaerythritol Tetranitrate (PETN) under Melt Conditions. *ACS Mater Au*., *2*(4), 464–473. Doi: 10.1021/acsmaterialsau.2c00022.
2. Li Zhuang et al. (2014). Laboratory and pilot-scale bioremediation of Pentaerythritol Tetranitrate (PETN) contaminated soil. *Hazard. Mater., 264*, 261–268. https://doi.org/10.1016/j.jhazmat.2013.11.035
3. Naresh et al. (2021). A Review on Biosensors and Recent Development of Nanostructured Materials-Enabled Biosensors. *Sensors*, *21*(4), 1109. Doi: 10.3390/s21041109.
4. Alvarado-Ramirez et al. (2021). Enzyme (Single and Multiple) and Nanozyme Biosensors: Recent Developments and Their Novel Applications in the Water-Food-Health Nexus. *Biosensors*, *11*(11), 410. Doi: 10.3390/bios1111.