Expression, Purification, and Characterization of Laccase from Bacillus paramobilis and Its Application in the Biotreatment of Carbonaceous Matter in Double Refractory Gold Ores

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Abstract

Laccase is a versatile enzyme classified in multicopper oxidase family. The property of laccase makes the enzyme labelled as the green-catalyst enzyme, which useful for industry and eco-friendly catalyst. This research is aimed to utilize laccase in environmental purpose, especially in gold-mining mitigation. The gene encoded laccase is isolated from *Bacillus paramobilis*. The isolated gene is amplified, and verified using gel electrophoresis. Further cloning is done using high copy number plasmid, resulting in transformant colonies. Firther validation is done using colony PCR and plasmid isolation. The gene is successfully isolated. Through the validations showing the 1.6 kb in size.

Keyword: Laccase, Recombinant, Green-Catalyst

Introduction

Laccase is an enzyme belonging to the multicopper oxidase (MCO) family and is classified as a benzenediol:oxygen oxidoreductase (EC 1.10.3.2). Due to its relatively broad substrate specificity, this enzyme is capable of oxidizing a wide range of both phenolic and non-phenolic compounds. In its catalytic reaction, laccase utilizes oxygen (O_2) as an electron acceptor, producing water (H_2O) as a by-product. Laccases are found in various organisms ncluding fungi, bacteria, plants, yeasts, and insects with structural characteristics and catalytic activities that vary depending on the source. This versatility makes laccase an important enzyme in numerous biotechnological processes, particularly in lignin biodegradation and waste treatment (Arregui et al., 2019).

Laccases widely used in industrial applications are typically derived from fungi; however, they often exhibit limited stability at high temperatures and under alkaline conditions. In contrast, bacterial laccases have the potential to be more stable under extreme conditions, such as high temperatures, alkaline pH, and high chloride ion (Cl^-) concentrations (Sharma et al., 2007; Brugnari et al., 2021). One promising source is the genus *Bacillus*, which is capable of producing a variety of enzymes including the thermostable CotA laccase found in *Bacillus subtilis* (Wang et al., 2015).

The potential for laccase production from the genus *Bacillus* specifically *Bacillus paramobilis* remains to be further explored. Therefore, this study focuses on the production potential, as well as the functional and structural characterization, of the laccase derived from this bacterium.

Research Method

Genomic DNA of *Bacillus paramobilis* was isolated using a standard bacterial genomic DNA extraction kit. The open reading frame (ORF) encoding the CotA-type multicopper oxidase (BpLac) was amplified by PCR using gene-specific primers incorporating EcoRI and NdeI restriction sites and a C-terminal hexahistidine (His_6) tag sequence. The PCR reaction consisted of denaturation, annealing (at 58°C), and extension step. The resulting ~1.6 kb fragment was confirmed by agarose gel electrophoresis.

The target DNA fragment was purified using a PCR purification or gel extraction kit, depending on the electrophoresis results. The purified DNA was then ligated into the pGEM-T Easy vector using a ligation kit. The ligation reaction was incubated for 16 hours at 4°C to generate recombinant plasmids.

Escherichia coli TOP10 competent cells were prepared using the calcium chloride ($CaCl_2$) method and transformed with the ligation product through heat shock. Following recovery in LB medium, the transformed cells were plated on selective LB agar supplemented with ampicillin, X-gal, and IPTG for blue-white screening. White colonies were selected as potential transformants carrying the recombinant plasmid and further confirmed by colony PCR using gene-specific primers. The PCR products were subsequently analyzed by agarose gel electrophoresis to verify the presence of the expected ~1.6 kb BpLac fragment.

PCR-positive colonies were subcultured, and recombinant plasmids were isolated using a miniprep kit. Plasmid concentration and purity were measured by NanoDrop, followed by Sanger sequencing using universal T7 and SP6 primers to analyze the BpLac insert.

Discussion & Result

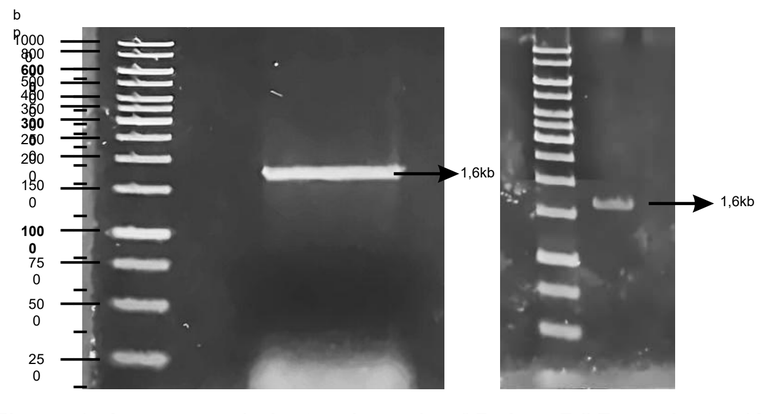


Figure 1. Agarose gel electrophoresis of BpLac PCR products. (A) PCR product. (B) Purified PCR product.

PCR amplification produced a single DNA band of approximately 1.6 kb, consistent with the expected BpLac ORF size. After purification, the fragment retained its expected size, with a concentration of 15 ng/µL, and no primer dimers were detected on the gel. The purified DNA was therefore suitable for subsequent cloning (Crossley et al., 2020).

The purified amplicon is used in 1:3 ligation with high copy number vector, pGEM-T Easy (Promega, USA). The ligation ratio was optimzed, 1:1; 1:3; and 1:5, thereby, the 1:3 is the best for this recombinant. Then, the transformation using the heat-shock method resulting in high number blue and white colonies.

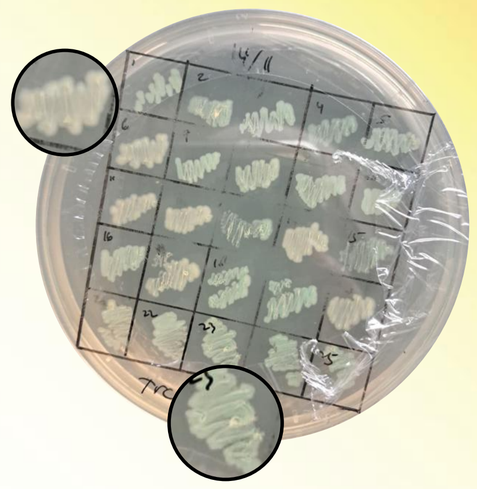


Figure 2. Transformant Master Plate consisted of blue and white colonies

This blue white screening happens based on the vector/plasmid properties that incorporated with short coding sequence of β-galactosidase that associated with carboxy-terminal of β-galactosidase from host, that is called α-complementation. insertion of fragment will block this expression resulted in white colony. while the clue colony is from genetic combination with chromogenic substrate X-gal and screened with antibiotic (Green & Sambrook, 2019).

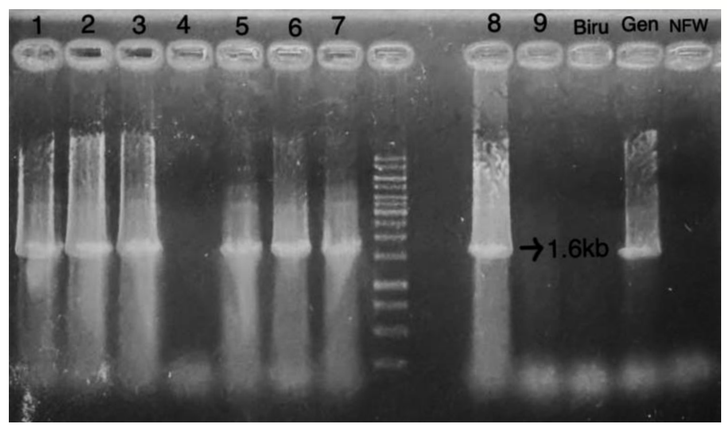


Figure 3. Agarose gel electrophoresis analysis of colony PCR. Lanes 1–9 represent white colonies; Blue: blue colony; Gen: genomic DNA of Bacillus paramobilis ; NFW: nuclease-free water.

Nine white colonies were selected as candidate transformants, while one blue colony served as a negative transformation control. Colony PCR was performed to confirm the presence of the insert based on the size of the DNA bands. A band of approximately 1.6 kb indicated an amplicon size consistent with the BpLac target (Bryksin & Matsumura, 2010). Plasmid isolation yielded a concentration of 211.8 ng/µL with an A260/A280 ratio of 1.97, indicating high DNA purity and supporting its use as a template for Sanger sequencing (Crossley et al., 2020).

Conclusion

The 1.6 kb amplicon of gene encoded laccase from *Bacillus paramobilis* is succeeded to be isolated. The gene is amplified using high copy number plasmid, pGEM-T Easy trrough heat-shock transformation. Further validation for transformed plasmid, BpLacc1-pGEM-T Easy is done with colony PCR and plasmid isolation.

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