Production and Characterization of Pullulan Polysaccharide from *Aureobasidium pullulans*

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Abstract- Pullulan is a water-soluble polysaccharide composed of maltotriose and maltotetrose units linked by α-(1→4) and α-(1→6) bonds. This extracellular polymer is produced by *Aureobasidium pullulans*, a saprophytic fungus found on diverse surfaces such as plant leaves. The present study focuses on isolating A. pullulans from guava leaves, identifying it through morphological and biochemical characterization, and evaluating its pullulan production capacity. The extracted polysaccharide was confirmed using Fourier Transform Infrared (FTIR) spectroscopy and analyzed for its flocculating, antioxidant, emulsification, and silver nanoparticle synthesis potential. The study highlights the multifaceted applications of pullulan in various industrial sectors.

Keywords: *Aureobasidium pullulans*, Pullulan, Nanoparticles, Flocculation, Antioxidants, Emulsification

I.INTRODUCTION

Aureobasidium pullulans is a yeast-like fungus categorized among "black yeasts" and is widely distributed in nature, thriving on plant surfaces, wood, and artificial substrates (Campbell & Siddique, 2004). It exhibits optimal growth between 10–35°C, with peak metabolic activity at 30°C. The organism's ability to produce pullulan, a linear, extracellular, water-soluble polysaccharide, makes it highly valuable for industrial applications. Pullulan consists of repeated maltotriose (α-1,6) and maltotetraose (α-1,4) units, which confer properties such as transparency, tastelessness, resistance to grease and oil, and high solubility in water (Kanmani, 2013).

Pullulan's ability to form films with high tensile strength, transparency, and oxygen impermeability has made it particularly attractive for applications in the food packaging and pharmaceutical industries (Leathers, 2003). Its non-toxic, non-immunogenic, and biodegradable nature further enhances its suitability for medical applications such as drug delivery systems, wound healing scaffolds, and tissue engineering matrices.

Several environmental and nutritional parameters influence pullulan biosynthesis, including carbon source, nitrogen availability, pH, temperature, and dissolved oxygen levels. Studies have demonstrated that optimizing these parameters can significantly improve yield and quality of pullulan (Catley, 1971). Therefore, this study aims to isolate a novel strain of A. pullulans, optimize its pullulan production, and characterize the produced polymer for potential industrial and biomedical applications.

In addition to conventional uses, microbial polysaccharides like pullulan are being actively investigated for their role in nanotechnology. Specifically, their role in the green synthesis of metal nanoparticles has drawn considerable attention. Pullulan's hydroxyl groups allow it to stabilize nanoparticles without toxic reducing agents, offering safe and sustainable nanomaterial alternatives (Islam et al., 2011; Kanmani, 2013). These properties not only broaden the scope of pullulan in biomedicine and catalysis but also align with the global shift toward greener technologies.

II.MATERIALS AND METHODS

1. Isolation of Fungus

Guava leaves were washed with sterile distilled water and cut into small fragments. A 5-gram sample was placed in sterile water and incubated at 30°C in a shaking incubator for three days. An aliquot of 0.2 mL was cultured in potato dextrose broth and plated onto potato dextrose agar for fungal isolation (Mishra & Vuppu, 2013).

2. Biochemical Characterization

Standard biochemical tests were conducted for sugar fermentation (glucose, sucrose, lactose) and nitrogen source assimilation (ammonium sulfate, sodium

nitrite) to confirm the identity of *A. pullulans* (Nagavarma et al., 2012).

3. Extraction of Pullulan

The fungal culture was inoculated in minimal salt media with glucose as the primary carbon source. After 120 hours of incubation at 28°C, the extracellular polysaccharide was precipitated using 95% ethanol and dried at 60°C (Nitta & Numata, 2013).

4. Analytical Tests

- FTIR Spectroscopy: Used to confirm the presence of characteristic functional groups in pullulan.

Figure 1: Flocculation Activity – Pullulan concentration vs. flocculating efficiency.

- Emulsification: Various concentrations of pullulan solutions were tested against olive oil to determine emulsification efficiency.

Figure 3: Antioxidant Activity – Pullulan activity compared with Vitamin C.

- Silver Nanoparticle Synthesis: Pullulan solutions were mixed with silver nitrate, heated, and analyzed using UV-Visible spectroscopy.

III.RESULTS AND DISCUSSION

The fungal isolates obtained from guava leaves exhibited morphological characteristics consistent with Aureobasidium pullulans, transitioning from white to yellow and finally to black pigmentation. Biochemical characterization confirmed the identity of the isolate based on its positive glucose fermentation and nitrogen assimilation patterns (Gaur et al., 2018). The FTIR spectra of the extracted polysaccharide exhibited characteristic peaks corresponding to hydroxyl (-OH) stretching and carbonyl (C=O) groups, confirming the presence of pullulan. The pullulan yield was approximately 2.3 g/L after 120 hours of incubation under optimized conditions, aligning with previous reports of wild-type strains (Roukas, 1998). Pullulan was obtained as a white, odorless, and tasteless powder highly soluble in water. Its flocculation efficiency increased proportionally with concentration, as shown in Figure 1, suggesting its potential as an eco-friendly bioflocculant (Zhu et al., 2010).

Emulsification studies revealed that pullulan enhanced emulsification with increasing concentration (Figure 2), supporting its application as a natural emulsifier, consistent with findings by Singh et al. (2008). Antioxidant assays demonstrated significant free radical scavenging activity comparable to vitamin C (Figure 3), indicating potential pharmaceutical and nutraceutical applications (Chen et al., 2012).

Furthermore, pullulan successfully mediated the green synthesis of silver nanoparticles, confirmed by a strong surface plasmon resonance peak at 420 nm in the UV-Vis spectrum (Figure 4). This finding corroborates previous work on the nanoparticle-stabilizing properties of microbial polysaccharides (Islam et al., 2011). Overall, these results underscore the versatile industrial potential of pullulan derived from A. pullulans.

GRAPHICAL REPRESENTATION

Figure 1: Flocculation Activity – Pullulan concentration vs. flocculating efficiency.

Figure 2: Emulsification Index – Emulsification capacity of pullulan over time.

Figure 3: Antioxidant Activity – Pullulan activity compared with Vitamin C.

Figure 4: UV-Vis Absorption Spectrum – Silver nanoparticle synthesis mediated by pullulan.

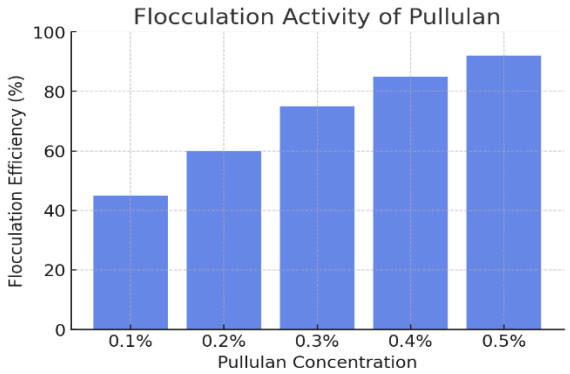

Figure 1: Flocculation Activity – Pullulan concentration vs. flocculating efficiency.

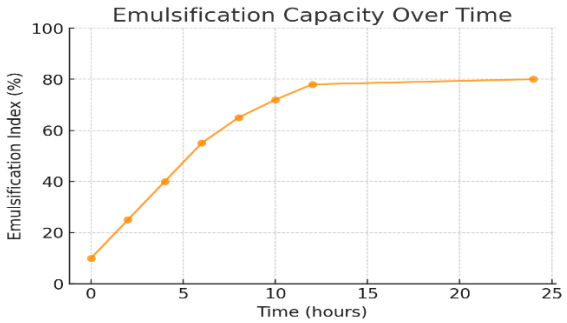

Figure 2: Emulsification Index – Emulsification capacity of pullulan over time.

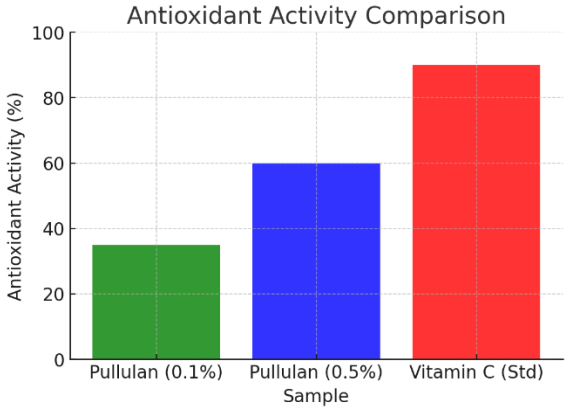

Figure 3: Antioxidant Activity – Pullulan activity compared with Vitamin C.

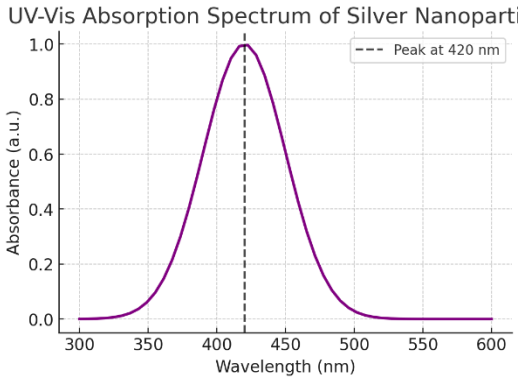

Figure 4: UV-Vis Absorption Spectrum – Silver nanoparticle synthesis mediated by pullulan.

IV.CONCLUSION

The present study successfully isolated and characterized Aureobasidium pullulans from guava leaves and confirmed its capacity to produce pullulan. The extracted polysaccharide exhibited promising flocculating, emulsifying, antioxidant, and nanoparticle-synthesizing activities, reinforcing its potential as a multifunctional biopolymer. These findings underscore the importance of microbial sources for eco-friendly and industrially relevant biomaterials. Further work focusing on optimization, scale-up processes, and in-depth structural analyses would be beneficial to fully harness the commercial viability of pullulan.

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