

Box-Wilson Design for Optimization of *in vitro* Levan Production and Levan Application as Antioxidant and Antibacterial Agents

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ABSTRACT

Background: Levan or fructan, a polysaccharide of fructose, is widely used in various commercial industries. Levan could be produced by many organisms, including plants and bacteria. The cloning of the gene from *Bacillus licheniformis*, which expressed levansucrase in *Escherichia coli* host, was carried out successfully. In the present study, we performed the *in vitro* production of levan and analyzed its potential application as antibacterial and antioxidant agents. **Methods:** *In vitro* levan production catalyzed by heterologous-expressed levansucrase Lsbl-bk1 and Lsbl-bk2 was optimized with BW design. The antibacterial activity of the produced levan was carried out using agar well diffusion method, while its antioxidant activity was tested by free radical scavenging assays. **Results:** The optimum conditions for levan production were observed at 36 °C and pH 7 in 12% (w/v) sucrose for levansucrase Lsbl-bk1, while the optimum catalysis of levansucrase Lsbl-bk2 was obtained at 32 °C and pH 8 in the same sucrose concentration. The *in vitro* synthesized levan showed an antibacterial activity within a concentration range of 10-20% (w/v) against *Staphylococcus aureus*, *E. coli*, and *Pseudomonas aeruginosa*. The same levan was also able to inhibit the DPPH radical scavenging activity with the antioxidant strength of 75% compared to ascorbic acid inhibition. **Conclusion:** Our study, therefore, shows that the optimized heterologous expression of levansucrases encoded by Lsbl-bk1 and Lsbl-bk2 could open the way for industrial levan production as an antibacterial and antioxidant agent. **DOI: 10.29252/ibj.25.3.202**

Keywords: Antioxidants, Fructans, *In vitro* technique, Levan

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