

## Potensi bakteri endofitik ICM<sub>3</sub> sebagai penghasil enzim pullulanase untuk proses hidrolisis pati = The potency of an endophytic bacteria ICM<sub>3</sub> as pullulanase producer for starch hydrolysis

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### Abstrak

Sugar is a very important carbon and energy source for human. The local production of sugar in Indonesia is not adequate and alternative sources should be found. Microorganisms (*Bacillus amyloliquefaciens*, *B. licheniformis*, *B. cereus*, *B. circulans*, *B. megaterium*, *B. polymyxa*, *B. stearothermophilus*, *Pyrococcus woesei*, *P. furiosus*, *Clostridium thermosulfurogenes*, *C. thermohydrosulfuricum*, *Aspergillus awamori*, *A. niger*, *A. oryzae*, *A. saitoi*, *Mucor rouxianus*, *Penicillium oxalicum*, *Rhizopus delemar*, *Aerobacter aerogenes*, and *Streptomyces*) are known as producers of  $\alpha$ -amylase, glucoamylase, and pullulanase enzymes through starch fermentation which may be converted into a sugar compound. A preliminary study on endophytic bacteria proved their ability to grow on soluble starch, glutinous rice, and pullulan. Pullulanase converts pullulan to maltotriose. This enzyme may work synergistically with  $\alpha$ -amylase and with glucoamylase for a better conversion of starch to glucose. An endophytic bacteria ICM<sub>3</sub> obtained from the Research and Development Centre for Biotechnology LIP at Cibinong, Bogor was examined on its ability to produce pullulanase. For this purpose, soluble starch 1%, cassava starch 1%, and pullulan 1% (all w/v), were used as carbon and energy source in Bakshi medium (Bakshi et al., 1993). The concentration of the inoculum was  $1.25 \times 10^8$  cells/ml. Incubation was carried out at 30°C (room temperature) and 37°C (Mapiliandari, 1999), at pH 7.0 (Bakshi et al., 1993) and pH 5.0 (Mapiliandari, 1999). The fermentation process was terminated after 24 - 26 hours. The growth of ICM<sub>3</sub> varied depending on carbon source, temperature, and pH. The best growth was found on pullulan at pH 7.0 and incubation temperature of 30°C. However, when the pH of the medium was lowered to 5.0 (Mapiliandari, 1999) and the incubation temperature 30°C a higher cell number ( $79.5 \times 10^8$  cells/ml) was obtained on pullulan as carbon source. The bacteria was grown on cassava starch medium and the pullulanase activity studied. The synergism of pullulanase with  $\alpha$ -amylase and with glucoamylase to degrade cassava starch was also studied. To obtain the crude enzyme extract, the cell mass was centrifuged with a Sorval RC - 26 Plus centrifuge. The filtrate was then concentrated with UHF, sedimented with  $(\text{NH}_4)_2\text{SO}_4$ , and dialyzed with buffer Na-acetate (pH 4.8). Activity of the crude enzyme was examined on cassava starch and on pullulan. The unit activity of enzyme was 1.374 U/ml on cassava starch, 1.290 U/ml on pullulan, and the protein content was 0.039 mg/ml. The activity of the crude enzyme, after treatment with UHF, was 2.225 U/ml for pullulan, 2.527 U/ml for cassava starch, and the protein content was 0.014 mg/ml. The activity of the crude enzyme obtained after sedimentation with 60% saturation of  $(\text{NH}_4)_2\text{SO}_4$ , was 1.156 U/ml for pullulan, 1.162 U/ml for cassava starch, the protein content 0.579 mg/ml. After dialyzed with buffer Na-acetate (pH 4.8) the activity was 6.25 U/ml for pullulan, 6.45 U/ml for cassava starch with the protein content of 2.997 mg/ml. To study the optimum pH and temperature for the enzyme production, the isolate ICM<sub>3</sub> was grown on Bakshi medium with various pHs, : 4.0, 4.5, 4.8, 5.0, 5.5, 6.0, 6.5, 7.0 and incubated at various temperatures 30°C, 40°C, 50°C, 60°C, 70°C, 80°C, 90°C. The

optimum pH for enzyme synthesis on pullulan was 5.0 (4.81 U/ml) and on cassava starch 4.8 (13.27 U/ml). The optimum temperature for enzyme synthesis on pullulan was 40°C (26416 U/ml) and on cassava starch 50°C (22.34 U/ml). The best synergism of pullulanase with  $\alpha$ -amylase for both C sources was 25% (dilution of enzyme), while the synergism with glucoamylase was 100% for pullulan and 50% for cassava starch to convert the starch (pullulan and cassava starch) to glucose.