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Potensi bakteri endofitik ICMe3 sebagai penghasil enzim pululanase untuk proses hidrolisis pati = The potency of an endophytic bacteria ICMe3 as pullulanase producer for starch hydrolisis

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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 amyfoiiquefaciens, B. Iicheniformis, B. cereus, B. circulans, B. megaterium, B. polymyxa, B. stearothermophilus, Pyrococcus woeseg P. furiosus, Clostndium thermosulfurogenes, C. thermohydrosulfuricum, Aspergillus awamorL A. nigen A. oryzae, A. saitoil Mucor rouxianus, Penicillium oxalicum, Rhizopus deleman Aerobacter aerogenes, and Streptomyces) are known as producer ot on-amylase, glucoamylase, and pullulanase enzymes through of 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 convert pullulan to maltotriosa. This enzyme may work synergistically with on-amylase and with glucoamylase for a better conversion of starch to glucose. An endophytic bacteria lCMe3 obtained from the Research and Development Centre for Biotechnology LIP! at Cibinong, Bogor was examined on its ability to produce pullulanse _ For this purpose, soluble starch 1%, cassava starch 1%, and pullulan 1% (all wlv), were used as carbon and energy source in Bakshi medium (Bakshi etal., 1993). The concentration of the inoculum_was 1.25 x 10° 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 lCNle3 varied depending on carbon source, temperature, and pH. The best growth was found on pullulan at pH 7.0 and incubation temperature of 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) x 108 cells/ml was obtained on pullclan as carbon source. The bacteri was grown on cassava starch medium and the pullulanase activity studied. The synergism of pullulanase with 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 Hltrate was then concentrated with UHF, sedimented with (NH4)2SO4, and dialized with buffer Na-acetat (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 mglml. The activity of the crude enzyme, after treatment with UHF, was 2.225 U/ml for pullulan, 2.527 U/mt 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 (NH4)2SO4, was 1.156 U/ml for pullulan, 1.162 U/mi for cassava starch, the protein content 0.579 mg/ml. After dialysed 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 temperaturefor the enzyme production, the isolate iCMe3 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 sinthesis on puliulan 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 on-amylase for both C sources was 25% (dilution of enzyme), while the synergism with glucoamylase was 100% for pulluian and 50% for cassava starch to convere the starch (pullulanand cassava starch) glucose.