SYNERGISTIC FERULIC ACID PRODUCTION FROM BANANA STEM WASTE BY CO-CULTURE

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ABSTRAK

Penghasilan asid ferulik (FA) menggunakan mikrob kini telah diperluaskan kerana kandungan antioksidan yang terdapat di dalamnya. Kulat dan bakteria telah digunakan dalam penghasilan asik ferulik daripada sisa pertanian. Namun, tidak banyak penyelidikan yang telah dijalankan terhadap bakteria berbanding kulat. Oleh itu, teknik fermentasi yang sesuai oleh ko-kultur bakteria menggunakan sisa batang pisang (BSW) sebagai substrat telah dilaksanakan untuk meningkatkan penghasilan FA. Mikrob daripada tanah telah dipencilkan dan kajian awal dilaksanakan untuk memilih strain bakteria yang terbaik. Setelah itu, 26 jenis ko-kultur bakteria telah dihasilkan dan dinilai keupayaannya berdasarkan kandungan FA yang dihasikan. Pengoptimuman penghasilan FA telah dijalankan menggunakan kaedah gerak balas permukaan menggunakan ko-kultur yang terpilih sebagai inokulum. Kajian kinetik telah dijalankan untuk menentukan pemalar kinetik menggunakan persamaan Michaelis-Menten. Manakala, pengasaian enzim telah dijalankan untuk mengkaji mekanisma penghasilan FA melalui enzim hidrolisis. Eksperimen telah dilaksanakan melalui fermentasi tenggelam dan sampel dianalisis oleh HPLC untuk mengukur kandungan FA. Hasil kajian mendapati bahawa 5 daripada 46 strain bakteria yang dipencil menunjukkan kecekapan dalam membebaskan FA daripada BSW. Namun, penghasilan FA yang paling tinggi telah diperhatikan dalam ko-kultur A (*Bacillus cereus* CCM 2010, *Bacillus pumilus* SAFR-032 dan *Bacillus thuringiensis* Bt407), diikuti oleh ko-kultur B (*Bacillus cereus* CCM 2010, dan *Bacillus thuringiensis* Bt407). Dalam usaha menambah baik proses fermentasi, penyaringan oleh rekabentuk pecahan faktorial telah dijalankan dan hasil yang diperoleh menunjukkan hanya empat faktor termasuk pH, kadar putaran, jenis ko-kultur, dan jumlah inokulum mempunyai kesan yang nyata terhadap penghasilan FA. Selanjutnya, kadar putaran pada 150 rpm dan jumlah inokulum 5 % adalah optimum dalam mempengaruhi peningkatan hasil FA sehingga 510.24 mg/kg dalam tempoh 24 jam menggunakan ko-kultur B sebagai inokulum. Sementara itu, pemalar kinetik V_{max}, K_m dan K_s telah dilaporkan masing-masing sebagai 0.0003 g L^{-1} h^{-1}, 1.636 g L^{-1} dan 0.0095 h^{-1} dengan menggunakan kaedah Runge-Kutta Keempat. Manakala, aktiviti esterase asid ferulik (0.046 mU/mL) adalah maksimum semasa keadaan fasa pegun pertumbuhan ko-kultur. Hasil yang diperoleh membuktikan bahawa hidrolisis enzim telah berlaku dengan menguraikan dinding sel BSW dalam proses membebaskan FA. Penemuan dalam kajian ini mencadangkan bahawa ko-kultur bakteria berupaya merangsang kesan sinergistik dalam meningkatkan penghasilan FA daripada sisa batang pisang semasa proses fermentasi.

ABSTRACT

Ferulic acid (FA) production using microbes has currently become extensive owing to its antioxidant properties. Both fungi and bacteria have been used in producing ferulic acid from agricultural waste. However, less work has been done on bacteria compared to fungi. Therefore, an appropriate bacterial co-culture fermentation technique using banana stem waste (BSW) as substrate was implemented to improve FA production. Soil microbes were isolated and preliminary study was performed to select the best bacterial strain. Subsequently, 26 type of bacterial co-cultures were formed and evaluated their ability dependent on FA production. Optimization of the FA production was conducted by employing response surface methodology using the selected co-culture as inoculum. A kinetic study was carried out to determine kinetic constants using Michaelis-Menten equation. Meanwhile, the enzyme assay was performed to investigate the mechanism release of FA by enzymatic hydrolysis. Ferulic acid production was accomplished through submerged fermentation and the sample was analyzed by HPLC to quantify the FA content. The result found that 5 out of 46 isolated bacterial strains were efficient in releasing FA from BSW. However, the highest production of FA was observed in co-culture A (*Bacillus cereus* CCM 2010, *Bacillus pumilus* SAFR-032 and *Bacillus thuringiensis* Bt407), followed by co-culture B (*Bacillus cereus* CCM 2010, and *Bacillus thuringiensis* Bt407). In improving the fermentation process, screening by fractional factorial design was performed and the result obtained presented four factors including pH, rotation rate, type of co-culture, and volume of inoculum have significant effects on FA production. Furthermore, rotation rate at 150 rpm and volume of inoculum of 5 % were found to be optimum in increasing FA yield up to 510.24 mg/kg within 24 h using bacterial co-culture B as an inoculum. Meanwhile, the kinetic constant of V_{max}, K_m and K_s were reported as 0.0003 g L^{-1} h^{-1}, 1.636 g L^{-1} and 0.0095 h^{-1}, respectively using Runge-Kutta Forth method. Besides, the maximum activity of ferulic acid esterase (0.046 mU/mL) was detected in the stationary phase of co-culture growth. The result proved that the enzymatic hydrolysis was taking place to break down the cell wall of BSW in releasing FA. The finding of this study suggests that co-culture could induce the synergistic work in improving the FA production from banana stem waste during the fermentation process.