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EFFECTS OF SIGNAL PEPTIDES ON SECRETION OF THE BACILLUS G1 β- CYCLODEXTRIN GLUCANOTRANSFERASE IN LACTOCOCCUS LACTIS NZ9000

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By

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Cyclodextrin glucanotransferase, CGTase is an enzyme used in food and pharmaceutical industries to catalyze the formation of cyclodextrin (CD) from starch. CDs are of great interest because of their ability to form inclusion complexes with a guest molecule such as drug which would result in the physiochemical modification of this guest molecule. Although *Bacillus* and *Escherichia coli* are known workhorses for expression of heterologous proteins, the production of CGTase in these hosts eventually reduces the quality of the products with the presence of impurities such as proteases and endotoxins. Therefore, the production of CGTase using the food-grade lactic acid bacterium is an attractive alternative and safer strategy to produce CGTase for industrial and pharmaceutical uses. This study was aimed to develop genetically modified *Lactococcus*

lactis NZ9000 strains harboring plasmids that secrete the β-CGTase into the exterior environment. CGTase secretion with the presence of signal peptides namely, SPK1 from *Paediococcus pentosaceus* K1, USP45 from *L. lactis* MG1363 and NSP from *Bacillus* sp. G1 were analysed using SignaIP 4.0 software. From the prediction, SPK1 shows the highest protein grand average of hydropathy, GRAVY (the sum of hydropathy values of all amino acids divided by the protein length) of 1.552 followed by USP45 and NSP with 1.174 and 1.089, respectively. Vectors with different signal peptides fused with CGTase gene were constructed and transformed into *L. lactis* NZ9000. The formation of halo zones by the transformants on starch plate assay after 24 hr incubation indicated the production and secretion of β-CGTase. The expression of this enzyme in the transformants was further confirmed by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS PAGE) and zymogram analysis. A band size of ~75 kDa corresponding to β-CGTase was identified in the extracellular environments of the host after medium optimization. Interestingly, the replacement of glucose by starch in the medium was shown to induce β-CGTase production in *L. lactis*. The secreted β-CGTase was quantified by the CGTase assay. The use of different signal peptides shows difference in the secretion efficiency of β-CGTase. Although β-CGTase production was comparatively low in NZ:SPK1:CGT, the signal peptide SPK1 used for this strain was shown to have higher secretion efficiency of 49 % compared to the other two signal peptides used in this study which is in agreement with the computational analysis. In shake-flask fermentation, a maximum of 4.23 U/ml of CGTase was obtained at 8 hr of cultivation by NZ:SPK1:CGT. Nevertheless, at 7 hr a higher CGTase yield of 6.21 U/g of starch by NZ:USP:CGT was observed which was two times higher than that achieved

by NZ:SPK1:CGT (3.45 U/g of starch) and three times higher than NZ:NSP:CGT (2.36 U/g of starch). Higher CGTase productivity was achieved at 0.53 U/ml.h for both strains NZ:USP:CGT and NZ:SPK1:CGT.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah master sains

KESAN ISYARAT PEPTIDA KE ATAS PEREMBESAN β-SIKLODEKSTRIN GLUKANOTRANSFERASE *BACILLUS* G1 DI DALAM *LACTOCOCCUS LACTIS* NZ9000

oleh

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Siklodekstrin glukanotransferase, CGTase adalah enzim yang digunakan secara meluas dalam industri makanan dan farmaseutikal. Enzim ini diguna untuk menghasilkan siklodekstrin, CD daripada kanji. CD mendapat perhatian yang tinggi disebabkan oleh keupayaannya untuk membentuk kompleks khas bersama molekul asing seperti dadah. Pembentukan kompleks khas ini menyebabkan perubahan ciri-ciri fizikal dan kimia pada molekul asing tersebut. *Bacillus* dan *Escherichia coli* digunakan secara meluas dalam penghasilan CGTase, tetapi enzim yang dihasilkan adalah berkualiti rendah disebabkan oleh kewujudan benda asing seperti protease. Oleh sebab itu, *Lactococcus lactis* sebagai 'bakteria gred makanan' boleh digunakan sebagai perumah untuk menghasilkan CGTase

yang berkualiti tinggi untuk industri dan farmaseutikal. Tujuan kajian ini adalah untuk menghasilkan CGTase melalui pengklonan gen ke dalam vektor daripada *L. lactis* dengan gabungan peptida isyarat yang berbeza. Peptida isyarat seperti SPK1 daripada *Paediococcus pentosaceus* K1, USP45 daripada *L. lactis* MG1363 dan NSP daripada *Bacillus* sp. G1 dianalisis melalui perisian SignalP 4.0. Daripada jangkaan tersebut, SPK1 mencatatkan GRAVY (hasil bahagi jumlah nilai hydrophilic asid amino kepada panjang peptida) peptida yang tinggi iaitu 1.552 manakala USP45 and NSP masing-masing mencatatkan GRAVY peptida sebanyak 1.174 dan 1.089. Pembentukan zon halo oleh strain rekombinan di atas piring kanji membuktikan penghasilan dan perembesan CGTase oleh bakteria ini. Pengekpresan CGTase disahkan melalui teknik SDS-PAGE dan zymogram. Selepas medium kultur dioptimumkan, satu jalur bersaiz 75 kDa didapati dirembes ke luar medium. Penggantian glukosa kepada kanji di medium kultur, membolehkan penghasilan CGTase oleh *L. lactis*. Rembesan CGTase yang berbeza direkod dengan kehadiran peptida isyarat yang berlainan. Kuantiti enzim rekombinan yang dirembes ke medium dianalisis melalui ujian CGTase. Walaupun penghasilan enzim yang rendah dicatatkan bagi NZ:SPK1:CGT, peptida isyarat SPK1 menunjukkan kecekapan rembesan yang tinggi iaitu 49% berbanding dengan rekombinan strain yang lain seperti didapati dalam jangkaan SignalP 4.0. Peptida isyarat untuk mengekspresi CGTase ke medium kultur dikenal pasti dalam kajian ini. Selain daripada peptida isyarat NSP dan USP45, SPK1 daripada *Paediococcus pentosaceus*, K1 dapat membantu perembesan CGTase oleh *L. lactis*. Penghasilan maksimum CGTase (4.23 U/ml) dicatatkan pada jam ke-8 pengkulturan oleh NZ:SPK1:CGT melalui fermentasi kelalang goncang. Manakala, hasil CGTase yang tinggi 6.21 U/g kanji , diperoleh bagi strain

NZ:USP:CGT pada jam ke-7 iaitu dua kali ganda daripada NZ:SPK1:CGT dan tiga kali ganda daripada NZ:NSP:CGT (2.36 U/g kanji). Produktiviti CGTase yang tinggi sebanyak 0.53 U/mL.h dicapai oleh kedua-dua strain rekombinan NZ:USP:CGT dan NZ:SPK1:CGT.

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APPROVAL

I certify that an examination committee has met on 9 January 2013 to conduct the final examination of Menaga A/P Subramaniam on her degree of Master of Science thesis entitled "Effects of signal peptides on the secretion of the *Bacillus* sp. G1 cyclodextrin glucanotransferase in *Lactococcus lactis* NZ9000" in accordance with Universiti Putra Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The committee recommends that the student be awarded the degree of Master of Science.

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DECLARATION

I declare that the thesis is based on my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at Universiti Putra Malaysia or at any other institutions.

MENAGA A/P SUBRAMANIAM

Date: 9 January 2013

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