



UNIVERSITI PUTRA MALAYSIA

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

MENAGA A/P SUBRAMANIAM

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CYCLODEXTRIN GLUCANOTRANSFERASE IN *LACTOCOCCUS LACTIS*
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By

MENAGA A/P SUBRAMANIAM

**This thesis submitted to the School of Graduate Studies, Universiti Putra Malaysia, in
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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfilment
of the requirement for the degree of Master of Science

**EFFECTS OF SIGNAL PEPTIDES ON SECRETION OF THE *BACILLUS* G1 β -
CYCLODEXTRIN GLUCANOTRANSFERASE IN *LACTOCOCCUS LACTIS*
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Chairman: Professor Raha binti Abdul Rahim, PhD

Faculty: Biotechnology and Biomolecular Sciences

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 SignalP 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 jangkauan 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. Pengekspresan 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 jangkauan 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

TABLE OF CONTENTS

	Page
ABSTRACT	ii
ABSTRAK	v
ACKNOWLEDGEMENTS	viii
APPROVAL	ix
DECLARATION	xi
LIST OF TABLES	xvi
LIST OF FIGURES	xvii
LIST OF ABBREVIATIONS	xix
CHAPTER	
1 INTRODUCTION	1
2 LITERATURE REVIEW	4
2.1 Cyclodextrin glucanotransferase	4
2.1.1 CGTase producer	5
2.1.2 General properties of CGTase	5
2.1.3 Catalytic reaction of CGTase	6
2.1.4 Applications of CGTase in Biotechnology	8
2.1.5 Cloning of CGTase gene	9
2.1.6 CGTase from <i>Bacillus</i> sp. G1	9
2.2 Cyclodextrin	10
2.2.1 Properties of CD	10
2.2.2 CD application	13
2.3 Lactic acid bacteria	16
2.3.1 <i>Lactococcus lactis</i>	17

2.3.2	Gene cloning and Expression in <i>L. lactis</i>	18
2.3.3	Application of the NICE system	21
2.4	Protein secretion in <i>L. lactis</i>	22
2.4.1	Protein secretion	22
2.4.2	Signal peptide	26
2.5	Fermentation: Large scale protein production	27
3	METHOD AND MATERIALS	29
3.1	Bacterial strains, plasmid, media and growth condition	29
3.2	Plasmid extraction	31
3.2.1	pET:CGT	31
3.2.2	pNZ8048	32
3.3	Genomic extraction	32
3.4	Agarose gel electrophoresis	33
3.5	Polymerase chain reaction	34
3.5.1	PCR amplification of CGTase genes	36
3.5.2	PCR amplification of signal peptide USP45 and SPK1	37
3.6	PCR product purification	37
3.7	Contig construction	38
3.8	Ligation of reporter cassettes in expression vector, pNZ8048	40
3.9	Competent cell preparation	45
3.10	Transformation into <i>L. lactis</i> NZ9000	46
3.11	Verification of recombinant plasmid	46
3.12	Starch plate assay	47
3.13	Growth study	47
3.14	Plasmid stability test	48
3.15	Medium optimisation	48
3.16	Nisin optimisation	49
3.17	Induction interval	49
3.18	Protein induction and extraction	49

3.18.1	Extracellular fraction	50
3.18.2	Intracellular fraction	50
3.19	Protein purification	50
3.20	Quantification of recombinant proteins	51
3.21	SDS-PAGE	51
3.22	Zymogram analysis	52
3.23	Assay of CGTase	52
3.24	Fermentation	53
3.24.1	OD Measurement	53
3.24.2	Residual starch analysis	53
3.24.3	Glucose analysis	54
4	RESULTS AND DISCUSSION	
4.1	Signal peptide prediction	55
4.2	Polymerase chain reaction (PCR)	58
4.2.1	PCR amplification of signal peptides USP45 and SPK1	58
4.2.2	PCR amplification of CGTase gene	59
4.3	Construction of secretion cassettes	60
4.3.1	Construction of secretion cassettes	60
4.4	Cloning of expression cassettes into pNZ8048 expression vector	63
4.4.1	Cloning of CGTase gene (without native signal peptide) into <i>L. lactis</i> by using pNZ8048	63
4.5	Verification of recombinant <i>L. lactis</i> NZ9000 clones harboring expression vector with secretion cassettes	64
4.5.1	Colony PCR	64
4.5.2	Recombinant plasmid extraction	66
4.5.3	Restriction enzyme digestion	66
4.5.3.1	RE digestion of pNZ:CGT and pNZ:NSP:CGT	66

4.5.3.2	RE digestion of pNZ:USP:CGT	67
4.6	Stability of recombinant strains	69
4.7	Starch plate assay	71
4.8	Protein expression analysis for recombinant clones	73
4.8.1	SDS-PAGE analysis of recombinant CGTase	73
4.8.1.1	Medium optimization	75
4.8.1.2	Nisin concentration	80
4.8.1.3	Induction interval	83
4.8.1.4	CGTase induction at optimum condition	85
	and Zymogram analysis	
4.9	Quantification of CGTase	87
4.10	Batch Fermentation	91
5	CONCLUSION AND RECOMMENDATIONS	96
	REFERENCES	98
	APPENDICES	111
	BIODATA OF STUDENT	135
	LIST OF PUBLICATIONS	136