

CONTENTS

	PAGE
ENGLISH ABSTRACT	ii
THAI ABSTRACT	v
ACKNOWLEDGEMENTS	viii
CONTENTS	ix
LIST OF TABLES	xiii
LIST OF FIGURES	xv
LIST OF ABBREVIATIONS	xix
CHAPTER	
1. INTRODUCTION	1
1.1 Background	1
1.2 Objectives	2
1.3 Scopes	2
1.4 Benefits	3
2. LITERATURE REVIEW	4
2.1 Biomass	4
2.1.1 Biomass overview	4
2.1.2 Biomass components	4
2.1.3 Biomass conversion	8
2.2 Plant cell wall	10
2.2.1 Plant cell wall compositions	11
2.2.2 Plant cell wall polysaccharide biodegradation	20
2.2.3 Microorganisms producing plant cell wall polysaccharide degrading enzymes	26
2.2.4 Plant cell wall polysaccharide degrading enzymes	30
2.2.5 Application of plant cell wall polysaccharide degrading enzymes	41
2.3 Prokaryotic cellulase enzyme system	62

	PAGE
2.3.1 Free enzymes	62
2.3.2 Cell-bound enzymes	63
2.3.3 Multi-functional enzymes	64
2.3.4 Multienzyme complexes	66
2.4 Concept of multienzyme complex	68
2.4.1 Cellulosome component	72
2.4.2 Cohesin-dockerin interaction	85
2.4.3 Bacterial adhesion to cellulose	86
2.4.4 Carbon source regulation of cellulosome production	88
2.4.5 Biotechnological uses of cellulosomes	91
2.4.6 Designer cellulosomes-construction of function-specific cellulosomes	95
2.4.7 Improving cellulosomal properties	98
2.5 Bioconversion of plant cell wall polysaccharide by microbial combination	98
3. MATERIALS AND METHODS	102
3.1 Culture medium	102
3.2 Carbon sources	102
3.3 Corn hull component analysis	102
3.4 Preparation of insoluble xylan	103
3.5 Sampling procedure	103
3.6 Screening and isolation of thermophilic anaerobic biomass degrading strains	103
3.7 Isolation of genomic DNA	104
3.8 Detection of Contamination by PCR Assay	104
3.9 16S rRNA gene analysis and phylogenetic analysis	104
3.10 Nucleotide sequence accession number	105
3.11 Bacterial strains and growth condition	105
3.12 Effect of temperature on cell growth	106
3.13 Characterization of <i>T. thermosaccharolyticum</i> NOI-1	106
3.14 Adhesion of bacterial bells to insoluble substances	106

	PAGE
3.15	Fermentation products analysis 107
3.16	Protein determination 107
3.17	Enzyme production 107
3.18	Enzyme assays 108
3.19	Gel electrophoresis and zymograms 109
3.20	Effect of pH and temperature on enzyme activity and stability 110
3.21	Binding of enzyme to insoluble substances 111
3.22	Isolation of multienzyme complex 111
3.23	Hydrolysis of xylan and biomass 112
3.24	Thin layer chromatography 112
3.25	Combination of cellulolytic enzyme <i>C. thermocellum</i> NKP-2 and xylanolytic enzymes from <i>T. thermosaccharolyticum</i> NOI-1 113
4.	STUDY ON COEXISTENCE OF TWO BACTERIAL STRAINS 115
4.1	Stable coexistence of cellulolytic and non-cellulolytic bacteria on biomass degrading enzyme production 115
4.1.1	Introduction 115
4.1.2	Results and discussion 116
4.2	Isolation and characterization of cultivable members of NKP and study of behavior symbiosis on corn hull cultivation 125
4.2.1	Introduction 125
4.2.2	Results and Discussion 126
5.	STUDY ON ENDOCELLULASE-FREE MULTIENZYME COMPLEX LIKE XYLANOSOME 147
5.1	Introduction 147
5.2	Results and Discussion 148
5.2.1	Characterization of bacterium, the strain NOI-1 148
5.2.2	Characterization of crude enzymes from <i>T. thermosaccharolyticum</i> strain NOI-1 152

	PAGE
5.2.3 Isolation, purification and characterization of endocellulase-free multienzyme complex from <i>T. thermosaccharolyticum</i> strain NOI-1	159
6. CONCLUSIONS AND SUGGESTIONS	166
REFERENCES	169
APPENDIX	211
Appendix A	211
Appendix B	213
CURRICULUM VITAE	218

LIST OF TABLES

TABLE	PAGE
2.1 Lignocellulosic constituents of some biomass	6
2.2 Representative cellulolytic microbes isolated from diverse natural ecosystems	27
2.3 Microorganisms having xylanolytic abilities	29
2.4 Microorganisms having pectinolytic enzymes	30
2.5 Enzymes involved in the hydrolysis of complex heteroarabinoxylans	34
2.6 The hemicellulolytic enzymes, their classification into glycosyl hydrolase (GH) and carbohydrate esterase (CE) families	38
2.7 Classification of pectinolytic enzymes	41
2.8 Applications of cellulases in various industries	51
2.9 Commercial preparations of xylanases	58
2.10 Cellulosome and cellulosome-like multienzyme complexes from anaerobic and aerobic microorganisms	71
2.11 CBM structures	76
2.12 CBM fold families	77
2.13 CBM types	77
2.14 Cellulosomal enzymes of clostridia	83
4.1 Growth and clear zone formation of anaerobic thermophilic biomass degrading bacteria	117
4.2 Comparison of cellulolytic-xylanolytic activities from the NPK	121
4.3 Characterization of coexistence bacteria (NKP) and cultivable members of its, cellulolytic bacterium strain NKP-2 and non-cellulolytic bacterium strain NOI-1	127
4.4 Homology with bacterium strain NKP-2 in a phylogenetic analysis based on the 16s rRNA sequence	130
4.5 Homology with bacterium strain NOI-1 in a phylogenetic analysis based on the 16s rRNA sequence	130
4.6 Cellulolytic and xylanolytic enzyme activities of <i>C. thermocellum</i> NKP-2 and <i>T. thermosaccharolyticum</i> NOI-1 on corn hull cultivation	134

TABLE	PAGE
4.7 Comparison of cellulolytic-xylanolytic enzyme activities from <i>T.thermosacchrolyticum</i> NOI-1 and <i>C. thermocellum</i> NKP-2	139
4.8 Relationships between ratio and synergism of enzymes from <i>T. thermosaccharolyticum</i> NOI-1 and from <i>C. thermocellum</i> NKP-2 on hydrolysis of corn hull	143
4.9 Reducing sugars released from lignocellulosic material hydrolysis by alone and combination	144
4.10 Composition of the lignocellulosic materials	145
5.1 Utilization of carbon sources for the strain NOI-1 compared with other strains of <i>T. thermosaccharolyticum</i>	151
5.2 Enzymatic activities of culture supernatant (extracellular protein) and pellet-bound protein from <i>T. thermosacharolyticum</i> strain NOI-1	154
5.3 Enzymatic activities of crude enzyme and isolated multienzyme complex	161

LIST OF FIGURES

FIGURE	PAGE
2.1 Structure of plant biomass	5
2.2 Main conversion options for biomass to secondary energy carriers	9
2.3 Microfibril cellulose in plant cell wall	12
2.4 Schematic structure of corn fiber heteroxylan	14
2.5 Composition of O-acetyl-4-O-methylglucuronoxylans	15
2.6 Composition of arabino-4-O-methylglucuronoxylans	15
2.7 Structure of xyloglucan; principal component of the hemicelluloses	16
2.8 The structure of locust bean gum	17
2.9 Structure of pectin	19
2.10 Partial structure of hardwood lignin	20
2.11 Enzyme systems involved in the degradation of cellulose	21
2.12 The basic structural components found in hemicellulose and the hemicellulases responsible for their degradation	23
2.13 Structure of pectin and enzymatic sites for pectin lyase	25
2.14 Overall folds of recently solved structures of hemicellulases and hemicellulose-binding modules	39
2.15 Hypothetic model for the attachment of exocellular proteins from <i>Thermoanaerobacterium thermosulfurigenes</i> EM1 to the cell envelope via their SLH domains	63
2.16 A very large, cell-surface enzyme	64
2.17 A very large, multimodular xylanase from <i>Caldicellulosiruptor</i>	66
2.18 Schematic representation of the hydrolysis of amorphous and microcrystalline cellulose by noncomplexed and complexed cellulase systems	67
2.19 Simplified schematic of the hydrolysis of amorphous and microcrystalline celluloses by non-complexed and complexed cellulase systems	68
2.20 Simplified schematic of general cellulosome components	69

FIGURE	PAGE
2.21 Schematical presentation of the cellulosome component of <i>Clostridium thermocellum</i>	73
2.22 The modular structure of scaffoldins from various microorganisms	74
2.23 The big picture: CBMs, shown as ribbon structures, grouped as fold families and functional types	78
2.24 Model for the interaction of families 1, 2 and 3 CBMs from Type A CBMs with cellulose	79
2.25 Schematic diagram of the family 4 CBM from the N-terminus of Cel9B from <i>Cellulomonas fimi</i>	79
2.26 Schematic diagram of family 13 CBM from <i>Sreptomycelividans</i>	80
2.27 Hypothetical model for attachment of EngE to the CbpA of <i>C. cellulovorans</i> and the cell surface	81
2.28 Three-dimensional crystal structure of the <i>C. thermocellum</i> cohesin-dockerin heterodimer	86
2.29 Adherence of mixed rumen bacteria to plant material	87
2.30 Ultrastructure of the <i>Clostridium thermocellum</i> cell surface	88
2.31 Scanning electron micrographs (x22000) of representative cells	89
2.32 Scanning electron micrograph (x22000) of representative cells of cellulose-grown <i>Eubacteriumcellulosolvens</i>	89
2.33 CBM-based expression and purification of recombinant proteins	92
2.34 CBM-based pathogen detection system	93
2.35 Designer cellulosome	96
2.36 A model of a designer mini-cellulosome	97
2.37 Organism development strategies and related fundamentals	101
4.1 Degradation pattern on corn hull and cellulose by NKP	117
4.2 Growth of the NKP on BM media contained 1% Avicel at 37 °C to 80 °C	118
4.3 Time courses of fermented products from the NKP on Avicel cultivation	119
4.4 Profiles of remained Avicel and enzyme production by the NKP	120
4.5 Zymogram analysis for CMCCase and xylanase activities in culture supernatants of day 7 culture produced by the NKP	122
4.6 Effects of temperature on activity and stability of CMCCase and xylanase of crude extracellular enzymes from the NKP	123

FIGURE	PAGE
4.7 Reducing sugar content of biomass after hydrolysis with crude extracellular enzymes from the NKP	124
4.8 Specific PCR for <i>C. thermocellum</i> and <i>T. thermosaccharolyticum</i>	128
4.9 Agarose gel electrophoresis of PCR product amplification with EUB8f and U1492r primer sets	129
4.10 Phylogenetic tree showing the phylogenetic position of the isolated strain NOI-1	132
4.11 Time courses of cell growth and remaining corn hulls of <i>C. thermocellum</i> NKP-2, <i>T. thermosaccharolyticum</i> NOI-1 and the co-culture of both strains	136
4.12 Thin layer chromatography of sugars from the culture supernatant	137
4.13 Fermentation end-products of individual-culture and co-culture of <i>T. thermosaccharolyticum</i> NOI-1 and <i>C. thermocellum</i> NKP-2 grown on corn hulls	138
4.14 Corn hull hydrolysis time of individual and combination of crude enzyme	141
5.1 SEM of morphology and cell surface structure of <i>T. thermosaccharolyticum</i> strain NOI-1	149
5.2 Effect of pH and temperature on cell growth using OSX as sole a carbon source	150
5.3 Fermented products in culture medium of OSX-grown cells at stationary phase	152
5.4 Time courses of xylanase production of the strain NOI-1	153
5.5 Binding abilities of crude enzyme from the strain NOI-1	155
5.6 Patterns of xylanase activities by zymogarm gel electrophoresis from pellet-bound protein and extracellular protein	156
5.7 Effect of pH and temperaturre on activity and stability of xylanase	157
5.8 Time course of hydrolysis of pure xylans by crude enzyme from <i>T. thermosaccharolyticum</i> NOI-1	158
5.9 Thin layer chromatography of the hydrolysis products of xylan by crude xylanase	159
5.10 Gel filtration chromatography on Sephacryl S-300 column of the isolated multienzyme complex of the strain NOI-1	160

FIGURE	PAGE
5.11 Time courses of the hydrolysis of OSX	162
5.12 Effect of pH and temperature on the activity and stability of the isolated multienzyme complex	163
5.13 Patterns of proteins and xylanase and CMCase activities by gel electrophoresis	164
A. 1 The 16S rDNA sequence of strain NOI-1	211
A. 2 The 16S rDNA sequence of strain NKP-2	212
B. 1 Standard curve of bovine serum albumin (BSA) solution	213
B. 2 Standard curve of xylose solution	214
B. 3 Standard curve of glucose solution	214
B. 4 Standard curve of <i>p</i> -nitrophenol solution	215
B. 5 Standard protein pattern of 10% SDS-PAGE	216
B. 6 Standard protein pattern of gel filtration	217
B. 7 Standard curve of Standard protein for gel filtration	217

LIST OF ABBREVIATIONS

°C	= Degree celcius
CBD	= Cellulose-binding domain
CBMs	= Carbohydrate-binding modules
CE	= Carbohydrate esterase
CMC	= Carboxymethylcellulose
CMCase	= Carboxymethylcellulase
Coh	= Cohesin
d	= Day
Da	= Dalton
DNA	= Deoxyribonucleic acid
DP	= Degree of polymerization
g	= Gram
GH	= Glycosyl hydrolase
h	= Hour
HLD	= Hydrophillic domain
kDa	= Kilodalton
µg	= Microgram
µl	= Microliter
M	= Molar
mg	= Milligram
min	= Minute
mM	= Millimolar
nm	= Nanometer
OD	= Optical Density
PBS	= Phosphate buffer saline
PCR	= Polymerase Chain Reaction