

**ISOLATION OF CELLULOSE-DEGRADING BACTERIA FROM
TERMITES *Microcerotermes* sp.**



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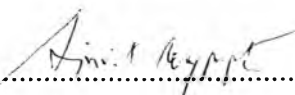

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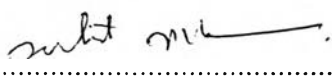
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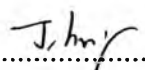

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ABSTRACT

5073003063: Petroleum Technology Program
Kitipong Taechapoempol: Isolation of Cellulose-Degrading Bacteria
from Termites *Microcerotermes sp.*

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Bacillus subtilis/ 1-Butyl-3-Methylimidazolium Chloride

The main problem of using cellulose as a feedstock for direct bio-hydrogen production by a fermentative microorganism is the inefficiency of the process, because the hydrogenase enzyme cannot access the internal surface of the cellulose and convert it to hydrogen. To solve this problem, the accessibility of the hydrogenase enzyme was increased by converting cellulose to glucose by using a cellulase enzyme, which is generated from bacteria, before the fermentation process. In this research, cellulase-producing bacteria were isolated from higher termites, *Microcerotermes sp.* Three effective strains of *Bacillus subtilis* (A 002, F 018, and M 015) were identified and studied for the specific cellulase activities—endoglucanase, exoglucanase, and β -glucosidase—at 37°C and pH 7.2. The results showed that strain F 018 had the highest specific exoglucanase activity and β -glucosidase activity; and strain M 015 had the highest specific endoglucanase activity. In addition, all three strains were also tested for their tolerance to the presence of the ionic liquid, 1-butyl-3-methylimidazolium chloride, or [BMIM]Cl, which was used to enhance the accessibility of the cellulase enzyme in the pretreatment step. All strains were able to tolerate the [BMIM]Cl in the range of 0.1 to 1.0 vol%.

บทคัดย่อ

กิติพงษ์ เตชะเพิ่มผล: การคัดแยกจุลินทรีย์ที่สามารถย่อยสลายเซลลูโลสจากปลวก สายพันธุ์ *Microcerotermes sp.* (Isolation of Cellulose-Degrading Bacteria from Termites *Microcerotermes sp.*) อ.ที่ปรึกษา: รศ. ดร.ปราโมช รังสรรค์วิจิตร, รศ. ดร.สุเมธ ชวเดช, ผศ. ดร. ชรรมนุญ ศรีทะวงศ์ และ รศ. ดร.ศิริรัตน์ เร่งพิพัฒน์ 101 หน้า

ปัญหาสำคัญที่เกิดจากการใช้เซลลูโลสเป็นวัตถุดิบในการผลิตก๊าซไฮโดรเจนโดยตรง ด้วยกระบวนการหมักของจุลินทรีย์คือประสิทธิภาพในการผลิตก๊าซไฮโดรเจนที่ต่ำ เนื่องจากเอนไซม์ไฮโดรจีเนสไม่สามารถเข้าถึงพื้นผิวภายในของเซลลูโลสและเปลี่ยนเซลลูโลสให้เป็นก๊าซไฮโดรเจนได้ การแก้ปัญหาที่เกิดขึ้นทำได้โดยการเพิ่มความสามารถในการเข้าถึงของเอนไซม์ไฮโดรจีเนสในขั้นตอนการหมักด้วยการเปลี่ยนเซลลูโลสให้เป็นน้ำตาลกลูโคสก่อนโดยอาศัยเอนไซม์เซลลูเลสที่สร้างขึ้น โดยแบคทีเรีย งานวิจัยนี้ศึกษาการคัดแบคทีเรียที่สร้างเอนไซม์เซลลูเลสจากปลวกชั้นสูงสายพันธุ์ *Microcerotermes sp.* โดยแบคทีเรียชนิด *Bacillus subtilis* ที่มีประสิทธิภาพดี 3 สายพันธุ์ คือ A 002 F 018 และ M 015 ได้ถูกพิสูจน์เอกลักษณ์และศึกษาค่าแอกทิวิตีจำเพาะของเอนไซม์เซลลูเลสที่ประกอบไปด้วยเอนไซม์ย่อย 3 ชนิดด้วยกันคือ เอนไซม์เอนโดกลูคาเนส เอนไซม์เอกโซกลูคาเนส และเอนไซม์เบตาไกลูโคซิเดส ที่อุณหภูมิ 37 องศาเซลเซียส และค่าความเป็นกรด-ด่างที่ 7.2 จากการทดลองพบว่า แบคทีเรียสายพันธุ์ F 018 มีค่าแอกทิวิตีของเอนไซม์เอกโซกลูคาเนสและเอนไซม์เบตาไกลูโคซิเดสสูงที่สุด แบคทีเรียสายพันธุ์ M 015 มีค่าแอกทิวิตีของเอนไซม์เอนโดกลูคาเนสสูงที่สุด นอกจากนี้ยังได้ทดสอบความทนทานของแบคทีเรียทั้ง 3 สายพันธุ์ต่อสารกลุ่ม ionic liquid คือ 1-butyl-3-methylimidazolium chloride หรือ [BMIM]Cl ที่ใช้ในขั้นตอนการเตรียมเบื้องต้น เพื่อช่วยให้เอนไซม์เซลลูเลสเข้าถึงพื้นผิวภายในของเซลลูโลสได้มากขึ้น จากการทดสอบพบว่า แบคทีเรียทั้ง 3 สายพันธุ์สามารถทนต่อสาร [BMIM]Cl ในช่วงความเข้มข้น 0.1 ถึง 1.0 เปอร์เซ็นต์โดยปริมาตร

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TABLE OF CONTENTS

	PAGE
Title Page	i
Abstract (in English)	iii
Abstract (in Thai)	iv
Acknowledgements	v
Table of Contents	vi
List of Tables	x
List of Figures	xi
CHAPTER	
I INTRODUCTION	1
II LITERATURE REVIEW	3
III EXPERIMENTAL	23
3.1 Materials	23
3.1.1 Media	23
3.1.2 Chemicals	23
3.1.3 Identification Kits	25
3.2 Instrument and Apparatus	26
3.3 Methodology	27
3.3.1 Termite Sample Collection	27
3.3.2 Nomenclature and Classification of Termites	27
3.3.3 Screening of Cellulose-Degrading Bacteria	27
3.3.3.1 Isolation of Cellulase-Producing Bacteria from Lower Termites	27
3.3.3.2 Isolation of Cellulase-Producing Bacteria from Higher Termites	29

CHAPTER	PAGE
3.3.3.3 Determination of Hydrolysis Capacity Value (HC value)	30
3.3.4 Cellulase Enzyme Activity Assay	30
3.3.4.1 Preparation of Crude Enzyme for Enzymatic Assay	30
3.3.4.2 Quantitative of Cellulase Enzyme Assay	31
3.3.5 Determination of the Specific Growth Rate (μ) and Tolerance of Cellulase-Producing Bacteria in the Presence of Ionic Liquid	35
3.3.6 Nomenclature and Classification of Bacteria from Determinative Bacteriology	36
3.3.6.1 Preliminary Identification by Microbiological Methods	36
3.3.6.2 Preliminary Identification by Biochemical Methods	36
3.3.6.3 DNA base composition	36
IV RESULTS AND DISCUSSION	38
4.1 Nomenclature and Classification of Termites	38
4.2 Screening of Cellulose-Degrading Bacteria	39
4.2.1 Isolation of Cellulase-Producing Bacteria from Termites	39
4.3 Determination of Hydrolysis Capacity Value (HC value)	41
4.4 Specific Cellulase Enzyme Activity Assay	47
4.4.1 Effect of Temperature on Specific Cellulase Enzyme Activity of Cellulase-Producing Bacteria	47
4.4.1.1 Strain A 002	47
4.4.1.2 Strain M 015	49
4.4.1.3 Strain F 018	51

CHAPTER	PAGE
4.4.2 Comparison of Specific Cellulase Activity between Three Effective Isolates at the Optimum Temperature	53
4.4.2.1 Specific endoglucanase activity	53
4.4.2.2 Specific exoglucanase activity	54
4.4.2.3 Specific β -glucosidase activity	55
4.5 Determination of the Specific Growth Rate (μ) and Tolerance of Cellulase-Producing Bacteria in the Presence of [BMIM]Cl	56
4.5.1 Tolerance of the Cellulase-Producing Bacteria in the Presence of [BMIM]Cl	56
4.5.2 Specific Growth Rate of Cellulase-Producing Bacteria in the Presence of [BMIM]Cl	59
4.6 Nomenclature and Classification of Bacteria from Determinative Bacteriology	60
4.6.1 Preliminary Identification by Microbiological Methods	60
4.6.2 Preliminary Identification by Biochemical Methods	62
4.6.3 DNA Base Composition	65
4.6.3.1 16S rDNA of strain A 002	65
4.6.3.2 16S rDNA of strain M 015	67
4.6.3.3 16S rDNA of strain F 018	69
V CONCLUSIONS AND RECOMMENDATIONS	72
5.1 Conclusions	72
5.2 Recommendations	73
REFERENCES	74

CHAPTER	PAGE
APPENDICES	77
Appendix A Media for microorganisms	77
Appendix B Reagent Preparations	80
Appendix C Standard Calibration Curves	85
Appendix D Calculations	87
Appendix E Experiment data of cellulose-degrading bacteria	89
CURRICULUM VITAE	101

LIST OF TABLES

TABLE		PAGE
2.1	Some cellulase-producing bacteria, which are classified by oxygen relationship	8
2.2	Physicochemical properties of some selected ion liquids sorted by anion	13
2.3	Solubility of cellulose in some ionic liquids	16
4.1	Number of pure isolates and number of clear-zone producing isolates from <i>Schedorhinotermes sp.</i>	40
4.2	Number of pure isolates and number of clear-zone producing isolates from <i>Microcerotermes sp.</i>	40
4.3	HC values of cellulase-producing bacteria isolated by the aerobic condition	42
4.4	HC values of cellulase-producing bacteria isolated by 2 steps anaerobic condition	43
4.5	HC values of cellulase-producing bacteria isolated by aerobic with anaerobic pretreatment step condition	44
4.6	Effective isolates classified by the isolation conditions	45
4.7	Preliminary identification of strain A 002, M 015 and F 018 by microbiological methods	61
4.8	API 50 CHB biochemical characteristics of three effective isolates	63
4.9	API 20 E biochemical characteristics of three effective isolates	65

LIST OF FIGURES

FIGURE		PAGE
2.1	Cellulose structure	3
2.2	Mechanistic scheme of enzymatic cellulose hydrolysis by <i>Trichoderma</i> , non-complexed cellulase system	6
2.3	Most commonly used cation structures and possible anion types	11
2.4	Structure of 1-butyl-3-methylimidazolium chloride, [BMIM]Cl	17
2.5	SEM micrographs of the initial dissolving pulp and after dissolution in [BMIM]Cl and regeneration into water	17
2.6	Thermal decomposition profiles of (i) regenerated cellulose and (ii) original dissolving pulp	18
2.7	Complex of 1-butyl-3-methylimidazolium chloride with cellobiose	19
2.8	XRD patterns for IL-treated and untreated-cellulose	20
2.9	Cellulose samples of 5% (□), 10% (○), 15% (Δ), and 30% (∇) were incubated for 30 min in [BMIM]Cl at 120°C, and precipitated with deionized water	21
3.1	Procedure of endoglucanase activity assay	32
3.2	Procedure of exoglucanase activity assay	33
3.3	Procedure of β-glucosidase activity assay	34
3.4	Procedure of protein determination of each supernatant	35
4.1	Lower termites, <i>Schedorhinotermes sp.</i>	38
4.2	Higher termites, <i>Microcerotermes sp.</i>	39
4.3	Bacterial colony of isolate strain F 002 on 65 modified DSMZ agar medium 2 before detecting the clear-zone	41

FIGURE	PAGE
4.4 Clear-zone around the bacterial colony of isolate strain F 002 on 65 modified DSMZ agar medium 2	42
4.5 Isolate strain A 002	45
4.6 Isolate strain M 015	46
4.7 Isolate strain F 018	46
4.8 Specific cellulase enzyme activity of strain A 002 at pH 7.2 at various temperatures (37, 40, 45, and 50°C)	48
4.9 Specific cellulase enzyme activity of strain M 015 at pH 7.2 at various temperatures (37, 40, 45, and 50°C)	50
4.10 Specific cellulase enzyme activity of strain F 018 at pH 7.2 at various temperatures (37, 40, 45, and 50°C)	52
4.11 Comparison of specific endoglucanase activity between strain A 002, M 015, and F 018 at 37°C, and pH 7.2 for 24 hours	54
4.12 Comparison of specific exoglucanase activity between strain A 002, M 015, and F 018 at 37°C and pH 7.2 for 24 hours	55
4.13 Comparison of specific β -glucosidase activity between strain A 002, M 015, and F 018 at 37°C for 24 hours	56
4.14 Growth curve at 37°C and pH 7.2 of strain A 002 in various concentrations of [BMIM]Cl	57
4.15 Growth curves at 37°C and pH 7.2 of strain M 015 at various concentrations of [BMIM]Cl	58
4.16 Growth curves at 37°C and pH 7.2 of strain F 018 at various concentrations of [BMIM]Cl	58
4.17 Relationships between specific growth rate (μ) of strain A 002, M 015, and F 018 with various concentrations of [BMIM]Cl	60