IDENTIFICATION OF CELLULOSE DEGRADING BACTERIA

MUHAMMAD AL AMIN BIN MOHAMED SAUPI

Final Year Project Report Submitted In Partial Fulfilment of The Requirements for the Degree of Bachelor of Science (Hons.) Biology In The Faculty of Applied Sciences Universiti Teknologi MARA

JULY 2016

ACKNOWLEGDEMENTS

Bismillahirrahmanirrahim,

In the name of Allah, The Most Gracious and The Most Merciful, Alhamdulillah, all praises to Allah for the strength and His blessing in completing this thesis. I am using this opprtunity to express my special appreciation and thanks to my lovely supervisor, Miss Siti Suhaila Bt Harith and Madam Marlina Bt Mohd Mydin, you have been a tremendous mentor for me. I would like to thank you for encouraging my research and for allowing me to grow as a good researcher. Your advice and guidance have been so priceless to me. You showed me the direct path in order to complete this final year project for two semesters. Only Allah can pay for all your kindness for everything you did to me. Besides that, I would like to acknowledge with much appreciation the crucial role of the staff of laboratory assistance, who gave the permission to use all required equipment and necessary materials to complete my project, Mr Hafidz and Mr Suhairi. A special thank goes to all my friends who help me always to assemble the parts and gave suggestion about the project.

Words cannot express how grateful I am to all people around me who always sacrifice and support for me. Lastly, i would like to thank all of my friends who supported me in writing and encourage me to strive towards my goal.

(Muhammad Al Amin Bin Mohamed Saupi)

i

TABLE OF CONTENT

CHAPTER 1: INTRODUCTION 1.1 Background Study 1 1.2 Problem Statement 2 1.3 Significance of the Study 3 1.4 Objective of the Study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2.1 Preparation of Agar 12 3.2.1.1 Preparation of Carboxymethyl Cellulose Agar 12 3.2.1.3 Preparation of Simmon Citrate Agar 13	ACKNOWLEDGEMENT TABLE OF CONTENT LIST OF TABLES LIST OF FIGURES LIST OF ABBREVIATION ABSTRACT ABSTRAK					
CHAPTER 1: INTRODUCTION 1.1 Background Study 1 1.2 Problem Statement 2 1.3 Significance of the Study 3 1.4 Objective of the Study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2 Methods 12 3.2.1.1 Preparation of Nutrient Agar 12 3.2.1.2 Preparation of Carboxymethyl Cellulose Agar 12 3.2.1.3 Preparation of Simmon Citrate Agar 13						
1.1 Background Study 1 1.2 Problem Statement 2 1.3 Significance of the Study 3 1.4 Objective of the Study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.1 Raw Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2.1 Preparation of Agar 12 3.2.1.1 Preparation of Carboxymethyl Cellulose Agar 13 3.2.1.3 Preparation of Simmon Citrate Agar 13	СНА	PTER	I: INTRO	DUCTION		
1.2 Problem Statement 2 1.3 Significance of the Study 3 1.4 Objective of the Study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.1 Raw Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2 Methods 12 3.2.1 Preparation of Agar 12 3.2.1.1 Preparation of Carboxymethyl Cellulose Agar 13 3.2.1.3 Preparation of Simmon Citrate Agar 13	1.1	Backg	ground St	udy	1	
1.3 Significance of the Study 3 1.4 Objective of the Study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2 Methods 12 3.2.1 Preparation of Nutrient Agar 12 3.2.1.2 Preparation of Carboxymethyl Cellulose Agar 13 3.2.1.3 Preparation of Simmon Citrate Agar 13	1.2	Proble	em Staten	the Study	2	
1.4 Objective of the study 4 CHAPTER 2: LITERATURE REVIEW 2.1 Cellulose 5 2.2 Microbial Cellulase 7 2.3 Application of Cellulase in the Industries 8 2.4 Cellulase Production by Biological 10 CHAPTER 3: METHODOLOGY 3.1 Material 11 3.1.1 Raw Material 11 3.1.2 Chemicals 11 3.1.3 Apparatus and instrument 12 3.2 Methods 12 3.2.1 Preparation of Agar 12 3.2.1.2 Preparation of Carboxymethyl Cellulose Agar 13 3.2.1.3 Preparation of Simmon Citrate Agar 13	1.5	Signii	icance of the	study -	5	
CHAPTER 2: LITERATURE REVIEW2.1Cellulose52.2Microbial Cellulase72.3Application of Cellulase in the Industries82.4Cellulase Production by Biological10CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar (CMC)133.2.1.3Preparation of Simmon Citrate Agar13	1.4	Objec	uve of the	e Study	4	
2.1Cellulose52.2Microbial Cellulase72.3Application of Cellulase in the Industries82.4Cellulase Production by Biological10CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar (CMC)133.2.1.3Preparation of Simmon Citrate Agar13	СНА	PTER	2: LITER	RATURE REVIEW		
2.2Microbial Cellulase72.3Application of Cellulase in the Industries82.4Cellulase Production by Biological10CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar133.2.1.3Preparation of Simmon Citrate Agar13	2.1	Cellul	ose		5	
2.3Application of Cellulase in the Industries82.4Cellulase Production by Biological10CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar123.2.1.3Preparation of Simmon Citrate Agar13	2.2	Micro	bial Cellu	ılase	7	
2.4Cellulase Production by Biological10CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar133.2.1.3Preparation of Simmon Citrate Agar13	2.3	Appli	cation of	Cellulase in the Industries	8	
CHAPTER 3: METHODOLOGY3.1Material113.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar (CMC)133.2.1.3Preparation of Simmon Citrate Agar13	2.4	Cellul	ase Produ	action by Biological	10	
 3.1 Material 3.1.1 Raw Material 3.1.2 Chemicals 3.1.3 Apparatus and instrument 3.2 Methods 3.2.1 Preparation of Agar 3.2.1.1 Preparation of Nutrient Agar 3.2.1.2 Preparation of Carboxymethyl Cellulose Agar (CMC) 3.2.1.3 Preparation of Simmon Citrate Agar 	СНА	PTER 3	3: METH	IODOLOGY		
3.1.1Raw Material113.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar (CMC)133.2.1.3Preparation of Simmon Citrate Agar13	3.1	Material			11	
3.1.2Chemicals113.1.3Apparatus and instrument123.2Methods123.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar133.2.1.3Preparation of Simmon Citrate Agar13		3.1.1	Raw Ma	aterial	11	
3.1.3 Apparatus and instrument123.2 Methods123.2.1 Preparation of Agar123.2.1.1 Preparation of Nutrient Agar123.2.1.2 Preparation of Carboxymethyl Cellulose Agar133.2.1.3 Preparation of Simmon Citrate Agar13		3.1.2	Chemic	als	11	
3.2Methods123.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar133.2.1.3Preparation of Simmon Citrate Agar13		3.1.3	1.3 Apparatus and instrument			
3.2.1Preparation of Agar123.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar133.2.1.3Preparation of Simmon Citrate Agar13	3.2	Metho	Methods			
3.2.1.1Preparation of Nutrient Agar123.2.1.2Preparation of Carboxymethyl Cellulose Agar (CMC)133.2.1.3Preparation of Simmon Citrate Agar13		3.2.1	Prepara	tion of Agar	12	
3.2.1.2 Preparation of Carboxymethyl Cellulose Agar (CMC) 13 3.2.1.3 Preparation of Simmon Citrate Agar 13			3.2.1.1	Preparation of Nutrient Agar	12	
3.2.1.3 Preparation of Simmon Citrate Agar 13			3.2.1.2	Preparation of Carboxymethyl Cellulose Agar	12	
5.2.1.5 I reparation of Smillion Citate Agai 15			3212	(CIVIC) Preparation of Simmon Citrate Agar	13	
3.2.1.4 Preparation of Starch A gar 13			3.2.1.3	Preparation of Starch Agar	13	
3.2.2.7 Preparation of Broth 14		322	Prenaration of Broth			
3.2.2.1 Preparation of Nutrient Broth 14		0.2.2	3.2.2.1	Preparation of Nutrient Broth	14	

		3.2.2.2 Preparation of Glucose Broth	14		
		3.2.2.3 Preparation of Lactose Broth	14		
		3.2.2.4 Preparation of Mineral Salt Medium Broth	15		
		3.2.2.5 Preparation of Urea Broth	15		
	3.2.3	Preparation of Congo Red Stain	15		
	3.2.4 Preparation of Trace Element				
	3.2.5	Sample Collection	16		
	3.2.6	Isolation of bacteria by enrichment method	16		
	3.2.7 Screening test of bacteria.				
	3.2.8 Microscopic observation				
	3.2.9	Biochemical test	18		
		3.2.9.1 Starch hydrolysis test	18		
		3.2.9.2 Catalase test	19		
		3.2.9.3 Glucose / Lactose fermentation test	19		
		3.2.9.4 Urea hydrolysis test	19		
CHA	PTER 4	4: RESULT AND DISCUSSION	21		
4.1	Isolati	on and enrichment of cellulose degrading bacteria (CDB)			
	from s	sample of bacteria.	21		
4.2	Recon	ifirmation test or screening of cellulose degrading bacteria			
4.3	Analy	sis of microscopic observation			
4.4	Bioch	ochemical characterization			
	4.4.1	Analysis of citrate utilization	28		
	4.4.2	Analysis of starch hydrolysis	30		
	4.4.3	Analysis of catalase	31		
	4.4.4	Analysis of glucose / lactose fermentation	32		
	4.4.5	Analysis of urea hydrolysis	33		
СНА	PTER 5	5: CONCLUSION AND RECOMMENDATION	34		
CITED REFERENCES APPENDICES					
					CUR

ABSTRACT

IDENTIFICATION OF CELLULOSE DEGRADING BACTERIA

Cellulose as the major component of plant tissues is the most abundant organic compound on the earth and the most abundant renewable bioresource produced in biosphere. This process bioconversion involved the cellulose enzyme. There are several microorganisms involves in production of enzyme called cellulase and commonly by bacteria and fungi. This study is done to identify the potential cellulose degrading bacteria, and to classify the bacteria based on their characteristics. A total of five bacteria were taken from previous study and growth on nutrient agar. The bacteria were screening by using mineral salt medium agar containing carboxymethyl cellulose (CMC) and stained with Congo Red and Iodine. All the five bacteria do not show any hydrolysis zone in Congo Red Test. Only Bacteria C and D show positive result for Iodine test. The selected bacteria are then further characterized with a few biochemical tested. Bacteria C and D show similar characteristic of colony morphology which are yellow in color, circular shape, convex and translucent with exception on their colony edge which either undulate or smooth respectively. Bacteria C and D are gram positive rod bacteria. Both are positive for catalase and urea test. Bacteria C and D are capable for utilize glucose and lactose. As a conclusion, Bacteria C and D are potentially from Bacillus group and capable to produce cellulose.