UNIVERSITI PUTRA MALAYSIA

OPTIMIZATION OF MEDIUM AND CULTURE CONDITIONS FOR PECTINASE PRODUCTION BY LOCALLY ISOLATED BACTERIA FROM KENAF STEM

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OPTIMIZATION OF MEDIUM AND CULTURE CONDITIONS FOR PECTINASE PRODUCTION BY LOCALLY ISOLATED BACTERIA FROM KENAF STEM

By

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Abstract of thesis presented to the Senate of Universiti Putra Malaysia in the fulfillment of the requirement for the degree of Master of Science

OPTIMIZATION OF MEDIUM AND CULTURE CONDITIONS FOR PECTINASE PRODUCTION BY LOCALLY ISOLATED BACTERIA FROM KENAF STEM

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Chairman : Assoc. Prof. Rosfarizan Mohamad, PhD

Institute : Tropical Forestry and Forest Products

Pectinases are complex and diverse group of enzymes involved in the degradation of pectic substances and have great potential to be applied in biological retting of kenaf plant (*Hibiscus cannabinus*) for high quality fibre production. In this study, bacterial isolates (*Bacillus cereus* T1 and *Enterobacter cloacae* T2) were isolated from rotten kenaf stem and the isolates were identified. Then the bacterial isolates were assayed for potential enzymes production. The best enzyme producer was selected for further medium composition and culture conditions optimization study based on conventional and response surface methodology (RSM) approaches.

The bacterial isolates were isolated from rotten kenaf stem which collected from Taman Pertanian Universiti Putra Malaysia and identified based on morphological analysis, biochemical characteristics and API kit. Then, the bacterial isolates were assayed for cellulolytic enzyme activities and pectinase production chosen. The highest pectinase activity (0.138 U/mL) at 16^{th} h of cultivation was exhibited by *B. cereus* T1.

The different media (P1, P2 and P3) were used to analyze pectinase production by *B. cereus* T1. As a result, P3 was chosen as the best medium as P3 showed higher pectinase activity (0.18 U/mL) as compared to P1 and P2 media. In the RSM study, thirty experiments of four factors (inoculum size, temperature, pH and weight of wheat) in response to pectinase biosynthesis were carried out in shake-flask. The estimated optimize conditions of the chosen factors for the growth of *B. cereus* T1 and pectinase biosynthesis as suggested by RSM are as inoculum size (7.6%), temperature (41.85°C), pH (3.41) and weight of wheat (3.67%). By using the optimized conditions as suggested by the RSM, an increment of pectinase activity (6.52 U/mL) by 47-fold was achieved as compared to non optimized conditions.

The optimized conditions were then applied in the cultivation process using 2 L stirred tank bioreactor. However, reduced pectinase activity (2.925 U/mL) was obtained in the bioreactor experiment. Further optimization study considering other bioprocessing factors should increase the pectinase production by the isolate.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia
sebagai memenuhi keperluan untuk Ijazah Sains Sarjana

PENGOPTIMUMAN MEDIA DAN KEADAAN KULTUR UNTUK PENGHASILAN PEKTINASE OLEH PENGASINGAN BAKTERIA DARIPADA BATANG KENAF

Oleh

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Ogos 2014

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Pektinase adalah kumpulan kompleks dan pelbagai enzim yang terlibat dalam degradasi bahan pektik dan mempunyai potensi yang sangat besar untuk diaplikasikan dalam pengeretan secara biologi bagi pokok kenaf (*Hibiscus cannabinus*) untuk penghasilan fiber yang berkualiti tinggi. Dalam kajian ini, jenis bakteria (*Bacillus cereus* T1 dan *Enterobacter cloacae* T2) telah diasingkan daripada batang kenaf yang reput dan bakteria tersebut telah dikenal pasti. Kemudian kedua-dua bakteria tersebut telah disaring untuk pengeluaran enzim yang berpotensi. Bakteria yang terbaik dalam penghasilan enzim telah dipilih untuk digunakan dalam kajian komposisi media dan pengoptimuman keadaan kultur berdasarkan kaedah konvensional dan kaedah ransangan permukaan (RSM).

Bakteria tersebut telah diasingkan daripada batang kenaf reput yang diperoleh dari Taman Pertanian Universiti Putra Malaysia dan dikenalpasti berdasarkan analisis ciri-ciri morfologi, analisis biokimia dan kit API. Kemudian, kedua-dua bakteria telah disaring untuk aktiviti enzim selulase dan pektinase dipilih sebagai enzim yang terbaik. Aktiviti pektinase tertinggi (0. 138 U/mL) pada jam ke-16 pengkulturan telah dihasilkan oleh *B. cereus* T1.

Media yang berbeza (P1, P2 dan P3) telah digunakan untuk analisis biosintesis pektinase oleh *B. cereus* T1. P3 dipilih sebagai media terbaik kerana P3 menunjukkan aktiviti pektinase tertinggi (0.18 U/mL) berbanding P1 dan P2. Dalam kajian RSM, tiga puluh eksperimen untuk empat faktor (saiz inokulum, suhu, pH dan berat gandum) yang bertindakbalas terhadap penghasilan pektinase telah dijalankan di dalam kelalang kon. Jangkaan keadaan faktor yang optimum untuk pertumbuhan *B. cereus* T1 dan penghasilan pektinase seperti yang disyorkan oleh RSM adalah seperti berikut; saiz inokulum (7.6%), suhu (41.85°C), pH (3.41) dan berat gandum (3.67%). Dengan menggunakan keadaan optimum seperti yang disarankan RSM,

peningkatan aktiviti enzim pektinase sebanyak gandaan 47 telah dicapai berbanding keadaan yang tidak optimum.

Keadaan optimum kemudiannya diaplikasikan dalam proses pertumbuhan dengan menggunakan bioreaktor 2 L yang berpengaduk. Bagaimanapun, penurunan aktiviti enzim pektinase telah diperoleh melalui eksperimen meggunakan bioreaktor. Kajian pengoptimuman lanjutan yang mengambil kira faktor bioproses lain sepatutnya dapat meningkatkan penghasilan pektinase oleh bakteria tersebut.

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APPROVAL SHEET

I certify that a Thesis Examination Committee has met on 6th August 2014 to conduct the final examination of Puvaneswary A/P Degarajan on her thesis entitled "Optimization of Medium and Culture Conditions For Pectinase Production by Locally Isolated Bacteria From Kenaf Stem" in accordance with the Universities and University Colleges Act 1971 and the Constitution of the Universiti Putra Malaysia [P.U.(A) 106] 15 March 1998. The Committee recommends that the student be awarded the Masters of Science.

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LIST OF ABBREVIATIONS

NA	Nutrient Agar
PGA	Polygalacturonic Acid
rpm	Rotation per minutes
m	meter
mm	Millimeter
RSM	Response surface methodology
CCD	Central composite design
h	Hour
min	Minutes
ml	milliliter
OD	Optical density
L	Liter
X_{max}	Maximum cell concentration (g/L)
P_{max}	Pectinase activity (U/mL)
$Y_{x/s}$	Growth yield coefficient (g/g)
$Y_{p/s}$	Pectinase yield per substrate utilized (U/mL/g)

CHAPTER 1

INTRODUCTION

Enzymes are known as biological catalysts with high selectivity, which has been used in the food industry and also important for other industries such as washing agents, textile manufacturing, pharmaceuticals, pulp and paper for hundreds of years. Recently, enzymes are becoming important in sustainable technology and green chemistry and according to many researchers and studies, 20% of world chemical products will be produced by biotechnological approach in a worth of 300 billion US dollar. Microorganisms, fungus or bacteria are commonly used as sources as enzyme producer industrially. Microorganisms have a few advantages; interaction or involved in various substrates and selection of method on biosynthesis through the suitable conditions cultivation (Bhardwaj and Garg, 2010). One of the industrially important enzymes and intensively studied is pectinase. Pectinases are complex and diverse group of enzymes involved in the degradation of pectic substances (Sathyanarayana et al., 2007).

Pectinases are declared as one of the upcoming enzymes of the commercial sector. According to (Jayani et al., 2005), the microbial pectinases account for 25% of global food enzymes. These pectinases are known as heterogeneous group related enzymes which breakdown pectin substances that present in plants. Pectin is complex polysaccharide consisting mainly of esterifies D-galacturonic acid resided in α(1-4) chain (Shembekar and Dhotre, 2009).

Pectinases or pectinolytic enzymes are naturally distributed in microorganisms and higher plants (Whitaker, 1990). They are crucial for plants because aid in extension of cell wall and also play the important role as softening the plants tissues during maturation and storage (Ward and Moo-Young, 1989; Sakai, 1992; Aguilar and Huirton, 1990). Besides, it helps to maintain the ecological balance by decomposition and recycling of waste plant materials (Jayani et al., 2005).

Pectinases have potential applications in cotton scouring and degumming of plant fibres to improve the quality of fibre (Salazar and Jayasinghe, 1999). Pectinases also widely used in industry for retting of natural fibre and extraction of oils from vegetables and citrus peels (Bacarat et al., 1989; Brumano et al., 1993). Treatment of jute, flax, ramie and kenaf with pectinase improves the mechanical properties of the fibres, increasing flexibility with good retention of tensile strength. Besides, pectinases also used for clarification of juices, increases of filtration efficiency (Brawman, 1981), in maceration and liquefaction (Arunachalam and Asha, 2010).

In Malaysia, kenaf is an emerging new crop to the agriculture industry (Kassim, 2001). Research on kenaf typically have been done by the Institute of Tropical Forestry and Forest Products (INTROP) and Malaysian Agricultural Research and Development Institute (MARDI). INTROP identified nine varieties of kenaf which can be categoried as early flowering varieties (V132, V36, V19, V12, Q-Ping, KK60), intermediate flowering varieties (V133, NS) and late peak varities (TK). In Malaysia, variety TK as the potential fibre source to be used in pulp and paper process, particle board and fibreboard (H'ng et al., 2009).

Currently, microbial pectinase production has been widely studied by researchers and experts (Kashyap et al., 2001; Torres et al., 2006). Pectinolytic enzymes have been reported to produce by bacteria and fungi such as *Bacillus* spp., *Clostridium* spp., *Pseudomonas* spp., *Penicillum* spp., *Aspergillus* spp., *Monilla laxa, Fusarium* spp., *Verticillium* spp., *Sclerotinia libertiana, Coniothyrium diplodiella and Polyporus squamosus* (Bhardwaj and Garg, 2010).

Higher cost of production is probably the critical constraint in commercialization of new sources of enzymes. The cost of enzyme production can be reduced in industrial processes due to several factors; using high producing strain, optimum fermentation conditions and cheap raw material (Murad and Azzaz., 2011) or organic nature of agro-industrial crops and residues as a carbon source (Pandey, 2001). In this study, wheat has been used as inexpensive carbon source for pectinase production by locally isolated bacteria of *Bacillus cereus* T1.

Optimization of fermentation conditions is an important step in the development of feasible industrial bioprocess. Response surface methodology (RSM) is a tool that used to analyze the optimized conditions which enables the experts or researchers to design the experimental and the RSM initially introduced by (Box and Wilson, 1951). Nowadays, researchers used this tool to provide maximum yield production through an experimental design that suggested by RSM (Raissi, 2009). Besides, RSM is a combination of good experimental design, regression modeling techniques and optimization for process improvement. Therefore, RSM is a most significant use approach prior to industrial level production (Montgomery and Myers, 1997)

The present study focuses on the search of local bacterial pectinase producer and strategies in improving the pectinase production via optimization of medium and culture conditions of the selected producer cultivation process. The optimized environmental conditions were applied in the bioreactor system cultivation equipped with a process control system. Hence, the specific objectives of this study were;

1. To screen, isolate and identify the bacterial pectinase producer isolated from rotten *Hibiscus cannabinus* (kenaf) stem.

2. To optimize the medium and culture conditions for pectinase production by the selected bacterial isolate via conventional and RSM approaches.

3. To evaluate the performance of the bacterial isolate in batch cultivation process for pectinase production using 2 L stirred tank bioreactor.

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