



UNIVERSITI PUTRA MALAYSIA

**ISOLATION AND SCREENING OF PECTINASE PRODUCING BACTERIA
FROM ROTTEN FRUITS**

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PENGESAHAN

Dengan ini adalah disahkan bahawa projek yang bertajuk “ISOLATION AND SCREENING OF PECTINASE PRODUCING BACTERIA FROM ROTTEN FRUITS” telah disiapkan serta dikemukakan kepada Jabatan Mikrobiologi oleh Amirah Adibah binti Abdul Wahid (162586) sebagai syarat untuk kursus BMY 4999 projek.

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ABSTRACT

Production of enzymes from bacteria for example pectinase has high demand from industry especially for cost-effective production process in food industry. Besides, redundancy of agricultural wastes is also a concern that need to be handled. Waste materials from agricultural activities might be one of the resources that we can take as in order to be used in the production of the enzymes such as pectinase. Hence, in this study, pectinolytic bacteria were isolated from four rotten local fruits which were watermelon, lime, banana and mango in yeast extract pectin (YEP) agar medium. The best pectinase producing bacteria isolated among the four was identified as *Bacillus* sp. and labeled as *Bacillus* sp. WM 3. The bacteria was then studied for its pectinase activity and specific pectinase activity. The maximum enzyme activity of the crude enzyme extract was 4.383 U/ml at 30°C with agitation and incubated for 5 days. From this study, we have discovered the potential bacterial species that can produce significant pectinase amount.

ABSTRAK

Penghasilan enzim daripada bakteria sebagai contoh enzim pektinase mempunyai permintaan yang amat tinggi terutama di dalam industri makanan yang ingin mengurangkan kos proses penghasilan produk mereka. Selain itu, bahan buangan pertanian yang semakin membimbangkan turut menjadi tumpuan. Oleh itu, di dalam kajian ini, bakteria yang dapat menghasilkan pektinase telah dipencilkan daripada empat buah-buahan tempatan buangan yang hampir rosak iaitu tembikai, limau nipis, pisang dan mangga di dalam agar yeast ekstrak pektin (YEP) dan telah diidentifikasi sebagai *Bacillus* sp. WM 3. Bakteria tersebut kemudiannya digunakan untuk kajian lanjut iaitu untuk mengetahui aktiviti enzim dan aktiviti enzim spesifik. Aktiviti enzim maximum daripada ekstrak enzim yang terhasil ialah 4.383 U/ml pada suhu 30°C dengan pengeraman berputar selama 5 hari. Melalui kajian ini, secara konklusif, kita telah diberi pendedahan terhadap spesies bakteria yang berpotensi menghasilkan pektinase selain dapat juga mengetahui keadaan optima bagi penghasilan pektinase yang terbaik.

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LIST OF ABBREVIATIONS

°C	degree Celsius
µl	micro litre
BSA	Bovine Serum Albumin
cfu	colony forming unit
g/L	gram per litre
mg	milligram
ml	millilitre
MR-VP	Methyl Red – Voges-Proskauer
MSA	Mannitol salt agar
NaCl	Sodium chloride
nm	nano metre
PG	Polygalacturonase
PGA	Polygalacturonic acid
t_n	time interval at n hour
U	Unit for enzyme activity where it is the amount of product produced in µmol per minute
WM 3	Isolate labelling for bacteria isolated from watermelon
YEP	Yeast-extract Pectin

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CHAPTER 1

INTRODUCTION

Pectinase is a type of enzymes that breaks down pectin structure. Inside plant like fruits and vegetables, holds a structure called as pectin in their tissues which has jelly-like matrix structure that helps to hold plant cells together. The degrading pectin because of presence of pectinase cause degradation in plant cells (Pasha et. al., 2013) especially in fruits and vegetables. This causes rises in amount of fruit wastes.

Pectinase usually presence in various plant pathogenic bacteria or fungi (Hasunuma et al., 2003) that need to utilize pectin for their metabolism. Despite its bad effect towards fruits and vegetables, pectinase present a lot of applications that useful industrially. The market demands of pectinase is 25% in global scale of food enzyme usage (Kumar and Sharma, 2012). Pectinase is widely used in increasing fruit juices quality, textile industries, fiber crop industries, and also in the fermentation of coffee and tea. Hence, this study basically wants to portray the idea of turning fruit wastes into something beneficial of producing valuable enzyme, pectinase.

Pectin is a jelly-like matrix structure which helps to hold the plant cells together (Pasha et al., 2013) due to its complex colloidal acid polysaccharides, with a backbone of galacturonic acid residues linked by (1,4) linkages. Pectic substances are classified into protopectin, pectic acid, pectinic acid and pectin (Miller, 1986).

Even though pectin has strong backbone structure pectinase, an enzyme can break down the complex structure of pectin into simpler molecules like galacturonic acids (Pasha et al., 2013). Components of pectinase includes pectolyase, pectozyme and also polygalacturonase which is the most studied pectinase type. Examples of bacteria that can produce pectinase are *Bacillus* sp., *Cocci* sp. and *Erwinia caratovora*.

Pectinase enzyme due to its degradable activity hence can increase the speed of extraction of fruit juices like in fruit juices and wine production. For example, acidic pectinase that is excreted by various types of bacteria is widely used industrially in bringing down the cloudiness and bitterness of fruit juices since forever (Alkorta et al., 1998; Kashyap et al., 2001; Kilara, 1982). Besides acidic one, there are also alkaline pectinase that is used widely in textile industries (Hoondal et al., 2000; Pasha et al., 2013), in retting and degumming of fiber crops (Kashyap et al., 2001; Pasha et al., 2013), in fermentation of coffee and tea, extractions of oil, and treatment of pectic water. They have also been reported to work on purification of viruses (Salazar and Jayasinghe, 1999) and in making of paper (Reid and Richard 2004; Viikari et al., 2001). Due to various applications of pectinase in industry, scientists and researchers are motivated in order to study and explore the cheapest and most efficient ways of producing massive amount of biological pectinase enzymes.

1.1 Problem Statement

Municipal and agricultural solid waste which is dumped by residential people, industry, and agriculture has become a very concerning issue right now. Moreover, most of the solid waste contains high percentage of food wastes including fruit wastes. Researchers nowadays are concerned in order to turn the wastes into wealth. In order to decrease the production of the wastes, it is brilliant to reprocess the wastes and extracting the beneficial values out of it. Since that, why do we need to isolate pectinase producing bacteria out of the fruit wastes? Besides, what can pectinase give as benefits towards the industry?

1.2 Hypothesis

Pectinase-producing bacteria can be isolated from fruit wastes and the pectinase produced can be characterized.

1.3 Objectives

- a. To screen and isolate pectinase-producing bacteria from the fruit wastes
- b. To identify the bacterial strain of the isolated bacteria by biochemical tests
- c. To determine the pectinase enzyme activity by the isolated bacteria

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