

ISOLATION AND ANTIBIOTIC SUSCEPTIBILITY SCREENING OF
PSEUDOMONAS SP. FROM SOIL.

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To my dearest mother, father and my beloved husband

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ABSTRACT

Pseudomonas is an aerobic Gram-negative, rod-shaped bacterium which emerged as one of the most problematic nosocomial pathogens. To characterize *Pseudomonas* species strains that are widespread in animal, plant, human, and environment especially soil and water, the soil sample was collected from T02 Building in Universiti Teknologi Malaysia. An isolated bacterium was cultured on nutrient agar and MacConkey agar plates. The results showed that using (MacConkey agar) at 37°C had highest recovery in the isolation of *Pseudomonas*. The colony produced white-pink pigment. Based on the Gram-staining method, *Pseudomonas* species was Gram negative with rod in shape, in pink color. The effects of temperature and incubation time, as well as their growth profiles in nutrient broth media, were studied. *Pseudomonas* can grow well in 37°C, which recorded maximum growth rate, and at 30°C *Pseudomonas* grow nearly similar to its growth rate at 37°C, while at 25°C growth of *Pseudomonas* was slow. Methods for isolation and characterization of *Pseudomonas* species based upon culture methods coupled with biochemical tests were used in this study. According to biochemical tests, *Pseudomonas* was catalase positive, citrate positive, gelatin liquefaction and oxidase positive whereas was negative for methyl red, urease, starch hydrolysis, triple sugar iron and Indole. However, to study the susceptibility of tested strain to different antibiotics using disk diffusion method the result showed that the isolated strain of *Pseudomonas* was resistance to Erythromycin, Gentamycin, Ampicillin and Penicillin, and susceptible to Ciprofloxacin, Imipenem, Lincomycin, Piperacillin and Tobramycin.

ABSTRAK

Pseudomonas adalah Gram-negatif aerobik, bakteria berbentuk rod yang muncul sebagai salah satu daripada patogen nosocomial yang bermasalah. Untuk mencirikan strain *spesies P.* yang meluas dalam haiwan, tumbuhan, manusia, dan alam sekitar terutamanya tanah dan air, sampel tanah dari Bangunan T02 di Universiti Teknologi Malaysia telah digunakan. Bakteria yang dipisahkan dikulturkan pada agar nutrien dan plat agar MacConkey. Keputusan menunjukkan bahawa, menggunakan (agar MacConkey) pada 37°C mempunyai pemulihan tertinggi di bawah pengasingan *Pseudomonas*. Ia telah menghasilkan pigmen putih-kemerahan jambu. Berdasarkan kaedah Gram-pewarnaan *Pseudomonas sp.* adalah Gram-negatif dengan berbentuk rod, dalam warna merah jambu. Kesan suhu dan masa penderaman, serta profil pertumbuhan mereka dalam sup medium nutrien, telah dikaji. *Pseudomonas* dapat tumbuh dengan baik pada suhu 37°C, yang mencatatkan kadar pertumbuhan maksimum, dan pada suhu 30°C *Pseudomonas* berkembang hampir sama dengan kadar pertumbuhan pada 37°C, manakala dalam pertumbuhan 25°C pertumbuhan *Pseudomonas* adalah lambat. Kaedah pengasingan dan pencirian *spesies Pseudomonas* berdasarkan kaedah pengkulturan dan ujian biokimia telah digunakan dalam kajian ini. Menurut ujian biokimia, *Pseudomonas* adalah positif bagi catalase, positif bagi citrate, pencairan gelatin dan positif bagi oxidase manakala negatif bagi methyl merah, urease, starch hydrolysis, triple sugar iron dan Indole. Walau bagaimanapun, untuk mengkaji kecenderungan ketegangan diuji kepada antibiotik yang berbeza menggunakan kaedah penyebaran cakera hasilnya menunjukkan bahawa *Pseudomonas* yang diasingkan mempunyai rintangan terhadap Erythromycin, Gentamycin, Ampicillin dan Penicillin, dan cenderung kepada Ciprofloxacin, Imipenem, Lincomycin, Piperacillin dan Tobramycin.

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LIST OF SYMBOLS

cm	Centimetre
g	Gram
µg	Microgram
h	Hour
min	Minute
sec	Second
ml	Millilitre
l	Litre
mm	Millimetre
°C	Celsius
rpm	Round per minute

LIST OF ABBREVIATIONS

100x	Time magnification
$A_{600\text{nm}}$	Absorbance at 600 Nanometre
DNA	Deoxyribonucleic acid
<i>et al</i>	And others
H ₂ O ₂	Hydrogen peroxide
HCl	Hydrogen chloride
HIV	Human immunodeficiency virus
AIDS	Acquired immunodeficiency syndrome
KI	Potassium chloride
mRNA	Messenger ribonucleic acid
N.A	Nutrient agar
NaCl	Sodium chloride
NB	Nutrient broth
NO ₃	Nitrate
OD	Optical density
O/F	Oxidation/ Fermentation
PHA	Poly hydroxyalkalanoate
rRNA	Ribosomal ribonucleic acid
<i>sp</i>	<i>Species</i>
TMPD	Tetra methyl-p- phenylenediamine
TSA	Tryptose Soya Agar
TSI	Triple Sugar Iron

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CHAPTER 1

INTRODUCTION

1.1 Introduction

Pseudomonas is the most heterogeneous and significant group of known bacteria. It is Gram-negative, rods (Ugur, *et al*, 2012), motile by mean of one or more polar flagella, obligate aerobes, while some species can grow in anaerobic conditions in the presence of nitrate (Zago *et al*, 2009). *Pseudomonas* is a free living organism, commonly found in soil, and water (Todar, 2006).

Pseudomonas is generally found in soil, and water environments that have methophilic, and neutral pH conditions, in nature *Pseudomonas* exist as saprophytes and parasites. *Pseudomonas* species are non-prominent in anaerobic environments, and they don't occur in extreme thermophiles or acidophilic habitats. Soil place may include the most complex of environmental place that are encountered by microorganisms. The heterogeneity of factors for growth in a soil presents the bacteria a challenge matrix of aggregate surface and structure (Moore *et al*, 2006).

Pseudomonas is characterized by high metabolic adaptability. The identification of the species of *Pseudomonas* was first described by Migula in 1894 (Franzetti *et al*, 2007). However some species of *Pseudomonas* are very important in the agricultural soil, which have a role in bioremediation and bio-control, thus given the great interest in the *Pseudomonas* and its primary role in the use as bio-control

agents in the soil (Uğur *et al*, 2012). While other has become increasingly recognizing as emerging opportunistic. *Pseudomonas* is generally resistant to many antibiotics (Todar, 2006).

The pathogenesis of *Pseudomonas* infections is multifactorial and complex. *Pseudomonas aeruginosa* are both troubling and toxigenic. The three stages of infection according to Pollack (2000) are (1) bacterial attachment and colonization, (2) local infection, and (3) blood stream dissemination and systemic disease. Production of extracellular proteases adds to the organism's virulence by assisting in bacterial adherence and invasion (Pollack, 2000). Acquisition and spread of the antibiotic resistance among pathogenic bacteria constitute a major threat in modern medicine (Alonso *et al*, 1999).

1.2 Problem Statement

Pseudomonas is becoming increasingly ubiquitous in the environment. Even though some species of *Pseudomonas* are being characterized as beneficial to the ecosystem, many negative effects have been attributed to their application as biological agents in the agricultural sector. Some *Pseudomonas species* are pathogenic and causes urinary tract infection, lung infection, skin infection and sore throats, whose pathogenicity are difficult to control. As a module to study pathogenic *Pseudomonas*, a *Pseudomonas* strain must be isolated and its properties analyzed. Isolation and characterization of the bacterial diversity from soil and the effects of antimicrobial agents in retarding the growth of these bacteria will be the important aspects to be investigated in order to improve the antimicrobial steps to eventually minimize the potential diseases caused by *Pseudomonas* in environment.

1.3 Scope of study

This research is mainly focused on isolation and characterization of *Pseudomonas* from the soil which are the residual deposits of agricultural applications. Moreover, this research was focus in detailing the basic investigation of the microbial activities of this specie before embarking into identification of their non-pathogenicity which serves a useful application in the field of medical sciences. After collecting the sample, *Pseudomonas* was propagated via serial dilutions to obtain pure colonies, which employs the technique of samples being cultured on nutrient agar mediums in the lab and streaked on nutrient agar and MacConkey agar for cultivating pure microbial colony and screen for Gram negative bacteria. Gram staining methods and biochemical analysis were characterized to identify the *Pseudomonas* specie and also study the effects of selected antimicrobial agents in inhibiting the proliferation of this bacteria. The microbial growth curve observed for *Pseudomonas* via optical density was analyzed with the aid of a spectrophotometric technique and the doubling time as well as the effect of different temperatures on the growth of *Pseudomonas sp.* was investigated.

1.4 Objective of Study

1. To isolate the *Pseudomonas* from the soil environment.
2. To investigate the effect of different temperatures in the growth of *Pseudomonas sp.*
3. To characterize the bacteria including Gram staining, biochemical tests and the effects of selected antimicrobial agents in inhibiting the growth of the bacteria.

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