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High Prevalence of *Pseudomonas* Species in Soil Samples from Ternate Island-Indonesia

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Abstract: In the present study, Ten soil samples were examined and the pH of the soil was recorded. For bacterial isolation, a sterile nutrient and blood agars were used. Gram stain and biochemical tests were done for identification. A total of 384 genus were isolated, 314 (81.8%) were identified as *Pseudomonas* species of which 245 (78.0%) were *Pseudomonas aeruginosa*, 42 (13.4%) were *Pseudomonas fluorescens*, 13 (4.2%) were *Pseudomonas mallei*, 10 (3.1%) were *Pseudomonas putida* and 4 (1.3%) were *Pseudomonas syringe* and are regarded as pathogenic and harmful to man, animal and plants. This study shows that *Pseudomonas aeruginosa* had a high adaptation capability to grow in soil samples from Ternate, Indonesia. The rest of the bacterial isolates (18.2%) were identified as follows*:* 24 samples (6.2%) were *Micrococcus,* 23 samples (6.0%) were *E. coli*, 12 samples (3.1%) were *Pasteurella* and 11 samples (2.9%) were *Staphylococcus. Pencillium* was also isolated.

Key words: *Pseudomonas*, high adaptation capability, ternate soil-indonesia

INTRODUCTION

Soil samples from Ternate Island, Indonesia has a very high percentage of *Pseudomonads*, one of them is *Pseudomonas fluorescens* nonpathogenic saprophytes that has a proper role in bio-control and has been applied directly to soils as a way of preventing the growth or establishment of crop pathogens such as *P. fluorescens* strains (CHAO or Pf-5) which induce systemic resistance in the host plant, (Haas and Defago, 2005). The *Pseudomonas fluorescens* produce pyoverdin (*fluorescein*) pigment, particularly under conditions of low iron availability. This pigment is a soluble, bluish-green fluorescent pigment that led to the group's name (Meyer *et al*., 2002). Certain *Pseudomonas* species may also produce additional types of siderophore, such as pyocyanin by *Pseudomonas aeruginosa* (Lau *et al*., 2004) and thioquinolobactin by *Pseudomonas fluorescens* (Matthijs *et al*., 2007). These bacteria are generally obligate aerobes; however, some strains can utilize NO_3 instead of O_2 as an electron acceptor. They have simple nutritional requirements; they grow well in mineral salts media supplemented with any of a large number of carbon sources. Some researches seek make use of *Pseudomonas fluorescens* to partially or completely degrade pollutants such as styrene, TNT and polycyclic aromatic hydrocarbons. Several strains of this bacteria also have the ability to suppress plant diseases by protecting the seeds and roots from fungal infection (DOE). This ability is due to secondary metabolites produced by these bacteria such as antibiotics, siderophores and hydrogen cyanide as well as the ability of these bacteria to rapidly colonize the Rhizosphere and out-compete some of pathogens (DOE).

The other species of *Pseudomonads* are the most pathogenic types which threatens the general health especially the patients that has been hospitalized for long period of time, the swimmers in common swimming pools, human with compromised host defense mechanisms, animals and also plants so they should be aware of its high fatality which is 50%. *Pseudomonas* bacteria can be found in many different environments such as soil, water and plant and animal tissue. The species of these bacteria are 80% of the opportunistic pathogens that affect humans (Brown, 1975), animals and plants. *Pseudomonas aeruginosa* infection is a serious problem in patients hospitalized with cancer, cystic fibrosis and burns; the case fatality is 50%. Other infections caused by

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Pseudomonas species include endocarditis, pneumonia and infections of the urinary tract, central nervous system, wounds, eyes, ears, skin and musculoskeletal system. *Pseudomonas aeruginosa*, called the epitome of opportunistic pathogens, almost never infects uncompromised tissues; however, it can infect practically any type of tissue if that tissue has some type of compromised defenses (Kenneth, 2004). *Pseudomonas mallei* affect animals specially horses and cause a serious disease called melioidosis and Glanders, respectively, *Pseudomonas syringe* is a plant pathogen.

Because of their widespread occurrence in nature, the *pseudomonads* were observed early in the history of microbiology it means 'false unit', being derived from the Greek pseudo (ψευδο false) and Monas (μονάς /μονάςδα a single unit). The term monad was used in the early history of microbiology to denote single-cell organisms. Gram-negative, rod-shaped, 0.5-0.8×1-3 μm, non-spore forming and polar-flagella bacteria (Cornelis, 2008). Some species of these bacteria, such as *Pseudomonas aeruginosa*, are opportunistic pathogens that secrete extra cellular proteases and adhere and invade host tissue (Ryan and Ray, 2004).

Pseudomonas species normally inhibit in soil, marshes, coastal marine and can be isolated from the skin, throat and stool of healthy persons, the plant and animal tissue. Spread is via contact with fomites or by ingestion of contaminated food and water. Generally, these bacteria can tolerate a variety of physical conditions.

Since, the mid 1980s *Pseudomonads* have been applied to cereal seeds or direct to the soil as a way of preventing the growth or establishment of crop pathogens e.g. *Pseudomonas fluorescens* which induce systemic resistance to the plant host or might contend other pathogenic soil microbes (Hass and Defago, 2005). *Pseudomonas chlororaphis* produce a phenazine type of antibiotic which is active against certain fungal plant pathogens (Chin-A-Woeng *et al*., 2000). Some species are able to metabolize pollutants in the environment and as a result can be used as a bioremediation, e.g., *Pseudomonas alcaligenes* degrades polycyclic aromatic hydrocarbons (O'Mahony *et al*., 2006), *Pseudomonas mendocina* which degrades toluene (Yen *et al*., 1991), *Pseudomonas veronii* which degrades a variety of simple aromatic organic compounds (Nam *et al*., 2003; Onaca *et al*., 2007), *Pseudomonas pseudoalcaligenes* which is able to use cyanide as a nitrogen source (Huertas *et al*., 2006) and *Pseudomonas putida* which has the ability to degrade organic solvents such as toluene of high capability to convert morphine in aqueous solution into hydromorphone which is the stronger and somewhat expensive to manufacture drug (Marques and Ramos, 1993). Among the fluorescent species of *Pseudomonads*, *Pseudomonas fluorescens* is the most important as a biocontrol and bioremediation and out of many researches done few of them refer to *Pseudomonas fluorescens* in relation to the pathogenic strains.

Pseudomonas aeruginosa is common in human with compromised host defense mechanisms and is the most common pathogen isolated from patients who have been hospitalized longer than one week (Qarah and Cunha, 2003). It is common for these bacteria to cause nosocomial infections like pneumonia, urinary tract infections and bacteraemia (Cornelis, 2008). Infections caused by *Pseudomonas* can become complicated and may even be life threatening. It is both invasive and toxigenic and has three stages of infection: the first is bacterial attachment and colonization, the second is local infection and the third is bloodstream dissemination and systemic disease. The bacteria produce extra cellular proteases that assist adherence and invasion and are important in the organism's virulence. *Pseudomonas* frequently cause out breaks of *Pseudomonas* dermatitis. This is a self-limiting rash about two week's duration, often associated with swimming pools and pool type saunas and hot tubes. When many people use these facilities, the alkalinity rises and the chlorine become less effective, at the same time, the concentration of the nutrients that support the growth of *Pseudomonads* increases. Hot water causes hair follicles to dilate, facilitating the entry of bacteria. Competition of swimmers is often troubled with otitis externa, or swimmer's ear, a pseudomonad infection of the external ear canal leading to eardrum. *Pseudumonas aeroginosa* is also a very common and serious opportunistic pathogen in burn patients causes blue-green bus this color is caused by the bacterial pigments (pyocyamin) (Roger *et al*., 1988).

This study carries one objective that is to isolate and characterize the bacteria in the soil samples from Ternate Island, East Indonesia.

MATERIALS AND METHODS

This study was conducted in the School of Bioscience and Biotechnology, Faculty of Science and Technology, UKM, Malaysia during the years 2007-2008.

Soil samples were collected from Indonesia (Ternate Island) from different areas of grasslands, non-agricultural soil; 50 mL of each sample were ground and passed through a 0.5 mm sieve and dissolved in 50 mL sterile distilled water, mixed throughly then left for 2 h then centrifuged at 3000 rpm for 10 min. One milliliter of the samples was diluted into eight serial tubes of 9 mL distilled water, pre-inoculation in blood agar plates or

eosin-methyl thionine blue agar or nutrient agar and incubated at 37°C for 24 h. Gram negative bacilli were identified according to the standard biochemical methods and Bergy's Manual of Determinative Bacteriology Holt *et al*. (1994) and Meyer *et al*. (2002). Especially the presence of flagella, inability to ferment lactose, the oxidase and the catalase tests, methyl-red test, glycogen hydrolysis, arginine dihydrolase and some produce insoluble pigments were also examined. The pH of the soil was determined by using pH meter (DEITA 320).

RESULTS AND DISCUSSION

The bacterial isolates from Ternate Island soil-Indonesia were identified as follows: 314 isolates (81.8%) were *Pseudomonads*; 24 isolates (6.2%) were *Micrococcus*; 23 isolates (6.0%) were *E. coli*; 12 isolates (3.1%) were *Pasteurella* and 11 isolates (2.9%) were *Staphylococcus* (Table 1).

The total of (314) isolates of *Pseudomonads* were isolated from 10 soil samples, with an average of 31 isolates per sample approximately. A high percentage of *Pseudomonas aeruginosa* (78.0%) was found within the total of species of *Pseudomonads* isolated.

Five species of the *Pseudomonads* were isolated e.g., *Pseudomonas aeruginosa* (78.0%), *Pseudomonas fluorescens* (13.4%), *Pseudomonas mallei* (4.2%), *Pseudomonas putida* (3.1%) and *Pseudomonas syringe* (2.9%) (Table 2). The pH of the soil samples varies from 5.2 to 8.7 with an average of 6.9.

The *Psedomonas* species were isolated in Blood agar (Fig. 1) colonies were white, round, convex, glistening and non-haemolytic and when cultured in Nutrient agar (Fig. 2) the colonies were mucoid, flat, grayish and lobulated, after 24 h of incubation at 37°C.

In the present study a beneficial bacteria to biotechnology and bio-control in the soil samples from Ternate Island, East Indonesia was isolated. A high prevalence of *Pseudomonas* species were found, with a high average when compared with other genera of bacteria isolated from the same samples. This may be due to moisture and warmness of the soil characteristics. A microbial richness was also observed such as *Micrococcus*, *E. coli*, *Pasteurella* and *Staphylococcus*.

Some species of *Pseudomonas* has been recently used as a bio-control and use as bioremediation which is able to clear the environmental pollution and improve the hygienic measures and partially or completely degrade pollutants such as styrene, TNT and polycyclic aromatic hydrocarbons. Several strains of this bacteria also have the ability to suppress plant diseases by protecting the seeds and roots from fungal infection (Hass and Defago, 2005).

Other species were described as a toxigenic (produce exo and endo-toxins) and cause 80% of the diseases that affect man, animals and plants. A high percentage of *Pseudomonads* were isolated from the soil samples, the reason of this high prevalence of *Pseudomonads* is unknown, but we considered that the physical, chemical or micro-biological characteristics of the grasslands and non-agricultural soil may affect (Coburn *et al*., 1989).

Table 1: The percentage of different Bacterial isolates from Ternate Island soil samples

Species name	No. of isolates out of 384	Percentage
Pseudomonas sp.	314	81.8
Micrococcus	24	6.2
E. coli	23	6.0
Pasteurella	12	3.1
Staphylococcus	11	2.9

Table 2: The percentage of different *Pseudomonads* species in Ternate Island soil samples

Pseudomonas species	No. of isolates out of 314	Percentage
P. aeruginosa	245	78.0
P. fluorescens	42	13.0
P. mallei	13	4.2
P. putida	10	3.1
P. syringe	4	2.9

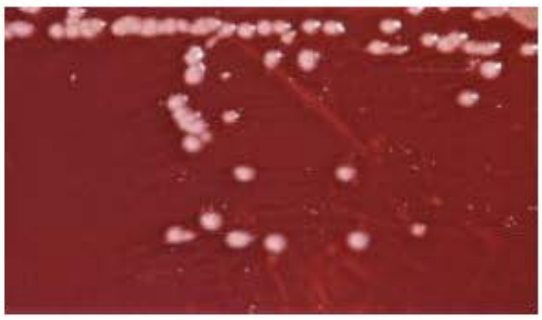

Fig. 1: *Pseudomonas aeruginosa* colonies on blood agar plate

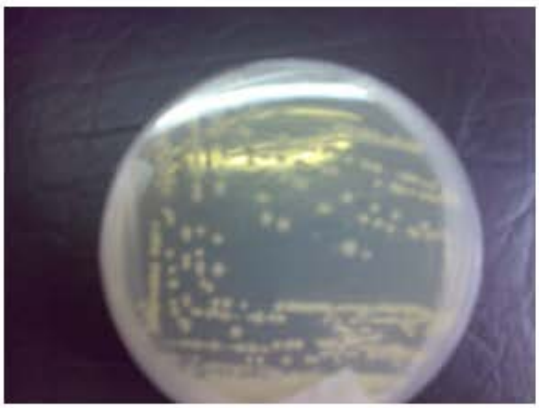

Fig. 2: *Pseudomonas aeruginosa* colonies on Nutrient agar plate

The pH does not affect on the presence of *Pseudomonas* in the soil because the pH had a broad range (5.2-8.7) with a medium of 6.9 closely related to the optimum neutral pH for the growth of *Pseudomonas*. In conclusion, this study demonstrates the presence of at least 5 species of *Pseudomonads,* one of them is *Pseudomonas fluorescens* which has a very effective role in bio-control and bioremediation. The other four species are the most pathogenic ones e.g. *Pseudomonas aeruginosa*, *Pseudomonas mallei*, *Pseudomonas putida* and *Pseudomonas syringe* with high percentage. We believe that this study gives a possible explanation of *Pseudomonas* related diseases in Ternate Island and will highlight the steps needed for the spread of pathogenic strains of *Pseudomonas*, which can best be controlled by observing proper isolation procedures, aseptic technique and careful cleaning and monitoring of all of the instruments in hospitals, the swimming pools for the efficiency of chlorine. Topical therapy of burn wounds with antibacterial agents such as mafenide or silver sulfadiazine, has dramatically reduced the incidence of *Pseudomonas aeruginosa* (Cross *et al*., 1980).

Pseudomonas is frequently resistant to many commonly used antibiotics, as penicillin and the majority of related beta-lactam antibiotics, although many strains are susceptible to piperacillin, imipenem, gentamicin, tobramycin, colistin, amikacin, or ciprofloxacin. Resistant forms have developed, making susceptibility testing essential. The combination of gentamicin and carbenicillin is frequently used to treat severe *Pseudomonas* infections, especially in patients with leukopenia. Several types of vaccines are being tested, but none is currently available for general use.

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