ANTIBACTERIAL ACTIV FFERENT PARTS OF *SWIETENIA MAHOGANI* AGAINST MULTIPLE-DRUG RESISTANT BACTERIA

TAN WEE YEONG

Report submitted in partial fulfillment of the requirements for the award of the degree of Bachelor of Applied Science (Honor) - Industrial Chemistry

Faculty of Industrial Science & Technology
UNIVERSITI MALAYSIA PAHANG

November 2012

ABSTRACT

This study concerned with the evaluation of antibacterial activity of crude extracts of three parts (leaf, fruit cover, seed cover and seed) of *Swietenia mahagoni* obtained by three solvents (acetone, ethanol and hexane). The seedoil obtained by solvent continuous extraction method (Soxhlet) from seed by using hexane as solvent. The antibacterial activity of these extracts was assessed against two multiple-drug-resistance bacteria strains namely, *Escherichia coli* (*E.coli)* and *Staphylococcus aureus* (*S.aureus*) by the well diffusion method. For the antibacterial bioassay, four concentrations (0.1, 0.2, 0.4 and 0.8%) of each solvent and part extract solutions were prepared; DMSO was used to solubilize the extract in water. A control using 1% dimethyl suldoxide (DMSO) used for comparison. The antibacterial activity among extracts was extremely broad against both test organisms. With higher concentration (0.8%) the acetone extracts from fruit cover and leaf displayed overall more potent activity than other parts against *S. aureus*; whereas ethanol extracts from fruit and seed cover showed more efficiency against *E. coli*. However, no inhibition activity of leaf acetone and ethanol against *E. coli* with concentration of 0.8%. Bioactive groups such as alkaloid and sesquiterpene lactones were screened by Thin Layer Chromatography (TLC); and the results obtained were positive. The experimental results obtained from this study suggest that *Swietenia mahagoni* extracts are promising as natural antibacterial and this may warrant further research to determine the bioactive compound(s).

ABSTRAK

Kajian ini berkenaan dengan penilaian aktiviti antibakteria dua belas ekstrak mentah tiga bahagian (daun, kulit buah-buahan penutup, kulit biji benih penutup dan benih) Swietenia mahagoni yang diperolehi daripada tiga pelarut (aseton, etanol dan heksana). Minyak dari benih yang diperolehi dengan menggunakan kaedah pengekstrakan pelarut berterusan (Soxhlet) dari benih dengan menggunakan heksana sebagai pelarut. Aktiviti antibakteria pati ini telah dinilai terhadap dua bakteria-dadah-rintangan iaitu *E. coli* dan *S. aureus* dengan kaedah resapan. Untuk menguji antibakteria, empat kepekatan (0.1, 0.2, 0.4 dan 0.8%) setiap ekstrak pelarut dan sebahagian telah disediakan; DMSO telah digunakan untuk melarut bahan di dalam air. Sampel Kawalan menggunakan 1% DMSO digunakan untuk perbandingan. Aktiviti antibakteria di kalangan bahan adalah sangat luas terhadap kedua-dua organisma ujian. Dengan kepekatan yang lebih tinggi (0.8%) ekstrak aseton daripada perlindungan buah-buahan dan daun yang dipaparkan aktiviti keseluruhan lebih tinggi daripada bahagian-bahagian lain terhadap S. aureus, manakala ekstrak etanol dari buah-buahan dan perlindungan kulit benih menunjukkan kecekapan yang lebih terhadap E. coli. Walau bagaimanapun, tiada aktiviti perencatan daun aseton dan etanol terhadap E. coli dengan kepekatan 0.8%. Kumpulan bioaktif seperti alkaloid dan bahantara lakton telah ditayangkan oleh TLC; dan keputusan yang diperoleh adalah positif. Keputusan eksperimen yang diperolehi daripada kajian ini mencadangkan bahawa ekstrak Swietenia mahagoni dapat dijadikan sebagai anti-bakteria semulajadi dan ini boleh menjamin penyelidikan lanjutan bagi menentukan kompaun bioaktif.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF SYMBOLS

cm	Centimeter
°C	Degree Celsius
g	Gram
H	Hours
mm	Millimeter
mL	Milliliter
mg	Milligram
%	Percentage

LIST OF ABBREVIATIONS

C. albcans	*Candida albcans*
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
E.coli	*Escherichia Coli*
MRD	Multiple-drug resistant
MIC	Minimal inhibition concentration
HIV	*Human Immunodeficiency Virus*
LD50	Lethe death of 50 %
S.aureus	*Staphylococcus aureus*
TLC	Thin layer chromatography
UV	Ultraviolet light

CHAPTER 1

INTRODUCTION

1.1 INTRODUCTION OF THE STUDY

In the 20 century, there are sharply improves of the science and advance in technology in the medical field to improve the health quality of human. However, there are still have a lot of human affected by some of dangerous bacterial, fungus, and virus. For example, *Human Immunodeficiency Virus (HIV), Rubella Virus, Stomatitis, Bacillus Anthrax, and Helicobacter pylori.*

Recently, hand, foot and mouth disease rash called *Stomatitis* have cause a lot baby affected and die (Chen et al., 2007). *Severe acute respiratory syndrome (SARS)* recently emerged as a human disease associated with pneumonia. This disease was first recognized in Guangdong Province, China, in November 2002. Subsequent to its introduction to Hong Kong in mid-February 2003 (Guan et al., 2003*)*. Moreover, *malaria* is one of the main public health problems which is an incredibly common and dangerous infections disease have killing approximately more than one million people each year especially young children in Sub-Saharan Africa and placing a strong burden on developing African countries (Djenontin et al., 2009).

There are a lot of vaccines, medicine, or antibacterial agents produced in the market to get rid of the diseases. However, many vaccines and antibacterial agents in the market nowadays are less efficiently. It is due to the global increase in resistance to antibacterial drug, including the emergence of bacterial strain that are resistant to all available antibacterial agents. The bacterial resist to drug though various alteration or mutations in their Deoxyribonucleic acid (DNA). Thus, the need for the development of

newer drugs to treat infections caused by these multiple-drug resistant (MDR) bacterial species has never been more paramount (Gislene et al., 2000).

The evolution drug scientists are tried to synthesis the antibacterial agents to against the bacterial. However, there are a lot of problems. For example, synthesis a new drug is too expensive, there produce many waste products in process, the drug is not stable and may be create toxic as by-products. Therefore, there is more favorable used national product to form drug. By this way, there are more safety, friendly environment, and cheaper in price.

In the global, there are many extractant from plant which are treated bacterial. Extracts from the following plants where utilized: *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa offficinalis* (lemon-balm), *Ocimun basilucum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygyum joabolanum* (jambolan) *and Thymus vulgaris* (thyme) *and Swietenia mahogani* (mahogany) (Nascimento et al., 1990).

Among these plants, *Swietenia mahogany* (mahogany) is the most potentially in plant extraction as antibacterial agent. *Swietenia mahogany* also called west India mahogany (John and Francis, 1991). Its fruit is also known as "sky fruit" (Tan et al., 2009). It comes from *Meliecease* family. It is large, semi-ever green tree form a loose rounded canopy and casts light. It is one south Florida's popular landscape and street trees. *Mahogany* can reach 75 feet in height with a 50-foot-spread.

The extracting *Swietenia mahogany* especially the plant seed have medical efficacy. It is proven traditionally and scientific used to cure malaria, anemia, diarrhea, fever, dysentery, hypertension, cancer, coughs, chest pains and intestinal parasitism (Sathish et al., 2010). The current study of *Swietenia mahogany* extractions are as antibacterial agents. There were evaluated against multiple-drug resistant (MDR) pathogenic bacteria strain such as *E.coli, Staphylococcus aureus*, and *Salmonella typhimurium*. The finding will contribute to the further research for the new antibacterial agent.

1.2 OBJECTIVES OF THE STUDY

There are three objectives of this study which are:

1. To crude extract from different parts of *Swietenia mahogany* by using different solvents (ethanol and acetone).
2. To test antibacterial activity of *Swietenia mahogany* extracts against two types of bacteria which are *Escherichia coli* and *Staphylococcus aureus.*
3. To determine lethal dose (LD50).

1.3 SCOPE OF THE STUDY

There are some important tasks to be carried out in order to achieve the objective of this study. Three important scopes have been identified for this study in achieving the objectives:

1. Study the general information of the *swietenia mahogany* plant and crude extraction of the plant in antibacterial activities.
2. Comparing antibacterial activity and crude extract from different parts of *Swietenia mahogany* by different solvents against *E.coli* and *S.aureus*.
3. Study the most effective crude extraction of *Swietenia mahogany* in antibacterial activity.

CHAPTER 2

LITERATURE REVIEW

2.1 SWIETENIA MAHOGANI PLANT

2.1.1 Species of *Swietenia Mahogani*

Swietenia mahogani also know as mahogany or West Indies mahogany in common name. It comes from *Meliacea* family (Carlos and Sergio, 2007). It is a tropical region of the America, mainly in Mexico, Bolivia and centre Americal. The genus consists of two other species, *Swietenia macrophylla* and *Swietenia humilis.* The three species are poorly defined biologically, in part because they hybridise freely. *Swietenia aubrevilleana* Stehle & Cusin is a putative hybrid between *Swietenia macrophylla* and *Swietenia mahagoni.* Figure 2.1 shows *Swietenia mahogany* plant and leaves part.

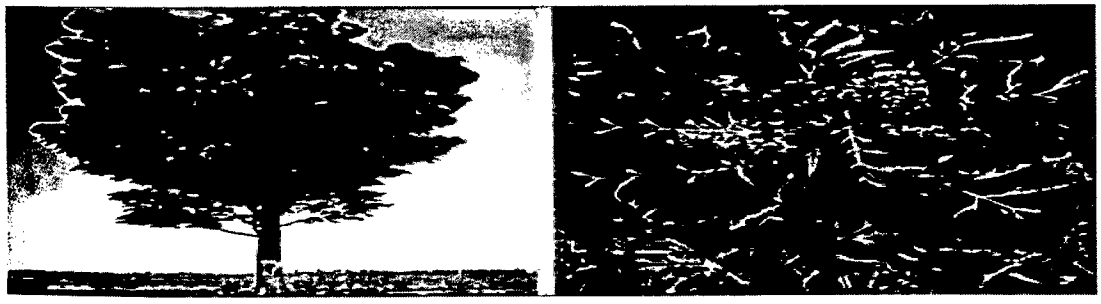

Figure 2.1: *Swietenia mahogani* plant and leave.

Source: Carlos and Sergio (2007)

2.1.2 Habitat

Swietenia mahagoni is a humid zone species, with natural distribution in the Caribbean region (S.Florida, Bahamas, Antilles, Haiti and Jamaica). The species is over exploited in much of its natural area of distribution. It has been extensively planted mainly in Southern Asia (India, Sri Lanka, Bangladesh) and in the Pacific (Malaysia, Philippines, Indonesia and Fiji), and has been introduced into cultivation in West Africa (Schmidt and Dorthe, 2000). The most important ecological characteristic that distinguishes *Swietenia mahogany* from *Swietenia macrophylla* is the ability to grow under dry conditions (Edward and Dennis, 1994). It occurs naturally in climates with annual rainfall of only 580-800 mm. The yields from plantations are generally lower than for *Swietenia macrophylla* but on dry sites it is superior and the wood quality is better.

2.1.3 Occurrence

This plant is Evergreen to semi-evergreen tree, up to 20-40 m height and more than 2 m in diameter. Mahogany species are medium to long lived, mid-succession species (John and Francis, 1991). The trunk of the plant is Bark grey and smooth when young, turning dark brown, ridged and flaky when old (Schmidt and Dorthe, 2000). Leaves clustered, glabrous, 12-15 cm long paripinnately compound with 2-4 pairs of leaflets. Leaflets ovate-lanceolate, 5-6 cm long, 2-3 cm wide, dark green, glabrous (Edward and Dennis, 1994).

2.1.4 Flowering and fruiting

The small greenish white flowers are borne in panicles attached at leaf axials near the ends of branches (John and Francis, 1991). Flowers are unisexual and the trees monoecious (Schmidt and Dorthe, 2000). The flowers are pollinated by insects and usually produce only 1 fruit (a capsule) per inflorescence. Flowering generally takes place during the spring, with fruits ripening 9 months later (Edward and Dennis, 1994). However, Flowering varies according to climate example geographical site; it usually takes place shortly before the rainy season. *Swietenia mahagoni* flowers in the

Caribbean Islands between April and July and the fruits are mature 8-10 months later, between January and March (Schmidt and Dorthe, 2000). However, season of fruiting varies between portions of the range and individual trees. Occasional trees can be found with fruits at any season of the year in Puerto Rico (Marrero, 1949).

Fruiting begins when trees are between 10 and 25 years old for open-grown and dominant or codominant trees. A few to more than a hundred capsules may be produced, depending on the size and vigor of the tree. Mahogany species produce good seedcrops nearly every year. The fruit sizes, seeds per fruit, and seeds per weight for members the genus are shown in **Table 2.1**.

	Median fruit dimensions		Seeds/weight	
Species	(cm)	Seeds/fruit	/kg	/lb
S.humilis	17 -- 11	50 +	1500	680
S.macrophylla	15 -- 8	50 - 70	1400 - 2400	640 - 1100
S.mahogani	7 -- 4	35 - 60	5400 - 7800	2500 - 3500
S.macrophylla mahognoni	12 -- 7	45 - 65	1900 - 3000	860 - 1400

Table 2.1: Lists of the fruit sizes, seeds per fruit, and seeds per weight for members the genus.

Source: (Schmidt and Dorthe, 2000)

2.1.5 Harvest, collecting, storage

As the fruits mature, they turn from gray-green to reddish brown. At maturity, the capsule walls split into 5 carpels from the bottom upwards and then fall off. The fruits are preferably collected from the trees just before opening or from the ground immediately after seeds fall.

Small quantities of seeds can be picked up from the ground near seed-bearing trees. Seeds can be collected in quantity by clipping the capsules from short-statured trees with pruning poles after the first few capsules on a tree have opened. Mature dry fruits or dry seeds collected from the forest floor can be stored for some days in sacks

without significant deterioration. However, in order to reduce bulk it is often preferable to initiate processing in the field. The fruits will split open when dried for 1-4 days, depending on maturity, after which the seeds are easily released by gentle shaking or raking of the fruits. Fruit parts (valves and columella) are removed by hand. Further reduction of bulk by manual dewing may be desired.

Storage at ambient room temperature is about 1-2 months. Storage at 15°C prolongs viability to 3-6 months. Cold storage (2-5°C) with 4-5% moisture content extends viability up to 1 or more years (Schmidt and Dorthe, 2000).

2.2 ANTIBACTERIAL ACTIVITY OF PLANT EXTRACTS ON ANTIBIOTIC RESISTANT BACTERIA

The problem of microbial resistance is growing and the outlook for the use of antimicrobial drugs in the future is still uncertain. Therefore, actions must be taken to reduce this problem, for example, to control the use of antibiotic, develop research to better understand the genetic mechanisms of resistance, and to continue studies to develop new drugs, either synthetic or natural. The ultimate goal is to offer appropriate and efficient antimicrobial drugs to the patient. For a long period of time, plants have been a valuable source of natural products for maintaining human health, especially in the last decade, with more intensive studies for natural therapies. The use of plant compounds for pharmaceutical purposes has gradually increased in Brazil. According to World Health Organization medicinal plants would be the best source to obtain a variety of drugs. About 80% of individuals from developed countries use traditional medicine, which has compounds derived from medicinal plants (Santos et al., 1995). Therefore, such plants should be investigated to better understand their properties, safety and efficiency (Ellof, 1998). The use of plant extracts and phytochemicals, both with known antimicrobial properties, can be of great significance in therapeutic treatments. In the last few years, a number of studies have been conducted in different countries to prove such efficiency (Kubo et al, 1993). Many plants have been used because of their antimicrobial traits, which are due to compounds synthesized in the secondary metabolism of the plant. These products are known by their active substances, for example, the phenolic compounds which are part of the essential oils (Jansen et al.,

1986), as well as in tannin (Saxena et al., 1994). The antimicrobial properties of plants have been investigated by a number of researchers world wide, especially in Latin America. In Argentina, a research tested 122 known plant species used for therapeutic treatments (Anesini and Perez, 1993). It was documented that among the compounds extracted from these plants, twelve inhibited the growth of *Staphylococus aureus*, ten inhibited Escherichia coli, and four inhibited *Aspergillus niger* and also reported that the most potential compound was one extracted from *Tabebuia impetiginosa.*

The antimicrobial properties of compounds obtained from *Parthenum argentatum* against *Candida albicans, Torulopsis, Hansemula, Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was detected (Martinez et al., 1994). Work done was observed that the substances extracted from nine known plants in Uruguai did not show any activity against *C. albican*s and *Saccharomyces cerevisiae*, but inhibited the growth of Bacillus subtilis, *E. coli and P. aeruginosa* (Alonso-Paz et al., 1995). Many studies have been conducted in Brazil. The inhibitory activity of *Vatairea macrocarpa* on *Klebsiella spp.* and *S. aureus* was observed (Matos et al., 1988) and the inhibitory activity of extracts from Eucaliptus spp. against soil fungi (Bruna et al., 1989). A more detailed study on antimicrobial compounds was done evaluating extracts from 120 plant species from 28 different families. It was documented that 81 extracts obtained from 58 plants were active against *S. aureus*, and five extracts from four other plants inhibited the growth of *P. aeruginosa.* Another study (Lemos et al., 1992) detected the antibacterial and antifungal (*C. albicans*) activity of essential oils obtained from Croton triangularis leaves. Extracts from *Lippia gracilis* and *Xylopia sericea* showed antifungal activity. The investigation of antimicrobial activity as well as cell toxicity of extracts from 30 plant species against five bacteria species and two fungi species was studied (Gislene et al., 2000). It was concluded that ethanol extracts from 70 % of the plants were toxic to cell and only one of the species of *Combretum duarteanum* showed antimicrobial activity. The toxicity of extracts from *Arthemus sativa*, which is known to have antimicrobial activity, was also studied (Carvalho et al., 1988). The antimicrobial activity from *Mikania triangularis*, known as "thin leaf *guaco*", was tested against five genera of bacteria and three genera of yeast, and showed it had activity against *Bacillus cereus, E. coli, P. aeruginosa, S. aureus and S. epidermidis* (Cruz et al., 1996). Effects of phytochemical were conducted (Kubo et al., 1993) and it was observed the

antimicrobial activity of anacardic acid on *S. aureus, Brevibacterium ammoniagenes, Streptococcus mutans and Propionibacterium acnes*. Later, it was tested the bactericidal activity of anacardic acid and totarol on *methicillin* resistant strains of *S. aureus* (MRSA) and the synergistic effect of these compounds associated with *methicillin* (Muroi and Kubo, 1996).

The antimicrobial activity of plant extracts and phytochemicals was evaluated with antibiotic susceptible and resistant microorganisms. In addition, the possible synergistic effects when associated with antibiotics were studied. Extracts from the following plants were utilized: *Achillea millifolium* (yarrow), *Caryophyllus aromaticus* (clove), *Melissa offficinalis* (lemon-balm), *Ocimun basilucum* (basil), *Psidium guajava* (guava), *Punica granatum* (pomegranate), *Rosmarinus officinalis* (rosemary), *Salvia officinalis* (sage), *Syzygyum joabolanum* (jambolan) and *Thymus vulgaris* (thyme). The phytochemicals benzoic acid, cinnamic acid, eugenol and farnesol were also utilized.

Even though pharmacological industries have produced a number of new antibiotics in the last three decades, resistance to these drugs by microorganisms has increased. In general, bacteria have the genetic ability to transmit and acquire resistance to drugs, which are utilized as therapeutic agents (Cohen, 1992). Such a fact is cause for concern, because of the number of patients in hospitals who have suppressed immunity, and due to new bacterial strains, which are multi-resistant. Consequently, new infections can occur in hospitals resulting in high mortality. From 1980 to 1990, there was documented a high incidence of resistant microorganisms in clinical microbiology in Brazil (Montelli et al., 1991). This fact has also been verified in other clinics around all over world.

Hence, more studies pertaining to the use of plants as therapeutic agents should be emphasized, especially those related to the control of antibiotic resistant microbes. The objective of this research was to evaluate the potential of *Swietenia mahogani* extracts on three bacterial strains as well as multi-drug resistant bacteria, which are *Escherichia coli, Staphylococcus aureus and Candida albcans.*

2.3 ANTIBACTERIAL AND ANTIMICROBIAL OF *SWIETENIA MAHOGANY* EXTRACTS

Medicinal plants are natural resources, yielding valuable herbal products which are often used in the treatment of various ailments (Dulger and Gonuz, 2004). In recent years, attempts have been taken to investigate the indigenous drugs against infectious diseases in order to help developing safer antimicrobial drugs (Rahman et al., 2001). In the continuation of this strategy there are new discovery have studied the different parts of the plant *Swietenia mahagony* for their antibacterial activities.

Swietenia mahagony, commonly known as the West Indian Mahogany, is a species of Swietenia which belongs to the family Meliaceae. The plant is native to Southern Florida and the islands of Cuba, Jamaica and Hispaniola. The plant under investigation has many traditional uses. The seeds have been used for leishmaniasis and abortion medicine by an Amazonian Bolivian ethnic group (Bourdy et al., 2000) and for the treatment of hypertension, diabetes and malaria as a folk medicine in Indonesia (Kadota et al., 1990). The bark has been used as an astringent for wounds and occasionally for tanning because of the rich red color (Falah et al., 2008).

Though the study of crude extracts from different parts (leaf, bark and seed) of *Swietenia mahagony* (Family: Meliaceae) were screened for their antibacterial activity. Among the crude extracts, chloroform and ethyl acetate extracts of leaf and bark showed good activity against all the tested organisms. The chloroform and ethyl acetate extracts of seed exhibited little or positive effect against most of the tested bacteria (Haque et al., 2009).

Besides that, there is the study of antibacterial activity of *Swietenia macrophylla* leaf extraction. The antibacterial activities of methanol, dichloromethane and *n*-hexane e xtracts of *S. macrophylla* leaves were evaluated. The antimicrobial activity of the extracts was tested against four species of bacteria, *Staphylococcus aureus*, *Bacillus subtilis*, *Escherichia coli* and *Pseudomonas aeruginosa*, and a fungus, *Candida albicans*. The methanol and the dichloromethane extracts were found to be active

against the Gram positive bacteria tested. The methanol extract also showed antifungal properties (Tan et al., 2009).

In addition, there is the study of seed *Swietenia mahogany* in antibacterial activity analysis of *Swietenia mahagoni* seed oil. The oil extracted from *Swietenia mahagoni* seed was studied with a view to finding out its suitability for ethnomedical uses with special focus on antibacterial activities. Some of its physical and chemical properties were examined and compared with those of standard oils: olive, sunflower, cotton seed, linseed, soybean, coconut, palm and castor. The refined oil was found to show good to moderate activity against disease causing bacteria *viz. Shigella dysenterial, Salmonella typhi, Staphylococcuss aureus* and fungal pathogens viz. *Macrophomina phascolma, Alternaria alternata, Curvularia lunat*a (Majid et al., 2004). For example previous study of antibacterial activity of *Swietenia mahagoni* crude methanolic seed extraction. This study was designed to evaluate the antibacterial activities of *Swietenia mahagoni* crude methanolic (SMCM) seed extract. The antibacterial activity of the oily extract against bacterial was evaluated based on the inhibition zone using disc diffusion assay, minimal inhibition concentration (MIC) and minimal bactericidal concentration (MBC) values. The Swietenia mahogany crude methonolic (SMCM) seed extract had inhibitory effects on the growth of *Candida albicans, Staphylococcus aureus, Pseudomonas aeroginosa, Streptococcus faecalis* and *Proteus mirabillase* (Sahgal et al., 2009).

As the result, crude extracts from different parts (leaf, bark and seed) of *Swietenia mahagony* (Family: *Meliaceae)* show well antibacterial activities. Different solvent extraction in different parts of *swietenia mahogany* will have different compounds and concentration. Therefore, the extraction of the plant can used as antibacterial reagent in inhibition of bacterial activities such as *Candida albicans, Staphylococcuss aureus, Escherichia coli* and others.

2.4 *ESCHERICHIA COLI*

2.4.1 Introduction

Escherichia coli (commonly abbreviated *E.coli*) is a Gram-negative, rod-shaped bacterium that is commonly found in the lower intestine of warm-blooded organisms. Most *E.coli* strains are harmless, but some serotypes can cause serious food poisoning in humans, and are occasionally responsible for product recalls. Some kinds of *E. coli* can cause diarrhea, while others cause urinary tract infections, respiratory illness and pneumonia, and other illnesses (Dippold and Vogt, 2005). The harmless strains are part of the normal flora of the gut, and can benefit their hosts by producing vitamin K_2 (Bentley and Meganathan, 1982) and by preventing the establishment of pathogenic bacteria within the intestine.

The genus *Escherichia* and *Salmonella* diverged 102 million years ago, which coincides with the divergence of their hosts: the former being found in mammals and the latter in birds and reptiles. This was followed by a split of the escherichian ancestor into five species (*E.albertii*, *E.coli*, *E.fergusonii*, *E.hermannii* and *E.vulneris*. The last *E. coli* ancestor split between 20 and 30 mya (Lecointre et al., 1998).

In 1885, Theodor Escherich, a German pediatrician, first discovered this species in the feces of healthy individuals and called it *Bacterium coli communic* due to the fact it is found in the colon and early classifications of Prokaryotes placed these in a handful of genera based on their shape and motility (Daegelen et al., 2009). *Bacterium coli* is the type species of the now invalid genus *Bacterium* when it was revealed that the former type species (Bacterium triloculare) was missing (Breed and Conn, 1936). Following a revision of *Bacteria* it was reclassified as *Bacillus coli* by Migula in 1895 and later reclassified in the newly created genus *Escherichia*, named after its original discoverer. The genus belongs in a group of bacteria informally known as "coliforms", and is a member of the *Enterobacteriaceae* family (the enterics) of the *Gammaproteobacteria* (George, 2005).

2.4.2 Biochemistry of *E.coli*

E. coli is Gram-negative, facultative anaerobic and non-sporulating. Cells are typically rod-shaped, and are about 2.0 micrometers (μm) long and 0.5 μm in diameter, with a cell volume of 0.6 – 0.7 $(\mu m)^3$. However, the volume of E.coli will be increase when shift to richer media (Kubitschek, 1990). It can live on a wide variety of substrates. *E.coli* uses mixed-acid fermentation in anaerobic conditions, producing lactate, succinate, ethanol, acetate and carbon dioxide. Since many pathways in mixed-acid fermentation produce hydrogen gas, these pathways require the levels of hydrogen to be low, as is the case when *E. coli* lives together with hydrogen-consuming organisms, such as methanogens or sulphate-reducing bacteria.

Optimal growth of *E. coli* occurs at 37°C (98.6°F) but some laboratory strains can multiply at temperatures of up to 49°C (120.2°F) (Fotadar et al., 2005). Growth can be driven by aerobic or anaerobic respiration, using a large variety of redox pairs, including the oxidation of pyruvic acid, formic acid, hydrogen and amino acids, and the reduction of substrates such as oxygen, nitrate, dimethyl sulfoxide and trimethylamine N-oxide (Ingledew and Poole, 1984).

Strains that possess flagella can swim and are motile. The flagella have a peritrichous arrangement (Darnton et al., 2006). *E.coli* and related bacteria possess the ability to transfer DNA via bacterial conjugation, transduction or transformation, which allows genetic material to spread horizontally through an existing population. This process led to the spread of the gene encoding shiga toxin from *Shigella* to E. coli O157:H7, carried by a bacteriophage (Brussow et al., 2004).

2.4.3 Diversity

Escherichia coli encompass an enormous population of bacteria that exhibit a very high degree of both genetic and phenotypic diversity. Genome sequencing of a large number of isolates of *E. coli* and related bacteria shows that a taxonomic reclassification would be desirable. However, this has not been done, largely due to its

medical importance and *Escherichia coli* remains one of the most diverse bacterial species: only 20% of the genome is common to all strains (Ussery et al., 2010).

In fact, from the evolutionary point of view, the members of genus shigella (*dysenteriae, flexneri, boydii, sonnei*) should be classified as *E. coli* strains, a phenomenon termed taxa in disguise (Reeves et al., 2000). Similarly, other strains of *E. coli* (e.g. the K-12 strain commonly used in recombinant DNA work) are sufficiently different that they would merit reclassification.

A strain is a sub-group within the species that has unique characteristics that distinguish it from other strains. These differences are often detectable only at the molecular level; however, they may result in changes to the physiology or lifecycle of the bacterium. For example, a strain may gain pathogenic capacity, the ability to use a unique carbon source, the ability to take upon a particular ecological niche or the ability to resist antimicrobial agents. Different strains of *E. coli* are often host-specific, making it possible to determine the source of faucal contamination in environmental samples. For example, knowing which *E. coli* strains are present in a water sample allows researchers to make assumptions about whether the contamination originated from a human, another mammal or a bird.

(i) Serotypes

A common subdivision system of *E. coli*, but not based on evolutionary relatedness, is by serotype, which is based on major surface antigens (O antigen: part of lipopolysaccharidelayer; H: flagellin; K antigen: capsule), e.g. O157:H7) (Orskov et al., 1977)

Serotypes were discovered by the American microbiologist Rebecca Lancefield in 1933. Serotype refers to distinct variations within a subspecies of bacteria or viruses. These microorganisms are classified together based on their cell surface antigens. Determining serotypes, the process of serotyping, can be based on a variety of factors, including virulence, lipopolysaccharides (LPS) in Gram-negative bacteria, presence of an exotoxin (such as pertussis toxin in *Bordetella pertussis*), plasmids, phages, genetic profile (such as determined by polymerase chain

reaction), or other characteristics which differentiate two members of the same species, allowing the epidemiologic classification of organisms to the sub-species level. A group of serovars with common antigens is called a serogroup. The *Salmonella* genus of bacteria, for example, has been determined to have over 4400 serotypes, including *Salmonella enterica* serovar Typhimurium, *S. enterica* serovar Typhi, and *S. enterica* serovar Dublin (Ryan and Ray, 2004).

(ii) Genome plasticity

Like all lifeforms, new strains of *E. coli* evolve through the natural biological processes of mutation, gene duplication and horizontal gene transfer, in particular 18% of the genome of the laboratory strain MG1655 was horizontally acquired since the diverged from *Salmonella* (Lawrence and Ochman, 1998). In microbiology, all strains of E. coli derive from E. coli K-12 or E. coli B strains. Some strains develop traits that can be harmful to a host animal. These virulent strains typically cause a bout of diarrhea that is unpleasant in healthy adults and is often lethal to children in the developing world. More virulent strains, such as O157:H7 cause serious illness or death in the elderly, the very young or the immunocompromised (Servin et al., 2001).

(iii) Neotype strain

E. coli is the type species of the genus (*Escherichia*) and in turn *Escherichia* is the type species of the family *Enterobacteriaceae*, where it should be noted that the family name does not stem from the genus *Enterobacter* + "i" (sic.) + "aceae", but from "enterobacterium" + "aceae" (Euzéby, 1997). The original strain described by *Escherich* is believed to be lost, consequently a new type strain (neotype) was chosen as a representative: the neotype strain is ATCC 11775, also known as NCTC 9001, which is pathogenic to chickens and has a O1:K1:H7 serotype. However, in most studies either O157:H7 or K-12 MG1655 or K-12 W3110 is used as a representative *E.coli*.