CHAPTER 2

LITERATURE REVIEW

2.1 *Escherichia coli*

2.1.1 Taxonomy

Domain	: *Bacteria*
Kingdom	: *Bacteria*
Phylum	: *Proteobacteria*
Class	: *Gamma Proteobacteria*
Order	: *Enterobacteriales*
Family	: *Enterobacteriaceae*
Genus	: *Escherichia*
Species	: *Escherichia coli*

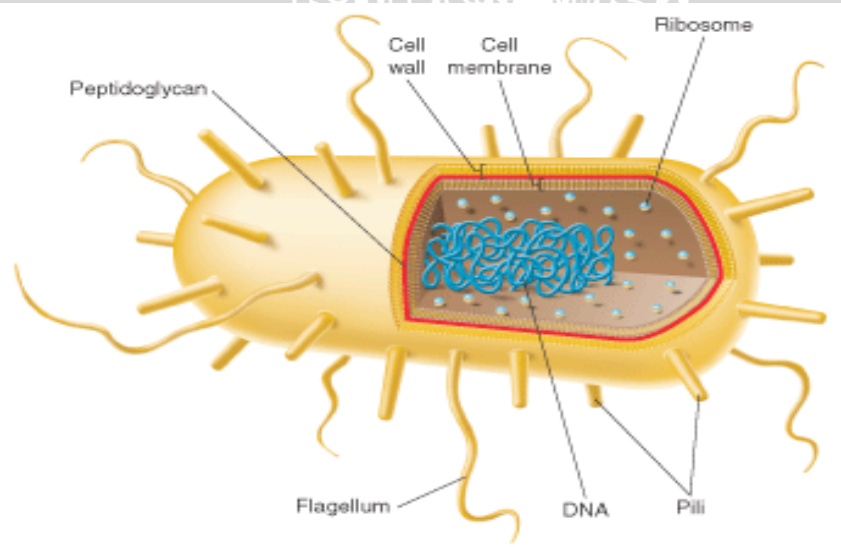

Figure 2.1 *Escherichia coli* Structure (Cosbiology, 2009)

Escherichia coli is the most abundant facultative anaerobic microorganism that is found in the gastrointestinal tract in humans and mammals. This bacteria is cylindrical in shape,grossly has a measurement of 0.4-0.8 x 1.0-1.4 µm, a gram negative bacteria, able to live independently or dependent. These bacteria appear in the body just few hours after birth. The *Escherichia coli* actually forms a mutualistic relationship with its host bacteria normally adhere to the mucus or the epithelium of the wall of the intestines. (Davis, 2010).

2.1.2 Morphology

Echerichia coli is a gram negative (bacili) from the family *Enterobacteriacea* found in the gastrointestinal flora. It has flagella, outer cell membrane, inner and outer plasma membrane. The capsule of *Escherichia coli* is made from polysaccharide gel which has a pathogenic factor that will protect it structure against any phagocytosis until opsonized. The outer membrane consists of phospholipids that made from lipopolysaccharide, lipid A and polysaccharide. The process of passive transport is done by the outer membrane and protein porins.The lipopolysaccharide from outer membrane is endotoxin and causes fever and inflammation. (Todar, 2011).

The cell wall consist of murein,lipoprotein,phospholipids and lipopolysaccharide(LPS) and arranged as layers. The murein-lipoprotein consist of 20% of the cell wall and is responsible of the cellular rigidity.The remaining 80% bonds with lipids from the lipoprotein to form lipid bilayer. (Dzen et al, 2010).

2.1.3 Culture and Biochemical Reaction

Escheriachia coli is a facultative anaerobic bacteria and during low oxygen concentration it will ferment carbohydrate. Besides that, *Escheriachia coli* can also ferment glucose, reduction of nitrate to nitrite but cannot lysis alginate. Eosin Methylene Blue(EMB) agar can be used to culture *Escherichia coli*. EMB contains lactose and sucrose with 2 indicators dyes, Eosin Y and Methylene Blue.This was developed by Holt-Harris and was further modified by Levine to give better differentiation by removing the sucrose and adding more lactose. *Eschericha coli* will grow as large, blue-black, green metallic sheen. (Kaiser, 2002).

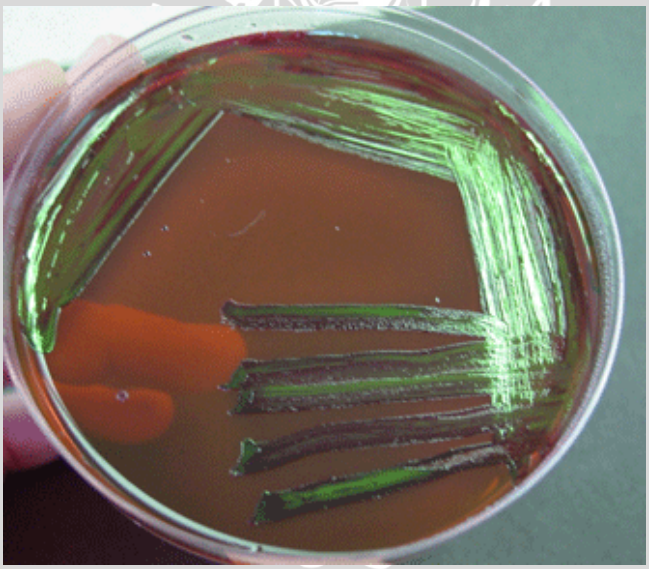

Figure 2.2 *Escherichia coli* Colonies in EMB agar (lifesci-rutgers)

Biochemical reaction on gram negative bacili from the Enterobacteriaceae are done frequently around the globe.Many conventional biochemical test media have been developed to identify and study bacterias.

The Gram Stain helps to distinguish gram positive and gram negative bacteria. This is based on the thickness of their respective cell walls as *Escherichia coli*,gram negative bacteria have a thinner cell wall and hence easier for the alcohol to penetrate.The steps of Gram stain is as follows,first crystal violet is added,then Lugol's iodine solution (a mordant) is added,then alcohol is added which extracts the blue dye complex from the thin wall of gram negative *Escherichia coli* and finally safranin is added and it stains the decolorized gram negative bacteria. Another test done to determine gram negative bacteria such as *Escherichia coli* is MacConkey agar where the bacteria will ferment the lactose and hence giving a red colour appearance on the agar. (Jawetz, 2003).

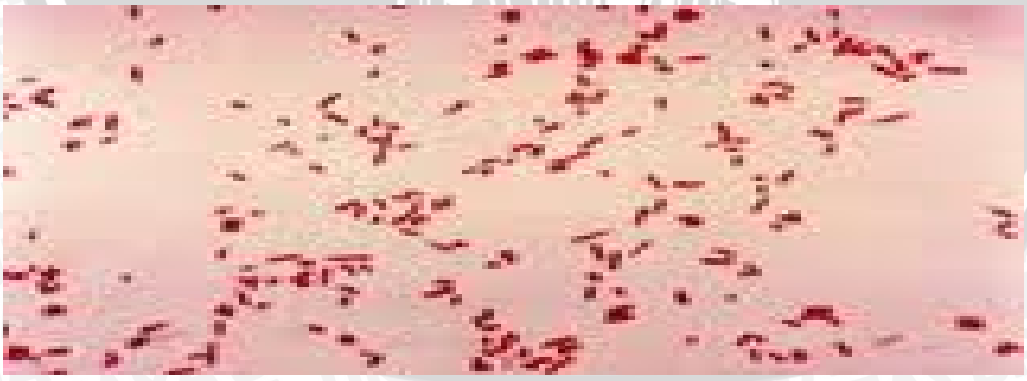

Figure 2.3 *Escheriachia coli* Gram Staining(Jawetz, 2003)

2.1.4 Pathogenicity

Escherichia coli which is an *Enterobacteriaceae* have a complex antigen structure. It consist of 3 different antigens such as O antigen, K antigen and H antigen. The O antigen (somatic antigen) is derived of lipoprotein from the cell wall,the K antigen (capsule antigen) from the polysaccharide capsule and the H antigen (flagella antigen) is derived from the flagella. There are more than 164 O antigens,100 K antigens and 50 H antigens.Most of these specific strains can be divided into three more categories based on how they infect mankind such as gastroenteritis,meningitis and urinary tract infection. (Dzen et al, 2010).

Gatstroenteritis (intestinal disease) enterovirulent *Escherichia coli* cause diarrhea and poisoning by producing toxins and causing inflammation.Types of *Escherichia coli* which can cause these conditions are EPEC (Enteropathogenic E. coli), EHEC (Enterohemorrhagic E. coli), ETEC (Enterotoxigenic E.coli),EIEC (Enteroinvasive E.coli) and EAEC (Enteroadherent E.coli). (Davis, 2010).

There are 2 types of Enterotoxin produced by *Escherichia coli* that are *heat-labile enterotoxin* (LT) and *heat-stable enterotoxin (ST).* Each enterotoxin have a slight different mechanism of pathogenicity. LT activates the adenylate cyclase enzyme of the intestinal mucosa leading to increased levels of intracellular cAMP and secretion of water, Na^+, K^+ into the lumen of the small intestine. This toxin causes cAMP to be produced at abnormal rate which in turn stimulates mucosal cells to pump large amount of Cl^- into intestinal content and hence water together with other electrolytes follow it due to osmotic gradient. This causes diarrhea, loss of electrolytes and dehydration. ST increase the cyclic GMP in host cell cytoplasm and

leading to same effects as increase in cAMP and bind to guanylate cyclase located on membranes of host cells and therefore leads to secretion of fluid and electrolytes resulting in diarrhea. (Todar, 2011).

Enterotoxigenic *Escherichia coli* (ETEC) are common cause of *traveller's diarrhea*(among tourist) and also very important cause of diarrhea in infants in the 3rd world countries.ETEC are aquired by the ingestion of contaminated food and water.People who live in areas where there is high prevalence,usually develop immunity towards ETEC. (Dzen et al, 2010).

Enteroinvasive *Escherichia coli* (EIEC) causes disease by penetrating and invade epithelial cells of the intestinal mucosa and results in large area of cell destruction. EIEC are very similar to Shigella in their path of symptoms which is diarrhea and fever. (Dzen et al, 2010).

Enterohemorrhagic *Escherichia coli* (EHEC) causes severe diarrhea and hemorrhagic collitis. EHEC are transmitted by infected dairy products and hamburger meat and they releases a verotoxin which attacks the intestinal system. Hemorrhagic collitis is an acute condition and usually resolves spontaneously with abdominal pain and watery-bloody stool. (Robson, 2002).

Enteroadherent *Escherichia coli* (EAEC) causes diarrhea in young children by adhering attaching themselves to intestinal wall (mucosal wall) and release enteroadherent toxin.The main difference between ETEC is that there is no inflammation or invasion. (Davis, 2010).

Escherichia coli is the main etiology of urinary tract infection and the main colonies of bacteria are the Uropathogenic E.coli (UPEC). This disease has a higher

prevalence among women because of their anatomical,maturity and the difference of urogenital tract during pregnancy and delivery.This colony of E.coli have type P fimbria which attaches it self to the urogenital system and hence causing the symptoms such as painful urination, frequent urination, and cloudy urination. (Dzen et al, 2010).

Meningitis is the inflamation of the meninges which affects 1 out of 2000 infants, is caused by strains of *Escherichia coli* invading the nasopharynx or the gastrointestinal tract and then travel through bloodstream and cross the BBB (Blood Brain Barrier) and infecting the meninges. (Robson, 2002).

2.2 *Origanum vulgare*

2.2.1 Taxonomy

Figure 2.4 Oregano (BBC,2014)

Kingdom :Plantae

Subkingdom :Tracheobionta

Subdivision :Spermatophyte

Divison :Magnoliophyta

Class :Magnoliopsida

Subclass :Asteridae

Order :Lamiales

Family :Lamiaceae

Genus :*Origanum L*.

Species :*Origanum vulgare L*.

(United States Department of Agriculture, 2014)

2.2.2 Content

The *oregano* plant contains many types of chemical compounds and has been done vast research on the pharmacological activity. The main components of *oregano* leaves were dominated by the *oxygenated monoterpenes fraction, carvacrol*, *p-cymene, pinene* and *α-terpinene*. (Afef , 2013).

There are also other research that had been done to find the actual components of *oregano* leaves and it shows *oregano* species are characterized besides than the presence of two main chemotypes, *thymol* and *carvacrol*,there is also high content of *two monoterpene hydrocarbons, g-terpinene, linalool* and other *monoterpenes* and *sesquiterpenes*.There are also volatile compounds such as *1,8 cineole,borneol,camphor.* (Busattal,2007).

Another research indicates that per 100g *oregano* extract contains about 13.5 g, 40 mg *sodium*, less than 0.1 g *fat*, 3.5 g *protein*, 11.5 g *total sugars*, 6.5 g *glucose*, 4.0 g *fructose*, 1.0 g *sucrose*, 4.0 g *total nutritional fibers*, and 7.0 g *water*. The extract contains about 24% *phenolics* and 3% *rosmarinic acid.* (Charrondiere, 2010).

The main active components of *oregano leaves* extract is *phenols* which consists of *phenolic acid* and *flavonoids*. *Caffeic acid,hispidulin,rosmarinic acid* and *hydroxycinnamic acid* have been demonstrated to possess strong antioxidant activity and they are the main antioxidant present in oregano.These components can exert anti inflammatory properties when react with isoprenoids such as tochopherols and caretenoids. (Zheng and Wang , 2001)

The flavonoids can be divided into 2 classes which are *free flavonoids* and *flavonoid glycosides*.*Free flavonoids* are often highly methylated.Methly ethers of *flavones*,*flavonols*, *flavanones* and *dihydroflavones* are insoluble in water and are found on the plant surface, in the waxy layer or in the glands. The other class of *flavonoids* includes *O-glycosides* and the *C- glycosides* that are water soluble and are accumulated in the vacoules of plants. (Kintzios, 2002).

Few studies has shown that *flavonoids* have antimicrobial activity although the specific mechanism havent been discovered yet but this component can cause the inhibition of nucleic synthesis, inhibition of cytoplasmic membrane function and also the inhibition of energy metabolisme. (Cushnie and Lamb, 2005).

The *monoterpenes* can act as an antimicrobial agent because it causes pertubation of the lipid fraction of the plasma membrane.This process actually alters membrane permeability and hence the leakage of intracellular materials happens. (Trombetta, 2005).

Phenolic acids such as *hydroxycinnamic acid* and *caffeic acid* prevents the adhesion of bacterial cells to their target such as urogenital tract. Besides that, *phenolic acid* also reduces the formation of biofilms and hence reducing the chances of the bacteria becoming resistance to drugs. Lastly, these components increase the permeability of the outer membrane of the bacteria. (Nohynek, 2006).

2.2.3 Usages of *Oregano vulgare*

There are many uses of *oregano* and it can be used daily. A few example of application of this plant is as follows,in cooking,medical therapy,in cosmetics and others uses. (Nordqvist, 2013).

1. Cooking

Oregano leaves are used vastly in cooking especially in western cuisine such as roasting and grillling usually dried oregano leaves are used as they can be kept longer.Oregano is also used in pizza to give the herbs aroma and helps digestion.

2. Medical therapy

The herb (*oregano* oil) is used to treat respiratory tract disorders, gastrointestinal disorders, menstrual cramps and urinary tract infection. Besides that,this herb is also used in tootache because it possesses anti-inflammatory properties.

3. Cosmetics

The herb is also applied topically to help treat a number of skin conditions such as dandruff. This herb works on hair follicles and hence stimulate the regrowth of hair. Acne can also be treated by using this herb.

4. Others

This herb are used frequently as garden decorating plants and some communities believe that this herb can ward of insects such as mosquitoes. (Nordqvist, 2013)

2.3 In Vitro Microbial Susceptibility Testing

The in vitro antimicrobial susceptibility testing is done to prove whether the tested antimicrobial extract can be used to overcome the bacteria's infection. This testing can be done by 2 methods which are dilution method and diffusion method.

1. Dilution method

Dilution method consist of 2 types, the first is broth dilution while the second is agar dilution. The Broth Dilution method is a simple procedure for testing a small number of isolates even single isolate. It has the advantage that the same tubes that were used to determine the MIC can be used to determined the MBC.

The Agar Dilution method is the second type and most often prepared in petri dish and have advantage that it is possible to test several organisms on each plate. This procedure was usually used if a particular extract contains active substances such as tannin since tannin has coagulative property.

Susceptibility testing methods are used to determine the minimal concentration of antimicrobial (MIC) to inhibit or the minimal bactericidal concentration (MBC).This method consist of 2 stages where the first stage is to determine the minimal inhibitory concentration (MIC) by using broth media while the second stage is using agar media to determine the minimal bactericidal concentration.

To determine the MIC, a series of test tube are filled with broth media and a fixed amount of bacteria. Then each test tube is added with different concentration of antimicrobial substance. The lowest concentration of antimicrobial substance which

causes the lack of turbidity or clear turbidity shows the MIC. More clearer turbidity shows lesser growth of bacteria.

To determine the MBC, cultures from each of the test tube is inoculated on agar media. The lowest concentration of antimicrobial substance which causes no colonies of bacteria shows the MBC. (Dzen et al, 2010)

2. Discs Diffusion method

This method was done by infusing antimicrobial with disc. The antimicrobial disc will then be dispended onto the surface of inoculation medium plate which has been mixed with the studied bacteria and incubated at 37^{o}C for 18-24 hours.. Observation was done on the clear zone around the disc which showed no bacteria growth. There are 2 methods to evaluate the effectiveness result. The first is Kirby Bauer method which compares the clear zone around the disc using the NCCLS standard table. With this table, it is possible to know the sensitivity criteria, intermediate sensitivity and resistance. The second method the Joan Stokes, compares the inhibition zone which occurs between a known effectiveness of control bacteria against the drug with the tested bacteria isolate.(Dzen et al,2010)