Probiotic Effect on *Proteus mirabilis* and its Adhesion Property

A thesis

Submitted to the College of Science / AL-Nahrain University

In partial fulfillment of the requirements for the degree of

Master of Science in Biotechnology

By

Ghyda'a Hussein Abdul-Raheem Al-Jeboury

B. Sc. Biotechnology Al-Nahrain University. 2002

May *2005*

بسم الله الرحمن الرحيم

ن وَالقَلم وَمَا يَسْطُرُون

صَدق الله العَظيم

القلم : ١

ألاهداء

اليكَ ...

يا من احتارت كلماتي في وصف حنانك ... ابي الغالي

اليكِ...

يا من حملتني وهنا على وهن...امي الحبيبة...

اليكم ...

يا خير الاخوة والاخوات...اخواني واخواتي الاعزاء...

الى مشرف البحث...

الدكتور عبد الواحد باقر الشيباني...

الى صديقتي ...

رفيقة دربي... ريم

اهدي ثمرة جهدي المتواضع

غيداء

Acknowledgements

Thank **GOD** the most merciful and gracious who gave me the power and health to complete this work.

I would like to express my thanks to my supervisor **Professor Dr. Abdul W. Baqir** for suggesting the project and for his advisement, and support and encouraged which enable me to accomplish my project and thesis.

Thanks are deviated to the head of Biotechnology Department and Dean of the College of Science for support and help.

I, also wish to thank my dear colleague Reem and friends; Miss Dafar, Rana, Hanaa, Wassen, Rahab, as well as my colleagues of graduate students.

Finally, deep thanks and respect should be introduced to my family patience, helps and support.

Ghyda'a

Committee Certification

We, the examining committee, certify that we have read this thesis and examined the student in its contents and that, according to our opinion, is accepted as a thesis for the degree of Master of Science in Biotechnology.

Signature:

Name:

Scientific Degree:

Date:

(Chairman)

Signature:

Name:

Scientific Degree:

Date:

(Member)

Signature:

Name:

Scientific Degree:

Date:

(Member)

I hereby certify upon the decision of the examining committee

Signature:

Name: **Dr. Laith A. Z. Al- Ani**

Scientific Degree: Assistant Professor

Title: Dean of College of Science

Date:

Supervisor Certification

I certify that this thesis was prepared under my supervision in the College of Science, Al-Nahrain University as a partial requirement for the degree of Master of Science in Biotechnology.

Signature:

Supervisor: **Dr. Abdul W. Baqir**

Scientific Degree: Professor

Date:

In review of the available recommendations, I forward this thesis for debate by the examining committee.

Signature:

Name: **Dr. Majed H. Al-Gelawi**

Scientific Degree: Assistant professor.

Title: Head of Biotechnology Department.

Date:

List of Contents

List o f Tables

List o f Plates

List o f Abbreviations

Abbreviation	Mean
UTI	Urinary Tract Infection
MRS	DeMan Regoza Sharpe
LAB	Lactic Acid Bacteria
LB	Luria-Bertonia
TSI	Triple Sugar Iron
PAD	Phenylalanin Deaminase
PBS	Phosphate Buffer Saline

List o f Figures

Chapter One

Introduction and Literature Review

1.1 Introduction:

In the last decades, microorganisms and their metabolic products were broadly used in treatment of various diseases and infections. Normal flora, such as lactic acid bacteria (LAB), found in the gastrointestinal tracts can produce different types of materials; organic acids, ammonia, hydrogen peroxide, diacetyl, bacteriocins and others which had been used as inhibitory means against pathogenic bacteria. LAB were used to treat gastric disturbance, colon irritation, diarrhea and even colon carcinoma (Donohue and Salminen, 1996). One of the most common causes of UTI is *Proteus mirabilis* which have many various virulence factors, such as adhesion, swarming, urease, hemolysin and protease production that causing infection (Mobley and Belas, 1995). Adhesion is the initial step of *Proteus mirabilis* infection (Mobley and Chippendle, 1990). Investigations suggested that pili are the adherence element responsible for binding uropathogenic *Proteus mirabilis* to uroepithelium (Wray *et al*., 1986). Adhesion involves complex interaction between pili and specific complex carbohydrate (as receptors) of host cell membrane (Jackson *et al*., 1977). It was found that probiotic (LAB isolates) have inhibitory effect of the adherence of bacteria, and can alter some surface structures of gram negative bacteria without killing it (Chan *et al*., 1984).

Aims of the Study:

- Isolation and identification of *Proteus* isolates from patients suffering from UTI.
- Isolation and identification of *Lactobacillus* isolates form yoghurt samples.

- Detecting the most antibiotics-resistant isolate of *Proteus mirabilis* to be used in the probiotic experiment.
- Investigation the inhibitory effect of LAB isolates against the pathogenic *Proteus mirabilis*.
- Determining the minimum inhibitory concentration of LAB filtrates on the adhesion property of *Proteus mirabilis* isolate.

1.2 Literature Review

1.2.1 Urinary Tract Infection (UTI):

Urinary Tract Infection is one of common diseases, occurring from neonate up to adult age groups. More than 80% of Urinary Tract Infection is due to the bacteria like *Escherichia coli* and *Proteus mirabilis* (Jonhnson and Stamm, 1989). Urinary Tract Infection defines a condition in which the urinary tract is infected with the pathogen causing inflammation (Kunin and White, 1993). The major cause of UTI is gram negative bacteria which belongs to the *Enterobacteriaceae* family (Mobley *et al*., 1994).

Walker (1999) stated that most common bacteria causing UTI is *Proteus mirabilis* Which is gram negative, motile, swarmer bacteria and this bacteria attached to the penetrated tissue, resists host defenses and induce change to the host tissue, while Sleigh and Timbury (1994) pointed out that symptoms of UTI one frequent urination, flank pain, dysuria, burning with urination and some time fever.

1.2.2 Etiology of Urinary Tract Infection:

The member of family Enterobacteriaceae is considered as the major causing organisms of UTI which are originating in the gut before entering the urethra (Stamey *et al*., 1971).

There is consensus that most uropathogenic microorganism such as *Escherichia coli* colonize the colon, perianal region, and in female vagina, facultatively they may further ascend to the bladder and / or kidneys (Clague and Horan, 1994).

Proteus mirabilis is the organism which infect a much higher proportion of patient with complicated UTI, and in the infected patient, *Proteus mirabilis* does not only cause cystitis and acute pyelonephritis but it also cause urinary stones

which complicate further the problems associated with urinary tract (Mobley and Belas, 1995).

Glauser (1986) in Philadelphia and Mims (1987) in Torento stated that *Klebsiella, Pseudomonas aeurogenosa, Enterobacter, Serratia* are more frequently found in hospital acquired UTI due to their selection in hospital patients, while Mitchell (1964) declared that gram positive species, mainly *Staphylococcus epidermidis* (which cause mild infection), *Staphylococcus aureus* and *Enterococci* are more associated with the UTI s in hospitalized patients.

Yeast, especially that belongs to *Candida,* could cause UTI. Viruses also can cause UTI such as *Herpes simplex* virus which produces an active urithritis (Stamm, 1998).

Table (1-1), which is assembled the most frequent microorganisms causing UTI s in human.

Table (1-1). Microorganisms causing UTI s in human.

Bacteria
Gram Positive
Corynebacterium spp.
Enterococcus spp.
Staphylococcus aureus
Staphylococcus epidermidis
Staphylococcus saprophyticus
Staphylococcus hominis
Staphylococcus hemolyticus
Streptococcus feacalis
Streptococcus milleri
Staphylococcus xylosis

Gram Negative
Acinotobacter spp.
Brucella spp.
Enterobacter spp.
Escherichia coli
Haemophilus influenzae
Klebsiella spp.
Nisseria gonorrhoeae
Proteus spp.
Pseudomonas spp.
Serratia marsecens
Salmonella spp.
Yeast
Candida spp.
Viruses
Herpes simlpex

(Maskell, 1988; Navarro *et al*., 1994; Terai *et al*., 1994)

1.2.3 Pathogenesis of UTI:

Many routes by which the bacteria cause UTI have been described, they are:

a- Ascending route: It's the most important mean by which the urinary tract became infected (Santoro and Kaye, 1978). It includes the ascend of infections agents from the external (genital and perineal region) to the urinary tract and cause infection (Ogra and Faden, 1985). Tanagh and McAninch (1995) stated that ascending infection is the most common cause of UTI in women than in man.

b- Haematogenous route: It's uncommon route, and includes the transfer of bacteria from blood which contains bacteria during bacteriemia to urine through the kidney (O'Gradyfwl, 1980).

c- Lymphatic route: Infection of urinary tract by means of lymphatic channels probably occurs, but this is rare (Mims *et al*., 1987; Sobel and Kaye, 1992; Tanagh and McAninch, 1995).

1.2.4 Genus *Proteus*:

Enterobacteriaceae is considered as one of the biggest family from the five groups of Bergy's key of classification 1994, *Proteus* is one of important medical genera which return to this family. Bacteria in this genus is gram negative rods, measuring (1.5-3 μm) in length and 0.5 μm in diameter, motile by peritrichous flagella, facutatively anaerobic, non-sporforming, uncapsulated, most isolates having fimbriae, it's oder is very strong (Gruikshank, 1975).

Mobley and Belas (1995) mentioned that *Proteus* applied its name by Hauser in 1885 for their different shapes, from short vegetative swimmer cell to elongated highly flagellated forms referred as swarmer cell. The most important feature which differentiated *Proteus* from other genera in the *Enterobacteriaceae* family is the swarming phenomena.

Jawetz *et al* (1998) pointed out that the genus *Proteus* has four species which are *P. mirabilis, P. vulgaris, P. penneria and P. myxofaciens.*

Proteus is found in the soil, polluted water, intestinal tract of many mammals including humans (Ananthanarayan and Pariker, 1988). It could be isolated from clinical specimen such as urine, wounds, and blood and it also found in normal flora in the intestine of healthy human, but it is considered as apportunistic pathogenes that causing many infections when moves from their normal site (Davis *et al*., 1990).

1.2.5 Virulence Factors of *Proteus mirabilis:*

Proteus mirabilis have many virulence factors which help in causing the infections these are:

1.2.5.1 Fimbriae and Adherence Ability of *Proteus mirabilis*:

Mobley and Chippendle (1990) mentioned that the ability to adhere to uroepithelium is considered as important virulence factor in *Proteus mirabilis*.

However, presence of fimbriae on the bacterial cell surface the bacterium to stick at the specific site on epithelium. This fimbriated cells can develop pylonephritis and considered as more virulent than that smooth one (Silverblatt, 1974).

Strains of *Proteus mirabilis* attached only to sequamous and not to transitional epithelial cells, where most of *E. coli* tested attached to both cell types (Eden *et al*., 1980).

1.2.5.2 Swarming of *Proteus*:

Swarming was also considered as an important phenomenon during life cycle of *Proteus*, it is a cyclical differentiation process in which typical vegetative rods (2-4 μm in length) differentiate into long (up to 8 μm), a septate filaments that posses up to 50 fold more flagella per unit cell surface area (Liaw *et al*., 2000).

Mobley and Belas (1995) stated that the swarming cell differentiation is a result of at least three separate phenomena including the production of elongated swarmer cells, the synthesis of vastly increased amount of flagellin and the coordinate multicellular interaction that result in a cyclic waves of cellular differentiation. There are many anti-swarming agents which can inhibit the swarming phenomena of *Proteus mirabilis* such as alcohol (6%), sodium azide, boric acid (Gupta, 1988) and poly nitrophenylglycerol (Liaw *et al*., 2000).

1.2.5.3 Hemolysin Production of *Proteus mirabilis*:

Two distinct hemolysins have been found among *Proteus* isolates which are HpmA and HlyA (Koronakis *et al*., 1987).

Swihart and Welch, (1990) found that HpmA which is calicum-independent hemolytic activities produced by all strains of *Proteus mirabilis* and most strain of *Proteus vulgaris*, while HlyA which is calcium dependent hemolysin activity is not found in *Proteus mirabilis* but it is found in some strains of *Proteus vulgaris*. In addition, hemolytic strains of *Proteus mirabilis* are more virulence than the non-hemolytic strains, when injected intravenously into mice.

1.2.5.4 Urease Production by *Proteus mirabilis*:

Urease is an important agent responsible for the pathogenesis of *Proteus* in the kidney, it is a cytoplasmic multimeric, nicked metalloenzyme which catalyzes the hydrolysis of urease to carbon dioxide and ammonia, elevated the pH and result in precipitation of magnesium ammonium phosphate and carbonate-apattite which form stones of kidney and bladder (Mobley and Chippendle, 1990).

Larrson (1978) found that the increase in pH due to hydrolysis of urea by urease results in decrease of the biological activity of antibodies and distraction of leukocytes. The presence of ammonia has been implicated as directly toxic to epithelium of kidney (Mobley and Chippendale, 1990).

1.2.5.5 Protease Production by *Proteus mirabilis*:

Strains belong to *Proteus mirabilis* associated with human urinary tract infections have previously been shown to secret an extracellular metaloproteinase which cleaves both subclasses of immunoglobulin proteins such as secretory component casein and bovine serum albumin (Leoomes *et al* ., 1990).

Senior (1990) stated that both IgA1 and IgA2 are cleaved by *Proteus mirabilis* protease, although the secretory IgA2 molecule was less readily cleaved than secretory IgA1. IgA protease cleaves IgA at specific sites of the β-amino acid proline rich sequence in the hinge region of the α-1 heavy chain. This amino acid sequence is absent in IgA2, so it is less cleaved by protease (Leoomes *et al*., 1990).

1.2.5.6 Other Virulence Factors of *Proteus mirabilis*:

There are many virulence factors produced by *Proteus mirabilis* which are serum resistant (Zunino *et al*., 1990). Production of proteocin is considered as an important virulence factor (Tracy and Thomson, 1972), natural resistance to polymexin and outer membranes protein (omps) (Sidovezyk and Zych., 1986).

1.2.6 Adhesion Property of *Proteus mirabilis*:

Studies on the Pathogenicity of UTI demonstrated that the ability of *Proteus mirabilis* to adhere to uroepithelium is of prime important in the initiation of infection (Zunino *et al*., 1994). Previous reports on other bacterial genera specify that adhesion could be mediated by structures present on the outer surface of the cells such as capsules and fimbriae (Bruce *et al*., 1983).

During an *in vitro* study, the investigators identified a protein from a uropathogenic isolates of *Proteus mirabilis* which adhere to desquamated human uroepithelial cells. This protein was then purified and found to be organized as flexible rods (Fimbriae), and so they suggest that these fimbriae are the adherence elements responsible for binding uropathogenic *Proteus mirabilis* to uroepithelium (Wray *et al*., 1986).

Silverblatt and Ofek (1978) showed that some strain of *Proteus mirabilis* appears heavily piliated but others appear lightly piliated.

Matsumoto (1998) found that the existence of adherence factor with bacterial cell surface enables the bacteria to adhere to the tissue and so develops

infection. This suggests the adhesion factors is an important virulence factors which assist the bacteria to colonize and cause infection.

Moreover, surface hydrophobicity is important factor for adhesion because bacteria and host cells commonly have net negative surface charges and therefore repulsive electrostatic forces will develops. These forces are overcome by hydrophobic property, the more hydrophobicity on the bacterial cell surface, the greater adherence to the host cell.

Different strains of bacteria within a species may vary widely in their hydrophobic surface properties and ability to adhere to the host cells (Jawetz *et al*., 1998).

Chabanon (1979) stated that the adherence factors in *E. coli* which adheres to human epithelial cells lies under control of gene carry on plasmid. While Matsumoto (1998) found that adherence factors of *Proteus mirabilis* are coded by the chromosomal genes. The adherence of bacteria to biological surface is a complex process which often involves lock and key type interaction between bacterial attachment fimbriae and specific complex carbohydrate structures of the host cell membrane (receptors). It was also found that bacteria which are high adherence are more virulent than that of less adherent (Jackson *et al*., 1977; Peter *et al*., 1988).

1.2.7 Fimbriae of *Proteus mirabilis*:

Fimbriae are hair like appendages attached to bacterial cells, similar to flagella, but fimbriae are considerably shorter and thinner than flagella, fimbriae (or pili) consist of protein called pilin arranged helically around the control core (Funk, 1989). Pili are of tow types each have different function, the first type called common pili, allows a cell to adhere to the surface including the surface of other cells, the second type of pili referred to as sex, functioning to join bacterial cells prior the transfer of DNA from one cell to another (Jawetz *et al*., 1980).

Silverblatt (1974) stated that the electron microscopic studies of *Proteus mirabilis* strains have been identified two types of fimbriae which are thick filament (approximately 7nm in diameter) and thin filament (about 4 nm in diameter), thick pili appear to be related to infection. Strains of *Proteus mirabilis* expressing 7nm thick fimbriae were more virulence in rate model of pylonephritis. Such strains of diverse origin grown under a verity of conditions producing more than one of the three kinds of haemoaglutinin. Each haemoagultinin is associated with the presence of distinct kinds of fimbriae (Adegbola *et al*., 1983) as follows:-

A- Mannose resistant *Proteus* like (MR / P) haemagglutinins were produces commonly agglutinin of fresh fowl, horse, sheep and human erythrocytes.

B- Mannose resistant *klebsiella* like (MR / K) haemagglutinin (agglutination of tannedox erythrocytes).

C- Mannose sensitive (MS) haemagglutinins which were rarely detected (agglutination of guinea pig erythrocytes).

Latta *et al*., (1999) found that hyper flagellated swarmer cells of *Proteus mirabilis* were found not expressing pili. As long as pili are often implicated in adhesion and flagella are used for locomotion.

1.2.8 Antibiotic Sensitivity of *Proteus mirabilis*:

Many properties should be considered to choose drug for UTI treatment, these are; the drug activity against the infecting organisms, toxicity, the tissue concentration obtained, the effect of pH and posses no or little effect on normal flora of intestine and other regions (Glauser, 1986).

There are different mechanisms by which microorganisms might exhibit resistance to drug, such as; production of enzymes that destroy the active drug, changing their permeability to the drug, developing an altered structural targets for the drug, and finally metabolic pathway which microorganisms produce an altered enzyme that can still perform its metabolic function but is much less affected by the drug (Jawetz *et al*., 1998).

There are many groups of antibiotics which are active against *Proteus mirabilis*:

1.2.8.1 β-Lactam Antibiotics:

They affect the cell wall by inhibiting enzymes that involve in the formation of peptidoglycan layer. β-lactam includes two groups which are penicillin and cephalosporin (Jawetz *et al*., 1998). Ampicillin and amoxicillin are active against *Proteus mirabilis* (Cercenado *et al*., 1990).

Pagani *et al* (1988) studied the activity of pipracillin / tozobactam against *Enterobacteriaceae* and found it highly active against *Proteus mirabilis*, while Prescott *et al* (1990) studied the activity of carbencillin and cephalexin which were active antibiotics against *Proteus mirabilis*.

1.2.8.2 Aminoglycosides Antibiotics:

They inhibit protein synthesis by attaching to and inhibiting the function of 30S subunit of the bacterial ribosome (Jawetz *et al*., 1998). Amikacin is the important drug of choice in treatment infection caused by *Proteus mirabilis* (Al-Talib and Habib, 1986). Gentamycin used widely in treatment of UTI (Merlin *et al*., 1988). Streptomycin is considered as the oldest aminoglycoside drug used to treatment UTI (Mingeot-Lectero *et al*., 1999).

1.2.8.3 Quinolones Antibiotics:

They inhibit the bacterial DNA synthesis by blocking DNA synthesis which acts on the DNA gyrase (Jawetz *et al*., 1998).

Ciprofloxacin and ofloxacin are drug of choice against UTI (Harnett *et al*., 1997; Briss *et al*., 1999).

1.2.8.4 Macrolides Antibiotics:

They bind to 23S rRNA on the 50S ribosomal subunit resulting in blocking of transpeptidation and / or translocation (Kawamura-Sato *et al*., 2000). The

antimicrobial activity of macrolides is broad spectrum antibiotic used against gram positive and some gram negative bacteria (Jawetz *et al*., 1998).

1.2.8.5 Other Antibiotics:

Like tetracycline which inhibits protein synthesis, and chloromphenicol a potent inhibitor of protein synthesis, and Trimethoprime-Sulfamethazol resistance among UTI. All were found to have antimicrobial activity against *Proteus mirabilis* (Jawetz *et al*., 1998).

1.2.9 Probiotic:

The word Probiotic is derived from the Greek and means (forlife). It was first used by Lilly and Stillwell (1965). Probiotic is a microbial dietary adjuvant that beneficially affects the host physiology by modulating mucosal and systemic immunity, as well as improving nutritional and microbial balance in the intestinal tract (Lister, 1973). Currently probiotic preparations contain, *Lactobacillus acidophilus, Lactobacillus Plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis, Lactobacillus brevis* are available (Tissier, 1905). Various nutritionl and therapeutic effects of lactic acid bacteria (LAB) are summarized as follow (Mckay, 1985):-

- Improvement of nutritional quality of food and feed.
- Metabolic stimuli of vitamin synthesis and enzyme production.
- Stabilization of gut microflora and competitive exclusion of enteric pathogene.
- Enhance innat host defenses by production of antimicrobial substances.
- Reduction of serum cholesterol by assimilation mechanism.
- Decrease risk of colon cancer by detoxification of carcinogenes.
- Tumor Suppression by modulation of cell mediated immunity.

Further more LAB have several properties of economic importance like; lactose utilization, proteinase activity, bacteriophage defense mechanism and bacteriocin production.

1.2.10 Genus *Lactobacillus*:

Lactobacillus is gram positive, non-sporeforming bacilli, single, paired, chain or tetrad, catalase negative, anaerobic or microaerophilic and stable in the acidity and salt (Stamer, 1976). This genus contain largest group of LAB. Hammes and Vogel (1995) mentioned that first namely by Beijernick as "Bacill" in 1901. They Classified by Orla-Jensen (1919) to thermobacterium, streptobacterium and Betabacterium. After that new classification appeared by Kandler and Weiss (1986) which classified LAB three groups; Obligate homo fermentative; facultatively heterofermentative, and obligatory hetero fermentative.

Lactobacillus have numerous inhibitory substances that produce through the fermentation of LAB (Bonestroo *et al*., 1993); organic acid, Hydrogen peroxide (H_2O_2), Diacetyl, acetaldehyde, carbon dioxide CO_2, Bacteriocin are some of these substances.

1.2.10.1 Nutritional Requirements of LAB:

LAB needs fastidious nutritional requirements (Skaar *et al*., 1956), it needs two broad spectrum of organic acid and inorganic acid, amino acid, vitamins (B-blex), carbohydrates, peptides, salts and fatty acid (Stanier *et al*., 1963; Stamer, 1976; Kandler and Weiss, 1986). Morishita *et al* (1981) stated that *Lactobacillus* needs amino acids and found that the absence of arginine, leucine, iso leucine, valine, phenylalanin, tryptophane, glutamine from the medium caused decrease the growth of *Lactobacillus acidophilus*, *Lactobacillus plantarum* and *Lactobacillus casei.*

Narendranath *et al* (1997) ensured that the requirements of LAB for growth are nucleotide, amino acid, vitamin B-12 and the biotin. They added that the

addition of Tween 80 and citrate to the medium caused increase and better growth, while the absence of this growth factor from the medium like citrate, and manganise lead to decrease the growth rate.

Addition of Yeast extract to the milk culture helped to stimulate the growth of LAB and protein synthesis (Smith *et al*., 1975).

1.2.10.2 Antimicrobial Effects of Lactic Acid Bacteria (LAB):

Several investigations have demonstrated that various species of LAB exert antagonistic action against intestinal and food-borne pathogenes (Gibson *et al*., 1997).

LAB are capable of preventing the adherence, establishment, replication and / or pathogenic action of specific enteropathogens (like *Proteus mirabilis*) (Saavedra, 1995).

These antagonist properties may be manifested by;

a) Decreasing the luminal pH through the production of volatile short chain fatty acids (SCFA) like acetic, lactic or propionic acid.
b) Rendering specific nutrients unavailable to pathogens.
c) Decreasing the redox potential of the luminal environment.
d) Producing hydrogen peroxide under anaerobic conditions and / or producing specific inhibitory compounds like bacteriocins (Havenaar *et al*., 1992).

1.2.10.3 Using Lactic Acid Bacteria as Probiotic in the Therapy:

LAB resambling large proportion from normal flora in the intestinal and the gut (Isolauri *et al*., 1991; Salminen and Deighton, 1992). LAB strains that demonstrate a wide spectrum of antimicrobial characteristics, including acid and bile resistance, anti-microbial systems (ex: bacteriocin, lactic acid, peroxide), and adhesion to various types of pathogens (Chan *et al*., 1984).

Lindgren and Dobrogosz (1990) stated that there are many mechanisms in which LAB protects the intestinal tract including; decreasing pH value,

adherence to the intestinal cell wall, production of inhibitory material (bacteriocin), production of antitoxin and ability to still life.

Salminen *et al* (1993) suggested that the minimum concentration from LAB in the product using in therapy should be ($1*10^5$) bacteria / ml or 1 gram like *Lactobacillus acidophilus, Lactobacillus plantarum* which are widely used in the industry (food preservation) and in the therapy.

Gorbach (1990) performed several studies on the LAB to control the intestinal infection like salmonellosis and shigellosis, some type of colon cancer, and cholesterol in serum.

Probiotic are extremely safe and are not associated with any significant or detrimental side effects (McFarland and Elmer, 1995).

Lactobacillus therapy seems to reduce the recurrence rate of uncomplicated lower urinary tract infections in women, so it is used against urinary tract infections (Reid *et al*., 1987).

Lactobacillus acidophilus has a superior capability of producing lactic acid, which is antimicrobial and helps the body protection from harmful bacteria adhering to the intestinal mucosa (cell lining the intestine) (Donohue and Salminen, 1996).

Winkelstin (1955) formulated "probiotic tablet" from *Lactobacillus acidophilus*, there are several studies that mentioned the activity of LAB as "antigen" to the mucosal intestine layer and named (mucosal vaccin) (Mercenier, 1999).

Lactobacillus spp. inhibits the activities and proliferation of pathogenic bacteria by several ways such as production of lactic acid, production of antibiotics. *Lactobacillus acidophilus* produces acidophilin, *Lactobacillus plantarum* produces lactocidin that have action and inhibited several bacterial like *E. coli*, *Helicobacter pulori*, *Proteus* (Hirayma and Rafter, 1999).

A *lactobacillus* strain was shown to competitively inhibit adhesion of enteropathogenic like *E. coli* and *Proteus* (Blomberg *et al*., 1993).

However, adhesion capacity and competitive inhibition for *Lactobacillus acidophilus* are not constant properties since certain *L. acidophilus* can attach *in vitro* to cells like enteropathogenic while other strains do not.

Although, *L. acidophilus* inhibits the adhesion of several enteric pathogens to human cells, when pathogen attachment preceded *L. acidophilus* treatment, no inhibitory interference occurred indicating that steric hindrance of site occupation is important in the inhibition of adhesion, thus therapeutic use is limited to preventive application and not to a curative goal once binding of the pathogen has occurred. In addition a dose-dependent inhibition against cell adhesion of several pathogens has been demonstrated only for one strain of *L. acidophilus* (Bernet *et al*., 1994).

Chapter Two

Materials and Methods

2.1 Materials:

2.1.1 Culture Media:

2.1.1.1 Ready to Use Media:

Medium	Company (Origin)
Blood agar base	Mast-diagnosis (England)
Brain heart infusion agar	Difco (U.S.A)
Litmus milk	Biolife (Italy)
MacConkey agar	Oxoid (England)
Modified Regoza agar (MRS)	Hi media (Italy)
Muller hinton agar	Biokit S. A (Spain)
Nutrient agar	Oxoid (England)
Nutrient broth	Oxoid (England)
Phenylalanine deaminase	Oxoid (England)
Simon citrate media	Difco (U.S.A)
Triple Sugar Iron (TSI)	Difco (U.S.A.)
Urea base agar	Oxoid (England)

2.1.1.2 Laboratory Prepared Media:

The following media were prepared (as will follow later) in the laboratory:

Luria-Bertonia broth, Modified regoza broth, Carbohydrate fermentation media, Gelatin medium, Arginin-MRS broth.

2.1.2 Chemicals:

Material	Company (Origin)
Acetic acid	BDH (England)
Agar-Agar	Difco (U.S.A)
Ammonium sulphate	BDH (England)
Chlorophenol (Red)	Fluka (Switzerland)
Diamine dihydrochloride	Difico (England)
Disodium hydrogen phosphate	Oxoid (England)
Ethanol	Riedel-DeHaeny (Germany)
Gelatin	Oxoid (England)
Glucose, Manitol, Xylose, Maltose	BDH (England)
Glycerol	BDH (England)
Hydrochloric acid	BDH (England)
Hydrogen peroxide	Fluka (Switzerland)
Isoamyle alcohol	BDH (England)
K_2HPO_4	BDH (England)
Lactose, arabinose, galactose, rafinose	Difco (England)
L-arginine monohydrochloride	Fluka (Switzerland)
Mannose	Fluka (Switzerland)
Meat extract	Oxoid (England)
Methanol	Riedel-DeHaeny (Germany)
Methylene blue	BDH (England)
$MgSO_4.7H_2O$	BDH (England)
$MnSO_4.4H_2O$	Riedel-DeHaeny (Germany)

Material	Company (Origin)
N,N,N,N-Tetramethyl-p-Phenylene	Difco (England)
Nessler's reagent	BDH (England)
p-dimethyl-amino-benzaldehyde	Riedel-DeHaeny (Germany)
Peptone	BDH (England)
Potassium Chloride	BDH (England)
Potassium dihydrogen phosphate	BDH (England)
Sodium acetate hydrate	BDH (England)
Sodium Chloride	Riedel-DeHaeny (Germany)
Sodium dihydrogen phosphate	BDH (England)
Sodium hydroxide	Fluka (Switzerland)
Sucrose	BDH (England)
Tryptone	Fluka (Switzerland)
Tween 80	Oxoid (England)
Urea	BDH (England)
Yeast extract	Fluka (Switzerland)

2.1.3 Equipment:

Equipment	Company (Origin)
Anaerobic Jar	Rod well (England)
Autoclave	Gallen kamp (England)
Balance	Ohans (France)
Compound Light Microscope	Olympus (Japan)
Distillator	Gallen kamp (England)
Electrical oven	Memmert (Germany)
Freeze-Dryer	Virtis (USA)
Glass pasture pipette	John poulten Ltd. (England)
Hot plate with magnetic stirrer	Gallen Kamp (England)
Incubator	Gallen Kamp (England)
Micropipette	Witeg (Germany)
Millipore filters (0.22 μm)	Millipore and Whatman (England)
pH-Meter	Metter-GmpH Tdedo (UK)
Portable Centrifuge	Hermle labortechnik(Germany)
Refrigerator Centrifuge	Harrier (UK)
Sensitive balance	Delta Range (Switzerland)
Spectrophotometer	Aurora instruments Ltd. (England)
Vortex	Buchi (Swissrain)
Water path	GFL (England)

2.1.4 Api 20E kit (Api Bio Merieux, Lyon, France):

Api 20E kit consist of :

a) Galleries: the gallery is a plastic strip with 20 microtubes containing dehydrated reactive ingredients.

b) Api 20E Reagents:

- Oxidase reagent (1% tetra-methyl-p-phenyle-diamine)
- Kovac's reagent (p-dimethyl aminobenzaldehyde at 4% in HCl isoamyl alcohol).
- Voges-Proskauer reagent:

  -Vp1 (40% potassium hydroxide).

  -Vp2 (6% alpha-nephthol).
- Ferric chloride 3-4%.

2.1.5 Antibiotics:

2.1.5.1 Antibiotic Disks:

Antibiotic	Code	Concentration (µg)	Source (origin)
Amikacin	AK	30	Oxoid (England)
Amoxicillin	AMX	10	Al-Razi (Iraq)
Cephalexin	KF	30	Al-Razi
Chloromphenicol	C	30	Al-Razi
Ciprofloxacin	CIP	5	Oxoid
Gentamycin	GM	10	Al-Razi
Nadilixicacid	NA	30	Al-Razi
Penicillin G	P	10	Al-Razi
Pipracillin	PIP	100	Oxoid
Streptomycin	S	10	Al-Razi

Antibiotic	Code	Concentration (µg)	Source (origin)
Tetracycline	TE	30	Al-Razi
Trimethazol	SXT	25	Al-Razi

(NCCLs, 1991)

2.1.6 Bacterial Strains:

Strain	Supplied by
Escherichia coli ATCC 25922	Department of Biotechnology College of Science / Al-Nahrain University / Iraq
Lactobacillus acidophilus	Department of Biology College of Science / Al-Mustanseria University / Iraq

2.1.7 Solutions, Buffers and Reagents:

– Physiological saline solution.

– Phosphate buffer saline.

– Staining solution.

– Fixative solution.

– Oxidase reagent.

– Kovac's reagent.

2.2 Methods:

2.2.1 Media Preparation:

2.2.1.1 Ready to Use Medium:

The media listed in (2.1.1.1) were prepared according to information fixed on their containers by the manufacturer.

2.2.1.2 Laboratory Prepared Medium:

2.2.1.2.1 Blood Agar Medium:

It was prepared by autoclaving blood agar base after adjusting pH=7.0 previously then cooling to 50˚C, and 5% blood was added, mixed well and poured into petridish (≈15 ml each), used for identification of *Proteus mirabilis* isolates.

2.2.1.2.2 Urea Agar Medium:

It was prepared by adjusted pH=7.0 and autoclaving urea agar base (Christensens media) then cooling to 50˚C, and proper amounts of filtrate urea added after the medium was sterilized, it was dispensed in test tube and let solidified as slants, this medium was used for identification of *Proteus mirabilis* isolates.

2.2.1.2.3 Triple Sugar Iron (TSI) Agar and Simon Citrate Agar Media:

They were prepared and adjust pH=7.0 and autoclaved. This medium was used for identification of *Proteus mirabilis* isolates.

2.2.1.2.4 Phenylalanine Deaminase (PAD) Medium:

It was prepared according to Holt *et al.*, (1994) by dissolving trypton (15g), L-phenylalanine (10g) in 1 liter of distilled water and adjusted pH=7.0 and autoclaved, this medium was used for identification of *Proteus mirabilis* isolates.

2.2.1.2.5 Indole Medium:

It was prepared according to Atlas *et al*., (1995) by dissolving (15g) trypton in 1 liter of distilled water and adjusted pH=7.0 and autoclaved, this medium was used for identification of *Proteus mirabilis* isolates.

2.2.1.2.6 Luria-Bertonia Broth (LB) Medium:

It was prepared according to Atlas *et al*., (1995) by dissolving trypton (10g), yeast extract (5g) and sodium chloride (5g) in 1 liter distilled water and adjusted pH=7.0 and autoclaved, this medium was used for identification of *Proteus mirabilis* isolates.

2.2.1.2.7 Modified Regoza Broth (MRS) Medium:

It was prepared according to DeMan (1960) by dissolving the following ingredients in 1 liter of distilled water, pepton (10g), meat extract (10g), yeast extract (5g), glucose (20g), Tween 80 (1ml), K_2HPO_4 (2g), sodium acetate hydrate (5g), triammonium citrate (2g), $MgSO_4.7H_2O$ (0.2g), $MnSO_4.4H_2O$ (0.05g). After pH was adjusted to 6.0 the medium was autoclaved, this medium was used for growing lactic acid bacteria (*Lactobacillus* spp.).

2.2.1.2.8 Carbohydrates Fermentation Media:

It was prepared according to DeMan (1960) by using sterilized MRS broth after elimination of glucose and meat extract source from it. Instead 1% of each of autoclaved sugars (glucose, sucrose, maltose, mannitol, rafinose and lactose) and membrane filtrated sugars (arabinose, galactose, mannose, xylose) was added and 0.004% of chlorophenol red reagent was added also, then pH was adjusted to (6.2-6.5), this medium was used for identification of *Lactobacillus* spp.

2.2.1.2.9 Gelatin Medium:

Brain-Heart infusion broth was used after adding 12% of dissolve gelatine to it, and pH was adjusted to 6 and autoclaved (Baron and Finegold, 1994). This medium was used for identification of *Lactobacillus* spp.

2.2.1.2.10 Arginin MRS Broth:

It was prepared by adding 0.3 % (w/v) of L-arginin-monohydrochloride to the MRS broth, pH was adjusted to 6 and autoclaved (Harrigan and MacCance, 1976), this medium was used for identification of *Lactobacillus* spp.

2.2.1.2.11 Litmus Milk Medium:

It was prepared by dissolving (100g) of skim milk and (5g) litmus in 1 liter of distilled water, pH was adjusted to 6 and autoclaved (Baron and Finegold, 1994), this medium was used for the growth of *Lactobacillus* spp.

2.2.2 Solutions, Buffers and Reagents Preparation:

2.2.2.1 Solutions and Buffers:

2.2.2.1.1 Physiological Buffer Saline:

It was prepared according to Atlas *et al*., (1995) by dissolving (0.85g) of NaCl in 1 liter of distilled water, pH was adjusted to 7.0 and sterilized by autoclave, it was used for dilution.

2.2.2.1.2 Phosphate Buffer Saline (PBS):

It was prepared according to Gruikshank *et al*., (1975) as follow:

After dissolving (8g) NaCl, (0.2g) KCl, (0.2g) KH_2PO_4, (1.15g) Na_2HPO_4 in 1 liter distilled water was sterilized by the autoclave, it was used for preserve the cells (uroepithelium and bacteria).

2.2.2.1.3 Fixative Solution:

It was prepared by mixing 30 ml of methanol with 10 ml of acetic acid, used for adhesion test (Iawhi *et al*., 1982).

2.2.2.1.4 Staining Solution:

It was prepared by dissolving (0.3g) of methylene blue powder in 30 ml of ethanol (95 % (v/v)) (Atlas *et al*., 1995).

2.2.2.2 Reagents Preparation

2.2.2.2.1 Oxidase-Test Reagent (Baron *et al*., 1994):

A solution of 1% N,N,N,N-Tetramethyl-p-phenylene diamine dihydro-chloride was prepared in sterile distilled water when needed.

2.2.2.2.2 Kovac's Reagent (Colle *et al*., 1996):

It was prepared by dissolving (1g) of P-dimethyl-aminobenzaldehyde in (15 ml) of isoamyle alcohol and then added (5ml) of concentrated HCl carefully and gradually and keep in refrigerator, use in indol test.

2.2.3 Sterilization:

Three method of sterilization were used:

2.2.3.1 Moist Heat Sterilization:

Media and solutions were sterilized by the autoclave at 121˚C (15 Ib/in^2) for 15 minutes, except that different.

2.2.3.2 Dry Heat Sterilization:

Electric oven was used to sterilize glassware and others at 160-180 ˚C for 2-3 hr.

2.2.3.3 Membrane Sterilization (Filtration):

Millipore filtering was used to sterilize antibiotics solution and the filterates of growth of *Lactobacillus acidophilus* and *Lactobacillus plantarum* by using (0.22 μm) in diameter millipore filters.

2.2.4 Urine Sample Collection:

Mid stream urine samples specimen were collected in sterile tubes from patient of AL-Karama hospital and AL-Yarmoq hospitals in Baghdad during the period from 1/10/2003 to 1/2/2004. A total of 150 samples were aseptically collected and transported to the laboratory as fastly as.

2.2.5. Yoghurt Sample Collection:

Yoghurt samples specimen were collected in sterile tubes under aseptic and cooled conditions from Baghdad markets from 1/12/2003 to 1/2/2004. A total of 15 samples were aseptically collected and transported to the laboratory as fastly as.

2.2.6 Bacterial Isolation from Urine Sample:

One loopfull of undiluted urine sample was spread on blood agar and MacConkey agar plates. Plates then were incubated over night at 37˚C. Single colonies which were non lactose fermenters, and gave negative reaction to oxidase test and making swarming on blood agar were transferred to blood and MacConkey agar. The process was repeated several times for purity before use for further diagnosis.

2.2.7 Bacterial Isolation from Yoghurt Sample:

Serial dilutions of samples were made. From the last dilution, 1 ml was transferred to the poured MRS plates and incubates over night at 37˚C under anaerobic conditions using gas generating kit. After incubation, colonies were surrounded by inhibition zone and G +ve and catalase –ve were selected and transferred to MRS broth and incubated (Harrigan and MacCane, 1976).

2.2.8 Maintenance of *Proteus mirabilis*:

Maintenance of bacterial isolates was performed according to Mainiatis *et al*., (1982) as follow:

2.2.8.1: Short-Term Storage:

Isolates of bacteria were maintained for few weeks on MacConkey agar plates. The plates were tightly wrapped with parafilm, and then stored at 4˚C.

2.2.8.2 Medium-Term Storage:

Isolates of bacteria were maintained as stab cultures for few months. Such cultures were prepared in small screw-capped bottles containing 2-3 ml of nutrient agar medium and stored at 4˚C.

2.2.9 Maintenance of Lactic Acid Bacteria:

2.2.9.1 Daily Working Cultures:

After inoculation of MRS broth by the lactic acid bacteria isolates they were incubated at 37˚C for 24 hr anaerobically and kept after that in refrigerator (Baron and Fingold, 1994).

2.2.9.2 Stock Cultures:

Ten ml of 20% glycerol were added to the screw cup tubes containing MRS. After autoclaving, inoculate with bacteria (lactic acid) and incubate at 37˚C for 24 hr anaerobically, then kept in freezer (Contreras *et al.,* 1991).

2.2.10 Identification of *Proteus mirabilis*:

2.2.10.1 Microscopic Examination of *Proteus mirabilis*:

A loopfull of *Proteus* isolates was fixed on a microscopic slide, then stained by gram stain to examine cells shape, grouping, reaction and non-spore forming (Atlas *et al*., 1995).

2.2.10.2 Biochemical Tests of *Proteus mirabilis* isolates:

– Oxidase Test (Atlas *et al*., 1995):

This test was done by using moisten paper with few drops of a freshly prepared solution of N,N,N,N-Tetramethyl-p-phenylene diamine dihydro-chloride. Aseptically a clump of cell was picked up from the slant growth with a sterile wooden stick and smeared on the moisten paper. The development of a violet or purple color within 10 seconds indicates a positive test.

– Urease Test (Atlas *et al*., 1995):

Urease activity was detected by inoculating the surface of Christensen urea agar slants with the bacterial growth and incubated at 37˚C for 24 hr. The appearance of a red-violate color indicates a positive test while a yellow-orange color indicates a negative result.

– Triple Sugar Iron Test (TSI) (Atlas *et al*., 1995):

Isolates were cultured on the TSI agar slants by stapping and streaking on surface, and then incubated for (24-48) hr at 37˚C. When the color of medium was changed from red to yellow it is an indication of acid formation, while appearance of precipitate indicated ferric sulfate formation. Pushing the agar to the top indicates CO_2 formation.

– Phenyl Alanine Deaminase Test (PAD) (Senior, 1997)

A portion of 2 ml of (PAD) was inoculated with 2 ml of isolate suspension. After incubation at 37˚C for 24 hour, positive results were recorded by color change from green to blue.

– Simon Citrate Test (Colle *et al*., 1996)

Simon citrate agar was streaked by the isolate then incubated at 37˚C for (24-48) hr. Changing the color of media from green to blue indicates positive result.

– Indole Test (Colle *et al*., 1996):

Peptone water was inoculated by the isolate and incubated at 37˚C for 48 hr. A quantity of 0.05 ml of kovac's reagent was added and mixed in gently. Positive result was recorded by the appearance of ring on the surface.

2.2.11 Identification of Lactic Acid Bacteria:

2.2.11.1 Microscopic Examination of LAB:

A loopfull of lactic acid bacteria culture was fixed on microscopic slide, then stained by Gram stain to examine cells shape, grouping, gram reaction and non-spore forming (Kandler, 1986; Garvie and Weiss, 1986).

2.2.11.2 Biochemical Tests of LAB:

– Gelatinase Test:

Gelatin medium agar was used to detect gelatin liquification in tubes, by inoculating with 1% of LAB, and incubating at 37˚C for 48 hr. After that it was put into the refrigerator (4˚C) for 30 minutes, and the positive result recorded by gelatin liquification (Baron and Fingold, 1994).

– Catalase Test:

The method of Atlas *et al*., (1995) was followed to detect presence of catalse enzyme by putting one drop of LAB on a microscope slide, after one drop of H_2O_2 (3%) was added, positive results was observed through formation of gas bubbles.

– Acid and Curd Production in Litmus Milk:

Tubes containing litmus milk were inoculated by (1%) of LAB cultures and incubated for 24 hr at 37˚C. Changing color, cured production and decrease in pH were observed as positive results (Kandler and Weiss, 1986).

– Production of Ammonia from Arginine:

Tubes containing arginine and MRS were inoculated with (1%) of LAB culture and incubated at 37˚C for 72 hr. After incubation 1 ml taken from it and add to it Nessler reagent. Changing color to orange was evidence of inability of the isolates to produce ammonia (Brigges, 1953).

– Carbohydrates Fermentation:

Tubes containing MRS broth were inoculated with (1%) of LAB culture and incubated (including the positive control which was MRS broth with sugars without glucose and meat extract and negative control which was MRS). Tubes were incubated at 37˚C for 5 days, changing color to red indicates (pH base) while changing to yellow indicates (pH acid) (DeMan *et al*., 1960).

– Growth at 45˚C:

Tubes containing MRS broth were inoculated with (1%) of LAB culture and incubated at 45˚C for 24 hr, positive results obtaining by the growth of LAB.

2.2.12 Api 20E Identification for *Proteus mirabilis* Isolates:

Identification was carried out by subculturing of selected colonies grown on MacConkey agar into Api 20E microtubes gallery. This system is designed for the performance of more than 20 standard biochemical tests from a single colony grown on plating medium. Each test in this minimized system is performed within a sterile plastic microtube which containing appropriate substrates and was fixed to an impermeable plastic strip (gallery) each gallery contain 20 microtubes including the biochemical test and sugar fermentation. Inoculation of the galleries was done with sterile pasture pipette and five ml of tap water dispensed into tray provide a humid atomsphere then incubated at 37 ˚C for 24 hr. After that reagent added for reading the galleries, each positive reaction was given a value 1,2 or 4 according to the position of the test in its group, so a value from 0 to 7 digit observed was then looked up in the index and the identification is determined (Appendix 1).

2.2.13 Sensitivity of *Proteus mirabilis* to Antibiotic:

Ten ml of nutrient broth were inoculated by each bacterial isolate, then incubated at 37 ˚C to log phase (O. $D._{600}$ about 0.35) giving ($1*10^8$) cell / ml of

broth. After that, 0.1 ml of the inoculated broth was transferred and spread by sterile cotton swab on Muller-Hinton agar plates surface in three different planes (by rotating the plate approximately 60˚ each time to obtain an even distribution of the inoculum). The inoculated plates were then placed at room temperature for 30 minutes to allow absorption of excess moisture. With a sterile forceps the selected antibiotic disks were placed on the inoculated plates and incubated at 37˚C for 18 hr in an inverted position. After incubation, the diameter of inhibition zones was measured by a ruler (mm). Results were determined and compared according to the National Committee for Clinical Laboratory Standards (NCCLs, 1991).

2.2.14 Determining Inhibitory Effect of LAB:

2.2.14.1 On Solid Medium (MRS Agar):

A culture of LAB previously grown in MRS broth was streaked on MRS agar, and then incubated under anaerobic conditions at 37˚C for 24 hr (Silva *et al*., 1987). After incubation a cock porer (5mm) was used to withdraw discs of LAB growth and put on surface of the nutrient agar that was inoculated (before) with 0.1 ml of pathogenic bacteria. After incubate, at 37˚C for 24 hr, the inhibition zone around the disc was estimated in (mm).

Same procedure was repeated by using different incubation times of LAB (18, 24, and 48 hr) to determine the optimum incubation time that gives greater inhibition effect.

2.2.14.2 In Liquid Medium (MRS Broth):

MRS broth was inoculated by 1% of LAB culture, then incubated anaerobically at 37˚C for different period of times (18, 24 and 48 hr) (Schillinger and Luck, 1989; Lewus *et al*., 1991). After incubation the culture was centrifuged at 6000 rpm for 15 minutes, the supernatant was obtained. After adjusting the pH of the filtrate to 6.5 by using NaOH O.4 N (1ml), it was filtered through Millipore filter unit (0.22 μm).

Then well diffusion method that mentioned by Vignolo *et al*., (1993) was used; when nutrient agar plates which was inoculated with 0.1ml of each pathogenic bacteria by a spreader. Then (5mm) wells were made by a cock porer. Each well were filled with the LAB filtrate, and then incubated at 37˚C for (18, 24 and 48 hr). The inhibition zone around the well was measured by (mm) and compared with that of the control which contained MRS broth without bacteria (Vignolo *et al*., 1993). The filtrate was concentrated by freeze-dryer and the well diffusion method was repeated to detect the effect of each concentrated filtrate against the pathogenic bacteria. Control was containing concentrated MRS broth without LAB.

The filtrates of LAB were concentrated by freeze dryer which has equal volume 100 ml of MRS broth inoculated with 1 ml LAB, concentrated to one fold (50 ml), two fold (25 ml) and three fold (12.5 ml).

2.2.15 Bacterial Adhesion Test (Iwahi *et al*., 1982):

2.2.15.1 Preparation of *Proteus mirabilis* Suspension:

Ten milliliter of nutrient broth medium was inoculated with bacterial growth, the culture was then incubated at 37˚C for over night (O. $D._{600}$ about 0.4) giving ($1*10^9$) cell / ml. cultures of bacteria were washed twice with PBS and centrifuged at 1000 rpm for 20 minutes and resuspended in PBS.

2.2.15.2 Preparation of Epithelial Cells:

Uroepithelial cells were isolated from the urine of some healthy females by centrifugation at 1000 rpm for 5 minutes then washed three times with PBS and centrifuged at 1000 rpm for 10 minutes before resuspended in PBS.

2.2.15.3 Adhesion Test:

- A mixture of 0.2 ml of the bacterial suspension, 0.2 ml of the epithelial cells suspension and 0.1 ml of PBS was incubated at 37 ˚C for one hour.

- Unattached bacteria to uroepithelial cells were removed by centrifugation in PBS at 1000g for 10 minutes.
- The final pellet was resuspended in PBS then a drop of it was put onto a microscope slide, air-dried fixed with methanol : acetic acid (3:1) and stained with methylene blue.
- The adherent bacteria to epithelial cells were observed by the compound light microscope.
- Control of only epithelial cells was included.

2.2.16 Determination of Minimum Inhibitory Concentration of *Lactobacillus* LAB Concentrated Filtrate:

Different dilution of each concentrated filtrate were made in tubes containing sterile nutrient broth each. The ratio were (10, 20, 30, 40, 50, 60, 70, 80 and 90%) giving final volume 10ml in each tubes.

Then each concentration was inoculated by 0.1ml culture previously of *Proteus mirabilis* grown in nutrient agar and incubated at 37 ˚C for 24 hr. After incubation the growth of tubes was observed and minimum inhibitory concentration was determined as the lower concentration of the filtrate that gave no growth of *Proteus mirabilis* in the tubes.

2.2.17 Effect of Concentrated Filtrate on Adhesion of *Proteus mirabilis*:

The minimum inhibitory concentration of the concentrated filtrate of LAB isolates was used to investigate the effect on adhesion property of *Proteus mirabilis*. For this purpose method described by (Iawhi *et al*., 1982) was applied which was mentioned previously in (2.2.15) unless:

- Nutrient broth medium containing minimum inhibitory effect of concentrated filtrate was dispensed in sterile tubes and incubated with a loopfull of liquid culture of *Proteus mirabilis*, then incubated for 24 hr.
- Bacterial adhesion test was done as indicated previously (2.2.15).
- Adhesion free concentrated filtrate was prepared as control.

Chapter Three

Results and Discussions

3.1 Isolation and Identification of *Proteus* Isolates:

One hundred and fifty midstream urine samples were collected from patients suffering from symptoms referred as urinary tract infection.

It was found that 116 (77.3%) out of the total 150 samples collected gave positive results on MacConkey agar and Blood agar.

These results were near to those reported by Younis (1986) and AL-Bayati (1999) who found that the percentage of positive cultures of urine samples were (84%) and (83%) respectively. But such results were in disagreement with those of Obi (1996) in Zymbaboy when found that percentage of positive culture of urine samples was (27%). The reason of the differences in percentage may be owed to differences in size and number of hospital surveyed as well as to the season and medications before sampling. Identification of isolates was carried out according to culture, morphological and biochemical tests. Result showed that 16 isolates belong to genus *Proteus* from the 116 positive cultures and, so the isolation percentage of *Proteus* from other bacteria of the UTI cases was (13.7%). This result was near that of Saeed (1993) who found that *Proteus* isolates were representing (10.5%) of the UTI cases tested. Moreover, Kareem (2001) found that isolation percentage of *Proteus* occurrence from out-patient and in-patient with UTI cases was (11.7%) and (10.8%), respectively. However higher percentage (28%) was obtained by Warren (1982) as causatives of UTIs. Results shown in figure (3-1) indicate that *Proteus* found in both sexes, but its isolation percentage in male samples was higher (62.5%) when 10 isolates were belonged to them, while (37,5%) isolation percentage in female when 6 isolates were belonged to them, this result agree with those reported by AL-Murjany (2002) who found that the isolation percentage of *Proteus* in male was (63.5%) and (36.5%) in female.

However, the killer effect of vaginal fluid (which has low pH) may act naturally as a selection pressure against *Proteus* (Stamey, 1975).

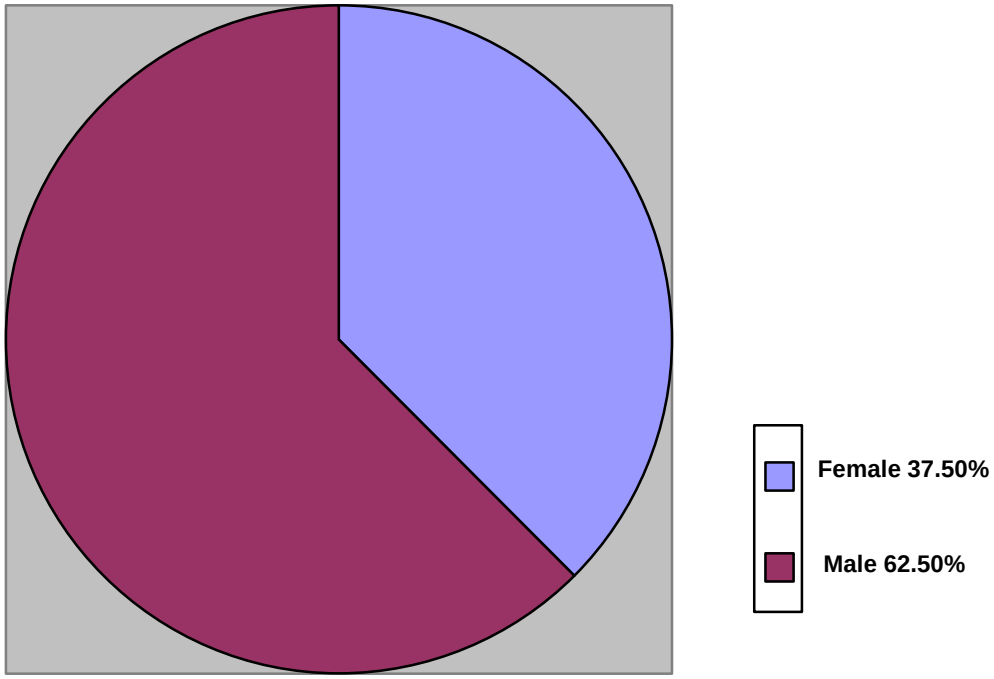


Figure (3-1). UTI by Infectivity of *Proteus mirabilis* among sexes.

3.2 Identification of *Proteus mirabilis*:

3.2.1 Cultural Characteristics:

In accordance to their pale colony appearance on MacConkey agar as non lactose fermenters, and swarming motility on blood agar after 24 hr incubation, the suspected isolates are considered to be as *Proteus* isolates which require more identification processes to be identified for species.

3.2.2 Morphological Characteristics:

The oil immersion lence objective of the compound light microscope, Gram staining examination, showed that cells of the suspected isolates appeared purple, non spore former, rods. Motility test of the cell indicated that they are motile.

3.2.3 Biochemical Tests:

Several biochemical tests were done to characterize *Proteus* isolates.

All the 16 isolates of *Proteus* gave positive results to the biochemical tests; phenylalanine-deaminase, catalase, urease and triple sugar iron (TSI) (by forming alkaline / acid reaction). But all were negative to the oxidase test.

Regarding phenylalanine deaminase test all the 16 isolates were positive to it. Indol test was used to differentiate between the *Proteus* isolates, 10 of the 16 isolate were unable to form indol ring which is one of the major property distinguishing the species of *Proteus mirabilis*, while the other remaining 6 isolates were able to form such ring. The isolates vary in their response to the Simon citrate test. Upon the former characterization of *Proteus* isolates, it appeared that 10 of the total 16 isolated may be classified as *Proteus mirabilis* and the remaining 6 were *Proteus vulgaris*. Results in figure (3-2) show that *Proteus mirabilis* resembled the higher percentage 10 isolate (62.5 %) than the *Proteus vulgaris* 6 isolates (37.5 %). This result was agreed by that of Al-Murjany (2000) who found that *Proteus mirabilis* percentage was 85.1 % as compared with *Proteus vulgaris* which was (14.8 %).

Table (3-1). Biochemical tests for characterization of *Proteus* isolates of UTI.

Test / No. of Isolate	PAD	TSI	Indole	Citrate Utilize	Urease	Oxidase	Catalase
10 isolates *P. m.*	+	ALK/A	–	+/–	+	–	+
6 isolates *P. v.*	+	ALK/A	+	+/–	+	–	+

P. m. = *Proteus mirabilis*
P. v. = *Proteus vulgaris*
\+ = Positive
– = Negative
+/– = Positive or negative
ALK/A = Alkaline / acid
PAD = Phenyl Alanine Deaminase
TSI = Triple Sugar Iron

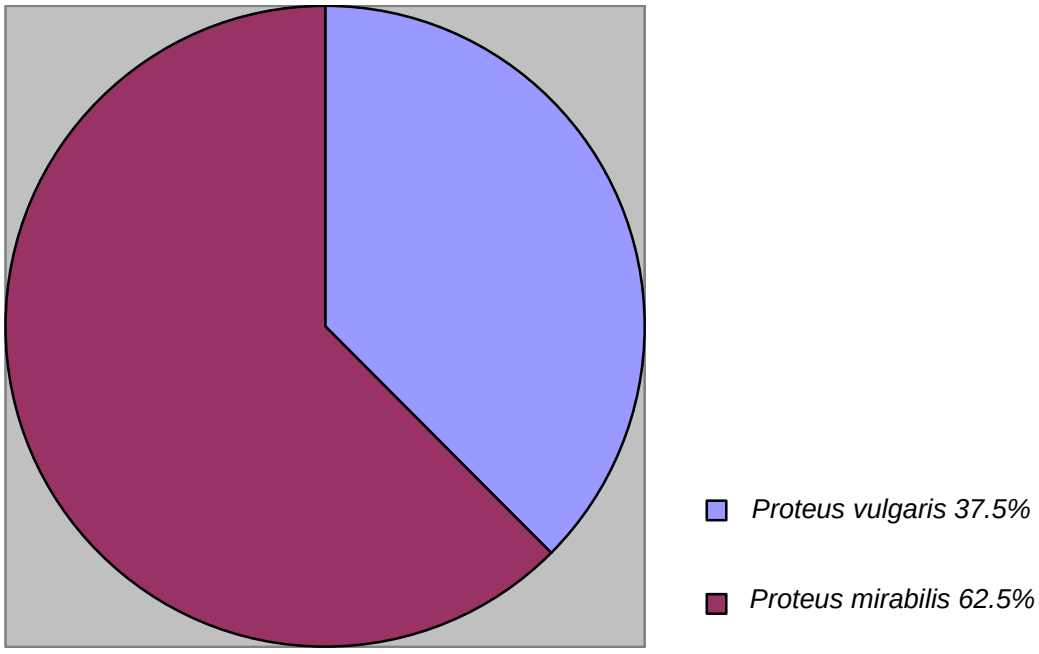


Figure (3-2). Percentage of isolates of *Proteus* spp. in 16 patients suffering from UTI.

Moreover, identification of the isolates was confirmed by using Api system (Api 20E) as shown in (plate 3-1) the findings obtained by the conventional biochemical tests.

3.3 Isolation of Lactic Acid Bacteria:

From a total of 15 yoghurt samples collected from local market of Baghdad city for the period of 1/12/2003 to 1/2/2004, 10 of them gave colonies surrounded by inhibition zone after culturing on MRS agar. Moreover, these colonies were negative to the catalase test. Such results concide with those stated by DeMan *et al* (1960) who insisted that MRS medium is the most selective medium for LAB.

3.4 Identification of Lactic Acid Bacteria:

3.4.1 Cultural Characteristics:

Colonies of LAB on MRS agar were pale, round shape, soft, mucoid, convex and surrounded by inhibition zone as a result of dissolving calcium carbonate.

3.4.2 Morphological Characteristics:

Lactobacillus appeared blue, bacilli, mainly grouped in long to chain containing (3-8 cells), non-sporformers in Gram stain.

3.4.3 Biochemical Tests:

All the suspected LAB when grown on gelatin medium were unable to produce the gelatinase enzyme, but were able to produce clot on the litmus milk medium and leading to decrease pH from 6.5 to 4.5 even to 4.

All the isolates gave negative results for the catalase test when not producing bubbles after the addition of hydrogen peroxide to the colonies grown on MRS agar. The isolates were also unable to produce ammonia from arginine

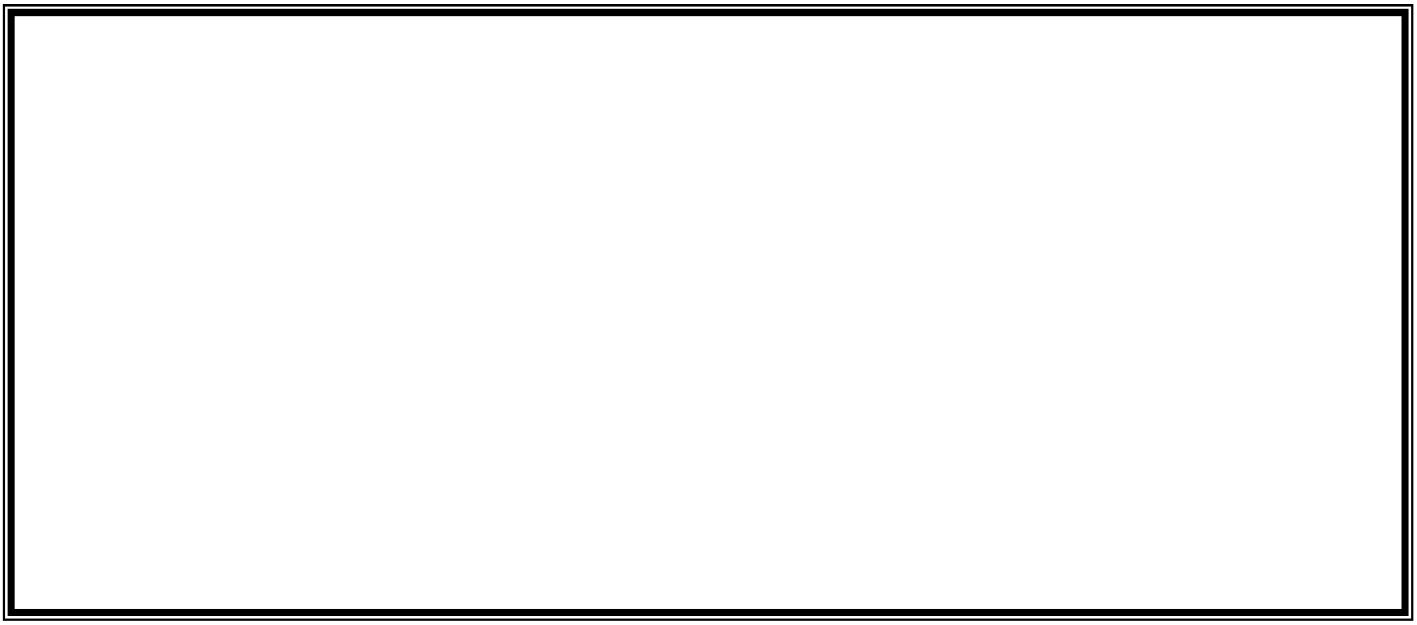


Plate (3-1). Api 20E system for characterization of *Proteus mirabilis* showing the results of reactions includes in the system.

A- Control **B-** Identification of *Proteus mirabilis*

Positive results (ODC, CIT, H_2S, URE, TDA, IND, VIP, GEL, GLU).

Negative results (ONPG, ADH, LDC and the entire rest sugars test in system).

when the color of the medium stay as it was (orange) without any change after the addition of Nessler's reagent. Moreover, the isolates were unable to grow in 45ºC. Carbohydrates fermentation test also was performed to identified the species of 10 isolates of *Lactobacillus*, the isolates were different in their ability to ferment the carbohydrate sources used in this study. Table (3-2) showed that all isolates were able to ferment glucose, fructose, sucrose and lactose. Isolates which fermented the previous four sources but not fermenting xylose were considered to be belonging to *Lactobacillus casei*. While those fermented the same sources but not mannitol and lactose were classified as *Lactobacillus brevis.* On the other hand, isolates which were able to ferment xylose and mannitol and fermenting the previous four sugars were identified as *Lactobacillus plantarum*, and isolate which were unable to ferment xylose and mannitol but fermenting the four sugars were identified as *Lactobacillus acidophilus* (Kandler and Weiss, 1986; Nigatu and Cashe, 1994).

3.5 Antibiotics Sensitivity of *Proteus mirabilis*:

The emergence of prevalence of antibiotic resistance strains is considered as a major therapeutic problem that could be explained by several hypothesis such as, the influence of excessive and /or inappropriate antibiotic use (Sotto *et al*., 2001), transmission of resistant isolates among people, consumption of food from animals that had received antibiotics, and greater mobility of individual world wide have also contributed to the extension of antibiotic resistance (Blanco *et al*., 1997).

In this study the effect of antibiotic on *Proteus mirabilis* was tested by using standard disk diffusion method, and results obtained were compared with those of NCCLs, (1991). Table (3-3) show that antibiotic resistance among *Proteus mirabilis* isolate varied according to the nature of the isolate or antibiotic. Among them no single antibiotic was resisted by all the isolates of *Proteus mirabilis* or sensitive to it.

Table (3-2). Ability of *Lactobacillus* isolates to ferment carbohydrate sources.

Sugar source / Isolate	Glucose	Fructose	Lactose	Sucrose	Mannitol	Xylose
Lactobacillus plantarum	(1) +	(1) +	(1) +	(1) +	(2) +	(1) +
Lactobacillus plantarum	(1) +	(1) +	(1) +	(2) +	(3) +	(2) +
Lactobacillus plantarum	(1) +	(1) +	(1) +	(1) +	(1) +	(3) +
Lactobacillus brevis	(1) +	(1) +	(1) –	(2) +	–	(3) +
Lactobacillus plantarum	(1) +	(1) +	(1) +	(2) +	(1) +	(2) +
Lactobacillus casei	(1) +	(1) +	(1) +	(2) +	(1) +	–
Lactobacillus brevis	(1) +	(1) +	(1) –	(3) +	–	(2) +
Lactobacillus brevis	(1) +	(1) +	(1) –	(2) +	–	(3) +
Lactobacillus casei	(1) +	(1) +	(1) +	(1) +	(3) +w	–
Lactobacillus casei	(1) +	(1) +	(1) +	(1) +	(2) +w	–
Lactobacillus acidophilus	(1) +	(1) +	(1) +	(1) +	–	–

+ = positive ferment

– = negative ferment

( ) = No. of days to change the color

w = weak ferment (delayed results)

However, amikacin was the most effective antibiotic when only one isolate (Pm4) of *Proteus mirabilis* resisted it, while all others were sensitive.

Ciprofloxacin was the second highly effective antibiotic when all isolates, expect two (Pm3 and Pm9), were resistant to it. Gentamycin was the third expect three (Pm1, Pm7 and Pm9), were resist to it. On the other hand, penicillin G was the least effective antibiotic because all isolate, expect (Pm7), were sensitive to it. Followed by amoxicillin, chloramphenicol and tetracycline which were sensitive by only two isolate each.

Moreover, results indicated in table (3-4) show the frequency of resistance *Proteus mirabilis* isolates, amikacin has the lowest resistance percentage (10%) when one isolate resist to it, followed by ciprofloxacin (20%), gentamycin (30%). On the other hand (90%) of *Proteus mirabilis* isolate were resistant to penicillin G, while (80%) were resist to amoxicillin and tetracycline, and the others were distributed between these range as show in the table.

Majority of *Proteus mirabilis* (90%) were resistant to penicillin G. same percentage of resistance was also found by Kareem (2001), while AL-Taiee (2002) registed 100% resistance to penicillin G. Results also shown that the resistant percentage to amoxicillin was (80%) which differ from the results obtained by AL-Taiee (2002) with (64%). High resistance to such antibiotics may be due to abuse and unwise which may develop bacterial resistant to them. The study also showed that pipracillin was the most active among the group of penicillins when only (40%) of the isolates resisted it, findings which were near those (50.7) obtained by AL-Murjany (2000).

Cephalexin, a first generation of cephalosporin group of antibiotics, was resisted by (50%) of the isolates, which was highly lower that those obtained by Al-Jebouri (2001) and Al-Taiee (2002) who found that the resistant percentages were (80%) and (72%) respectively. Resistance of *Proteus mirabilis* isolates to the β-lactam antibiotics used was evident in this study, it may be due to the possessing of β-lactamase by the isolate they may be encoded by transferable plasmids and found in various Enterobacterceae members such as *E. coli*, *Proteus mirabilis, Klebsiella pneumonia* and *Salmonella typhimurium* (Bret *et al*., 1998).

Resistant to aminoglycosides in this study was found to be variable among *Proteus mirabilis* isolates when most of them were sensitive to amikacin with resistance percentage of only (1%). Startchounski *et al*. (1998) also found in a study performed in Russia that resistance percentage of the isolated to amikacin was only (1%).

Table (3-4). Frequency of antibiotic resistance of *Proteus mirabilis* isolates.

Antibiotic	Symbol	Resistant isolates	
		Number	Percentage (%)
–β-lactam penicillin			
Penicillin G	P	9	90
Amoxicillin	AMX	8	80
Pipracillin	PIP	4	40
–Cephalosporins			
Cephalexin	CL	5	50
– Aminoglycosides			
Streptomycin	S	7	70
Gentamycin	GM	3	30
Amikacin	AK	1	10
– Tetracyclines			
Tetracycline	TE	8	80
– Quinolons			
Naldixic acid	NA	5	50
Ciprofloxacin	CIP	2	20
– Others			
Chroamphenicol	C	7	70
Tri-methoprim-sulphamethazol	SXT	7	70

Relatively high resistance (70%) to the streptomycin (an aminoglycoside antibiotic) was recorded in this study. Al-Bayati (1999) and Al-Murjany (2000) were also found similar results with (78%) and (73%) respectively. On the other hand, higher resistance (92%) to this antibiotic was found by Talib and Habib

(1986), while lower one (45%) was recorded by Kareem (2001). Resistance of *Proteus mirabilis* isolates to gentamycin was found to be low as (30%), this result was agreed with those of AL-Talib and Habib (1986) and AL-Taiee (2002) who found that the resistance to gentamycin were (32%), but this result as disagreed with (45%) resistance percentage as Kareem (2001) was found. In general bacteria resist aminoglycoside by producing modifying enzymes which alter the aminoglycoside and prevent it from binding to ribosome (Jawetz *et al*., 1998). High resistance to tetracycline was found among the isolates as (80%) was recorded, and this was due to resistant gene which carried by plasmid (Merlin *et al*., 1988). Other investigation, AL-Talib and Habib (1986), AL-Murjany (2000) were found that the *Proteus mirabilis* isolates were very resistant to tetracycline and its resistance percentage was (96%). *Proteus mirabilis* isolates were found to be sensitive to quinolones antibiotic like ciprofloxacin which have (20%) resistance while AL-Murjany (2000) and Startchouski *et al* (1988) were found that *Proteus mirabilis* resisted as (4.7%) and (5%) respectively. This difference was due to abuse and currently use of antibiotic was associated with development of resistant also the shelve life (Rice *et al.*, 1992). Naldixic acid has moderate resistance (50%) against the *Proteus mirabilis* isolates, AL-Talib and Habib (1986) found that the resistance to naldixic acid was only (36%) and this due to development of resistant gene carried by conjugative plasmid (Martinez-Martinez *et al*., 1998). Resistance to trimethoprin / sulphamethazol was (70%) and this result was agreed with Barret *et al* (1999) which found that resistance ratio (75%).

So from the results reported and shown in table (3-3) that the isolate *P. m* 9 was the strong isolate which was resisted to almost all antibiotics, so it selected from other isolates to study the adhesion property and the inhibitory effect of LAB.

3.6 Inhibitory Effect of LAB:

3.6.1 On Solid Medium (MRS Agar):

Propagating LAB isolates on MRS agar under anaerobic conditions was the most efficient method for production their inhibitory metabolites against tested pathogenic bacteria. Despite that all LAB isolates, exhibited serious inhibitory effect on *Proteus mirabilis* isolates, an inhibitory effect of LAB isolates *Lb. a.*11 was the most effect against *P. m.* 9, where as Lb. p.3 also have effect on *P. m.* 9 but less than *Lb. a.* 11, when inhibitory zone reached 18.5 mm after 24 hr incubation time, while *Lb. p. 3* have 17 mm after the same incubation time. Moreover, such LAB isolate (*Lb. a.* 11) was effective against *P. m.* 9 it also gave highest inhibition zone against the other *P. m.* isolates for both incubation time (18 and 24 hr). Results in table (3-5) showed in general that almost all LAB isolates exhibited better inhibitory effect on *P. m.* isolates after incubation for 24 hr.

Generally incubation period of (24 hr) resulted in production of more inhibitory effect by almost all LAB isolates against all *P. m.* isolates than the incubation period of 18 hr. However, when longer incubation periods (30 hr and more) were tested on some isolates result in no difference in inhibition zone were recorded or, in some times lower. For such thing they were not receded in the table. The LAB varied in there inhibitory zone diameter (9-18.5) mm in diameter, *Lb. c*. and *Lb. b*. gave an inhibitory effect after 18 hr and increased for 24 hr incubation periods but not any noticed increase after more incubation, this results agreed with AL-Obidy (1997) who found that each LAB gave inhibitory effect after 24 hr. But AL-Dulemy (2000) was found that the inhibitory effect increased after (48 hr). The differences on the above results of LAB effected the pathogenic bacteria was related to the type of bacteria, type of inhibitory substance, its quantity and its ability for distribution in the media (Egorov, 1985).

Lb. p. have also inhibitory effect against tested isolates and its effect increased after (24 hr) incubation (24 hr) and this was it could be due to lactocidin which produce from *Lb. p.* (Speck, 1971) and due to plantaracin which was active against the tested isolates (Olasupo *et al*., 1995). While the *Lb. a*. have the highest inhibitory effect after (24 hr) incubation and this was due to acidophilin produce from *Lb. a.* (Gilliland and Speck, 1972).

LAB have an inhibitory effect against gram negative and positive bacteria (Nigatu and Gash, 1994). Fang *et al* (1996) stated that LAB has a high inhibitory effect against enteropathogenic bacteria. Plate (3-2) show the inhibitory effect of *Lb. p* 3 and *Lb. a.* 11 against *P. m*. 9 which have the highest inhibitory effect, they were selected as the strong LAB isolates depending on the results shown in table (3-5) previously.

3.6.2 In Liquid Medium (MRS Broth):

Inhibitory effect of LAB isolates grown in MRS broth was evaluated against the tested isolates of *Proteus mirabilis*. Well diffusion method was used by filling the wells which made in nutrient agar plates which is cultured by the *P. m.* 9 with the filtrate of two LAB isolates (*Lb. p.* 3, *Lb. a.* 11). Selection of these two isolates depended on their ability in production best inhibitory effect. Maximum inhibition zone diameters reached (20) mm which is a highest than that recorded by solid medium, this may be due to the existence in MRS broth exhibited a wide spectrum inhibitory effect against gram positive bacteria (*Staphylococcus aureus, Bacillus subtilis*) and gram negative bacteria (*E. coli, Klebsiella spp., Proteus spp.*) when the inhibition zone diameter ranged between (13-19) mm (Gupta *et al*., 1998). To study the effect of incubation time period in the liquid medium the two isolates of LAB were grown for (18, 24 and 48) hr. Incubation period of 24 hr that gave the best inhibitory effect by *Lb. p.* 3 the inhibition zone diameter reached to (18 mm) against tested *Proteus mirabilis* isolates. Increasing incubation period to 48 hr resulted in least inhibitory effect for *Lb. p.* 3 isolates.

Plate (3-2). Inhibitory effect of *Lactobacillus plantarum* 3 and *Lactobacillus acidophilus* 11 against *Proteus mirabilis* 9 isolate on solid medium (MRS agar).

A- *Lactobacillus acidophilus* 11 after 24 hr. of incubation on MRS agar resulted zone of inhibition with (18.5) mm diameter ***B-*** *Lactobacillus plantarum* 3 after 24 hr of incubation on MRS agar resulted zone of inhibition with (17) mm diameter.

*Lb. a.*11 also gave optimum inhibitory effect after 24 hr incubation and not after 48 hr, the reason for such two LAB isolates may be that the inhibitory materials (acidophilin, plantaracin) loose activity when secreted outside the cells after increasing the incubation time. Results shown in plate (3-3) and (3-4) show the inhibitory effect of *Lb. p.* 3 and *Lb. a.* 11 respectively against the tested isolate *P. m.* 9 liquid media for different incubation time (18, 24 and 48) hr.

The investigation also includes the inhibitory effect of concentrated filtrates on tested isolate. The filtrates of *Lb. p.* 3 and *Lb. a.* 11 were concentrated three fold by using freeze-dryer. The first and second fold of concentrated filtrates of *Lb. p*. 3 have zone diameter (20) and (21) mm, respectively against *P. m.* 9 while first and second fold of concentrated filtrates of *Lb. a.* 11 have zone diameter (21) and (23) mm, respectively against *P. m.* 9, while the third fold has the highest inhibitory effect after 24 hr incubation because all the inhibitory substances was concentrated, zone diameter of *Lb. p.* 3 against *P. m.* 9 reached to 27 mm and *Lb. a.* 11 against *P. m.* 9 have zone diameter reached to 30 mm.

Incubation time of 18 hr and 48 hr gave inhibitory effect less than effect after 24 hr incubation so Pfeiffer and Radler (1982) stated that there is a relationship between the diameter of inhibition zone and the concentration of inhibitory substances. On the other hand Barefoot and Klaenhammer (1983) mentioned that death of tested bacteria increased by the increasing of inhibitory substances like bacteriocin and acidophilin and plantaracin of LAB.

Results shown in plate (3-5) was include the inhibitory effect of *Lb. p.* 3 and *Lb. a.* 11 against the test isolate *P. m.* 9 for 18 hr and 24 hr incubation which is gave the best inhibitory effect.

Plate (3-3). Inhibitory effect of *Lactobacillus plantarum* 3 against *Proteus mirabilis 9* isolate in liquid medium (MRS broth).

A–After 18 hr. incubation in MRS broth resulted zone of inhibition with (12) mm diameter. **B–**After 24 hr. incubation in MRS broth resulted zone of inhibition with (18) mm diameter. **C–**After 48 hr. incubation in MRS broth resulted zone of inhibition with (15) mm diameter.

Plate (3-4). Inhibitory effect of *Lactobacillus acidophilus* 11 against *Proteus mirabilis 9* isolate in liquid medium (MRS broth).

A–After 18 hr. incubation in MRS broth resulted zone of inhibition with (15) mm diameter. **B–** *A*fter 24 hr. incubation in MRS broth resulted zone of inhibition with (20) mm diameter. **C–** *A*fter 48 hr. incubation in MRS broth resulted zone of inhibition with (16) mm diameter.

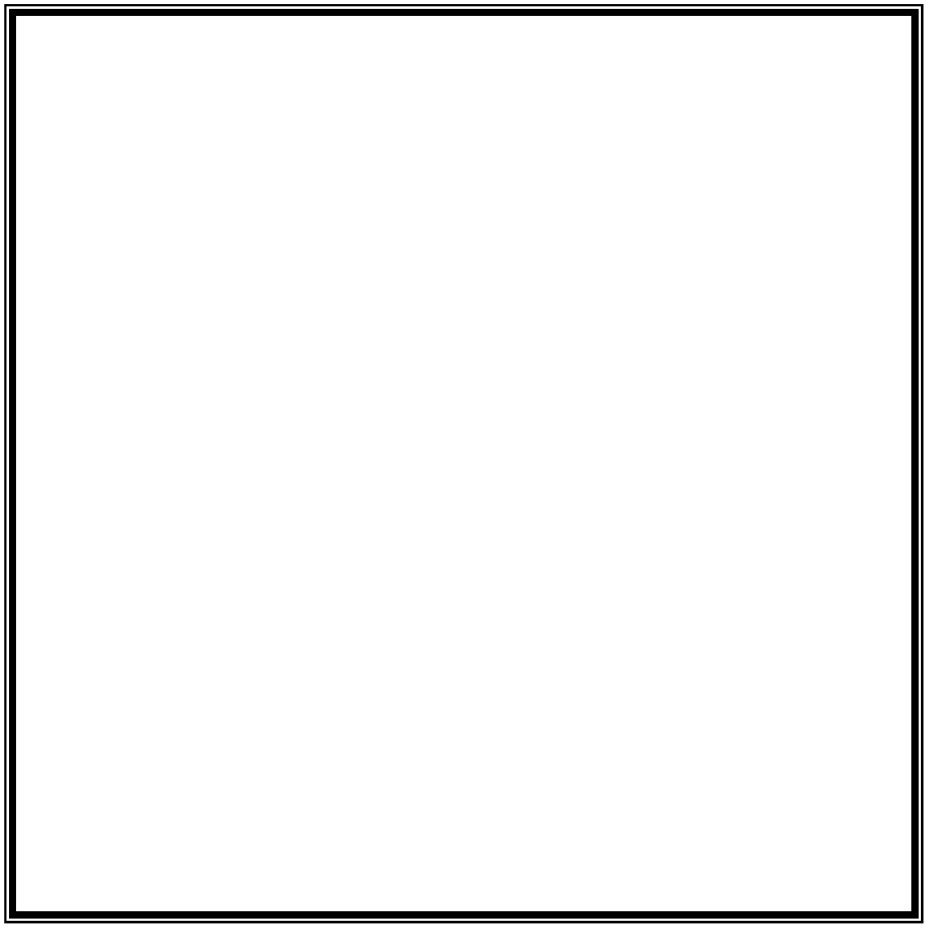


Plate (3-5). Inhibitory effect of concentrated filtrates for *Lactobacillus plantarum* 3 and *Lactobacillus acidophilus* 11 against *Proteus mirabilis* 9 isolate.

A– Control (contain concentrated MRS). **B–** *Lactobacillus acidophilus* 11 concentrated filtrate after 18 hr. incubation in MRS broth resulted zone of inhibition with (25) mm diameter. **C–** *Lactobacillus plantarum* 3 concentrated filtrate after 18 hr. incubation in MRS broth resulted zone of inhibition with (23) mm diameter. **D–** *Lactobacillus acidophilus* 11 concentrated filtrate after 24 hr. incubation in MRS broth resulted zone of inhibition with (30) mm diameter. **E**– *Lactobacillus plantarum* 3 concentrated filtrate after 24 hr. incubation in MRS broth resulted zone of inhibition with (27) mm diameter.

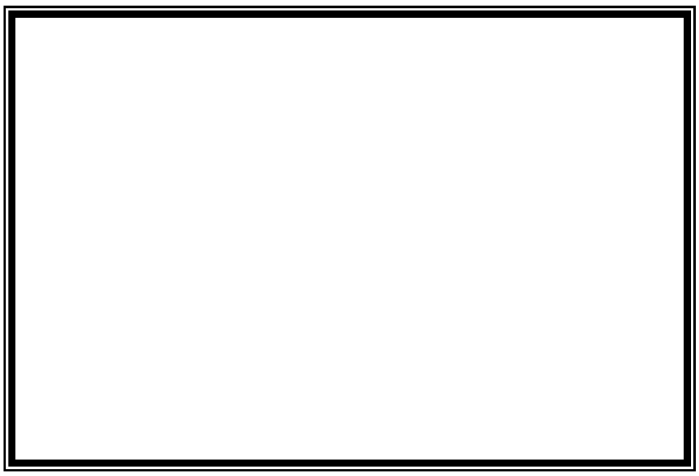
3.7 Adhesion of *Proteus mirabilis*:

Ability of *Proteus mirabilis* to adhere to uroepithelial cells is considered as an important virulence factor in pathogenesis of urinary tract infections (Mobley and Chippendle, 1990). In this study, adherence property of *Proteus mirabilis* as well as how this property may be affected by LAB isolates was investigated.

Adherence ability of *Proteus mirabilis* to uroepithelium which observed by viewing under oil-immersion of the compound light microscope is shown in plate (3-6) which was include the uroepithelium from healthy female and the infected uroepithelium (UEP), where the *P. m.* 9 appeared as rod and adhere to the uroepithelium (by pili), the results show that the highest number of adhering bacteria to UEP (rang from 45-55 bacteria / cell) recorded by isolate *P. m.* 9, this result come almost in agreement with that recorded by Ramphal *et al* (1984) who found that the highest number of mucoid strain of *Pseudomonas aeroginosa* to the tracheal epithelium was 45 bacteria / cell, but disagreed the result of Wray *et al* (1986) who found that the highest number of adherent of *Proteus mirabilis* to UEP was 29 bacteria / cell.

Proteus mirabilis usually has fimbriae which are considered as adherence elements responsible for binding of uropathogenic *Proteus mirabilis* to uroepithelial cell (Wray *et al*., 1986). Ramphal *et al* (1984) stated that mucoid strains of *Pseudomonas aeroginosa* contain two adherence elements namely fimbriae and alginate.

-A-



-B-

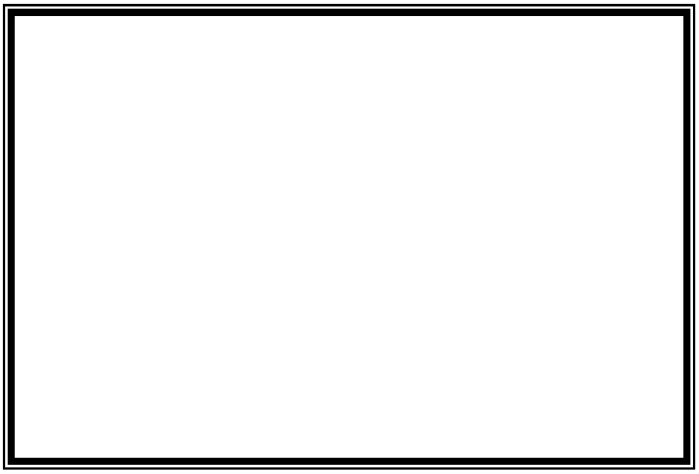


Plate (3-6). Microscope examination of Adhesion property of *Proteus mirabilis* 9 to uroepithelium under oil immersion lens (1000x).

A- Normal uroepithelial cell. **B-** Adherence of *Proteus mirabilis* 9 to uroepithelial cell.

3.8 Minimum Inhibitory Concentration of LAB Filtrates against *Proteus mirabilis*:

3.8.1 Determining MIC,s of LAB Against *Proteus mirabilis* Growth:

To determine MIC,s of the filtrates of LAB isolates which inhibit or minimize adhesion property of *Proteus mirabilis*, serial dilutions were prepared from the three-fold filtrates of isolates *Lb. p.* 3 and *Lb. a* 11. Result in table (3-6) show that concentration 10% and 20% Filtrate had no effect on *P. m.*9 when clear growth of this test bacteria was observed. Concentration 30% and 40% of filtrates led to minimize growth of the test bacteria.

Filtrate of *Lb. p.*3 in the concentration 70% and above also caused total inhibition for the test bacteria when growth of isolate *P. m.*9 was completely inhibited by such diluents. On the other hand concentration 60% and above of *Lb. a.*11 filtrate completely inhibited growth of *P. m.*9 isolate.

Depending on the just mentioned findings, concentration 60% of *Lb. p.* 3 and 50% of *Lb. a.*11 were selected and recorded as the MIC,s of the two LAB filtrates against growth of the test bacterial isolate *P. m.* 9.

Table (3-6). Minimum Inhibitory Concentrations (MIC) of Lactic Acid Bacteria (LAB) against *Proteus mirabilis* isolate (*P. m.*9).

MIC / LAB isolate	%								
	10	20	30	40	50	60	70	80	90
Lb. p. 3	+	+	+	+	+	+	–	–	–
*Lb. a.*11	+	+	+	+	+	–	–	–	–

+ = growth

– = no growth

3.8.2 Adhesion Inhibition by LAB Filtrates:

The initial step in the infection of host cells by *Proteus mirabilis* to the host cell is the adhesion (Mobley and Chippendle, 1990), so interference with adhesion process cause a prevention of infection.

Effect of concentrated filtrates of LAB against adhesion property of *Proteus mirabilis* was investigated using Iawhi method (1982). Results obtained showed that the three-fold concentrated filtrated of LAB isolates (*Lb. p.* 3 and *Lb. a.*11) against tested *P. m.*9 was observed to minimizing adhesion of *P. m.* 9 to the uroepithelial cells reaching an average of (3-10) bacteria / cell as shown in plate (3-7). It could be observed by the plate that adhesion of *P. m.* 9 to UEPCs was clearly minimized. This was may be due to the effect of the inhibitory substances found in the filtrates of the LAB isolates and to the acidic pH which aSffect growth of the gram negative bacteria by altering some surface structures (like pili), leading to prevent bacterial cells form adhesion to UEPCs with out killing the bacteria (Chan *et al*., 1984). Some authors were reported partial and complete inactivation of adherence of several gram negative uropathogenes.

Reid *et al* (1985) investigated the inhibitory effect produced by *Lactobacillus casei* on *E. coli* (which is also uropathogenic), and found that the inhibitory effect was not caused by the bacteriophage or hydrogen peroxide but due to the coaggregation of *E. coli* and *Lb. c.* in urine which was occurred after 20 hr at 37ºC.

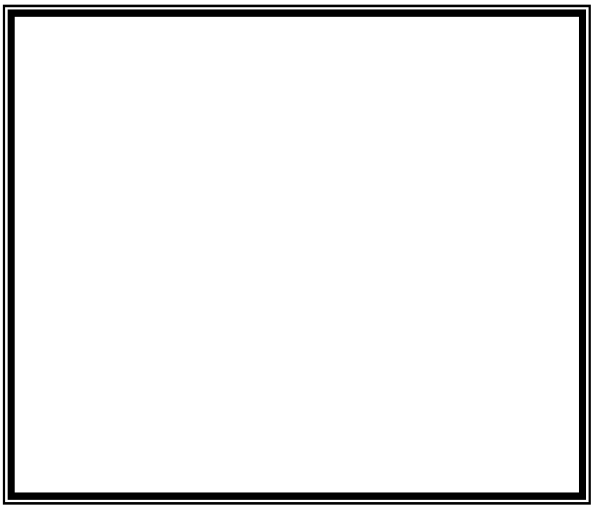
The prevalence of inhibitory-producing LAB on the uropathogenes and the ability of LAB to interact closely with the uropathogenes seem to constitute an important host defense mechanism against infection (Reid *et al*., 1990).

Hawthorn and Reid (1995) reported that precoating of LAB strains reduced the binding of uropathogenic coagulase-negative *Staphylococci* and *E. coli* to 8 bacteria / cell.

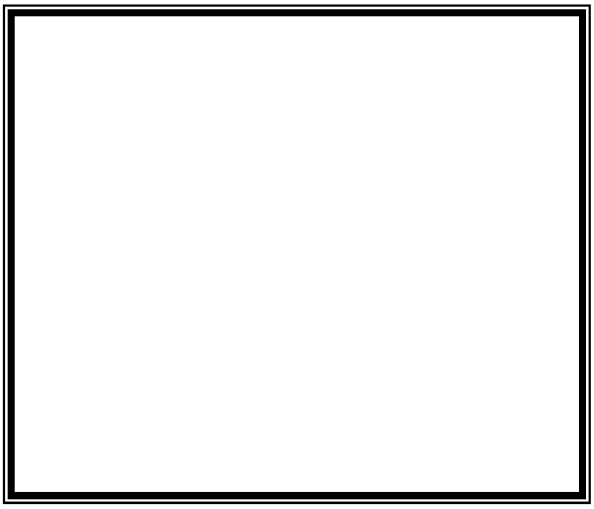
Velraeds *et al* (1996) found that the biosurfactant surlactin as released by *Lactobacillus* isolates may open the way to the development of anti-adhesive

biologic coating against *Enterococcus faecalis*, they reported a decrease in the number of adhering *Enterococcus* reaching to approximately 70%.

A



B



C

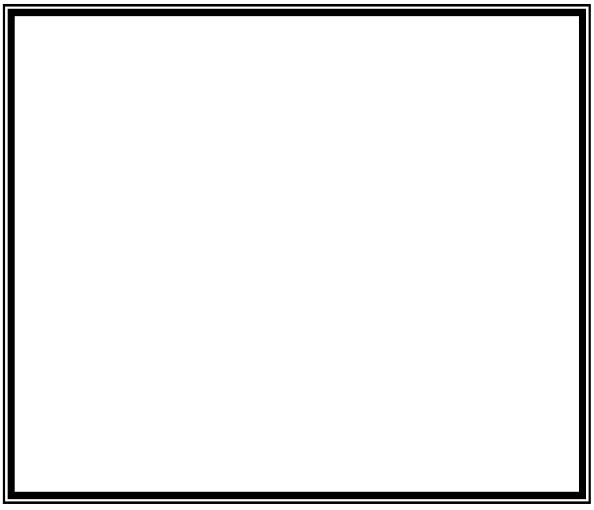


Plate (3-7). Microscopic examination of adhesion of *Proteus mirabilis* cells to uroepithelium cells (1000x).

A: after treating *Proteus mirabilis* (*P. m.* 9) with three-fold filtrate of probiotic LAB (isolate *Lb. p.* 3), **B:** *Proteus mirabilis* (*P. m.* 9) with probiotic treating three-fold filtrate of LAB (isolate *Lb. a.* 11), **C:** Adhesion of untreated *Proteus mirabilis* (isolate *P. m.* 9).

Chapter Four

Conclusions and Recommendations

Conclusions:

1. *Proteus mirabilis* isolates different in their antibiotic resistance considerable, when amikacin, ciprofloxacin and gentamycin were the most effective, while penicillin, amoxicillin and tetracycline were the least effective ones.
2. *Lactobacillus* filtrates had considerable effects against the test *Proteus mirabilis* isolate.
3. Three-fold concentrated filtrates of LAB gave the highest inhibitory effect on the growth and adhesion property of *Proteus mirabilis* in comparison to the one and two- fold filtrates.

Recommendations:

Further studies are needed for:

1. Extraction, purification and characterization of the inhibitory substances produced by the probiotic LAB isolates.
2. Investigating the effect of LAB inhibitory substances against other virulence factors of *Proteus mirabilis* such as urease or swarming.
3. *In vivo* studies about the effect of LAB isolates against *Proteus mirabilis.*

References

-A-

Adegbola, R. A; Old, D. C. and Senior, B. W. (1993). The adhesion and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. J. Med. Microbiol. 16: 427-431.

Adler, J. L.; Burke, J. P.; Martin, D. F. and Finland, M. (1971b). *Proteus* infection in a general hospital; Biochemical characteristic and antibiotic susceptibility of the organism. Annals. Intern. Med 75: 517-530.

Al-Bayati, Z. G. F. (1999). Identification of bacterial strains isolated from patients with urinary tract infection and the role of plasmids in their antibiotic resistance. M. Sc. Thesis, College of Science. Al-Nahrain University. Baghdad. Iraq.

Al-Jebouri, K. K. W. (2001). Study on antibiotic resistance bacteria isolated from patients with urinary tract infection. M. Sc. Thesis, College of Scienc. University of Baghdad. Baghdad. Iraq.

Al-Murjany, M. F. S. (2000). Study the multiple antibiotic resistance and some of virulence factor for *Proteus mirabilis* which cause UTI, and study plasmid contain. M. Sc. Thesis, College of Science. AL-Mustansiryah University. Baghdad. Iraq.

AL-Samak, M. M. A. (2003). Inhibition of adherence of *Proteus mirabilis* to uroepithelial cells by sub minimum inhibitory concentration of antibiotic. M. Sc. Thesis, College of Science, Al-Nahrain University. Baghdad. Iraq.

AL-Talib, A. M. and Habib. H. M. (1986). *Proteus mirabilis* isolated from four bacteriological studies. J. Fac. Med. 29: 391-396.

Ananthanaryan, R. and Paniker, C. K. (1988). Enterobacteriaceae: Coliform, *Proteus* in The Textbook of Microbiology. (3rd ed.): 259. Orient Longman.

Atlas, R. M.; Brown, A. E.; Parks, L. C. (1995). Laboratory Manual of Experimental Microbiology. (1st ed). Mosby. Inc. Missouri.

-B-

Barefoot, S. F. and Kaenhammer, T. R. (1983). Detection and activity of Lactocin B. A bacteriocin produced by *Lactobacillus acidophilus*. Appl. Environ. Microbiol., 45 (6): 1808-1815.

Baron, E. J.; Finegold, S. M. and Peterosn, L. R. (1994). Bailey and Scott's Diagnostic Microbiology. (9th ed.). Mosby Company. Missouri: 389-395.

Bernet, M. F.; Brassart, D.; Neeser, J. R.; Servin, A. L. (1994). *Lactobacillus acidophilus* LA1 binds to cultured human intestinal cell lines and inhibits cell attachment and cell invasion by enterovirulent bacteria. J. Gut 35: 483-439.

Barret, S. P.; Savage, M. A.; Robec, M. P.; Guyot, A. and Shrimpton, S. B. (1999). Antibiotic sensitivity of bacteria associated with community-acquired UTI in Britain. J. Antimicrob. Agents chemother. 44: 359-365.

Blomerg, L., Henmikkson, A., Conway, P. L. (1993). Inhibition of adhesion of *Escherichia coli* k88 to piglet ideal moucus by *Lactobacillus spp.* Appl. Environ. Microb. 59: 34-39.

Boestroo, M. H.; Wit, J. C.; Kusters, B. J. M. and Rombouts, F. M. (1993). Inhibition of the growth of yeast in fermented salads. Inte. J. Food Microbiology; 17: 311-320.

Bret, L.; Chanal, C. C.; Sirot, D.; Chaibi, E. D.; Labia, R. and Sirot, J. (1998). Chromosomally encoded ampC-type beta-lactamase in clinical isolate of *Proteus mirabilis*. Antimicrob. Agents Chemother. 42 (5): 1110-1114.

Briggs, M. (1953). The classification of *Lactobacilli* by means of physiological tests. J. Gen. Microbiol., 9: 234-248.

Bruce, A. W. Chan, R. C. Y.; Pikerton, D.; Morales, A. and Chadwick, P. (1983). Adherence of gram-negative uropathogenes to human uroepithelial cells. J. Urol. 130: 293-298.

-C-

Cercenado, E.; Gacia-Leoni, M. E.; Rodena, P. and Rodriguez-Creixems, M. (1990). More Extended-spectrum beta-lactamase. J. Antimicrob. Agents. Chemothera. 35: 1697-1704.

Chabanon, G.; Hartley, C. and Richmond, M. H. (1979). Adhesion to human cell line by *E. coli* strains isolated during UTI. J. Clin. Microbiol. 10: 563-566.

Chan, R. C.; Bruce, A. W. and Reid, G. A. (1984). Adherence of cervical, vaginal and distal urethral normal microflora to human uroepithelial cells and the inhibition of adherence of gram-negative uropathogens by

competitive exclusion, J. Urol. 131: 596-601. (Cited by Fergus M. C. (1999).

Clague, J. E.; Horan, M. A. (1994). Urine culture in the elderly: Scientifically doubtful and practically useless. Lancet,; 344: 1035-1036. (Cited by Fergus M. C. (1991)).

Colle, J. G.; Fraser, G. A.; Marmion, P.B.; Simmoms, A. (1996). Practical Medical Microbiology. (14th ed). Chrchill living-Stone, New York.

Contreras, B. G. L.; Vuyst, L.; Devreese, B.; Busanyova, K.; Raymaeckers, J.; Bosman, F.; Sablon, E. and Vandamme, E. J. (1991). Isolation, purification and amino acid sequence of lactobin A, one of the two bacteriocins produced by *Lactobacillus amylovorus* LMGP. 13139. Appl. Environ. Microbiol., 63 (1): 13-20.

-D-

Davis, B. D. Dulbecoo, R.; Eisen, H. N. and Ginsberg, H. S. (1990). Microbiology (4th ed.). Philadelphia.

Degbola, R. A.; Old, D. C. and Senior, B. W. (1993). The adhesion and fimbriae of *Proteus mirabilis* strains associated with high and low affinity for the urinary tract. J. Med. Microbiol. 16: 427-431.

DeMan, J. C.; Rogosa, M. and Sharpe, M. E. (1960). A medium for the cultivation of *Lactobacilli*. J. Appl. Bact., 23 (1): 130-135.

Donohue, D. C. and Salminen, S. (1996). Safely of probiotic bacteria. Asia Paciffic J. Clin. Nutr., 5: 25-28.

-E-

Eden, L. S.; Haussons, U. and Janson, G. L. (1988). Host-parasite interaction in urinary tract. J. Infec. Dis. 157: 412-426.

Egorov, N. S. (1985). Antibiotics a scientific approach. Mir Publishers, Moscow.

-F-

Fang, W.; Shi, M.; Huang, L.; Chen, J. and Wang, Y. (1996). Antagonism of lactic acid bacteria towards *Staphylococcus aureus* and *Escherichia coli* on agar plates and in milk. Vet. Res., 27 (1): 3-12. (Abst.).

Fergus M. C. (1999). Probiotic Spectra of Lactic Acid Bacteria (LAB). Food Sci. and Nut. 39 (1): 17-43.

Funke, B. R. and Case, C. L. (1989). Microbiology An Introduction. (3rd ed.). The Benjamin / Cummimgs Publishing Company. Inc. Redwood City. California.

-G-

Garvie, E. I. and Weiss, N. (1986). Genus *Leuconostoc.* In: Bergeys Manual of Systematic Bacteriology. 2: 128-134.

Gibson, G. R., Saavedra, J. M., Macfarlane, S., and Macfarlane, G. T. (1997). Probiotics and Intestinal infections. In: Probiotics 2: Applications and Practical aspects, Chapman and Hall, New York; 10-39. (Cited by Fergus M. C. (1991)).

Gilliland, S. E. and Speck, M. L. (1972). Interaction of food starter cultures and food born pathogens: Lactic *Streptococci versus*, *Staphylococci* and *Salmonella*. J. Miller Food Technol., 35 (5): 307-310.

Gluser, M. P. (1986). Urinary tract infection and pyelonephritis. In medical microbiology and infection disease. Saunders. West. Washington, Squares, Philadelphia.

Gorbach, S. L. (1990). Lactic acid bacteria and human health. Ann. Med., 22 (1): 37-41. (Abst.).

Gruickshank, R. H. A.; Dugend J. P.; Dugenud, B. P.; Marmion, B. P. and Swain, R. H. A. (1975). Medical Microbiology. 2 (12^{th} ed.). Logram Group Limited. Britain.

Gupta, S. (1988). The short textbook of medical microbiology. (3^{rd} ed.). Daryagant. Indian.

Gupta, V.; Radramma; Rati, E. R. and Joseph, R. (1998). Nutritional quality of lactic acid fermented better gourd and fenugreck leaves. International Journal of Food Science and Nutrition. 49 (2): 101-108.

-H-

Hammes, W. P. and Vogel, R. F. (1995). The genus *Lactobacillus*. In: The Genera of Lactic acid Bacteria.

Harnett, N.; Brean, S.; Riley, G.; Terro, R. (1997). Analysis of *Nisseria gonorrhoeae* in Ontario Canda, with decrease susceptibility to quinolones by pulsed-field gel electrophoresis, auxotyping, serotyping and plasmid content. J. Med. Microbiol. 64: 383-388.

Harrigan, W. F. and MacCance, M. E. (1976). Laboratory Methods in Food and Dairy Microbiology. Academic Press. London.

Havenaar, R.; Brink, B.; and Huisin't Veld, J. H. J. (1992). Selection of strains for probiotic use. In: Probiotic, the scientific basis, Chapman and Hall, London, 209-224. (Cited by Fergus M. C. (1991)).

Hawthorn, L. A. and Reid, G. (1990). Exclusion of uropathogen adhesion to polymer surfaces by *Lactobacillus acidophilus*, J. Biomed. Mater. Res. 24: 39-46. (Cited by Fergus M. C. (1999)).

Hirayama, K. and Rafter, J. (1999). The role of lactic acid bacteria in colon caner prevention: Mechanistic considerations. Antonie Van Leeuwenhoek International Journal of General and Molecular Microbiology., 56 (1): 391-394. (Abst).

Holt, J. G.; Krieg, N. R.; Sneath, P. H. A.; Staley, J. A. and Williams, S. T. (1994). Bergy's Manual of Determinative Bacteriology. (19th ed.). Williams and Wilkins.

-I-

Isolauri, E.; Juntunen, M; Rantanen, T.; Sillanaukee, P.; Koivula, T. (1991). A human *Lactobacillus* strain *Lactobacillus casei* spp. strain GG promotes recovery from acute diarrhea in children. Pediatrics., 88 (1): 90-97. (Abst).

Iwahi, T., Abc, Y. and Tsuchiya, K. (1982). Virulence of *E. coli* in a sending urinary tract infection in mice. J. Med. Microbiol. 15: 303-316.

-J-

Jackson, E; Fowler, J. R. and Thomas, A. Stamey. (1997). Studies of introital colonization in women with recurrent urinary infections UTI: The role of bacterial adherence, J. Urol. 117: 472-607.

Jawetz, E. J.; Melinick, J. L. and Adelberg, E. A. (1987). "Review of Medical Microbiology". (17th ed.). Prenticehall international. USA.

Jawetz, E.; Melinick, J. L. and Adelberg, E. A. (1998). "Medical Microbiology". Middle east edition appelton and hongenor walk Connecticut / San Matea. California.

Jawetz. E.; Melinick, J. L. and Adelberg, E. A. (1980). Review of Medical Microbiology. (14th ed.). Middle East Edition. London.

Jonhnson, J. R.; Stamm, W. E. (1989). Urinary tract infections in women: diagnosis and treatment. Ann Intern. Med.; 111: 906-917.

-K-

Kandler, O. and Weiss, N. (1986). Genus *Lactobacillus.* In: Bergy's Manual of Systematic Bacteriology. (Sneath, P. H. A.; Mair, N. S. and Hold, J. G. ed.). 2 William and Wilkins Co., Baltimore. M. D. USA.

Kareem, I. J. (2001). A study of pathogenesis of *Proteus mirabilis* isolated from human urinary tract infection by tissue culture technique and laboratory animals. M. Sc. Thesis, Biotechology Department. University of Baghdad. Iraq.

Kawamura-Sato, K.; Linuma, Y.; Hasegawa, T.; Horii, T.; Yamashino, T. and Ohta, M. (2000). Effect of subihbitory concentration of macrolides on

expression of flagellin in *Pseudomonas aeroginosa* and *Proteus mirabilis*. Antimicrob. Agents and Chemother. 44 (10): 2869-2872.

Koranakis, V; Cross, M.; Senior, B.; Koronakis, E. and Hughes, C. (1987). The secreted hemolysins of *Proteus mirabilis*, *Proteus vulgaris* and *Morganella morganii* are genetically related to each other and to the alpha-hemolysin of *Escherichia coli*. J. Bacteriol. 169 (4): 1509-1515.

Kunin, C. M.; White, L. V.; Tong, H. H. (1993). Area assessment of the importance of "low-count" bacteria in young women with acute urinary symptoms. Ann. Intern. Med.; 119: 454-460.

-L-

Larsson, P. (1978). Comparison of the host parasite relation in UTI caused by *Proteus* and *E. coli* in animal and children: 12. Ggoeteborg. Sweden.

Latta, R. K.; Grondin, A.; Jarrell, H. C.; Nicholls, G. R. and Berub, L. R. (1999). Deferential expression of non agglutinating fimbriae and MR/P pili in swarming colonies of *Proteus mirabilis*. J. Bacteriol. 181 (10): 320-325.

Leoomes, L. M.; Senior, B. W. and Kerr, M. A. (1990). A proteolytic enzyme secreted by *Proteus mirabilis* degrades immunoglobulins of the immuno-globulin (IgA1), (IgA2) and (IgG) isotypes. Infect. And Immun. 58: 1979-1985.

Lewus, C. B.; Kaiser, A. and Montville, T. J. (1991). Inhibition of food borne bacterial pathogens by bacteriocins from lactic acid bacteria isolated from meat. J. Appl. Environ. Microbiol., 57: 1683-1688. (Abst).

Liaw. S. J.; La, H. C. Ho, S. W.; Luh, K. T. and Wang, W. B. (2000). Inhibition of virulence factor expression and swarming differentiation in *Proteus mirabilis* by P. nitrophenylglycerol. J. Med. Microbiol. 49: 725-731.

Lilley, D. M. and Stillwell, R. H. (1965). Probiotics : growth promoting factors produced by microorganism, Science,; 147: 747-748. (Cited by Fergus M. C. (1991)).

Lindgren, S. E. and Dobrogosz, W. J. (1990). Antagonistic activities of lactic acid bacteria in food and feed fermentation. FEMS Microbiology Revi., 87: 149-164.

Lister, J. A. (1873). Further contribution to the natural history of bacteria and the germ theory of fermentative changes, Quart. J. Microsc. Sci 13: 380-408. (Cited by Fergus 1999).

-M-

Maniatis, T.; Fritch, E. F. and Sambrook, J. (1982). Molecular cloning. A Laboratory Manual, Cold Spring Harbor Laboratory, New York.

Martinez-Martinez, L.; Pascnal, A. and Jacoby, G. A. (1998). Quinolone resistance from a transferable plasmid. Lancet. Mch. 351 (14): 797-800.

Maskell, R. (1988). Urinary tract infection in clinical and laboratory practice. Bedford square, London.

Matsumoto, T. (1998). Virulence factors and host response in UTI Sangyoika. Daigiaku. Zassihi. B: 45-60. (Abst).

McFarland, L. V., Elmer, G. W. (1995). Biotheraputic agents: Past, present and future, Microecol. Ther.; 23: 46-73.

McKey, L. L. (1985). Roles of plasmids in starter cultures, in bacterial starter culture for foods, CRC press Inc., Boca Raton, Florida, 159-147. (Cited by Fergus 1999).

Mercenier, A. (1999). Lactic acid bacteria as live vaccines. In: Probiotic: Acritical Review. Edited by Tannock, G. W.

Merline, T. L.; Corvo, D. L.; Gill, J. H. and Griffith, J. K. (1988). Notes: enhanced gentamycin killing of *E. coli* by tel gene expression. J. Antimicrob. Agents. Chemother. 33: 230-232.

Mims, C. A.; Playfair, J.; Ratt, I. M.; Walkelin, D. and Williams, R. (1987). "Medical Microbiology". M. Mosoly, Torento.

Mingeot-Lectero, M. P.; Glupezynski, Y. and Tulkens, P. M. (1999). Mini review aminogilcosides activity and resistance. J. Antimicrob. Agents. Chemother. 43: 727-737.

Mitchell, R. G. (1964). Urinary tract infection due to coagulase negative *Staphylococcus*. J. Clin. Path. 17: 105.

Mobley, H. L. T. and Belas, R. (1995). Swarming and pathogenicity of *Proteus mirabilis* in the urinary tract. Tends in Microbiol. 3 (7): 280-284.

Mobley, H. L. T. and Chippendle, G. R. (1990). Hemaglutinin urease and hemolysin production by *Proteus mirabilis* from clinical sources. J. Infec. Dis. 161: 525-530.

Mobley, H. L. T. and Warren, J. W. (1994). Urease-positive bacteria and obstruction of long-term urinary catheters. J. Clin. Microbial. 25 (11): 2216-2217.

Morshita, T.; Kegushi, Y.; Yajima, M.; Sakurai, T. and Yura, T. (1981). Multiple nutritional requirements of *Lactobacilli*: genetic lesion affecting amino acid biosynthetic pathways. J. Bacteriol., 148: 64-71. (Cited from Neto and Yokoya, 1997).

-N-

Narendranth, N. V.; Hynes, S. H.; Thomas, K. C. and Ingledew, M. W. (1997). Effect of *Lactobacilli* and yeast. Catalyzed ethanol fermentations. J. Appl. Environ. Microbiol., 63 (11): 4158-4163.

National Committee for Clinical Laboratory Standrs (1991). Performance Standard for Antimicrobial Susceptibility Testing. Third informational supplement. Document M 100-41; 11 (17), NCCLs, Villanva. Pa.

Navarro, E. E.; Almario, J. S.; King, C.; Bacher, J.; Pizzo, P. A. and Walsh, T. J. (1994). Detection of *Candida casts* in experimental renal cndidiasis for the diagnosis and pathogenesis of upper UTI. J. Med. Mycol. 32 (6): 415-426.

Nigatu, A. and Gashe, B. A. (1994a). Inhibition of spoilage and food borne pathogene by lactic acid bacteria isolated from fermenting tef (eragrostis tef) dough. Ethiop. Med. J., 32: 223-229.

Nigatu, A. and Gashe, B. A. (1994b). Survival and growth of selected pathogens in fermented kocho (ensete ventricosum). J. East African Medical. J. 71 (1): 514-518.

-O-

Obi, Cl.; Traupiwa, A. and Simango, C. (1996). Scope of urinary pathogens isolates in the public health bacteriology laboratory, Harare. Antibiotic susceptibility pattern of isolates and incidence of hemolytic bacteria. Cent. Afr. J. Med. 42: 244-249.

O'Gradyfwl (1980). Factors Affecting Urinary Tract Infection. In: Tutorial in Past Graduated Medicine Urology. Chisholm G. D.

Olasupo, N. A.; Olukoya, D. K. and Odunfa, S. A. (1995). Studies on bacteriocinogenic *Lactobacillus* isolates from selected Nigerian fermented foods. J. Basic Microbiol, 35 (5): 319-324. (Abst).

Orga, P. L. and Faden H. S. (1985). Urinary tract infection in childhood, an update. The J. Pediator. 106 (6): 1023-1028.

-P-

Pagani, L.; Migliavacca, R.; Luzzaro, F.; Giacobone, E.; Perilli, M.; Micheletti, P. and Amicosante, G. (1998). Comparative activity of pipracillin / trazobactam against clinical isolates of extended spectrum beta-lactamase producing Enterobacteriaceae, Chemotherapy-Basel. 44 (6): 377-384.

Pfeiffer, P. and Radler, F. (1982). Purification and Characterization of extracellular and intracellular killer toxin of *Saccharomyces cerevisiae* strain 28. J. Gen. Microbiol., 128: 2699-2706.

Prescott, L. M.; Haarley, J. P. and Klein, D. A. (1990). Microbiology (1st ed.). Brown publisher. New York.

-R-

Ramphal, R.; Sadoff, J. C.; Pyle, M. and Silipigni, J. D. (1984). Role of pilin in the adherence of *P. aeroginosa* to injured tracheal epithelium. Infec. Immun. 44: 38-40.

Reid, G.; Cook, K. L. and Bruce, A. W. (1987). Examination of strains of *Lactobacilli* for properties that may influence bacterial interference in the urinary tract. J. Urol. 138: 330-335. (Cited by Fergus M. C. (1999)).

Reid, G.; Tieszer, C. and Lam, D. (1995). Influence of *Lactobacilli* on the adhesion of *Staphylococcus aureus* and *Candida albicans* to fibers and epithelial cells. J. Ind. Microbiol: 15: 248-253. (Cited by Fergus M. C. (1999)).

Rice, L. B.; Marshall, S. H. and Carias, L. L. (1992). Tn 5381, a conjugative trasposon identifiable as a circular from in *Enterocuccus faecalis*. J. Bacreriol. 174: 7308-7318.

Rogosa, M. (1970). Characters used in the classification of *Lctobacilli*. Inter. System. Bacteriol., 20: 519.

-S-

Saavedra, J. M. (1995). Microbes to fight microbes: a not so novel approach to controlling diarrheal disease. J. Pediatr. Gastroenterol, Nutr., 21 (2): 125-129. (Cited by Fergus 1999).

Saeed, R. S. (1993). *Proteus* and other microorganisms causing UTI in children. M. Sc. Thesis, College of Medicine. University of Baghdad. Iraq.

Salminen, S.; Deighton, M. and Gorbach, S. (1993). In: Lactic acid bacteria. Marcel Dekker. (Cited from Lee and Salminen, 1995).

Santaro, J. and Kaye, D. (1978). Recurrent UTI pathogenesis and management. Med. Clin. North AM. 62: 1005.

Schillinger, U. and Luck, F. K. (1991). Antibacteial activity of *Lactobacillus sake* isolated from meat. Appl. Environ. Microbiol., 55 (8): 1901-1905.

Senior, B. W. (1997a). Media and tests to simplify the recognition and identification of members of the protease. J. Med. Microbiol. 46: 39-44.

Senior, B. W. (1990). Investigation of the types and characteristic of the proteolytic enzymes formed by diverse strains of *Proteus* spp. J. Med. Microbiol. 48: 623-628.

Sidovezyk, Z. and Zych, K. (1986). Lipopolysaccharides of flagellated and non-flagellated *Proteus vulgaris* strains. Arch. Immun. Ther. Exp. 34: 461-469.

Silva, M.; Jacobus, N. V.; Deneke, C. and Gorbach, S. L. (1987). Antibacterial substances from a human *Lactobacillus* strain. Antimicrob. Agent and Chemother.; 31 (8): 1231-1233.

Silverblatt, F. (1974). Host-parasite interaction in the rat renal pelvis. J. Exp. Med. 140: 1696-1711.

Silverblatt, F. J. (1975). Host-parasite interaction in the rat renal pelvis: a possible role of pilli in the pathogenesis of pylonephritis. J. Exp. Med. 140: 1695-1711.

Skaar, R; Duggan, D.; Elliker, P. R.; Anderson, A. W. and Hannesson, C. (1959). An agar culture medium for lactic acid *Streptococci* and *Lactobacilli*. J. Dairy Sci., 39: 1611-1612.

Sleigh, J. D. and Timbury, M. C. (1994). Notes on medical bacteriology. Churchill living Stone Inc. New York.

Smith, H. W. and Halls, S. (1975). The transmissible nature of the genetic factor in *E. coli* that controls hemolysin production. J. Gen. Microbiol. 47: 153-161.

Sotto, A.; Boever, C. M.; Fabbro-peray, P.; Gouby, A.; Sirot, D. and Jourdan, J. (2001). Risk factors for antibiotic-resistance *Escherichia coli* isolated

from hospitalized patients with urinary tract infections: a prospective study. J. Clin. Microbiol. 39 (2): 438-444.

Speck, M. L. (1971). Control of food born pathogens by starter cultures. J. Dairy Sci., 55 (7): 1019-1022.

Stamer, J. R. (1979). The lactic acid bacteria: microbes of diversity. Food Technology., 33: 60-65.

Stamey, T. A. (1975). A clinical classification of UTI based upon origin sothern. Med. J. 68: 934-939.

Stamey, T. A.; Sexton, C. S. and Chung, H. K. (1971). The role of vaginal colonization with Enterobacteriaceae in recurrent urinary infection. J. Urol. 113: 213-218.

Stamm, W. E. (1998). Urinary tract infection and pylonephritis. In: Harrison's principle of Interval Medicine. (14^{th} ed.).

Stamm, W. R. and Turck, M. (1980). Causes of the acute urethral syndrome in women. New. Eng. J. Med. 303: 409-412.

Stanier, R. Y.; Ingraham, J. L.; Wheelis, M. L. and Painter, P. R. (1986). The microbial world. (5^{th} ed). Prentice Hall. Jersey.

Stratchounski, L. S.; Kozlove, R. S.; Rechedko, G. K.; Stetsiok, O. U. and Chavrikova, E. P. (1998). Antimicrobial resistance patterns among aerobic gram-negative bacilli isolated from patients in intensive care units: results of multicenter study in Russia. Clinical microbial. And Infect. 4 (9): 497-507.

Swihart, K. G. and Welch, R. A. (1990). The HPMA Hemolysin is more common than HlyA among *Proteus* isolates. Infec. and Immun. 58 (6): 1853-1860.

-T-

Tanagh, E. A. and McAninch, J. W. (1995). "Smith's general Urology". (14th ed.). Appleton and Lange, Norwalk, Connecticut.

Terai, A.; Arai, Y. and Okada (1994). Urinary bacteriology of continent urinary reservo and calculus formation. J. Urol. 1 (4): 332-336.

Tissier, H. (1905). Taxanomy and ecology of *Bifidobacteria*, *Bifidobacteria* microflora. 3: 11-28. (Cited by Fergus 1999).

Tracy, O. and Thomson, E. J. (1972). A evaluation of three methods of typing organisms of the genus *Proteus*. J. Clin. Path. 25: 69-72.

-V-

Velraeds, M. M., Van der Mei, H. C., Reid, G. and Busscher, H. J. (1996). Inhibition of initial adhesion of uropthogenic *Enterococcus faecalis* by biosurfactants from *Lactobacillus* isolates. Appl. Environ. Microbiol. 62: 1958-1963. (Cited by Fergus M. C. (1999)).

Vignolo, G. M.; Suriani, F., Halgado, A. P. R. and Oliver, G. (1993). Antibacterial activity of *Lactobacillus* strains isolated from dry fermented sausages. J. Appl. Bacteriol., 75: 344-349.

-W-

Walker, K. E., Moghaddame-Jafari, S.; Lockatell, C. V.; Johnson, D. and Belas, R. (1999). Zap A, the IgA degrading metalloprotease of *Proteus mirabilis,* is a virulence factor expression specifically in swarmer cells.

Warren, J. W.; Tenney, J. H.; Hoops, J. M.; Muncie, H. L. and Antony, W. C. (1982). A prospective microbiologic study of bacteria in patients with chronic indwelling uretheters. J. Inf. Dis. 146: 719-723.

Winkelstin, D. M. (1955). The characteristics of *Lactobacillus acidophilus* and *Lactobacillus bulgaris*. J. Gen. Microbiol., 12: 123-132.

Wray, S. K. Hull, S. I. Cook; R. C. Barrish, J. and Hall, R. A. (1986). Identification and characterization of a uroepithelial cell adhesion from a uropathogenic isolate of *Proteus mirabilis*. Inf. Immun. 54 (1): 43-49.

-Y-

Younis, A. T. (1986). Effect of the heamolysin of *E. coli* causing UTI on phagocyte cells. M. Sc. Thesis, College of Medicine. University of Baghdad. Iraq.

-Z-

Zunino, P.; Piccini, C. and Leganni-Fajardo, C. (1999). Growth, cellular differentiation and virulence factor expression by *Proteus mirabilis in vitro* and *in vivo*. J. Med. Microbiol. 48: 527-534.

Zunio, P.; Piccini, C. and Legnani-Fajardo, C. (1994). Flagellate and non-flagellate *Proteus mirabilis* in the development of experimental urinary tract infection. Microbial. Path. 16: 379-385.

المصادر العربية:

الدليمي، جيهان عبد الستار سلمان. (٢٠٠٠). استخدام الكحول الاثيلي لعزل بكتريا حامض اللاكتيك ودراسة تاثيرها التازري مع خميرة الخبز ضد بعض انواع البكتريا. رسالة ماجستير. كلية العلوم. الجامعة المستنصرية.

العبيدي، نبراس نزار محمود. (١٩٩٧). تاثير البكتريوسين المنتج من بكتريا حامض اللاكتيك على بعض انواع البكتريا المرضية. رسالة ماجستير. كلية العلوم. الجامعة المستنصرية.

Appendix (1)

Interpretation of Api 20 E Reactions.

Microtube	Positive	Negative
ONPG	Yellow	Colorless
ADH	Red/Orange	Yellow
LDC	Orange	Yellow
ODC	Red / Orange	Yellow
CIT	Blue-Green	Pale green / Yellow
H_2S	Black deposit	Colorless / Grayish
URE	Red / Orange	Yellow
TDA	Dark brown	Yellow
IND	Red Ring	Yellow Ring
VP	Pink / Red	Colorless
GEL	Diffusion of Black Pigment	No diffusion
GLU	Yellow	Blue / Blue green
MAN	Yellow	Blue / Blue green
INO	Yellow	Blue / Blue green
SOR	Yellow	Blue / Blue green
RHA	Yellow	Blue / Blue green
SAC	Yellow	Blue / Blue green
MEL	Yellow	Blue / Blue green
AMY	Yellow	Blue / Blue green
ARA	Yellow	Blue / Blue green
OX	Violet / Dark purple	Colorless / Light purple

الخلاصة

❖ جمعت (١٥٠) عينة ادرار مرضى يعانون من التهاب المجاري البولية ممن راجعوا مستشفيين في بغداد (مستشفى اليرموك والكرامة)، اظهرت النتائج بعد الزرع البكتيري على وسط اكار المكونكي واكار الدم لمدة ٢٤ ساعة بدرجة حرارة ٣٧ °م، الكشف عن (١١٦) عزلة بكتيرية، كانت منها (١٦) عزلة تعود للجنس *Proteus* والذي تمثل بنسبة عزل (٦٢,٥%) للنوع *Proteus mirabilis* وبنسبة عزل (٣٧,٥%) للنوع *Proteus vulgaris*.

❖ تضمن الجزء الاخر من الدراسة عزل عصويات اللاكتيك *Lactobacillus* لاستخدامها في تثبيط نمو والتصاق بكتريا *Proteus mirabilis*، فقد امكن الحصول على (١٠) عزلات تعود لبكتريا حامض اللاكتيك العصوية *Lactobacillus* من (١٥) عينة لبن جمعت من اسواق مدينة بغداد المحلية بعد تنميتها على وسط ركوزا اكار المحور وحضنها لمدة ٢٤ ساعة بحرارة ٣٧°م.

❖ بعد اجراء اختبار فحص الحساسية لبكتريا *Proteus mirabilis* ضد ١٢ مضاد حيوي، اظهرت النتائج ان الاميكاسين، السبروفلوكاسين والجنتامايسين هم اكثر المضادات حساسية حيث بلغت نسب العزلات الحساسة لها ٩٠%، ٨٠% و٧٠% على التوالي. فيما كانت كل من مضادات البنسلين، الاموكيسلين والتترسايكلين الاقل حساسية ضد هذه البكتريا عندما تجاوزت نسبة الحساسية ١٠%، ٢٠% و٢٠% على التوالي.

❖ اعتمادا على نتائج فحص الحساسية فقد اختيرت احدى عزلات بكتريا *Proteus mirabilis* وهي (*P. m.* 9) لكونها مقاومة لجميع المضادات (باستثناء الاميكاسين)، فيما اختيرت عزلتين من بكتريا حامض اللاكتيك وهما *Lactobacillus acidophilus* (*Lb. a.* 11), *Lactobacillus plantarum* (*Lb. p.* 3) اعتمادا على فعاليتها التثبيطية العالية ضد بكتريا *Proteus mirabilis* لاستخدامها في التجارب اللاحقة.

❖ اجري اختبار التركيز المثبط الادنى لرواشح مزروع بكتريا حامض اللاكتيك المركز لثلاث مرات على ظاهرة التصاق بكتريا *Proteus mirabilis*. اظهرت النتائج فعالية الراشح المركز في التقليل من التصاق اعداد البكتريا المرضية هذه بالخلايا الطلائية بمعدل وصل الى(٣-١٠) بكتريأ/ خلية بدلا من (٤٤-٥٥) بكتريا /خلية لمعاملة السيطرة.

تاثير Probiotic على بكتريا *Proteus mirabilis* وصفتها الالتصاقية

رسالة

مقدمة الى كلية العلوم جامعة النهرين

وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانة الاحيائية

من قبل

غيداء حسين عبد الرحيم الجبوري

بكلوريوس تقانة احيائية جامعة النهرين ٢٠٠٢

ربيع الثاني ١٤٢٦

ايار ٢٠٠٥

Table (3-3). Antibiotics sensitivity of *Proteus mirabilis* isolates.

Antibiotic / Isolate No.	AMX	KF	C	GM	NA	PIP	AK	S	TE	SXT	P	CIP
***P. m*.1**	R	S	R	R	S	S	S	R	R	R	R	S
***P. m*.2**	R	R	R	S	S	S	S	R	S	S	R	S
***P. m*.3**	S	R	R	S	S	R	S	R	R	S	R	R
***P. m*.4**	R	R	S	S	S	S	R	R	R	R	R	S
***P. m*.5**	R	S	R	S	R	S	S	R	R	R	R	S
***P. m*.6**	R	R	S	S	R	R	S	S	R	R	R	S
***P. m*.7**	R	R	R	R	R	S	S	S	R	S	S	S
***P. m*.8**	S	S	S	S	S	R	S	S	S	R	R	S
***P. m*.9**	R	S	R	R	R	R	S	R	R	R	R	R
***P. m*.10**	R	S	R	S	R	S	S	R	R	R	R	S

S : Sensitive
R : Resistance
AMX : Amoxicillin
KF : Cephlaxin
C : Chloroamphenicol
NA : Naldixic acid
PIP : Pipracillin
AK : Amikacin
S : Streptomycin
TE : Tetracycline
GM : Gentamycin
P : Pencillin G.
SXT : Tri-methoprin sulphamethazole
CIP : Ciprofloxacin

Table (3-5). Inhibitory effect of LAB against *Proteus mirabilis* isolates (10 isolates) on solid medium (MRS agar) after incubation periods.

Isolate No.	Incubation period (hr.)	Inhibition zone (mm) of:										
		***Lb. p*1**	***Lb.p*.2**	***Lb.p*.3**	***Lb.b*.4**	***Lb.p*.5**	***Lb.c*.6**	***Lb.b*.7**	***Lb.b*.8**	***Lb.c*.9**	***Lb.c*.10**	***Lb.a*.11**
***P. m*.1**	18	10	11	12	10.5	10.5	12	11	13	14	12	15
	24	13	12	16	12	13	13	13	14	15	12.5	16
***P. m*.2**	18	11	12	10	12.5	13	10	11.5	11	10	12	13
	24	12	13	13	14.5	14	11	12	13	13	12.5	14
***P. m*.3**	18	9	10	12	10	11	11	12	12	9	12	10
	24	12	11	13.5	12	11.5	12	12.5	14	12	13	13
***P. m*.4**	18	12	12	10	11	10	11	11.5	10	12	10.5	12
	24	12.5	13	13	13.5	12	12.5	13	11	12.5	12	13
***P. m*.5**	18	10	10.5	10	11	12	10	13	10.5	12	11	13
	24	12	12	13	12	13	12	13.5	10.5	13	12.5	14

Isolate No.	Incubation period (hr.)	Inhibition zone (mm) of:										
		***Lb. p*1**	***Lb.p*.2**	***Lb.p*.3**	***Lb.b*.4**	***Lb.p*.5**	***Lb.c*.6**	***Lb.b*.7**	***Lb.b*.8**	***Lb.c*.9**	***Lb.c*.10**	***Lb.a*.11**
***P. m*. 6**	18	10	11	9	12	13	12	11	10	11.5	12	14
	24	12	13	12	13.5	14	13	11	10.5	122	13	15
***P. m*.7**	18	10	10	9	10.5	12	11	12	12	13	11	12
	24	13.5	10	13	11.5	13.5	13	13	14	13	12	14
***P. m*.8**	18	12	11	12	10	10.5	11	12.5	11	10	10	12
	24	12.5	12.5	13	12	13	12	13	12.5	12	13	14
***P. m*.9**	18	11	9	14	10	12	13	11.5	12	11	10.5	16
	24	13	12	17	13	12.5	14	13	14	13.5	11.5	18.5
***P. m*.10**	9	12	10	9	12	11	13	12	11	11.5	11	13
	24	13.5	12	14	14	15	14	15	13	12.5	14	15

P.m. = *Proteus mirabilis*
Lb. p. = *Lactobacillus plantarum*
Lb.b. = *Lactobacillus brevis*
Lb.c = *Lactobacillus casei*
Lb.a. = *Lactobacillus acidophilus*