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Marwa

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.

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1.1. Introduction:-

Dermatophytes are fungi that require keratin for growth. These fungi can cause superficial infections of the skin, hair and nails. Dermatophytes are spread by direct contact from other people, animals and soil, as well as indirectly from formats. (Barry and Hainer, 2003).

Tinea pedis is the most common dermatophytosis which is found to affect up to (70%) of the adults worldwide. Also it is referred to as athlete's foot or ringworm of the foot. It involves the plantar surface and interdigital spaces of the foot which includes inflammatory and non inflammatory lesion. (Drake *et al*., 1996). Tinea pedis appears to be a disease of civilized human, having evolved in the late 19^{th} and early 20^{th} centuries. It occurs throughout the United States but is the most prevalent in warm, humid climates. (Masir-Fridling, 1996).

A great attention was made to use microorganism or their metabolites in treatment of various infection and diseases. Bacteria and yeast are considered as the most and first two types of microorganisms used in this approach. Among bacterial groups comes lactic acid bacteria (LAB) to be used as a probiotic by too many investigators to treat several diseases through excreting numerous metabolites that possess inhibitory activity on microorganisms causing these diseases. It present as normal microflora in the mucus membrane of the intestine and digestive tract of human. The microbial action of LAB is based on both competition for nutrient and production of various compound, such as organic acids, hydrogen peroxide, bacteriocins and low molecular weight metabolites (Ouwehand, 1998). Lactic acid and phenyl acetic acid, in combination, have shown to exhibit

good antifungal properties (Lavermicocca *et al.*, 2003). More recently, the use of lactic acid bacteria to inhibit growth of fungi has gained serious attention due to the widespread of diseases caused by numerous types of such filamentous microorganisms. In Iraq, most of the previous probiotic studies were focused on using lactic acid bacteria (as probiotic) against bacteria causing diseases. For such reason, this study was designed to investigate the effect of local isolates of LAB, especially those belonging to the genus *Lactobacillus*, against fungi causing the dermatophytic disease tinea pedis. For such purpose, different concentration of filtrates of more than one species of *Lactobacillus* was tested growth of fungi isolated from scraped samples of patients infected with tinea pedis disease.

Aims of the study:

- Isolation and identification of fungal causatives of Tinea pedis.
- Detecting the inhibitory activity of probiotic isolates (or their filtrates) against fungal isolates.

1.2. Literature Review:-

1.2.1. Dermatophytoses:

The dermatophytoses cause tinea and related diseases. The word tinea comes from a Latin root meaning a gnawing worm; a common name for some forms of tinea is ring worm. The ring worm was coined to describe the circular lesion produced by dermatophytes on the skin or scalp. The circular form of tinea is due to the fungal infection spreads in an expending circular manner resulting in a ring-shaped, scaly, itching area on the skin (Ajillo, 1974; Myrvik and Weiser, 1988).

Dermatophytes are infection of keratinized tissues, that is, the epidermis, hair and nails. The history of human medical mycology started before one hundred and fifty years ago peculiar microscope structures of rods and buds when noted in the diseased skin of patients suffering from tinea favosa (Kown-Chung and Bennett, 1992; Martin and Kobayashi, 1999). Later it was found that the fungal cause of tinea favosa, was first recognized by Schonlein and now known as *Trichophyton Schoenleinii* (Padhye and Weitzman, 1998).

Sabouraud began his systematic studies of dermatophytoses in 1892, and he published a series of papers correlating earlier studies with his own astute observations, then Sabouraud in 1910 classified dermatophytes in to four genera (*Achorion, Epidermatophyton, Microsporum and Trichophyton*) and the culture media have been developed for isolation of dermatophyte (Aiello, 1977, Padhye and Weitzman, 1998). Emmon in 1934 established current taxonomic system of dermatoyphyes according to mycological standards, he

classified the dermatophytes into three genera :(*Trichophyton*, *Microsporum* and *Epidermophyton*) on the bases of spore morphology and accessory structure of the fungi.

Dermatophytes can be divided into three groups, anthropophilic, zoophilic and geophilic depending on their natural habitat. The primary reservoirs of them are man, animal and soil, respectively. Fungi in all three categories, however, cause human infection (Mackenize *et al.*, 1986). Cutaneous fungal infection are mostly caused by dermatophytes which include the fungi in genera *Trichophyton*, *Microsporum* and *Epidermophyton*, in addition to a great variety of yeast, molds and other fungi which sometimes infect the skin and all together are grouped under the name dermatomycosis (Edwardo, 1984).

Dermatophytes are capable of infecting nails and the skin from almost any body sites. One of the frequently encountered dermmatophyte infections is athlete's foot or tinea pedis. This can be recurring infection and is most often caused by the dermatophytes species *Trichophyton rubrum* and *Trichophyton mentugrophytes* (Howell *et al*., 1999).

Tinea pedis occurs mostly among people who wear shoes, the warmth and moisture provided by the shoes are the key factors in establising and maintaining tinea pedis (Nickerson-Irving and Mchmert, 1945). In 1973, Gentles and Evans noticed a definite relationship between the incidence of tinea pedis and the use of communal bath. The incidence among bather and non-bather was (21) % and (28) %, respectively.

1.2.2. Cutaneous Mycosis Infection:

Fungal infections of the skin can be classified according to their sites of infection, into the following five groups: (superficial, cutaneous,

subcutaneous, systemic and opportunistic) (Presscot *et al.*, 1996; Mitchell, 1998).

The cutaneous mycoses are caused by fungi that invade only superficial keratinized tissue (skin, hair and nails) and not the deeper tissues. The most important of these are the dermatophytes, a group of closely related fungi classified into three genera: *Epidermophyton* (infect skin and nails), *Microsporum* (infect skin and hair) and *Trichophyton* (infect skin, hair and nails) (Hunter *et al*., 1995; Jawetz *et al*., 1995). These fungi invade only the keratinous layer of skin because most are unable to grow at 37 ˚C except *Trichophyton verrucosum.* Growth may be enhanced at 35-37 ˚C (Arthur *et al*., 1986) or in presence of serum for the presented antibodies against the fungi (Mitchell, 1998).The cutaneous mycoses depend on the site and species of fungi causing the infection.

1.2.3. Types of Tinea:

1.2.3.1. Tinea Capitis

Tinea capitis is dermatophytosis or ringworm of the scalp and hair. The infection begin with hyphal invasion of the skin of the scalp, with subsequent spread down the keratinized wall of the hair follicle by *Microsporum audouinil* , *Microsporum canis* and *Trichophyton tonsurans* (Hay and Moore,1998; Odom *et al*. 2000).

1.2.3.2. Tinea Barbe

Tinea barbe, is a ringworm of the beard. Its affects the hairs of the beard, neck and mustache area of adult men who work with animals (Lesher *et al*., 1994; Goldstein *etal*., 2000). The most common zoophilic fungi that's cause

tinea barbe *Trichophyton mentagrophytes* and *Trichophyton verrucosum* (Website1; De-vroey, 1995).

1.2.3.3. Tinea Faciei

Tinea faciei, tends to occur in the nonbearded area of the face. Common causative organisms are *Trichophyton rubrum* and *Trichopyton mentagrophytes* or *Trichophyton tonsurans*. (Emanuel and Mcneely, 1996; Drake *et al*., 1996).

1.2.3.4.Tinea Corporis :

Tinea corporis is properly applied to lesions originating on the glabrous (relatively hairless) skin of the human body except the foot, hand and groin. (Ravine *et al*., 1980; Kown-chung and Bennett, 1992).Tinea corporis is caused by all dermatophytes fungi and the moste common types are: *Trichophyton rubrum, Microsporam canis* and *Trichophyton mentagrophytes* (Matsumoto, 1996).

1.2.3.5. Tinea Cruris

Tinea cruris is an acute or chronic infection occurs in the groin area and involving the perineum, scrotum and perianal area. (Prescott *et al*., 1996)**.** Most such infections involve males and the most fungi that's causes the tinea cruris are: *Trichophyton mentagrophtes*; *Epidermophyton floccosum* and *Trichophyton rubrum*. (Hay, 1993; Martin and Kobayshi, 1993).

1.2.3.6. Tinea Manuum

Tinea manus refers to ring worm of the hands and the fingers. (Martin and Kobayash, 1999).

All dermatophytes fungi are causes the tinea manuum but the most fungi is *Trichophyton rubrum* (Hay and Moore, 1998).

1.2.3.7. Tinea Uniguium

Tinea uniguium is a dermatophyte infection of the nails. It's a subset of onychcomycosis, which includes dermatophyte, nondermatophyte and yeast infection of the nails (Bergus, 1993).The most common causative fungi are *Trichophyton rubrum* and *Trichophyton mentagrophytes*. (Website1; Prescott *et al*., 1996).

1.2.3.8. Tinea Pedis

Tinea pedis, other wise known as Athlete's foot, is a fungal infection of the feet caused by superficial dermatophytes (Jahss, 1991; Tortora and Grabowski, 1996). It is the most common fungal infection and seems to be a higher incidence in males and it is the least common in children. (Rodger *et al*., 1996; Evans, 1997).

Initially it attacks the web of skin between the 3rd and 4th toes and then may progress to other area of the feet including the soles and the heels.

Athlete's foot is called at any infection occurs between the toes either to be bacteria or *Candida* or nondermatophyte molds (Ginsberg, 1997; Hay and Moore, 1998).

1.2.4. Epidemiology of Tinea Pedis:-

Tinea pedis infection is distributed in all worlds wide and it is considered the most common dermatophyte infection (Aly, 1994), and it is estimated to affect about (15) % of the population (Maruyama *et al*., 2003). The tinea pedis infection is more common during summer months and in semi-tropical and tropical climates. It is usually related to sweating and warmth, and use of occlusive footwear (Aly, 1994).

Most researchers insist that the most important place from which man may get the infection is the communal bath and swimming pools. Vanbreuseghem in 1955 showed that (30) % of persons whose main recreation was swimming in public baths had tinea pedis compared with (4.5) % of gymnasts. The infection is also common in areas such as college dormitory shower rooms where many people share showers, locker rooms, bathing facilities and gymnastic floors. (Conklin, 1990; Auger *et al*., 1993).

1.2.5. Etiology of Tinea Pedis:-

Tinea pedis is most commonly caused by *Trichophyton rubrum* (60) %, *Trichophyton mentagrophytes* (20) % and *Epidermophyton floceosum* (10) % (Midgley *et al*., 1997; Joseph, 2004).

Trichophyton rubrum is the most common pathogen cases of tinea pedis throughout the world (Arnold *et al*., 1990). The zoophilic species most commonly produce more inflammatory type of tinea pedis, (Hay and moore,1998; Website2), *Trichophyton violaceum*, however, is probably the most prevalent of the zoophilic dermatophytes that causes tinea pedis and this infection is considered a resistant for treatment .(Pock-steen,1967).

1.2.6. Pathogenesis of Tinea Pedis:-

Tinea pedis infection is considering the most important dermatophytes infections in the North America and United States (Rothman, 1957). *Trichophyton rubrum*, *Trichophyton mentagrophytes* and *Epidermophyton floccosum* most commonly causes of tinea pedis, with *Trichophyton rubrum* being the most common cause worldwide. (Rippon, 1988).

Tinea pedis spreads via the transfer of interdigital skin fragments in various environments, such surface include bathroom floors and communal areas or by towels, sock and shoes, due to this cross infection among family members is common (Rook *et al*., 1972; Burton, 1996). Dermatophyte fungi invade the superficial keratin of the skin, and the infection remains limited to this layer. Dermatophyte cell walls contain mannans, which can inhibit the body's immune response**;** *Trichophyton rubrum* in particular contains mannans that reduce keratinocyte proliferation, and the concomitant decrease in the rate of sloughing results in a chronic state of infection (Elewski, 1994; Ninomiya *et al*., 1998).

Enzymes and other diffusible substances produced by the dermatophyte, including the transepidermal leukocyte chemotaxis and protolytic enzymes, reach the viable layers of the skin and induces the inflammatory response, this is called the colonisation phase and occurs in the stratum corneum (Chan and Peterar, 1981; Nichiolas and Badery, 1994).

1.2.7. Clinical Feature of Tinea Pedis:-

A tinea pedis infection is more common in the adult young's and there seems to be a higher incidence in males more than females, and rather than it is the least common in the children (Frey *et al.,* 1985), but when it dose occur in children it is often misdiagnosed (Mcbride and Cohen, 1992;

Rodger *et al.,* 1996).

Tinea pedis may present in several ways, varying from mild chronic scaling to acute lesions that are exfoliative, pustular, or bullous. The vesicobullous disease is more occurs in the children from other types of tinea pedis (Geary and Lucky, 1999). There are four typical presentations of Athletes foot (Martin and Kobayashi, 1999):-

1.2.7.1. Chronic Interdigital Infection:-

Interdigital tinea pedis is the most common form and can be caused by any of three anthropophilic dermatophytes (*Trichophyton*, *Microsporam* and *Epidermphyton*). This form typically affects the toe webs and subdigital areas producing chronic scaling, fissuring and macerated skin. It frequently occurs between the lateral toe spaces especially third, fourth and fifth toe interspaces and may spread to the underside of the toes (Leyden and Kligman, 1978; Hay and Moore, 1998). It can be itchy and give a burning or painful sensation; also amalodour can often be noticed. Wet condition along with fungal invasion increases the incidence of bacterial infection in these patients by breaching cutaneous integrity by several types of bacteria such as *Staphylococcus aureus, Brevibacterium epidermids* and *Corynbacterium minutissimum,* (Reeves and Malbach, 1990; Leyden and Aly, 1993; Leyden, 1994). These pathogenic bacteria sometimes produce sulphur compounds which are potent antifungal and the fungus in the infected area may die, but the continued bacterial growth leads to sever, macerated interdigital infections. This type of infection, is not curable by antifungals alone, but requires antibiotics to eradicate the infection (Martin and Kobayashi, 1999).

1.2.7.2. The Chronic Papulosequomous Type:-

Chronic plantar scaling is also known as plantar hyperkeratosis or moccasin type of tinea pedis. The term moccasin is drived from the appearance the fungus imparts to the foot and also known as dry type infection (Habif, 1990).

The moccasin type is a more sever prolonged form of tinea pedis that covers the bottom and lateral aspects of the foot. Both feet are usually affected and the surface presents as a heavy scale of silvery white flakes on a red thickened base. Papules may also be noted around the demarcation line of erythema that surrounds the foot (Fitzpatrick *et al.*, 2001). *Trichophyton rubrum* is most commonly associated with moccasin type tinea pedis and other possible causative organisms include *Trichophyton mentagrophytes* and *Epidermophyton floccosum* (Martin and Kobayashi, 1999).

1.2.7.3. Vesiculobullous Tinea Pedis:-

Vesicobullous infection may be subacute but can be highly inflammatory; this infection is noticed on the arch and side of the foot and is usually caused by *Trichophyton mentagrophytes* (Black *et al.*, 1969). It causes small confluent vesicles or vesiculopustules surrounded by scaling and lacks the macerated weepy skin seen in interdigital type (Roldan *et al.*, 2000), and also vesicles in the company or absence of fissures span from the dorsal web space to the sole and arch of the foot.

The intense inflammation is noticed with this varity of tinea pedis is secondary to a host T- cell immune response, triggered by the generation of a sufficient number of organisms into the stratum cornoum. (Jones *et al.*,

1974), and this immune reactions is a contact-type dermatitis caused by contact of the epidermis with specific fungal antigens (Blak *et al.*, 1969).

1.2.7.4. Ulcerative Tinea Pedis:-

This type causes more acute and symptomatic picture characterized by maceration and ulceration of large areas of the soles, and also white hyperkeratosis and a strong heady odor are characteristic. It accompanied by a secondary bacterial infection, usually with gram-negative organisms. This type is commonly seen in immunocomprimised and diabetic patients (Martin and Kobaysh, 1999; Robbins, 2005).

1.2.8. Laboratory Diagnosis of Dermatohytes :-

Tinea pedis infections are typically easy to distinguish and diagnose. Usually, diagnosis of tinea pedis is based on history and clinical appearance of the feet. However, the following laboratory tests are important to ensure the infections and to know the causative fungi (Philpot, 1977; Weinstein and Berman, 2002).

1.2.8.1. Microscopical Examination:-

Specimens consist of scrapings from the infected skin, place on a slide where a few drops of (10-20) % potassium hydroxide (KOH) solution is added. After the cover slip is applied, the slide is generally heated (Jawetz *et al*., 1980; Hay and Moore, 1998). Care should be taken not to overheat the slide because boiling was precipitates KOH crystals (Kwon-Chung and Bennett, 1992). It can then be examined, immediately the branching hypha of the superficial dermatophytes can be recognized as well as the

pseudohyphae, chains of arthrospores and budding cells of yeast (Jawetz *et al*., 1982; Ajello and Padhye, 1998).

1.2.8.2. Cultural Examination:-

It is essential to isolate a dermatophyte in culture because direct examination dose not allow species identification. In some times it gives negative results. Sabourands agar is the most common agar medium used to culture the dermatophyte fungi (Habif, 1990; Kwon-Chung and Bennett, 1992). Medium containing cycloheximide and chloramphenicol are used to suppress mold and bacterial growth (Bailey and Scott, 1974; Jawetz *et al*., 1995). Incubation of cultures at 30 ºC for at least 4 weeks before discard negative cultures. Some dermatophytes sporulate within 5 days; whereas others take longer or seldom produce spores.

Macro conidia are more typical in morphology in young than old cultures, so that must be examined at this period (Tilton, 1992; Kwon-Chung and Bennett, 1992). When colonies appear, a small agar block containing hyphal tips may be cut out and transferred to sabouraud dextrose agar and incubation at 30 ºC to avoid contamination by other microorganisms. Species determination is made on the basis of colonial morphology, pigmentation, microscopic examination and in some cases biochemical tests (Jawetz *et al*., 1995).

1.2.9. Treatment of Tinea Pedis Infection:-

Treatment of tinea pedis infection may consist of topical and systemic treatment, in addition to some others:-

1.2.9.1. Topical Treatment:-

Topical antifungal agent is effective against most form of tinea pedis. The most antifungal drugs act by prevent synthesis vital compound in cell membrane of fungi. (Smith, 1993).

There are numerous topical agents available for the treatment of tinea pedis:-

1.2.9.1.1. Topical Imidazoles:-

Effective in all form of tinea pedis but are excellent treatments for interdigital tinea pedis these drugs are antifungal agents and have antibacterial activity and their mechanism of action is act by binding to the cytochrom system that's lead to inhibits the biosynthesis of cell membrane of fungi which leads to death of fungal cell (Bergus and Johnson, 1993; Martin, 2002).

1.2.9.1.2. Topical Pyridones:-

They are broad-spectrum agents with antidermatophytic, antibacterial and anticandidal activity and are therefore useful in all forms of tinea pedis but especially effective in interdigital tinea pedis and their mechanism of action is act by interferes with synthesis of DNA, RNA and protein (Martin, 2002).

1.2.9.1.4. Topical Allylamines:-

Effective in treating all forms of tinea pedis especially that's will be treatment resistance for example Moccasin type (Martin, 2002).

The mechanism of action of these compounds is act by inhibits squalen

epoxidase enzyme which decreases ergosterol synthesis, a vital compound of cell membranes of fungi that causes fungal cell death (Evans, 1997; Patel *et al*., 1999).

1.2.9.1.4. Topical Benzylamines:-

Sometime classified as a subset of allylamines, and useful for treating patients with refectory tinea pedis for example chronic hyperkeratotic (Savin *et al*., 1997; Saple *et al*., 2001).

1.2.9.1.5. Thiocarbamates:

Tolnaftate is a thiocarbamates, used principally in tinea pedis treatment, that's will be occurs on powder or solution form (Yamaguchi *et al*., 2001).

1.2.9.1.6. Keratolytic agents:-

These compounds are working to eliminate the keratin layer which dermatophytes lies on it, that's leads to inhibit the fungal growth (Kwon-Chung and Bennett, 1992).

1.2.9.1.7. Griseofulvin:-

It's use as powder in concentration (1) %, effective in all form of tinea pedis but is excellent treatment for interdigital tinea pedis (Aly *et al*., 1994).

1.2.9.1.8. Oil of Bitter Orange:-

It's considering very cheep treatments; however, it's able to prevent the fungal growth (Ramadan *et al*., 1996).

1.2.9.2. Systemic Treatments:-

Systemic treatments are taken by mouth in tinea pedis infection especially treatment of moccasin-type tinea pedis or inflammatory/ vesicular tinea pedis, and also may be required for patients who have failed with topical antifungal therapy, it can also be used as a first line therapy in patients with severe disease like hyperkeratotic lesions or patients with diabetes or peripheral vascular disease and patients with immunosuppressed (Xu *et al*., 1999; Martin, 2002; Markova, 2002), and this compounds are involves:-

1.2.9.2.1. Terbinafine:-

Terbinafine is synthetic allyamine derivative that has in vitro activity against dermatophytes and some molds (Faergeman *et al*., 1993; Noguchi *et al*., 1999).

1.2.9.2.2. Triazol Compounds:-

This compounds are belongs to azoles group, synthetic triazole antifungal agent that inhibits fungal cell growth by inhibiting the cytochrome P-450 dependent synthesis of ergosterol, a vital compound of fungal cell membranes (Hay and Moore, 1998; Nozickova *et al*., 1998).

1.2.9.2.3. Griseofulvin compound:-

Griseofulvin is an antibiotic produced by a *penicillium* species, this agent acts by inhibits fungal mitosis, perhaps by interaction with polymerized microtubules and disruption of the mitotic spindle, and it is fungistatic and

requires long courses of therapy (Tanz *et al.*, 1985; Kwon-chung and Bennett, 1992).

1.2.9.3. Another Treatment is Involves:-

1.2.9.3.1. Aluminum Acetate Solution (Burrow's sol.):-

It is used at concentration (1/10 to 1/40) for treatment vesicular tinea pedis and for decrease humidity (Martin, 2002).

1.2.9.3.2. Ammonium Lactate Lotion:-

It is used at concentration (12) % or (5%) to decrease scaling in patients with hyperkeratotic soles (Martin, 2002).

1.2.9.3.3. Drying Powders:-

It is put in the shoes for reduce foot humidity (Martin, 2002).

1.2.10. Important Fungi causing Tinea Pedis:-

1.2.10.1. *Tirchophyton rubrum*:-

Tirchophyton rubrum is a cosmopolitan, anthropophilic species. It has recently become the most frequent etiologic agent of human ringworm especially of tinea cruris through out the world. It is reported to cause tinea pedis in children as young as ago two years; it dose not invade the nail plate.

Tirchophyton rubrum is chronic and in some individuals become lifetime carriers once infected by this fungus, although it usually dose not invade hairs (John *et al*., 1996).

Skin lesions caused by *Tirchophyton rubrum* often have a red margin; the

central portion may appear to be relatively clear, although scaling may be apparent. On rare occasions, this organism may cause tinea capitis. Infected hairs do not fluoresce and hair invasion is endothrix. Because this fungus is quite variable, identification can be frustrating. However, most mycologists believe that identification is important because rigorous and extensive therapy is often required. One form of this fungus is very fluffy, white and exhibits many aerial hyphae. Other form of *Tirchophyton rubrum* is called the "granular". This white colonies are very flat, lacks aerial hyphae and have pronounced granular appearance; the undersurfaces colonies of *Tirchophyton rubrum* are usually red which is in sometimes is very bright color. It appears to have brown mixed with it (Bulmer, 1979). *Tirchophyton rubrum* is the most common isolate from all regions except the scalp. Tinea pedis usually of the moccasin type is the most frequently seen form of disease (Dompmartin *et al*., 1990).

1.2.10.2. *Trichophyton mentagrophytes*:-

This fungus is a worldwide cause of athlete's foot. It also can cause tinea capitis, tinea corporis, tinea barbae and tinea cruris. In many instances these infection are sporadic. According to many authorities, infections by *Trichophyton mentagrophytes* are not difficult to cure. There are several different variants of this organism; some are anthropophilic, while others are zoophilic. If hair is infected, the infection is endothrix (Bulmer, 1979). *Trichophyton mentagrophytes* is a cosmopolitan species and is the most commonly isolated dermatophyte from man and animal.

Typically colonies of *Trichophyton mentagrophytes*, are flat with a cream

to buff or tan, powdery surface. It tends to produce deeply pigmented colonies (Kwon Chung and Bennett, 1992).

Trichophyton mentagrophytes produces several different colonial forms. One major form is called “downy” because the culture is white, very fluffy and grossly has downy appearance, usually this form is anthropohilic. Other major form is “granular”, usually this colonial type is zoophilic in origin and when it infected man, may induce considerable inflammation (Bulmer, 1979).The reverse side of the colony is rose brown, occasionally orange to deep red in color (Bailey and Scott, 1974).

Trichophyton mentagrophytes is the species of *Trichophyton* most commonly isolated in clinical laboratories and it produces positive urease reaction within two or three days (Koneman *et al*., 1978).

1.2.10.3. *Candida albicans:-*

Candida albicans appears as unicellular, yeast like fungi, reproduced by budding and it is polymorphic, able to change reversibly between round budding and elongated hyphae or filamentous growth. This morphological flexibility appears to be a key contributor to virulence (Lodder, 1974; El-Barkani *et al*., 2000). The optimal temperature for growth of *Candida albicans* is 37 ºC and the optimal pH is 7. The favored temperature for germ tube formation is 37 ºC and pH 7 with exception obtained by Zhang who showed that *Candida albicans* able to produce germ tube in special medium has pH (5.4) (Sims, 1986; Zhang, 1995).

Candida albicans is a component of the normal skin floral and also the chief cause of mucocutaneous fungal disease in humans (Hay, 1993). Candida can also infect fingernails, producing onychomycosis and

paronychia and is more common with advanced human immunodeficiency virus (HIV) disease (Conant, 1994).

Candida albicans is the yeast pathogen most frequently isolated from patients with fungemia: Oropharyngeal candidiasis is the most common opportunistic infection in human immunodeficiency virus infected patients (Heelan *et al.*, 1996 and Wu *et al*., 1998).

Candida albicans is the yeast species most frequently isolated from human clinical specimens and causes a spectrum of superficial and systemic infections. The superficial infections such as oral candidosis which affect a large proportion of population including neonates and elderly individuals and candidal vaginitis which afflicts more than 30% of all woman (Nimi *et al*., 2001). Superficial infections by *Candida albicans* are common diseases in immunocompromised patients (Schaller *et al*., 2001).

1.2.11. Probiotics Definition and History:-

Early definition of probiotics by Lilly and Stillwall (1965) is substance produced by microorganisms which inhibit the growth of other microorganisms. While Havenaar and Hais (1992) defined them as mono or mixed cultures of live microbes that when applied to animal or human possess beneficial effect on health of the host. These beneficial effects include disease treatment and prevention as well as improvement of nutrients digestion and absorption (Fuller, 1991).

Probiotic microorganisms are generally (but not only) lactic acid bacteria (LAB) including *Lactobacillus acidophilus, Lactobacillus casei, Lactobacillus plantarum, Lactobacillus bulgaricus* and *Lactobacillus rhamnsus*. Other such as *bacillus* and *bifidobacterium* spp. as probiotic

strains is also used. Several microbes have been used unintentionally in food production such as dairy products and fermented vegetable. Such fermentend foods are popular due to their distinct characteristic in term of taste and aroma. Probiotic microorganisms are used to improve food flavor, for their beneficial aspects in health restoration and for disease treatment. They proposed and used in a wide range of clinical trials, ranging from diarrhea disease to cancer prevention (Fuller, 1994; Kaur *et al*., 2001).

The meaning of this term has now been redefined and restricted to aviable microbial agents which when used in animal or man; beneficially affect the host possibly by improving the balance of the indigenous microflora (Fuller, 1991; Salminem *et al*., 1999).

Based on this meaning, several term such as "friendly", "beneficial", "healthy" bacteria are also commonly know for probioties. The first study regarding beneficial effect of probiotics was carried out in the early of ninth century when a scientist that reported the favorable effects of soured milk in human and suggested that consumption of live microbes (possibly LAB) in such fermented milk may help improve the balance of the gut micro flora, since then, microbial probiotics have gained an increasing interest and their use in now widely accepted (Fuller, 1994).

The newest definition of probiotics is live microbial food supplements or components of bacteria which have been shown to have beneficial effects on human health (Isolauri *et al*., 2002).

1.2.12. Lactic Acid Bacteria (LAB):-

The term lactic acid bacteria (LAB) firstly are associated with the milk souring organism. It was classify by Oral-Jensen in 1919 into four main spp. (*streptococcus*, *Pediococcus*, *Leuconostoc* and *Lactobacillus*). They have common properties of gram-positive, non-spore forming bacilli,cocci or single, paired, chain or tetrad, catalase negative, anaerobic or aerotolarate, fastidious bacteria and produce lactic acid as main product of fermentation process (Holzapfel *et al*.,1998).

LAB reguires fastidious nutritional requirements. Its media should contain broad spectrum of organic acids and inorganic acids, amino acid, vitamins (B-blex), carbohydrates, peptides, salts and fatty acid (Kandler and Weiss, 1986). So that, deMan Rogosa Sharpe (MRS) medium was selected as a medium capable of supporting growth of all lactic acid bacteria (Teuber; 1995). LAB classified by Bergy's manual in 1994 into: *Aerococcus*, *Lactoabacillus*, *Leuconstoc*, *Pediococcus* and *Streptococcus.*

Later, it is found that there are new kinds of LAB which have the ability to moving, so it is put into a new genus called *Vagococcus.* Other newest *Tetragenococcus* have the ability to tolerate high salt concentration (18% NaCl). After that, new genus of LAB not tolerates high acid concentration was named *Ornobacterium.*

But in recent years, the modern classification of LAB was according to the sequence of rRNA and hybridization, for this reason lactic streptococcus was divided into two genera: *streptococcus* and *Lactococcus* (Axelsson, 1995).

1.2.13. Fungal Inhibition by LAB:-

Lactic acid bacteria (LAB) are of particular interest as biopreservation organisms. Their preserving effect mainly relates to the formation of lactic acid, acetic acid, hydrogen peroxide; competition for nutrients; and production of bacteriocins (Stiles, 1996). The bacteriocins from LAB are bioactive peptides, derived from ribosomally synthesized precursors and with a bacteriocidal effect on a number of different gram-positive bacteria (Klaenhammer, 1993). While many studies have assessed their antibacterial effects (Dodd and Gasson, 1994). There are very few reports on specific antifungal compounds from LAB.

A mixture of *Lactobacillus* spp. from a commercial silage inoculum was found to reduce mold growth and spore germination, as well as aflatoxin production by *Aspergillus flavus* subsp. *parasiticus* (Gourama and Bullerman, 1995). An antifungal *Lactobacillus sanfrancisco* CBI, isolated from sour dough inhibited bread spoilage molds from the genera *Fusarium*, *Penicillium*, *Aspergillus*, and *Monilia*.

Early research suggested antifungal activities from a *Lactobacillus casei* strain that inhibited both the growth and the aflatoxin production of *Aspergillus parasiticus* (El-Gendy and Marth, 1981).

It has been reported that antifungal activity of LAB might be due to the combination of lactic acid production and acetic acid in the MRS medium (Cabo *et al.*, 2002).

One such specific inoculum could be a LAB possessing antifungal properties in order to decrease fungal deterioration of silage and to improve aerobic storage stability.

1.2.14. Substances Produced by LAB:-

As mentioned above, LAB can produce a number of antimicrobial substances. Some of them (e.g. lactic acid and reuterin) inhibit both bacteria, yeasts and filamentous fungi, whereas others, such as the bacteriocins, only affect closely related bacteria.

Studies of the antifungal potential of LAB have identified a number of compounds with inhibitory effects against different mould and yeast species (Lavermicocca *et al*., 2000; Magnusson, 2003; Sjögren, 2005).

Apart from the actual inhibition of fungal growth, LAB can also specifically inhibit production of mycotoxins or immobilize mycotoxins through binding to their surface (El-Nezami *et al*., 2004).

They found a mixture of organic acids (acetic, caproic, formic, propionic, butyric and n-valeric acid) synergistically responsible for the inhibitory effect of this strain, where caproic acid seemed to be the most important. All these identified substances are low molecular weight compounds, but there are also reports of unidentified proteinaceous compounds with broad antifungal activity (Magnusson & Schnürer, 2001).

Studies on antifungal LAB reveal that the production of fungal inhibitory substances occurs among many different species. Species belonging to the genus *Lactobacillus* are reported in the majority of the studies. It is not known if this is due to the fact that they generally possess more antifungal capacity than other species, or whether it is due to a higher prevalence in the type of environments studied.

Antifungal LAB could probably be used in food or feed systems to enhance quality, for example by, reducing the use of chemical additives and preventing growth of spoilage yeasts and mycotoxigenic fungi (Wessels *et al*., 2004).

Substances that inhibit fungal growth are of importance both in the control of human and animal pathogens, and in the prevention of fungal growth in food and other materials. Besides the environmental, pH-decrease, there are other, probably more important effects of these compounds on fungal growth. General mechanism behind the inhibition is suggested to be due to the passage of undissociated acid molecules across the cell membrane. Once inside the cell, the higher pH of the cytoplasm will lead to the dissociation of acid. This will generate an accumulation of the anion of the acid together with protons, and consequently a decrease in the intracellular pH. In yeasts, lowering the pH has been shown to inhibit glycolysis (Krebs *et al.*, 1983). Thus, intracellular acidification directly affects growth.

Apart from the lowering of pH, other actions, such as anion accumulation and disordering of cell membrane structure have also been proposed as being responsible for the weak acid inhibition (Piper *et al.*, 2001). Cheng *et al.*, (1999) reported on the accumulation of trehalose and inhibition of macroautophagy (Hazan *et al.*, 2004), as well as aromatic amino acid uptake in *Saccharomyces cerevisiae* by weak organic acids (Bauer *et al.*, 2003).

Weak acid inhibition of filamentous fungi has not received as much attention as effects on yeast. However, Plumridge *et al.*, (2004) showed that sorbic acid causes intracellular acidification and inhibition of conidial germination and mycelia growth in *Aspergillus niger*.

Most studies of the effect of weak acids on fungal growth have established that a certain pH is necessary for the inhibitory action, whereby the acid is undissociated, leading to diffusion across the membrane. Moreover, the actual mode of action seems to be different, depending both on the type of organic acid and target organism. In conclusion, this group of compounds shares common features important for inhibition of fungal

growth, but some specific actions by different acids also occur.

Niku-Paavola *et al*. in 1999 reported the production of antimicrobial low-molecular-weight compounds other than organic acids from *Lactobacillus plantarum*. The active fraction containing, for example, benzoic acid, methylhydantoin, mevalonolactone, and cyclo-(glycyl-L-leucyl), and acting synergistically with lactic acid, against both *Fusarium avenacum* and the gram-negative bacterium *Pantoea agglomerans*.

3.1. Isolation and Identification of Fungi from Clinical Samples:-

Fifty scraping samples were collected from patients suffering from Tinea pedis. Microscopic examination illustrates that 26(52%) of them were infected with fungi. Further cultural and biochemical identification showed that 13(26%) isolates belonged to the molds *Trichophyton* spp. (Dermatophytic fungi), 8(16%) to *Trichophyton rubrunm* and 5(10%) to *Trichophyton mentagrophyt*, while 13(26%) isolates belonged to the yeasts *Candida***,** 7(14%) to *Candida albicans*, 3(6%) to *Candida tropicals***,** 2(4%) to *Candida parapsiolosis* and 1(2%) isolate to *Candida krusei*. Other molds found were 7(14%) isolates of *Aspergillus* spp and 3(6%) *Pencillium* spp, (as opportunistic fungi). The rest 14 samples showed no growth for any fungi which were considered as healthy. Table (3-1).

Table (3-1). Types of fungi isolated from patient with Tinea pedis infection.

Type of fungus	Male	Female	Total	Percentage%
Trichophyton mentagrophyte	3	2	5	10
Trichophyton rubram	5	3	8	16
Candida albicans	4	3	7	14
Candida tropicls	1	2	3	6
Candida krusei	/	1	1	2
Candida parapsilosis	1	1	2	4
Pencillium spp.	1	2	3	6
Aspergillus spp.	5	2	7	14
No. growth	6	8	14	28
Total	26	24	50	100

3.1.1:- Dermatophytic Molds Occurance:-

When samples that obtained by scraping the interdigital foot of suspected patients were put on sterile Petri dishes containing sabouraud dextrose agar modified with chloramphenicoal and cyclohexamide, also 13(26%) of the 50 samples were found to be positive for dermatophytic fungi. Direct KOH examination and biochemical tests listed in Table (3-2) below, confirmed that two Tinea pedis causative species of dermatographytic fungi were present; *Trichophyton rubrum* as the main species with 8 isolates (16%), and 5 isolates (10%) of *Trichophyton mentagrophyte*.

Table (3-2). Results of morphological and biochemical tests of Dermatophytic fungal isolates obtained from foot scraping samples.

isolates	Hair perforation	Growth at different temperature		Culture on PDA (red color produce)	Culture on corn meal agar (red color produce)	Urease test
		28	**37**			
T. rubrum	-	+	-	+	+	-
T. mentagrophyt	+	+	+	-	-	+

+ (Positive result) = growth appearance
- (Negative result) = no growth appearance

3.1.1.1. *Trichophyton rubrum*:

Upon culturing on Sabauroud dextrose agar, colonies of *Trichophytone rubrum* were slow-growing, white, and cottony to velvety with a radish to purple pigment on the reversed side of the colonies which spread into marginal hyphae and diameters reaching 4.5cm after 14 days of incubation at 30 ˚C (Fig. 3-1). They developed numerous micro conidia which were oval and born along only the sides of hyphae. Few macroconidia had cylindrical shapes with smooth-thin walls. After 7 days inoculation on urease medium, no change was observed on the color of the medium and dose not perforate hair when grown in hair culture in vitro, while, produce red pigment when grown on corn meal agar and on PDA medium. Table (3-2), (Koneman and Roberts, 1985).

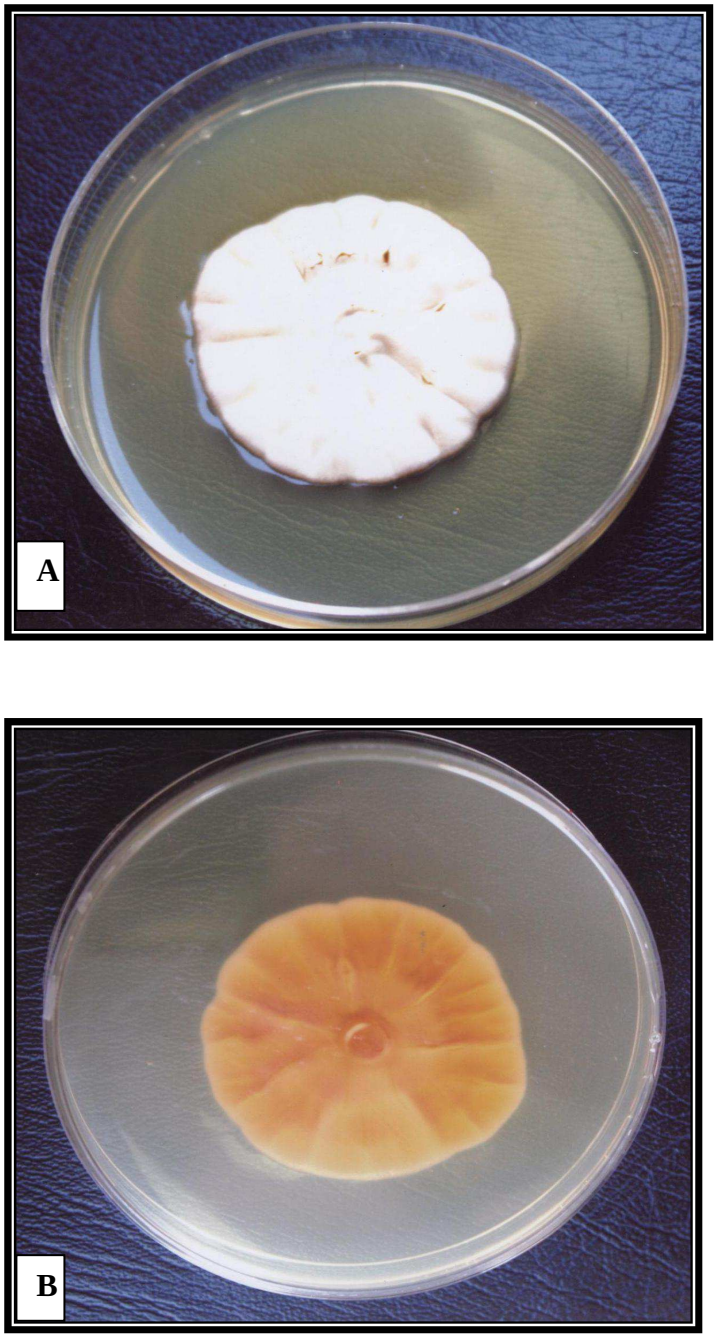

Figure (3-1): *Trichophyton rubrum* grown on SDA at 30°C for 7-14 days

A- Top view B- Reversed view

3.1.1.2. *Trichophyton mentagrophyte*: -

The colonies of *Trichophyton mentagrophytes* grown very quickly on (SDA) with diameter reaching 6 cm after two weeks of incubation at 30˚C. They were powdery to granular, light buff to rose-tan in color, and vary from a fluffy, cottony type to velvety and pure white. The reverse of the colonies was brown, (Fig.3-2). On corn meal agar the colonies were yellow. They were urease-positive within 7 days and also produce hair perforations when grown in hair cultures in vitro, Table (3-2).

Macroconidia were produced in few numbers and they were cylindrical with thin, smooth walls. While microconidia were spherical, abundant and born both in clusters along the hyphae, and spiral hyphae were often present. (Hironaga *et al.*, 1980).

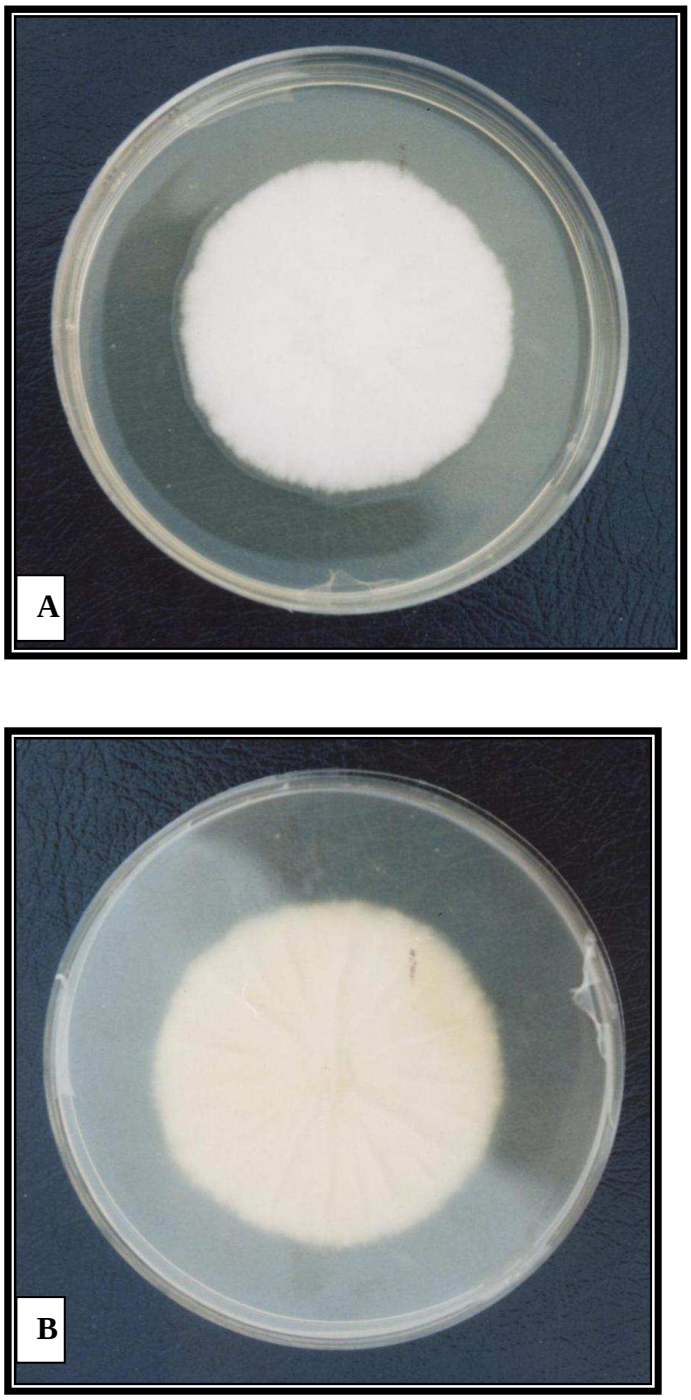

Figure (3-2): *Trichophyton mentagrophyte* grown on SDA at 30°C for 7-14 days

A- Top view B- Reversed view

3.1.2. Dermatophytic Yeasts Occurrence:-

3.1.2.1. Cultural examination:-

Cultural examination of the suspected isolates showed that their colonies appeared on (SDA) as white to creamy, glossy, smooth, soft and circular. Such characteristics come in accordance with those belonged to genus *Candida.*

3.1.2.2. Microscopic examination:-

After Gram staining of smears obtained from colonies grown on SDA, cells appeared under the oil-immersion objective as violet, oval having short extension shape, budding and spore former.

3.1.2.3. Biochemical Tests:-

Table (3-3) shown that the results of *Candida* isolates to the biochemical and morphological tests performed (surface growth on SDB, formation of germ tube when cultured in human serum at 2-4 hrs. and chlamydospore formation). Depending on such characteristics, and in accordance with Ping (2002**),** the following species of yeasts were detected: *Candida albicans*, *Candida tropicals*, *Candida krusei* and *Candida parapsiolosis*

Table (3-3). Morphological characteristics of *Candida* isolated from scraping samples.

Candida spp.	Surface growth	Germ-tube formation	Chlamydospore formation
C. albicans	-	+	+
C. tropicals	+	-	-
C. krusei	+++	-	-
C. parapsiolosis	-	-	-

+ = Slight growth +++ = Heavy growth - = No growth

Results of Table (3-4) describe the ability of *Candida* isolates to ferment (glucose, lactose, maltose and sucrose). While all four species were able to ferment glucose, none were to do so for lactose. Regarding maltose, only *Candida albicans* and *Candida tropicals* fermented it, while all, except *Candida albicans***,** can not ferment this sugar.

Table (3-4). Biochemical Tests.

Candida isolate	Glucose	Lactose	Maltose	Sucrose
C. albicans	+	-	+	-
C. tropicals	+	-	+	+
C. krusei	+	-	-	-
C. parapsiolosis	+	-	-	-

+ = Positive result - = Negative result

Candida albicans grew very quickly 1-2 days on SDA media, and its colonies appeared smooth, glossy, circular and white to creamy with specific yeast odor. It also produced germ-tube after 3 hr. While on corn meal agar containing tween 80, thick-walled chlamydospores singly or in clusters were

formed along or at the tip of pseudohyphae, and it can not able to produced surface growth on SDB Table (3-3). On the other hand, it was able to ferment (glucose and maltose) but not (lactose and sucrose) Table (3-4).

The colonies of *Candida tropicals* appeared white or creamy, soft and smooth, and when cultured on corn meal agar, it formed long and branched pseudohyphae bearing blastospores (singles, chain and clusters) at the septa, but was unable to produced chlamydospores, and germ-tube, also it produced surface growth on SDB Table (3-3). On the other hand, it was able to ferment (glucose, maltose and sucrose) but not lactose Table (3-4).

The colonies of *Candida krusi* were smooth, soft and white to creamy, while the cells possessed avoid, elongated or cylindrical shape. On corn meal agar, this species formed pseudohyphae with clusters of blastospores, but neither chlamydospores nor germ-tubes were produced on such medium. However, heavy surface growth was produced in SDB Table (3-3), in addition to ferment only glucose but not (lactose, maltose and sucrose), Table (3-4).

On SDA, the colonies of *Candida parapsiolosis* appeared smooth, soft and white to creamy, while it's were oval, elliptical and elongated. On corn meal agar, it formed pseudohyphae consisting of branched chains of elongated cells with clusters of blastospores at or near the septa. Also it was unable to produced germ-tube and surface growth Table (3-3), while in the sugar fermentation, it gave positive result for glucose only and negative for the others Table (3-4).

3.2. Distribution of Tinea pedis infection according to Age and Gender:-

The infection of tinea pedis is to be similar between male and female in the before teenage but it's to be more prevalence in male than female during and after teenage period (Perea *et al*., 2000). Results of Table (3-5) show that tinea pedis infection was more prevalent in males 20 patients than in females 16 patients. Despite that age group of 10-19 showed no difference between both sexes; infection was higher in male of age groups of 20-29; 30-39 and 50-59 year when the recorded numbers were 10; 3; and 3 for males compared to 8; 2; and 1 for females, respectively. Females were prevalence in only one age group 40-49 year with a very slight difference when their number was 3 compared to 2 for males.

Table (3-5). Distribution of Tinea pedis patients according to age and gender

Age-Group (year)	Infected Patients				Total
	Male	%	Female	%	
10-19	2	4	2	4	4
20-29	10	20	8	16	18
30-39	3	6	2	4	5
40-49	2	4	3	6	5
50-59	3	6	1	2	4
Total	20	40	16	32	36

The reason for higher occurrence of Tines pedis in males than females may be due to their using of occlusive foot ware for long time which leads moisture accumulation and warm environment between toes. Such conditions usually encourage the dermatophytic fungi to grow and cause the infection. In addition, males through more frequent attending the swimming pools, they may contact the infected towels , shores, and changing rooms that lead to increase their chances of infection. These results came almost in agreement with Martin (2002) when he found that the

disease was more commonly affects male than female due to swimming for long time. Rashid *et al.*, (2004) found that percentage of tinea pedis infection was generally higher in males due to wearing more occlusive and heavier shoes for long times during the year than females do. Results of Table (3-5) also show that age group of 30-39 contained the highest case of Tinea pedis compared the rest groups. However, Robbins, (2005) found that the prevalence of tinea pedis increased with age. Table (3-6) shows that the most important fungi which isolated from patients of tinea pedis were *Trichophyton rubum* and *Trichophyton mentagrophyte* at percentages of 16% and 10 %, respectively. This result agreed with that of lorimer *et al.*, (1997) when he found that the main microorganisms responsible for tinea pedis infections were *Trichophyton rubum* and *Trichophyton mentagrophyte*, and we found that the most common causative fungi of tinea pedis in the studying was *Trichophyton rubum*_and this result was agreed with Geary and Lucky (1999) .

Table (3-6). Percentage of occurrence of dermatophytic fungal isolates.

fungi isolate	Male	%	Female	%	Total	Percentage %
T. mentagrophyte	3	6	2	4	5	10
T. rubrum	5	10	3	6	8	16
Total	8	16	5	10	13	16

3.3. Antifungal Sensitivity of fungi: -

3.3.1. Determination of minimum inhibition Concentration (MIC):-

In this study, ketazole was used as an antifungal agent due to its remarkable inhibitory effect on *Trichophyton rubrum*, *Trichophyton mentagrophyte* and *Candida albicans* isolate (C1), which highly producing to the germ tubes. Table (3-7) shows that among the seven isolates of *Candida albicans***,** (C1) isolate was the highest producer of germ tubes from the budding cell with a number of ($75*10^{-4}$). For this reason, it was chosen for further studies.

Table (3-7):- Ability of *Candida albicans* isolates to form germ tube in human serum.

Candida albicans isolate	No. of germ tube formed
C. 1	75×10^4
C. 2	64×10^4
C. 3	17×10^4
C.4	52×10^4
C.5	42×10^4
C. 6	48×10^4
C. 7	20×10^4

The minimum inhibitory concentration (MIC) of ketazole were 64, 128 and 1024 µg/ml in SDB against (*Candida albicans*, *Trichophyton rubrum* and *Trichophyton mentagrophyte*) respectively. Ketazole was able to inhibit growth of dermatophytes fungi including *Trichophyton* spp. and *Candida albicans*. Daniel stated that there is a general agreement that it prevents the

synthesis of ergosterol (a major component of fungal plasma membrane) by inhibiting the cytochrome P-450-dependent enzyme lanosterol demethylase.

Upon fungal exposure to ketazole, depletion of ergosterol and accumulation of 14α-methylated sterols will take place. This interferes with the functions of ergosterol in fungal membranes and disrupts both the structure of the membrane and several of its functions, such as nutrient transport and chitin synthesis, leading to inhibit fungal growth (Daniel *et al.*, 1999). Same authors added that ketazole inhibit fungal growth by preventing 14-demethylation of lanosterol and effectively block synthesis of ergosterol. Also, some of these agents, at higher concentrations, destroy membranes directly, causing rapid killing. Depending on the former results, it can be concluded that *Candida albicans* yeast was more sensitive to the ketaconazole than *Trichophyton* spp. fungus. Inder and Davids (1985) obtained similar finding when they noticed that the antifungal activity of ketazole was better against yeast than fungi.

3.4. Inhibitory effect of LAB:-

3.4.1. On solid media:-

Studying the effect of LAB on fungi is complicated by the fact that fungi are sensitive to the normal by-products of LAB metabolism, like acetic and lactic acids.

Agar-diffusion method on SDA was used for detecting the effect of LAB on fungi. This method is usually used because antimicrobial synthesis is sometimes dependent on direct contact between the indicator and test strain (Rees, 1997). In this study, *Lactobacillus plantarum* and *Lactobacillus acidophilus* propagation on MRS agar were used against *Trichophyton*

rubrum, Trichophyton mentagrophyte and *Candida albicans*, previously propagated on SDA. No inhibition zone was observed when LAB isolated was propagated on MRS agar against the fungi isolate cultured previously on SDA. This result was agreed with that of Ress (1997) who found that agar diffusion method was less effective method in investigated the inhibitory effect of LAB on fungi because LAB may not be given sufficient time to express its antimicrobial activity and the action to replace the inverted agar tends, to smear the streak of the test culture.

Suzuki *et al.***,** (1991) stated that agar-diffusion method is ineffective in such regard because lactic acid bacteria when added, as starters, in cheese making will not inhibit the growth of contaminated fungi in the product due to it's fast growing that reaches maximum before fungi grow. Moreover, growth rate of molds such as *Trichophyton mentagophyte* and *Trichophyton rubrum* is usually slower than that of bacteria. So at the time that fungi growth after one week or more of incubation, grown bacteria in the infusion disc agar was loss its inhibition activity. This may be referred to the exhausting of the nutrient in the medium that will be lead to decrease LAB ability to exert its bioactivity and producing antifungal compounds.

3.4.2. In liquid Media:-

Well diffusion method was used to determine the inhibition activity of filtrates of *Lactobacillus acidophilus* and *Lactobacillus plantarum* grown for different incubation periods against fungi: *Trichophyton mentagrophyte*, *Trichophyton rubrum* and *Candida albicans* causing Tinea pedis. Unconcentrated filtrates of LAB isolates showed no inhibitory activity at all incubation periods 24, 48 and 72 hr against any of the three fungi tested.

Table (3-8) shows that at the time that one-fold concentrated filtrates activity against the tested fungi, all other folds of concentration exhibited such activity. In general, the two isolates of molds (*Trichophyton mentagrophyte and Trichophyton rubrum*) were more affected than the yeast isolates (*Candida albicans*). Moreover, as the filtrates of both LAB isolates (*Lactobacillus acidophilus* and *Lactobacillus plantarum*) be more concentrated, as their activity improved.

Table (3-8):- Effect of Concentrated filtrates of lactic acid bacteria on mold and yeast isolates grown on sabourade dextrose agar in order of inhibition zones (mm).

LAB isolate	Concentrated filtrate	Diameter of inhibition zones(mm)		
		T.mentagrophyte	*T. rubrum*	*C. albicans*
L. plantarum	One fold	-	-	-
	Two fold	١٦	١٠	-
	Three fold	١٨	١٧	١٠
	Four fold	٢٢	١٩	١٢
L. acidophilus	One fold	-	-	-
	Two fold	١١	١٠	-
	Three fold	١٦	١٥	-
	Four fold	٢١	١٧	١٠

Two-fold filtrate of *Lactobacillus acidophilus* gave inhibition zone diameters of 11 and 10 mm against *Trichophyton mentagrophyte* and *Trichophyton rubrum* isolates, respectively while, *Lactobacillus plantarum* showed noticeable inhibitory effects with zone diameters of 16 and 10mm against *Trichophyton mentagrophyte* and *Trichophyton rubrum* isolate (Fig. 3-3 and 3-4), respectively. Two-fold filtrates of both LAB isolates were unable to produce any inhibitory effect on *Candida albicans* (Fig. 3-5). Asimilar result was found by Ogunbanwo *et al.*, (2003) who reported that *Lactobacillus plantarum* and *Lactobacillus acidophilus* exhibited no inhibitory activity against *Candida albicans.*

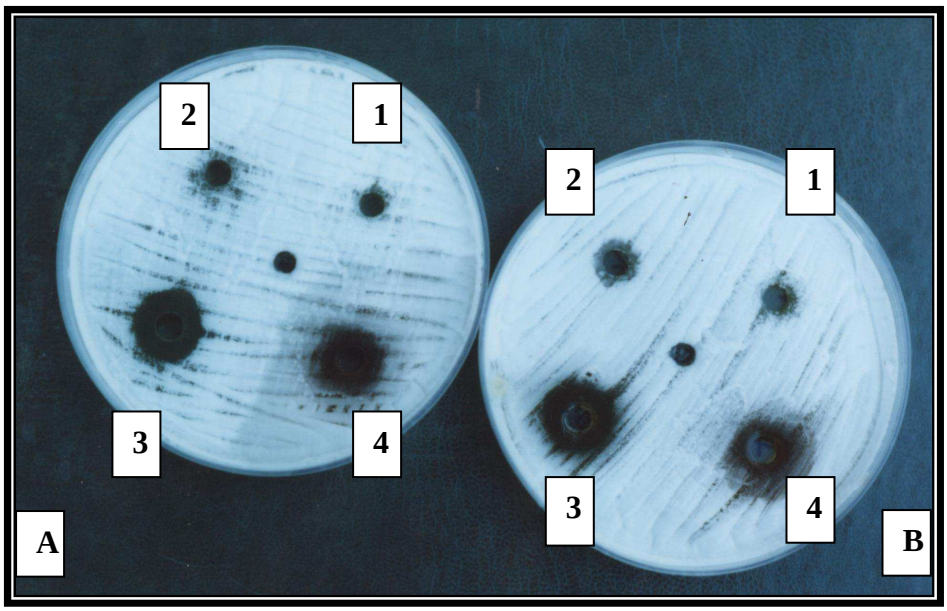

Figure (3-3): The effect of concentrated filtrate obtained from *L. acidophilus* and *L. plantarum* on *T. rubrum* grown on SDA at 25°C for 7-14 days.
(A), effect of *L. acidophilus* on *T. rubrum*, 1. (75%), 2. (50%), 3. (25%), 4. (12.5%).
(B), effect of *L. plantarum* on *T. rubrum*, 1. (75%), 2. (50%), 3. (25%), 4. (12.5%).

The three-fold filtrates of LAB isolates exhibited better inhibitory activity against fungal isolates after 24 hr incubation. Inhibition zones diameters of

the *Lactobacillus acidophilus* three-fold filtrate against *Trichophyton mentagrophyte* and *Trichophyton rubrum* isolates reached to 16 and 15 mm, respectively, without any activity on yeast isolate. Such filtrate of *L. plantarum* showed higher inhibitory activity against all three fungi tested with zone diameters of 18, 17 and 10 mm for *Trichophyton mentagrophyte*, *Trichophyton rubrum* and *Candida albicans*, respectively. The four-fold of *L. acidophilus* reached to 21, 17 and 10mm for *Trichophyton mentagrophyte*, *Trichophyton rubrum* and *Candida albicans*, respectively, while *Lactobacillus plantarum* gave higher activity with inhibition zone diameters of 22, 19, and 12 mm against *Trichophyton mentagrophyte* **,** *Trichophyton rubrum* and *Candida albicans*, respectively.

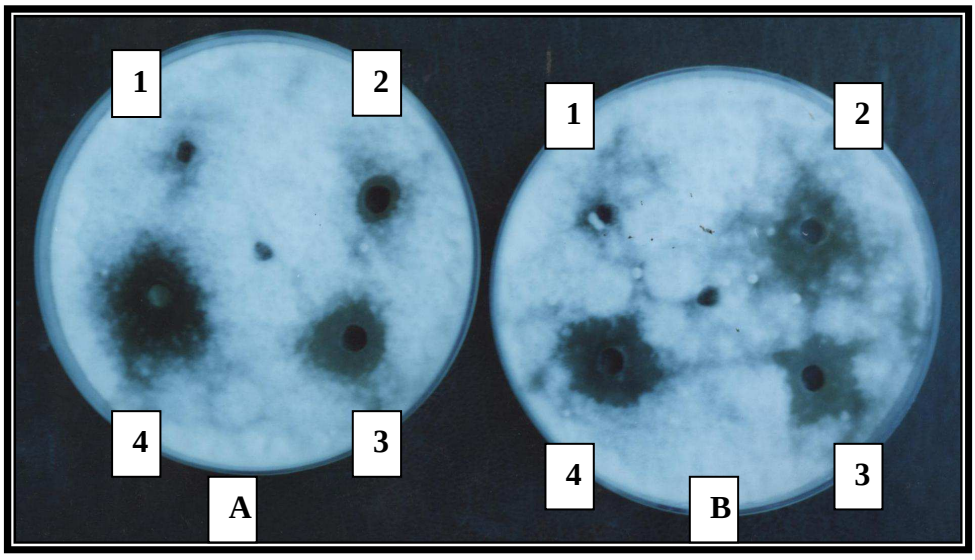

Figure (3-4): The effect of concentrated filtrate obtained from *L. acidophilus* and *L. plantarum* on *T. mentagrophyte*, grown on SDA at 25°C for 7-14 days.

(A), effect of *L.acidophilus* on *T. mentagrophyte*, 1. (75%), 2. (50%), 3. (25%), 4. (12.5%)

(B), effect of *L. plantarum* on *T. mentagrophyte*, 1. (75%), 2. (50%), 3. (25%), 4. (12.5%)

As illustrated in (figures 3-3, 3-4 and 3-5), LAB isolates were different in the intensity of their inhibitory activity against the fungal isolates used in this study. This may be related to specificity of compounds produced against fungal isolates (AL-Dulaimy, 2000).

From the above results, inhibitory activity of lactic acid bacteria filtrates was effective against molds than yeasts. Suzuki *et al*.**,** (1991), obtained similar finding when they noticed that the antifungal activity of lactic acid bacteria was better against molds than yeasts. Reasons for the good inhibitory activity of *Lactobacillus plantarum* may be due to its heterofermentative pattern which enable it to produce metabolites, like organic acid (formic, acetic, propionic, butyric) and CO_2, where fungi are very sensitive to them. Despite that the antifungal compounds of lactic acid bacteria vary, they are found mainly in the obligate heterofermentative *lactobacillus* spp.

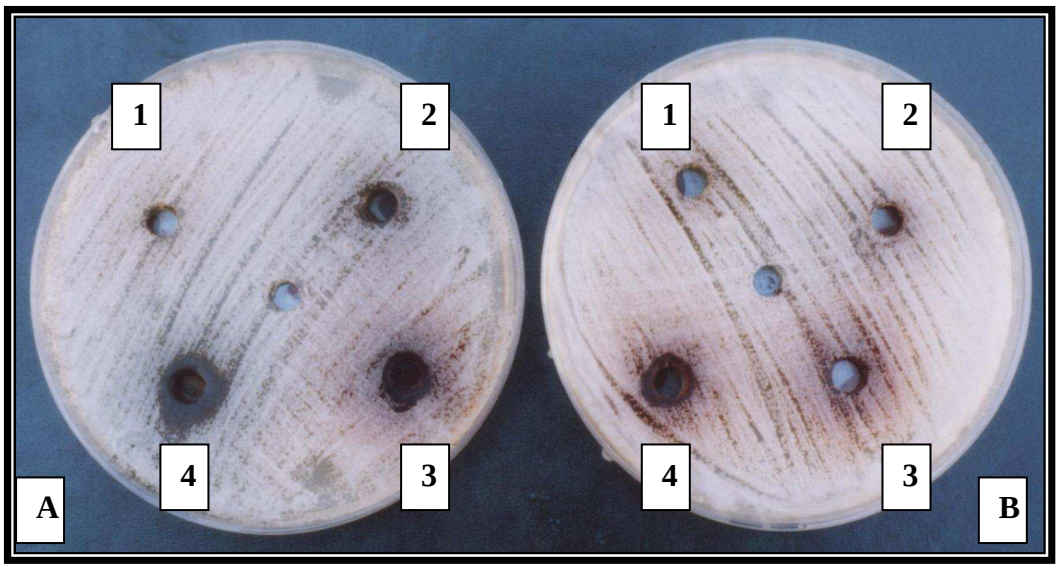

Figure (3-5): The effect of concentrated filtrate obtained from *L. acidophilus* and *L. plantarum* on *C. albicans*, grown on SDA at 30°C for 24-48 hr.
(A), effect of *L. plantarum* on *C. albicans* , 1. (75%), 2. (50%), 3. (25%), 4. (12.5%)
(B), effect of *L. acidophilus* on *C. albicans*, 1. (75%), 2. (50%), 3. (25%), 4. (12.5%)

Ström (2005) showed that all fungal growth can be inhibited by the presence of weak organic acids since, at a certain concentration, can lower the pH to a level where many fungi cannot grow. He added that lactic acid does not inhibit fungi, while acetic acid seems to be more strictly related to the antifungal activity. Depending on the former results, *Lactobacillus plantarum* was selected as the most effective isolate as a probiotic due its wide spectrum of inhibitory activity against fungal isolates causing Tinea pedis disease. (Lavermicocca et al., 2000).

3.5. Minimum inhibitory concentrations (MICs) of filtrates against fungi: -

Minimum inhibitory concentration (MIC) was determined applying serial ratios from the four-fold concentrated filtrates of *Lactobacillus acidophilus* and *lactobacillus plantarum* against the three fungal isolates. Regarding filtrates of *Lactobacillus acidophilus***,** results in Table (3-9) declare that ratios 1:9 and 2:8 had no observed effect against *Trichophyton mentagrophyte* and *Candida albicans* isolates when heavy growth of these fungi was noticed after 7 days (for molds) and 24 hr (for yeast) of incubation, while *Trichophyton rubrum* growth decreased to the medium level at these concentrations.

Table (3-9): Minimum Inhibitory concentration of concentration filtrates of *L. acidophilus* against fungi isolates.

Isolates	Concentration of filtrate: (Concentration : Medium)									
	0:10	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
C. albicans	+++	+++	+++	++	+	-	-	-	-	-
T. rubrum	+++	+++	++	+	+	-	-	-	-	-
T.mentagrophyte	+++	+++	+++	++	++	+	+	-	-	-

+++ = Heavy growth ++ = Medium growth + = Light growth - = No growth

Coming next, ratios 3:7 and 4:6 which decreased *Trichophyton mentagrophyte* growth to the medium level ++, but growth of *Candida albicans* decreased to the medium level at ratio of 3:7. Sharp decrease in growth (to the light level) was record for *Candida albicans* and *Trichophyton rubrum* at ratios of 3:7 and 4:6. With the ratio of 5:5, the situation was different when no any growth was observed for *Candida albicans* and *Trichophyton rubrum.* On the other hand, light level growth was observed for *Trichophyton mentagrophyte* at ratios 5:5 and 6:4. The last three ratios of *Lactobacillus acidophilus* 7:3, 8:2, 9:1 were quite enough to retard any growth of fungi isolates due to the same above mentioned reasons. It may be concluded that filtrate ratio of 4:6 is the MIC for *Candida albicans* and *Trichophyton rubrum*, that of 4:6 for *Trichophyton mentagrophyte*.

Regarding filtrates of *Lactobacillus plantarum***,** result in table (3-10) declare that ratios 1:9 and 2:8 had no observed effect against *Trichophyton mentagrophyte* isolate when heavy growth of this fungi was noticed after 7

days of incubation. While *Candida albicans* and *Trichophyton rubrum* growth decreased to the medium level at these concentrations.

Table (3-10): Minimum Inhibitory concentration of concentration filtrates of *Lactobacillus plantarum* against fungi isolates.

Isolates	Concentration of filtrate: (Concentration : Medium)									
	O:10	1:9	2:8	3:7	4:6	5:5	6:4	7:3	8:2	9:1
C. albicans	+++	+++	++	++	+	-	-	-	-	-
T. rubrum	+++	+++	++	+	-	-	-	-	-	-
T. mentagropyte	+++	+++	+++	++	++	+	+	-	-	-

+++ = Heavy growth ++ = Medium growth + = Light growth - = No growth

Coming next, ratios 3:7 and 4:6 which decreased *Trichophyton mentagrophyte* growth to the medium level ++, but growth of *Candida albicans* decreased to the medium level at ratios of 2:8 and 3:7.

Sharp decrease in growth (to the light level) was recorded for *Trichophyton rubrum* at ratio of 3:7; while *Candida albicans* at ratio of 4:6. With the ratio of 4:6, the situation was different when no any growth was observed for *Trichophyton rubrum*, while *Candida albicans* was observed no growth at ratio of 5:5. On the other hand, light level growth was observed for *Trichophyton mentagrophyte* at ratios 5:5 and 4:6. The last three ratios of *Lactobacillus plantarum* 7:٣, 8:2, 9:1 were quite enough to retard any growth of fungi isolates relating to the just mentioned finding.

It may be concluded that filtrate ratio 3:7 is the MIC for *Trichophyton rubrum*, while the MIC of *Candida albicans* at ratio 4:6, and 6:4 ratio for *Trichophyton mentagrophyte*. Similar results were recoded by Lavermicocca

et al., (2000) who found that the MIC of lactic acid bacteria concentrated filters were ranging from 50 to 166 mg /ml with various fungi.

Ström ***et al***., (2002) found that the MIC of LAB required to retard any growth of fungi was at 40% and 60% concentration.

2.1. Materials

2.1.1. Equipment and Apparatus:-

The following equipment and apparatus were used throughout the study:-

Equipment	Company – Country
Autoclave	GallenKamp-England
Anaerobic Jar	Rod well-England
Distillator	GallenKamp
Incubator	GallenKamp
Compound Light Microscope	Olympus-Japan
Micropipette	Witeg-Germany
Millipore Filters	Millipore and Whattman - England
Oven	Memmert-Germany
Pasture Pipette	Witeg
pH-meter	Meter-Gmp H Tdedo-England
Refrigerated Centrifuge	Harrier-Germany
Sensitive Balance	Delta Range-Switzerland
Shaking Incubator	GallenKamp
Spectrophotometer	Aurora Instruments Ltd-England
Vortex	Buchi-Sweden
Water Bath	GFI-England

2.1.2. Chemicals:-

Material	Company-Country
Peptone Glucose $MgSO_4.7H_2O$ Sodium acetate trihydrate Phenol red Sucrose Maltose Dextrose Glycerol Lactose Lactic acid Phenol crystals KOH Hydrogen peroxide 10%	BDH-England
Gelatin Tween-80 Meat extract Urea agar base Sabouraud dextrose agar	Oxoid-England
Ethanol $MnSO_4.4H_2O$ Sodium chloride Triammonium citrate	Riedel-DeHaen-Germany
Agar-Agar Corn meal agar Beef extract	Difco-England

Material	Company-Country
Yeast extract	**Biolife-Italy**
Nutrient agar	

2.1.3. Antimicrobial:-

Antibiotic	Company-Country
Cycloheximide	Fluka-Switzerland
Chloramphenicol	Troge-Germany
ketazole	Medipharm-Syria

2.1.4. Solutions, Stains and Reagents:-

2.1.4.1. Physiological saline solution (NaCl):-

It was prepared according to Atlas *et al*., (1995) by dissolving 8.5 g of NaCl in 950 ml of distilled water. After pH was adjusting to 7, it was sterilized by the autoclaving.

2.1.4.2. Potassium hydroxide-glycerin solution KOH (10%):-

It was prepared according to Emmons *et al*., (1977) by dissolving 10 g of KOH and 10 ml of glycerin in 80 ml of distilled water. It was used for microscopic detection of fungi in clinical specimens.

2.1.4.3. Chloramphincol stock solution:-

It was prepared by dissolving of 100 mg of chloramphenicol in 10 ml distilled water (AL-Janabi, 1999).

2.1.4.4. Cychloheximide stock solution:-

It was prepared by dissolving 100 mg of Cychloheximide in 3 ml absolute ethanol, and then the volume was adjusted to 10 ml by sterile distilled water. (Frazier, 1985).

2.1.4.5. Sugars stock solutions:

Sugars stock solutions were prepared by dissolving 2 g of glucose, lactose, maltose and sucrose in 100 ml of distilled water, and then it was sterilized by autoclaving.

2.1.4.6. Ketazole stock solution:-

It was prepared by dissolving of 200 mg of ketazole in 10 ml of sterile distilled water. Then the solution was sterilized by filtration.

2.1.4.7. Phenol red reagent:

It was prepared by dissolving 0.1 g of phenol red in 10 ml of distilled water.

2.1.4.8. Lactophenol cotton blue stain:

It was prepared according to Ellis (1994) from the following ingredients:-

Cotton Blue (Aniline Blue)	0.05 g
Phenol	20 g
Glycerol	40 ml
Lactic acid	20 ml
Distilled water	20 ml

By:-

- Dissolving the cotton blue in the distilled water. Then left to stand overnight to eliminate insoluble dye.
- The phenol crystals was added to the lactic acid in glass beaker, and with the aid of magnetic stirrer, the phenol was dissolved, then Glycerol was added.
- The cotton blue was filtered into the (phenol – glycerol – lactic acid) solution.
- After mixing, it was stored at room temperature to be used for staining and microscopic identification of fungi.

2.1.5. Culture Media:

2.1.5.1. Ready-to use (Powdered) media:

The following media were used, and prepared according to the instructions listed on their containers. After pH was adjusted, they were sterilized by autoclaving :- Nutrient agar, Yeast extract, Beef extract, Cornmeal agar, Peptone, Agar-Agar, Sabouraud dextrose agar and Urea agar base.

2.1.5.2. Laboratory-prepared media:

2.1.5.2.1. Sabouraud Dextrose Broth medium (SDB):

(Buffo *et al.* **1984):-**

(SDB) contain:-

Peptone	10 g
Dextrose	20 g
D.W.	90 ml

It was prepared by dissolving this compound in distilled water and the pH was adjusted to 7, then it was sterilized by autoclaving. This medium was used for growth and identification of fungi.

2.1.5.2.2. Sabouraud Dextrose agar with Antibiotic (SDA): (Jong, 1981).

(SDA) contain:

Peptone	10 g
Dextrose	20 g
Agar	15 g
D.W.	950 ml

It was prepared by dissolving these compounds in distilled water and the pH was adjusted to 7, after autoclaving the medium was supplemented with chloromphenical and cycloheximide was added at concentrations of 0.04 mg/ml and 0.5 mg/ml, respectively. This medium was used for primary isolation of dermatophytes and it was used as selective media for dimorphic fungi and dermatophytes.

2.1.5.2.3. Potato Dextrose agar (PDA) medium: (Pitt and Hocking, 1997)

Potato Dextrose agar contains:-

Potato infusion	200 g
Dextrose	20 g
Agar	15 g
D.W.	950 ml

It was prepared by dissolving these compounds in distilled water and the pH was adjusted to 6.3, and then sterilized by autoclaving. This medium was used for growth and identification of filamentous fungi.

2.1.5.2.4. Corn-Meal Agar with Dextrose medium: (Kane and Summerbell, 1999)

Corn – Meal agar	17 g
Dextrose	10 g
D.W.	950 ml

It was prepared by dissolving these compounds in distilled water and the pH was adjusted to 7, and then sterilized by autoclaving. This medium was used for differentiation between *Trichophyton mentagrophyte* and *Trichophyton rubrum*

2.1.5.2.5. Urea Agar medium: (Marshall *et al.*, 1984)

It was prepared by adding 5 ml of sterile 40 % of urea solution to 95 ml of a cool sterile urea agar base at 50 ºC. The medium mixed well, 5ml aliquots were dispensed into sterile test tubes and left to solidify as slants, then kept tightly closed in a cool dry place. This medium was used for urease production.

2.1.5.2.6. Gelatin media: (Baron and Finegold, 1990)

It was prepared by dissolving of 12% w/v of gelatin in MRS broth. After adjusting the pH to 6, the medium was sterilized by autoclaving. This media was used for identification of *Lactobacillus* spp.

2.1.5.2.7. Corn-Meal Agar with 80% Tween medium: (Forbes *et al*., 1998).

Corn-Meal Agar	**17 g**
Tween	**10 ml**
D.W.	**950 ml**

After adjusting the pH to 7, the medium was sterilized by autoclaving. This medium was used for morphological identification of yeast.

2.1.5.2.8. Fermentation basal medium: (Hassan *et al*., 2004)

Yeast extract	**5 g**
Peptone	**15 g**
Phenol red indicator	**10 ml**
D.W.	**950 ml**

It was prepared by dissolving 2% of sugars (glucose, lactose, maltose and sucrose) in the fermentation basal medium; pH was adjusted to 7 and

sterilized by autoclaving. This media was used for identification of Candida spp.

2.1.5.2.9. DeMan Rogosa Sharp broth medium (Harrigan and McCance, 1976) (MRS):

DeMan Rogosa Sharp broth contains:

Yeast extract	**5 g**
Peptone	**10 g**
Meat extract	**10 g**
D. Glucose	**20 g**
Sodium acetate trihydrate	**5 g**
Triammonium citrate	**2 g**
Mg SO4.7H2O	**0.2 g**
MnSO4.4H2O	**0.5 g**
Tween 80	**1 ml**
D.W.	**950 ml**

It was prepared by dissolving these compounds in distilled water and the pH was adjusted to 6.0 then it was sterilized by autoclaving. This media was used for growth of *Lactobacillus* spp.

2.1.6. Normal human plasma (Oliver *et al*., 1982)

Human plasma obtained from single donor was taken from Blood Bank center of Alyarmouk hospital in Baghdad, sterilized by filtration and kept frozen at -20 ºC. Used for germ tube formation.

2.1.7. Bacterial Isolates:-

Isolate	Source
Lactobacillus plantarum	Biotechnology Department Al-Naharin University
Lactobacillus acidophillus	

2.1.8. Sterilization Methods:-

2.1.8.1. Moist-Heat Sterilization:-

Media and solutions were sterilized by autoclaving at 121 °C (15 lb / in^2) for 15 min.

2.1.8.2. Dry-Heat sterilization:-

Electrical oven was used to sterilize glassware at 180 °C for 2 hr.

2.1.8.3. Membrane Sterilization (Filtration):-

Millipore filters 0.22 μm in diameter were used to sterilize the filtrates of lactic acid bacterial cultures (Probiotics), ketazole (antifungal) and sugar solutions.

2.2. Methods:-

2.2.1. Samples collection:

Samples were collected from 50 patients (18 to 66 years old of both sexes) suffering from Tinea pedis, (as clinically identified by a physician), during the period of November/2005 to March/2006 from the Medicale City

and College of physical Education in Baghdad governorate. The samples were obtained by scraping the infected area of skin with unsharped blade, and examining under microscopic KOH and culturing on the sabouraud dextrose agar.

2.2.2. Microscopic Examination:-

Specimen were placed on a microscope slide, and few drops of 10% KOH were put on the specimen, and then a cover slip was applied and warming over a small flame just before boiling because that leads to precipitates KOH crystals. The slide was examined under the low power and high dry objectives to detect fungi and their septet hyphae and/or barrel-shaped or rounded arthrospores (Emmons *et al*., 1977).

2.2.3. Samples Culturing:

Scraped samples were cultured on sabouraud's dextrose agar (SDA) supplemented with 0.05 mg/ml cycloheximide and 0.04 mg/ml chloramphenicole (to inhibit the growth of saprophytic fungi and bacteria), then incubated at 30 °C (for molds) and 37 °C (for pathogenic yeast) and examined every 4 to 7 day for at least 4 weeks before it was discarded. (Midgley *et al*., 1997).

2.2.4. Identification of Molds:

2.2.4.1. Scotch tape preparation:-

A small piece of transparent-adhered tape was touched to the surface of the suspected colony, and then adhered to the surface of a microscope slide to which a drop of lactophenol cotton blue was added. Shape and

arrangement of the spores were examined under microscopically. (Jawetz *et al*., 1991; Forbes *et al*., 1998).

2.2.4.2. Hair Perforation test:-

Hair from healthy humans was cut into short pecies (approximately 15-20 mm) and divided into two portions to be autoclaved in glass Petri plates (10 to 20 hair pecies per plates). After the plates were cooled, 25 ml of sterile distilled water and 3 drops of sterile yeast extract solution 10% were aseptically added to each plate.

A small portion of growth for 14 days old culture of mold isolate was added to one plate, and fragments from a similar culture of another mold isolate were added to the other plates. The plates were then incubated at 27 °C for 20 days. Hair segments and associated fungal hyphae were removed at 5 days intervals, mounted in lactophenol cotton blue, and examined microscopically for the presence or absence of perforation (Deshmukh and Agrawal, 1983; Fathi and AL- samarai, 2000).

2.2.4.3. Urease test:-

This test, which differentiates between the dermatophyte isolates, was performed Inoculating surface of the urea agar slant with the fungal isolate, then incubated at 28 °C for 3-7 days.

After incubation, pink-red color was considered as positive result, and yellow-orange color as negative. (Baron *etal*., 1994; Kane and Summerbell, 1999).

2.2.4.4. Culturing on Potato Dextrose Agar (PDA):-

This test, which distinguishes between dermatophyte isolates, was done by culturing part of the fungal colony on the PDA and incubated at 28 °C for 7 days. Indicate positive result by red stain production. (Collee *et al.*, 1996).

2.2.4.5. Culturing on Corn Meal Agar with Dextrose:-

This test, which distinguishes between dermatophyte isolates, was done by culturing part of the fungal colony on the corn meal agar media supplemented with 1% dextrose and incubated at 28 °C for 7 days. Indicate positive result by red color production. (Forbes *et al*., 1998; Kane and Summerbell, 1999).

2.2.4.6. Growth at 28 ºC and 37 ºC:-

A culture of mold isolates were incubated at two incubation temperatures (28ºC and 37ºC) for 14 days. Appearance of fungal growth was recorded as positive results (Kane and Summerbell, 1999).

2.2.5. Preparing *T. mentagrophyte* and *T. rubrum* spore suspension:

After the isolates were cultured on potato dextrose agar at 25 °C for 6-10 days, fungal colonies were covered with 10 ml of sterile saline solution, and the suspensions were made by gently rubbing of the surface with the tip of a pasture pipette. The mixture of conidia and hyphae fragments was filtered through sterile gauze, and then the filtration was transferred to a sterile test tube. Inoculums quantification was made by counting the spores using

haemocytometer by added one drop of the suspension to haemocytometer by Pasteur pipette, spores were calculated under high power 40X of light microscope using the following Equation: - (Faraj, 1990).

Concentration of spores = (Z x 4 x 10^6) /n spores / ml

Where n: total No. of small squares

Z: total No. of spores.

The concentration of spores which was modified to 1.5×10^6 spore/ml for *Trichophyton rubrum* and 1.8×10^6 spore/ml for *Trichophyton mentogrophyte*. The spore suspension was stored in the refrigerator for 4 weeks (Santos and Hamdan, 2005).

2.2.6. Identification of Yeast:-

2.2.6.1. Morphology of colony:-

After cultivation of suspected isolates on SDA and incubated at 37 °C for 48 hr, morphology of colonies was examined and described according to (Savage and Balish, 1971) as follows:-

2.2.6.2. Microscopic examination:-

Part of the colony was fixed on a glass slide, stained by gram's stain, then cell were examined under oil-immersion lens of a compound light microscope (Kreger-van, 1984).

2.2.6.3. Germ tube formation test:-

Formation of germ tube was examined by adding 1 ml of human serum in a sterile test tube and resuspended with part of yeast colony then incubated at 37 ºC for 2-4 hrs. After incubation, a drop from the suspension was put by a pasture pipette on a slide. Result was recorded under light

microscope by observing germ tube formation (Evans and Richardson, 1989).

2.2.6.4. Chlamydospores formation test:-

Yeast isolates were cultured on corn-meal agar with tween 80 and incubated at 37 °C for 5-7 days. Presence of round blastoconidia bunched together with pseudohyphae or true hypha under light microscope indicates positive result for yeast identification. (Barnett *et al*., 2000).

2.2.6.5. Surface growth:-

This test was to detect the ability of yeast isolate to grow on surface of SDB by transferring part of a colony to tubes contained this medium. After incubation at 37 °C for 25-72 hr, presence of gas bubbles indicates appositive result. (Forbes *et al*., 1998).

2.2.6.6. Sugar fermentation test:-

This test was used for detecting the ability of *Candida* spp. to ferment certain sugars (glucose, sucrose, lactose and maltose) and production of CO_2. Yeast isolates were incubated in the fermentation basal media contained 2% of each sugar in test tubes at 37°C for 48-72 hr. Positive result was recorded by changing the color of medium and production of Co_2 gas bubbles. (Forbes *et al*., 1998).

2.2.7. Preservation of *Candida* spp:-

Yeast isolates were inoculated on SDA slants contained 0.5 mg/ml chloramphenical. After incubation at 37 °C for 48 hr, the slants were placed

in 4 °C as stock cultures. These slants were subcultured every three months (Oliver *et al*., 1982).

2.2.8. Lactic Acid Bacteria (LAB):-

2.2.8.1. Identification of (LAB):-

2.2.8.1.1. Microscope Examination:-

A loop full of LAB culture was fixed on a microscopic slide, then stained by Gram stain to examine cells shape, grouping, gram reaction and non-spore forming (Kandler, 1986).

2.2.8.1.2. Catalase test:

This test was performed by adding few drops of H_2O_2 3% on a single bacterial colony grown on MRS agar. Production of gaseous bubbles indicates a positive result. (Baily *et al*., 1990).

2.2.8.1.3. Gelatin Hydrolysis test (Harely and Prescott, 1996):

Tube containing gelatin medium was inoculated with isolate culture by stabbing and then incubated at 30 ºC for 5 days. This test was performed to demonstrate the ability of bacteria to hydrolyze gelatin.

2.2.8.2. Maintenance of Lactic Acid Bacteria:-

2.2.8.2.1. Daily working cultures:-

After inoculating MRS broth with LAB culture, it was incubated anaerobically at 37 °C for 24 hr. then the cultures stored at ~ -4 ºC in the refrigerator. (Baron *et al*., 1994).

2.2.8.2.2. Stock Cultures:-

Screw cup tubes containing MRS broth with 10 ml of 20% glycerol were inoculated with LAB isolates, and incubated at 37 °C for 24 hr. under anaerobic condition, then kept at -20 ºC in the freezer. (Contreras *et al*., 1991).

2.2.9. Sensitivity of fungi to antifungal Ketazole and its minimum inhibition concentration (MIC):-

Ten ml of SDB was inoculated by yeast isolate and incubated at 37 °C for 24-48 hr. after incubation, stationary phase was determined by spectrophotometer 20 through estimating the optical density (O.D.$_{490}$) which was fixed to 1.2.Then serial dilution were made for yeast to obtain 10^5 cell/ml and mold isolate was prepared according to (2.2.5). On the other hand, different concentrations (4, 8, 16, 32, 64, 128, 256, 512, 1024, 2048 µg/ml) were prepared from Ketazole (as antifungal) stock solution in test tubes in which each contained 5 ml of SDB medium.

A portion of 0.05 ml of each of fungal and yeast culture was added to each tubes, then incubated at 25°C for 7-10 days (for mold), and 37°C for 48 hr. (for yeast). Antifungal activity of ketazole on the mold and yeast isolates was determined. The suitable concentration was cultured on SDA. After incubation period (at 25°C for 7-10 days for mold isolate and 37°C for 48 hr. for yeast isolate) the result was recorded and compared with control (SDB with fungi) to determine the minimum inhibitory concentration of Ketazole (NCCL, 2002; Santos and Hamdon, 2004).

2.2.10. Determination of inhibitory Effect of (LAB):-

2.2.10.1. On solid medium (MRS Agar):-

Lactic acid bacteria (LAB) was cultured on MRS broth and incubated at 37 °C for 24 hr. (Piard *et al*., 1992). Then, LAB was streaked on MRS agar by the spreader and incubated under anaerobic condition at 37 °C for (48) hrs. Agar disc diffusion method was used to determine the antimicrobial activities of LAB on yeast isolate and mold isolate. After incubation, and with the aid of cork porer 5 mm, discs of the grown culture were put on the surface of SDA which was previously inoculated with 0.1 ml of mold isolate and yeast isolate, then incubated at 25°C for 7-10 days (for molds) and 37°C for 24-48 hr (for yeast). After that, the diameters of inhibition zone a round the discs were measured in mm. (Bektas *et al*., 2004).

2.2.10.2. In Liquid media (MRS broth):-

Lactic acid bacteria was inoculated in MRS- broth and incubated at 37 °C for 24, 48 and 72 hrs. After incubation, cell-free supernatant was obtained after centrifugation at 3000 rpm for 15 min and filtered through 0.22 Millipore filter (piard *et al*., 1990; Sreekumar and Hosono, 1999).Well diffusion method was used for detect inhibitory effect of cells- free filtrates of LAB on fungal isolates. For this purpose, 0.1 ml of each isolate of mold isolate and yeast isolate was spreader on the surface of plates containing solidified SDA by a sterile spreader (Cupta *et al*., 1998). With the aid of a cork porer 5 mm, wells were made in agar and filled by filtrate of LAB. Then incubated at 30 °C for 24-48 hr (for yeast) and 25 °C for 7-14 days (for molds). Diameters of inhibition zones around the wells were measured in millimeter and compared with control, which contain MRS broth without bacteria (Bektas *et al*., 2004).

Filtrates of LAB isolates were concentrated by the freeze-dryer. Four concentrations were prepared for the isolates after inoculating 0.1 ml of each in a volume of 100 ml of MRS broth and incubated under the above conditions. One-fold concentrated filtrate was made by reducing the volume to 75, two-fold to 50 ml, three-fold to 25ml and four-fold to 12.5ml. The inhibitory activity of each fold of the filtrates was tested against the fungal isolates as illustrated in the unconcentrated filtrates.

2.2.11. MIC Determination of LAB filtrates on Fungal isolates:

A portion of 10 ml of each different ratios of filtrate: SDB medium (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, and 9:1) were prepared in test tubes from the four fold concentrated filtrates of *Lactobacillus acidophilus* and *Lactobacillus plantarum.* They were inoculated with 0.1 ml of mold isolate and yeast isolate cultures, then incubated at 25 ºC for 7-14 days (for molds) and 37 ºC for 24 hr (for yeast). Growth intensity of each tube was observed and recorded as; slight (+), medium (++), heavy (+++) and no growth (-). (Midolo *et al*., 1995).

4.1. Conclusion:

1**.** Three species of fungi were isolated from Tinea pedis patients including: *Trichophyton rubrum, Trichophyton mentagrophyte* and *Candida albicans*.

2. Tinea pedis was more prevalence in males than females.

3. Unconcentrated filtrate of lactic acid bacteria (LAB) had no observable inhibitory effect against any of the isolated fungi.

4. Generally, molds isolates were more affected by concentrated LAB filtrates than the yeast isolates.

5. Concentrated filtrates of the LAB isolates exhibited more inhibitory effect on molds isolates of *Trichophyton* spp. than did the antifungal agent (ketazole), while yeast isolates of *Candida albicans* were more affected by ketazole than by the filtrates.

4.2. Recommendations:

1. Determination the effect of LAB on fungal isolates in vivo.

2. More studies are required on the extraction, purification and identification of inhibitory substance and metabolites produced by LAB isolates to be used as probiotic.

3. Investigation the mechanisms of action of LAB against fungi causing tinea pedis.

Dedication

To the great Allah for all his giftness

To my lovely……mother

To my dearest……father

To my aunt……Alia

To my lovely……husband

To my dearest……brothers and sisters

I dedicated this Work

Marwa

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List of Abbreviations

Abbreviation	Mean
HIV	Human Immunodeficiency Viruse
LAB	Lactic Acid Bacteria
MRS	DeMan Regosa Sharpe
MICs	Minimum Inhibition Concentration
PDA	Potato Dextrose Agar
SDB	Sabouraud Dextrose broth
SDA	Sabouraud Dextrose Agar

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Thesis Name: Effect of Lactic Acid Bacteria as Probiotics on Locally Isolated Fungi Causing Tinea Pedis

Date: 6/5/2007

الاسم: مروة مشتاق صالح
العنوان: العامرية – شارع العمل الشعبي
الهاتف: لايوجد
اسم الاطروحة: تاثير بكتريا اللاكتيك على عزلات محلية من الفطريات المسببة لمرض سعفة القدم
يوم المناقشة: ٢٠٠٧\٥\١٦

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Web Site:

1. **Web site 1: Aquatic Pathogenes.**

   WWW. Env. Gov. bc. Ca/wat/wa/reference/fungi. Ht ml.

2. **Web site 2: Tinea Pedis.**

   WWW. Continuing Education. Com/pharmacy/foot/intro. Ht ml.

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١. الدليمي، جيهان عبد الستار سلمان (٢٠٠٠). استخدام الكحول الاثيلي لعزل بكتريا حامض اللاكتيك ودراسة تأثيرها التازري مع خميرة الخبز ضد بعض انواع البكتريا رسالة ماجستير- جامعة بغداد- كلية الزرعة.

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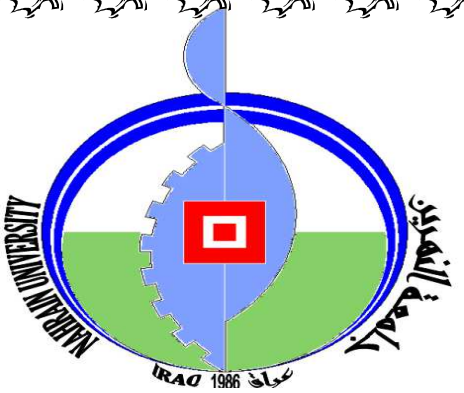

Effect of Lactic Acid Bacteria as Probiotics on Locally Isolated Fungi Causing Tinea Pedis

A thesis
Submitted to the College of Science /AL-Nahrain University
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Master Science in Biotechnology

By

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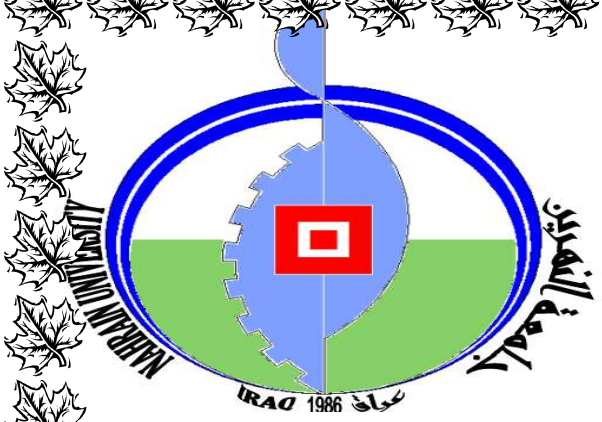

جمهورية العراق

وزارة التعليم العالي والبحث العلمي

جامعة النهرين

كلية العلوم

تاثير بكتريا اللاكتيك على عزلات محلية من الفطريات المسببة لمرض سعفة القدم

رسالة
مقدمه إلى كلية العلوم جامعة النهرين
وهي جزء من متطلبات نيل درجة ماجستير علوم في التقانه الاحيائيه

من قبل

مروة مشتاق صالح
بكالوريوس تقانه احيائية/ جامعة النهرين(٢٠٠٤)

جماد الاولى ١٤٢٨

ايار ٢٠٠٧

Summary

This work aimed to study the effect of some probiotic microorganisms on fungi causing tinea pedis disease. Total of 50 scraped samples were collected patients (18 to 66 years old of both sexes) suffering from Tinea pedis, during the period of November/2005 to March/2006 from the Medicale City and College of physical Education in Baghdad governorate. After subjecting samples to cultural, microscopic and biochemical tests, the following causative fungi were identified; *Trichophyton rubrum* 8(16%), *Trichophyton mentagrophyte* 5(10%) and 7(14%) of isolates were belong to *Candida albicans.* Other identified species of *Candida* were: - *Candida tropicals, Candida krusi* and *Candida parapsiolosis*. Minimum inhibitory concentration of ketazole against *Candida albicans, Trichophyton rubrum* and *Trichophyton mentagrophyte* were (64, 128, 1024) µg/ml, respectively.

Upon such results, one isolate of *Candida albicans* (C1) was chosen due to its ability to form high number of germ tube among others isolates. On the other hand, two isolates of lactic acid bacteria (LAB): *Lactobacillus acidophilus* and *Lactobacillus plantarum* were used as probiotics to investigate their inhibitory effect in liquid and on solid media against fungi causing tinea pedis. It was found that unconcentrated filtrates of LAB isolates exhibited no inhibitory effects against any of the three pathogenic fungal isolates for all incubation periods, while the one-fold concentrated filtrate exhibited a slight effect. But upon concentrating the filtrates to (two-fold, three-fold and four-fold), remarkable inhibitory effects, especially the four-fold, were recorded against all tested fungi.

Regarding minimum inhibitory concentration (MIC) experiment, several ratios were prepared by reconstituting the four-fold concentrated filtrate with the SDB (0:10, 1:9, 2:8, 3:7, 4:6, 5:5, 6:4, 7:3, 8:2, 9:1). Results showed that the MICs of *Lactobacillus acidophilus* were (4:6, 4:6, 6:4) for (*Candida albicans, Trichophyton rubrum* and *Trichophyton mentagrophyte)* and those of *Lactobacillus plantarum* were (4:6, 3:7 and 6:4) for (*Candida albicans, Trichophyton rubrum* and *Trichophyton mentagrophyte)*, respectively.

الخلاصة

هدفت الدراسة للتحري عن تأثير بعض النبيت المايكروبي المعوي على الفطريات المسببة لسعفة القدم. لذا جمعت (٥٠) عينة قشطات من المرضى المصابين بسعفة القدم. بعد تشخيص هذه العزلات بزراعتها على وسط السابروید والفحص المجهري والفحوصات الكيموحيوية، وجد ان تلك العينات التي عزلت من القدم تعود لثلاث انواع من الفطريات هي٨ (١٦%) عزلة*T. rubrum* و٥ (١٠%) عزلة *T. mentagrophyte* و٨ (١٦%) عزلة *Candida albicans*، وهناك أنواع اخرى من الخمائر تعود الى جنس *Candida* قد عزلت من القدم ولكن بنسب قليلة جدا وهي *Candida parapisolosis* , *Candida krusi* and *Candida tropicals*.

كان اقل تركيز مثبط من الكيتازول ضد(*T. rubrum* , *Candida albicans*، *T.mentagrophyte*) هو (٦٤، ١٢٨، ١٠٢٤) على التوالي اعتمادا على ماورد آنفا من نتائج، فقد اختيرت إحدى عزلات خميرة *Candida albicans* هي (C1) وذلك لقابليتها على تكوين أعداد كبيرة من الأنابيب الجرثومية مقارنة مع العزلات الأخرى، كما واختيرت عزلتان من بكتريا حامض اللاكتيك العصوية وهي: *L. plantarum* and *L. acidophilus* واستخدمت كمضاد حيوي لمعرفة مدى تأثيرها المثبط في الوسط السائل والصلب ضد الفطريات المسببة لسعفة القدم. لم تظهر الرواشح غير المركزة لعزلات البكتريا أي تأثير مثبط ضد جميع الفطريات المعزولة لجميع فترات الحضن، لكن عند تركيز راشح البكتريا إلى أربع مرات لم يعطي التركيز الأول أي تأثير تثبيطي ضد الفطريات المرضية بينما التركيز الثاني والثالث والرابع للراشح البكتيري أعطى تأثيرا ملحوظا خاصتا التركيز الرابع حيث أعطى تأثيرا تثبيطيا ملحوظا ضد جميع الفطريات المعزولة. اجري اختبار التركيز المثبط الأدنى لرواشح بكتريا حامض اللاكتيك، حيث حضرت عدة نسب للتركيز الرابع للراشح البكتيري مع وسط السابروید السائل وكانت النسب كالأتي: (١:٩، ٢:٨، ٣:٧، ٤:٦، ٥:٥، ٦:٤، ٧:٣، ٨:٢، ٩:١) اظهرت النتائج أن التركيز المثبط الأدنى لبكتريا *L. acidophilus* هو (٦:٤، ٤:٦، ٤:٦) ضد الفطريات (*C. albicanc*, *T. rubrum and T. mentagrophyte*) بينما أن التركيز المثبط الأدنى لبكتريا *L. plantarum* هو (٦:٤، ٣:٧، ٤:٦) ضد الفطريات (*C. albicanc*, *T. rubrum and T. mentagrophyte*) على التوالي .

سورة الإسراء

بسم الله الرحمن الرحيم

قُل كُلٌ يَعمَلُ عَلَى شَاكِلَتِهِ
فَرَبُّكُم أعلَمُ بِمَن هُوَ أهدَى
سَبِيلاً وَيَسئَلُونَكَ عَنِ الرُّوحِ قُلِ
الرُّوحُ مِن أمرِ رَبِّي وَمَا أُوتِيتُم
مِن العِلمِ إلاَّ قَلِيلاً

صدق الله العظيم

الآية ٨٤ـ٨٥