Acknowledgment

Praise to Allah, Mercy and Peace are to the prophet Mohammed and his relatives.

I wish to reach the real expression of my sincere thanks to my supervisor Dr.Abdul W. Baqir and Dr.Makki H. Fayat for their continues support to finish this study.

My deep gratitude and appreciation to Nada Al-Mudallal and Mohammed Abdul Wahab.

A word of thanks is due to the staff of Biotechnology department of Al-Nahrain University.

Gratefull thank to all my friends Montaha, Rafal, Zainab, Sora, Maha, Ranya , Marwa and Sinan.

Special deep of gratitude to my family and every one gave me a hand of support.

Zahraa

Supervision Certificate

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5.1 Conclusions :-

1- Isolates of *Lactobacillus acidophilus* (31.4%) were isolated from stool samples and 35% isolates of also *Lactobacillus acidophilus* were isolated from dairy products .

2- All *Lactobacillus acidophilus* isolates have the ability to produce β-galactosidase enzyme .

3- For stool isolates, MRS broth medium with pH 6.5 and incubation at 37˚C could be used as an optimum condition for β-galactosidase activity from cells grown to mid log phase ($O.D._{600}$ =0.6).

4- For yoghurt isolates , MRS broth medium with pH 6.5 and incubation at 40˚C could be used as an optimum condition for β-galactosidase activity from cells grown to mid log phase ($O.D._{600}$ =0.6).

5- *Lactobacillus acidophilus* bacteria that was isolated from Yoghurt had a higher β-galactosidase activity than *Lactobacillus acidophilus* from stool .

6- *Lactobacillus acidophilus* ($28.5x10^{9}$ cell/ml) was able to consume about (1)g of lactose when incubate for 48hrs.

5.2 Recommendations :-

1- Use of chemical mutagenesis to obtain β-galactosidase over production from mutants of *Lactobacillus acidophilus.*

2- Evaluate the ability of *Lactobacillus acidophilus* to digest lactose *invivo* .

3- Using of protein engineering and genetic manipulation to obtain recombinant enzyme and strains with higher efficiency of enzyme synthesis .

4- Studying the influence of bile salts on β -galactosidase activity .

1.1 Introduction:

Lactose intolerance is due to intestinal lactase deficiency is a most common public health problem of intestinal carbohydrate digestion (Ayyildiz , 1999 ; Saltzman *et al.,* 1999).

Lactose maldigestion has been under intensive research since its discovery in the 1960 (Vesa *et al.,* 2000).

Lactose is primary carbohydrate of mammalian milk, the disaccharides, is hydrolyzed to the monosaccharide glucose and galactose by the intestinal brush border enzyme lactase (Lin *et al*., 1991). In most mammals , intestinal lactase concentration are highest at birth , decrease after weaning , and drop to very low concentration in adults (Simoons , 1978).

Lactose that is not digested by small intestine becomes a substrate for fermentation by bacteria in the colon resulting in the production of gas and short chain fatty acids , lactose maldigestion is confirmed by measuring the concentration of hydrogen in breath following an oral challenge with lactose (Arola , 1994).

Lactose maldigestion can lead to symptom of intolerance including abdominal pain, flatulence, bloating and diarrhea (Montes, 1995)

The condition occur in three main types , primary which is the more common and usually occur after weaning , secondary can follow any intestinal illness and congenital which is the complete absence of lactase (Swagerty *et al*., 2002)

Lactose in yoghurt with live bacteria is better tolerated than lactose in other dairy food , partly because of the activity of microbial β-galactosidase which digest lactose *in vivo* (Martins *et al.,* 1991).

The degree of lactose malabsorption varies widely among patients, but most patients do not require a totally lactose-free or severely restricted diet. Dairy products should not be totally eliminated because they provide key nutrients such as calcium, vitamin A and D, riboflavin, and phosphorus.(Swagerty *et al* ., 2002)

From the past century the beneficial role of non pathogenic bacteria in the intestinal lumen were described and many clinical benefits to these specific non pathogenic organism (probiotic) were studied like diarrhea treatment , antimicrobial activity , anticarcenogenic activity, immune modulation , reduction of cholesterol level and others (Oyetayo and Oyetayo , 2005).

Lactobacillus acidophilus are one of the most strain used as probiotic and specially in treatment of lactose intolerance , it has been hypothesized that ingestion of *Lactobacillus* strain that properties with high β-galactosidase activity an avid intestinal adherence would lead to prolong intestinal survival of *Lactobacilli* and possibly the conversion from lactose intolerant to lactose tolerant state (Saltzman *et al*., 1999).

1.2 Aim of The Study :-

1- Isolation and identification of *Lactobacillus acidophilus* bacteria from local dairy products and stool of children.

2- Studying the ability of locally isolate *Lactobacillus acidophilus* in β-galactosidase production.

3- Determination the optimum pH and temperature for enzyme production.

4- Estimation the ability of the bacterial isolate for lactose consumption.

List of Abbreviations

LI	**LACTOSE INTOLERANCE**
AAD	**Antibiotic-Associated Diarrhea**
IBS	**Irritable Bowel Syndrome**
LAB	**Lactic Acid Bacteria**
β-gal	**β-galactosidase**
CLD	**Congenital Lactase Deficiency**
LPH	**Lactase Phlorizin-Hydrolase**
ONPG	**O-Nitrophynel-β-D-Galactosidase**
ONP	**O-NitroPhenol**
X-Gal	**5-bromo-4-Chloro-3-indodyl- β-D-Galactopyranoside**
IPTG	**IsoPropyl-β-D-Thiogalactopyranoside**
DNSA	**Dinitrosalicylic acid**
SDS	**Sodium Dodecyl Sulphate**
DMF	**Dimethylformamide**
MRS	**de Mann's Rogosa and Sharp**
D.W.	**Distilled Water**
BSA	**Bovine Serum Albumin**

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2.1 Probiotic

2.1.1 Definition and history

Probiotics, a term derived from Latin and Greek meaning literary “for life” has been defined in many ways since it was first coined 50 years ago (Hamilton and Miller,2001). The most recent consensus is defined as Live microorganisms administrated in adequate amounts which confer a beneficial physiological effects on the host(Reid *et al.*,2003).

Probiotics are usually bacterial components of the normal human intestinal flora , for example Lactobacilli and Bifidobacteria , that produce as end product of metabolism , lactate and short-chain fatty acids such as acetate and butyrate (Hamilton and Miller,2001). Mono or mixed culture of live microorganism effect the host by improving the properties of the indigenous microflora (Holzapfel, *et al.,* 2001). By its implantation or colonization in a compartment of the host exert there beneficial effects (Schrezenmeir and De Vrese,2001).

The mechanisms by which probiotics exert their effects on the host are still speculative (Koop-Hoolihan, 2001). Their beneficial effects may be mediated by direct antagonistic effect against specific groups of organisms, resulting in a decrease in numbers, or by an effect on their metabolism, or by stimulation of immunity. Probiotics antagonize pathogens through production of antimicrobial and antibacterial compounds such as cytokines and butyric acid (DeVuyst and Vandamme, 1994; Kailasapathy and Chin, 2000); reduce gut pH by stimulating the lactic acid producing microflora (Langhendries *et al.,* 1995); compete for binding and receptor sites that pathogens occupy (Fujiwara *et al*., 1997; Kailasapathy and Chin, 2000); improve immune function and stimulate immunomodulatory cells (Isolauri *et al.*, 1995; Rolfe, 2000); compete with

pathogens for available nutrients and other growth factors (Rolfe , 2000); or produce lactase which aids in lactose digestion.

Ecological controls which involve the normal body flora exist in a balance state and thereby protect the host from invasion by pathogens (Waaij *et al.*, 1982). The home guard in the digestive tract are what is called 'friendly' bacteria. These are bacteria that fight off the potentially pathogenic ones such as *Escherichia coli* and keep our intestinal tracts 'in balance'. When friendly bacteria are not at the appropriate levels, and when unfriendly bacteria dominate, health problems such as production of gas, bloating, intestinal toxicity, constipation, and malabsorption of nutrients can occur. (Oyetayo and Oyetayo,2005) .

The powerful health benefits that friendly bacteria can provide, coupled with the development of new supplements that provide a greater natural balance of bacteria, will easily make this class of nutrient one of the most important parts of a healthy regimen (Int1).

Conceptually, the use of these nonpathogenic probiotic agents constitutes a purposeful attempt to modify the relation with our immediate microbial environment in ways that may benefit our health. Probiotics may also have a prophylactic effect in terms of decreasing the incidence of illness when taken regularly, the effect of which appears to be greater in high-risk populations (eg, children who are hospitalized, non-breast-fed, or living in underprivileged conditions) (Saavedra , 2001).

The resistance of people in developing countries to diseases can be improved upon by promoting the consumption of locally fermented foods that are rich in probiotic organisms. (oyetayo and oyetayo ,2005).

Microorganisms have been essential to food and alcohol fermentation for thousands of years. Over the last century, different microorganisms have been

used for their supposed ability to prevent and cure diseases, leading to the coining of the term probiotics, or ‘pro-life’ (Lilly and Stillwell, 1965). The

concept of probiotic progressed around 1900, when Elie Metchnikoff hypothesized that the long and healthy lives of Bulgarian peasants were the outcome of their consumption of fermented milk product (Eduardo *et al.,* 2003) .At this time Henry Tissier, a French Paediatrician in (1906), observed that children with diarrhoea had in their stools a low number of bacteria characterized by a peculiar, Y-shaped morphology. These “bifid” bacteria were, on the contrary, abundant in healthy children (Oyetayo and Oyetayo ,2005).

The works of Metchnikoff and Tissier were the first to make scientific suggestions about the probiotic use of bacteria. The first clinical trials were done in the 1930s on the effect of probiotics on constipation (Koop- Hoolihan, 2001). Many researched on probiotics had been carried out after that time and it is increasing steadily since then, but much of it is in Europe, Asia, America, and of recent in South Africa. Presently, probiotics are available in a variety of food products and supplements. In the U.S.A., the food products containing probiotics are almost exclusively dairy products, fluid milk and yoghurt, due to the historical association of lactic acid bacteria with fermented milk . The most frequently used bacteria in these products belong to the genera *Lactobacillus, Bifidobacterium,* and *Streptococcus* (Oyetayo and Oyetayo ,2005).

2.1.2 Characteristics of Probiotics:

It should be a strain, which is capable of exerting a beneficial effect on the host animal, e.g. increased growth or resistance to disease. It should be also non-pathogenic and non-toxic resistance to low pH and organic acids (Fuller, 1989).The culture should adhere to the intestinal wall and proliferate (Berent *et al.*, 1994 ; Ouwehand *et al*., 1999). The potency of probiotic was losed when they are exposed to oxygen, moisture, and heat. For that reason, probiotic supplements should be freeze dried, nitrogen-packaged and refrigerated to maintain their potency and viability (Goldin and Gorbach , 1984).The supplement is tested for viable microorganisms at the time of manufacturing and at the expiration date (Dash , 2003).

Antagonism (*in-vivo*) towards pathogenic bacteria, Production of antimicrobial substances and have the ability to modulate immune responses and also gnetically stable (Mercenier *et al* . , 2003) .

2.1.3 Beneficial Health Effects of Probiotics

Several beneficial effects could obtained upon the use of probiotics. Mercenier *et al* (2003) summarized those effects as :

Health benefit : - proposed mechanism(s)

- **Alleviation of lactose intolerance :**
    - Bacterial β-galactosidase acts on lactose

-Positive influence on intestinal flora :

- Lactobacilli influence activity of overgrowth flora, decreasing toxic metabolite production
- Antibacterial characteristics

-Prevention of intestinal tract infections :

- Adjuvant effect increasing antibody production
- Stimulation of the systemic or secretory immune response
- Competitive exclusion
- Alteration of intestinal conditions to be less favorable for pathogenicity (pH, short chain fatty acids, bacteriocins)
- Alteration of toxin binding sites
- Gut flora alteration
- Adherence to intestinal mucosa, preventing pathogen adherence
- Competition for nutrients

-Improvement of the Immune system :

- Strengthening of non-specific defense against infection
- Increased phagocytic activity of white blood cells
- Increased serum IgA after attenuated *Salmonella typhimurium* challenge
- Increase in IgA production
- Proliferation of intra-epithelial lymphocytes
- Adjuvant effect in antigen-specific immune responses
- Regulation of the Th1/Th2 balance, induction of cytokine synthesis

- Reduction of inflammatory or allergic reactions :

- Restoration of the homeostasis of the immune system
- Regulation of cytokine synthesis
- Prevention of antigen translocation into blood stream

- Anti-colon cancer effect

- Mutagen binding
- Carcinogen deactivation

- Alteration of activity of colonic microbes
- Immune response
- Influence on secondary bile salt concentration

-Blood lipids, heart disease :

- Assimilation of cholesterol
- Alteration of activity of bile salt hydrolase enzyme
- Antioxidative effect

-Antihypertensive effect:

- Peptidase action on milk results in antihypertensive tripeptides (angiotensin converting enzyme inhibitors)
- Cell wall components act as angiotensin converting enzyme inhibitors

Urogenital infections :

- Adhesion to urinary and vaginal tract cells
- Competitive exclusion
- Inhibitor production (H2O2, biosurfactants)

-Infection caused by *Helicobacter pylori* :

- Competitive exclusion
- Lactic acid production
- Decreased urease activity of *H. pylori* in humans after administration of a supernatant of a *Lactobacillus* culture

-Regulation of gut motility (constipation)
-Feeling of well-being

2.1.4 Clinical applications of probiotics :-

2.1.4.1 Lactose Intolerance

Lactose intolerance is a problem for people who have a low amount of intestinal β-galactosidase activity and for whom lactose behave like non-digestible carbohydrate. Lactose is a substance found in milk and milk products. Probiotics have been shown to improve lactose digestion by reducing the intolerance symptoms.(Lin *et al.,* 1991; Montes , 1995).

2.1.4.2 Diarrhea and Gastrointestinal disorders

Diarrhea is one of the most common health problems in the world; gastro-enteritis is the main cause of acute diarrhea. Nonpathogenic living organisms (such as selected strains of *Lactobacillus acidophilus, L. Bulgaricus, Bifidobacterium longum*), capable of re-establishing the equilibrium of the intestinal ecosystem, the prevention of (Naguib , 2000).*Lactobacillus* GG was found to be effective in the prevention of traveler's diarrhea in some studies (Hilton *et al.*,1997), but the effect may not be uniform or consistent, depending on the geographic area or populations studied (Oksanen , 1990), mild bacterial overgrowth can also treated with *lactobacilli*(Mercenier *et al.,* 2003).

Irritable Bowel Syndrome is a multifactorial gastrointestinal disorder affecting 15 - 20% of the population in industrialized countries and 25-50% of all patients in gastro-enterological ambulatory services. IBS is not associated with an organic disease. Some studies with probiotics have shown improvement in pain and flatulence or in relieving constipation (Swagerty *et al.,* 2002).

2.1.4.3 Antibiotic-Associated Diarrhea (AAD)

Approximately 20% of the patients treated with antibiotics will develop AAD because their intestinal flora, responsible for the natural colonization resistance, is disturbed or reduced. The intestinal flora modification (in particular in the LAB population) could be the cause of diarrhea, dehydration and electrolytic imbalance. Also, the fermentation in the colon can be reduced. Many preparations have been tested for their preventive efficacy against AAD. However, more studies need to be performed using well controlled conditions and strains, before we can finally understand which prophylactic probiotics should be taken against secondary effects of specific antibiotics, applied at a specific dose in a specific type of patient (Kelly *et al.*, 1994).

2.1.4.4 *Helicobacter pylori* Infection

Helicobacter pylori infection of the stomach is associated with gastritis, gastric or duodenal ulcers and possibly with gastric cancer. Although antibiotic therapy for gastritis is quite effective, eradication is not always achieved and reinfection may occur. *In vitro* and *in vivo* inhibitory effects of *Helicobacter pylori* are reported for several lactic acid bacteria (Mercenier *et al* , 2003).

2.1.4.5 Viral Infections

The possible effect of probiotics on viral infections has most logically been related to a stimulating effect of the probiotic agent on the immune system of the host (Isolauri , 2001).

2.1.4.6 Lowering Cholesterol

Hypercholesteremia has been linked with increased risk for coronary heart disease, one of the leading causes of death today. The use of probiotics to reduce this risk seems very attractive, especially if consumed as a part of a normal daily nutrition. (Mercenier *et al* ,2003 ; Liong and Shah,2005).

2.1.4.7 Cancer

The anticancer activities of probiotics have been demonstrated in a test-tube study. Live cells of probiotics, six strains of *L. acidophilus* and nine strains of *Bifidobacterium*, showed higher anti -mutagenic activity against potent chemical mutagens, and their efficiency in inhibiting the mutagens was better than killed bacterial cells. (Sanders , 2000 ; Roos and Katan ,2000)

2.1.4.8 Immune System

Lactic acid bacteria, *Bifidobacterium, Lactobacillus acidophilus (L. acidophilus), L. bulgaricus, L. casei, L. gasseri, and L. reuteri* were shown in vitro to stimulate macrophages and possibly other immune cells to produce proinflammatory cytokines and nitric oxide (Naguib , 2000).

2.2 Probiotic Microorganisms:

Prpbiotics can be obtained from foods , primarily dairy products (eg.yoghurt and milk) and commercial supplements. Foods are a better choice due to the synergistic effects between foods compounds and probiotic culture (Int2).

Lactic acid bacteria are among the most important probiotic microorganisms typically associated with the human gastrointestinal tract. A beneficial association of microorganisms on the human host was pastly suggested, which

proposed that vaginal bacteria produced lactic acid from sugars to prevent or inhibit the growth of pathogenic bacteria (Holzapfel *et al*., 2001).

There are dramatic increase in scientific work to investigate the benefits of ingestion specific nonpathogenic organisms (probiotics) (saavedra, 2001).

Members of the genera *Lactobacillus* and *Bifidobacterium* are mainly used, but not exclusively, as probiotic microorganisms and a growing number of probiotic foods are available to the consumer. Some ecological considerations on the gut flora are necessary to understand the relevance, for human health, of the probiotic food concept (Araya *et al*., 2001). Table (2-1) contains microorganism which are considered to be used as probiotic.

Table (2-1) Microorganisms considered as probiotics (Holzapfel *et al.*, 1998).

Lactobacillus **species**	***Bifidobacterium*** **species**	**Other lactic acid bacteria**	**microorganisms**
L. acidophilus	*B. adolescentis*	*Enterococcus faecalis2*	*Bacillus cereus var.* toyoi*2,3*
L. amylovorus	*B. animalis*	*Enterococcus faecium*	*Escherichia coli* strain nissle
L. casei	*B. bifidum*	*Lactococcus lactis4*	*Propionibacterium freudenreichii2,3*
L. crispatus	*B. breve*	*Leuconstoc mesenteroides*	*Saccharomyces cerevisiae3*
L. delbrueckii	*B. infantis*	*Pediococcus acidilactici4*	*Saccharomyces boulardii3*
L. gallinarum2	*B. lactis5*	*Sporolactobacillus inulinus2*	
L. gasseri And other	*B. longum*	*Streptococcus thermophilus4*	

2.2.1 Lactic Acid Bacteria (LAB):

Lactic acid bacteria are gram-positive, nonsporing, catalase-negative organisms that are devoid of cytochromes and of nonaerobic habit but are aerotolerant, fastidious, acid-tolerant, and strictly fermentative; lactic acid is the major end product of sugar fermentation (Axelsson , 1998). However, exceptions from this general description do occur because some species can form catalase or cytochromes on media containing hematin or related compounds (Whittenbury ,1964; Meisel ,1994). The production of a nonheme catalase, called pseudocatalase, by some lactobacilli can also cause some confusion in the identification of LAB (Engesser and Hammes ,1994).

Lactic acid bacteria are associated with habitats that are rich in nutrients, such as various food products and plant materials. They can be found in soil, water, manure, sewage, and silage and can ferment or spoil food. Despite that particular LAB are inhabitants of the human oral cavity, the intestinal tract, and the vagina, and may have a beneficial influence on these human ecosystems (Holzapfel *et al.*, 2001), they have been used for centuries in fermentation processes and recently are gaining increased attention due to their probiotic properties (Altermann *et al.*, 2004).

2.2.1.1 *Lactobacillus acidophillus:*

Lactobacillus is gram positive , have a rod shape with a regular dimension of 0.5-1.5x10nM , single , paired , or small chain , non-sporeforming , faculititvily anaerobe or microaerophilic , catalase negative , and stable in acid media and salt (Stamer , 1976). They are classified by Oral-Jensen (1919) to thermobacterium streptobacterium and betabacterium. Lactobacilli are also classified as lactic acid bacteria, and derive almost all of their energy from the conversion of glucose to lactate during homolactic fermentation. In this process

85-90% of the sugar utilized is converted to lactic acid. They generate ATPs by nonoxidative substrate-level phosphorylation.

Lactic acid produces by Lactobacilli used for many different things, including yoghurt production and the maintenance of healthy intestinal microflora. Lactobacilli are commonly associated with the gastrointestinal tract of humans (Int7). *Lactobacillus acidophilus* is generally considered to be beneficial because it produces vitamin K, lactase, and anti-microbial substances such as acidolin, acidolphilin, lactocidin, and bacteriocin.

Studies report few side effects from *L. acidophilus* when used at recommended doses. Some experts recommend limiting the daily dose to fewer than 10 billion living *L. acidophilus* organisms to reduce the side effects. (Adolfsson *et al.,* 2004). Oral supplements of *L.acidophilus* in human have resulted in synthesis of B-complex vitamins and adsorption of calcium amilelioration of diarrhea and constipation and immunity activation (Reddy *et al.,* 1983) , also saltzman *et al* (1999) hypothesized that ingestion of a strain of *Lactobacillus acidophilus* with properties of high β-galactosidase activity and avid intestinal adherence would lead to prolong intestinal survival of lactobacilli and possibly the conversion from a lactose intolerant to a lactose-tolerant state .

Consumption of unfermented milk containing live *Lactobacillus acidophilus* was reported to improve lactose digestion .This finding suggests that the beneficial effect could have occurred in the digestive tract after consumption of milk containing *L. acidophilus*. Cultures may continue to produce *β*-gal in the intestinal tract after milk digestion , so to alleviate lactose intolerance. It is important to select cultures capable of producing large amounts of *β*-gal. One approach is mutagenesis of existing strains to obtain overproducing mutants.

Chemical mutagenesis has been used extensively in bacteria, including lactic acid bacteria and is considered food grade, because it does not involve the introduction of heterologous DNA or the manipulation of existing DNA by recombinant means (Ibrahim and Sullivan , 2000).

2.3 Lactose intolerance (LI) :-

Lactose is an important disaccharide in the human diet since it is the primary carbohydrate in mammalian breast milk and is found as the primary sugar in common dairy products. Composed of the two monosaccharides joined by a β glycosidic bond, galactose and glucose, lactose must be hydrolyzed into its respective monosaccharides for absorption through the villi of the small intestine to occur. The enzyme lactase (β-galactosidase) is responsible for hydrolysis of the disaccharide for proper absorption (Int5). Lactose appears to enhance the absorption of several minerals, including calcium, magnesium, and zinc. It also promotes the growth of *Bifidobacterium* and is a major source of galactose, which is an essential nutrient for the formation of cerebral galactolipids (Marteau et al., 1992 ; Int6).

Despite that lactose intolerance was first described by Hippocrates, only in the past 50 years has this condition been recognized and diagnosed medically **(**Cuatrecasas *et al.,* 1965; Jarvis and Miller, 2002). It is important to distinguish between hypolactasia, a low level of lactase, and clinical lactose intolerance.

2.3.1 Pathophysiology:-

Lactose maldigestion is the most common disorder of intestinal carbohydrate digestion in humans. (Saltzman, *et al.*, 1999).This disease is usually occurring as a result of a deficiency of the enzyme lactase, and an inability to properly absorb the sugar lactose. The excess lactose that was unable to be hydrolyzed will continue to travel through the small intestine creating an osmotic gradient for water loss into the intestine. This process can result in a watery, osmotic diarrhea. The enriched carbohydrate medium now present in the intestine promotes growth of the intestinal flora, often creating overgrowth out of the large intestine and invading into the distal ileum. These bacteria use anaerobic fermentation to metabolize the lactose present, creating hydrogen gas as a by-product of their metabolism. This excess gas production causes the symptom of lactose intolerance (Int5) .

Among the physiological factors that affect the amount of lactose digested and its tolerance are gastrointestinal transit, intestinal lactase activity, visceral sensitivity and the presence of functional bowel disorders , and possibly the composition of the colonic microflora . In addition, factors related to the sensory and central nervous system modify symptom perception. (Vesa *et al* .,2000). These symptoms can occur 30 minutes to 2 or more hours after eating foods with lactose in them. Some people with LI can eat small amounts of lactose without any problems. Others may get many of the symptoms mentioned from very small amounts of lactose. (Int3).

Burton and Tannock (1997) reported that Lactose-intolerant individuals tolerate fermented milks better than fresh milk with the equivalent amount of lactose. A generally accepted explanation for this is that the lactose-hydrolyzing enzyme (β-galactosidase) contained within the microbial cells of the yogurt

substitutes for the paucity of lactase (β-galactosidase) in the small bowel mucosa of lactose-intolerant individuals.

Lactase activity is genetically programmed to decline at the age of 2 years. Signs and symptoms usually do not become apparent until after the age of 6 years and may not be apparent until adulthood, depending on dietary lactase intake and rate of decline of intestinal lactase activity. Lactase enzyme activity is highly correlated with age, regardless of symptoms. (Int6). It has been hypothesized that ingestion of a strain *Lactobacillus* with properties of high β-galactosidase activity an avid intestinal adherence would lead to prolonged intestinal survival of Lactobacilli and possibly the conversion from a lactose-intolerant to lactose-intolerant state (Saltzman *et al.,* 1999).

2.3.2 Maldigestion of Lactose (Hypolactsia):

Hypolactasia and lactose maldigestion accompanied by clinical symptoms is termed lactose intolerance. (De Verse *et al* ., 2001) . Some patients may have improved tolerance of lactose over time if lactose – containing foods are provided slowly and consistently (Pribila *et al.,* 2000). De Vrese *et al* (2001) found that this does not mean the small bowel adapts to produce more lactase in response to increased consumption . This adaptation occurs in the colon. Over time, the colonic flora may adapt to the lactose load, resulting in less gas production, this along with adaptation in motility and pH may decrease or eliminate the symptom of lactose intolerance.

The degree of lactose malabsorption varies widely among patients, but most patients do not require a totally lactose-free or severely restricted diet. Dairy products should not be totally eliminated because they provide key nutrients

such as calcium, vitamins A and D, riboflavin, and phosphorus.(Swagerty *et al* ., 2002).

Three main types of lactose maldigestion may occur :

- Primary type : -

This is the most form in which the genetically determined reduction of lactase activity occurs soon after weaning in almost all animals and in many human groups (Johnson *et al.,* 1981). The activity drops to about one tenth or less of the suckling level, and this situation is referred to as hypolactasia, (adult-type) lactase deficiency or lactase non-persistence (Vesa *et al.*,2000). Level of lactase expression may drop low enough to create symptoms and intolerance (Int5).

- Secondary (acquired) type:-

Secondary hypolactasia or maldigestion can result from small intestinal resections, from gastrectomy (Welsh and Griffiths, 1980) and from diseases that damage the intestinal epithelium, e.g. untreated coeliac disease or intestinal inflammation (Bode *et al.,* 1988 ;Pironiet *et al.*, 1988) . Arrigoni *et al* (1994) stated that when the epithelium heals, the activity of lactase returns. However, secondary maldigestion does not automatically lead to severe symptoms of intolerance. This type can appear at any age; however, children younger than 2 years are very susceptible because of many factors, including a high sensitivity of the gut to infectious agents, low reserve because of the small intestinal surface area, and high reliance on milk-based products for nutrition (Int6).

- Congenital type: -

Human congenital lactase deficiency (CLD) condition where is an extremely rare autosomal recessive disorder and is associated with a complete absence of lactase expression (Vesa *et al.* ,2000). Congenital lactose intolerance

involve not expressing the mRNA or translating the enzyme lactase even from birth , this is a more serious deficiency (Savilahti *et al* .,1983; Int5).

2.3.3 Clinical features of Lactose intolerance:-

The symptoms patients experience vary, however, according to the quantity of lactose ingested and patients' ability to digest lactose. In patients with common adult-type hypolactasia, the amount of ingested lactose required to produce symptoms varies but is reported to be about 12 to 18 g, of lactose. Several factors affect the severity of symptoms after lactose ingestion, including the patient's ethnic origin and age; older patients are more susceptible (Swagerty *et al.,* 2002 and Vesa *et al.,* 2001). Saltzman *et al* (1999) and Vesa *et al* (2000) reported that ingestion of small to moderate amounts of lactose usually produces bloating, cramps, and flatulence but not diarrhea. Ingestion of larger amounts of lactose, a faster gastric emptying time, and a faster intestinal transit time all contribute to more severe symptoms. Conversely, increased lactase activity in the small intestine reduces symptoms.

2.3.4 Symptoms of Lactose Intolerance

The mechanism of loose stools induced by unabsorbed carbohydrate is well documented. The osmotic load of the carbohydrate causes secretion of fluid and electrolytes until osmotic equilibrium is reached Dilatation of the intestine, caused by the osmosis, induces an acceleration of small intestinal transit, which increases with the degree of maldigestion (Ladas *et al.*, 1982). The accelerated transit further reduces the hydrolysis of lactose, because the contact time between lactose and the residual enzyme is decreased. The origin of abdominal distension and cramps has been suggested that these symptoms originate from the small intestine and are not caused by colonic fermentation. On the other

hand, Bouhnik *et al* (1996) a recent study showed that symptoms seemed to originate from the colon, since lactose both ingested orally and introduced directly to the colon caused similar symptoms . In the study of Hammer *et al* (1996) gas seemed to serve as a trigger for symptoms, as suggested by the significant correlation between the time of the occurrence of peak symptoms and the time of peak breath hydrogen concentration.

The production of hydrogen depends on colonic acidity. Reduced hydrogen excretion was seen after continuous ingestion of the non-digestible sugar, lactulose, which resulted in increased colonic acidity (Flourie´*et al.*, 1993) . Reduced hydrogen excretion and symptoms have been reported after continuous lactose consumption (Hertzler *et al*., 1996) .

2.3.5 Diagnosis of Lactose intolerance:-

Four tests are available for positive diagnosis of lactose intolerance according to (Arola H , 1994 ;Carol ,2003; Int5) .They are briefly explained below from least to more invasive :-

2.3.5.1 Hydrogen breath test:

Excess hydrogen gas produced by the intestinal flora from metabolism of lactose is passed into the blood stream and exits the body through lung interface. Amounts of hydrogen present upon exhalation can be measured and confirm lactose intolerance. Often a test load of lactose is administered and results are compared to baseline exhalation.

2.3.5.2 Acid stool test:

The stool is analyzed for pH since metabolites of bacterial fermentation contribute to producing an overly acidic stool excreted through the GI tract . A pH of less than 5.5 is considered a positive result. This test is appropriate for infants and children .

2.3.5.3 Lactose tolerance test (Lactose challenge) :

An amount of 2g/Kg of lactose is administered after fasting. Blood sugar levels carefully monitored, and upon proper hydrolysis and absorption, blood sugar should increase. If blood sugar does not rise more than 20mg/dl and corresponding symptoms appear, lactose intolerance is confirmed.

2.3.5.4 Endoscopic biopsy:

An endoscope is used to section a small piece of the small intestinal mucosa. Lactase activity can be measured directly from this sample.

2.3.6 Genetics Of lactose intolerance:-

Childhood- and adult-onset lactase deficiency is extremely common and inherited in an autosomal recessive manner, and persistent lactase activity into adulthood is inherited in an autosomal dominant manner (Int4). The gene for lactase is located on chromosome 2. There is a suggestion of no differences exist in the DNA from individuals with low and high levels of lactase activity. Differences do exist in messenger RNA (mRNA), suggesting the primary regulation of this enzyme occurs during translation (Int4). While Lactose intolerance was known to be genetic, caused by a recessive gene , this meaning a person has to inherit a" faulty" copy from each parent to be lactose intolerant.(Int5).

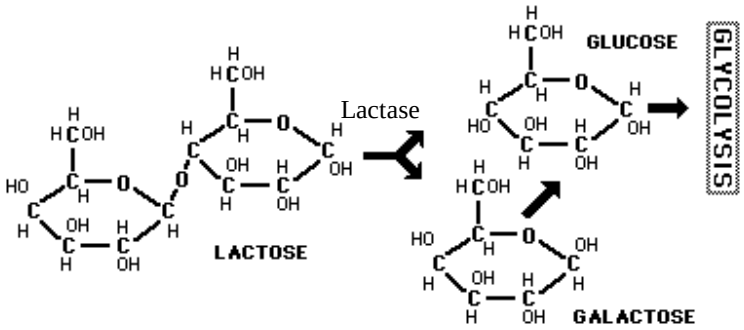
The molecular mechanism causing this is unknown. It is not attributable to polymorphisms within the lactase gene itself or within its promoter (55 kb within 70 kb, long arm of chromosome 2 (2p.21q) 17 exons**)** (Swallow, 2003). However, there is a close correlation between lactase persistence and two polymorphisms, C/T_{13910} and G/A_{22018} upstream from the lactase gene, CC/GG

being associated with lactase non-persistence and lactose intolerance **(**Enattah, 2002; Matthews *et al*., 2005**)**.

2.4 β-galactosidase Enzyme (Lactase):-

Chemical reactions are at the heart of all biological processes. The body must regulate precisely all the chemical reactions going on in order to maintain life. Much of this regulation is done by changing the activity of enzymes, which are biological catalysts.(Int6).

β-galactosidase catalyzes the breakdown of the substrate lactose, a disaccharide sugar found in milk, into two monosaccharide sugars, galactose and glucose (as shown below). The oxygen bridge connecting the two sides of the lactose molecule is cleaved through the addition of a water molecule. The addition of the water molecule is known as hydrolysis.(Int6).



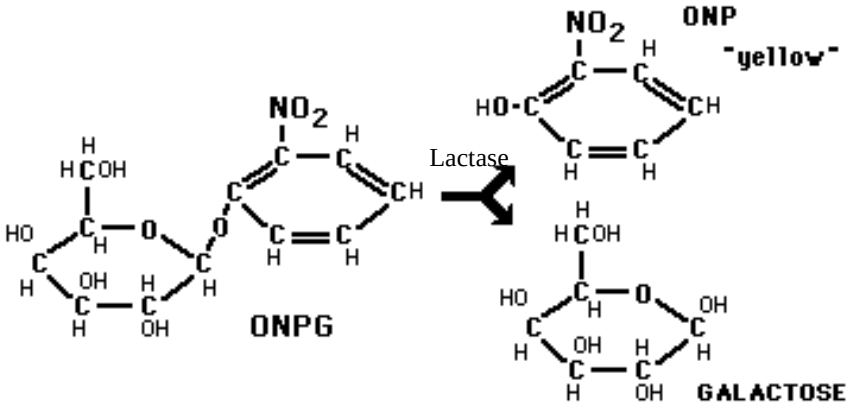
Lactase is a unique enzyme in its formation, location and enzymatic activity (Mantei,1988). It is formed as a 1927 amino acid protein, and then processed, leaving a final protein of 1059 amino acids as a dimer of 320 kDa. Lactase (lactase phlorizin-hydrolase—LPH, EC 3.2.1.62/108; but ß- galactosidase is EC 3.2.1.23, mistakenly used for lactase in some publications) is highly unusual, having two active sites within one polypeptide chain, one hydrolysing lactose,

the other aryl and aliphatic glycosides such as phlorizin into glucose and phloretin, the latter being a potent diabetic agent. Two important natural substrates for this latter site are cerebrosides, a crucial source of sphingosine, and glycosyl-pyridoxal, **(**a vital source of vitamin B6. Lactase has no sequence similarity to its bacterial counterpart ß galactosidase **(**Wuthrich *et al*., 1996 ;Mackey *et al*., 2002). Suppose a chemical was somewhat similar to lactose, but when it splits, one portion becomes a colorful dye. (Int7).

Lactase is found most abundantly in the jejunum (at the beginning of the small intestine), and it specifically only hydrolyses lactose. It is found at the tip of the intestinal villi and is therefore more vulnerable to intestinal diseases that cause cell damage than other disaccharidases, which are located deeper. The enzyme activity and the transit time of lactose through the jejunum mucosa are important for proper absorption. (Alavi and Squillante ,2002 ; Swagerty *et al*.,2002; Seyis and Aksoz ,2004 ;) .

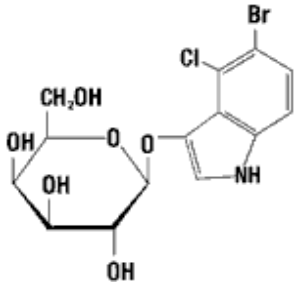
2.5 Analogs of Lactose.

A commonly used one is ortho-nitro-phenyl-β-D-galactoside (ONPG), this analog has an O-nitrophenoll "ONP" instead of the monosaccharide glucose. When the bond is snipped between ONP and Galactose, the ONPG is colorless while the resulting ONP is a bright yellow water-soluble dye as shown below (Int7).



The advantage of using ONPG as the substrate is that it is relatively easy to determine the amount of ONPG cleaved by using a spectrometric assay. So that as the β-galactosidase continues to work, more and more ONPG is degraded, and the solution turns more and more yellow. By measuring the rate at which the color intensity increases the activity of the enzyme can be calculated (Int6).

Another analog is 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal) a noninducing chromogenic substrate for beta-galactosidase, which hydrolyzes X-Gal forming an intense blue precipitate. X-GaI is most frequently used in conjunction with Isopropyl-β-D-thiogalactopyranoside (IPTG) in blue/white colony screening to detect recombinants (white) from non-recombinants (blue). It is also utilized for selection of beta- galactosidase reporter gene activity in transfection of eucariotic cells and for detection of beta-galactosidase in immunology and histochemistry applications. (Sambrook *et al.*,2001) .



X-Gal

Formula:	$C_{14}H_{15}Br\ Cl\ N\ O_6$
Molecular Weight:	408.6

3.1 Materials: -

3.1.1 Apparatus

Apparatus	Company(Origin)
An aerobic jar	Rodwell (England)
Autoclave	Gallenkamp (U.K.)
Balance	Ohans (France)
Compound light microscope	Olympus (Japan)
Cooling centrifuge	Harrier (U.K.)
Distillater	GFL (Germany)
Electrical incubator	Gallenkamp
Electrical oven	Gallenkamp
Gas generating kit	Rodwell
Glass Pasteur pipettes	John poulten Ltd. (England)
Micropipette	Oxford (U.S.A.)
pH-Meter	Meter-GmpH Tdedo (U.K.)
Refrigerated centrifuge	Harrier (U.K.)
Sensitive balance	Delta Range (Switzerland)
Spectrophotometer	Aurora instruments Ltd.(England)
Vortex	Buchi (Swissrain)
Water bath	GFL

3.1.2 Chemical and Biological materials:-

Material	Company
Agar-Agar	Difico (U.S.A.)
Ammonium ferric tricitrate	BDH (England)
Beef extract	Oxoid (England)
Calcium carbonate	BDH
Casein	Fluka (Switzerland)
Chlorophenol (Red)	Fluka
Dimethylformamide (DMF)	Oxoid
Dinitrosalcilic acid (DNSA)	Fluka
Dipotassium phosphate	BDH
Disodium hydrogen phosphate	Oxoid
Esculine	BDH
Ethanol	Riedel-DeHaeny (Germany)
Gelatin	Oxoid
Glucose, Manitol , Xylose, Maltose, Cellobiose	BDH
Glycerol	BDH
Hydrochloric acid	BDH
Lactose ,arabinose,galactose,rafinose	Difco
L-arginine monohydrochloride	Fluka
Magnesium sulfate (hydrated)	BDH
Manganese Sulfate(hydrated)	Riedel-DeHaeny
O-nitrophynel-β-D-galactoside(ONPG)	Sigma (U.S.A.)
Peptone	BDH

Potassium Chloride	BDH
Sodium acetate hydrate	BDH
Sodium carbonate	BDH
Sodium dihydrogen phosphate	BDH
Sodiumdodecyl sulphate SDS	BDH
Sodium hydroxide	Fluka
Sodium-potassium tartrate	May.and Baker (England)
Trypton	Fluka
Tween 80	Oxoid
X-GAL	Biochemical corporation(U.S.A.)
Yeast extract	Fluka

3.1.3 Culture Media:

3.1.3.1 Ready to use powdered media:

Medium	Company (Origin)
Mann' s Rogosa and Sharp (MRS) agar	Himedia (India)
Litmus milk broth	Biolife (Italy)

3.1.3.2 Laboratory prepared media:

- MRS broth
- MRS-$CaCO_3$ agar
- Aesculine-Cellobiose agar
- MRS-Raffinose agar
- Milk agar
- Arginine-MRS broth

- Indole broth
- Starch agar
- Gelatin broth
- Carbohydrate fermentation broth

3.1.4 Solutions, Buffers ,Reagents, and Dye:

3.1.4.1 Ready to use reagents:

Reagent	Company (origin)
Kovacs reagent	Department of chemistry of Al-Nahrain university
Nesslar reagent	Chemical department of Al-Nahrain university
Catalase reagent	Al-mansor company(Iraq)

3.1.4.2 Laboratory prepared Solution, Buffers, Reagents and Dye:

- Physiological saline solution
- z-buffer
- Sodium-Phosphate buffer
- Chlorophenol red reagent
- Starch hydrolysis reagent
- Dinitrosalcilic acid (DNSA) reagent
- Coomassie Brilliant Blue (G250)

3.2 Methods:

3.2.1 Media Preparation:

3.2.1.1 Ready to use powdered media:

The media listed in (3.1.3.1) were prepared according to the information fixed on their containers by the manufacture. After pH was adjusted, they were sterilized in the autoclave unless otherwise stated.

3.2.1.2 Laboratory prepared medium:

3.2.1.2.1 MRS Broth:

It was prepared according to Harrigan and McCance (1966) from the following components:

Peptone	20g
Beef extract	10g
Yeast extract	5g
Sodium acetate trihydrate	5g
Glucose	20g
Tween80	1ml
Triammonium citrate	2g
$MgSO_4.7H_2O$	0.2g
$MnSO_4.7H_2O$	0.05g
D.W.	1liter

After pH was adjusted to 6, the medium was autoclaved.

3.2.1.2.2 MRS-$CaCO_3$ Agar:

This medium contain all MRS broth components plus 1.5%(w/v) agar and (1)g of $CaCO_3$ (القصاب، ١٩٨٨).It was prepared and sterilized as in MRS broth .

3.2.1.2.3 Asculine-Cellobiose Agar:

This medium was prepared according to Hunger (1986) from the following components:

Casein	20g
Yeast extract	5g
Cellobiose	20g
Sodium Chloride	4g
Sodium acetate trihydrate	1.5g
Tween80	0.5ml
Asculine	1g
Ammonium ferric tricitrate	0.5g
Chlorophenol red	6.5ml
Agar	15g
D.W.	1liter

After pH was adjusted to 6, the medium was autoclaved.

3.2.1.2.4 MRS-Raffinose Agar:

This medium was prepared and autoclaved as in MRS broth (item 3.2.1.2.1) but with using raffinose sugar instead of glucose and 1.5% agar.

3.2.1.2.5 Arginine MRS Broth:

It was prepared by adding 0.3% (w/v) of L-arginine-monohydrochloride to the MRS broth, after pH was adjusted to 6, it was autoclaved (Harrigan and McCance, 1966).

3.2.1.2.6 Starch medium:

This medium was prepared by dissolving (10)g of soluble starch ,(3)g of beef extract and (12)g of agar in 1liter of D.W., gently heated and brought to boiling , then autoclaved (Atlas *et al*., 1995).

3.2.1.2.7 Indole Medium:

It was prepared by dissolving (15)g tryptone in 1liter of D.W., pH was adjusted to 7, then autoclaved (Atlas *et al*., 1995).

3.2.1.2.8 Gelatin Medium:

The medium was prepared from MRS broth after adding 10% of dissolve gelatin to it, pH was adjusted to 7.2, then autoclaved (Harrigan and McCance ,1966).

3.2.1.2.9 Milk Agar Medium:

It was prepared by dissolving 2% agar in 1liter of D.W. and autoclaved, then 10% skim milk was added to it under aseptic condition before let for solidification (Harrigan and McCance ,1966).

3.2.1.2.10 Carbohydrate fermentation Medium:

Previously sterilized MRS broth free from glucose and beef extract was prepared, 1% of each of autoclaved sugar solutions (glucose, mannitol, raffinose, asculine, cellobiose and lactose) and membrane filtrated sugar solutions (xylose and galactose) was added and 2% chlorophenol red reagent also added , then pH was adjusted to (6.2-6.5).This medium was used for identification of *Lactobacillus* spp. (Cowan, 1974).

3.2.1.2.11 X-Gal MRS Agar:

A quantity of (40)µg/ml of x-gal solution was sterilized by Millipore filter (0.2µm), after cooling down it was aseptically added to previously autoclaved MRS agar before let for solidification (Ibrahim and Sullivan, 2000).It was used for detection the bacterial ability for production of β-galactosidase in the medium.

3.2.2 Preparation of Solution , Buffer, Reagents and Dye :

- Physiological Saline Solution :

In 1 liter of D.W, 0.85g of NaCl was dissolved, then autoclaved after pH was adjusted to 7 (Atlas *et al* , 1995).

- z-buffer:

It was prepared according to Atlas *et al* (1995) by dissolving 0.08g of Na_2HPO_4, 0.0012g $MgSO_4$ in 20ml of D.W , pH was adjusted to 7 then 54µl of β-mercaptoethanol was added.

- Sodium-Phosphate Buffer :

It was prepared by dissolving 0.108g of NaH_2PO_4 and 0.582g of Na_2HPO_4 in 50ml D.W. then autoclaved (Noh and Gilliland , 1994).

- Dinitrosalicylic acid (DNSA) Reagent :

It was prepared by dissolving 1g of DNSA in 50 ml D.W. and 20ml of (2µ) NaOH solution, then 30g of sodium-potassium tartrate was added , after that volume was completed to 100ml by D.W. and stored in dark container(Whitaker and Bernhard , 1972).

– Starch-hydrolysis Reagent:

This reagent was prepared by dissolving 10g of potassium iodide in 25ml D.W. , 5g of iodide was added with stirring until dissolving completely . The volume was completed to 100ml with D.W. and kept in a dark bottle (Fad , 1976).

- Coomassi Brilliant Blue G250 (Protein Dye reagent):

A quantity of 0.1g of coomassi brilliant blue (G250) was dissolved in 50ml of 95% ethanol then 100ml of 85% orthophosphoric acid was added . The volume was completed to 1 liter by D.W. (Bradford , 1976).

3.2.3 Sterilization:

3.2.3.1 Autoclave Sterilization:

Media and solutions were sterilized by the autoclave at 121˚C (15Ib/inch^2) for 15minutes .

3.2.3.2 Oven Sterilization:

Electrical oven was used to sterilized glasswares and others at 160-180˚C for 2-3 hrs.

3.2.3.3 Membrane Sterilization (Filtration):

Millipore filters (0.22µm) was used to sterilize some sugars and x-gal solution.

3.2.4 Samples Collection :

3.2.4.1 Stool samples:

A total of 35 samples were obtained from infants of Al-Mansor hospital in Baghdad governorate from the period between 1/10 and 31/12/2004. Each sample was collected in a sterile glass tube containing (5)ml of peptone water, then samples were transferred to the laboratories for analysis within 2hr. after collection .

3.2.4.2 Yoghurt Samples:

Twenty samples of Yoghurt were collected from local markets of Baghdad from 1/11 to 31/12/2004. The samples were obtained as part of fermented yoghurt.

3.2.5 Bacterial Isolation

3.2.5.1 From Stool Samples:

Isolation method was performed in four steps; at first loopfull of stool sample in peptone water was inoculated in to MRS broth and incubated overnight at 37˚C for three times to increase the bacterial number, then serial dilutions were made and 1ml from the last one was transferred and plated on MRS agar and incubate overnight at 37˚C under anaerobic condition using gas generating kit. In the second step colonies were picked and grown on MRS-$CaCO_3$. After incubation, colonies surrounded by clear zones (due to the production of acid hydrolyzing $CaCO_3$) were collected. Morphological and microscopical examinations were performed (Harrigan and McCance , 1966). A third step , suspected isolates were transferred to aesculine-cellobiose agar medium to obtain *Lactobacillus spp.*, only which have the ability to hydrolyze and convert aesculine to aesculietine, and then combines with iron ion to form dark green color that characterizing *Lactobacillus* colonies (Hunger, 1986).Fourth step was performed by transferring green colonies to MRS-raffinose agar medium in which only the *Lactobacillus acidophilus* have the ability to ferment raffinose for their growth (Holt and Krieg , 1986).

3.2.5.2 From Yoghurt Samples:

Serial dilutions of samples were made , 1ml from the last dilution was transferred and poured on MRS plates and incubated overnight at 37˚C under anaerobic condition , then same isolation steps of bacterial cells from stool sample were followed .

3.2.6 Maintenance of isolates:

-Working culture:

Maintenance of bacterial isolates was performed according to Baron and Fingold , (1994) in which sterile MRS broth was inoculated with bacterial isolates and incubated anaerobically at 37˚C for 24hr., then kept in refrigerator and reactivated weekly .

- Stock Culture :

Sterile glycerol (20%) was added to an exponential growth of bacterial isolates in screw-caped tubes and stored at -18˚C (Contreras *et al*., 1991).

3.2.7 Identification of *Lactobacillus acidophilus* isolates :

3.2.7.1 Microscopical Examination:

A loopfull of suspected colonies was fixed on a microscopic slide, then stained by Gram stain to examine cells grouping, shape, gram reaction and non-sporeforming (Kandler and Weiss , 1986 ; Garvie and Weiss ,1986).

3.2.7.2 Biochemical Tests:

- Catalase Test:

This test was performed by adding 2-3 drops of hydrogen peroxide (3%)H_2O_2 to the mass of bacterial cells placed on the microscopic slide .Production of gaseous bubbles indicate a positive result .

- Gelatinase Test :

Gelatine liquefaction was detected by using gelatin medium inoculated with 1% of bacterial culture and incubated at 37˚C for 48hr. After that, it was put in the refrigerator (4˚C) for 30 minutes . The positive result was observed by gelatin liquefaction (Baron and Fingold , 1994).

- Indole Test :

Tryptone broth was inoculated with 1% of the bacterial isolate and incubate at 37˚C for 3 days , after that, (0.5)ml of Kovacs reagent was added and shaked . Positive result was recorded by the appearance of red layer at the top of the broth (Atlas *et al.*, 1995).

- Starch Hydrolysis Test:

Starch agar medium was inoculated by a loopfull of bacterial isolate and incubated at 37˚C for 3-5 days, after that the plate was flooded by iodine solution . Positive result was indicated by the formation of clear zone around the colonies while the medium stained by blue color (Atlas *et al*., 1995).

– Casein Hydrolysis Test :

Milk agar medium was inoculated by the bacterial isolate and incubated at 37˚C for 7 days under anaerobic condition , positive result is by the formation of clear zone around the growing colonies (Harrigan and McCance , 1966).

- Acid and Curd Production in Litmus Milk :

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

- Production of Ammonia from Arginine :

Arginine-MRS broth was inoculated with 1% of bacterial culture and incubated at 37˚C for 72hr. Then to 1ml of it Nesslar reagent was added, and positive result was detected by the appearance of red color (Brigges , 1953).

- Carbohydrate Fermentation Test :

Carbohydrate fermentation medium (MRS free from glucose and beef extract) was inoculated with 1% of bacterial culture and incubated at 37˚C for 5 days. Color changing from red to yellow indicates positive result (Atlas *et al*.,1995) .

- Growth at 15 and 45˚C :

Tubes containing MRS broth were inoculated with 1% bacterial culture and incubated at 37˚C for 24hr. positive result obtained by the appearance of bacterial growth.

3.2.8 growth curve *of Lactobacillus acidophilus*

200ml of MRS broth was inoculated with 1% exponentionaly growing culture of *Lactobacillus acidophilus* and optical density was read in spectrophotometer at wave length of 600nm at the time of inoculation and then each 2hrs. for 24hr.

3.2.9 Preparation of O-Nitrphenol Standard Curve :-

Standard curve of O-nitrphenol (ONP) was prepared to determined the β-galactosidase activity (Noh and Gilliland , 1994).

A stock solution of o-nitrophenol was prepared in a concentration of 120µg/ml in a total volume of 100ml by dissolving 0.012g of o-nitrophenol in 10ml of 95% ethanol, then volume was completed to 100ml by 1%Na_2CO_3. From this solution , different concentrations was prepared (10-120µg/ml), the optical density was read in spectrophotometer at 420nm for each concentration (Figure 3-1).

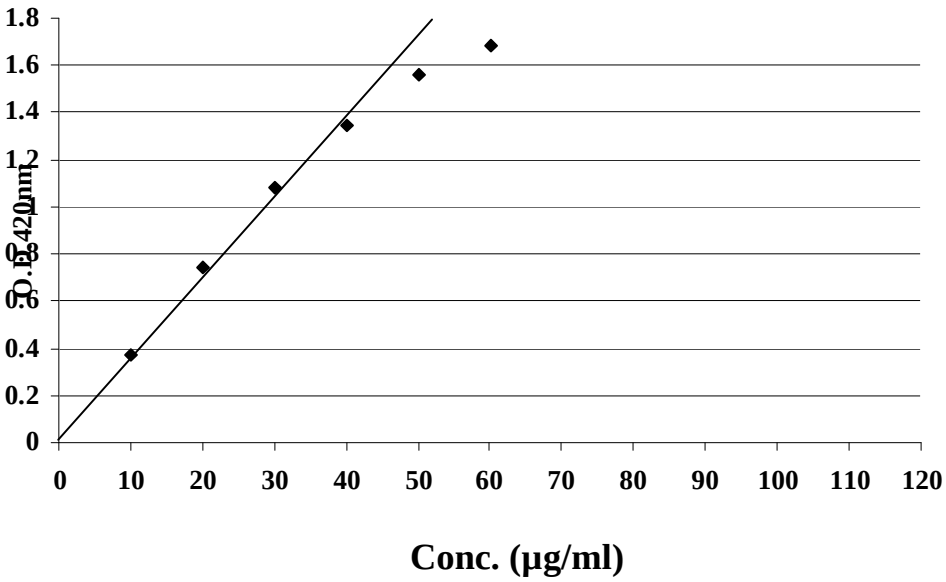


Figure (3-1): O-nitrophenol standard curve

3.2.10 Activity of β-galactosidase produced by locally isolated Lactobacillus acidophilus:-

A portion of 25ml of MRS broth was inoculated by 1% exponentially growing culture, and incubated at 37˚C for 18hr. The cells were harvested by centrifugation (6000rpm for 20min. at 2˚C), then washed with cold (6˚C) sodium phosphate buffer, and resuspended in the same buffer (Noh and Gilliand , 1994). The following steps were performed to determine the enzyme activity according to Atlas *et al* (1995):

a- At first two tubes containing the following ingredients were prepared :

Component	Tube 1	Tube 2
Z-buffer	0.5ml	0.5ml
Culture	0.5ml	0ml

b- Tubes were placed in a water bath at 30˚C for 3 minutes .

c- One drop of (1%) SDS was added to each tube and quickly mix by flicking the bottom of the tube.

d- Two drops of chloroform were added and mix by vortex for 20 second.

e- Tubes were placed in water bath, then 200µl of 4mg/ml ONPG was added to each tube and mixed . After that tubes were incubated at 30˚C for 30 minute.

f- Two milliliters of Na_2CO_3 were added and tubes were transferred to an ice bath.

g- Contents of the tubes were centrifuged at 5000rpm for 10 minute.

h- Supernatants were taken and the optical density was recorded by spectrophotometer at 420nm.

Unit of activity was defined as the quantity of enzyme that will liberate o-nitriphenol at a rate of 1µmol/min under the condition of the experiment

(30min. incubation at 30˚C). Enzyme activity was determined by returning to the o-nitrophenol standard curve and using the following equation: µM of librated o-nitrophenol / TxV , while T= time of reaction and V= volume of culture.

3.2.11 Determination of Protein concentration :

Bradford method (1976) was used for protein estimation, different concentration of bovine serum albumin (2-20)µg/ml were prepared, aportion of 20µl was transferred from each concentration in test tubes , and then 50µl of NaOH (1M) and 1ml of commassie blue reagent were added to the tubes. After well mixing, it was left at room temperature for 5 min. then the optical density was drawn against protein concentration.

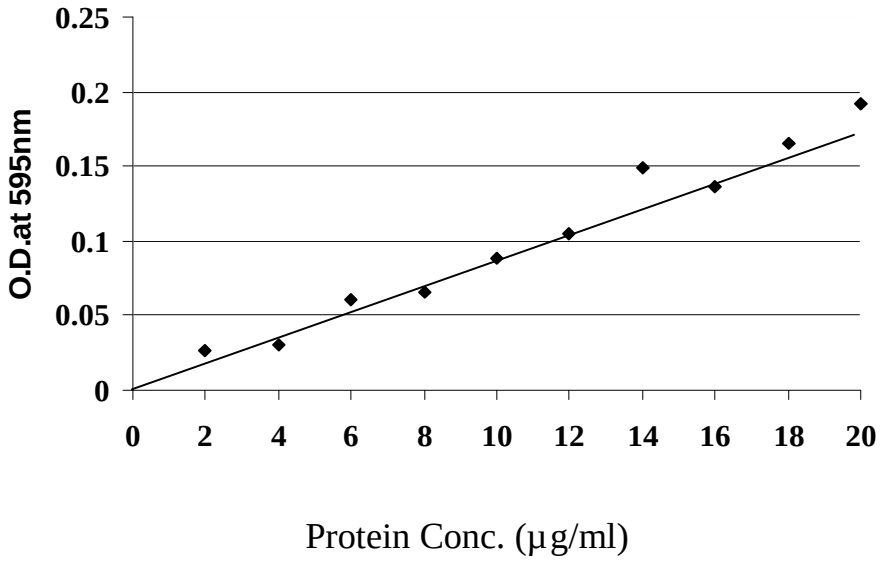


Figure (3-2): Standard curve of Bovine Serum Albumin

3.2.12 Determination of Optimal pH for β-galactosidase Activity:

In conical flasks 25ml volumes of MRS broth containing lactose instead of glucose with different pH (3.5 , 4 , 4.5 , 5 , 5.5 , 6 , 6.5 , 7 , 7.5) were prepared and inoculated with 1% exponentially growing culture of *Lactobacillus acidophilus* isolated from stool and yoghurt samples then incubated at 37˚C under anaerobic condition . When cultures were reached an $O.D_{600}$ of (0.6), enzyme activity was determined according to the method described in (3.2.10).

3.2.13 Determination of Optimal Temperature for β-galactosidase Activity :

After 1% of each of stool and yoghurt isolate cultures was propagated in 25ml MRS broth containing lactose instead of glucose of their optimal pH, incubated at different temperatures (25, 30, 35, 37, 40 , 45˚C), cultures were let to reached an $O.D_{600}$ of 0.6. Then enzyme activity was determined according to the method described in (3.2.10).

3.2.14 Selection The Best Isolate for β-Galactosidase Activity:

All the isolates of stool and yoghurt samples were growing at their optimum pH and temperature, when the ($O.D._{600}$ = 0.6), β-galactosidase activity was determined to each isolate to choose the most active one according to the method described in (3.2.10).

3.2.15 Isolates Production of β-galactosidase on xgal-MRS medium :-

One loopfull of each bacterial isolates was separately inoculated in X-gal MRS medium and incubated at 37˚C for 2-3 days. Isolates showed changing to blue were selected and considered to be β-galactosidase producers.

3.2.16 Estimation of Lactose Consumption by *Lactobacillus acidophilus* :

3.2.16.1 Preparation of Lactose Standard Curve :-

Stock solution of lactose was prepared by dissolving 2g of lactose in 100ml of D.W. Different concentrations were then prepared from it as follow:

Tube number	Lactose volume(ml)	Volume of added D.W.(ml)	Lactose conc.(g/ml)
1	0	1.0	0
2	0.1	0.9	0.002
3	0.3	0.7	0.006
4	0.5	0.5	0.01
5	0.7	0.3	0.014
6	0.9	0.1	0.018
7	1.0	0	0.02

Then 1ml of DNSA reagent was added to each tube and placed in boiling water bath for 5min. After cooling directly under tab water, 10ml of D.W. was added and optical density was recorded by spectrophotometer at 540nm. First tube was set as blank to zero the instrument (Whitaker and Bernhard,1972). Figure (3-3) represents the obtained standard curve of lactose.

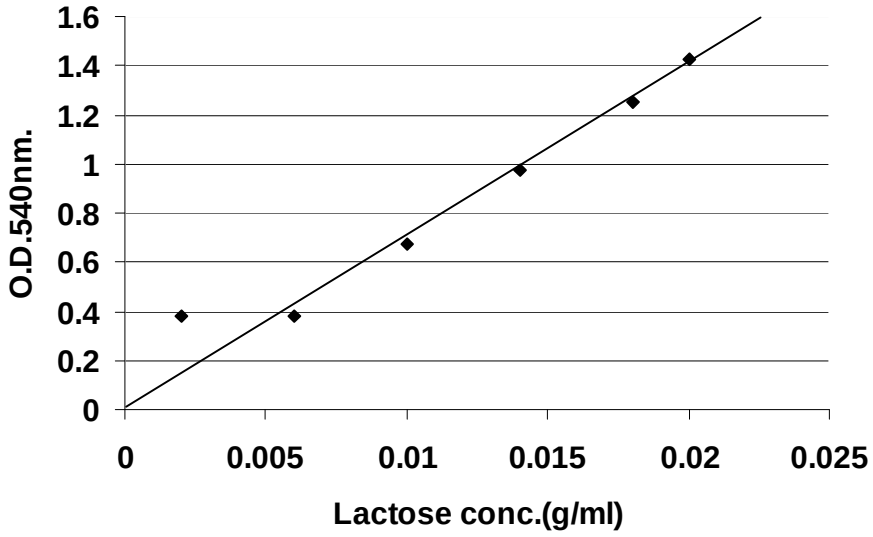


Figure (3-3) Lactose standard curve

3.2.16.2 Sample Estimation:

Five flasks containing MRS broth had lactose in defined concentration (0.02g/ml) were inoculated with five inoculums size (1, 1.25, 1.5, 1.75, 2)% of exponentially growing bacteria (the most active one) and incubated for different incubation periods (18 , 24 , 36 , 48hr) . Another one was set as a control (without) inoculum. After each incubation period total viable count was performed, and then bacterial cells were precipitated by centrifugation at 5000rpm for 20min. From each supernatant, (2)ml were taking and (1)ml of D.W. was added to it , then (1)ml of DNSA reagent was added and placed in boiling water bath for 5min. followed by the addition (10)ml D.W. After that optical density was read in spectrophotometer at 540nm and lactose remaining in the medium was determined by returning to the lactose standard curve.

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Int 3:- http://www.aboutibs.org/Publications/dietaryGuidelines.html#LI Lactose intolerance treatment

Int4:-http://digestive.niddk.nih.gov/ddiseases/pubs/lactoseintolerance/index.htm β-galactosidase activity

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المصادر العربية:

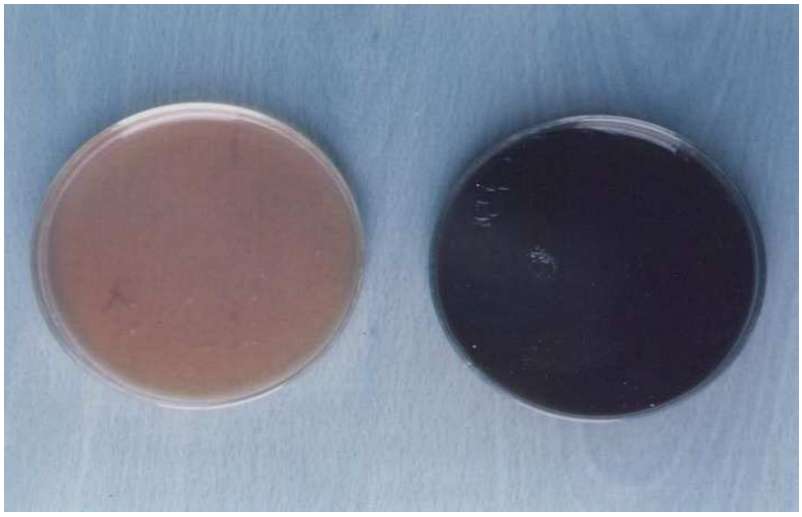
القصاب ، عبد الجبار عمر. (١٩٨٨). التأثير المضاد لبكتيريا حامض اللبنيك على بعض انواع البكتريا المرضية، رسالة ماجستير ،كلية الزراعة-جامعة بغداد.

4.1 Isolation of *Lactobacillus acidophilus* from Stool Samples :-

From a total of 35 stool samples collected, 30 of them gave pale, round shape , soft , mucoid colonies on MRS agar in the first isolation step . Such result coincide with those mentioned by De Man *et al* (1960) who insisted that MRS medium is the most selective medium for *Lactobacillus* spp. In the second step after transferring colonies to MRS-$CaCO_3$ agar medium some of them gave clear zones surrounding them as a result of acid production and $CaCO_3$ dissolving. Microscopic examination declared that cells were gram(+) , bacilli, groups mainly in chain, non-sporeforms. The third step which included transferring colonies to the Aesculine-cellobiose medium, all undesirable microorganisms other than *Lactobacillus* spp. were excluded .Hunger(1986) stated that the following species of *Lactobacillus*(*Lb. acidophilus, casei* , *rhumnosus* , *planetarium*) have the ability to hydrolyze asculine converting it to asculitine which be detected by dark green color (plate 4-1).

To isolate *Lactobacillus acidophilus* from other species, fourth step was applied by growing on MRS raffinose when results shown that only *Lactobacillus acidophilus* grow due to its ability to utilize raffinose on this medium (Holt and Krieg, 1986).

Depending on the above results, 12 isolates of *Lactobacillus spp* were obtained to be used for further experiments.



A= Aesculine –cellobiose medium (control).

B= converting the color of the medium by *L. acidophilus* .

Plate (4-1): Ability of *Lactobacillus* spp hydrolyze asculine.

4.2 Isolation of *Lactobacillus acidophilus* from yoghurt samples:-

From a total of 20 yoghurt samples collected only 8 isolates of *Lactobacillus acidophilus* were obtained after using the same previous screening method for stool samples.

4.3 Identification of *Lactobacillus acidophilus*:-

4.3.1 Cultural characteristics :-

Colonies of *Lactobacillus acidophilus* on MRS agar were small, pale, round shape , soft , mucoid , and convex , and appeared to be just below the surface of the medium . Almost similar characteristic were mentioned for the bacteria by Briggs (1953) .

4.3.2 Microscopic Characteristics :

Cells of *Lactobacillus acidophilus* appeared after staining by gram method as rods either single or in pairs but mostly grouped as chain. They were gram positive and non-sporeforming.

4.3.3 Biochemical Tests :-

All the *Lactobacillus* suspected isolates gave negative results in the catalase test due to their lack for catalase , which was detected when bubbles were appeared after the addition of hydrogen peroxide. Also the isolates were unable to produce ammonia from arginine and convert the orange color to red, while most of the isolates had the ability to hydrolyze starch on starch agar medium (Holt and Krieg , 1986).More over all isolates were unable to produce gelatinase after grow on gelatin medium which agreed with Buchanan and Gibbons (1974).

The isolates were, also, unable to hydrolyze casein when grow on milk agar medium, due to their inability to produce protease as mentioned by Robinson (1990). Morever, they gave negative results in the indole test because of inability to produce tryptophanase which hydrolyzes tryptophan to indole and forming a red layer (Holt and Krieg , 1986).

When various temperatures were used to grow the lactic acid isolates, all were unable to grow in 15˚C while they grew in 45˚C. except two isolates . Carbohydrates fermentation test also was performed to detect and identify *Lb.acidophilus* . Results showed that all the isolates were able to ferment (glucose , cellobiose , fructose , lactose , raffinose , and aesculine) but were unable to ferment mannitol and xylose , except two isolate which were xylose ferment excluded (Kandler and Weiss , 1986 ; Holt and Kriege ,1986).Table (4-1) shows biochemical tests.

Table (4-1) Biochemical Tests for Characterization of *Lactobacillus acidophilus* :-

isolates / Test	Lbs 1	Lbs 2	Lbs 3	Lbs 4	Lbs 5	Lbs 6	Lbs 7	Lbs 8	Lbs 9	Lbs 10	Lbs 11	Lbs 12	Lby 1	Lby 2	Lby 3	Lby 4	Lby 5	Lby 6	Lbs 7	Lby 8
Catalase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Indole	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Starch hydrolysis	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Casein hydrolysis	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
NH_3 from arginin	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Acid and curd production	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
Growth at 15˚C	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Growth at 45˚C	+	+	+	+	+	-	+	+	+	+	-	+	+	+	+	+	+	+	+	+
Acid production from carbohydrates sources																				
Glucose	(1) +	(1) +	(1) +	(2) +	(1) +	(1) +	(2) +	(1) +	(1) +	(1) +	(1) +	(1) +	(2) +	(1) +	(1)+	(1) +	(1) +	(1) +	(1) +	(1) +
Lactose	(1) +	(2) +	(1) +	(1) +	(1) +	(2) +	(1) +	(1) +	(1) +	(2) +	(1) +	(1) +	(1) +	(1) +	(1)+	(1) +	(1) +	(1) +	(1) +	(2) +
Fructose	(2) +	(1) +	(1) +	(2) +	(2) +	(1) +	(1) +	(2) +	(2) +	(1) +	(1) +	(1) +	(2) +	(1) +	(2)+	(2) +	(1) +	(1) +	(1) +	(1) +
Mannitol	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Raffinose	(1) +	(1) +	(1) +	(2) +	(1) +	(1) +	(2) +	(1) +	(2) +	(1) +	(1) +	(1) +	(1) +	(2) +	(1)+	(1) +	(1) +	(1) +	(1) +	(2) +
Xylose	-	-	-	-	-	-	(2) +	-	-	-	-	-	-	-	-	-	-	(1) +	-	-
Aesculine	(1) +	(1) +	(2) +	(2) +	(1) +	(1) +	(1) +	(1) +	(1) +	(1) +	(1) +	(1) +	(2) +	(1) +	(2)+	(1) +	(2) +	(3) +	(2) +	(1) +
Cellobiose	(1) +	(2) +	(1) +	(2) +	(1) +	(1) +	(2) +	(2) +	(1) +	(1) +	(3) +	(2) +	(1) +	(3) +	(1)+	(3) +	(3) +	(2) +	(1) +	(1) +

Lbs1 to Lbs12 = stool isolates

Lby1 to Lby8 = yoghurt isolates

+ = positive result , - = negative result

( ) = no. of days to change the color.

4-4 Growth curve of *Lactobacillus acidophilus*:

Growth characterization of selected isolates were done to determine the extent of each growth phase in order to be used as basic information required for measuring β-galactosidase activity .

When a bacterium is inoculated in to a new culture medium, it exhibits a characteristic growth curve which consisted in the normal growth from four phases: the lag phase, the log or exponential growth phase, the stationary phase and the death phase.

Figure (4-1) shows that lag phase took about 2 hours. During this phase there no increase in cell number was detected; which may be related for the cells to prepare for reproduction, synthesizing DNA and various inducible enzymes needed for cell division (Atlas *et al*., 1995) .This phase was followed by the log phase when number of cells was increase and bacterial biomass increases linearly with time. After about 17 hours, bacterial cells entered the stationary phase, during which there was no considerable further net increase in bacterial cell numbers, when the growth rate is equal to the death rate.

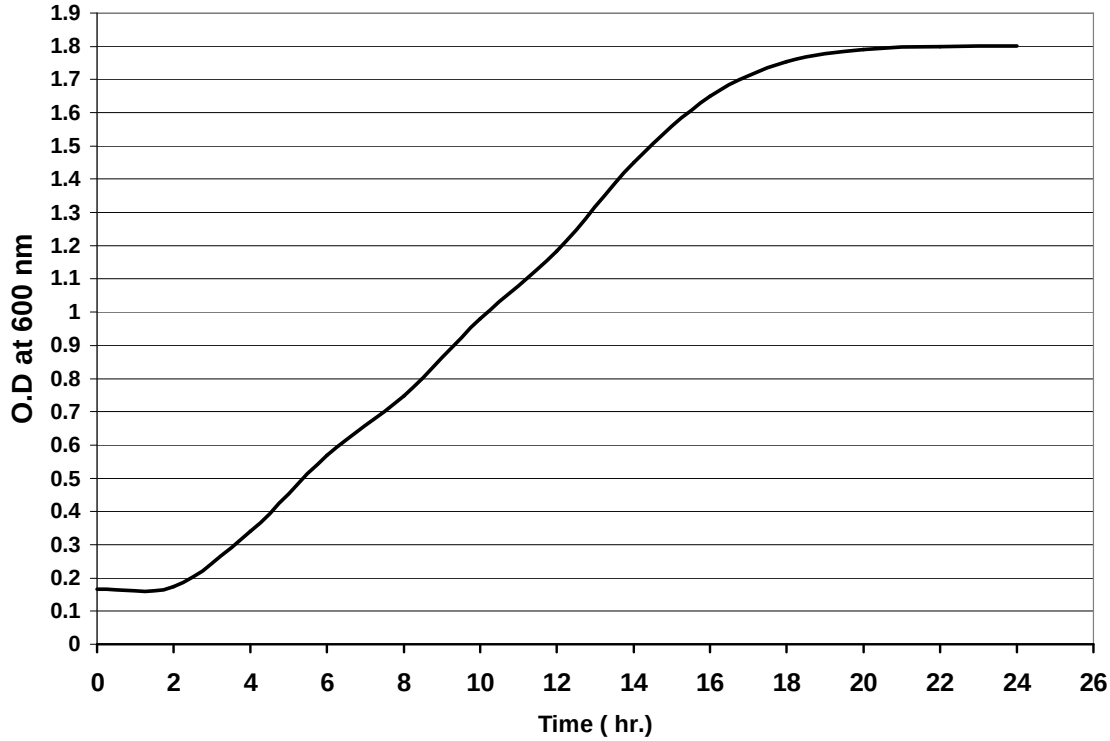


Figure (4-1) Growth curve of *Lactobacillus acidophilus* in MRS medium.

4.5 Optimal Conditions for β-galactosidase Activity:-

4.5.1 Optimal pH for β-galactosidase production :-

To investigate the effects of the initial medium pH on β-galactosidase activity , one stool isolate and another yoghurt isolate were grown in MRS broth containing lactose instead of glucose with different pH values (3.5 , 4 , 4.5 , 5 , 5.5 , 6 , 6.5 , 7 , 7.5) then incubated at 37˚C overnight .

Results in figure (4-2) and (4-3) showed that the two isolates had recorded their highest activities at pH 6.5 and less activities at pH 3.5. Jiang and Savaiano (1997) reported that *Lactobacillus acidophilus* exhibited maximum β-galactosidase activity at pH 6.7 and this is likely in agreement with the results of this study. While Noh and Gilliland (1994) found β-galactosidase activity of *Lactobacillus delbrueckii* and *Lactobacillus bulgaricus* in an optimal pH of 7.

The effect of pH on enzyme production resulted from its role in the solubility of the nutritional substances of the medium, its effect on the ionization of the substrates and its availability to the microorganism in addition to its effect on the stability of the produced enzyme (Bull and Bushnell , 1976).

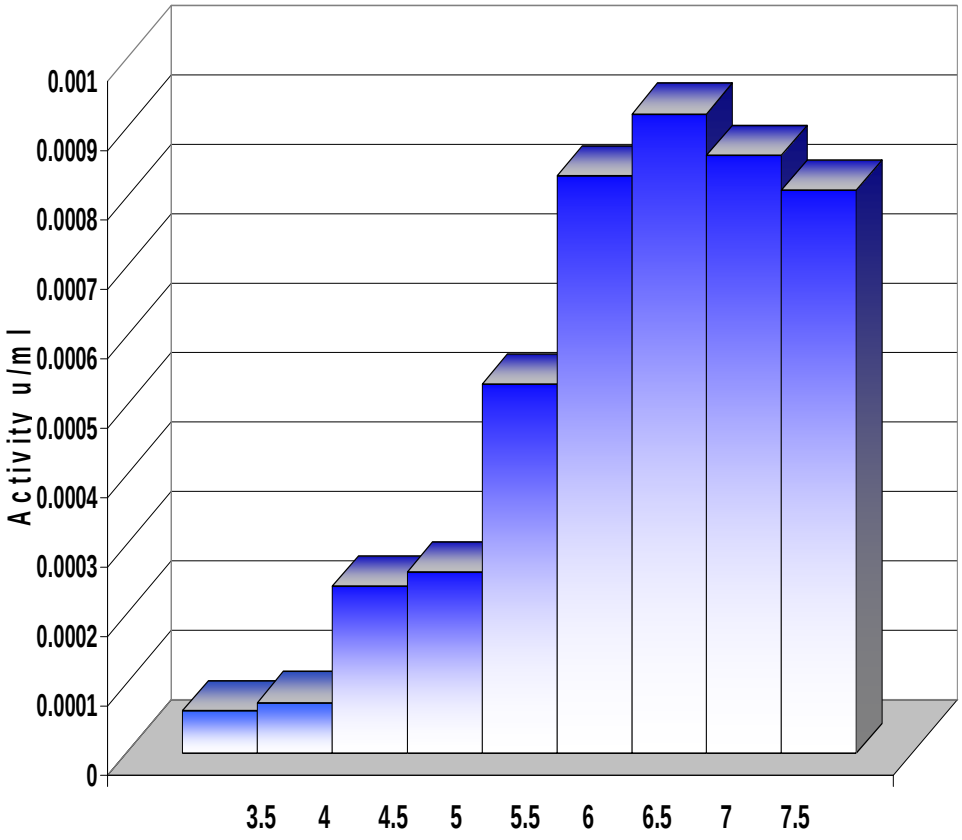


Figure (4-2): Effect of initial pH on β-galactosidase activity of *Lactobacillus acidophilus* (Lbs1) grown in MRS medium at 37˚C overnight.

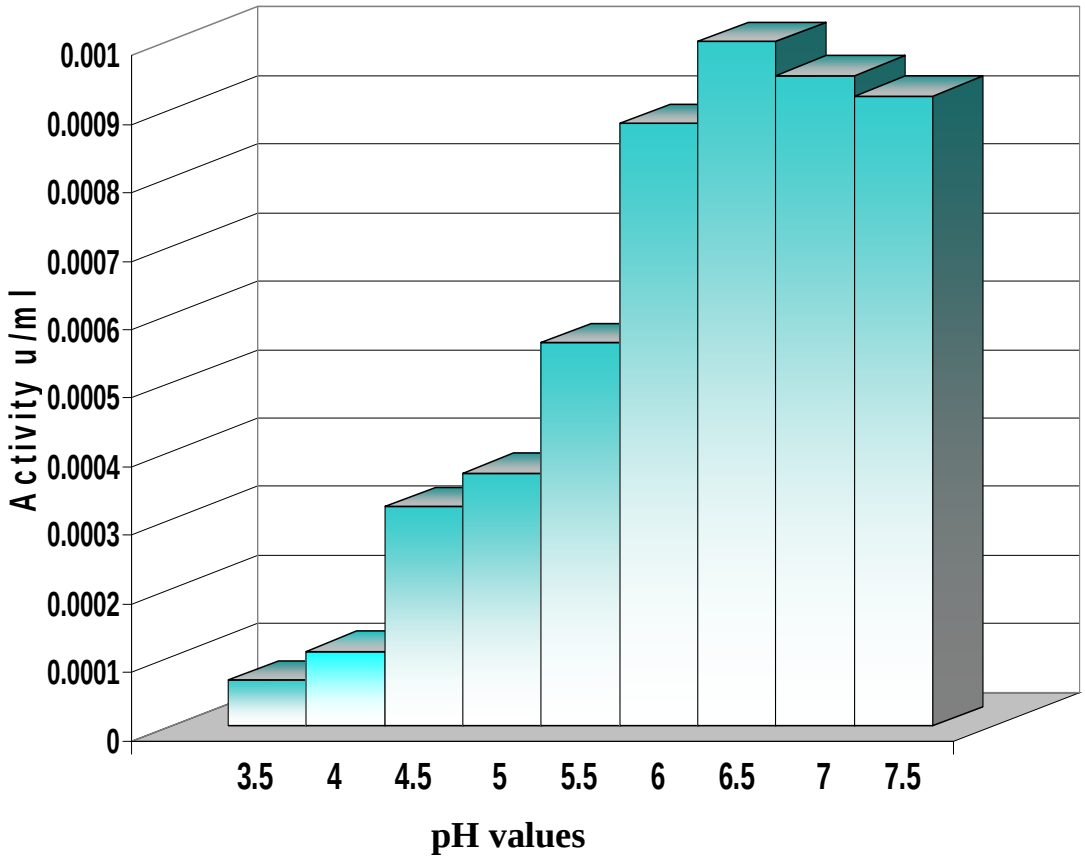


Figure (4-3) Effect of initial pH on β-galactosidase activity of *Lactobacillus acidophilus* (Lby1) grown in MRS medium at 37˚C overnight.

4.5.2 Optimal Temperature for β –galactosidase production :–

The temperature dependence of the β-galactosidase activity of cell extract , which was obtained from cells grown at different temperatures (25 , 30 , 35 , 37 , 40 , 45˚C) in MRS medium containing lactose instead of glucose with optimal pH 6.5 , was estimated, after incubation overnight .

Results in figure (4-4) showed that the stool isolate had highest β-galactosidase activity when incubated at 37˚C and less activity at 25˚C. Results in figure (4-5) on other hand showed that the yoghurt isolate had highest β-galactosidase activity at 40˚C and less activity at also 25˚C for the same period of time .

Jiang and Savaiano (1997) found that maximum β-galactosidase activity of *Lactobacillus acidophilus* was achieved at 37˚C , Noh and Gilliland (1994) stated that growing *Lactobacillus delbrueckii and Lactobacillus bulgaricus* at 50˚C gave the maximum β -galactosidase activity .

Generally, the decay of enzyme activity with increasing or decreasing temperature is attributed to thermal effects on the growth of the microorganism and velocity of the enzymatic reaction inside the cells reflecting the properties of the enzyme and its local environment (Cornish –Bowden , 1979).

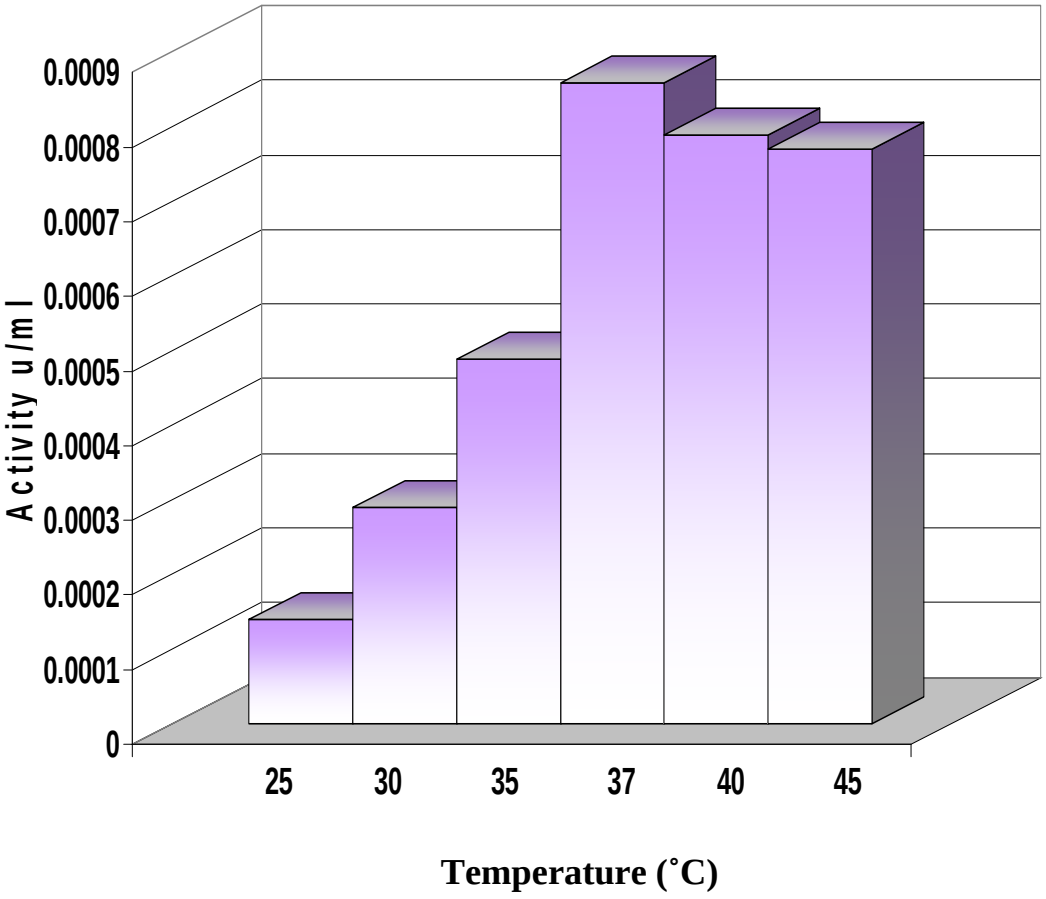


Figure (4-4): Effect of temperature on β-galactosidase activity that produced by *Lactobacillus acidophilus* (Lbs1) which grew in MRS medium at 37˚C overnight.

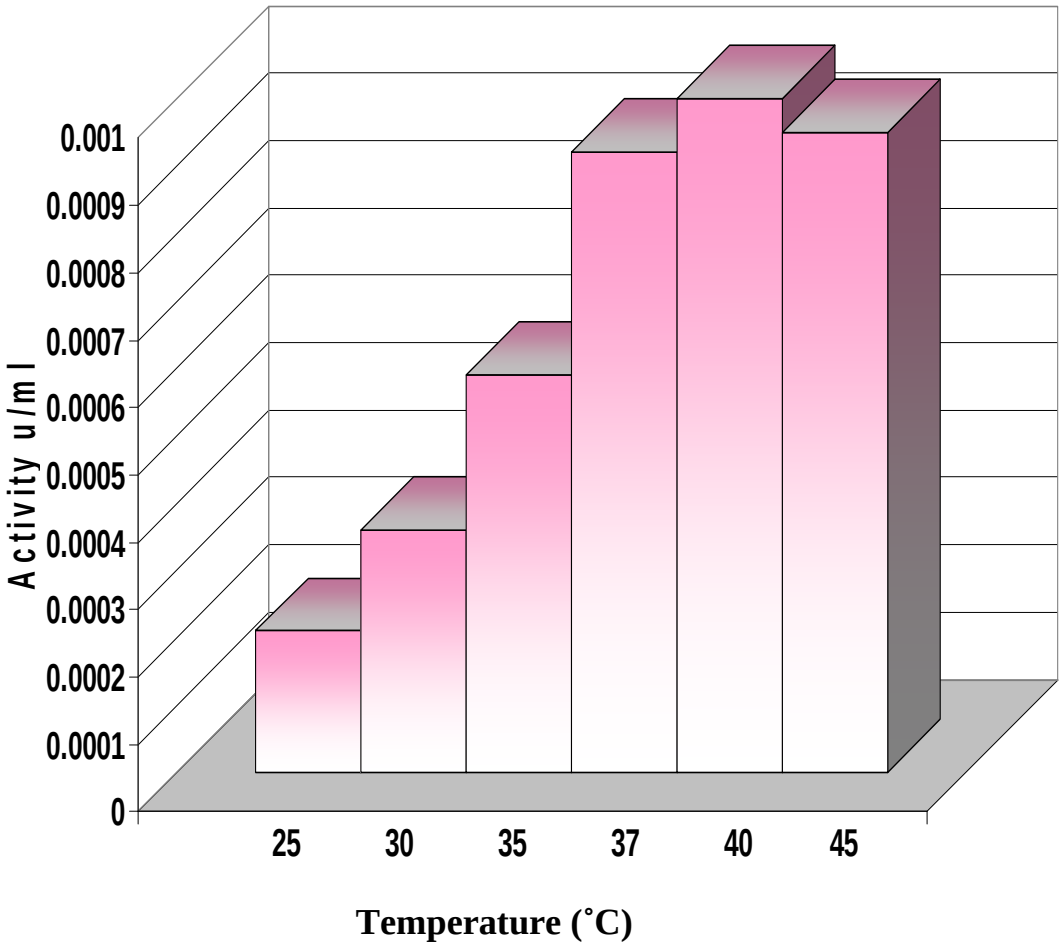


Figure (4-5): Effect of temperature on β-galactosidase activity that produced by *Lactobacillus acidophilus* (Lby1) which grew in MRS medium at 37˚C overnight.

4.6 Selection of most efficient isolate for β-galactosidase activity:

When all stool and yoghurt isolates have been grown in their optimal pH and temperature to select the most active one, results in table (4-2) showed that yoghurt isolate (Lby4) exhibited highest β-galactosidase activity among all other yoghurt and stool isolates about (0.0014)U/ml of 2.3µg/ml protein concentration. This result came in agreement with the result of De Verese (2001) who found that the yoghurt culture is more efficient than other cultures in treatment of lactose intolerance.

Table (4-2) β-galactosidase activity of *Lactobacillus acidophilus* isolates obtained from stool and yoghurt samples at their optimum conditions in MRS broth medium.

Isolates symbol	B-galactosidase activity (U/ml)
Lbs1	0.00056
Lbs2	0.00053
Lbs3	0.00074
Lbs4	0.0006
Lbs5	0.00077
Lbs6	0.00092
Lbs8	0.00068
Lbs9	0.00088
Lbs10	0.00092
Lbs11	0.00047
Lbs12	0.00089
Lby1	0.00095

Lby2	0.00099
Lby3	0.001
Lby4	0.0014
Lby5	0.00099
Lby7	0.001
Lby8	0.001

Lbs1 to Lbs12 are stool isolates.

Lby1 to Lby8 are yoghurt isolates.

4.7 production of β-galactosidase by *Lactobacillus acidophilus* isolates on Xgal-MRS medium:-

Bacterial culture was grown on Xgal-MRS medium to investigate the ability of the bacterial isolate for production of β-galactosidase. Results in plate (4-2) showed that the isolate was able to produce β-galactosidase after hydrolyzing their chromogenic substrate X-gal and forming blue color. The turning to blue color took more than two days due to the need for oxygen and because the bacteria was incubated under anaerobic conditions , Ibrahim and Sullivan (2000) used this chromogenic substrate to select the over producing β-galactosidase enzyme *Lactobacillus* mutant .

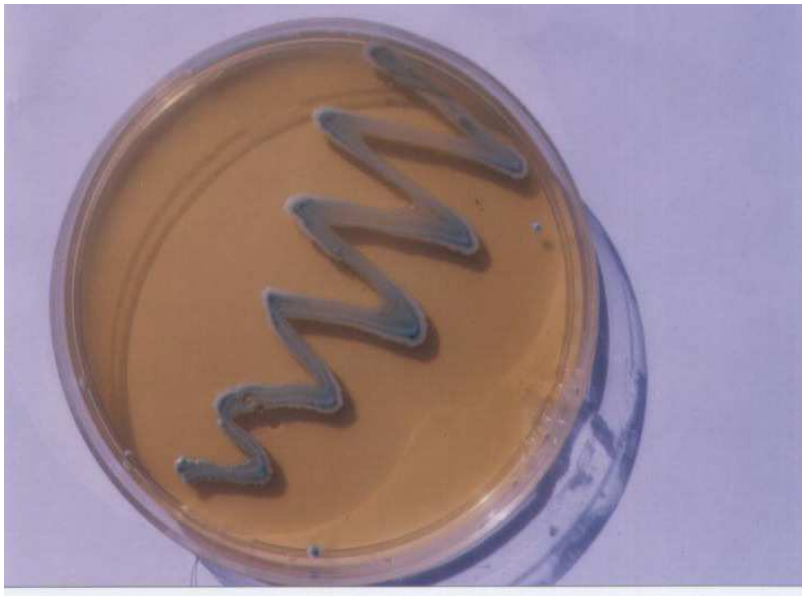


Plate (4-2) : Ability of *Lactobacillus acidophilus* isolate for production of β-galactosidase on Xgal-MRS agar medium .

4.8 Lactose consumption (utilization) by *Lactobacillus acidophilus*:-

Five inoculums sizes (1 , 1.25 , 1.5 , 1.75 , 2%) and four incubation periods(18 , 24 , 36 , 48hr.) were used for more utilization of lactose(0.02g/ml) by the most active isolate(Lby4) in the culture medium . Results in table (4-3) showed that lactose utilization was increased with increasing of inoculums size (from 1 to 2%) and with increasing of incubation period (from 18-48hr.). In the inoculation with 1% , the lactose consumption by bacterial growth was (0.61)g from total (1)g of lactose in MRS medium after incubation from 18 to 48hr. , while bacterial count was $22.5x10^{8}$ cell/ml . By increasing the inoculum size to 1.25% the consumption only increased (0.06)g with bacterial number of

$24.2x10^{8}$ cell/ml . Also by increasing the inoculum size to 1.5% and bacterial number to $29.7x10^{8}$ cell/ml lactose utilization reach (0.77)g , and with inoculation by 1.75% ,(0.16)g of lactose more consumed by $22.1x10^{9}$ cell/ml than previous inoculation. Maximum utilization of lactose obtained by inoculation with 2% for 48hr.,when $28.5x10^{9}$ cell/ml able to reduce about (1)g of lactose in the culture media .Results in 50ml of culture medium.

Lactose concentration in MRS broth without inoculation was ranged from (0.92 to 0.96)g, small amount of lactose was lost may be by sterilization process .

The lactose in the medium act as an inducer for production of more β-galactosidase from the bacteria that consumed the lactose by hydrolyzing it to glucose and galactose.

However lactose consumption by bacterial culture was rather slow which may be because the incubation of culture was made without shaking which used to help the bacteria growing fast and consumed more nutrients as lactose.

Bodun *et al* (2001) found that the incubation of cell-associated β-galactosidase (probiotic microorganisms) with high concentration of lactose increases the lactose reduction with time , and the effect was most marked for *Lactobacilli* and *Bifidobacteria*.

Result by Jiang and Savaiano (1997) showed that 5g of lactose reduced in 7 days by continues culture.

Table (4-3) Effect of inoculum size of *Lactobacillus acidophilus* (Lby4) on Lactose consumption in MRS broth after incubation for(18,24,36.48)hr. at 40˚C.

Control	0.96g
Stock	$4.5x10^6$ cell/ml
Inoculation with 1% (500µl)	

Time of incubation/hr.	Remaining Lactose/g	cell/ml
18	0.67	$12.5x10^7$
24	0.54	$18.7x10^7$
36	0.46	$19.5x10^8$
48	0.39	$22.5x10^8$

Inoculation with 1.25% (625µl)		
Time of incubation/hr.	Remaining Lactose/g	cell/ml
18	0.6	$14.5x10^8$
24	0.48	$16.9x10^8$
36	0.4	$22.1x10^8$
48	0.33	$24.2x10^8$

Inoculation with 1.5% (750µl)		
Time of incubation/hr.	Remaining Lactose/g	cell/ml
18	0.51	$17.8x10^8$
24	0.42	$21.5x10^8$
36	0.31	$26.1x10^8$
48	0.23	$29.7x10^8$

Continue of table (4-3)

Inoculation with 1.75% (875µl)		
Time of incubation/hr.	Remaining Lactose/g	cell/ml
18	0.39	$13.1x10^9$
24	0.32	$15.3x10^9$
36	0.19	$17.7x10^9$
48	0.07	$22.1x10^9$
Inoculation with 2% (1000µl)		
Time of incubation/hr.	Remaining Lactose/g	cell/ml
18	0.32	$14.1x10^9$
24	0.16	$18.8x10^9$
36	0.04	$24.1x10^9$
48	0.003	$28.5x10^9$

Summary:

A total of 35 sample of infants stool collected from Al-Mansor hospital for children and 20 sample of dairy products collected from Baghdad markets. Eleven isolate of *Lactobacillus acidophilus* were obtained from stool sample and seven isolates of also *Lactobacillus acidophilus* were obtained from yoghurt , the bacteria isolated and identified according to screening method and using specialized growth media.

Isolates tested for their ability to produce β-galactosidase by using O-Nitrophynel-β-D-Galactosidase (ONPG) which is lactose analog , and the ability of these isolates to enzyme production were detected in the medium by using X-gal as chromogenic substrate for β-galactosidase which turn the color of colony from white to blue.

Optimal pH and temperature for enzyme activity were determined and found that the stool isolates gave highest β-galactosidase activity at pH 6.5 and 37˚C while the yoghurt isolates gave a highest enzyme activity at pH 6.5 and 40˚C .

Enzyme activity was determined for all stool and yoghourt isolates under their optimal condition, and found that the yoghurt isolates is more active in enzyme production than stool isolates, and from the yoghurt isolates the most active one was selected for further study.

The ability of the most active yoghurt bacterial culture for lactose consumption was estimated by using five inoculums size (1 , 1.25 , 1.5 , 1.75 , 2%) and for four incubation periods (18 , 24 , 36 , 48hr.) with defined sugar concentration (1g/50ml) , total viable count was performed after each incubation periods , and found that by inoculation with 2% and incubation for 48 hour , 28.5×10^9 cell/ml was able to consume about 1g of lactose.

الأهداء

الى من لا قدرة لي على وصفها وكيل المديح والثناء لها

أمي

الى خير مستخلف حفظ الأمانة وصانها

أبي

الى الغائب الحبيب الذي سأبقى أفتقده الى آخر يوم من حياتي

أخي الشهيد الوحيد

الى من أحتضنتني وهي بعد بحاجه الى من يحتضنها

أختي الكبيره

الى اللواتي متعت معهن براحة النفس ومعشر الود والمحبة

أخواتي

الى أول من علمني حرفا وحتى آخر من تلقيت على يده علما

أهدي ثمرة جهدي المتواضع

زهراء

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College of Science
Department of Biotechnology

Isolation and Characterization of *Lactobacillus acidophilus* and Its Ability in β-galactosidase Production

A thesis submitted to the college of science of Al-Nahrain University

as partial fulfillment of the requirements for the degree of Master Of Science in Biotechnology

By

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B.Sc. 2003

Al Nahrain University

September 2006 **Shaban 1427**

جمهورية العراق
وزارة التعليم العالي و البحث العلمي
جامعة النهرين
كلية العلوم
قسم التقانة الاحيائية

عزل وتشخيص بكتريا الـ *Lactobacillus acidophilus* وقابليتها على إنتاج أنزيم الـ *β-galactosidase*

رسالة مقدمة الى كلية العلوم في جامعة النهرين جزأ من متطلبات نيل درجة ماجستير علوم في علوم التقانات الحياتية

من قبل

زهراء عبد المنعم عبد الهادي شربه

بكلوريوس تقانة احيائية ٢٠٠٣
جامعة النهرين

أيلول ٢٠٠٦ شعبان١٤٢٧

الملخص:

تم جمع ٣٥ عينة من براز الاطفال الرضع من مستشفى المنصورللأطفال و٢٠ عينة من منتجات الالبان المأخوذة من الاسواق المحلية في بغداد وقد تم الحصول على ١١ عزلة من بكتريا الـ *Lactobacillus acidophilus* من عينات براز الاطفال و٧ عزلات من منتجات الألبان ، شخصت العزلات على اساس الحذف بأستخدام أوساط زرعية متخصصة.

تم التحري عن قابلية هذه العزلات لانتاج أنزيم الـ β-galactosidase وقياس الفعاليه لها باستخدام الـ ONPG كما اختبار قدرة العزلات على انتاج الأنزيم وتحليل سكر اللاكتوز(*in vitro*) وذلك بتنميتها على وسط الـ MRS الحاوي على مادة أساس ملونة (chromogenic substrate) X-gal عند تحللها من قبل الأنزيم تحول المستعمرات إلى اللون الأزرق ، ووجد ان كل هذه العزلات لها القدره على ذلك.

درست الظروف المثلى لإنتاج الأنزيم من قبل بكتريا الـ *Lactobacillus acidophilus* لكل من العزلة المأخوذة من البراز والمأخوذة من منتجات الألبان على حدا بتنميتها إلى الطور اللوغارتمي ($O.D_{600}=0.6$)في وسط الـ MRS ووجد أن العزلة المأخوذة من البراز أعطت أعلى فعالية أنزيمية لها عند رقم هيدروجيني ابتدائي ٦,٥ والحضن بدرجة حرارة ٣٧م° بينما العزلة المأخوذة من منتجات الألبان أعطت أعلى فعالية أنزيمية لها عند رقم هيدروجيني ابتدائي ٦,٥ والحضن بدرجة حرارة ٤٠م° الفعالية الإنزيمية عند الظروف المثلى حسبت لكل العزلات ووجد أنها أعلى في العزلات المأخوذة من منتجات الألبان من تلك المأخوذة من براز الأطفال. قابلية العزلة ذات

الفعالية الأنزيمية الأعلى على استهلاك سكر اللاكتوز (١غرام\٥٠ مل) من الوسط الزرعي حسبت باستخدام تراكيز مختلفة من اللقاح البكتيري(١%،١,٢٥%،١,٥%،١,٧٥%،٢%)وبفترات حضن مختلفة لكل لقاح(١٨ ،٢٤ ،٣٦ ،٤٨ساعة) عند الظروف المثلى لها من رقم هيدروجيني ابتدائي ٦,٥ والحضن بدرجة حرارة ٤٠م° .وتم حساب عدد البكتريا بعد كل مدة حضن ووجد انه عند التلقيح بـ ٢% وبعد فترة حضن ٤٨ ساعة ، ٢٨,٥x ١٠ ^{9}- خلية\مل لها القدرة على استهلاك واحد غرام من سكر اللاكتوز.

بسم الله الرحمن الرحيم

يُؤتي الحِكمَةَ مَن يَشَاءُ وَمَن يُؤتَ الحِكمَةَ فَقَد أُوتي خَيراً

كَثيراً وَمَايَذَّكَّرُإِلّآأُوْلُواْ الأَلبَبِ

صدق الله العلي العظيم

سورة البقرة الأيه :(٢٩٦)