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Journal of Bioscience and Applied Research
www.jbaar.org

Antimicrobial activity of *Lactobacillus acidophilus* against pathogenic *Escherichia coli* isolated from diarrhea patients

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DOI : 10.21608/jbaar.2022.255697

Abstract

Lactobacillus acidophilus represents a probiotic bacterium that may be found in the mouth, gut, and vaginal canal. *Lactobacillus acidophillus* may help to relieve diarrhea, bloating, and cramps caused by bacteria that may cause everything from diarrhea to life-threatening colon inflammation. Escherichia coli (hencefourth *E. Coil*) bacteria are found in healthy people's intestines as natural flora. Only a very small percentage, like the *E. coli* O157:H7 variety, can lead to severe stomach discomfort, bloody diarrhea, and vomiting; the rest of the *E. coli* variety is harmless.

Keywords: *Lactobacillus acidophilus*, Escherichia coli, probiotics, diarrhea patients

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1. Introduction

Diarrhea is responsible for 2.5 million fatalities globally each year (Kosek *et al.*,2003). In undeveloped nations, infectious results from acute diarrhea are commonly associated with contaminated food and water sources (DuPont,1995). *Salmonella*, *Campylobacter*, *Shigella*, and *E. coli* that produce Shiga toxin are some of the most usual reasons for acute diarrhea in the US (enterohemorrhagic E. coli), all of which are pathogenic bacteria (Centers for Disease Control and Prevention, 2010). In children, acute diarrhea represents the most prevalent gastrointestinal ailment, and it is also the major reason behind dehydration (Farthing *et al.*, 2013). It is defined by the existence of three or more watery or loose stools each day over an extended period (Guarino *et al.*, 2008). In addition, anorexia, vomiting, stomach discomfort, and a raised body temperature are common symptoms of the illness during its earliest phase of development (Farthing *et al.*, 2013).

E. coli strains implicated in diarrheal disorders are one of the most significant of the different etiological agents of diarrhea, with strains evolving through the acquisition of a specific set of features effectively retained in the host via horizontal gene transfer (Croxen *et al.*,2013; Kaper *et al.*, 2004). 3, 5, and 6 three types of E. coli are linked to diarrheal illnesses. Enterotoxigenic *E. coli* are strains of Escherichia coli that generate enterotoxins (ETEC). Enterotoxins come in a variety of forms. Certain toxins are cytotoxic, destroying mucosal cells, while others are cytotonic, causing just the loss of water and electrolytes. The second group of *E. coli* bacteria has invasion factors that promote tissue death and inflammation like Shigella (EIEC). The third group of serotypes, known as enteropathogenic E coli (EPEC), is related to diarrhea epidemics in newborn nurseries but does not generate any toxins or invasion

Received: June 14, 2022. Accepted : August 2, 2022. Published: August 22, 2022

factors (Turner *et al*., 2006; Chattopadhyay *et al*., 2012). Anti-diarrheal drugs should be avoided since they slow down your digestion and prevent your body from removing toxins. Antibiotics aren't normally recommended since they might have serious negative effects (Nachamkin and Ung,2002).

2. *Lactobacillus acidiphillus*

Probiotics refer to a term taken from the Greek word probioticos, which means "for life." Around 100 years ago, Nobel laureate Elie Metchnikoff argued that consuming specific lactic bacilli might be useful to humans by increasing the health-promoting activities of the gut microbiota while reducing their potentially negative consequences. (Casas and Dobrogosz, 2000). Moreover, the notion of probiotics was broadened, as "a viable mono- or mixed- culture of microorganisms which applied to animal or man, beneficially affects the host by improving the properties of the indigenous microflora" (Havenaar and Huisin't Veld, 1992). Probiotics are now officially defined by the Food and Agriculture Organization (FAO) and the World Health Organization (WHO) as "live microorganisms that, when administered in suitable proportions, impart a health benefit on the host" (Araya *et al*., 2001). Probiotics are gaining popularity across the world, and as more information becomes available, the notion of probiotics will undoubtedly enter a new age (Salminen *et al*., 1999). There are already probiotic formulations available that contain *Lactobacillus acidophilus, Lactobacillus plantarum, Lactobacillus casei, Lactobacillus fermentum, Lactobacillus lactis,* and *Lactobacillus brevis* (Tissier, 1905; Auclair *et al*., 2015*). Lactobacillus acidophilus* points out as a homofermentative, microaerophilic species that ferments carbohydrates into lactic acid. It grows well at low pH (below pH 5.0) and a temperature of roughly 37 °C (Bâati*et al*.,2000). These strains are commercially employed in the manufacturing of acidophilus-type yogurt and acidophiline, often in combination with Streptococcus thermophilus and Lactobacillus delbrueckii subsp. bulgaricus (Fijan, Sabina, 2014). Lactose consumption, proteinase activity, bacteriophage resistance, and the generation of bacteriocins are only a few examples of the economic value of LABs (Araya *et al*, 2001).

2. Materials and Methods

2.1. sampling

For microbiological testing, samples were gathered from individuals with diarrhea illnesses of diverse ages and both sexes. (Each patient's name, gender, age, sampling date, and past therapy were recorded on a separate form.) The samples were collected in disposable containers and cultivated on blood agar and McConkey's agar before being submitted to last identification.

2.2 Identifying pathogenic bacterial isolates

2.2.1. Blood Agar Medium (Atlas *et al*., 1995)

It was made by autoclaving the blood base agar (pH 7.0) and cooling it to 45°C before adding 5% blood plasma and mixing thoroughly. Clear or green zones formed around the colonies to symbolize blood hemolysis.

2.2.2Vetic2 Identification

The microbe should be suspended in 3.0 mL of sterile salt solution (NaCl 0.45% to 0.50%, pH 4.5–7.0) in a 12 x 75 mm clear plastic (polystyrene) test tube with appropriate numbers of pure culture colonies transferred with the use of a sterilized brush or applicator stick. A turbidity meter named the DensiChek is used to alter and measure turbidity. The microorganism suspension is deposited in a specific rack (cassette) while the transfer tube is inserted into the corresponding suspension tube, and the identification card is placed in the adjacent slot. A vacuum chamber station is used to place the filled cassette. Micro-channels fill all the test wells when the vacuum is released and the air is reintroduced into the station, driving the organism suspension via the transfer tube into the test wells. Using distinct wavelengths in the visible spectrum, a transmittance optical system can evaluate test reactions. As a result of these computations, thresholds are established for each test. Test response findings on the VITEK 2 Compact are shown as +, –, (–), or (+). Weak responses are indicated by reactions that appear in parenthesis.

2.2.3. Carbon source fermentation medium: (Harrigan and MacCance, 1976).

Originally isolated from the vagina of healthy women, *Lactobacillus acidophilus* was obtained from the Biology Department, College of Science at Al-Mustansiriyah University. This medium was used for the identification of *Lactobacillus* spp. and prepared by using MRS broth containing 0.005% (v/v) bromocresol purple. After pH was adjusted to 6.2, the medium was distributed into test tubes (10 Ml each), then sterilized by the autoclave. Later, each carbon source (glucose, mannitol, xylose, lactose, sucrose, fructose, and sorbitol) was particularly added, separately, to the medium to obtain a final concentration of 2%.

2.2.4 Preparation of *Lactobacillus* filtrates:

2.2.4.1 Preparation of unconcentrated filtrate:

To obtain unconcentrated filtrates of *Lb. acidophilus*, each isolate culture was cultured in 9ml MRS broth for 24h at 37°C, and then 2% of the inoculum was added to 100ml of MRS broth and incubated at 37°C for another 24h in a candle jar at the same temperature. Then the isolates were centrifuged at 6000rpm for 10min. and the suspension was taken and filtrated through autoclaved Whatman filter paper No.1 (Moncada*et al.*, 2012; Izgü and Altinbay, 1997). This filtrate is considered the unconcentrated filtrate.

2.2.4.2 Preparation of concentrated filtrates:

Take 100ml of the unconcentrated filtrate and condense in the vacuum oven at 40-45°C until volume fell to 50ml, at which point a one-fold filtrate was formed. Two-fold concentrated filtrate (25ml) and the three-fold concentrated filtrate (12.5ml) were produced by repeating this experiment on the one-fold concentrated filtrate (12.5ml) (Izgü and Altinbay, 1997).

3. Results and Discussion

3.1 Isolation of bacteria:

There was a total of 60 samples taken from Fatima Alzarhraa Hospital for Diarrhea patients. It was found that 55(91.7%) from 60 samples show bacterial growth on culture media while 5 samples have no bacterial growth as shown in the table (3-1). The most common reason for this result is that the source culture was dead and dead bacteria generally look the same as live bacteria which leads to cannot assume the cells on an agar surface (Goodman AL, *et al.* 2011).

Figure (3-2) show that the age group from 1-12 years is the most common people are prone to getting diarrhea at a percentage of 50% while the age from 12-20 years is the less likely to get diarrhea and the age group from 20-80 they are less diarrheal infection this is agreed with Talan D, *et al.*, in (2001) who recorded that children are more prone than adults to develop symptoms, according to the researchers, and symptoms generally appear 3 to 4 days after exposure with E. coli. Some people are oblivious to any signs.

As it is displayed in a table (3-1). *Escherichia coli* was the most common bacterial species causing diarrhea with a total isolate of 25 and a percentage of 41. 7%, followed by 15 isolates (25%) for *Shigella,*10 *(*16.7%) *Salmonella* and 5 (8.3%) *Campylobacter*.The

Percentage of E.coli found in patients have diarrhea disease include 10% - 25% and other types of bacteria found in diarrhea patients are Salmonella include 3%, Shigella 10% and campylobacter 3-6% (Marder E.P, *et al*., 2017) while in another country, for example, the U.S the bacteria of diarrhea reach to 31% of all diarrheas and the reason of bacteria pathogen lead to foodborne diarrheal illness is Salmonella 15.4%, campylobacter 11.8%, Shigella 4.6% and Shigella toxin-producing E.coli (STEC) around 3% (Riddle MS, *et al*.,2016).

Table (3-1): The number and proportion of bacterial isolates acquired from Diarrhea patients

Number of Samples	Types of bacteria	percentage
25	*Escherichia coli*	41.7%
15	*Shigella*	25%
10	*Salmonella*	16.7%
5	*campylobacter*	8.3%
5	No growth	8.3%
60		100%

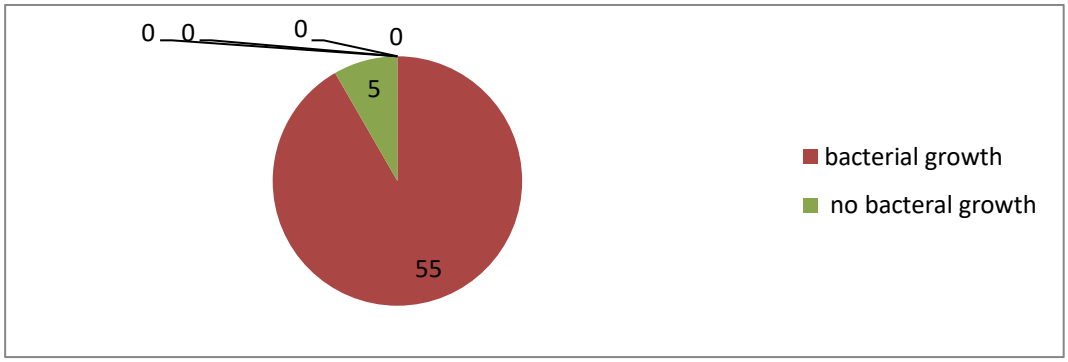

Figure (3-1): Percentage of bacterial growth

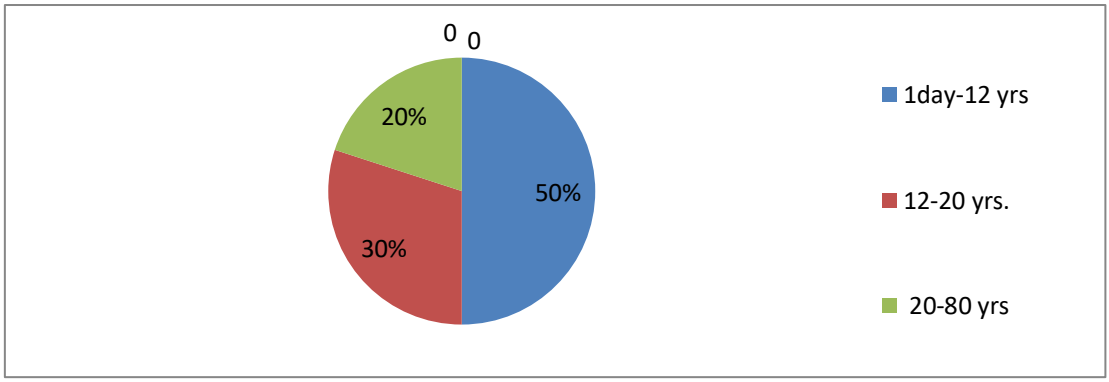

Figure (3-2): Percentage of diarrhea between age groups

3.2 Identifying bacterial isolates:

The suspected bacterial isolates were identified., primarily by cultural and microscopic examinations, then the species by the biochemical tests. The results obtained are illustrated as follows:

3.2.1Characterization on a cultural and microscopic level:

The suspected (55) bacterial isolates were identified first by colony growth on MacConkey and Blood agar surfaces, and then by microscopic properties based on Gram response. In MacConkey agar, bile salts and crystal violet are added to promote the growth of Enterobacteriaceae and related enteric Gram-negative rods while inhibiting Gram-positive bacteria and certain fastidious Enterobacteriaceae. When blood is added to the medium, it serves as an enrichment agent for the development of bacteria and other microorganisms. Based on the hemolysins they generate, hazardous bacteria can be differentiated from non-threatening ones (which lyse red blood cells) (Atlas *et al*.,1995).

3.2.2 Biochemical characterization (VITEK 2**):**

The bacterial isolates were identified by using VITEK 2 system by gram-positive and gram-negative cards in the Central Health Laboratory/Ministry of Health

3.3 Probiotics microorganism's characterizations:

3.3.1 *Lactobacillus acidophilus*

The *Lactobacillus acidophilus* bacteria isolates were reidentified by using the microscope and biochemical test and the result of their characterization is as follows.

3.3.1.1 Cultural characteristics:

After propagating the shape of *Lactobacillus acidophilus* colones on the MRS agar is white, round, mucoid, convex, and soft with smooth edges, and these characteristics are concluded by Jawetz *et al.* (2010)

3.3.1.2 Microscopic characteristics:

Gram stain was used to examine the *Lactobacillus* colony under the microscope where the bacterial cells are gram-positive like bacilli were the cluster found in a long and short chain, non-spore-forming and the same characteristics were determined by Christine *et al.* (2016).

3.3.1.3 Biochemical characteristics:

Lactobacillus acidophilus isolate was found to be negative for catalase and oxidase production as described by Christine *et al.* (2016). These isolates were unable to ferment xylose, sorbitol, and mannitol but fermented Glucose, Fructose, Lactose, and Sucrose came following those of Neamtu *et al.*, (2014)**.**

Table (3-2) Ability of *Lactobacillusacidophillus* (as probiotic) to ferment carbon sources.

Carbon source / Isolate	Glucose	Fructose	Lactose	Sucrose	Mannitol	Xylose	sorbitol
Lb. acidophilus	+	+	+	+	–	–	–

3.4 Probiotics filtrate effect on pathogenic bacteria

After performing the primary experiments, it was found that the three-fold concentrated filtrate of *Lb. acidophilus* filtrate exhibited the highest inhibitory (40 mm) effect against *E. coli*. As reported by Ouweh and Vest (2004), probiotic microorganisms produce a wide range of anti-microbial metabolites that may contribute to microbiological safety by limiting the growth of other microorganisms as well as inhibiting pathogenic bacteria, which may, in turn, reduce the risk of foodborne illness and disease. However, Oskar et al. (2004) discovered that anaerobic Lactobacillus isolates on MRS agar produced the most inhibitory metabolites against pathogenic bacteria.

3.5 Antibiotics' effect on pathogenic bacteria

Antibiotic resistance is a severe therapeutic issue caused by the overuse or misuse of antibiotics (Sotto *et al*., 2001). The study's *E. coli* strain had the highest antibiotic resistance. Possibly *E. coli* is readily acquired. Antibiotic resistance was greatest in *E. coli* This may be because *E. coli* quickly develops antibiotic resistance from the environment. Ampicillin and penicillin derivatives (Wazait *et al*., 2003). This resistance occurs because of the activity of β- lactamase enzymes (penicillinase and cephalosporinase) which can cancel the activity of these antibiotics during the break of the β-lactam disc of the medicine (Levinson and Jawetz, 2000). Several authors found that Levofloxacin and ciprofloxacin greatly impacted E. coli and S. aureus adherence. This is true for both older and newer quinolones (oxolinic acid, ciprofloxacin, pefloxacin, enoxacin, lomefloxacin, and rufloxacin) (Jones *et al*., 1999; Braga and Piatti, 1992**).**

3.6 Comparison between the effect of probiotics and antibiotics on pathogens

When the effect of probiotics compared with antibiotics coppered it was shown that probiotics give a highly inhibitory effect against pathogenic bacteria than antibiotics. Probiotics may have this impact owing to a variety of processes, including the increased synthesis of harmful chemicals such as lactic acid, hydrogen peroxide, and bacteriocins in L. acidophilus rather than L. rhamnosus(Todorov*et al*., 2011). Furthermore, *Lactobacilli* have a great potential to prevent pathogenic development and multiplication by competing for nutrients with other harmful microbes (Andreu*et al*., 1995; Cadieux *et al*., 2002). Lactobacilli have been shown to have stimulatory actions on cells of the innate immune system in vitro, including macrophages and natural killer cells, in addition to their antibacterial capabilities (Mc-Cracken and Gaskins, 1999).

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