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ANTAGONISTIC EFFECT OF *LACTOBACILLUS ACIDOPHILUS* AGAINST SOME PATHOGENIC BACTERIA

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Received: 1 February 2023; **Accepted:** 15 March 2024

ABSTRACT

Aim: This study intended to ascertain whether probiotics added to various food products exhibited antimicrobial and antibiofilm properties against common microbial pathogens. **Materials and methods:** Two hundred samples were collected (130 from Assiut and 70 from New Valley) from different sources including yogurt (90), rayeb (80), and milk powder (30). *Lactobacilli* strains were isolated and biochemically identified. Then genotypic characterization of the samples was done. The *lactobacilli* CFS's (CFS) antimicrobial activity was measured by agar well diffusion. The ability of these pathogens to form biofilms was tested. Furthermore, the microplate titer method was employed to analyze the antibiofilm characteristics of CFS. **Result:** *Lactobacillus* strains were isolated from different milk products and genotypically investigated and nineteen *L. acidophilus* have been isolated. Most concentration CFS show antibacterial activity against tested microorganisms with an inhibition zone of diameter greater than 25 mm. Moreover, The CFS of lactobacilli inhibited the biofilm formation by these pathogens.

Keywords*: Lactobacilli*, antibacterial, antibiofilm.

INTRODUCTION

Probiotics are viable microorganisms that, when supplied in enough doses, profit the host's health. Probiotic microorganism that has been used safely in both fermented and non-fermented foods for a long time is named Lactic Acid Bacteria (LAB) (Quigley, 2019). It is commonly known that probiotics have antibacterial properties against harmful microorganisms. The synthesis of organic acids (which antagonize bacteria, bind to bacteria, and decrease bacterial adherents), bacteriocin, and hydrogen peroxide is what gives probiotics their antibacterial properties (Mahdavi and Isazadeh, 2019). Furthermore,

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LAB and their ingredients have been recommended as a possible biofilm biocontrol agent (Khiralla, *et al*., 2015).

Staphylococcus is the reason for many disorders in people and affects animals. Because *Staphylococcus aureus* produces a variety of endotoxins when it grows on different food commodities, it is well-known as a common food poisoning pathogen (Lee, *et al.*, 2015). Furthermore, the infection of the urinary system impacts 150 million people globally each year. UPEC is the most popular microorganism linked to urinary tract infections (UTTIs), accounting for 90% and 50% of community-acquired and nosocomial infections of the urinary tract (oval *et al*., 2014). E. coli creates microcolonies called biofilms on the urethral catheters, and also on the urinary bladder mucosa. By encasing them in an extracellular matrix, biofilm shields bacteria from the immune system and drugs. Furthermore, the easy transmission of resistance factors amongst the bacteria in the biofilm is made possible by close bacterial association. Drug resistance will increase as a result, making UTI treatment more challenging (Eberly *et al.*, 2017).

The rise of new strains that are not affected by antibiotics, new therapy protocols away from antibiotics have become increasingly popular in recent years. Probiotic use is one of these treatment modalities that shows promise for infection control.

MATERIALS AND METHODS

Preparation of samples (APHA, 1992):
Preparation of pasteurized yogurt, rayeb milk and milk powder for isolation of *Lactobacillus* by mixing 1gm of the sample with 9 ml peptone physiological saline solution.

Isolation of *Lactobacilli* (Lamiaa, 2014):
The diluted test sample was streaked on the surface of De Man Rogosa Sharpe (MRS) agar and incubated for 40 – 42 hours at 37ºC in a 10% Co2 incubator. Colonies appear as white smooth and convex with regular edges varying in size 1-5 mm. After being purified by recurred subculturing, *Lactobacillus spp.* were confirmed depending on Gram staining, sporulation, morphology and biochemical tests. A copy of the strains was cultured on MRS agar slants at 4 °C and subcultured every four weeks (Shruthy *et al.,* 2011).

Identification of Lactobacillus Sp. by PCR (Kim *et al*., 2020):
To identify the bacteria's species, Bacterial genomic DNA was extracted by boiling method. The supernatant was then taken as a template and preserved at 20° C. Amplified of the 16S ribosomal RNA gene was done by using *L. acidophilus* 16S-23S rRNA **(**F:5'-CCT TTC TAA GGA AGC GAA GGA T-3'\R:3'- ACG CTT GGT ATT CCA AAT CGC-5') primer**.** The PCR was done as follows: Primary denaturation for 5 min at 94˚C, then 35 cycles of at 94˚C for 30 sec, at 60˚C for 30 sec and at 72˚C for 30 sec and a final extension for 7 min at 72˚C.

Preparation of pathological bacterial strains:
The used bacterial strains were *Escherichia coli* and *Staph. aureus*. All strains were gained from the High-Quality Media unit (HQM) in the Animal Health Research Institute in Dokki, Egypt. Three to five colonies were emulsified from each pathogenic strain in 3-4ml tryptone soya broth. The turbidity was adjusted to approximately 10^8 cfu/ml by comparing it to a McFarland barium sulfate standard 0.5 incubated for 24 hours at 37ºC.

Antibiotic susceptibility testing of pathological agents (CLSI, 2017):
Susceptibility of *E. coli* and *S. aureus* bacteria to antibiotics involving, Aztreonam, Ampicillin, Amikacin, Gentamicin, Nitrofurantoin, Ceftriaxone, Ceftazidin, Imipenem, Meropenem, Tetracycline, Cefoxitin, Sulpha\Trimethoprim, Cefoperazone and Cefpodoxime were examined using the disc diffusion method, and the inhibition zone diameters were

evaluated and contrasted with the zones that had been previously reported.

Method for Making Cell-Free Supernatants:

Probiotics were cultured anaerobically in MRS broth for 24 h at 37°C. For 20 minutes at 4 °C and 5000 rpm, cultures were centrifuged to produce a cell-free supernatant. subsequently used after being passed through a filter with a pore size of about 0.2 um.

The antibacterial activity of *L. acidophilus* (Njoki *et al*., 2015):

Two plates were done, one for *E.coli* and the Other for *S. aureus*. Using 1 N NaOH, the supernatant was neutralized (pH adjusted to 4) in order to eliminate the organic acid's antibacterial properties. A sterile cork borer was used to create 10 mm wells in the Muller-Hinton agar after each overnight cultures of the indicator strains of *S. aureus* and *E. coli* were swabbed. The sterile neutral supernatant was added to the agar wells in approximately 100 ul aliquots. For every test isolate, this was done twice. The plates were kept at 48 hours at 34 °C, that is the ideal condition for indicator microorganisms, after being kept at 4 °C for two hours to permit for prediffusion. Inhibition zones were noted, along with their diameters and presence or absence.

Inhibition of biofilm formation (Plyuta *et al*., 2013):

After ascertaining that the tested microorganisms form biofilm according to Stepanović *et al*. (2007). The assessment of biofilm inhibition involved employing the spectrophotometric method. In 96-well microtiter plates, 180 µL of various concentrations of the probiotic extract was prepared and distributed. Subsequently, 20 µL of overnight growth cultures of the tested strains were added to each well and kept at 37 °C for 24 hours. Following the removal of the suspensions, 200 µL of phosphate buffer saline (PBS) was added to the wells in order to wash away any free-floating bacteria. Adherent cells forming biofilms on the plate were fixed with 200 µL of methanol for 20 minutes, followed by staining with 200 µL of 0.1% crystal violet at room temperature for 15 minutes. A thorough PBS wash was used to remove any remaining stains. After air-drying, we added 200 µL of ethanol (96%) were added to each well to solubilize the biofilm dye and the mixture was incubated for 15 minutes. The resulting reaction was assessed spectrophotometrically at 570 nm. Each treatment was triplicated, and the experiment was run three times. Control samples, consisting of wells inoculated with bacteria without any treatment, were included in each case. The percentage of biofilm inhibition = [(OD control – OD treatment)/OD control] × 100.

RESULTS

Isolation and Identification of Lactobacilli:

Various yogurt, Rayeb, and milk powder samples were gathered. The colonies that Gram-positive rod-shaped morphology, catalase and oxidase activity, cell motility, and sporulation are considered *Lactobacillus spp. Lactobacillus spp.* were the predominant organisms among all bacterial isolates. Further identification of *Lactobacillus spp*. was achieved using 16S ribosomal RNA, revealing 19 isolates of *L. acidophilus* in this study. In every experiment, the CFS was made from a single pure culture of *L. acidophilus* to maintain consistency and reproducibility.

Table 1: Susceptibility of tested pathogens to antimicrobial agents

Antimicrobial agent	*E. coli*	*S. aureus*
Aztreonam (AT)	R	R
Ampicillin (AM)	R	R
Amikacin (AK)	R	S
Nitrofurantoin (NIT)	R	I
Imipenem (IMP)	R	S
Meropenem (MRP)	S	S
Sulpha /trimethoprim (TBD)	I	R
Cefoxitin (FOX)	R	S
Cefpodoxime (CPD)	R	R
Cefoperazone(CEP)	S	S
Tetracycline (TE)	S	I
Gentamycin (CN)	S	I
Ceftriaxone (CTR)	R	S
Ceftazidime (CAZ)	R	R

Antibacterial Activity of CFS

Lactobacilli's CFS demonstrated antibacterial action against 94.74% and 84.21% of tested *S. aureus* and *E. coli* respectively but with variable degrees. The average diameter of the inhibition zone was more if *S. aureus* in comparison to the *E. coli* isolates.

Table 2: Antibacterial effect of the isolated *L. acidophilus* on the growth of pathogenic bacteria (diameter of the inhibition zone).

Samples No.	The inhibition zone's diameter (mm) (mm)	
	S. aureus	*E. coli*
1	16	25
2	19	24
3	20	22
4	24	25
5	20	25
6	18	22
7	10	20
8	15	18
9	13	19
10	19	22
11	12	17
12	10	12
13	16	19
14	11	13
15	9	10
16	13	15
17	11	13
18	18	20
Mean±SE	15.22±1.02	18.94±1.12

(Minimum – Maximum)	(9.00-24.00)	(10.00-25.00)

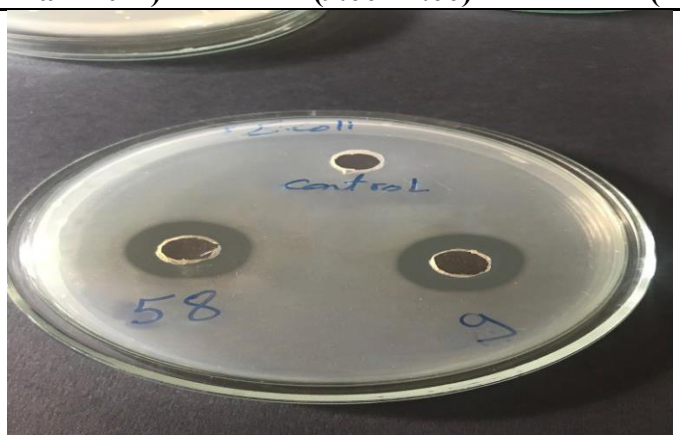

Photo (١): Antibacterial activity of *l. acidophilus strains* against *E.coli*.

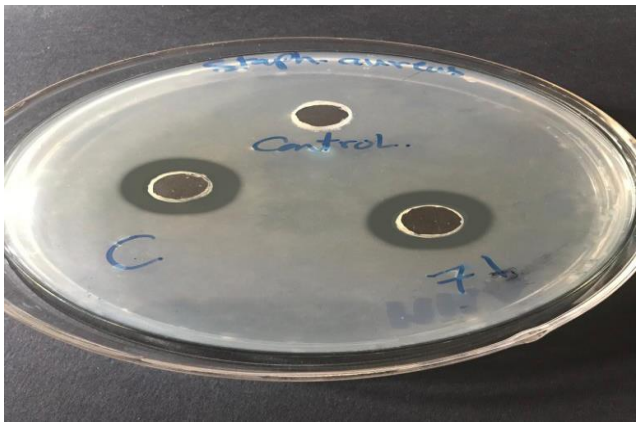

Photo (٢): Antibacterial activity of *lactobacilli* strains against *S. aureus.*

Assessment of the Antibiofilm Activity

The CFS of ten *L. acidophilus* strains resulted in preventing the formation of biofilms by *E. coli* isolates (Table3). twelve out of 18 strains showed an antibiofilm effect against *S. aureus* and the greatest inhibition was observed by LAB 6 (93.75%).

Table 3: The antibiofilm effect of *L. acidophilus* strains against some pathogenic organisms.

Isolates	Antibiofilm activity (%)	
	E. coli	***S. aureus***
1	70%	92.5%
2	76.05%	74%
3	70%	89%
4	81.69%	92.5%
5	76.05%	74%
6	88.73%	93.75%
7	70%	90%
8	73.239%	89%
9	87.32%	92.5%
10	88.73%	86.25%
11		90%
12		89%

Photo (٣): Antibiofilm activity by MTP.

DISCUSSION

Harmless bacteria known as lactobacilli are frequently used as probiotics and are intimately linked to the human microbiota. Their probiotic effect depends on their capacity to produce antimicrobial molecules, improve the function of the epithelium or modify the human immune system to prevent pathogen colonization through rivalry (Raheem *et al.,* 2021). The utilization of antimicrobial agent probiotics and even its products for battling infectious diseases is reinforced by the reality that these health-promoting bacteria produce inhibitory effects against resistant bacteria; moreover, it is predicted that the inability to fight against the intended pathogens is an uncommon instance, contrary to the often creation of resistance by pathogenic organisms in humans towards antibiotics often employed in the clinic (Simons *et al.,* 2020). Therefore, the potential use of probiotics as antimicrobial substances in the age of antibiotic resistance with a rapidly usage become of great importance (Silva *et al.,* 2020). A variety of pathogens, such as *L. monocytogens, E. faecalis, Salmonella typhimurium, S. typhimurium, E. cloacae, P. aeruginosa, Cl. difficile, H. pylori, E. coli O157:H7, S. aureus, S. epidermis, B. subtilis, and Campylobacter jejuni,* were demonstrated to be susceptible to antimicrobial effects by *lactobacillus* CFS. (Muhammad *et al.,* 2019). Indeed, the noteworthy aspect is that the antimicrobial activity of probiotics extends beyond bacteria. Probiotics were shown by Yasui *et al.,* (1993) to offer immunity to diarrhea caused by rotavirus. Furthermore, it was shown that neither environmental variables nor storage circumstances influence the inhibitory properties of lactobacilli supernatants (Koohestani *et al.,* 2018), which is advantageous if CFS is marketed as a medicinal agent. The majority of research on probiotics' antimicrobial effects now has concentrated on CFS (Jeyanathan *et al.*, 2021).

The *lactobacilli* used in this investigation were taken out of industrial items to test strains that had already received human use permission.

Generally, Greater antibacterial and antibiofilm effects against *E. coli* and *S. aureus* were demonstrated by the CFS of the *L. acidophilus* and the result indicated that 94.74% and 84.21% of *L.acidophilus* strains have antibacterial effects on *S.aureus* and *E.coli*. This was in agreement with Ebrahim (2017) who showed that 94.4% and 87.0% of *lactobacillus* strains have antibacterial effects on *S.aureus* and *E.coli* and a similar result was obtained by Karthikeyan and Santhosh (2009) they declared that *L.acidophilus* was inhibitory to both tested pathogens. Also, Ghane *et al.* (2020) improved despite neutralization, the CFS of every lactobacillus strain was still capable of suppressing UPEC microbes.

In line with Bassyouni *et al.* (2012), reported that isolated *Lactobacillus* have antimicrobial properties against *E. coli.* Another study introduced by Soltani *et al.* in (2022), it was found that *L. acidophilus* extraction resulted in a 16 mm diameter zone of inhibition when tested against UTI-isolated *E. coli*. Additionally, Koohestani *et al.* (2018) demonstrated a 16 mm

diameter zone of inhibition in opposition to *S. aureus*. against *S. aureus*. Tigu *et al.* (2016) demonstrated that *Lactobacillus* effectively suppressed the *E. coli* grwoth, with inhibition zones measuring between 10 to 14 mm in diameter. Similarly, Haghshenas *et al.* (2017), observed potent antibacterial activity in *Lactobacillus* strains derived from fermented dairy industries in Iranian, particularly against *E. coli*, where inhibition zones reached diameters of 12.3 mm.

In agreement with this, study by Kim *et al.* (2009) the exopolysaccharide of *L. acidophilus* removed 87.00% of *E. coli* biofilm created on surfaces. Similarly, Koohestani *et al.* (2018) approved that *L. acidophilus* removed 70.60 of *S. aureus* biofilm.

Consistent with our results, Rao *et al.* (2015), stated that the CFS from *Lactobacillus* exhibited significant antibiofilm activity. Additionally, Khiralla *et al.* (2015), recommended three *Lactobacillus* strains isolated from conventional products as effective biocontrol agents, emphasizing their potential for inhibiting the formation of biofilms by pathogens. Soltani *et al.* (2022) indicated that *L. acidophilus* can impede the formation of biofilms by *E. coli* isolates. However, Ghane *et al.* (2020) found that the CFS from all kefir isolates led to a decrease in biofilm formation by uropathogenic strains. However, the effectiveness of antibiofilm activity varied notably among different *lactobacilli* strains. Likewise, the CFS from all LAB led to over 30% inhibition of biofilm formation by *E. coli* isolates. Notably, from twelve strains only seven showed more than 50% inhibition against all four urinary strains.

In light of our findings, we propose utilizing the CFS of *L. acidophilus* to manage or stop infection and colonization brought on by pathological microbes, such as *S. aureus* and *E. coli*.

REFERENCE

A.P.H. An American Public Health Association (1992): Standard Methods for the Examination of Dairy Products. 16th Ed., American Public Health Association, New York.

Bassyouni, R.H.; Abdel-all, W.S.; Abdel-all, M.G. and Kamel, Z. (2012): Characterization of lactic acid bacteria isolated from dairy products in Egypt as a probiotic, Life Sci. 9 4–9.

Bin Masalam, M.S.; Bahieldin, A. and Alharbi, MG. (2018): Isolation, molecular characterization and probiotic potential of lactic acid bacteria in Saudi raw and fermented milk. *Evid Based Complement Alternat Med*.; 2018: 7970463. doi:10.1155/2018/7970463

Eberly, A.; Floyd, K.; Beebout, C.; Colling, S.; Fitzgerald, M. and Stratton, C. (2017): Biofilm formation by uropathogenic Escherichia coli is favored under oxygen conditions that mimic the bladder environment. Int J Mol Sci; 18 (10): 2077.

Ebrahim, A. (2017): Characterization of probiotic bacteria isolated from food and dairy products, Thesis submitted for fulfillment of the degree of ph. D. V. Sc. Of Veterinary Medicine, Microbiology and Immunology department, Assiut University.

Ghane, M.; Babaeekhou, L. and Sepideh S.K. (2020): Antibiofilm Activity of Kefir Probiotic Lactobacilli Against Uropathogenic Escherichia coli (UPEC), Journal of Medical Biotechnology. Vol. 12, No. 4.

Haghshenas, B.; Nami, Y.; Almasi, A.; Abdullah, N.; Radiah, D. and Rosli, R. (2017): Isolation and characterization of probiotics from dairies, Iran. J. Microbiol. 9 (4) 234.

Jeyanathan, A.; Ramalhete, R.; Blunn, G.; Gibbs, H.; Pumilia, C.A.; Meckmongkol, T.; Lovejoy, J.; Coathup, M.J. (2021): *Lactobacillus* Cell-Free Supernatant as a Novel Bioagent and Biosurfactant

against *Pseudomonas aeruginosa* in the Prevention and Treatment of Orthopedic Implant Infection. *J. Biomed. Mater. Res. B Appl. Biomater. 109*, 1634–1643.

Karthikeyan, V. and Santhosh, S.W. (2009): Study of Bacteriocin as a Food Preservative and the L. acidophilus Strain as Probiotic. Pakistan Journal of Nutrition, 9 (3): 224-232.

Khiralla, G.M.; Mohamad, E.A.H. and Farag, A.G. (2015): Antibiofilm effect of *Lactobacillus pentosus* and *Lactobacillus plantarum* cell-free supernatants against some bacterial pathogens. J Biotech Res; 6: 86-95.

Khiralla, G.M.; Mohamed, E.A.; Farag, A.G. and Elhariry, H. (2015): Antibiofilm effect of Lactobacillus pentosus and Lactobacillus plantarum cell-free supernatants against some bacterial pathogens, J. Biotech. Res. 6 86.

Kim, E.; Yang, S-M.; Lim, B.; Park, S-H.; Rackerby, B. and Kim, H-Y. (2020): Design of PCR assays to specifically detect and identify 37 Lactobacillus species in a single 96-well plate. BMC Microbiology 20(96):1-14.

Kim, Y.; Oh, S. and Kim, SH. (2009): Released exopolysaccharide (r-EPS) produced from probiotic bacteria reduce biofilm formation of enterohemorrhagic Escherichia coli O157:H7. Biochem Biophys Res Commun; 379(2): 324-329.

Koohestani, M.; Moradi, M.; Tajik, H. and Badali, A. (2018): Effects of cell-free supernatant of Lactobacillus acidophilus LA5 and Lactobacillus casei 431 against planktonic form and biofilm of Staphylococcus aureus, Veterinary Research Forum. 9 (4) 301 – 306.

Koohestani, M.; Moradi, M.; Tajik, H. and Badali, A. (2018): Effects of cell-free supernatant of Lactobacillus acidophilus LA5 and Lactobacillus casei 431 against planktonic form and biofilm of Staphylococcus aureus. *Vet Res Forum*. 9(4): 301–306. doi:10.30466/vrf.2018.33086.

Lee, J.S.; Bae, Y.M. and Lee, S.Y. (2015): Biofilm formation of Staphylococcus aureus on various surfaces and their resistance to chlorine sanitizer. J Food Sci; 80(10): M2279-M2286.

Mahdavi, S.; Isazadeh, A. (2019): Lactobacillus casei suppresses hfq gene expression in Escherichia coli O157:H7, Br. J. Biomed. Sci. 76 (2) 92–94.

Muhammad, Z.; Ramzan, R. and Abdelazez, A. (2019): Assessment of the antimicrobial potentiality and functionality of Lactobacillus plantarum strains isolated from the conventional inner Mongolian fermented cheese against foodborne pathogens. Pathogens. 8(2):71. doi:10.3390/pathogens8020071.

Njoki, W.J.; Boga, H. I.; Kutim, P.M.; Maina, M.J. and Kadere, T.T. (2015): Probiotic potential of lactic acid bacteria isolated from coconut (Cocos Nucifera) wine (Mnazi) in Kenya.International Journal of Life Sciences Research 3(1): 113-120.

Oval, F.; Köhler, C.D.; Vogel, U.; Wagenlehner, F.; Mellmann, A. and Fruth, A. (2014): Characterization of Escherichia coli isolates from hospital inpatients or outpatients with urinary tract infection. J Clin Microbiol; 52(2): 407- 418.

Patel, J.; Cockerill, III F.; Eliopoulos, G.; Jenkins, S. Lewis, J. and Limbago, B. (2017): M100 Performance standards for antimicrobial susceptibility testing. United State: Clinical and Laboratory Standards Institute.:240.

Plyuta, V.; Zaitseva, J.; Lobakova, E.; Zagoskina, N.; Kuznetsov, A. and Khmel, I. (2013): Effect of plant phenolic compounds on biofilm formation by *Pseudomonas aeruginosa*. Apmis 121(11), 1073-1081.

Quigley, EMM. (2019): Prebiotics and probiotics in digestive health. Clin Gastroenterol Hepatol;17(2):333-344.

Raheem, A.; Liang, L.; Zhang, G. and Cui, S. (2021): Modulatory Effects of Probiotics During Pathogenic Infections with Emphasis on Immune Regulation. *Front. Immunol. 12*, 616713.

Rao, K.P.; Chennappa, G.; Suraj, U.; Nagaraja, H.; Raj, A.C. and Sreenivasa, M.Y. (2015): Probiotic potential of Lactobacillus strains isolated from sorghum-based traditional fermented food, Probiotics Antimicrob. Proteins 7 (2) 146–156.

Shruthy, V.V.; Pavithra, M.; Gowri, S. and Ghosh, A.R. (2011): Probiotic potentials among lactic acid bacteria isolated from crud. Inter. J. Res. Ayurveda Pharm, 2 (2):602-609.

Silva, D.R.; Sardi, J.d.C.O.; Pitangui, N.d.S.; Roque, S.M.; Silva, A.C.B.D. and Rosalen, P.L. (2020): Probiotics as an Alternative Antimicrobial Therapy: Current Reality and Future Directions. *J. Funct. Foods*, *73*, 104080.

Simons, A.; Alhanout, K. and Duval, R.E. (2020): Bacteriocins, Antimicrobial Peptides from Bacterial Origin: Overview of Their Biology and Their Impact against Multidrug-Resistant Bacteria. *Microorganisms* , *8*, 639.

Soltani, N.; Abbasi, S.; Baghaeifar, S.; Taheri, E.; Farhoudi, M.S.J.; Emami, P.; Abolhasani, K. and Aslanshirzadeh, F. (2022): Antibacterial and antibiofilm activity of Lactobacillus strains secretome and extraction against Escherichia coli isolated from urinary tract infection, Biotechnology Reports 36: e00760.

Stepanović, S.; Vuković, D.; Hola, V.; Bonaventura, G.D.; Djukić, S. and Ćirković, I. (2007): Quantification of biofilm in microtiter plates: Overview of testing conditions and practical recommendations for assessment of biofilm production by staphylococci. APMIS; 115(8): 891-899.

Tigu, F.; Assefa, F.; Mehari, T. and Ashenafi, M. (2016): Probiotic property of lactic acid bacteria from traditional fermented condiments: datta and awaze, Int. Food Res. J. 23 (2) 770.

Yasui, H.; Kiyoshima, J. and Ushijima, H. (1995): Passive protection against rotavirus-induced diarrhea of mouse pups born to and nursed by dams fed Bifidobacterium breve YIT4064. *J Infect Dis*. 172(2): 403–409. doi:10.1093/infdis/172.2.403.

التأثير التثبيطي للملبنة الحمضية على بعض البكتريا الضارة

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زيادة انتشار البكتريا المقاومة للمضادات الحيوية أدي الي ترسيخ الجهود لاكتشاف طرق بديلة آمنة للعلاج منها البروبيوتك.

تهدف هذه الدراسة إلى عزل الملبنة والتحقيق جينيا من وجود الملبنة الحمضية. وتهدف أيضا الي التحقق من وجود أنشطة مضادة للميكروبات ومضادة للأغشية الحيوية ضد مسببات الأمراض الميكروبية الشائعة بين البروبيوتيك والتي تدخل في صناعة منتجات ألبان مختلفة.

قمنا بجمع مائتي عينة (١٣٠ من أسيوط و٧٠ من الوادي الجديد) من مصادر مختلفة وتتضمن هذه العينات الزبادي (٩٠٪) والرايب (٨٠٪) ومسحوق الحليب (٣٠٪).

بلغ معدل ظهور الملبنة في الوادي الجديد ٢٢,٨٥٪ و ٢٥,٧١٪ في الزبادي والرايب على التوالي، بينما بلغ معدل ظهور الملبنة في أسيوط ١٨,٤٦٪ و ١٦,١٥٪ و ٦,٩٢٪ في الزبادي والرايب و الحليب المجفف على التوالي.

تم فحص ٧٧ عينة إيجابية من الملبنة بواسطة تفاعل البلمرة المتسلسل، وتم التحقق من وجود ١٩ عينة إيجابية من الملبنة الحمضية.

تم تقييم تثبيط الملبنة الحمضية للبكتيريا الضارة عن طريق نشر آجار. أظهرت خلايا السطح للبكتيريا الحامضة اللبنية نشاطًا مضادًا للبكتيريا ضد ٩٤,٧٤٪ و٨٤,٢١٪ من المكورات الذهبية والبكتريا كولاي القولونية على التوالي ولكن بدرجات متفاوتة. كان متوسط قطر منطقة التثبيط أكبر في حالة المكورات الذهبية مقارنة بعزلات الكولاي القولونية.

علاوة على ذلك، منعت خلايا السطح للبكتيريا الحامضة اللبنية تكوين الأغشية الحيوية بواسطة هذه المسببات وفقا لمقايسة لوحة Microtiter. أدت خلايا السطح لعشرة سلالات من الملبنة الحمضية إلى تثبيط تكوين الأغشية الحيوية بواسطة عزلات الكولاي القولونية بينما أظهر اثني عشر من بين ١٨ سلالة نشاطًا مضادًا للأغشية الحيوية ضد المكورات الذهبية .