Antimicrobial Activity of Egyptian *Lactobacillus* spp. Isolated from Fecal Flora of Healthy Breast-Fed Infants against Food Borne pathogens

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Abstract: The last years have shown an interest to replacement the administration of antibiotics with probiotics. The aim of our study was to screen the antimicrobial activity and effect of some enviromential factors on this activity. The fifity *Lactobacillus* isolates were experimented to investigate the inhibitory activity against four pathogens (*Staphylococcus aureus, Escherichia coli, Salmonella typhi and Candida albicanes*) which were separately inoculated in MRS medium (de Man, Rogosa and Sharpe medium) for 48 hours at 37 °C and pH 6.8. Our results showed that enteropathogens growth was stopped in the presence of cell free supernatant (CFSC) of most *Lactobacillus* and only six isolates had anticandidal activity. The inhibition zone was ranged between 13 and 34 millimeter. Regarding environmental factors they are key parameters which have pronounced influence on the antimicrobial content productivity. The highest production was obtained in addition glucose or fructose to MRS broth adjusted to pH 6.8, at 37 °C. Our findings show that *Lactobacillus* strains with human origin had inhibitory activity against pathogens and these strains may be useful as probiotic candidates in prevention of intestinal infections caused by pathogenic microorganisms.

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1. Introduction

Most foods are commonly non sterial, they may contaminated with saprophytic and pathogenic microorganism. Food borne illness such as peptic ulcer, gastirites and enteritis which caused by these pathogen that contaminate food can be prevented by probiotic bacteria. The assurance of microbial food safety to public health occure by promising a trend to enrich food with lactic acid bacteria (LAB) that having potent antimicrobial activity against these pathogenic microorganisms **(Samot and Badet, 2013).** LABS are normal flora in the human intestinal tract which having along history for use in many industrial and fermentation food. The beneficil health effects were showen for some LAB strains which commonly marketed as probiotics **(Hamon *et al.*, 2011).** The maintenance of ecological balance is main significant role of probiotics due to production of metabolites such as lactic acid, propionic acid and actic acid which lowering the local pH and inhibit the growth of a wide range of pathogenic bacteria. Probiotics produce also other inhibitory substance such as bacteriocin, hydrogen peroxide. The mechanism (s) of the antimicrobial activity of probiotic *Lactobacillus* strains showen to be multifactorial **(Servin, 2004)**. Inculding pH- lowering capacity, bacteriocin production, hydrogen peroxide production, competition for nutrition and at adhesion sites and stimulation of the immune system (**Voravuthikunchai *et al.*, 2006).** Additionally, they have antioxidant activity so may prevent cancer, detox and protect from toxins, reduce the risk of inflammatory bowel movements and various gasterio - intestinal and extra-intestinal disorders, synthesize vitamins. Moreover, the beneficial health effects of LAB are strain specific **(Hobbs, 2000; Hamon et al., 2011).**

Lactobacilli inhibit the growth of many pathogens, including *Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Candida albicans* **(Verdenelli *et al.*, 2009)**. The aim of the work was to determine the antimicrobial activity of *Lactobacillus* isolates and effect of enviromential factors on its activity.

2. Materials and Methods:

Reference strains:

- *Staphylococcus aureus* (ATCC 29231).
-*Salmonella typhi* (ATCC 14028).
- *Escherichia coli* (ATCC 25922).

These strains were kindly obtained from department of pharmaceutical microbiology faculty of pharmacy Tanta University**.**

-Two isolates of *Candida albicans* were obtained from bacterial culture collection faculty of pharmacy Tanta University.

Sample Collection

A total of 50 lactobacilli isolates were recovered from 250 fecal samples from 3 days to1.5 years' old healthy breast – feed infants (191 natural milk feeding and 24 artificial and natural milk feeding infants). These samples were collected from Tanta and Kafer El Zyate cites Egypt during the period between Decembers to Augustus 2014_2015.

Bacterial Isolation and Identification

The isolation of *Lactobacillus* from the collected samples was done according to **Adnan and Tan (2007).** In tube containing samples were vigorously checked on vortex for homogenization and then 1ml was inoculated into MRS broth and incubated at 37°C for 48 h in anaerobic jars. The resulted cultures were streaked onto MRS agar plates. The developed white creamy colonies suggested to be lactobacilli were picked up and sub cultured for more purification. These colonies primerally identified by both Gram staining and biochemical tests. the identity of the lactobacilli isolates was conformed by Matrix-assisted laser desorption/ionization mass spectrometry (MALDI-TOF MS)**.**

Antimicrobial Activity

Antimicrobial activity of *Lactobacillus* isolates toward the microbial indicators was performed using agar well diffusion method according to **Schillinger and Luke (1989)**. The cell free supernant cultures (CFSCs) of lactobacilli grown in De man, Rogosa and Sharpe (MRS) media were obtained by centrifugation at 8000 rpm for 15 min at 4°C, sterilized through 0.45 µm Millipore filter membrane. A hundred µl of supernatants was transferred delicately into 9 mm holes drilled into muller hinton agar and Sabouraud which were previously inoculated with 100 µl of bacterial and fungal indicators, respectively. The plates were incubated aerobically at 37°C for 18-24 h. Antimicrobial activity was positively recorded in millimeters if there was a clear halo zone around the well **(Cadirci and Citak, 2005)**.

Effect of environmental factors on the productivity of antimicrobial content produced by *Lactobacillus* isolates (Kheirallah e*t al*., 2013).

1- Effect of pH:

The effect of pH on the antimicrobial activity produced by *Lactobacillus* isolates grown in MRS medium was determined. Flasks of 100 ml of MRS broth were adjusted to initial pH values 5.8, 6.4, 6.8 and 7.2 using 1 N HCl or 1N NaOH.

Each one was inoculated with overnight culture of lactobacilli isolates and incubated anaerobically at 37°C for 18-24 h. Values of antimicrobial activity (mm) of CFSCs against the microbial indicators were determined at the end of incubation period.

2- Effect of incubation temperature:

To determine the effect of incubation temperature on the antimicrobial activity produced by *Lactobacillus* isolates grown in MRS medium adjusted to pH 6.4 as the optimal pH, flasks containing 100 ml MRSbroth were inoculated with overnight culture of lactobacilli and incubated anaerobically at 25, 37 and 40°C for 18-24 h. The antimicrobialactivity (mm) of CFSCs against the indicators were determined at the end of incubation period.

3- Influence of medium component on the antimicrobial activity:

The effect of the addition of some nutrients including: fructose 20gm/l, glucose 20gm/l, starch 20gm/l and yeast extract 4gm/l to MRS broth as a basal medium on the production of antimicrobial activity of CFSCs of lactobacilli against the aforementioned microbial pathogenic indicators was investigated in comparison to MRS as a reference medium. All cultures were incubated at 37°C for 24h and The antimicrobialactivity (mm) of CFSCs against the indicators were determined.

3. Results

1-Isolation and identification of *Lactobacillus* Species

A total of 50 colonies were isolated from 185 stool samples (2 - 3 colonies per sample). All the isolates were Gram positive and catalase negative. Primary identification of 50 *Lactobacillus* isolates was performed on the basis of biochemical profiles. Finally, the identification of these isolates was confirmed by MULDI TOF mass spectroscopy. According to MULDI TOF mass spectroscopy *Lactobacillus casei, Lactobacillus paracasei* with percentage 26%, 24%, respectively were the most common species in fecal samples, together accounting for half of recovered isolates while *Lactobacillus rhamnosus*, *Lactobacillus fermentum* and *Lactobacillus zeae* obtained were ranged from 20% to 12%. Only one isolates of *Lactobacillus gasseri* and *Lactobacillus curvatus* with percentage2% were recovered.

2-Assessment of antimicrobial activity of *Lactobacillus* isolates:

The antimicrobial activities of different CFSCs of the tested isolates grown at 37°C for 48h were determined by well diffusion method against 4 microbial indicators; *S. aureus, E. coli, S. typhi*, and *C. albicans*.

The antimicrobial activities of CFSCs of the 50 tested *Lactobacillus* isolates were expressed as ascending order *S. aureus* ATCC29231 > *S. typhi* ATCC14028 > *E. coli* ATCC 25922 > *C.albicans*. The greatest inhibition zone diameters were recoded against *S. aureus* ATCC29231 it was ranged between 34 and 19 mm, aganist *S. typhi* ATCC14028 ranged between 16 and 25 mm while against *E. coli* ATCC

25922 were ranged between 13 and 22 mm. It worth mention that non detectable effects were observed for three isolates against *E. coli*. In contrast limited effects were observed against *C.albicans*. Only six isolates which exihibited antifungal activity and its inhibition zone diameters were ranged between 16 and 18 mm.

3-Effect of incubation temperature on the productivity of antimicrobial content against indicator strains.

The effect of incubation temperature on the productivity of antimicrobial content against indicator strains was carried out by incubation of the tested bacteria on MRS broth at 25°C, 37°C and 40°C. Data shown that the antimicrobial activities of CFSCc of lactobacillus isolates incubated at 37°C > 40°C > 25°C against the four indicator strains.

As shown in table (1) the effect of incubation temperature on the productivity of antimicrobial content against *S. aureus*, at 37°C & 25°C was highly significant effect than observed at 37 °C & 40 °C which had significant effect in contrast the effect which were observed at 25°C & 40°C was non significant. Against *S. typhi* the significant effect was observed only at 37°C & at 25 °C while at 37°C & 40°C and at 25 °C & 40°C non significant effect as shown in table (2). Against *E. coli* had the same results which were observed against *S. typhi* as shown in table (3).

Interestingly the CFSCs of the tested lactobacilli although at 37°C all CFSCs of the tested isolates were expressed antibacterial activity in contrast when the lactobacilli grown at 25°C, 8 of CFSCc of the tested isolates were shown non detectable effect against *E. coli* and two isolates against *S. typhi*. Upon the incubation temperature increased at 40°C, non detectable effect were showed for 7 of CFSCs of the tested isolates against *E. coli*, two isolates against *S. typhi* and one isolate against *S. aureus*. Five of the six CFSCs of lactobacilli which exhibited anti. Candidal activities lost their effect at 40°C and 25°C.

Inhibition zone as a functional activity of CFSCs of the tested *Lactobacillus* isolates at differarnt incubation temperature against standared bacteria and fungi are presented on figure (1).

Table (1): Effect of incubation temperature on the productivity of ntimicrobial content of CFSCs of lactobacilli against *S. aureus*.

Incubation temperature	*S. aureus* ATCC29231			ANOVA	
	Range	Mean ± SD	% of change	f	P-value
At 37°C	19 - 34	29.90 ± 3.30		6.656	0.002*
At 25 °C	18 - 32	26.84 ± 3.58	10.2		
At 40 °C	16 - 33	27.78 ± 4.33	7.1		

Tukey's test		
At 37°C & At 25 °C	At 37°C & At 40 °C	At 25 °C & At 40 °C
0.002*	0.041*	0.526

Non significant >0.05 significant <0.05* high significant <0.001*

Table (2): Effect of incubation temperature on the productivity of antimicrobial content of CFSCs of lactobacilli against *S. typhi*.

Incubation temperature	*S. typhi* ATCC 14028			ANOVA	
	Range	Mean ± SD	% of change	f	P-value
At 37°C	16 - 25	20.13 ± 2.53		8.091	<0.001*
At 25 °C	12 - 24	17.76 ± 2.76	11.8		
At 40 °C	14 - 24	18.78 ± 2.46	6.7		

Tukey's test		
At 37°C & At 25 °C	At 37°C & At 40 °C	At 25 °C & At 40 °C
<0.001*	0.066	0.216

Non significant >0.05 significant <0.05* high significant <0.001

Table (3): Effect of incubation temperature on the productivity of antimicrobial content of CFSCs of lactobacilli against *E. coli*.

Incubation temperature	*E. coli* ATCC 25922			ANOVA	
	Range	Mean ± SD	% of change	f	P-value
At 37°C	13 - 21	16.23 ± 1.78		6.696	0.002*
At 25 °C	12 - 17	14.85 ± 1.64	8.5		
At 40 °C	12 - 17	15.50 ± 1.30	4.5		

Tukey's test		
At 37°C & At 25°C	At 37°C & At 40°C	At 25°C & At 40°C
<0.001*	0.140	0.234

Non significant >0.05 significant <0.05* high significant <0.001.

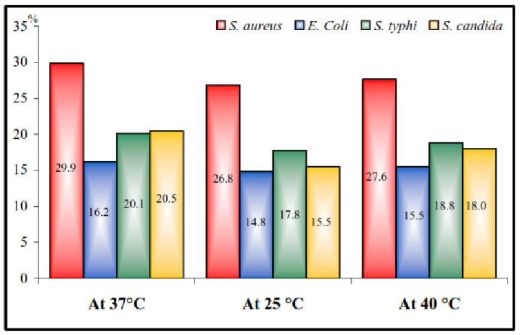

Figure (1): The antimicrobial activities of CFSCs of *Lactobacillus* isolate against indicator strains *S. aureus, E. coli, S. typhi* and *C.albicans* at different incubation temperature**.**

4- Effect of pH on the productivity of antimicrobial content against indicator strains.

The effect of pH on the productivity of antimicrobial content of the tested *Lactobacillus* isolates against indicator strains was carried out by adjusted the MRS broth to initial pH values 5.8, 6.4, 6.8 and 7.2. Data shown that the antimicrobial activities of CFSc of the tested isolates grew at initial pH values 6.4 > 6.8 > 5.8 >7.2.

As shown in table (4) the effect of pH on the productivity of antimicrobial content against *S. aureus* at pH 6.4 & 7.4 was highly significant effect while at pH 6.4 & 5.5 had significant effect in contrast non significant effect was observed at pH 6.4 & 6.8. Against *S.typhi* and *E. coli* at pH 6.4 & 7.4 and at 6.4 & 5.5 was highly significant effect while at pH 6.4 & 6.8 had significant effect as shown in table (5) and table (6).

Interestingly the CFSCs of the tested lactobacilli although at pH 6.4 all CFSCs of the tested lactobacilli were expressed antibacterial activity in contrast when the lactobacilli grew at initial pH7.2, 19 of CFSCs of the tested isolates were observed

non detectable effect against *E. coli* and six isolates against *S. typhi* and one isolate against *S.aureus*. At pH 5.5 non detectable effects was observed for 8 isolates against *E. coli* and one isolates against *S. typhi* and at initial pH 6.8, five isolates aganist *E. coli* were not observed effect. The six isolates which exihibiting activity aganist C. *albicans* lost this effect at initial pH 5.5, 6.8and at pH7.2 as shown it Table (VI-3).

Inhibition zone of CFSCs of the tested isolates grew at different initial pH values against standared bacteria and fungi were presented on figure (2).

Table (4): Effect of pH on the productivity of antimicrobial content of CFSCs of lactobacilli against *S. aureus*.

	S. aureus ATCC29231			ANOVA	
	Range	Mean ± SD	% of change	f	P-value
PH 5.5	18 - 32	27.44 ± 3.63	10.6	14.851	<0.001*
PH 6.4	19 - 34	30.68 ± 3.26			
PH 6.8	21 - 34	29.10 ± 3.50	5.1		
PH 7.4	14 - 30	24.67 ± 4.28	19.6		

Tukey's test			
	PH 6.4	PH 5.5	PH 6.8
PH 5.5	0.004*		
PH 6.8	0.333	0.283	
PH 7.4	<0.001*	0.019*	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001

Table (5): Effect of pH on the productivity of antimicrobial content of CFSCs of lactobacilli against *S. typhi*.

	***S. typhi* ATCC 14028**			**ANOVA**	
	Range	Mean ± SD	% of change	f	P-value
PH 5.5	12 - 20	16.41 ± 2.28	18.5	22.953	<0.001*
PH 6.4	16 - 25	20.13 ± 2.46			
PH 6.8	14 - 23	18.44 ± 2.27	8.4		
PH 7.4	13 - 19	15.96 ± 1.86	20.7		

Tukey's test

	PH 6.4	PH 5.5	PH 6.8
PH 5.5	<0.001*		
PH 6.8	0.014*	<0.001*	
PH 7.4	<0.001*	0.862	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001.

Table (6): Effect of pH on the productivity of antimicrobial content of CFSCs of lactobacilli against *E. coli*.

	***E. coli* ATCC 25922**			**ANOVA**	
	Range	Mean ± SD	% of change	f	P-value
pH 5.5	13 - 16	14.42 ± 0.97	12.1	29.131	<0.001*
pH 6.4	13 - 21	16.57 ± 1.52			
pH 6.8	12 - 18	15.41 ± 1.38	6.1		
pH 7.4	12 - 15	13.30 ± 1.03	19.0		

Tukey's test

	PH 6.4	PH 5.5	PH 6.8
PH 5.5	<0.001*		
PH 6.8	0.004*	0.029*	
PH 7.4	<0.001*	0.025*	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001.

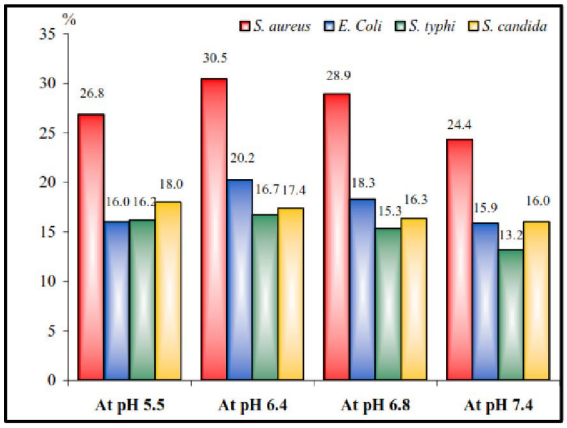

Figure (2): Antimicrobial activity of CFSC of lactobacilli against the indicator strains *(S. aureus, E. coli, S.typhi* and *C. albicans*) at different intial pH.

5-Influence of nutrient supplements on the antimicrobial productivity.

The antimicrobial activity of CFSCs of the tested lactobacilli using well diffusion method, were determined after growth in MRS broth, supplemented with some different carbon source including: glucose, fructose, starch and nitrogen source (yeast extract) against the aforementioned microbial standard indicators in comparison to MRS media as a reference medium. The antimicrobial activity results were presented in Figure (4).

From the results observed in table (7) the CFSCs for the tested isolates showed the highest inhibition zone (40mm) against the most sensitive strain *S. aureus* ATCC29231when medium supplemented with fructose or glucose as a carbon source, it's significantly increased the antibacterial activity in contrast addition of starch or yeast enhanced the antibacterial activity of lactobacilli but this enhancement non- significant. A similar result was observed aganist *E. coli* ATCC 25922 and *S. typhi* ATCC 14028 as shown in table (8) and table (9).

Table (7): Effect of change in medium component on antibacterial activity of CFSCs for lactobacilli against standard *S.auraus*.

	***S. aureus* ATCC29231**			**ANOVA**	
	Range	Mean ± SD	% of change	f	P-value
Culture medium	19 - 34	31.30 ± 2.13		14.216	<0.001*
Dextrose addition	27 - 40	34.96 ± 3.17	10.5		
Yeast addition	28 - 35	31.56 ± 1.83	0.8		
Fructose addition	30 - 40	34.74 ± 2.65	9.9		
Starch addition	25 - 36	31.69 ± 2.63	1.2		

Tukey's test

	Culture medium	**Dextrose addition**	**Yeast addition**	**Fructose addition**
Dextrose addition	<0.001*			
Yeast addition	0.996	<0.001*		
Fructose addition	<0.001*	0.998	<0.001*	
Starch addition	0.979	<0.001*	1.000	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001.

Table (8): Effect of change in medium component on antibacterial activity of CFSCs for lactobacilli against standard *S. typhi*.

	***S. typhi* ATCC 14028**			**ANOVA**	
	Range	Mean ± SD	% of change	F	P-value
Culture medium	16 - 25	20.35 ± 1.87		12.224	<0.001*
Dextrose addition	19 - 25	22.35 ± 2.00	8.9		
Yeast addition	17 - 23	19.65 ± 1.94	3.4		
Fructose addition	18 - 25	22.15 ± 1.91	8.8		
Starch addition	14 - 23	19.54 ± 2.14	4.0		

Tukey's test

	Culture medium	**Dextrose addition**	**Yeast addition**	**Fructose addition**
Dextrose addition	0.002*			
Yeast addition	0.713	<0.001*		
Fructose addition	0.009*	0.997	<0.001*	
Starch addition	0.579	<0.001*	1.000	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001.

Table (9): Effect of change in medium component on antibacterial activity of CFSCS for lactobacilli against standard *E. coli*.

	***E. coli* ATCC 25922**			**ANOVA**	
	Range	Mean ± SD	% of change	F	P-value
Culture medium	13 - 21	16.27 ± 1.46		14.183	<0.001*
Dextrose addition	15 - 20	17.88 ± 1.40	9.0		
Yeast addition	12 - 18	16.15 ± 1.69	0.7		
Fructose addition	15 - 21	18.04 ± 1.57	9.8		
Starch addition	14 - 17	15.61 ± 1.03	4.1		

Tukey's test

	Culture medium	**Dextrose addition**	**Yeast addition**	**Fructose addition**
Dextrose addition	<0.001*			
Yeast addition	0.999	<0.001*		
Fructose addition	<0.001*	0.996	<0.001*	
Starch addition	0.505	<0.001*	0.685	<0.001*

Non significant >0.05 significant <0.05* high significant <0.001.

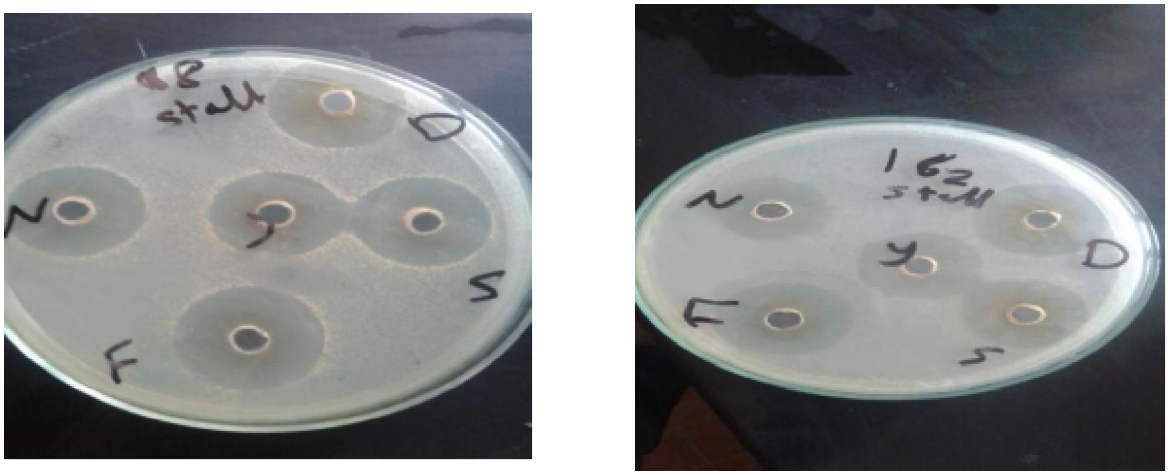

Figure (3): The effect of addition of some different carbon sources (glucose, fructose, starch) and nitrogen source (yeast extract) in the culture medium on antimicrobial activityof CFCS of lactobacilli against standard bacteria.
-N (normal MRS) - D (dextrose addition) - F (fructose addition) - S (starch addition)-Y (yeast addition).

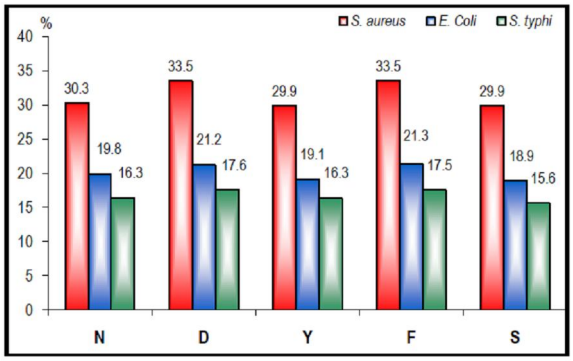

Figure (4): antibacterial activity CFSCs of *Lactobacillus* isolates at different condition of carbon and nitrogen source.

4. Discussion

Probiotics are defined as live, non-pathogenic microorganisms that confer health benefits to the host, and are increasingly being employed as an option for preventing and treating bacterial infections (**Gareau *et al.*, 2010**). Lactic Acid Bacteria (LAB) are regarded as a major group of probiotic bacteria there are one of the most important groups of microorganisms to mankind, being part of normal flora, contained antimicrobial substance that has inhibitory effect on growth of pathogens **(Darsanaki *et al.*, 2012)**. Main genera of LAB are *Lactobacillus*, *Enterococcus*, *Lactococcus*, *Bifidobacterium*, *Leuconostoc*, *Pediococcus*, and *Streptococcus* **(Tafvizi *et al.*, 2012)**. These bacteria cause reduction of gastrointestinal diseases by increasing benefit microorganisms growth and reducing pathogens population mechanisms (**Hawaz *et al.*, 2014)**. LAB are widely distributed in the environment (plant surfaces, decaying plant material, and the mammalian intestine, vagina and oral cavity) that can prevent the growth of pathogenic microorganisms by producing particular substances. According to scientific reports, antiallergic and anticancer effects, increasing fat loss and immune response of the host, improvement symptoms of irritable bowel syndrome, intestinal inflammation, and antibiotic-induced diarrhea are other useful effects of probiotics **(Nsofor *et al.*, 2014)**.

Lactobacilli are the most common used probiotic bacteria; they have an important role in controlling undesirable microflora in the gut and are able to prevent the rise of pathogenic bacteria by producing antimicrobial metabolites. They can be used as biological preservatives and are raised naturally in foods **(Oyetyo.2004)**.

In the present study, out of 250 collected samples, including 215 fecal samples from 3 days to 1.5 years' old healthy breast – feed infants and 35 breasts of milk healthy women. 85 isolates exihibited colony, cell morphologyand biochemical activity of lactobacilli. MULDI TOF mass spectroscopy showed that only 50 (83.3%) of these isolates were lactobacilli.

The capability of MULDI TOF mass spectroscopy to accomplish species identification in less than one hour makes it particulary suitable for routine clinical laboratories **(Benagli, Rossi *et al.*, 2011)** and it can be used for precise species identification of the recovered *Lactobacillus* isolates. Among 50 *Lactobacillus* tested isolates, *Lactobacillus casei* were the most common bacterial species (26%) followed by *Lactobacillus paracasei* (24%),

Lactobacillus rhamnosus (20%), *Lactobacillus fermentum* (14%), *Lactobacillus zeae* (12%). In contrast *Lactobacillus curvatus* and *Lactobacillus gasseri* were the least identified species (2%). **Arici *et al*. (2004)** reported that the most frequently isolated *Lactobacillus* species in the feces of infants and children less than 2 years of age were *L. rhamnosus, L. paracasei and L. fermentum*, respectively. In the present study, in accordance with **Arici *et al.* (2004)** *L. paracasei* and *L. rhamnosus* were isolated from infant faces, but in **Arici *et al*. (2004)** study *L. rhamnosus* was the most recovered species. In another study by **Shokryazdan *et al.* (2014**) the *L. casei and L. fermentum* were the most recovered species from the feces of healthy infants between 1 to 19 months. Variations in lactobacilli flora in infant feces may be due to differences in feeding (breast or formula) and the geographical zone. Also, variations in methodology may account for the differences, since identification of lactobacilli by traditional biochemical methods is very difficult **(Mitsou *et al*., 2008).**

Good probiotics should present their antimicrobial actions particularly to the pathogens in the gasterointestinal system **(Samot and Badet, 2013).** Accordingly; standared ATCC of *S.aureus, E.coli* and *S.typhi* were used as indicator bacteria in the present study because they are occasionally found as food-borne microorganisms that cause gastroenteritis. *C.albicans* was also used as indicator fungi. The results of agar well diffusion test showed that the antimicrobial activities (evaluated by inhibition zone diamters) of CFSCs of the 50 tested *Lactobacillus* isolates were expressed as deascending order *S. aureus* > *S. typhi* > *E. coli* > *C.albicans*. The CFSCs of the tested isolates activities against *S. aureus* were ranged betwean 19 - 34 mm and had a mean 29.9 mm, against *S. typhi* were ranged betwean16 - 25 mm and had a mean 20.13 mm and against *E. coli* were ranged betwean13 - 21 mm and had a mean 16.23 mm. This results in aggrement with results of **Al- Madboly and Abdallah. (2015), Georgieva *et al*. (2015), Nyazi *et al*. (2015)**, **Tebyanian *et al*. (2017), Bibalan *et al*. (2017)** and **Savadogo *et al*. (2004)** which indicated that the *Lactobacillus* activity against *S.aureus* were significantly more than *S.typhi* and *E coli* which could be duo to related closely activity of bacteriocin and this explain closely related activity of *Lactobacillus* Grame- positive bacteria than Gram -negative bacteria**.** In contrast, **Venkatesan *et al*. (2012)** found that the CFSCs of *Lactobacillus* isolate more active against *S. typhi* than *S. aureus* and *E. coli*. Although **Al- Madboly and Abdallah. (2015)** reported *Lactobacillus* activity against *S. aureus* were more than other indicators, it's disagreed with this study in its activity against *S. typhi* > *E. coli*, **Davoodabadi *et al*. (2015)** researched on antimicrobial activity of *Lactobacillus* strains against five diarrheagenic *E. coli* pathotypes and they found that *Lactobacillus* strains with human origin had a mild inhibitory activity against the diarrheagenic *E. coli*. Only six isolates exhibited antifungal activity against *C. albicans* and ranged between 15-18mm. This result simmeler to **Rossoni *et al*. (2018)** and in agreement with astudy performed by **Atanassova *et al.* (2003)** which found that the antifungal activity is very low. On the other hand, **Bulgasem *et al*. (2016)** found that the antifungal activity of CFSCs of *Lactobacillus* isolates was moderate while **Kheirallah *et al.* (2014)** was reported potant anti- candidal activity for probiotic bacteria.

Antimicrobial activity is one of the most important selection criteria for probiotics. The mechanism (s) of the antimicrobial activity of *Lactobacillus* strains appears to be multifactorial **(servin 2004)** In particular, by producing metabolites such as acetic acid and lactic acid and thus lowing pH, leading to inhibition of Gram- negative pathogenic bacteria **(Alakomi *et al*.,2000)** and Gram- positive bacteria. Organic acids are also considered one of the main LAB compounds exerting antifungal effects **(Russo *et al*., 2016)**. Lactobacilli may also incur antimicrobial effect by producing some substances such as carbon dioxide, hydrogen peroxide, diacetyl, low molecular weight antimicrobial substances such as bacteriocins, which may be continuously excreted by the colonies to generate the inhibitor activity against the indicator organism.

In the present study the effects of incubation temperature, media compositions and its pH on the antimicrobial productivity of the tested *Lactobacillus* isolates were studied. The obtained results revealed that the antimicrobial productivity (expressed as inhibition zone diamters) of the tested lactobacilli growen at 37^{o}C were greater than measured at 25°C and 40°C, the productivity of the tested isolates grown at 40^{o}C were greater than measured at 25°C. It worth mention that although at 37 ^{o}C all CFSCs of the tested lactobacilli were expressed antibacterial activity in contrast when the lactobacilli grown at 25^{o}C, 8 CFSCs of the tested isolates were shown non detectable effect against *E. coli* and two isolats against *S. typhi* and upon the incubation temperature increased at 40^{o}C, 7 CFSCs of the tested isolates were showed non dectectable effect against *E. coli*, two isolates against *S. typhi* and one isolate against *S. aureus.* Only one from six isolates which exhibiting antifungal activity kept this activity at 40^{o}C and 25^{o}C. This result in accordance to result obtained by **Kheirallah *et al*. (2014), Yang *et al*., (2018)**. In contrast **Sadiq *et al*. (2014)** and **Souza *et al*. (2017)** reported maximum antimicrobial activity of *L. lactis* PI-2 was at 25–

30°C. **Naz and Rasool. (2013)** found that maximum production of bacteriocin were at 29 °C.

These results declared that maximal production of antibacterial and antifungal activity of *Lactobacillus* metabolites coincided with optimal incubation temperature for cell growth. The temperature might affect microorganism through its effect on oxygen solubility in the medium, kinetic energy of molecules and reaction velocity in the cell, and these factors might affect the production of antimicrobial compounds specially bacteriocin **(Al Jumaily *et al.*, 2013)**. **Favaro *et al.* (2012)** supported the obtained results as they reported that the highest levels of antibacterial activity of *Enterococcus faecium* were recorded at 37°C. The reduction in antimicrobial activity due to the produced metabolites at higher and lower temperatures may be referred to slow growth leading to retardation of antimicrobial compounds production, as reported by **Khay *et al.* (2013)**. Temperature also played an important role in LAB growth, particularly influenced the latency time **(Yang *et al.*, 2018)**. **Carlos *et al.* (2009)** reported that *Enterococci* sp growth curve at 30 °C presented longer lag phase compared to that obtained at 37 °C and **Gardini *et al.* (2001)** found that the most important factor influencing the lag phase of *E. faecalis* was temperature, although its influence on the final cell yield was low.

Some research found that the produced antimicrobial metabolites were pH-dependent **(De Muynck *et al.* 2004, Elsanhoty 2008)**. **Olson. (1993)** observed the dependence of bacteriocin production on pH suggested that the expression of the biosynthetic genes may be regulated by pH. The obtained results revealed that the productivity of antimicrobial content (expressed as inhibition zone diamters) of the tested *Lactobacillus* isolates growen at different intial pH were expressed as deascending order at pH 6.4 > pH 6.8> pH 5.5 > pH 7.2 against all indicator strains. It worth mention that although all CFSCs of the tested lactobacilli isolates grown at pH 6.4, were expressed antibacterial activity in contrast when the lactobacilli grown at initial pH 7.2, 19 of CFSCs of the tested isolates were shown non detectable effect against *E. coli* and six isolats against *S. typhi*, when tested isolates grew at pH 5.5, non detectable effect were shown of eight isolates aganist *E. coli* and one aganist *S. typhi* while at initial pH 6.8, five isolates were not shown detectable effect aganist *E. coli*. The 6 isolates which exihibited ant-candidal activity lost this effect at pH 5.5, 6.8and 7.2.

This results in this study were comparable to that reported in Egypt **by Kheirallah *et al.* (2014)** which observed higher activity at 6.4, 6.8 than, 7.4, **Mahrous *et al.* (2013)** who reported maximum activity of CFSCs of probiotic bacteria was noted at pH 6.0 and in aggrement wih results of **Hassanzadazar *et al.* (2012)**. **Jozala *et al.* (2011)** also established that the highest production of antibacterial activity produced by *Lactococcus lact* is in milk whey was at initial pH 6.5 and also a medium pH of 5.5, there was a reduction in the specific growth rate of lactobacilli and a corresponding decrease in the lactic acid produced **(Neelakantam *et al.*, 2005)**. **Favaro *et al.* (2012)** reported that the highest antibacterial activity level produced by *Enterococcus faecium* was achieved with initial pH 6.5. On the ther hand **Kim *et al.* (2000)** reported that the *Lactobacillus* species had their highest antibacterial activity at pH 7 and **Penna *et al.* (2006**) results reveald that a strong influence of pH of the culture medium on nisin release by *L. lactis* ATCC 11454, that was, at pH <6, more than 80% of it was released extracellularly, while at pH >6, it was mainly linked to cell membrane or intracellularly trapped. **Sadiq *et al.* (2014)** reveald that the nisin production by *L. lactis* subsp. lactis IT-4 achieved a maximum at pH 5 and decreased at higher pH. **Bibalan *et al.* (2017)** results showed that pH alteration between 4 to 7 had no effect on antibacterial activity but in the alkaline range (8 to 10), these activities were reduced. The pH is known to be important for biomass as will as, because aggregations, adsorption of bacteriocin to the cells and /or proteolytic degradation depend on pH and can affect bacteriocin activity **(Cheigh *et al.* 2002)**.

Production of bacteriocin depended on type of culture medium, and influenced by media compostion, Different sugars has been successfully used in many studies for optimization of bacteriocin production in different bacterial strains, The nitrogen source was the most important factor affecting for *Lactobacillus* growth **(Wood. 1997)**. However, high levels of nitrogen in the extract can cause cell death **(De Lima *et al*,. 2009)** and carbon source are also essential for growth of *Lactobacillus* **(Laxmi *et al*.,2011)** so that this study were examined infulance of nutrient supplement to MRS broth on the antimicrobial productivity.

The obtained results showed that the strongly antimicrobial activity was produced when growth nutrients are available for metabolism. The greatest values of inhibition zones (mm) were obtained when the MRS was enriched by fructose or dextrose was measured 40 mm against *S. aureus* as the most sensitive strains. the observed results showed that enhancement in antimicrobial productivity against *S. aureus* by 10.5%, 9.9%, 0.8% and by 1.2% when used dextrose, fructose, yeast and starch respectively, aganist *S. typhi* the elevated antibacterial activity were done by 8.9%, 8.8%, 3.4% and 4.0% when used dextrose, fructose, yeast and starch respectively and against *E. coli* the increasment antibacterial activity

were done by 9.0%, 9.8%, 0.7% and 4.1% when used dextrose, fructose, yeast and starch respectively.

This result was agreed with results of **Chooklin *et al* (2011)** they could observed that *Lactobacillus casei* using glucose and fructose had the maximum production of lactic acid. The results indicated that the microorganism used simple sugars easier and produced more lactic acid. Similarity, **Delgado *et al*. (2007)** which enshured that the glucose is considered the main carbon source by most of microorganisms due to its rapid utilization and cellular energy conversion **(Zinedine and Faid., 2007)** however some bacteriocins producer bacteria revealed high yield of bacteriocins. In contrast **Abbasiliasi *et al*. (2011)** reported that non significant chang in bacteriocin like inhibitory substances production and bacterial growth when glucose concentration up to 10g/L in growth media and slight decreased at glucose concentration up to 20g/L (conc of glucose in MRS media) which could be due to osmotic stress.

The results from the effects of carbon sources on *Lactobacillus* inhibitory activity of the postbiotic metabolites produced by *Lactobacillus* isolates are also in agreement with findings from other studies, where glucose was reported to be the preferential carbon source for bacteriocin production by *Lactobacillus sakei* **(Todorov *et al*., 2005)** and *Streptococcus bovicin* **(Carvalho *et al*., 2008)**. However, other carbon sources which support optimal bacteriocin production and antibacterial activity have also been reported for other LAB strains **(Cheigh *et al*., 2002), (Drosinos *et al*., 2005).** Production in association to inclusion of other sugars to growth media rather than glucose, may they have a complex enzymatic system that allow them to use other carbohydrates, for example *Enterococcus faecium* showed a variable sugar utilization rather than glucose for bacteriocins production **(Barnes *et al*., 1994)**, that suggest specific nutrients are required some times for production of bacteriocins. The similar effect of sucrose was also confirmed in nisin production by *lactococcus lactis* subsp Moreover, **Hayek and Ibrahim, (2013)** reported that LAB growth and their metabolic activity were influenced by different concentrations of carbon source**.**

The supplementation of MRS broth with starch has limited effect (non significant change) on the productivity of *Lactobacillus* this was possibly due to the microorganism was requiring longer time for the enzymatic degradation of the starch polysacharid form as compared to glucose **(Ohkouchi and Inoue, 2006**), or perhaps because the bacteria were adsorbed to the surface of starch molecules that could ceased their good utilization **(Dominguez *et a*l. 2007**).

Yeast extract was identified as the best nitrogen source for the production of bacteriocin-inhibitory compounds by *L. plantarum I-UL4*. Indeed, the effect of yeast extract as a single organic nitrogen source on the improvement of bacteriocin activity has been reported for *Lactobacillus sakei* (**Todorov *et al*., 2012**), *Lactobacillus rhomanus* (**Sarika *et al*., 2010**) and *Streptococcus bovis* (**Carvalho *et al*., 2008**). It is likely that the free amino acids and growth factors present in the yeast extract (**Aasen *et al*., 2000**) contributed to the stimulatory effect on the production of bacteriocin-inhibitory compounds by *L. plantarum I-UL4*. Most of human indigenous bacteriocins producer's microorganisms could not grow well and produce bacteriocins only with organic nitrogen sources, possibly for the absence in vitamins and DNA precursors, which was rich in yeast extract **(Karthikeyan and Santosh. 2009)**. The obtained results showed that the antibacterial activity of CFSCs of the tested *Lactobacillus* isolates was non significant change when increased amount of nitrogen source (yeast extract). Similarity, **Abbasiliasi *et al*. (2011)** found enhancement in the antibacterial activity of *Lactobacillus* upon increase amount of yeast extract and this chang non significant. In Iraq **Al- Wendawi and Al- Saady. (2012)** reported that yeast extract was not good nitrogen source for production of bacteriocin. In contrast in Malaysia **Ooil *et al*. (2015)** found that the maximum bacteriocin-inhibitory activity of postbiotic metabolites was achieved 36.20 g/L of yeast extract were added in the modified MRS medium.

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