Evaluation of antimicrobial activity of *Lactobacillus acidophilus*NA-5 grown in cheese whey against foodborne pathogens.

Z. M.H. Kheiralla[1], M.M.Abou El Nour[1], M.I. Mabrouk[٢] A.Y. Okda[٣], and Ebtehag .A.E. Saker[1]

[1]Botany Dept., Fac. of Girls for Science, Education and Art, Ain Shams Univ., Cairo, Egypt.
[٢]National Organization of Drug Control and Research (NODCAR), Cairo, Egypt.
[٣]Lab of Dairy Microbiology, Dairy Sci. Dept. Food Industries and Nutrition Division, National Research Centre (NRC), Cairo, Egypt.

Abstract:

The antimicrobial activity of metabolites produced by *Lactobacillus acidophilusNA-5* was tested *in vitro* against some food pathogenic microorganisms; it was determined by agar well diffusion method. *Bacillus cereus*, *Sallmonella typhimurium* no.22 and *Candida albicans* were selected from seven food borne pathogens for their sensitivity towards *L. acidophilusNA-5* metabolites when grown in Man Regosa Sharpe media (MRS). *Lactobacillus acidophilus*NA-5 was grown in different dilution of the selected waste material (cheese whey) as the sole carbon source to determine the concentration required for the induction of highest antimicrobial activity against the tested pathogenic organisms. The different dilutions of cheese whey suggested were (25:75, 50:50,75:25 and the original cheese whey). As well as the original cheese whey which showed the most inhibitory effect against the selected pathogens.

Further experiments were done to optimize the cheese whey media, by adding different concentrations of nutritional elements. pH and temperature were also tested for optimization. Results revealed that 1% peptone, 0.4% beef extract, 0.6% yeast extract and 0.5% sorbitol were the optimum concentrations which showed highest activities against the growth of selected pathogens.

The optimum pH and temperature showed that the most antimicrobial activity were at 6.2 and 37°C, respectively. From the results obtained, the formulated cheese whey waste material might be used as a less expensive culture media for the production of beneficial metabolites by *L. acidophilusNA-5* with remarkable antimicrobial activities.

Key words: *Lactobacillus acidophilus*, Cheese whey, Antimicrobial activity, Optimization, Nutrient supplements, Pathogens**.**

1. Introduction:

L. acidophilus strains have been widely utilized as a dairy starter culture for their therapeutic activities associated with an intestinal microbial balance, and has been used in fermented foods, and as a probiotic in dietary supplements (**Sanders and Klaenhammer, 2001**). **Oda *et al*. (1994**) also showed that *L. acidophilus* increased iron bioavailability of fermented milk in animal model. *In vitro* studies showed that *L. acidophilus* is a strong Th1 cytokine (IL-12, IFN -γ) inducer (**Gackowska *et al.*, 2006**). *L. acidophilus* strains exhibiting antagonistic activity towards certain types of psychrotrophic microorganisms such as *Listeria monocytogenes*, *Bacillus cereus*, and *Clostridium* sp. are especially important as these microorganisms even at low levels in food pose a significant spoilage and public health threat (**Kanatani *et al*., 1995; Bogovic-Matijasic *et al.*, 1998).**

Corresponding Author: Ebtehag .A.E. Saker, Botany Dept., Fac. of Girls for Science, Education and Art, Ain Shams Univ., Cairo, Egypt
E-mail:ebtehagsakr@yahoo.com

The *Lactobacillus acidophilus* also exhibited antimicrobial activity against many food born harmful bacteria that cause various diseases (**Bharal and Sohpal., 2013).**

Lactic acid bacteria (LAB) are reported to be extremely fastidious organisms with numerous growth requirements. They need rich media containing compounds such as amino acids, peptides, vitamins and nucleic acids (**Narayanan *et al.* 2004; Dumbrepatil *et al.* 2008**). **Hofvendahl and Hahn-Hagredal, (2000)** reported that fermentation medium can represent almost 30% of the cost for a microbial fermentation. Therefore, it is important to reduce the cost of growth medium; low-cost (but effective) medium components must be identified (**Rashid and Altaf 2008; Ortiz *et al.*, 2012**). Pertinent studies reported in the literature have been conducted to find less expensive media supporting *Lactobacillus* growth. Those found contained carbohydrate such as lactose and whey permeate (**Fu and Mathews, 1999**), whey solely (**Mondragón-Parada *et al.*, 2006**). It is essential to note that all of those studies were aimed to optimize the medium for lactic acid production. Only a few reports can be found in the literature on a high density processes to culture lactic acid biomass *e.g. Lactobacillus casei* (**Aguirre-Ezkauriatza *et al.* 2010**) and *Lactobacillus delbrueckii* ssp. *bulgaricus* (**Schiraldi *et al.* 2003**).

Cheese whey (CW) is the liquid by-product effluent generated during the cheese making process (**Ghaly *et al.*, 2003**). Among the most abundant of these nutrients are lactose (4.5-5% w/v), soluble proteins (0.6–0.8% w/v), lipids, and mineral salts (**Yang *et al.*, 1994**). Unfortunately, whey and its associated nutritional qualities and quantities have traditionally been considered as waste, simply dumped in watercourses without any previous treatment and represent an important disposal, pollution issue and serious environmental problem because of its high biological and biochemical oxygen demand (**Liu *et al.*, 2004; Jozala *et al.*, 2011 ; Leite *et al.*, 2012).** The work reported on this paper attempts to find low-cost medium by using waste material (cheese whey) instead of MRS media and to obtain an optimized medium then to determine the biomass, pH and antimicrobial activity of *L. acidophilusNA-5*.

2. Materials and Method

Microorganisms used:

Lactobacillus acidophilusNA-5 (*L. acidophilusNA-5*) was obtained from Lab of Dairy Microbiology, Dairy Science Department, Food Industries and Nutrition Division, National Research Centre, Cairo, Egypt. *L. acidophilusNA-5* was activated and subcultured weekly in the lactobacilli MRS broth (Difco laboratories, Detroit, MI).

The pathogenic microorganisms, *Staphylococcus aureus, Bacillus cereus, Bacillus pumilus, E. coli O157, Salmonella typhimurium no. 22, Pseudomonas aeruginosa, and Candida albicans* were obtained from National Organization of Drug Control and Research (NODCAR), Egypt. The bacterial species were cultivated and maintained in nutrient agar slants in screw-capped tubes. The agar slants were preserved in a refrigerator at 4°C until use (**Hassan *et al.*, 2011),** while *C. albicans* was maintained and propagated in potato dextrose broth and agar (PDA) (**Jain *et al.*, 2008**).

Preparation Cheese whey media

It was treated through protein precipitation induced as follows: after adjusting the pH to 4.5 with 5 N HCl, it was heated at 121 ˚C for 15 min to denature the proteins. The precipitates were removed by centrifugation at 4 ˚C and 8000×*g* for 10 min. The supernatants were adjusted to pH 6.5, sterilized at 121 ◦C for 15 min and used as culture media (**Rodrigues *et al.*, 2006**). Cheese whey was supplied from Dairy Sci. and Tech. Dept., Fac. of Agric., Cairo Univ., Egypt. Cheese whey was diluted with sterile water as follows;

1- 25% volume whey + 75% H_2O.
2- 50% volume whey +50 % H_2O.
3- 75% volume whey+ 25%H_2O.
4- 100% volume whey+ 0 %H_2O.

Antimicrobial activity measurement:

L. acidophilusNA-5 grew on tested media including MRS and on different dilutions of cheese whey. Antimicrobial activity of *L. acidophilusNA-5* toward the tested organisms was performed using agar well diffusion method according to **Schillinger and Luke (1989**). It was prepared as follows. A 1 mL amount was inoculated into 50 mL MRS broth and grown at 37°C for 24 h. Culture free cells supernatant was obtained by centrifugation at 8000 rpm for 15 min at 4˚C, sterilized through 0.45 µm Millipore filter membrane. Hundred µl of supernatants was transferred delicately into 9 mm holes drilled into nutrient agar and PDA which were previously inoculated with 100 µl of bacterial and yeast indicators, respectively. The plates were incubated aerobically at 37˚C for 24 h.

Influence of addition of nutrient supplements to cheese whey media on the growth and antimicrobial activity of *L. acidophilusNA-5*

Peptone (0.25-1.5%), beef extract (0.2-1.2%), yeast extract (0.2-0.6%) and (0.5-3%) sorbitol were added to the selected cheese whey dilutions. pH, bacterial cell density (optical density at 620nm) and antimicrobial activity of cell free culture supernatant (CFCS) of the probiotic *L. acidophilusNA-5* against the (*B. cereus, S. typhimurium* no.22 and *C. albicans*) was investigated in comparison to MRS as a reference medium. All chemicals were obtained from (Fluka, Sigma-Aldrich Chemical Co., USA).

Optimization of growth parameters

Different growth parameters such as pH, temperature and incubation period were optimized by varying the respective parameters to enhance biomass, pH and the antimicrobial activity of *L. acidophilusNA-5* grown on whey medium.

Statistical analysis

The obtained data were exposed to analysis of variance. Duncan multiple ranges at 5% level of significance was used to compare between means. The analysis was carried out using PROC ANOVA procedure of Statistical Analysis System **(SAS, 2003).**

3. Results and discussion

Detection of antimicrobial activity of *L. acidophilusNA-5* supernatant

Figure 1 showed that *L. acidophilusNA-5* had antimicrobial effect against the tested microorganisms. The highest inhibition diameter zone with *Bacillus cereus, S. typhimurium no. 22 and C. albicans* with inhibition zones (IZ) 24.4, 25.67 and 24.67, respectively. While the lowest inhibition effect was obtained with *L. acidophilusNA-5* against *Staphylococcus aureus* and *B. pumilus.*

Among the probiotic properties, antimicrobial activity is one of the important criteria of selection of suitable strain of probiotic. With the emergence of antibiotic resistant bacteria and natural ways of suppressing pathogens, the concept of probiotic has attracted much attention. Lactobacilli produce various antimicrobial substances, which causes the inhibition of pathogenic microorganism's growth and activities **(Saran *et al.*, 2012)**.

Some authors observed antagonistic activities of *Lactobacillus* spp. against undesirable microorganisms like *E. coli, S. aureus* and *Salmonella* spp. (**Chaves *et al.*, 1999, Chioda *et al.*, 2007; Pereira and Gómez, 2007; Barros *et al.*, 2009; Pribul *et al.*, 2011). Bharal and Sohpal. (2013)** reported that the *Lactobacillus acidophilus* also exhibited antimicrobial activity against many food borne harmful bacteria that cause various diseases. **Aween *et al.* (2012)** also reported that all isolates of *Lactobacillus acidophilus* from honey showed very good inhibitory activity against target Gram negative bacteria as indicated by the diameter of inhibition zone: *Salmonella typhimurium* (23-30mm), *Escherichia coli* (7-18 mm) and *Enterobacter aerogenes* (10-18 mm) after 24 h incubation at 30°C. *Lactobacillus acidophilus* isolated from milk was found to display a higher antagonistic effect with zones of inhibition of 6 and 15 mm against *E. coli* and *Pseudomonas aeruginosa* respectively, (**Oyetayo, 2004).**

From the results shown in **figure (1)**, it could be concluded that *B. cereus, S. typhimurium no. 22 and C. albicans* were the most sensitive food borne pathogens towards *Lactobacillus acidophilus* metabolites so, we select them for further experiments as (indicators) to study the effects of low cost medium (cheese whey) for attainment of high levels of antimicrobial activity, cell mass and pH of the production medium.

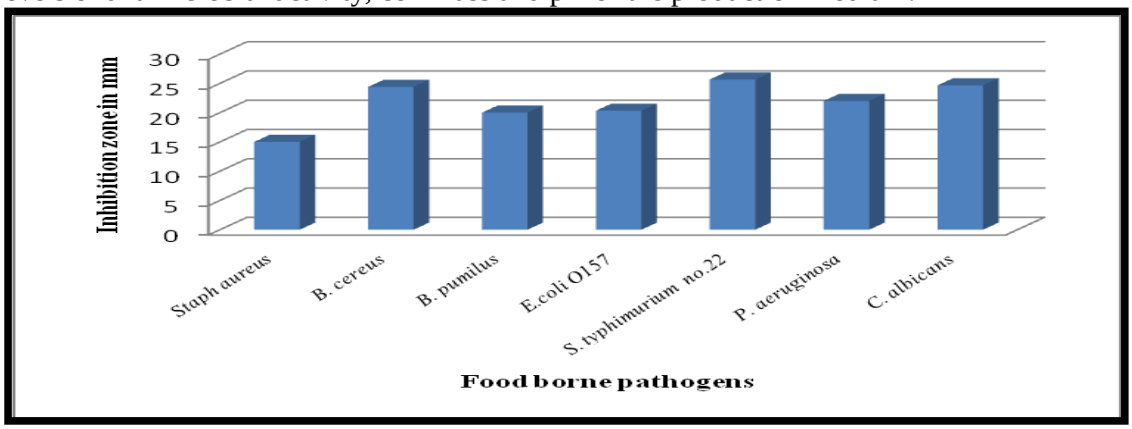

Figure (1): Detection of antimicrobial activity of *L. acidophilusNA-5* metabolites.

Effect of culture media on antimicrobial activity of *Lactobacillus acidophilusNA-5:*

The antimicrobial activity of *L. acidophilusNA-5* culture free cell supernatant (CFCS) using well diffusion method, cell density and final pH were determined after growth of probiotic *L. acidophilusNA-5* in different dilutions of cheese whey and in MRS as a control.

MRS broth is commonly used for small-scale cultivation of *L. acidophilusNA-5*. However, if industrial-scale growth of *L .acidophilus* is contemplated, it is necessary to develop a new low-cost medium. We tested inexpensive growth media containing diluted whey. Refined sugars (glucose or sucrose) are commonly used as a carbon source for the growth of probiotics, but these are economically not feasible due to high cost of pure sugars whereas the product (lactic acid) is relatively cheap (**Oh *et al.*, 2005**). *L. acidophilusNA-5*, according to **Table.1,** had the ability to grow in diluted cheese whey (CW), produce antimicrobial activity, although the cell density and inhibitory activity of the CFCS were lower than obtained in the control MRS.

Utilization of whey by *L. acidophilusNA-5* significantly inhibited the growth all tested organisms. Results were in according to the findings of **Ghasemi *et al* (2009**) who reported that the whey contains 50 g/l lactose, 0.6 g/l phosphate, 0.02 g/l calcium and 1 g/l chloride. Analysis shows the ultra filter whey had insufficient nutrients for growth of *Lactobacillus bulgaricus.* **Guerra *et al.*, (2001)** and **Perez *et al.*, (2013**) also reported that deproteinized diluted whey medium without supplementation was capable of promoting the growth of some probiotic lactic acid bacteria. **Enan and Alamri, (2006)** found that *L. plantarum* UG1 was also able to grow in whey permeate and produce the antimicrobial substances, but the biomass and antibacterial activity were lower than those obtained in the control MRS and were also less than those obtained when whey was enriched. **Ghasemi *et al* (2009)** reported when the whey percent in the medium increases (nutrient percent decreases) the lactose consumption increases and higher lactic acid is produced.

The results showed that the highest antimicrobial activity, the lowest pH values and the highest optical density were exhibited with original CW (4%) (**Table. 1).**

Table1: Influence of culture media on antimicrobial activity, final pH and growth (OD_{620nm}) of *Lactobacillus acidophilus*NA-5 after growth for 24 h at 37°C.

Culture medium	Final pH	OD_{620nm}	Antimicrobial inhibition zone (mm) Indicator microorganism		
			B. cereus	*S. typhimurium no. 22*	*C. albicans*
Control medium MRS	**3.610E**	**2.657A**	**24.4B**	**25.67A**	**24.67B**
Diluted CW(25+75)	5.630A	0.5237E	15.00HI	11.67M	12.33KL
Diluted CW (50+50)	5.483B	0.5610D	16.40EF	14.67I	14.17J
Diluted CW(75+25)	5.357C	0.5783C	16.00G	15.17H	12.67K
Normal CW	**5.253D**	**0.6763B**	**17.33D**	**19.00C**	**16.50E**
LSD	0.001693	0.001693		0.4215	

Values in this table are means of three replicates.
+ Optimum concentration for growth and antimicrobial activity production by *L. acidophilusNA-5*.
MRS (DeMan, Rogosa and Sharpe, 1960).
CW (Cheese whey).
OD: optical density.

LSD: less significance difference.

Additional minerals are occasionally required when the carbohydrate and nitrogenous sources lack sufficient quantities (**Narayanan *et al.*, 2004**). The effect of adding different sources of growth factors on whey fermentation, as measured by the antimicrobial activity of *L. acidophilusNA-5*, was investigated (**Table.2)**

It is clear from the results that nitrogen sources improved the antimicrobial activity when compared to the original cheese whey. The greatest values of inhibition zones (mm) were obtained when the CW was enriched with 1% peptone, 0.4% beef extract, 0.6 % yeast extract and 0.5% sorbitol forming 22.8, 23.7 and 23.30 mm of inhibition zones against *Bacillus cereus, S. typhmurium22* and *C. albicans* as the most sensitive strains, respectively (**Table.2).**

The obtained results were supported by many investigations. **Horn *et al.* (2005)** observed the growth of *L. plantarum* NC8 in hydrolysates of fish viscera that containing low amounts of yeast or fish peptones produced biomass. **Favaro *et al.*, (2012**) found that growth of *Enterococcus faecium* in CW was similar to the growth observed in MRS. Other similar results were obtained by **Ghaly *et al.*, (2003**) who reported that cheese whey enriched with 1% of both of yeast extract and lactamine AA gave the highest cell growth of *Lactobacillus helveticus*, lactose utilization and lactic acid yield. Some workers reported that improvement of CW as a medium can be achieved by addition of only yeast extract even in low concentrations. **Eldeleklioglu *et al.*, (2013)** reported that the highest production of lactic acid was achieved from whey when supplemented only with 0.5% yeast extract and without other nutrient supplements mentioned in literature. The above trend supporting the idea that the low concentrations of nutrient supplements could be attributed to the toxicity caused by the high concentration (1.5%) of yeast extract which led to the inhibition of cell growth (**Aeschlimann and von Stockar, 1990**). Although the culture medium is normally enhanced by the addition of supplements, it can be easily engorged by nutrients which can lead to the inhibition of growth and product formation. **Taleghani *et al* (2014)** reported that the cell dry weight concentration of *Lactobacillus bulgaricus* (ATCC 8001) is related to substrate concentration. *In-vitro* experiment using ruminal-based inoculum showed that sorbitol acted as a substrate that supported the growth of luminal bacteria which competitively inhibited growth of *E. coli*, suggesting the potential of sorbitol as a prebiotic (**De Vaux *et al.*, 2002**). Using pig and human cecal digesta, Sorbitol was also found to be slowly fermented and stimulated the production of butyrate and propionate (**Mortensen *et al.*, 1988; Kiriyama *et al.*, 1992**). This has been a desirable attribute as rapid fermentation often causes rapid growth of Gram-positive cocci, such as streptococci in the bowel (**Dawson and Allison, 1988).**

These results seem likely normal because lactic acid bacteria and bifidobacteria require many nutrients and rich media containing meat extract, yeast extract and protein hydrolysates (**Khay *et al.*, 2013**). On the other hand, yeast extract or peptones are needed to optimize the unbuffered whey composition (**Jozala *et al.*, 2011**). In addition, peptone and beef extract as nitrogen sources and yeast extract as a substrate containing various amino acids and various vitamins are of ability to enhance the growth of *L. acidophilusNA-5* or other probiotic lactic acid bacteria and stimulate the production of antimicrobial compounds from CW containing lactose as a carbonaceous source.

Table 2: Influence of culture media and nutrient supplements on antimicrobial activity of *L.acidophilus* after 24 h of incubation at 37°C.

Culture medium	Antimicrobial inhibition zone (mm) Indicator microorganism		
	B. cereus	*S. typhimurium. no.22*	*C. albicans*
Control medium MRS	**24.40abc**	**25.67a**	**24.67ab**
Normal Cheese Whey (CW) (4% lactose)	**17.33ps**	**19.00lp**	**16.50s**
a. different peptone concentrations (%):			
0.25	16.33s	17.00qs	17.67os
0.5	19.33ko	14.00t	14.33t
1	**20.00in**	**19.00lp**	**20.00in**
1.5	16.83rs	19.00lp	12.83t
b. CW+1%Peptone + diff. conc. of beef extract (%):			
0.2	22.43dg	20.33hm	20.67gl
0.4	**22.67df**	**21.00fk**	**21.67ei**
0.8	22.00dh	20.00in	19.33ko
1.2	19.00lp	17.00qrs	19.67jn
c. CW+1%Pep +0.4% beef +diff. conc. of yeast extract			
0.2	19.67jn	21.67ei	20.00in
0.4	20.00in	21.00fk	21.00fk
0.6	**22.70def**	**22.33dg**	**22.67def**
d. CW+1%Pep + 0.4%beef +yeast + diff. conc.Sorbitol			
0.5	**22.80cf**	**23.7bcd**	**23.30be**
1	18.33nr	21.00fk	20.67gl
1.5	18.67mq	21.33fj	20.33hm
2	16.00s	20.00in	16.67rs
2.5	17.33ps	20.67gl	16.00s
3	17.00qrs	17.33ps	18.33nr

LSD = 1.524

Values in this table are means of three replicates.
+ Optimum concentration for growth and antimicrobial activity production by *L.acidophilus*.
MRS (De Man, Rogosa and Sharpe, 1960).
CW (Cheese whey).

OD: optical density.

LSD: less significance difference

Some authors studied the effect of less expensive nitrogen source such as peptone malt sprout. They reported that yeast extract and peptone affect the cell concentration significantly (**Manteagudo *et al.*, 1995; Hujanen and linko, 1996). Fung *et al.* (2008**) showed that meat extract, vegetable extract and peptone significantly influenced the growth of *Lactobacillus acidophilus*. Nutrient supplements such as yeast extract, corn steep liquor, and lactamine AA (Casein hydrolysate) can improve the nutritional quality of the medium, because they contain growth promoting compounds, in addition to organic nitrogen, and carbonecious compounds (**Norton *et al.*, 1994**). **Ghaly *et al.* (2003**) found that the addition of nutrient supplements lactamine AA and yeast extract gave rapid cell growth due to the presence of most bacterial growth factors including amino acids, lipids, nucleotides (purines and pyrimidines), and vitamins (in case of yeast extract) which promote rapid propagation of the cells, enhance metabolism and stimulate the physiological activity of the cells.

Through the results shown in **Table (2) and Figure (2)**, it could be concluded that the higher the production of cell biomass of the *L. acidophilusNA-5*, the lower the pH values and the greater the antibacterial activity along the aforementioned supplementation steps. The above results supported the use of CW supplemented with1% peptone, 0.4% beef extract, 0.6% yeast extract and 0.5% sorbitol as the best medium for producing *L. acidophilusNA-5* metabolites with high inhibitory activity.

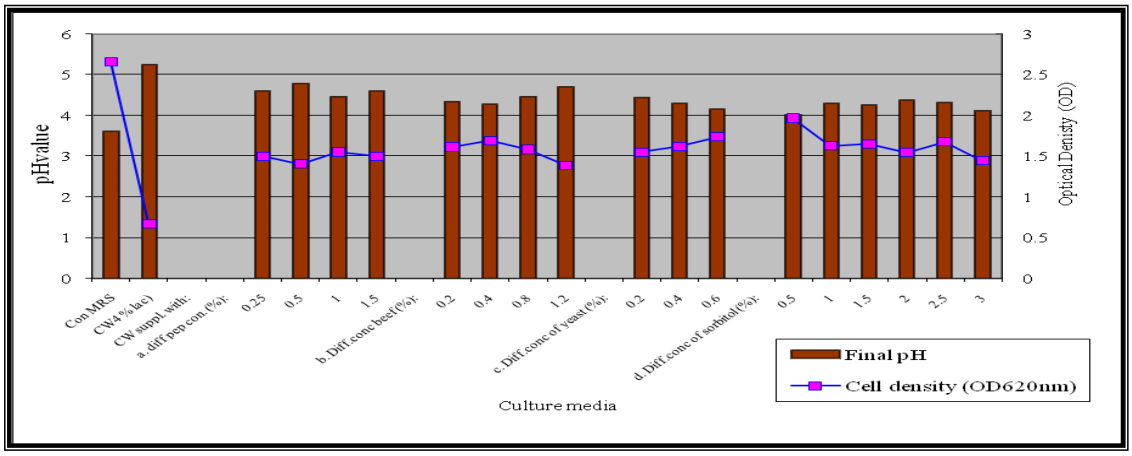

Figure 2: Influence of culture media and nutrient supplements on final pH and growth of *Lactobacillus acidophilus*NA-5 after 24 h of incubation at 37°C.

Optimal pH for antimicrobial activity:

Results in **Table (3)** indicate that the fermentation of whey proceeds at a faster rate at pH 6.2 with inhibition zones of 23.00, 23.83 and 23.43 mm against the most sensitive pathogens, namely *B. cereus*, *S. typhimerium no. 22* and *C. albicans* respectively. The cell growth reached 2.042 OD and was able to decrease the initial pH from 6.2 to 3.983. While the fermentation less efficiently at pH 5.8 and 7.2.

Our results are, thus, practically identical to those obtained by **Jozala *et al.*, (2011)** established that the highest production of antibacterial activity produced by *Lactococcus lactis* in milk whey was at initial pH 6.6. Additionally, **Favaro *et al.*, (2012)** reported that the highest antibacterial activity levels produced by *Enterococcus faecium* was achieved in supplemented CW with initial pH 6.5 and was comparable to those detected in MRS.

Table 3: The effect of initial pH on growth, final pH and antimicrobial activity of *Lactobacillus acidophilusNA-5* grown in supplemented cheese whey after 24 h of incubation at 37°C.

Initial pH	Final pH	OD_{620nm}	Inhibition zone (mm) Indicator Microorganisms		
			B. cereus	*S. typhimurium 22*	*C. albicans*
5.8	4.680b	1.392b	16.33d	15.00ef	16.00de
6.2	**3.983d**	**2.042a**	**23.00a**	**23.83a**	**23.43a**
6.4	4.043d	1.958a	21.30b	21.80b	20.83b
6.8	4.403b	0.9863c	17.67c	18.33c	17.67c
7.2	4.910a	0.7643d	14.33f	15.00ef	15.33def
LSD	0.2147	0.1883		1.110	

LSD: less significant difference.

Values in this table are means of three replicates.

OD: optical density.

Optimal incubation temperature and time course for antimicrobial activity:

This work examined the optimum temperature for microbial growth, and antimicrobial production. Bacterial growth under different temperature regimes were studied. Better understanding of the temperature effects on antimicrobial production will facilitate improvement of the production process. The temperatures were monitored and controlled during the fermentation process at elevated temperatures of 32, 37 and 45 °C with fermentation time 24 h as shown in **Figure (3).** The results showed that optimum temperature for the antimicrobial production was 37°C on the basis of inhibition zones of 23.30, 23.87 and 23.63 mm against the most sensitive pathogens, namely *B. cereus*, *S. typhimerium no. 22* and

C. albicans respectively. Higher temperature stimulated the slowly growth of *L. acidophilusNA-5* resulted in a slightly decrease in pH. Although the growth of *L. acidophilusNA-5* may occur at temperatures as high as 45 °C, the optimum growth occurs within 40-42°C. (**Korbekandi *et al.*, 2011**). **Krischke *et al.* (1991**) used 37°C temperature for lactic acid production using *L. casei*. **Favaro *et al.*, (2012**) supported the obtained results as they reported that the highest levels of antibacterial activity of *Enterococcus faecium* grown into enriched CW were recorded at 37°C. 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, as stated by **Al Jumaily *et al.*, (2013).**

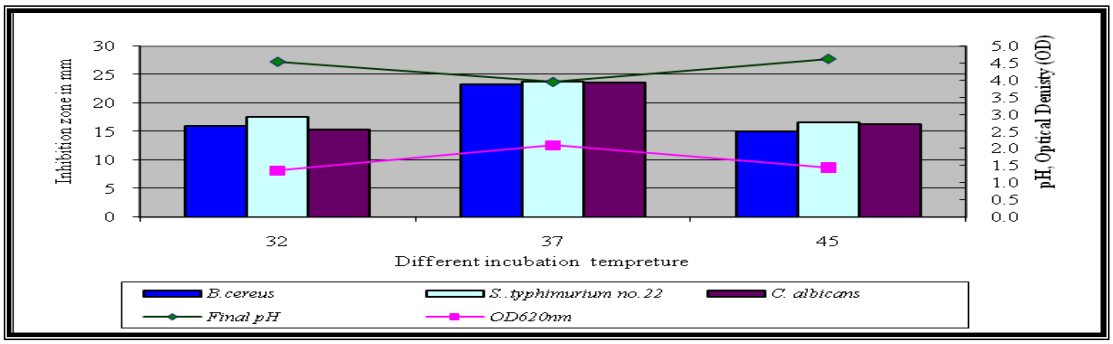

Figure 3: The effect of incubation temperature on growth, final pH and antimicrobial activity of *Lactobacillus acidophilusNA-5* grown in supplemented cheese whey after 24 h of incubation.

The time course of antimicrobial activity production of *L. acidophilusNA-5* metabolites produced in the supplemented CW medium and growth for 72 hrs is shown in **Figure 4**. Growth of the test organism reached a maximum after 24h of cultivation. Thereafter, the growth decreased. During the fermentation, the pH of the medium declined from 6.2 at the beginning to 3.857 after 3 days of fermentation. On the other hand, the antimicrobial activity was 23.47, 24.00 and 23.73 mm of inhibition zone diameter towards *B. cereus*, *S. typhimurium no. 22* and *C. albicans*, respectively after 24h fermentation, after which the level of antimicrobial activity decreased.

The obtained results were supported by many investigations. **Panesar *et al.* (2010)** reported that this could be attributed to the growth of the culture reached to the stationary phase and as a consequence of metabolism, microorganisms continuously change the characteristics of the medium and the environment. The reduction in fermentation period is additionally advantageous to improve the economics of the process. **Al Jumaily *et al.* (2013)** reported that maximum production of antimicrobial compounds, acetic acid, lactic acid and bacteriocin may occur at different phases of growth cycle, depending on the type of bacteria.

Contrarily, **Eldeleklioglu *et al.* (2013**) reported the highest lactic acid concentration was achieved from yeast extract-supplemented CW medium after 48 h of fermentation. 12 h incubation period was the best for antibacterial activity production of *Lactobacillus acidophilus* from whey according to findings of **Fatma *et al.*, (2013**).

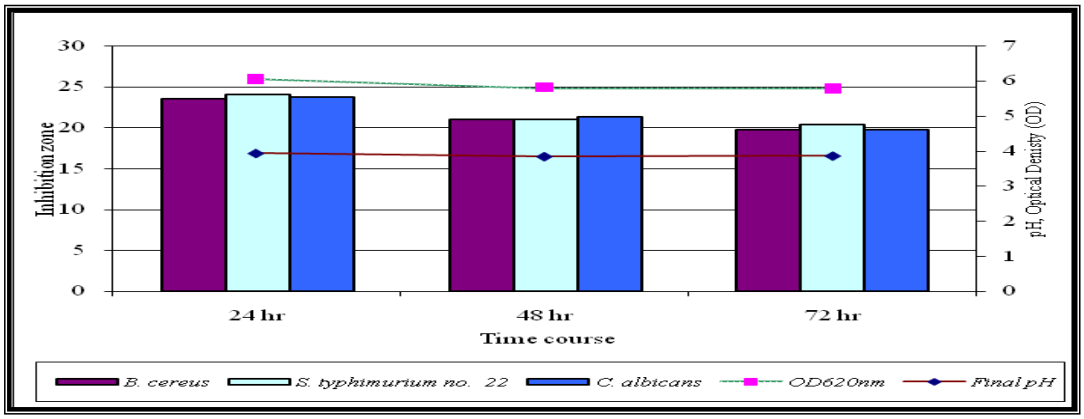

Figure 4: The effect of time course on growth, final pH and antimicrobial activity of *Lactobacillus acidophilusNA-5* grown in supplemented cheese whey at 37°C.

4. Conclusion:

Looking through the results along the current and previously related researches, it could be stated that the levels of antibacterial activity and growth of *L. acidophilusNA-5* in supplemented CW under optimally optimized conditions of inoculum size, initial pH and incubation temperature and period were comparable to those obtained in MRS as a reference medium. Supplementing CW with nutrients including peptone, beef extract, yeast extract and sorbitol gave reasonable cell mass and improve the nutritional quality of the medium which induce the antimicrobial efficiency of the tested *L. acidophilus NA-5*.

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الملخص العربي

تقييم نشاط التضاد الميكروبي لبكتيريا لاكتوباسلس اسيدوفيلس النامية علي مخلف شرش الجبنة ضد بعض مسببات الأمراض التي تنقلها الأغذية

أ.د.زينب محمد حسن خيرالله[١]، د.مني ابو النور[١]، أ.م.د مني مبروك[٢] ،د. أحمد يوسف عقدة[٣] وابتهاج عبدالفتاح صقر[١]

[١] قسم النبات- كلية البنات للآداب والعلوم و والتربية - جامعة عين شمس

[٢] الهيئة القومية للبحوث الدوائية

[٣] قسم ميكروبيولوجيا الالبان - شعبة الصناعات الغذائية والتغذية – المركز القومي للبحوث.

تم اختبار تأثير نواتج الأيض التي تنتجها بكتيريا لاكتوباسلس اسيدوفيلس في المعمل ضد بعض الكائنات الحية الدقيقة المسببة للأمراض الغذائية. النشاط المضاد للبكتيريا يتم تحديده بطريقة Well Diffusion method . *Bacillus cereus, Sallmonella typhimurium* no.22 و *Candida albicans* تم اختيارهم من سبعة كائنات مسببات الأمراض التي تنقلها الأغذية عن طريق حساسيتها تجاه نواتج الأيض التي تنتجها بكتيريا لاكتوباسيلس اسيدوفيلوس عندما نمت في بيئة (MRS). بكتيريا لاكتوباسيلس اسيدوفيلوس نمت علي تخفيفات مختلفة من مخلف (الشرش) كمصدر وحيد للكربون لتحديد افضل تخفيف لديه أكبر نشاط مضاد للميكروبات ضد الكائنات المسببة للأمراض التي تم اختبارها. وقد أظهرت النتائج أن الشرش الغير مخفف له تأثيرا عالي علي مسببات الأمراض المختارة.

وقد أجريت تجارب إضافية لتحسين بيئة الشرش، وذلك بإضافة تركيزات مختلفة من العناصر الغذائية. تم اختبار الرقم الهيدروجيني ودرجة الحرارة أيضا لتعظيم الاستفادة. وقد كشفت النتائج أن ١٪ ببتون، ٠.٤٪ مستخلص لحم ا، و ٠.٦٪ مستخلص الخميرة و ٠.٥٪ السوربيتول كانت التركيزات المثلى التي أظهرت الأنشطة الهامة ضد نمو الميكروبات المختارة.

أظهرت درجة الحموضة ودرجة الحرارة المثلى النشاط الأكثر لمضادات الميكروبات ٦.٢ و ٣٧ درجة مئوية. من النتائج التي تم الحصول عليها، يمكن استخدام بيئة مخلف الشرش كبيئة منخفضة التكلفة لإنتاج نواتج أيض مفيدة من بكتيريا لاكتوباسيلس اسيدوفيلوس.