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PROTOLYTIC ACTIVITY OF CERTAIN STRAINS Of BACILLUS SPP. USED AS SUBSTITUTE FOR BUFFALOES MILK CLOTTING

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ABSTRACT

The protolytic activity of 14 strains of *Bacillus spp* isolated from milk samples collected from different governorates in Egypt was evaluated . The strains were inoculated in pasteurized buffaloes milk incubated at 30 ^{o} C for 5 hours .Acidity, pH ., soluble nitrogen (SN) and non protein nitrogen (NPN) as % of total nitrogen (TN) were determined during the incubation period . The tested strain showed a wide range of protolytic activity as indicated by the rate of soluble nitrogen (SN) and non protein nitrogen (NPN) contents during incubation . The strain No 4,6 and 8 coagulated milk after 2 hours of incubation and showed the highest levels of both SN and NPN content . So these strains could be recommended the production of milk clotting enzyme .

The inhibitory affect of *Lactobacillus acidophilus , Lactobacillus delbrueckii subsp. bulgaricus* and *Lactobacillus casei subsp. casei* on *Bacillus* were evaluated use by diffusion method on nutrient agar media for the fourteen *Bacillus spp*. It was found that the inhibtory of *Lactobacillus acidophilus* was more than *Lactobacillus delbrueckii subsp. bulgaricus* and *Lactobacillus casei subsp. casei* except on *B . sphaercus* . The inhibitory effects of *L. acidophilus ; L. delbrueckii subsp. bulgaricus* and *Lactobacillus casei subsp. casei* was the same. Further mores the effect of heat treatment within the temperature range of 85-135 ^{o} C. , and various exposure times was studied . The highest resistance was found in case of *B . amyloliquficans* spores , which survived the temperature of 135 ^{o} C. the greatest reduction of spore numbers was found for *B .cereus* .

INTRODUCTION

Raw milk represents a very suitable medium for the growth of bacteria. Also the quality of product milk depends on its microflora.

Aerobic, spore forming, Gram positive bacteria of *Bacillus spp.* represents the important contaminate of the raw milk which plays an important role in the hygienic and technological points of view in dairy products .

Bacillus spp .are responsible for serious problem in dairy industry due to the heat resistance of spores and ability of regulative cell to produce extra cellular enzymes which cause deterioration of milk and milk products. *Bacillus spp*. are quite common in the agriculture environmental condition. So it easy to contaminate milk from various sources during production, storage, and processing. Moreover raw milk most frequently contaminated under condehons of inadequate hygiene, from soil, feed, dust, and fasces (Christiansen *et al* 1999)

Spores of *Bacillus spp*. appear regularly instable environmental and usually represent a secondary contamination of milk during milking process. Some species such as *B licheniformis* , *B subtilis* and *B cereus* are most commonly isolates from row milk (Crielly *et al* 1994).

B. licheniformis together with *B. subtilis* and *B. pumilus* belong to mesophilic , whereas *B. cereus* is rather psychrophilic and able to grow in milk and milk products at cold ,storage and its transportation .It is well known that B. spp were found in both raw milk and during pasteurizing process (Christianssess *et al*. 1999) .

B. cereuse is a predomination microorganism influencing the maintenance of pasteurized milk . It causes sweet curdling of milk and changed milk odour (meer *et al.*, 1991).These types of *B.spp*. produce (100%) photolytic and (61-82 %) lipoletic enzymes . All B.spp. strains produce highly protolytic enzymes. (Vyletelova *et al* 2001) .The present study aimed to evaluate the photolytic activity of 14 strains of B. spp. isolated from milk samples collected from different governorates of Egypt by(Nasr2007). These strains were shown in (Table 1) .

Table (1): 14 *Bacillus spp*. isolated from raw and heat treated milk

Bacillus amyloliqufaciens	***(SH1e)***	**(1)**
Bacillus amyloliqufaciens	***(SH2e)***	**(2)**
Bacillus amyloliqufaciens	***(SH3e)***	**(3)**
Bacillus amyloliqufaciens	**(D2c)**	**(6)**
Bacillus amyloliqufaciens	**(D3c)**	**(7)**
Bacillus amyloliqufaciens	**(K1a)**	**(8)**
Bacillus amyloliqufaciens	**(K2a)**	**(9)**
Bacillus amyloliqufaciens	**(B2a)**	**(12)**
Bacillus amyloliqufaciens	**(B3d)**	**(13)**
Bacillus cereus	**(SH4a)**	**(4)**
Bacillus cereus	**(D1a)**	**(5)**
Bacillus cereus	**(K3a)**	**(10)**
Bacillus cereus	**(B1b)**	**(11)**
Bacillus sphericus	***(B3e)***	**(14)**

In this study the proteolysis activity of these 14 strains of *Bacillus spp* . in pasteurized buffalo's milk to coagulate it were evaluated during the incubation for 5 hours at 30 ^{o}C.

MATERIALS AND METHODES

Milk samples were collected from different locations in Egyptian governorates (Giza - Kafr ElShikh - BaniSueif - Sharquia - Domiatta -North Sinai)

Pour plate technique was used for isolation of bacterial strains. Plates were incubated at 30ºC for 24h. Different isolates were sub cultivated on slants, medium used for isolation and purification on nutrient agar "oxoid"

The 14 isolates were identified according to "Holt *et al*;1994"and Murry *et al* - 1999", after that the identification of bacterial isolates up to species level were confirmed using BIOLOG system .

The well diffusion assay described by (Jin *et al* ; 1996) was used to detect the antagonistic effect of the 3 *Lactobacillus sp (L. acidophilus , L. delbrueckii subsp. bulgaricus* and *Lactobacillus casei subsp. Case i*) against the identified bacterial isolates. Seeding Petri dishes with the

bacterial isolates and then introduced 0.1 ml of Lactobacillus spp,. Into the holes bare with 5 mM carburet .Plates were incubated aerobically at 37 ºC for 74 h after that the plate examined far clear zone of inhibition .

Determination of thermo resistance of *Bacillus* isolates spores. The spore suspension were prepared according to the procedure described by Bradshaw *et al* (1975) . 0.5 ml from each spore suspension of given strains was submitted to heating in water bath at (85 and 95°c) and in glycerol bath at (100,105,110,115;120 and 135°c)exposure periods determined depend upon the heating temperature.

The enzyme activity (IUs/ml) was assayed for the cell-free filtrates of both sets using the method of Thangam and Rajkumar (2000) which is a modified method of Anson (1938) as follows:

1. The reaction mixture consisted of 1 ml of 1% (w/v) casein solution as a substrate and 1 ml cell-free filtrate. The casein is dissolved in 0.2 M sodium phosphate buffer at pH 7.2.
2. The reaction mixture was incubated at 37°C for 30 min to allow the action of enzyme on substrate.
3. The reaction terminated by the addition of 2 ml of 10% TCA (w/v) trichloroacetic acid or 0.5 ml of 20% TCA (Ghorbel *et al.,* 2003). Let stand in crushed ice for at least 1 hr (Gallop *et al.,* 1957). After separation of unreacted casein, precipitate by centrifugation. The amount of soluble protein in the supernatant was estimated by the use of Folin Ciocalteau's reagent according to Lowery *et al.* (1951). The optical densities of the samples were measured at 750 nm in a 20 D spectrophotometer. All the readings were corrected for the values of blanks which were prepared by first mixing casein solution with TCA and then adding enzyme preparation. The slope of the standard curve determined with L-tyrosine was used in the calculation of protease activity (Fig. 1).One unit of protease activity (U)was defined as the amount of enzyme required to liberate 1 μg of L-tyrosine/ml/min at 37°C. protein was estimated by the method of lowery *et al.* (1951) using bovine serum albumin (BSA) as a standard (Fig. 1).

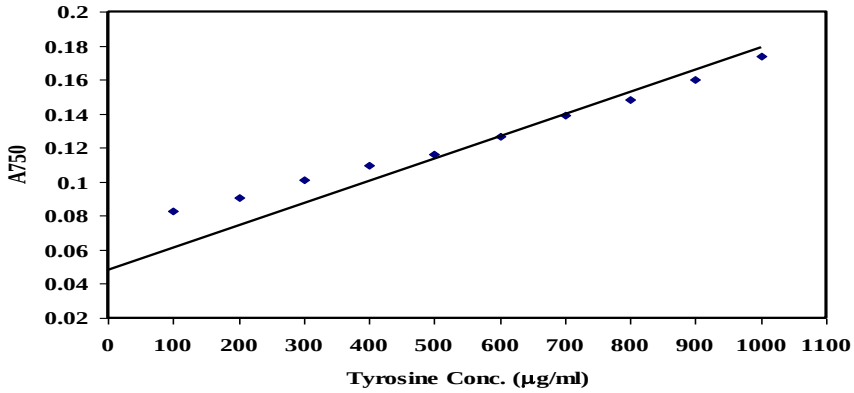

Fig. (1): The standard curve of L-tyrosine.

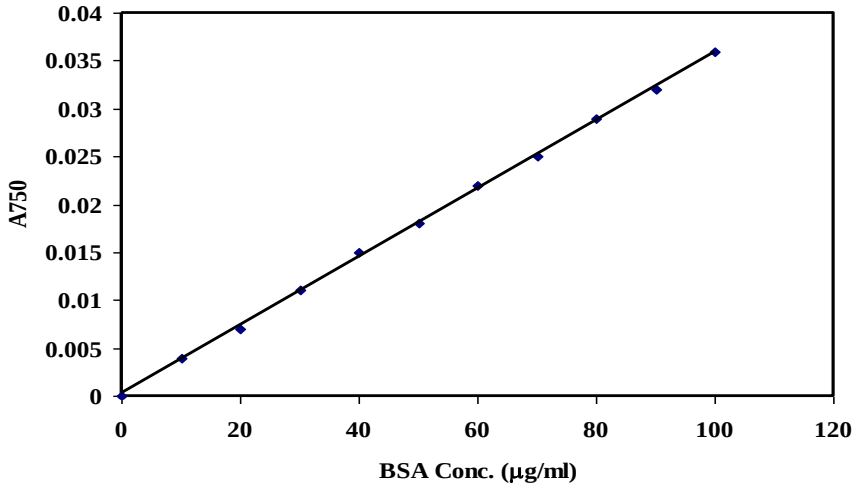

Fig. (2): The standard curve of bovine serum albumin (BSA).

Determination of protein content: Protein was estimated by the method of Lowery *et al.* (1951 a)

Chemical analysis

The titratable acidity, total nitrogen, soluble nitrogen and Non-protein nitrogen contents were determined to Ling 1963

pH value : -

pH value was measured directly using a digital pHmeter model 201 Orion Research , Japan

RESULTS AND DISCUSSION

Table (2), represented the coagulation time of pasteurized buffalo's milk inoculated with 14 strains of Bacillus spp. individually . The strain No 2, 3, 4, 6, 8, 10 and 13 were coagulated pasteurized buffalo's milk after 2hr. Whereas , the strains No.5and 11 coagulated the buffalo's milk after 3 h. Strains No. 7 and 9 coagulated milk after 4h. On the other hand, the control and strains No 1, 12 and 14 did not observed any coagulation of the tested milk.

This could due to the production of rennet-like enzymes by some strains Bacillus spp which may coagulate pasteurized buffalo's milk .This is commonly known as sweet curdling.

Results presented in Table (2) also showed the development of acidity and pH in head-treated buffalo's milk inoculated with 14 strains of Bacillus spp. during incubation at 30 C for 5 hr.. Results indicated that acidity of buffalo's milk gradually increased at slow .rate during the incubation period .However the control sample did not show developing of the acidity up to 2 hr. of incubation . The strains showed different rates for acidity development after 2 hr. of incubation. Strains No. 1 , 2 , 3 , 4. 5 , 6 , 8 , 10 , and 13

developed the milk acidity to .195 , 0.190 ,0 .195 , 0.20 , 0.195 , 0.20 , 0.198 , 0.20 , and 0.20 % respectively after 2 hr. of incubation . However the strains No. 7 , 9 , 11 , 12 , and 14 showed very slower rate of acidity development after 2 hr. of incubations reaching 0.184 , 0.175 , 0.185 , 0.173 , and0.185 % respectively. The trend of pH changed were associated with trend observed for acidity which were at the obesity side for the acidity .

Table (2): Effect of Bacillus spp. on the titratatable acidity , pH and coagulation of pasteurized buffalo‘ s milk during incubation of 30º C for 5 hrs.

No. of samples	Incubation time (h.)							
	0	1/4	1/2	1	2	2	2	2
Control	0.15	0.15	0.155	0.16	0.16	0.165	0.17	0.18
	6.70	6.70	6.66	6.60	6.57	6.57	6.50	6.40
1	0.15	0.155	0.165	0.18	0.195	0.21	0.23	0.25
	6.70	6.68	6.53	6.44	6.31	6.25	6.21	6.19
2	0.15	0.16	0.17	0.18	0.19			
	6.70	6.59	6.50	6.41	6.37	-	-	
3	0.15	0.165	0.173	0.180	0.195			
	6.70	6.57	6.38	6.30	6.19	-	-	-
4	0.15	0.173	0.180	0.197	0.20			
	6.70	6.60	6.46	6.40	6.36	-	-	-
5	0.15	0.160	0.173	0.180	0.195	0.220		
	6.70	6.60	6.46	6.40	6.36	6.320	-	-
6	0.15	0.158	0.164	0.182	0.20			
	6.70	6.62	6.58	6.42	6.38	-	-	-
7	0.15	0.160	0.167	0.17	0.184	0.190	0.215	
	6.70	6.61	6.58	6.46	6.32	6.28	6.30	-
8	0.15	0.167	0.77	0.185	0.198			
	6.7	6.50	6.48	6.41	6.34	-	-	-
9	0.15	0.155	0.160	0.167	0.175	0.183	0.190	
	6.70	6.67	6.70	6.52	6.48	6.40	6.35	-
10	0.15	0.160	0.168	0.18	0.20			
	6.70	6.60	6.43	6.40	6.33	-	-	-
11	0.15	0.160	0.168	0.175	0.185	0.195		
	6.70	6.57	6.45	6.42	6.37	6.32	-	-
12	0.15	0.164	0.17	0.173	0.173	0.175	0.18	0.192
	6.70	6.60	6.53	6.49	6.42	6.38	6.38	6.30
13	0.15	0.160	0.177	0.195	0.20			
	6.70	6.55	6.46	6.34	6.34	-	-	-
14	0.15	0.165	0.167	0.174	0.185	0.192	0.210	0.220
	6.70	6.355	6.50	6.47	6.36	6.10	5.58	5.40

- = Milk was coagulated

The changes in soluble nitrogen (SN) and non protein nitrogen (NPN) as % of total nitrogen (TN) in the pasteurized buffalo's milk inoculated with *Bacillus spp* were taken as indicates for effect of protolytic enzymes produced by these strains on milk protein degradation

The results presented in table (3) showed the soluble nitrogen (SN) values as % of total nitrogen in the pasteurized buffalo's milk inoculated with

14 strains of *Bacillus spp.* each single only and incubated at 30 ° C . Studying these data, it could be noted that the samples of pasteurized buffalo's milk inoculated with strains NO. 4 , 6 , 8 and 13 showed the higher content of SN/TN being 37.80, 38. 20, 38.00 and 36.00 after 2 hours incubation at 30°C. respectively. This indicated that strains produce high amount of protealytic enzymes. The corresponding values on the other samples were 23.00 , 27.00 , 31.00 , 27.50 , 38.00 , 23.50 , 31.40 ,26.50 , 21.90 and 21.90 for samples No. 1 , 2 , 3 , 5 , 7 , 9 , 10 , 11 , 12 and 14 in order . The lowest amount of SN was noticed for control sample which was 16.80 after2 hours incubated at 30 ° C

Table (3): Effect of *Bacillus spp.* on the soluble nitrogen /total Nitrogen (SN/TN) of pasteurized buffalo's during incubation of 30° C for 5 hrs.

No. of samples	Incubation time (h.)							
	0	1/4	1/2	1	2	3	4	5
Control	16.43	16.43	16.5	16.6	16.8	17.0	17.2	17.3
1	16.43	17.90	19.7	21.5	23.0	24.5	25.5	26.3
2	16.43	18.00	20.2	25.0	27.0			
3	16.43	19.25	14.5	28.7	31.0			
4	16.43	20.70	28.9	35.0	37.8			
5	16.43	18.00	21.5	25.7	27.5	29.3		
6	16.43	18.55	24.9	33.0	38.2			
7	16.43	17.90	19.0	21.5	24.0	26.4	27.7	
8	16.43	19.00	25.5	24.0	38.0			
9	16.43	17.55	19.0	20.8	23.5	26.5	28.0	
10	16.43	19.50	22.3	27.0	31.4			
11	16.43	17.50	19.0	23.0	26.5	30.0		
12	16.43	19.54	18.7	20.0	21.9	23.5	25.2	26.7
13	16.43	19.50	25.0	32.9	36.0			
14	16.43	17.25	18.5	20.5	21.9	23.0	24.2	25.0

Table (4): Effect of *Bacillus spp*. On the nonparties nitrogen /total nitrogen (NPN/TN) of pasteurized buffalo‘ s milk of pasteurized buffalo‘ s milk

No. of samples	Incubation time (h.)							
	0	1/4	1/2	1	2	3	4	5
Control	6.26	6.26	6.40	6.55	6.70	6.90	7.00	7.10
1	6.26	6.50	7.50	9.88	10.50	11.2	12.0	12.57
2	6.26	7.50	10.9	13.0	15.60			
3	6.26	9.50	14.0	17.0	19.5			
4	6.26	10.50	15.0	18.5	21.57			
5	6.26	8.00	11.7	15.5	17.00	18.7		
6	6.26	10.00	14.5	18.7	21.29			
7	6.26	7.00	9.50	10.5	11.75	12.5	13.7	
8	6.26	11.00	17.0	20.0	22.00			
9	6.26	7.90	10.0	11.0	11.50	12.0	13.2	
10	6.26	10.50	15.0	17.5	19.00			
11	6.26	8.75	12.0	15.5	17.00	18.0		
12	6.26	8.00	12.0	12.2	13.50	14.7	15.5	15.80
13	6.26	10.55	14.0	16.5	18.52			
14	6.26	7.00	8.00	8.50	8.95	9.50	9.95	10.00

Results presented in Table (4) showed the changes in the Non-protein nitrogenous compounds as percent of total nitrogen for buffalo's milk inoculated with 14 strains of *Bacillus spp*. each single only and incubated at 30 ^{o} C . Data indicated higher rate of increase in (NPN/TN) in milk inoculated with strains No2, 3, 4, 5, 6, 8, 10, 11, and 13being 15.60, 19.5, 21.57 ,17.0, 21.29, 22.00, 19.00, 17.0 and 18.52 %respectively after 2 hours of incubation . The highest values were reported for strains No4 ,6, and 8 being 21.57 , 21.29 , and 22.00% respectively .

The lowest values were observed for strains No 1, 9, and 14 being 10.50 , 11.50, and 8.95 % respectively after 2 hours incubation .

The obtained results indicated that the tested 14 strains of Bacillus spp. Showed a wide range of protolytic activity on heated buffolos milk proteins . The strains No. 4 , 6 , and 8 showed the highest rates of protolytic activity .These strains coagulated the buffalo's milk after 2 hours of incubation.

These results are in agreement with those reported by other different studies .(Gessesse *et al* 2003 ; Bromme *et al* 2004 and setyorini *et al* 2006) .

The inhibitory effect of some *Lactobacillus sp (L. acidophilus , L bulgaricus and L. casei*) on selected strains of *Bacillus* were studied and tabulated in (Table 5).Results illustrated that the effect of *Lactobacillus sp (L. acidophilus , L bulgaricus and L. casei*) was equal on *Bacillus* sphericus isolates but the effect of L.*acidophilus* was more inhibitor against *Bacillus* isolates than *L bulgaricus and L. casei* . These result obtained are in agreement with those reporteds (Balasubramanyam and Varadaraj 1995 and Uraz *et al* 2001).

Heat resistance of *Bacillus sp.* spores.

Heat resistance of spores was evaluated using the D-value . Mean, maximal and minimal D-value are plotted in (Table 6) which shown the great variability of spore resistance of individual species as well as individual strains of the genus *Bacillus* at different heating temperatures . The rise of heating temperature from 95 to 135°c led to decreasing the D-value of all isolates spores and this indicate that heating for 135°c was adequate for inactivation of spores even in high initial concentration (up to $10^{6}ml^{-1}$) of all examined strains except for *Bacillus amyloliquefaciens* spores which were able to germinate even after 135°c , but all isolates spored detected active at 115°c ,and at heating for 120°c the isolated spores of *Bacillus amyloliquefaciens* was found to be the most resistance isolates if compared with other isolates . The respective D-value at heating temperatures of (85, 95 , 100 , 105 , 110,115, 120 and 135°c) were (5.6, 4.6, 2.4, 1.25 , 0.67, 0.37, 0.17 and 0.02 min) these obtained results were in agreement with data recorded by (Pendurkar *et al* ; 1989).

Table (5): The antagonistic effect of *Lactobacillus sp.* against *Bacillus* isolates

***Lactobacillus* "0.1ml"** / **NO.of *Bacillus* isolates**	***L.acidophilus***	***L.Delbrueckii subsp.bulgaricus***	***L.casii subsp. casi***
1	0.8	0.4	0.2
2	0.4	0.2	0.3
3	0.5	0.3	0.4
4	0.5	0.4	0.4
5	0.5	.04	0.4
6	0.5	0.3	0.4
7	0.4	0.2	0.3
8	0.4	0.2	0.3
9	0.5	0.3	0.4
10	0.5	0.3	0.3
11	0.5	0.3	0.3
12	0.4	0.2	0.3
13	0.4	0.2	0.3
14	0.5	0.5	0.5

From the recorded data it could be concluded that the spores of *Bacillus amyloliquefaciens were* able to survive even after UHT processing for milk depending upon the spore concentration but *Bacillus amyloliquefaciens* had no effect on the UHT milk quality .Spores of other *Bacillus* surviving at temperature ranged from 95 to 120°c may play an important role in the contamination of cream and high pasteurized milk for the production of some foods.Results of resuscitation of *Bacillus* isolates spores plotted in Table (7) indicated that , heating temperature of 135°c was sufficient for the destruction of spores, these results were in agreement with those reported by Janstova and Lukasova (2001).

Table (6): Heat resistance D value (min). of *Bacillus sp.* spores.

Isolates	**Heat temperature (°C)**								
		85	**95**	**100**	**105**	**110**	**115**	**120**	**135**
Bacillus amyloliqufacienes	Mean	5.6	4.6	2.4	1.25	0.67	0.37	0.17	0.02
	Max.	3.01	2.04	1.11	0.52	0.26	0.12	0.02	0.00
	Min.	12.9	11.75	5.1	3.40	1.82	1.62	0.46	0.17
Bacillus cereus	Mean	3.12	2.03	0.78	0.25	0.12	0.06	0.02	0.00
	Max.	2.79	1.78	0.67	0.76	0.06	0.02	0.00	0.00
	Min.	3.77	2.80	1.50	0.53	0.42	0.18	0.07	0.00
Bacillus sphericus	Mean	5.40	4.43	1.87	0.98	0.37	0.17	0.03	0.00
	Max.	4.4	3.31	2.20	0.74	0.32	0.04	0.00	0.00
	Min.	7.9	6.83	1.95	1.11	0.42	0.28	0.05	0.00

Table (7): Effect of different combination on surviving of *Bacillus amyloiqufacien* and *Bacillus cereus*.

Temp (°C)	Heat time	*B. amyloliqufcanes* %	*B. cereus* %
95	1 (min)	47.0	31.9
	2	24.5	10.2
	3	13.6	4.0
	4	8.2	1.3
	5	5.0	0.4
100	1	52.0	9.0
	2	28.0	1.0
	3	9.0	0.4
	4	4.0	0.04
	5	2.0	0.01
105	5 (sec)	81.0	56.0
	10	65.0	35.0
	20	45.0	14.0
	30	29.0	9.0
	60	12.0	2.0
110	5	65.0	17.05
	10	46.0	7.03
	20	24.0	2.20
	30	13.0	0.83
	60	4.0	0.08
115	5	42.0	7.0
	10	23.0	2.0
	20	11.2	0.2
	30	5.0	0.03
	60	1.0	0.00
120	5	22.0	0.00
	10	9.3	0.00
	20	4.0	0.00
	30	2.0	0.00
	60	0.3	0.00
135	5	2.4	0.00
	10	0.0	0.00
	20	0.0	0.00

Screening of bacterial isolates for their protease productivity : -

Screening of the activity of protease enzymes by selected Bacillus strains is tabulated in Table (8) .

The most potent isolate is *Bacillus amyloliqufaciens* (D2c and K1a) . The proteolytic activity determined using the standard curve of L-tyrosine that was constructed for such purpose , as represented graphically in Fig. (2)

The *proteolytic activity of Bacillus amyloliqufaciens* (D2c) was 118.89 U
Bacillus amyloliqufaciens (k2a) was 122.23 U

This obtained results were agreement withthose reportedby (Abd el-hady 2002 , Janstova *et al* 2004 and Labena 2004)

Table (8):Protease activity of *Bacillus spp*. isolated from raw and heat treated milk

***Bacillus spp*. isolates**	**Clear zone / cm**
Bacillus amyloliqufaciens (SH1e)	2.0
Bacillus amyloliqufaciens (SH2e)	1.7
Bacillus amyloliqufaciens (SH3e)	2.4
***Bacillus amyloliqufaciens* (D2c)**	3.0
***Bacillus amyloliqufaciens* (D3c)**	1.9
***Bacillus amyloliqufaciens* (K1a)**	3.1
***Bacillus amyloliqufaciens* (K2a)**	2.8
***Bacillus amyloliqufaciens* (B2a)**	2.5
***Bacillus amyloliqufaciens* (B3d)**	2.7
***Bacillus cereus* (SH4a)**	2.5
***Bacillus cereus* (D1a)**	2.1
***Bacillus cereus*(10) (K3a)**	2.9
***Bacillus cereus* (11) (B1b)**	1.7
Bacillus sphericus*(14) *(B3e)	1.5

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تقييم نشاط بعض السلالات للبكتيريا العصويةالتابعة لجنس.Bacillus Spp على تحليل البروتين فى اللبن الجاموسالمعامل بالحرارة.

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تم عزل و تحديد وتصنيف وتعريف طبقا للقواعد العامة للتقسيم لعرلات من البكتيريا العصوية..Bacillus spp السائدة فى الألبان المعاملة حراريا والمجمعة من بعض المحافظات حيث وجد أن ١٤ عزلة منتشرة فى هذه العينات والتأكد منها بأستخدام نظام البيولوجى – وبصمة البروتين والبصمة الجينية للخلايا البكتيرية وعرفت تلك العزلات على الوجه الأتى : -

تسع عزلات من *Bacillus amyloliqufaciens* و أربع عزلات من *Bacillus cereus* و عزلة واحدة من *Bacillus sphericus* ورتبت من ١-١٤ حسب نسبة أنتشارها فى العينات

تهدف هذة الدراسةالى تقيم مقدرة تلك العزلات على تحليل البروتين اللبن الجاموسى المعامل بالحرارة كما تم التأثير المثبط لبعض سلالات بكتريا حمض اللاكتيك العصوية من جنس *Lactobacillus* على هذه العزلات فوجد أن تأثير *Lactobacillus acidophilus* كان أقوى هذه السلالات تأثيرا ماعدا على عزلة *Bacillus sphericus* فكان التأثير متساويا .

كما تم دراسة تأثير المعاملات الحرارية على جراثيم هذه العزلات وقد كانت درجات الحرارة المستخدمة فى *المعاملات الحرارية للبن التى* تتراوح بين (٨٥-١٣٥°س) وجد أن جراثيم بعض هذه العزلات تتحمل البقاء حية حتى عندما تصل درجة الحرارة إلى١٣٥°س

و تم دراسة قدرة هذه العزلات على أنتاج انزيم البروتيز وذلك بتلقيح هذه العزلات على بيئة اجار اللبن وتحضينها على ٣٧ درجة مئوية لمدة ٢٤ ساعة ودراسة المنطقة الرائقة للتعرف على مدى انتاج انزيم البروتيز وتم بعد ذلك قياس النشاط الأنزيمى لأفضل سلالاتين تنتج الأنريم وهما

Bacillus amyloliqufaciens (D2c) = 118.89 U

Bacillus amyloliqufaciens (k2a) = 122.23 U

ومن هذه الدراسة وجد أنه يمكن تنشيط هذه السلالات ومعاملتها للأستفادة من من انتاجها للأنزيمات لأمكانية أستخدامها كبدائل لتجبن اللبن وأسراع تسوية الأجبان . وهذا يحتاج الأستكمال هذه الدراسة

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