Zagazig J. Agric. Res., Vol. 35 No. (4) 2008 867-883

EVALUATION OF THE ANTIBACTERIAL ABILITY OF *BIFIDOBACTERIUM BIFIDUM* AGAINST *STAPHYLOCOCCUS AUREUS IN VITRO* AND DURING DOMIATI CHEESE MANUFACTUING

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Accepted 20/8/2008

ABSTRACT: **Cell free supernatants (CFS), cell suspensions (CS) as well as disintegrated cells (DC) of *Bifidobacterium bifidum* ATTC 15696 and *Bifidobacterium bifidum* Bb12 were examined for their antibacterial activity. *Staphylococcus aureus* NCTC 6571 was chosen as a test microorganism using disc assay technique.**

Ammonium sulphate precipitates of concentrated CFS as well as Methanol-chloroform extracts (1 : 1 v/v) were examined for their antimicrobial activity while methanol- chloroforem extracts were assessed for their molecular weight cut off (MWCO) in Datons (3KD), minimal effective concentration and heat stability.

Domiati cheese milk containing approximately 10^6 organisms/mL was incorporated with either 1, 2 and 3% of bififobacterial culture or antibacterial substance extracted from *Bifidobacterium bifidum* then converted to Domiati cheese. The produced cheese was analysed for titratable acidity, bifidobacterial counts, survival counts of *Staphylococcus aureus* and the remained antibacterial activity in cheese, when fresh and during pickling period of 8 weeks.

The attained results showed that neither CS nor DC had antibacterial activity. The antibacterial activity was detected in CFS.

The antibacterial activity in the precipitated pellet using ammonium sulphate, was without antibacterial activity, while the methanol-chlorform extracted substances had a strong antibacterial activity against *Staphylococcus aureus,* it has a molecular weight

lower than 3 KD and was heat-stable (72°C/15 second), the minimal effective concentration was 50μg/mL.

Using 50, 100, 150 μg/mL of the CFS dried extract to control the growth of *Staph. aureus* during cheese making did not control the growth of *Staph. aureus* but it was found that the inoculation of cheese milk with 3% bifidobacterial culture resulted in producing Domiati cheese with good bacteriological quality and healthy benefits.

Key words: *Bifidobacteria*, pathogenic bacteria, minimal effective concentration, antibacterial activity, disc assay technique, healthy benefits

INTRODUCTION

Bifidobacteria are indogenous gut organisms in gastrointestinal tract of humans. They are thought to have number of advantageous effect on the health of the host in both infants and adults (Miytsuok, 1990, Hughes & Hoover, 1991 and Salminen *et al.,* 1996) as they have potential benefits including inhibition of pathogenic microorganism (Duffy *et al.,* 1994 and Rodriguez *et al.,* 2000) alleviation of lactose intolerance reduction of cholesterol level, (Rasic *et al.,* 1992 and Mamdouh, 2005), as well as tumer inhibitory effect (Reddy and Rivenson, 1993).

The use of bifidobacteira as dietary adjunct in commercial dairy products was increased since the importance of maintaining balance among organisms has become widely recognized (Shimamura, 1982, Yuguchi, 1984 and Shehata *et al.,* 2004).

Bifidobacteria showed antibacterial activity towards enteropathogenic bacteria (Anand *et al.,* 1984 and Okamura *et al.,* 1986).

Because of its nutritional effects many efforts have been devoted to incorporate bifidobacteria in dairy products, e.g. yoghurt (Ariga *et al.,* 1989 and Torre *et al.,* 2003) probiotic ice-cream (Younis *et al.,* 1998), cheese with healthy benefits (Arunachalam, 1999, Shehata *et al.,* 2004 and Mamdouh, 2005) fermented milk (Bozanic *et al.,* 2002), Ras cheese (El-Abbbassy *et al.,* 2000). Detailed studies have been performed to demonstrate the antibacterial activity among

bifidobacterial strains (Anand *et al.,* 1984 and Yildrim & Johanson, 1998).

On the hand, *Staphylococcus aureus* is pathogen of major concern of dairy industry. Its survival in different cheese varieties has been well documented (Ibrahim *et al.,* 1981, Reitsama and Henning, 1996, Nunez *et al.,* 1997 and Rodriguez *et al.,* 2000).

Controlling of *Staphylococcus aureus* during cheese manufacturing well studied by (Rodrigues *et al.,* 2000).

Generally the presence of *Staphylococcus* in cheese could be attributed probably to the use of unsatisfactory conditions under which cheeses are produced (El-Basiony and Ahmed, 1979).

The aim of the present work was to evaluate the antibacterial ability of *Bifidobacterium bifidum* against *Staphylococcus aureus* in vitro and during Domiati cheese manufacturing.

MATERIALS AND METHODS

Microorganisms and Culture Media

Bifidobacterium bifidum ATTC 15696 (from Cairo Microbial, Resources Center, MIRCEN Faculty of Agriculture, Ain Shams University, Egypt) and *Bifidobacerium bifidum* Bb12 (from Chr. Hansen's lab. A/S Horsholm, Demmark) were propagated in TPYG (trypticase/ peptone yeast extract/glucose broth (Scardovi, 1986) at 37°C for 24 hours, and subcultured twice in sterile skim milk before being used (the media were subjected to water-bath at 80°C then cooled at 37°C before inoculation to remove the dissolved oxygen). TPYG agar (1.5%) was used during bifidobacterial counts. TPYG broth, skim milk medium and TPYG agar plates were incubated under anaerobic conditions using Gas Pack (H_2 + CO_2). *Staphylococcus aureus* NCTC 6571 (from the Mycological Ref. Lab. London, England) was maintained on nutrient agar slants at 5°C, it was propagated in nutrient broth at 37°C and incubated for 24h. Baired Parker agar was used during counting *Staph aureus* counts (Baired Parker, 1962). The nutrient agar plates seeded with *Staph. aureus* were used during disc assay technique as a test organism cell free supernatant (CFS), cell suspension (SC) and disintegrated

cells (DC) of bifidobacterial cultures were prepared according to Marth & Hussong, (1963). TPYG broth media were inoculated with active cultures of bifidobacteria at a level of 1, 2, 3%, incubated at 37°C for 48 hours, the cultured media wer taken for the preparation of CFS, CS, DC immediately after inoculation and after 4, 8, 12, 24 and 48 hours of incubation.

Preparation of CFS, CS and DC

TPYG broth cultured media were centrifuged at 5000 rpm for 30 min., the supernatants were concentrated under vacuum (2.5 fold) and adjusted to pH 6 using 1 N NaOH and treated with catalase (1mg/mL) to eliminate the inhibition action of organic acids and hydrogen peroxide as recorded by (Ivanova *et al.,* 1998). The obtained precipitated cells were taken for the preparation of CS and DC.

Cells obtained were washed twice with saline solution and resuspended in distilled water CS & DC were prepared by taking a part of precipitated cells with a few distilled water and mixed with powdered glass, grounded, centrifuged, the resultant supernatant (DC) was made as described by (Collins and Patricia, 1976).

Evaluation of Antibacterial Activity

CFS, CS and DC were evaluated for their ability to inhibit *Staph. aureus* using disc assay procedure (Pulusani *et al.,* 1979), as follow:

Melted nutrient agar were inoculated with 0.5% of an overnight old broth culture of *Staph. aureus*. Tens mL of this seeded agar were poured into sterile Petri dishes and allowed to solidify, then a steril filter paper discs (6 mm diameter) was placed on the agar plates and loaded with 30ml of the tested preparations (CFS, CS and DC), and lefted at room temperature for 1 hour, to make the tested material diffuse into the agar plates, then incubated at 37°C for 24 hours. The agar plates were examined for the inhibition zones.

Characterization of Antibacterial Activity

The remaining CFS of bifidobacterial cultures (when the inoculation level was 3% and the incubation period was 24 hours) were treated with solid ammonium sulphate which was stirred in the CFS until the solution reached

60% saturation. This solution was kept at refrigerator temperature overnight to allow complete precipitation of proteins and then centrifuged at 5000 rpm for 30 minutes. Antibacterial activity was determined in the pellets, which were resuspended in sterile 0.02N HCl, the antibacterial activity was assessed as mentioned above. The antibacterial fractions were extracted from the concentrated supernatant with chloroform-methanol (1 : 1 v/v). the obtained chloroform layers were dried and weighted. The purified fractions were resuspended in sterile 0.02 N HCl and tested for their antibacterial activity. The CFS of the bifidobacterial cultures were tested for their molecular weight using molecular weight cut off 3 KD.

Minimal Inhibitory Concentration

Minimal inhibitory concentration of the purified extracts were examined for attaining information on the minimal effective concentration. Serial concentrations namely 10, 50, 100, 150, 200, 250 µg/mL were prepared in ependorf tubes. The concentrations were assessed for their antibacterial activity.

Heat Stability

The effect of pasteurization temperature (72°C/15s). was examined by heating 1 mL of the purified fractions of bifidobacteria when the concentrations were 50, 100 and 150 µg/mL. The treated concentrations were examined for their antibacterial ability after heat treatment (72°C/15s).

Incorporation of Either Bifidobacterial Cultures or Purified Fraction during Domiati Cheese Manufacturing

Cheese manufacturing

Mixed baffaloe's and cow's milk (1:1) 4% fat, 8.5% SNF obtained from local market was pasteurized at 72°C/15 seconds Calcium chloride 0.02% and salt 8% were added then cooled at 37°C. The milk was divided into 7 equal parts. The first part was inoculated with *Staph. aureus* at a level of approximately 10^6 orgnism/mL, the 2^{nd}, 3^{rd}, 4^{th} parts were inoculated with *Staph. aureus* at the same level then inoculated with *Bifidobacterium bifidum* NTTC 15696 which was chosen to complete this work.

The 5^{th}, 6^{th} and 7^{th} parts of milk were inoculated with *Staph. aureus* at the same level and treated with the minimal effective concentration (50mg/ml) and 2, 3 fold of its active extract obtained from NTTC 15696 bifidobacteria CFS. All treatments were

converted to Domiati cheese according to (Fahmi and Sharara, 1950). The cheeses were pickled into its own whey (10% salt) for 8 weeks.

Cheese samples were analysed for titratable acidity % according to Ling (1963).

Total bacterial counts of cheese

Cheeses were sampled as two 5 g samples from two different sectors were pooled and homogenized with 90mL of sterile sodium citrate solution (2%) and decimal diluted in sterile 0.1% peptone water (Nunez *et al.,* 1985).

Bifidobacterial counts were determined on duplicate plates of TPYG agar (the colonies in this medium were round and white *Staph. aureus* counts were determined on duplicate of Baird Parker medium.

Estimation of the remained antibacterial activity in cheese

Cheese samples (5g) were homogenized with 10 mL of sterile 0.02 N HCl at 50°C. Homogenates were centrifuged at 5000 rpm for 30 min. The supernatants were frozen at -20°C in ependorf tubes, after thawing, pH of supernatants was adjusted to pH 6 using 1N NaOH, a volume of 30 µL of each was evaluated for its antistaphyloccocal activity (Nunez *et al.,*1985).

RESULTS AND DISCUSSION

Antibacterial Activity of CFS, CS and DC

Table 1 shows that cell suspension of bifidobacterial cultures did not show antibacterial activity, meanwhile the disintegrated cells had no inhibitory activity but in some cases they had an slight stimulation effect on the growth of *Staph. aureus* and this observation could be explained in the light of the possibility of releasing some nutritional growth factors as a result of disintegration of bifidobacterial cells. The general trend of the obtained results agreed with the foundation of Marth & Hussong (1963) and Branen *et al.* (1975).

Regarding the inhibition effect of cell free supernatant (CFS) obtained from different cultured TPYG broth at different level of inoculation, it was found that they were with a strong antibacterial activity after 12 hours of incubation and this effect began to increase sharply after incubation

Table 1. Antibacterial activity of bifidobacteria strains of CFS, CS and DC

Bifodobacterium bifidum strains	Tested preparations	Inoculation level %	Incubation period (hours)					
			0	4	8	12	24	48
			Inhibition zone (mm)					
ATTC 15696	CFS	1	-	-	8	16	21	20
		2	-	-	9	17	21	21
		3	-	16	17	18	23	23
	CS	1	-	-	-	-	-	-
		2	-	-	s	-	-	-
		3	-	-	-	-	-	-
	DC	1	-	-	-	-	-	-
		2	-	-	-	-	s	-
		3	-	-	-	-	-	-
Bb12	CFS	1	-	-	-	15	20	20
		2	-	-	13	17	19	19
		3	-	13	14	18	23	23
	CS	1	-	-	-	-	-	-
		2	-	-	-	-	-	-
		3	-	-	-	-	-	-
	DC	1	-	-	-	-	-	-
		2	s	-	-	-	s	-
		3	-	-	-	-	-	s

(-): Not detected , (S) : Stimulation

period of 24 hours. Also, the inhibition activity was considerably increased when the level of inoculation increased, the obtained results agreed with Muriana *et al.* (1991).

The obtained results indicated that the antibacterial substances produced by bifidobacterial cultures are extracellular substances. The same observation was recorded by Marth and Hussong (1963).

The antibacterial activity of both studied strains of *Bifidobacterum bifidum* were nearly the same.

Characterization of Bifidobacterial Antibacterial Activity

The evaluation of the antibacterial ability of NTTC 15696 and Bb_{12}, bifidobacteria CFS were assessed when the inoculation % was 3 and incubation period was 24 hours and *Staph. aureus* NTCC 6571 was chosen as a test organisms (Table 2).

The pellet of proteins present in the bifidobacterial CFS obtained with ammonium sulphate did not affect the activity of *Staph. aureus.* Antibacterial activity was found in the nonpolar fraction extracted from bifidobacterial CFS with chloroform-methanol (1:1). After microfiltration of CFS using microfiltration micocone, the activity against *Staph. aureus* was found in the resultant filtrates. It was found that NTTC 15696 and Bb12 bifidobacteria produced an antibacterial non polar factors with molecular weight lower than 3000D. The evaluation of bifodobacteria antibacterial factos resemble to those of the antibacterial factors produced by some lactobacilli strains (Vandenbergh, 1993) and *Streptococcus thermophilus* ST strain (Abdel-Baky, 2004).

500 mg of the dried crude antibacterial substance could be obtained from every 200 ml of TPYG broth cultured with 3% of bifodobacteria and incubated at 37°C for 24 hours.

Minimal Effective Concentration

Results of Table 3 show that *Staph. aureus* was affected with a concentration of 50 µg/mL, it had an inhibition zone 17 mm diameter. The increasing concentration of the partially purified fractions was found to give inhibition zone of 20, 22, 27 and 30 mm for the concentration of 100, 150, 200 and 250 µg/mL respectively. The extract of NTTC 15696 bifodobacteria was chosen to complete this work because the other strain was nearly the same.

Table 2. Characterization of bifidobacterial CFS

Treatments	Inhibition zone (mm)
Ammonium sulphate pellet, ATTC 15696	ND
Methanol-chloroform extraction, ATTC 15696	23
Ammonium sulphate pellet, Bb12	ND
Methanol-chloroform extraction Bb12	22
Microfiltration filtrate, ATTC 15696	20
Microfilitration filtrate, Bb12	19
Microfiltration retentate ATTC 15696	ND
Microfiltration retentate Bb12	ND

ND: Not detected

Table 3. Minimal effective concentration of antibacterial substance produced from CFS of ATTC 15696 bifidobacteria

Concentration (µg/mL)	Inhibition zone (mm diameter)
10	7
50	17
100	20
150	22
200	27
250	30

Heat Stability

Table 4 showed the effect of pasteurization temperature on the antibacterial activity of the partially purified extract obtained from bifidobacteria CFS.

It could be concluded that the antibacterial activity of this extract was stable when the extracted substance was heated at 72°C for 15 second.

Cheese Acidity and Bifidobacterial Counts during Pickling Periods

Cheese acidity (Table 5) was influenced by *Bifidoabcterium bifidum* ATTC 51696 and pickling periods. In control cheese, values of acidity were between 0.88 and 1.12% in the second week of pickling and increased to be 1.25% at the end of the picking period, while the corresponding values of acidity in the cheese made using 1% bifidobacterial culture were 0.92, 1.13 and 1.41. The acidity of bifidobacterial cheese was found to increase with increasing the inoculation levels and along the pickling. Regarding to Table (6) it could be concluded that the change in acidity values should not influence the incidence of *Staphlococcus aureus*. Next to the cheeses made using CFS extract at different concentration namely 50, 100 and 150 µg/mL, it was found that the acidity values were nearly the same comparing with the control one. The bifidobacterial growth was increased gradually to reach the maximum count at the end of 4 weeks of pickling and began to slightly decrease up to the end of pickling. The increasing of inoculation level of bifidobacteria resulted in increasing the bifidobacterial counts during pickling period.

Survival of *Staph. aureus* and Inhibition Activity of Domiati Cheese as Affected by Either Bifidobacteria or its Antibacterial Extracted Fractions

As shown in Table 6 fresh control Domiati cheese contained 2.2×10^6 cfu/g of *Staph. aureus*, remained at approximately 23×10^6 after the first two weeks of pickling and decreased to 33×10^5 after four weeks, then began to decrease to reach 99×10^3 at the end of pickling.

Also, *Staphylococcus aureus* were 22×10^4 after 2 weeks of pickling in the cheese of 1% bifidobacterial level (Tl) and gradually decreased (2×10^2) at the end of pickling, while the inhibition

Table 4. Effect of heat treatment on the extract of *Bifidobacerium bifidum* NCTC 15696

Concentration (µg/mL)	Inhibition zone (mm diameter)
50	18
100	20
150	21
200	26
250	29

Table 5. Titratable acidity (TA) and bifidobacterial counts (BC) in Domiatic cheese incorporated with either bifidobacterial culture or its CFS extrated substance

Pickling period (weeks)	Treatments									
	Control	T1		T2		T3		T4	T5	T6
	TA	TA	BC	TA	BC	TA	BC	TA	TA	TA
Fresh	0.88	0.92	$5.8x10^6$	0.93	$5.8x10^6$	1.10	$13x10^6$	0.89	0.90	0.90
2	1.12	1.13	$18x10^6$	1.17	$37x10^6$	1.19	$59x10^6$	1.15	1.13	1.10
4	1.40	1.22	$97x10^6$	1.32	$13x10^7$	1.33	$18x10^7$	1.37	1.15	1.15
6	1.15	1.31	$78x10^6$	1.63	$18x10^6$	1.73	$28x10^6$	1.62	1.35	1.50
8	1.25	1.41	$19x10^6$	1.71	$31x10^6$	1.82	$21x10^7$	1.71	1.61	1.60

T1 : Cheese milk inoculated with *St.aureus* + bifidobacteria 1%.
T2 : Cheese milk inoculated with *St.aureus* + bifidobacteria 2%.
T3 : Cheese milk inoculated with *St.aureus* + bifidobacteria 3%.
T4: Cheese milk inoculated with *St.aureus* + 50 µg/mL of the extracted substance.
T5: Cheese milk inoculated with *St.aureus* + 100 µg/mL of the extracted substance.
T6: Cheese milk inoculated with *St.aureus* + 150µg/mL of the extracted substance.

activity which extracted from cheese samples gained 11, 12 mm inhibition zone after 2 and 4 weeks of pickling and not detected after that, the same attitude was studied by Rodrguez *et al.* (2000) in cheese manufacturing with nisin producing *Lactococcus lactis* TAB50 as starter culture.

Domiati cheese made from milk inoculated with bifidobcaterial culture at a level of 2% (T2) contained less count of *Staph. aureus* comparing with Tl, it contained 13 x 10^3 after 2 weeks of pickling and decreased to be 2 x 10^2 at the end of pickling period of 4 weeks, it had inhibition zone of 15, 17 and 13 mm at the end of 2, 4 and 6 weeks of pickling and not detected after that. Using bifidobacterial culture at a level of 3% (T3) resulted in the disappearance of *Staph. aureus* starting from the end of 3 weeks of pickling period up to the end of pickling. Also, the inhibition zones were 15, 17, 16 and 15 mm inhibition zone at the end of 2, 4, 6 and 8 weeks of pickling respectively.

Due to the application of different concentration of purified substance extracted from CFS of bifidobacterium bifidum ATTC 51696, the counts of *Staph. aureus* decreased at the end of two weeks and begen to increase again. This observation could be explained on the basis that the antibacterial substance was not sufficient to inhibit the growth of *Staph. aureus*.

Differences in the antibacterial activity between treatments could be attributed to the amount of antibacterial substances produced during pickling, also the antibacterial substances may be persisted during cheese making and along pickling with released proteinases. These results agreed with the foundation of Buyong *et al.* (1998) while they attributed the decreasing of recoverable activity of bacteriocines for its sensitivity of pediocins to proteinases and peptidases. Also Nunez *et al.* (1997) and Farius *et al.* (1999) observed that enterocins were stable during the ripening of Manchego cheese.

In conclusion the highest inhibition of *Staph. aureus* during Domiati cheese making and along pickling period was when the inoculation level of *Bifidobacterium bifidum* ATTC 51696 was 3%. So because of the strong antibacterial activity against staphylococcal growth it should be applied in Domiati cheese manufacturing.

Table 6. Counts of *Staph. aureus* (A) cfu/g and inhibition activity (B) in Domiati cheese incorporated with either bifidobacterial culture or its CFS extracted substance

Pickling period (weeks)	Treatments													
	Control cheese		T1		T2		T3		T4		T5		T6	
	A	B	A	B	A	B	A	B	A	B	A	B	A	B
Fresh	$2.2x10^6$	ND	$2.3x10^6$	ND	$25x10^5$	ND	$22x10^5$	ND	$25x10^5$	ND	$50x10^4$	ND	$23x10^4$	11
2	$23x10^6$	ND	$22x10^4$	11	$13x10^3$	15	$2x10^3$	15	$18x10^5$	ND	$13x10^4$	ND	$12x10^2$	12
4	$33x10^5$	ND	$13x10^3$	12	$2x10^2$	17	Nil	17	$23x10^4$	10	$12x10^2$	11	$22x10^2$	ND
6	$12x10^5$	ND	$9x10^3$	ND	Nil	13	Nil	16	$18x10^4$	ND	$35x10^4$	10	$25x10^3$	ND
8	$99x10^3$	ND	$2x10^2$	ND	Nil	ND	Nil	15	$23x10^5$	ND	$13x10^4$	ND	$38x10^3$	ND

ND : Not detected
T1 : Cheese milk inoculated with *St.aureus* + bifidobacteria 1%.
T2 : Cheese milk inoculated with *St.aureus* + bifidobacteria 2%.
T3 : Cheese milk inoculated with *St.aureus* + bifidobacteria 3%.
T4: Cheese milk inoculated with *St.aureus* + 50 µg/mL of the extracted substance.
T5: Cheese milk inoculated with *St.aureus* + 100 µg/mL of the extracted substance.
T6: Cheese milk inoculated with *St.aureus* + 150µg/mL of the extracted substance.

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تقييم القدرة التثبيطية لميكروب الـــ *Bifidobacterium bifidum* ضد ميكروب الـ *Staphylococcus aureus* معمليا وأثناء تصنيع الجبن الدمياطى

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تم اختبار التأثير المثبط لمعلق الخلايا وناتج تكسيرها وكذا الرائق الناتج بعد ترسيب الخلايا لمزارع ميكروبـى *Bifidobacterium bifidum* ATTC 15696، Bb_{12} فـى حين تم استخدام ميكروب *Staphylococcus aureus* كميكروب أختبار وذلك بأتباع تقنية Disc assay.

وبنفس التقنية تم تقييم التأثير المثبط لكل من مترسب البروتين من الرائق السـابق اختباره وكذلك مستخلص الميثانول- كلورفورم بعد تجفيفه بالإضافة إلى تقدير كل من الثبات الحرارى وأقل تركيز مثبط كذا الوزن الجزيئى للمواد التى ثبت تأثيرها المثبط على ميكروب الـ *Staphylococcus aureus*

كما تناولت الدراسة متابعة بقاء ميكروبات الـ *Staphylococcus aureus* أثنـاء إنتـاج الجـبن الـدمياطى وذلـك فـى حـالتى اسـتخدام ميكـروب ATTC 15696 *Bifidobacterium bifidum* أو النواتج المثبطة منه بإضافتة للجبن وقد أشـارت أهـم النتائج إلى الأتى:

لم يكن لمعلق الخلايا أو ناتج تكسيرها تأثيراً مثبطاً. وأحتوى الرائـق النـاتج بعـد ترسيب الخلايا على المواد المثبطة كما كان البروتين المترسب من الرائق لا يحتوى علـى تأثير مثبط وكان التأثير المثبط فى الشق الذائب فى الكلورفورم. وقد أشارت النتائج إلى أن هذه المكونات المثبطة والمنقاه جزئيا لها ثبات حرارى على درجة حرارة البسترة وكان أقل تركيز مؤثر لها كان ٥٠ميكروجرام/مل كما أن الوزن الجزيئى لها أقل من ٣ كيلو دالتون.

أما بالنسبة لاستخدام هذا التأثير الناتج سواء بإضافة مزارع البيفيدو أو المستخلص الناتج عند صناعة الجبن الدمياطى فقد أوضحت النتائج أنه يلزم للسيطرة على ميكروبـات *Staphylococcus aureus* *استخدام الـ* *Bifidobacterium* بمسـتوى ٣%. وحفـظ الجبن فى محلول التخليل لمدة تزيد عن ٨ أسابيع قبل السماح باستهلاكها.