Antibacterial activity of *Lactobacillus delbreukii* subspecies *bulgaricus* isolated from Zabady

Seham Abdel-Shafi[1], Abdul-Raouf Al-Mohammadi[2], Sally Negm[1] and Gamal Enan[*1]

[1]Department of Botany and Microbiology, Faculty of Science, Zagazig University, Zagazig, Egypt.
[2]Department of Science, King Khalid Military Academy, Riyadh 11495, Box. 22140, Saudi Arabia
gamalenan@ymail.com

Abstract: Lactic acid bacteria are used recently as probiotics and as protective cultures in fermented food products. This is due to their ability to produce proteinacous antimicrobial agents, lactic acid and diacetyle. The prime objective of this work was to select a probiotic bacterium which could be used as protective culture. Characterization of bacteriocin by physicochemical techniques and inhibition of food-borne pathogens were carried out. Molecular methods were used for identification of bacterial strains used. Out of 100 lactic acid bacterial strains, the most inhibitory strain (Z_{55} strain) isolated from Arabian yoghurt (Zabady) inhibited other lactic and bacteria and some food-borne pathogens. This Z_{55} strain was identified as *Lactobacillus delbreukii* subspecies *bulgaricus* and designated *Lb. bulgaricus* Z_{55}. The inhibitory activity of cell free supernatant (CFS) of *Lb. bulgaricus* Z_{55} was lost by proteolytic enzymes, heat resistant. Consequently it was characterized as a bacteriocin. This bacteriocin was shown to consists of protein but has no lipidic or glucidic moieties in its active molecule. Its activity was stable in the pH range 2.0 to 7.0. Maximum bacteriocin production was obtained in MRS broth adjusted initially at pH 6.5 and incubated for 18 h at 35^0C when the producer organism was in the late logarithmic and early stationary phase of growth. A bacteriocin producer strain (*Lb. bulgaricus* Z_{55}) inhibited many food-borne pathogens. The antimicrobial agent was showed to be protein and characterized as a bacteriocin

[Seham Abdel-Shafi, Abdul-Raouf Al-Mohammadi, Sally Negm and Gamal Enan. **Antibacterial activity of *Lactobacillus delbreukii* subspecies *bulgaricus* isolated from Zabady.** *Life Sci J* 2014;11(8):264-270]. (ISSN:1097-8135). http://www.lifesciencesite.com. 36

Keywords: Lactic acid bacteria, probiotics and bacteriocin.

1. Introduction:

Lactic acid bacteria are commonly found in foods including fermented dairy product, vegetables, fruits meat products and beverages. Also in intestinal and respiratory tracts of humans and animals**[1].** They are used as probiotics to prevent or limit the growth and colonization of potentially pathogenic bacteria and to improve many nutritional functions in humans**2].** This clearly shows that there is a need to ingest fermented dairy products such as yoghurt and cheese; and a continued need is necessary to characterize a starter cultures for dairy fermentations with good capabilities such as bacteriocin production**[3].** *Lactobacillus delbruekii* is an industrially important organism, belonging to lactic acid bacteria. It is a natural inhabitant of the intestinal tract of humans and animals**[4].** This organism was reported to produce antimicrobial bacteriocins which are macromolecular complexes active against closely related species**[5].** This clearly suggests that there is a need to continue research in obtaining lactic cultures which could produce antimicrobial compounds active against many food-borne pathogens. *Lactobacillus delbreukii* subsp. *bulgaricus* is used in Zabady (Arabian yoghurt) making in Egypt which is made traditionally with or without starter culture by mixing (1:1) fresh buffaloes' and cow's milk[**6].**

The aim of the present study was to select and characterize a probiotic bacterium isolated from Arabian yoghurt (Zabady) which could be used also as protective and starter culture for dairy fermentations.

2. Methods

The *Lb. bulgaricus* Z_{55} strain was selected by screening of 100 lactic acid bacterial strains for bacteriocin activity. It was isolated from Arabian yoghurt (Zabady) made without starter culture; using MRS agar media**[7].** The strain was identified by physiological and biochemical tests**[8,9,10],** and API 20 carbohydrate galleries (Biomerieux, Marcy-1, Etoile, France). To complete the identification of Z_{55} strain, molecular techniques of 16 S rRNA cataloging analysis were used**[11,12,13].**

The indicator organisms are listed in Table (1). Lactic acid bacteria were maintained as frozen stocks at -20°C in brain heart infusion broth (Oxoid) plus 20% glycerol**[14,15]** and were propagated in the same media. All other indicator strains were maintained as frozen stocks at -20°C in glass beads (Oxoid) and were propagated in brain heart infusion broth.

Cell free supernatant (CFS) was collected by centrifuging *Lb. bulgaricus* Z_{55} culture (10000 xg for 15 min at 4°C) growing in MRS adjusted initially at pH 6.5 and incubated at 35°C for 18 h.**[16].** The pH of CFS was adjusted to pH 6.5 with 1M NaOH and was

sterilized by filtration (Amicon, 0.45 µm, Milipore). The agar well diffusion method was used for bioassay of inhibitory activity of CFS**[17,18].** The quantitative estimation of the antibacterial titre of CFS preparation was performed as described previously**[19.20, 21].** One arbitrary unit (AU) was defined as 5 µl of the highest dilution of filtrate yielding definite zone of growth inhibition in the lawn of indicator organisms. The highest dilution was multiplied by 200 (1ml/5µl) to obtain the arbitrary units per ml (AU/ml).

Listeria monocytogenes LMG 10470 was used as the indicator organism in these experiments. Heat stability of the antibacterial activity of CFS was determined by heating 1 ml aliquots of CFS (1860 AU/ml) at 100°C for 5:30 min. and at 70, 80, 90°C for 30-40 min. The heated CFS were tested for their residual antibacterial activity**[4,16].** To test the effect of pH values on the bacteriocin stability, aliquots of CFS (1860 AU/ml) and samples of fresh MRS broth (controls) were adjusted to different pH values listed in Table 2 and incubated for 24 h at 25°C. After setting pH to 6.5 with 10mM potassium phosphate buffer, the samples and controls were tested for remaining antibacterial activity.

The sensitivity of the antibacterial activity to proteases, lipase and amylase was assayed by incubating CFS with 1 mg/ml pepsin, trypsin, α-chemotrypsin, lipase, amylase (All from Sigma) in 1 mol/ml Tris HCl pH 7.6. After stopping the reaction by heating at 100 for 20 min, the residual inhibitory activity was determined as described above.[4.16] Also the sensitivity of the inhibitory substance of the CFS preparation to organic solvents was studied**[23].**

To study the influence of the initial pH of the medium and time and temperature during the incubation on the bacteriocin activity in CFS collected from *Lb. bulgaricus* Z_{55}, a series of 500 ml Erlenmeyer flasks, each containing 250 ml MRS broth were adjusted initially with either HCL or NaOH (1M) to various pH values (pH 5.0, 5.5, 6.0, 6.5, 7.0, 7.5 and 8.0) and inoculated with 1% log phase cells of *Lb. bulgaricus* Z_{55} at a concentration of about 2 x 10^3 CFU/ml. The flasks were then incubated at 25°C, 35°C and 42°C for 96 h. Every 6 h, samples were removed and examined for growth (CFU/ml) and antibacterial activity (AU/ml) using *Listeria monocytogenes* LMG 10470 as the indicator organism.

3. Results

One hundred isolates of lactic acid bacteria isolated from Arabian yoghurt (Zabady) were screened for production of inhibitory activity against many sensitive bacteria. The Z_{55} strain inhibited other lactic acid bacteria tested and food-borne pathogens. Results are given in Figure 1. The inhibition zones around wells measured ≥ 30 mm in Petri plates containing lawns of indicator organisms such as *Listeria monocytogenes, Bacillus cereus, Staphylococcus aureus, Streptococcus pyogens* and *Lactobacillus plantarum* (Figure 1). The antibacterial activity showed distinctive titre(s) against sensitive organisms (Table 1). Highest titer was showed against *Listeria monocytogenes* but the lowest titer was showed against *Lactobacillus sake*. Gram negative bacteria were not inhibited (Table 1).

It was necessary to identify the Z_{55} strain which produced inhibitory activity. The Z_{55} strain was Gram positive, catalase negative and showed rod cells under light microscope, grew in MRS broth incubated at 45°C and showed also the following characteristics: production of DL-isomers of lactic acid, homofermentation of glucose and characteristic sugar fermentation pattern using API 20 (API Streps, Montalieu, Vercieu, France) as described by manufacturer's instructions. Further identification of the Z_{55} strain was carried out by comparison of the sequence of 16 S rRNA gene of DNA with that of Gene Bank (Figure 2). The similarity assignment showed that the Z_{55} strain was classified and identified as belonging to *Lactobacillus delbreukii* subsp. *bulgaricus* and designated *Lb. bulgaricus* Z_{55}.

The effect of different treatments on the antibacterial activity of CFS was investigated. Results are given in Table 2. The inhibitory activity was almost heat resistant, lost by proteolytic enzymes and did not affect by organic solvents and either lipase or amylase. This indicated on the proteinaceous nature of the active substance(s). It also indicated on absence of lipidic or glucidic moieties in the active substance. Such properties coupled with criteria applied for bacteriocin properties.[22.23.16] Consequently the inhibitory activity was due to bacteriocin. This bacteriocin was thermostable and its activity did not affect by heating at 100°C for 30 min; was stable at acidic and neutral pH values but its activity was decreased or lost at alkaline pH values (Table 2).

The effect of initial pH values of the medium on the bacteriocin production in CFS by *Lb. bulgaricus* Z_{55} is shown in Figure 3a, b. Optimal values of growth ($2x10^7$ – $2x10^8$ CFU/ml) and bacteriocin activity (1860 AU/ml) were achieved in MRS broth adjusted initially at pH 6.5, when the producer organism was in the late exponential phase and in the early stationary phase (after 18 h of incubation). However other initial pH values pH 5.0, 5.5, 6.0, 6.5, 7.0 and 7.5) showed lower growth and bacteriocin activity than that obtained in MRS broth adjusted initially at pH 6.5.

The effect of different incubation temperatures on growth and bacteriocin activity of *Lb. bulgaricus* Z_{55} was studied. Results are given in Figures 4a, b. In *Lb. bulgaricus* cultures adjusted at an initial pH 6.5 and

incubated at 25°C, 35°C and 42°C, it was shown that the best incubation temperature for growth and bacteriocin activity was 35°C. At this incubation temperature, maximal values of bacteriocin activity of about 1860 AU/ml were obtained in CFS after 18-24 h when the producer organism was in the late exponential and the early stationary phase of growth. The growth and bacteriocin activity were rather low by prolonged incubation at either 25°C or 42°C.

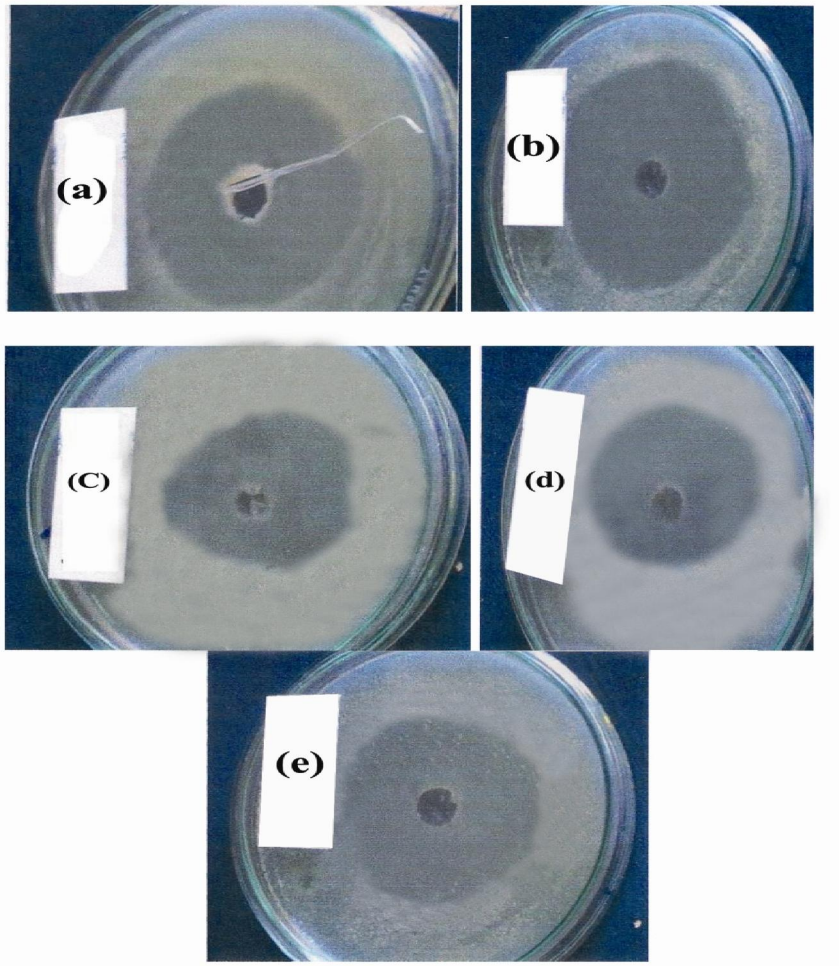

Figure 1. Antibacterial activity of CFS from *Lb. bulgaricus* Z_{55} against (a) *Listeria monocytogenes*, (b) *Bacillus cereus*, (c) *Staphylococcus aureus*, (d) *Streptococcus pyogens,* (e) *Lactobacillus plantarum* by the agar well diffusion assay.

CTCCTAAAGGTTACCTCACCGACTTCGGGTGTTACAAACTCTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTC
ACCGCGGCGTGCTGATCCGCGATTACTAGCGATTCCGACTTCATGTAGGCGAGTTGCAGCCTACAATCCGAACTGAGATTGGCTTT
AAGAGATTTGCTTGCCGTCACCGACTCGCGACTCGTTGTACCAACCATTGTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGA
TGATTTGACGTCATCCCCACCTTCCTCCGGTTTATTACCGGCAGTCTCGCTAGAGTGCCCAACTGAATGATGGCAACTAACAATAG
GGGTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTCACCGATGTTCCGA
AGAAACTTCCTATCTCTAGGAATAGCATCGGGATGTCAAGACCTGGTAAGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCC
ACCGCTTGTGCGGGCCCCCGTCAATTCCTTTGAGTTTCAACCTTGCGGTCGTACTCCCCAGGCGGAGTGCTTAATGCGTTAGCTGC
GGCACTAAGCCCCGGAAAGGGCCTAACACCTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTGTTTGCTCCCC
ACGCTTTCGAGCCTCAGCGTCAGTTACAGACCAGAGAGCCGCTTTCGCCACCGGTGTTCCTCCATATATCTACGCATTTCACCGCT
ACACATGGAATTCCACTCTCCCCTTCTGCACTCAAGTCTAACAGTTTCCAAAGCGAACAATGGTTAAGCCACTGCCTTTAACTTCA
GACTTATTAAACCGCCTGCGCTCGCTTTACGCCCAATAAATCCGGACAACGCTCGGGACCTACGTATTACCGCGGCTGCTGGCACG
TAGTTAGCCGTCCCTTTCTGGTAAGTTACCGTCACTGTGTGAACTTTCCACTCTCACACACGTTCTTCTCTTACAACAGAGCTTTAC
GATCCGAAAACCTTCTTCACTCACGCGGCGTTGCTCGGTCAGGGTTGCCCCCATTGCCGAAGATTCCCTACTGCTGCCTCCCGTAG
GAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTATGTATCGTCGCCTTGGTGAGCCGTTACCTCA
CCAACTAGCTAATACAACGCAGGTCCATCTACTAGTGAAGCAATTGCTCCTTTCAAGCATCTAACATGGGTTAAATGCTGTTATGC
GGTATTAGCTATCGTTTCCAATAGTTATCCCCCGCTAGTAGGCAGGTTACCTACGCGTTACTCACCCGTTCGCAACTCTTCAAACTT
TAGCAAGCTAAAGTTTCAGCGTTCTACTGC

Fig. 2. Sequence of 16 S r RNA gene of the eluted PCR product of isolate No. Z_{55} and showing a similarity to *Lactobacillus delbreukii* subsp. *bulgaricus* (*Lb. bulgaricus* Z_{55}) category of about 98.0%.

Table 1. Indicator strains and their sensitivity to CFS from *Lb. bulgaricus* Z_{55}.

Indicator strain	Source of strain	AU/ml
Listeria monocytogenes	LMG 10470	1860
Bacillus cereus	LMG 14579	1220
Staphylococcus aureus	DSM 1104	680
Escherichia coli	MIR 302	0
Pseudomonas aeruginosa	MIR 122	0
Klebsiella pnemoniae	MIR 603	0
Streptococcus pyogens	MIR 622	840
Lactococcus lactis	ATCC 11454	1020
Lactobacillus plantarum	HL 2	1060
Lactobacillus alimentarius	LMG 10630	780
Lactobacillus sake	Lb 706	830

LMG, Laboratorium voor Mikrobiologie, Gent culture collection, Gent University, Belgiumi; DSM, Deutsche Sammlung von Mikroorganisms and Zeukulturen, GmbII, Braunschweig, Germany; ATCC, American Type Culture Collection, Rockville, Maryland, USA; MIR, Mircen Culture Collection, Ain Shams Faculty of Agriculture, Cairo, Egypt; Lb 706, a bacteriocin producer strain provided kindly by Dr. U. Schillinger, Karlsruch, Germany; HL, Chr. Hansens Laboratorium, Horsholm, Denmark.

Table 2. Effect of different treatment on the stability of the inhibitory activity produced by *Lb. bulgaricus* Z_{55}.

Treatment	Residual antibacterial activity (AU/ml)
Temperature	
100°C for 5 min.	1860
100°C for 10 min.	1860
100°C for 20 min.	1860
100°C for 30 min.	820
90°C for 30 min.	1860
80°C for 30 min.	1860
70°C for 40 min.	1860
Enzymes	
Pepsin	0
Trypsin	0
α-chemotrypsin	0
Lipase or amylase	1860
Organic solvents	
Acetone	1860
Methanol or ethanol	1860
Chloroform	1860
Petroleum ether	1860
pH-stability	
pH 2.0	1860
pH 4.0	1860
pH 6.0	1860
pH 7.0	1860
pH 8.0	820
pH 9.0	600
pH 10.0	0

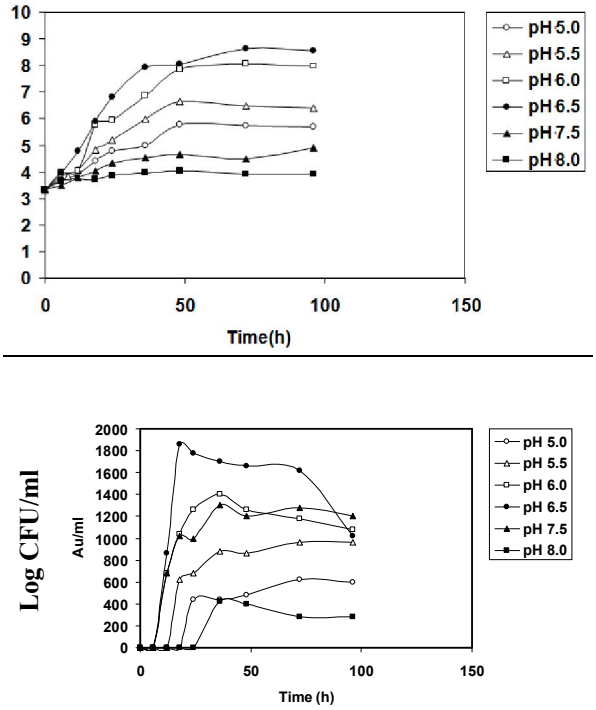

Figure. 3. Growth (CFU/ml) (a) and baceteriocin activity (AU/ml) (b) in MRS broth adjusted at initial pH 5.0 (0), 5.5 (Δ), 6.0 (□), 6.5 (•), 7.5 (▲), 8.0 (■) and inoculated with *Lb. bulgaricus* Z_{55} and incubated at 35°C.

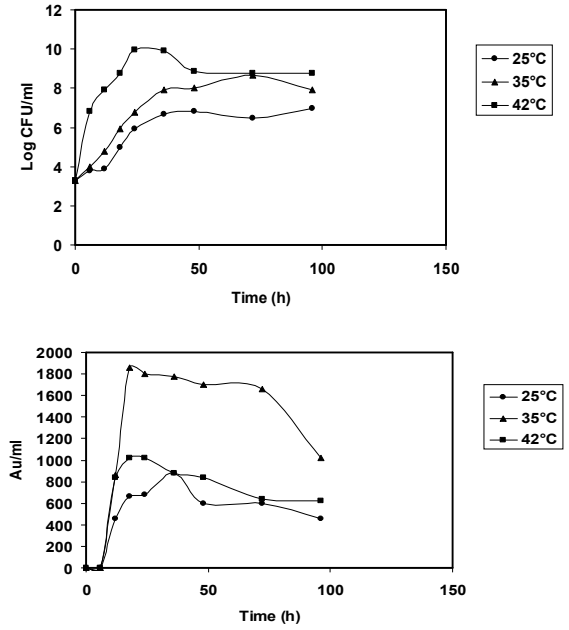

Figure 4. Growth (CFU/ml) (a) and bacetriocin activity (AU/ml) (b) in MRS broth adjusted at an initial pH 6.5 and inoculated with *Lb. bulgaricus* Z_{55} and incubated at 25°C (•), 30°C (▲) and 42°C (■).

4. Discussion

The study employed herein was coincided by clear success since one strain of lactic acid bacteria isolated from Arabian yoghurt (Zabady) was showed to produce inhibitory activity against other lactic acid bacteria and against food-borne pathogens. This is a promised result because the isolated Z_{55} strain could be used as starter and protective culture for Zabady with extended shelf-life.[24] The antibacterial activity of Z_{55} strain is of great importance since it could be used in controlling growth of pathogenic bacteria and could be a safety agent of any fermented fairy product made by such bacteria. This is in agreement **[24,25,26,15,16].**

It was necessary to characterize the Z_{55} isolate. Cultural, morphological and biochemical properties were studied using traditional and recent technique using API 20 carbohydrates kits. By surveying literature, isolate Z_{55} was classified and identified as belonging to *Lactobacillus delbreukii* subspecies *bulgaricus* (*Lb. bulgaricus* Z_{55})**[27,15,16,24].** Growth at 42°C and production of D- & L-lactate isomers proved that the Z_{55} strain was followed subspecies *bulgaricus***[9].** Since the identification of *Lb. bulgaricus* Z_{55} at the subspecies level was based on phenotypic and biochemical tests, molecular characterization and identification was mandatory**[28].** This is because culture conditions can give speculative results**[29].** The 16 S r RNA cataloging analysis was followed**[30].** The sequence of 16S rRNA gene was submitted to Gene Bank under accession number Q158828 and was compared to stored ones using Basic Local Alignment Search Tool and showed similarity > 98% to *Lactobacillus delbreukii* subspecies *bulgaricus* (*Lb. bulgaricus* Z_{55})**[11].**

The antibacterial activity of CFS from *Lb. bulgaricus* Z_{55} could not be attributed to organic solvents or due to catalase since it was treated with catalase and was neutralized with 1 M NaOH; was inactivated by proteolytic enzymes; and was heat resistant. Consequently, it has been coupled with most definitions of bacteriocins**[22].** It was, therefore, characterized as a bacteriocin.

The activity of *Lb. bulgaricus* Z_{55} in CFS was not affected by organic solvents and lipase; probably because of the absence of lipid moieties in the active molecule. The same was observed for some bacteriocins of *Lactobacillus delbreukii* bacteriocins.**[25,31,32].** and differs from the bacteriocin UD004 produced by *Lb. delbreukii* subsp. *lactis* UD004 **[4].** The activity of *Lb. bulgaricus* Z_{55} bacteriocin was not affected by amylase indicating on absence of glucidic moieties in the bacteriocin molecule. This is similar to some bacteriocins produced by lactic acid bacteria**[31,16].** and differs from the bacteriocin UD004 **[4].** The bacteriocin

employed herein was stable at acidic and neutral pH values and this can give wider application of the producer organism in dairy fermentations at acidic and neutral pH values. This is similar to bacteriocin S50 and differs from helveticin V and pediocin ACH **[25].**

Optimum production of *Lb. bulgaricus* Z_{55} bacteriocin was obtained in MRS broth adjusted initially at pH 6.5 and incubated for 18 h at 35°C, when the producer organism was in the late logarithmic and the early stationary phase of growth of the producer organism and declined thereafter. Similar results in bacteriocin activity have been reported for other bacteriocins **[33].** The decrease in the bacteriocin activity at latter stages of growth may be due to adsorption of the bacteriocin to live and dead cells of the producer organism**[25,6;16].**

In view of bacteriocins produced by *Lactobacilus delbreukii*, *Lb. bulgaricus* Z_{55} differs from the bacteriocin UD004 produced by *Lactobacillus delbreukii* subsp. *lactis* UD004 which contain lipidic and glucidic moieties in its active molecule and differs from some *Lb. delbreukii bacteriocins* which were active at only acidic pH values,**[31,32]** but similar in its thermostability and pH stability to other bacteriocins produced by lactic acid bacteria.[16] Generally comparison of bacteriocins based upon biological characters is elusive as it is strongly depend on the variability of strains used and culture conditions. Further work will be necessary to find out wheather the antibacterial activity noticed herein is due to one or more substances and to purify this bacteriocin and analyse its molecular mass, its amino acid composition; and its actual classification.

In conclusion, the strain of lactic acid bacteria (Z_{55}) isolated from Arabian yoghurt made without starter culture inhibited other lactic acid bacteria and many food-borne pathogens. This strain was identified as *Lb. bulgaricus* Z_{55}. The inhibitory activity of CFS was due to bacteriocin which was stable at acidic and neutral pH values; was produced optimally in MRS broth adjusted initially at pH 6.5 and incubated for 18 h at 35°C. *Lb. bulgaricus* Z_{55} could be used as probiotic, protective and starter culture for dairy fermentations.

Corresponding author:
Prof. Dr. Gamal Enan, Chairman of the Research Group of Bacteriology, Faculty of Science, Zagazig University, Zagazig, Egypt,
E.mail/ gamalenan@ymail.com

References:

1. Vandenbergh PA. (1993): Lactic acid bacteria: Production and interference with microbial growth. FEMS Microbiology Reviews; 12: 221-237.
2. Buyukourk S, Cibik R, Cetinkaya F, Goksog O and Kirkan S. (2010): Isolation, phenotypic and molecular identification of lactococcus lactis isolates from traditionary produced village cheesses. J. of Animal and Veterinary Advances 9: 2154-2158.
3. Moreno L, Lerayer ASL, Baldini VLS and Def-Leitao ME. (2000): Characterization of bacteriocins produced by *Lactococcus lactis* strain. Brazilian J. of Microbiol ; 31: 184-192.
4. Boris S, Jimenez R, Caso JL, Barbe C. (2001): Partial characterization of a bacteriocin produced by *Lactobacilus delbreukii* subsp. Lactis UO004, an intestinal isolate with probiotic potential. J. of Applied Microbiol; 91: 328-333.
5. Simova ED, Beshkova DM, Angelov MP.(2008): Bacteriocin production by a strain of *Lactobacillus delbreukii* ssp. *bulgaricus* BB18 during continuous prefermentation of yoghurt starter culture and subsequent batch coagulation of milk. J. of Ind Microbiol. and Biotechnol,; 35: 559-587.
6. Enan G, Al-Amri A.(2006): Novel plantaricin UG1 production by *Lb. planetarium* UG1 in enriched whey permeate by batch fermentation processes. J. of Food agriculture and Environment; 4: 84-88.
7. De Man JC, Rogosa M, Sharpe ME.(1960): A medium for cultivation of lactobacilli: Journal of Appl. Bacteriology; 23: 130-138.
8. Grover KI, Mariana M. (1993): Detection and characterization of bacteriocin producing lactic acid bacteria from retail food products. Intern. J. Food. Microbiol; 19: 241-258.
9. Wadhai VS, Dhawas VK (2011): Characterization and study of *Lactobacillus bulgaricus* as probiotic bacteria. Intern. Interdisciplinary Research J; 11: 55-60.
10. Enan G, Awny N, Abo Zeid A, Abdou MA.(2012): Incidence and virulence of *Bacillus cereus* isolated from Egyptian foods during four seasons. African J. of Microbiol. Research; 22: 4816-4824
11. Altschul SF, Madden TI, Schaffer AA, Zhang J, Miller W, Lipman DJ.(1997): Gapped Blast and PSI-BLAST: a new generation of protein databte we search programs. Nuclic Acids Research; 17: 389-402.
12. Chenbey D, Philippot L, Hartmann A, Henalul C, German JC. (2000): 16 S r RNA analysis for characterization and identifying bacterial isolates from three agricultural soils. FEMS Microbial Ecology; 24: 121-128.

13. Sambrook J, Russel D. (2001): Molecular cloning: a Laboratory Mannual, 3rd ed.,; Cold Spring Habour Press.
14. Joerger MC,Klaenhammer TR. (1986): Characterization purification of helveticin J and evidence chromosomally determined bacteriocin produced by *lactobacillus helveticus*. J. Bacteriol; 167: 439-446.
15. Enan G, Abdel-Shafi S, Ouda SM, El-Bolat I. (2013a): Genetic linkage of the antibiotic resistance ability in the *E. coli* UR4 strain isolated from urine. J. of Medical Sciences; 4: 261-268.
16. Enan G, Abdel-Shafi S, Ouda S, Negm S. Novel antibacterial activity of *Lactococcus lactis* subsp. *Lactis* Z11 isolated from Zabady. International Journal of Biomedical Sceince. 2013b; 3: 174-180.
17. Biswas SR, Ray P, Johnson MC, Ray B. (1991): Influence of growth condition on the production of bacteriocin, pediocin ACH, by Pediococcus acidiloctici H. Appl. Environ. Microbiol; 57: 1265-1267.
18. Enan G. (2006a): Inhibition of *Clostridium perfringens* LMG. 11264 in meta samples of chicken, turkey and beef by the bacteriocin plantaric in UG1. Intern. J. of Poultry Science; 5: 5195-200.
19. Pucci MJ, Vedamuthu ER, Kunka BS, Vandenbergh PA. (1988): Inhibition of *Listeria monocytogens* by using bacteriocin PA-L by pediococcus acidilactici PACL.O. APPl. Environ. Microbiol; 59: 2349-2353
20. Enan G. (2006b): Behaviour of *L. monocytogenes* LMG 10470 in poultry meat and its control by the bacteriocin plantaricin UG1. Intern. J. of Poultry Science; 5 : 355-359.
21. Enan G. (2006c): Control of the regrowing bacteriocin resistant variants of *L. monocytogenes* LMG 10470 *in vitro* and in food by nisin. Plantaricin UG1 mixture. Biotechnol; 2: 143-147.
22. Tagg JR, Dajani AS, Wannamaker NW. (1976): Bacteriocin of Gram positive bacteria. Bact. Rev; 40: 722-756.
23. Enan G. (2000): Inhibition of *Bacillus cereus* ATCC 1457g by plantaricin UG1 *in vitro* and in food. Die Nahrung; 44: 364-387.
24. Enan G, Abdel-Shafi S, Ouda S, Negm S. (2013c): Characterization of probiotic lactic acid bacteria to be used as starter culture and protective cultures for diary fermentations. Intern. J. of Probiotics and Prebiotics. 8 (4): 157-163.
25. Nettles CG, Barefoot SF. (1993): Biochemical and genetic characteristics of bacteriocins of food associated lactic acid bacteria. Journal of Food Protection; 56: 338-356.
26. Kumari A, Makin K, Garg AP, Marotta F, Divya,G. (2009): Effect of bacteriocin produced by *Lkactococcus lactis* subsp. *Lactis* CC SUB 202 on mode of action of *L. lactis* subsp. *lactis* MTCC 3038. J. Probiotics and Prebiotics; 4: 1-5.
27. Pot B, Janssens S (1993): The potential role of culture collection for identification and maintenance of Lactic acid bacteria pp.81-87. In: the lactic acid bacteria conference (E.L.Foo, Grittin HG, Mollby R, Heden CG, eds) Norfolk scientific Press.
28. Kelly WJ, Ward LJH, Leahy SC. (2010): Chromosomal diversity in *Lactococcus lactis* and the origin of dairy cultures. Genome Biological Evolution; 2: 729-744.
29. Grade S, Babin M, Goya P, Nunez M, Medina M. (1999): PCR amplification of gene acm differentiates *Lactococcus lactis* subsp. *Cremories*. Appl. and Environ. Microbiol; 65: 5151-5153.
30. Turner S, Preyer KM, Mias VPW Palmer DJ. (1999). Investigation of phylogenetic relationships among cyanobacteria and plastids by small subunit rRNA sequence analysis. Journal of Eucarytic Microorganisms; 46: 327-338.
31. Jack RW, Tagg JR, Ray B. (1995): Bacteriocins of gram-positive bacteria. Microbiological Reviews; 59: 171-200.
32. Parada JL, Caron CR, Pianche A, Medeiros P, Soccol CR. (2007): Bacteriocins from lactic acid bacteria: Purification, properties and sue as biopreservatives, Brazilian Archives of Biology and Technology; 50: 521-542.
33. Klaenhamme TR. (1988): Bacteriocins of lactic acid bacteria. Biochimie; 70: 337-349.

5/2/2014