Antimicrobial Activity of Bacteriocin Produced by *Weissella cibaria* NRIC0136

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Abstract

A total of 1070 isolates of lactic acid bacteria were obtained from 50 samples of different sources (fermented foods 30 samples, chicken intestine (10 samples) and human intestine and vagina (10 samples). Three hundred isolates were isolated from Baghdad, Iraq (220 isolates from human intestine and vagina and 80 isolates from fermented foods) and the others from Bangkok (Thailand). These isolates were screened for bacteriocin production using the agar well diffusion method and *Shigella dysenteriae* DMST 15110 was used as an indicator strain. Seventy two isolates gave clear inhibition zones against growth of indicator strain.

One isolate , F14 (from Thailand fermented fish) that produced largest inhibition zone against the indicator strain was chosen for further study, and identified as a *Weissella cibaria* NRIC0136 by morphological, physiological properties and PCR amplification, sequencing and comparison of 16S rRNA gene. The neutralized cell free supernatant of *W. cibaria* NRIC0136 inhibited growth of a number of standard strains of pathogenic microorganisms including *Listeria monocytogenes* DMST 17303, *Enterococcus faecalis* ATCC 29212, *Salmonella* sp DMST 22842 and *Shigella dysenteriae* DMST 15110. The optical density at 660nm (2.253) and inhibition zone (18 mm) against the indicator strain, *Shigella dysenteriae* DMST 15110 were maximum when growth temperature employed was 30°C.

Introduction

Pathogenic bacteria permanently threaten the health of human and animal. Microorganisms are the major cause of food-related diseases and spoilage during production and storage of food and beverages (Koponen, 2004). Lactic acid bacteria play an important role in producing many antimicrobial substances that are active against other bacteria such as food contaminating and pathogenic bacteria. The most interesting antimicrobial substance is bacteriocin (Wittayacom, 2004). Bacteriocins are proteinaceous antibacterial compounds exhibit bactericidal activity against species closely related to the producer strain. These substances are produced by many species of bacteria and among them are the lactic acid bacteria (Riley and Chavan, 2007).

Weissella cibaria is a member of the lactic acid bacteria (Salminen *et al.*, 2004).Very little information about *Weissella* bacteriocins are available. The purpose of this study was to investigate the antimicrobial activity of bacteriocin produced by lactic acid bacteria against some common human pathogens.

Materials and Methods

Sample collection and bacterial isolation: A total of 1070 isolates were from 50 samples obtained from fermented food (30 samples), chicken intestine (10 samples) and human intestine and vagina (10 samples) collected from Baghdad, Iraq (220 isolates from human intestine and vagina and 80 isolates from fermented food)

and Bangkok, Thailand (550 isolates from chicken intestines and 220 isolates from fermented food) during the period from January to July 2007.

A 10% (w/v) sample made in diluent (0.85% NaCl) mixed well and 10 fold serially diluted. Pour plates were made with the serially diluted samples (l ml aliquot) in De Man, Rogasa and Sharpe medium (MRS medium) (De Man *et al*., 1960) and incubated under anaerobic condition (anaerobic generating Kit, Merck, USA) at 30 °C for 18 -24 hr**.**

Detection of bacteriocin producing lactic acid bacteria: Single colony from MRS agar plate was inoculated in 5 ml MRS broth and incubated under anaerobic condition at 30 °C for 18 -24 hr. Cell-free supernatant was obtained by centrifugation at 10.000 xg for 10 min with adjustment of pH to 6 (2N NaOH) to eliminate the inhibitory effect of organic acids, and catalase was added at a final concentration of 50 U/ml to eliminate the potential inhibitory effect of H_2O_2 produced by isolates then filtered through 0.45µm filter. Inhibitory activity was detected by the agar-well diffusion method of Tagg and McGiven (1971), with some modifications. Portions (100 µl) of cell-free supernatant were added to wells (5mm) cut into the plate, which was inoculated with *Shigella dysenteriae* DMST 15110 (as the indicator strain) and the plate was incubated for 18 hr at 37°C. The isolates that showed clear inhibition zones were purified by streaked from the broth and restreaked for single colony.

Identification of the efficient bacteriocin-producing isolate:Isolate with which gave the largest inhibition zone against *Shigella dysenteriae* DMST 15110 was purified and identified to species-level using cultural and biochemical characteristics (Collins *et al*., 1993; MacFaddin, 2000). A concrete identification for the isolates was also done using 16S rRNA gene sequencing for characterization of the strain (Chagnaud *et al*., 2001).

Sequencing and comparison of 16S rRNA gene: PCR condition included: denaturation at 94 ºC for 3 min.,followed by 30 cycles of denaturation at 94 ºC for30 sec., annealing at 52 ºC for 30 sec., and extension at 72 ºC for 45 sec. and the final extension at 72 ºC for 2 min. PCR reaction composed of 50 µl of 1x reaction buffer with 25mM MgCl2, 2.5 mM deoxynucleoside triphosphate, 1 U of Taq polymerase , 20 pM of each primer, UFUL(5′-GCCTAACACATGCAAGTCGA3′) and URUL(5′-CGTATTACCGCGGCTGCTGG 3′),and a genomic DNA of 50-200 ng which was used as a template for amplification (Martinez-Murcia *et al.*, 1995; Christine *et al.*, 2002).The 16S rRNA gene was subjected to nucleotide sequencing with an automated DNA sequencing machine at Bio-Service unit. National Science and Technology Development Agency, Thailand.The 16S rRNA gene sequences determined were aligned along with the sequences of type strains obtained from the GenBank by using the program CLUSTAL X (version 1.82) **(** Björkroth *et al*., 2002).

Determination of optimum temperature for growth and bacteriocin production A portion of 100 µl of the overnight bacterial culture of *W. cibaria* NRIC0136 was inoculated into 5 ml of MRS broth and incubated at 15, 20, 25, 30, 37, and 44°C for 18 h. After incubation, growth was determined by measuring the optical density at 660 nm and bacteriocin activity was determined by the agar well diffusion method (Srionnual *et al*., 2007)**.**

Spectrum of bacteriocin activity of *W.cibaria* NRIC0136: Spectrum of bacteriocin activity was determined by using cell free supernatant and number of standard indicator strains of pathogenic microorganisms(*Listeria monocytogenes* DMST 17303, *Enterococcus faecalis* ATCC 29212 , *Salmonella* sp.DMST 22842 , *Shigella dysenteriae* DMST 15110 ,*Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, *Vibrio*

harveyi 639 , *Candida albicans* ATCC 10231 and *Bacillus cereus* ATCC 14579) with the agar well diffusion method .The plates were incubated for 18 hr at 37 °C (Jumrianrit, 2004).

Bacteriocin activity assay: Bacteriocin activity was assayed based on the critical dilution of antagonistic activity caused by *W.cibaria* NRIC0136 culture. Generally, a two-fold series (1-512) of dilutions of the bacteriocin sample were prepared and the agar well diffusion method was used for evaluating the antagonistic effect of aliqouts from each dilution using the indicator strain of *Shigella dysenteriae* DMST 15110 as the test organism, after incubation for 18 hour at 37°C, bacteriocin activity was expressed in arbitrary units (AU). One unit was defined as the reciprocal of the highest dilution showing inhibition of the indicator lawn and was expressed as activity units (AU) per mililiter. (Hoover and Harlander, 1993), Bacteriocin activity (AU/ml) = (the highest dilution exhibiting inhibition zone X 1000) / Volume (µl).

Results:

Detection and isolation of bacteriocin-producing lactic acid bacteria: A total of 1070 bacterial isolates from 50 various samples were screened for bacteriocin production by the agar well diffusion method using *Shigella dysenteriae* DMST 15110 as the indicator strain. The results showed that 72 isolates gave positive inhibition zones (11-18 mm) (Table 1). The detection rate was 6.73%. One isolate (F14) from Thailand fermented fish that gave the largest inhibition zone against the indicator strain was selected (Figure1) for identification and further study.

Table (1): Antimicrobial producing isolates obtained from various samples

TYPES OF SAMPLES	TOTAL ISOLATES	NUMBER OF ISOLATES THAT PRODUCED CLEAR ZONE*
Fermented food	300	19
Chicken intestine	550	41
Human intestine and vagina	220	12
Total	1070	72

*****clear zone (11-18mm)

Identification of the selected isolate (F14)

Morphological, cultural and biochemical characteristics

Morphological and cultural characteristics of the selected isolate (F14) are summarized in Figures (2) and Table (2). Colonies of the isolate F14 on MRS agar plates were small, grayish white, circular, low convex with entire margin, and non-pigmented.

Cells were Gram positive, non-motile, nonspore former, short rods in shape and appeared either singly or in pairs.Growth occured at pH 4.4 and 8.0 but not at pH 9.6. The isolate could not grow in presence of 8% NaCl but grew in 6.5% NaCl .Growth occured at 15, 37 and 45 °C, but not at 4°C. Catalase negative, Arginine is hydrolysed. CO_2 is produced from glucose. In order to confirm the results of the morphological and biochemical identification, the 16S rRNA gene sequence was determined since this method is more discriminating and allows precise identification at the species level.

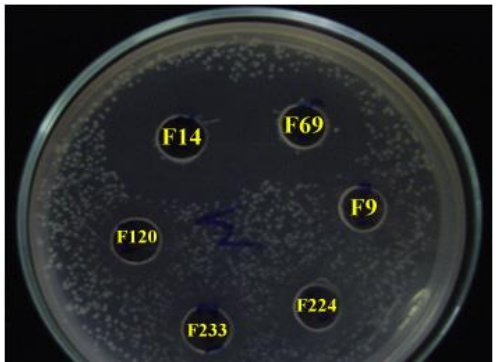

Figure (1). Inhibition zones around cell free supernatants of different tested isolates detected by using the agar well diffusion method, *Shigella dysenteriae* DMST 15110 was used as the indicator strain(TSA medium ,37 °C, 18hr, aerobic condition).

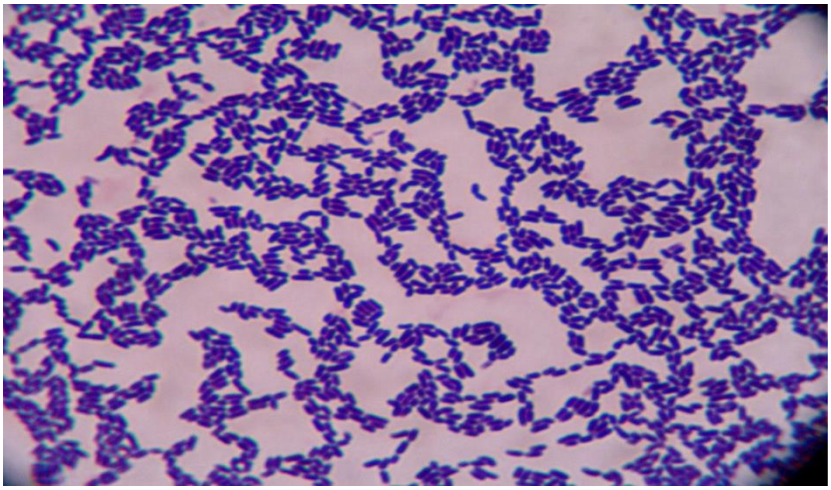

Figure (2) Morphology of the isolate F14 stained with Gram stain under light microscope (X1000).Cells showed Gram positive rods.

Table (2): Biochemical characteristics of the selected isolate (F14)

CHARACTERISTICS	RESULTS
Catalase	-
Growth at 4 °C	-
Growth at 15 °C	+
Growth at 37 °C	+
Growth at 45 °C	+
Growth at pH 4.4	+
Growth at pH 8.0	+
Growth at pH 9.6	-
Growth at 6.5% NaCl	+
Growth at 8% NaCl	-
Gas from glucose	+
Arginine hydrolysis	+

16S rRNA gene sequencing: The 16S rRNA gene sequence was investigated because this method is more discriminating (Chagnaud *et al.*, 2001). Thus, 830 bp of 16S rRNA gene was amplified using PCR. From comparison of the sequence with that in the database in GenBank by BLAST program, the microorganism was identified with 100 % certainty to be *Weissella cibaria* (accession no. AB362617.1). Therefore, the strain was designated as *Weissella cibaria* NRIC0136 (Figure 3).

Query *Weissella cibaria* NRIC 0136 gene for 16S rRNA, partial sequence.
(dbj|AB362617.1|)
Sbjct Fragment of F14 isolate
Score = 1157 bits (626), Expect = 0.0 Identities = 626/626 (100%), Gaps = 0/626 (0%) Strand=Plus/Plus

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Query 1   TGCTCAGATATGACGATGGACATTGCAAAGAGTGGCGAACGGGTGAGTAACACGTGGGAA 60
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 90  TGCTCAGATATGACGATGGACATTGCAAAGAGTGGCGAACGGGTGAGTAACACGTGGGAA 149
Query 61  ACCTACCTCTTAGCAGGGGATAACATTTGGAAACAGATGCTAATACCGTATAACAATAGC 120
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 150 ACCTACCTCTTAGCAGGGGATAACATTTGGAAACAGATGCTAATACCGTATAACAATAGC 209
Query 121 AACCGCATGGTTGCTACTTAAAAGATGGTTCTGCTATCACTAAGAGATGGTCCCGCGGTG 180
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 210 AACCGCATGGTTGCTACTTAAAAGATGGTTCTGCTATCACTAAGAGATGGTCCCGCGGTG 269
Query 181 CATTAGTTAGTTGGTGAGGTAATGGCTCACCAAGACGATGATGCATAGCCGAGTTGAGAG 240
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 270 CATTAGTTAGTTGGTGAGGTAATGGCTCACCAAGACGATGATGCATAGCCGAGTTGAGAG 329
Query 241
ACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGG 300
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 330 ACTGATCGGCCACAATGGGACTGAGACACGGCCCATACTCCTACGGGAGGCAGCAGTAGG 389
Query 301
GAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGTGTGATGAAGGGTTT 360
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 390 GAATCTTCCACAATGGGCGAAAGCCTGATGGAGCAACGCCGCGTGTGTGATGAAGGGTTT 449
Query 361 CGGCTCGTAAAACACTGTTGTAAGAGAAGAATGACATTGAGAGTAACTGTTCAATGTGTG 420
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 450 CGGCTCGTAAAACACTGTTGTAAGAGAAGAATGACATTGAGAGTAACTGTTCAATGTGTG 509
Query 421
ACGGTATCTTACCAGAAAGGAACGGCTAAATACGTGCCAGCAGCCGCGGTAATACGTATG 480
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 510 ACGGTATCTTACCAGAAAGGAACGGCTAAATACGTGCCAGCAGCCGCGGTAATACGTATG 569
Query 481 TTCCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGACGGTTATTTAAGTCTG 540
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 570 TTCCAAGCGTTATCCGGATTTATTGGGCGTAAAGCGAGCGCAGACGGTTATTTAAGTCTG 629
Query 541 AAGTGAAAGCCCTCAGCTCAACTGAGGAATTGCTTTGGAAACTGGATGACTTGAGTGCAG 600
          ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Sbjct 630 AAGTGAAAGCCCTCAGCTCAACTGAGGAATTGCTTTGGAAACTGGATGACTTGAGTGCAG 689
Query 601 TAGAGGAAAGTGGAACTCCATGTGTA 626
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Sbjct 690 TAGAGGAAAGTGGAACTCCATGTGTA 715

Figure (3). Aligment of *Weissella cibaria* NRIC 0136 gene for 16S ribosomal RNA and 16S rDNA amplified fragment of F14 strain.

Optimum temperature for growth and bacteriocin production : From the results shown in Table (3), the maximum O.D and inhibition zone (18mm) against the indicator strain, *Shigella dysenteriae* DMST 15110 were maximum when growth temperature employed was 30°C.

Table (3): Effects of growth temperature on bacteriocin production from *W. cibaria* NRIC0136

GROWTH TEMPERATURE (°C)	OD_{600} OF *W. CIBARIA* NRIC0136 CULTURE	DIAMETER OF INHIBITION ZONE (MM)
15	0.187	-*
20	2.046	-
25	2.128	17
30	2.253	18
37	2.091	16
44	0.253	-

***** No inhibitory zone observed

Antimicrobial spectrum of bacteriocin produced by *W.cibaria* NRIC0136: The cell free supernatant of *W. cibaria* NRIC0136 was tested for antimicrobial spectrum activity. Spectrum of activity (13-18mm) of the cell free supernatant was determined using the agar well diffusion method against a number of indicator strains (Table 4).

Table (4): Antimicrobail activity of cell free supernatant of *W. cibaria* NRIC0136 against a number of microbial strains

MICROBIAL STRAIN	INHIBITION ZONE(MM)	CULTURE MEDIUM	GROWTH TEMPERATURE(°C)
Bacillus cereus ATCC 14579	0	TSA	37
Enterococcus faecalis ATCC 29212	13	TSA	37
Staphylococcus aureus ATCC 25923	0	TSA	37
Listeria monocytogenes DMST 17303	16	BHI	30
Escherichia coli ATCC 25922	0	TSA	37

Pseudomonas aeruginosa ATCC 27853	0	TSA	37
Salmonella sp.DMST 22842	14	TSA	37
Shigella dysenteriae DMST 15110	18	TSA	37
Vibrio harveyi 639	0	TSA+3% NaCl	37
Candida albicans ATCC 10231	0	Sabouraud dextrose	25

Determination of bacteriocin activity: Bacteriocin activity of *W.cibaria* NRIC0136 was determined by the agar well diffusion method .Two-fold serial dilutions of cell-free supernatant were tested. After 18 hr at 30°C, the inhibition zone of the undiluted cell-free culture supernatant was observed with a diameter of 18 mm as shown in Figure (4). The inhibition zone was still clear even after 512-fold of dilution. Therefore, the bacteriocin activity of *W.cibaria* NRIC0136 was calculated as 5120 AU/ml.

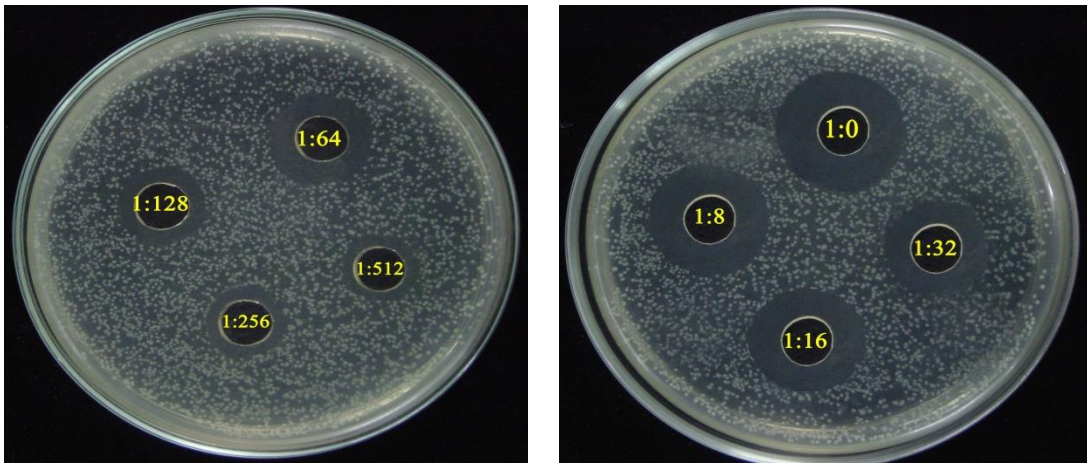

Figure (4) Bacteriocin activity assay. Crude extract of *W.cibaria* NRIC0136 was 2-fold serially diluted and 100 µl aliquot of each dilution was added to every well in TSA plate inoculated with indicator strain of *Shigella dysenteriae* DMST 15110. The plate was incubated at 30°C, 18 hr under aerobic conditions.

Discussion

Weissella cibaria is a member of the lactic acid bacteria (Salminen *et al*., 2004).Very little information about *Weissella* bacteriocins are available. Only weissellicin 110 produced by *W. cibaria* 110 had been investigated by Srionnual *et al*., (2007).

The first aim of this study was isolation and detection of bacteriocin-producing lactic acid bacteria of 50 samples from different sources (fermented foods (30 samples), chicken intestine (10 samples) and human intestine and vagina (10 samples)). From 1070 isolates, 72 isolates were able to produce bacteriocin by formation of clear inhibition zones against growth of indicator strains of *Shigella dysenteriae* DMST 15110. The rate of detection was 6.73% which was about 34 fold higher than detection rate of Coventry *et al*.,(1997) who screened about 600,000 colonies from dairy products (0.2% detection). The higher incidence of percent detection rate could be due to the differences of indicator strains used (4 indicator strains of lactic acid bacteria) and samples sources (fermented food, fresh meat, packaged meat, unflavored and flavored milk and cream) .However, other studies have reported 0.2 -32 % detection rate (Garver and Muriana, 1993:Coventry *et al*. 1997). Antimicrobial activity of the cell-free supernatants of the isolates had stable activity after neutralization and treatment with catalase as well as sensitive to

proteinase K and trypsin, suggesting that these antimicrobial substances were bacteriocins (de Vuyst and Vandamme, 1992; Riley and Chavan, 2007)..

One isolate (from Thailand fermented fish) that gave the largest inhibition zone (18mm) was selected for further study and identified as *Weissella cibaria* NRIC0136 by morphological, biochemical properties then by sequencing and comparison of 16S rRNA gene.As many LAB have similar nutritional and growth requirements, it is often difficult to use classical microbiological methods to identify them even to genus level. Research has focused on the application of molecular biology technique for the rapid detection and differentiation of LAB. The use of primers and probes that target genes encoding ribosomal ribonucleic acid (rRNA) is promising. Due to the high interspecies variability of this region, rDNA sequences coding for the 16S and 23S rRNA has been validated as a means of identification (Dubernet *et al*., 2002)

Cell-free supernatant of *W.cibaria* NRIC0136 inhibited growth of *L. monocytogenes* DMST 17303, *En.faecalis* ATCC 29212 , *Salmonella* sp.DMST 22842 and *Shigella dysenteriae* DMST 15110 .But could not inhibit *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *V. harveyi* 639 , *C. albicans* ATCC 10231 and *B. cereus* ATCC 14579. These results indicate that the bacteriocin had broad antimicrobial spectrum. However, several observations may be made on the antimicrobial activity of bacteriocins (i) some strains within a given species may be sensitive and others may be resistant to a particular bacteriocin; (ii) a strain that appears to be sensitive to a bacteriocin may also have some cells in the population that are resistant to it; (iii)a strain can be sensitive to one bacteriocin while resistant to a similar type of bacteriocin; (iv) cells of a strain producing one bacteriocin can be sensitive to another bacteriocin; (v) although the spores of a strain whose cells are sensitive to a bacteriocin are resistant to that bacteriocin, they become sensitive following germination; and (vi) under normal conditions, Gram-negative bacteria are not sensitive to LAB-bacteriocins. As regards the last observation, it is interesting to note that Gram-negative bacteria are only inhibited by purified LAB-bacteriocins provided conditions that weaken cell wall integrity, such as the presence of chemical agents (e.g., organic acids, EDTA and other quelants) or stressing environmental conditions (e.g., pH, freezing, mild heating or high hydrostatic pressure) (Cintas *et al*.,2001)

References

Björkroth, K. J.; Schillinger, U.; Geisen, R.; Weiss, N.; Hoste, B.;Holzapfel, W. H.;

Korkeala, H. J.; and Vandamme, P. (2002). Taxonomic study of *Weissella confusa* and description of *Weissella cibaria* sp. nov., detected in food and clinical samples. Int. J. Syst. Evol. Microbiol. 52: 141–148.

Chagnaud, P.; Machinis, K.; Coutte, L.A.; Marecat, A.; and Mercenier, A. (2001).Rapid PCR-based procedure to identify lactic acid bacteria: application to six common *Lactobacillus* species. J.Microbiol.Methods.44: 139-148.

Christine, F.; Vaughan, E.E.; De Vos, W.M.; and Akkermans, A.D.L. (2002).Molecular monitoring of succession of bacterial communities in human neonates. Appl. Environ.Microbial. 68:219-226.

Cintas, LM.; Casaus, P.; Herranz, **C.; Nes, IF.;and Hernandez PE.** (2001).Bacteriocins of lactic acid bacteria.Food Sci. Tech. Int. 7:281-305.

Collins, M. D.; Samelis, J.; Metaxopoulos, J.; and Wallbanks, S. (1993). Taxonomic studies on some leuconostoc-like organisms from fermented sausages: description of a new genus *Weissella* for the *Leuconostoc paramesenteroides* group of species. J. Appl. Bacteriol. 75: 595–603.

Coventry, Ml; Gordon, lB.; Wilcock, A.; Harmark, K.; Davidson, BE.; Hickey,MW.; Hickey, AJ.; and Wan J.(1997). Detection of bacteriocins of lactic acid bacteria isolated from foods and comparison with pediocin and nisin. J. Appl . Microbiol. 83:248-258.

De Man, J. D.; Rogosa, M.; and Sharpe, M. E. (1960). A medium for the cultivation of lactobacilli. J. Appli. Bacteriol.23: 130–135.

De Vuyst, L. and Vandamme, EJ. (1992). Influence of the carbon source on nisin production in *Lactococcus lactis* subsp. *lactis* batch fermentations. J. Gen. Microbiol. I38 :571-578.

Dubernet, S.; Desmasures, N.; and Guēguen. M.(2002). A PCR-based method for identification of lactobacilli at the genus level FEMS Microbiology Letters. 214: 271-275

Garver, KI,; and Muriana, PM. (1993). Detection, identification and characterization of bacteriocin-producing lactic acid bacteria from retail food products. Int. J. Food Microbiol. 19:241-258.

Hoover, D.G.; and Harlander, S.K.(1993). Screening methods for detecting bacteriocin activity. In Bacteriocins of lactic acid bacteria (Hoover, D.G. and Steenscr, L.R.); pp.23-39.Academic press, California, USA.

Jumrianrit, P. (2004). Charecterization and purification of a bacteriocin produced by *Lactobacillus plantarum* PMU33 strain from fermented fish products. Bangkok: Mahidol University.

Koponen, O. (2004). Studies of producer self-protection and nisin biosynthesis of *Lactococcus lactis*. Doctoral dissertation. University of Helsinki, Finland.

MacFaddin, J.F.(2000). Biochemical tests for identification of medical bacteria. Lippincott Williams & Wilkins. Philadelphia, USA.

Martinez-Murcia, A. J.; Acinas, S. G.; and Rodriguez-Valera, F. (1995). Evaluation of prokaryoticdiversity by restrictase digestion of 16S rDNA directly amplified from hypersaline environments.FEMS Microbiol. Ecol. 17: 247-256.

Riley, M.A.; and Chavan, M.A. (2007). Bacteriocins: Ecology and Evolution. Springer Berlin Heidelberg. New York.

Salminen, S.; Wright, A.; and Ouwehand, A. (2004).Lactic Acid Bacteria. Marcel Dekker, Inc. New York.

Srionnual, S.; Yanagida,F.; Lin. L.; Hsiao,K.; and Chen,Y. (2007). Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110, Isolated from Plaa-Som, a fermented fish product from Thailand .Appl and Environmental Microbiology.73: 2247–2250.

Tagg, J.R. and McCiven, A.R. (1971)**.** Assay system for bacteriocins. Appl. Microbiol. 21: 943- 948.

Wittayacom, K. (2004). Identification and purification of a bacteriocin secreted by lactic acid bacteria isolated from traditional Thai fermented food.Master thesis. Mahidol University.Thailand.

الفعالية المضادة للبكتريوسين المنتج من بكتريا *Weissella cibaria* NRIC0136

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الخلاصة :

عزلت 1070 عزلة من بكتريا حامض اللاكتيك من 50 عينة جمعت من مصادرمختلفة [اغذية متخمرة (30 عينات)،امعاءالدجاج(10عينات)،امعاءومهبل الانسان(10 عينات)]،عزلت 300 عزلة من بغداد، العراق(220 عزلة من امعاء ومهبل الانسان و80 عزلةمن الاغذية المتخمرة) اما العزلات المتبقية (770 عزلة) فقد عزلت من بانكوك (تايلند). غربلت هذه العزلات لانتاجها للبكتريوسين باستعمال طريقة الانتشار بحفر الاكار واستعملت السلالة *Shigella dysenteriae* DMST 15110 كسلالة حساسة للبكتريوسين.واظهرت 72 عزلة قطر تثبيط واضح لنمو السلالة الحساسة. اختيرت عزلة واحدة F14 (من الاسماك المتخمرة في تايلند) التي اظهرت اكبر قطر تثبيط ضد السلالة الحساسة وشخصت العزلة بانها *Weissella cibaria* NRIC0136 عن طريق الصفات المظهرية والفسلجية، وباستعمال تقنية التفاعل التسلسلي للبوليميريز Polymerase chain reactionومعرفة تسلسل ومقارنة جين الحامض النووى الرايبوسومى 16SrRNA .اظهر العالق الخالى من الخلايا للبكتريا المشخصة بعد معادلة رقمه الهيدروجينى تثبيطا لنمو عددا من السلالات الميكروبية المرضية القياسية والتي شملت *Listeria monocytogenes* DMST 17303 . *Shigella dysenteriae* DMST 15110, *Salmonella* sp DMST 22842 *Enterococcus faecalis* ATCC 29212 .وجد ان الكثافة البصرية باستعمال 660 نانوميتر (2.253) وقطر التثبيط (18 ملم) للسلالة الحساسة كانتا باعلى قيمة عندما كانت درجة حرارة النمو 30 درجة مئوية.