PAPER

ISOLATION AND CHARACTERIZATION OF LACTIC ACID BACTERIA FROM *XI-GUA-MIAN* (FERMENTED WATERMELON), A TRADITIONAL FERMENTED FOOD IN TAIWAN

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ABSTRACT

Young watermelon fruit was peeled and pickled for fermentation to produce a unique fermented food named *xi-gua-mian* (fermented watermelon) in Taiwan. In this study, we investigated the LAB microflora in *xi-gua-mian*. A total of 176 LAB isolates were identified; 118 cultures were isolated from the *xi-gua-mian* sample collected from three different farmers markets and 58 from six young watermelon fruit samples. These isolates were characterized phenotypically and then divided into seven groups (A to G) by restriction fragment length polymorphism analysis, sequencing of 16S ribosomal DNA and other genotypic analysis. *Lactobacillus plantarum* was the most abundant LAB found in *xi-gua-mian* samples collected in southern Taiwan, Tainan City and *Pediococcus pentosaceus* was the most abundant LAB in northern Taiwan, Taoyuan County. We found that LAB stains are similar in samples collected in the same geographic region but significant variations were observed between samples collected among different regions. On the other hand, a greater LAB diversity was observed in the young watermelon fruit samples. In addition, 10 *Lactococcus lactis* subsp. *lactis* showed inhibitory activity against the indicator strain *L. sakei* subsp. *sakei* JCM 1157^T. This is the first report describing the distribution and varieties of LAB existing in the *xi-gua-mian* and the young watermelon fruits.

- Keywords: lactic acid bacteria, *xi-gua-mian*, fermented watermelon, Taiwan -

INTRODUCTION

Watermelon (*Citrullus lanatus*) is a popular fruit in Taiwan. The farming area dedicated to watermelon production in Taiwan is reported to be largest among all fruits (LIN *et al.*, 2009). To have a better harvest, surplus fruits are eliminated and only one fruit is retained for every stock in the early phase of watermelon cultivation. In Taiwan, farmers use the eliminated young watermelon fruits to produce a unique fermented food named *xi-gua-mian* (fermented watermelon).

These immature watermelon fruits are peeled, cut, mixed with salt (NaCl) and then placed in a bucket. Salt is added to a final concentration of approximately 3-6%, and the bucket is sealed with heavy stones placed on the top of the cover. This process usually continues for 3 days and then the exuded water is drained. The bucket is sealed again with heavy stones and the fermentation process continues for at least 2 weeks at room temperature. Because of the contribution of the lactic acid bacteria (LAB), it has a special sour and sweet flavor. *Xi-gua-mian* is usually applied as a seasoning for various pork, seafood and poultry dishes in order to add a slightly acidic taste. Although the product is very popular, it has not been studied in detail.

Lactic acid bacteria (LAB) has been frequently found in various Taiwanese fermented foods such as *yan-tsai-shin* (fermented broccoli stems), *yan-jiang* (fermented ginger), *jiang-sun* (fermented bamboo shoot), *suan-tsai* (fermented mustard), *dochi* (fermented black beans), *jiang-gua* (fermented cucumbers), *yan-dong-gua* (fermented wax gourd) and *pobuzihi* (fermented cummingcordia) (CHANG *et al.*, 2011; CHEN *et al.*, 2006a, 2006b, 2010, 2012, 2013a, 2013b; LAN *et al.*, 2009). In these cited studies, various LAB species, such as *Enterococcus faecium*, *Lactobacillus plantarum*, *Lactococcus lactis* subsp. *lactis*, *Weissella cibaria* and *W. paramesenteroides*, were frequently found in the Taiwanese fermented products. However, there has been very little research reported on LAB distribution in fermented watermelon (*xi-gua-mian*).

One important attribute of LAB is the bacteriocin-producing abilities to inhibit food spoilage bacteria and many LAB strains isolated from the Taiwanese fermented foods were found to produce various bacteriocins. Some bacteriocins produced by these strains were further identified as novel bacteriocins in the later studies such as enterocin TW21, weissellicin L and enterocin T (CHANG *et al.*, 2013; CHEN *et al.*, 2013c; LIANG *et al.*, 2013).

The objectives of this study were to isolate LAB from the *xi-gua-mian*, to identify the isolates to the species level and to detect the antibacteri-

Table 1 - Analysis results and characteristics of isolates.

						16S rDNA RFLP groups						
						A	B	C	D	E	F	G
Sample No.	Location	pH	Salt con. (g/L)	Viable acid-producing cells (log CFU/mL)[α]	Lactic acid (g/L)	*L. plantarum*	*L. pentosus*	*P. pentosaceus*	*Lc. lactis* subsp. *lactis*	*Leu. mesenteroides*	*W. paramesenteroides*	*E. casslifiavus*
Fermented watermelon												
S1	Tainan	4.6	3.8	7.36±0.18	35.5	30	8					
S2	Tainan	3.9	3.8	6.77±0.17	95.0	36	4					
S3	Taoyuan	4.1	6.0	8.00±0.05	80.0			40				
Fresh watermelon												
W1	Hualien	–	–	1.84±0.09	–				7 (1[β])	1		2
W2	Hualien	–	–	3.77±0.01	–	2	1		7 (7)	2	1	
W3	Hualien	–	–	3.25±0.03	–	3	1		3 (2)	1	2	
W4	Tainan	–	–	3.04±0.06	–							10
W5	Tainan	–	–	3.51±0.08	–							12
W6	Chiayi	–	–	1.48±0.03	–				3			
Total						71	14	40	20	4	3	24

[α] The data are expressed as the mean±SD (n=3). [β] Number of BLIS-producing strains.
Abbreviations: L., *Lactobacillus*; P., *Pediococcus*; Lc., *Lactococcus*; Leu., *Leuconostoc*; W., *Weissella*; E., *Enterococcus*.

al activities of the isolates. Our results provide an example to understand the rich resources of LAB strains in the traditional Taiwanese fermented food.

MATERIALS AND METHODS

Xi-gua-mian and the young watermelon fruit samples

A total of 3 *xi-gua-mian* samples (S1-S3) were collected at three traditional farmers markets located in Tainan City and Taoyuan County (Table 1, Fig. 1A). In addition, six young watermelon fruit samples (W1-W6, approximately 8-15 cm in size) were collected from Hualien County, Tainan City and Chiayi County (Table 1, Fig. 1B). Samples were stored at 4°C and analyzed within 24 h of acquisition from the markets and the watermelon fields. The salt concentration and pH of *xi-gua-mian* juice was measured by using a model SK-5S salt meter (Sato Keiryoki, Tokyo, Japan) and a model B-112 compact pH meter (Horiba, Kyoto, Japan), respectively. Lactic acid in each *xi-gua-mian* samples was determined with a D-/L-Lactic Acid test kit (R-Biopharm AG, Darmstadt, Germany), according to the manufacturer's instructions.

Isolation of LAB

An initial analysis results showed that the *xi-gua-mian* samples S1 and S2 contained 3.8 % NaCl and sample S3 contained 6 % (Table 1). Therefore, MRS agar (Difco™ Lactobacilli MRS Broth; Sparks, MD, USA) containing 3 % NaCl was used for the isolation of LAB from *xi-gua-mian* samples S1 and S2. On the other hand, MRS agar containing 6 % NaCl was used for isolation from sample S3 and MRS agar without adding NaCl was used for isolation from young watermelon fruit samples. To distinguish acid-producing bacteria from other bacteria, 1% $CaCO_3$ was added to the MRS agar, and only colonies with a clear zone around them were selected (KOZAKI *et al.*, 1992). 0.5 g of crushed young watermelon fruit samples, and 0.5-mL aliquot of each *xi-gua-mian* juice samples were taken for LAB isolation. The isolation procedures of LAB were performed according to the methods described by CHEN *et al.* (2013a).

RFLP and sequence analysis of 16S rDNA

RFLP and sequence analysis of 16S rDNA were used to classify and identify the bacterial isolates. A colony PCR method described by SHEU *et al.* (2000) was performed in this study. PCR reactions were carried out using a Genomics *Taq* gene amplification PCR kit (Genomics, Taipei, Taiwan) and performed on a Gene Amp PCR System 9700 (PerkinElmer Corp., Boston, MA, USA) under the following conditions: 95°C for 3 min, 30 cycles of 95°C for 30 s, 60°C for 30 s and 72°C for 90 s, a final extension of 72°C for 10 min, and completion at 4°C (CHEN *et al.*, 2013b). 16S rDNA gene was amplified using the 16S rDNA universal primers 27F (5'- AGAGTTTGATCCTGGCTCAG -3') and 1492R (5'- GGTTACCTTGTTACGACTT-3') (CHEN *et al.*, 2013b). RFLP analysis of 16S rDNA was also performed, as described by CHEN *et al.*, (2013b). In this study, three restriction enzymes, *Acc*II (CG/CG), *Hae*III (GG/CC) and *Alu*I (AG/CT) (Chen *et al.*, 2013b), were mainly used for grouping. For sequence analysis of 16S rDNA, the PCR products were purified and then sequenced with the following primer: 5´-GTCAATTCCTTTGAGTTT-3´ (920R). Sequence homologies were examined by comparing the obtained sequences with those in the DNA Data Bank of Japan (DDBJ; http://www.ddbj.nig.ac.jp/) using BLAST.

Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum*

A multiplex PCR assay with *recA* gene-derived primers was performed using the methods and conditions described by TORRIANI *et al.* (2001).

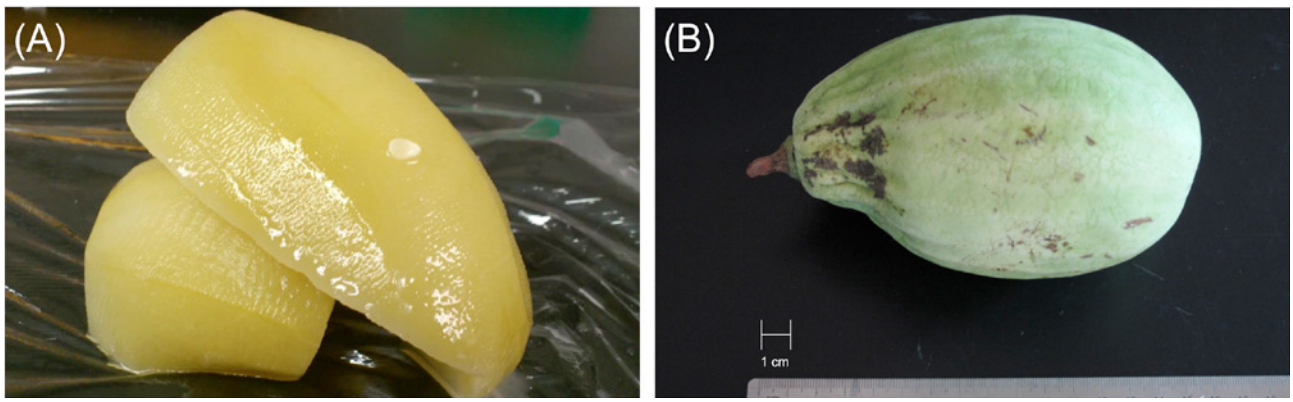

Fig. 1 - Pictures of (A) *xi-gua-mian* and (B) young watermelon fruit.

Differentiation of *Leuconostoc mesenteroides* and *Leu. pseudomesenteroides*

A rapid identification method described by JANG *et al.* (2003) was used to distinguish *Leuconostoc mesenteroides* and *Leu. pseudomesenteroides* isolates. Briefly, a PCR product of the isolate was amplified by using *Leuconostoc*-specific primers and then digested by using the restriction enzyme *Tsp509*I (/AATT) (JANG *et al.*, 2003). Restriction fragments were visualized on a 2% agarose gel in 1× TAE.

Differentiation of *W. paramesenteroides* and *W. hellenica*

In this study, isolate which showed high sequence homology to *W. paramesenteroides* and *W. hellenica* was further confirmed by using the restriction enzyme *Hha*I (GCG/C) described by CHEN *et al.* (2012).

Effect of NaCl on growth of isolates

Effect of NaCl on growth of isolates was assessed, as described by KOZAKI *et al.* (1992), by testing isolates for growth in MRS broth containing 0, 3 and 6% NaCl.

Detection of antibacterial activity

The agar well diffusion method as described by SRIONNUAL *et al.* (2007) was used to detect and determine the antibacterial activities of isolates. *Lactobacillus sakei* subsp. *sakei* JCM 1157^T was used as the indicator strain in this study. Antibacterial activity was further confirmed by pH adjustment and proteinase K treatment (SRIONNUAL *et al.*, 2007).

To determine whether nisin is the antibacterial substance, a PCR assay with the nisin-specific PCR primers, NISL: 5'-CGAGCATAATAAACGGC-3' and NISR: 5'-GGATAGTATCCATGTCTGAAC-3', described by VILLANI *et al.* (2001), were used for amplification in this study. In addition, a nisin Z producing strain, *Lc. lactis* subsp. *lactis* C101910 (YANAGIDA *et al.*, 2006) was used as the positive control and a non-BLIS (bacteriocin-like inhibitory substance) producing strain was used as the negative control.

RESULTS

In the *xi-gua-mian* samples collected from different markets, analyses of *xi-gua-mian* juice revealed different salt concentrations from 3.8 to 6.0% and lactic acid concentrations from 35.5 to 95.0 g/L (Table 1). The average number of viable acid-producing cells was 7.36±0.18, 6.77±0.17 and 8.00±0.05 log CFU/mL from the *xi-gua-mian* samples S1, S2 and S3, respectively (Table 1). The detailed analysis values of each sample are shown in Table 1 and a total of 118 acid-producing bacteria were isolated from these samples.

On the other hand, a total of 58 acid-producing bacteria were isolated from the young watermelon fruit samples. The number of viable acid producing cells on the six different young watermelon fruit samples was listed in Table 1.

The total 176 isolates were initially divided into six groups (R1-R6) according to cell morphology and the results of the 16S rDNA RFLP analysis. Of these isolated strains, 85 were placed in group R1, 40 in group R2, 20 in group R3, 4 in group R4, 3 in group R5, and 24 in group R6, according to RFLP patterns observed following digestion of their DNA with *Acc*II, *Hae*III, and *Alu*I. To identify the isolates, representative strains were randomly selected from each group, and 16S rDNA sequencing analysis was performed. The results identified group R1 isolates as *Lactobacillus plantarum*-related species, group R2 as *Pediococcus pentosaceus*, group R3 as *Lactococcus lactis* subsp. *lactis*, group R4 as *Leuconostoc mesenteroides*, group R5 as *Weissella paramesenteroides*, and group R6 as *Enterococcus cassliflavus*.

The identification of group R1 isolates was further verified using a multiplex PCR assay with *recA* gene-derived primers (TORRIANI *et al.*, 2001). An expected amplification band located at 318 bp and one at 218 bp (Fig. 2, lane 1 and 2) was respectively obtained from 71 and 14 isolates. Seventy-one isolates were therefore identified as *L. plantarum* and re-classified into group A. The remaining 14 isolates were identified as *L. pentosus* and re-classified into group B. All 4 isolates in group R4 were confirmed as *Leu. mesenteroides* based on *Tsp509*I digested fragments of the PCR product of *Leuconostoc*-specific primers and re-classified into group E (JANG *et al.*, 2003) (Fig. 2, lane 3; Table 1). Isolates in group R5 were further verified based on *Hha* I digested fragments of their 16S PCR product (CHEN *et al.*, 2012). All 3 strains were identified as *W. paramesenteroides* and re-classified into group F (Fig. 2, lane 4; Table 1). Following the re-classification of groups R1, R4 and R5, isolates in the remaining groups were also re-classified with a new code. The detailed distributions of LAB species are shown in Table 1.

Effect of NaCl on growth of all 176 isolates was estimated. All *P. pentosaceus*, *E. cassliflavus*, *L. plantarum*, *L. pentosus*, *W. paramesenteroides* and *Lc. lactis* subsp. *lactis* isolates grew well in MRS broth containing 0, 3 and 6 % NaCl except *Leu. mesenteroides* isolates. Growth of *Leu. mesenteroides* isolates was observed neither in 3 nor 6 % NaCl MRS broth.

Ten isolated *Lc. lactis* subsp. *lactis* strains showed antibacterial activity against *L. sakei* subsp. *sakei* JCM 1157^T (Table 1). The BLIS produced by all 10 strains maintained their antibacterial activities after neutralization (pH 6.5)

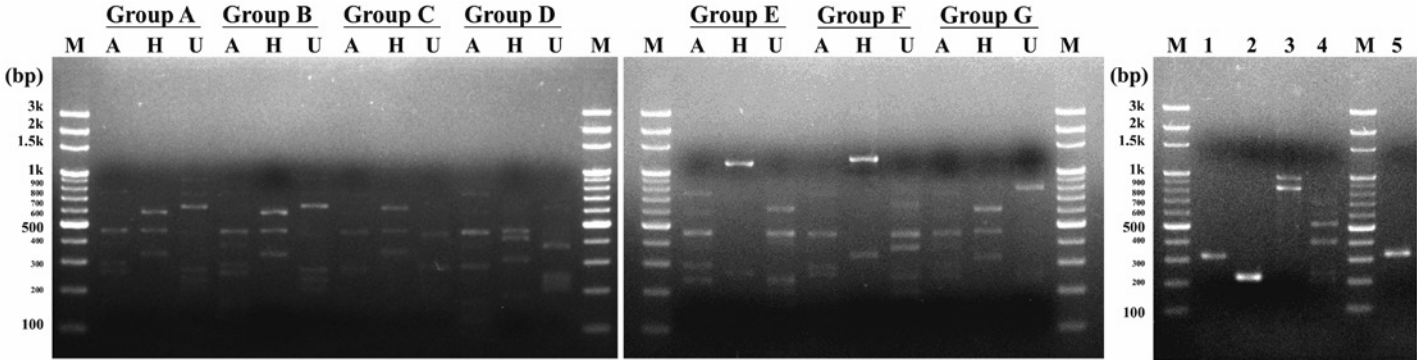

Fig. 2 - 16S rDNA RFLP patterns of *Acc*II, *Hae*III and *Alu*I digests from Groups A to G. Lane M, size marker; A, *Acc*II digested patterns; H, *Hae*III digested patterns; U, *Alu*I digested patterns; 1, amplification products obtained from the *recA* multiplex assay of *L. plantarum* isolates; 2, amplification products obtained from the *recA* multiplex assay of *L. pentosus* isolates; 3, *Tsp509*I digested patterns of *Leuconostoc*-specific PCR products from Group E isolates; 4, *Hha*I digested patterns of Group F isolates; 5, PCR products using nisin-specific primers.

but lost their antibacterial activities completely after treatment with proteinase K. In addition, nisin-specific primers were used to amplify a PCR fragment and identify the BLIS from these 10 strains. An expected amplification band located at 320 bp (Fig. 2, lane 5) was obtained from all *Lc. lactis* subsp. *lactis* isolates and the nisin Z producing strain, *Lc. lactis* subsp. *lactis* C101910 (YANAGIDA *et al.*, 2006). No amplification band was observed from the negative control strain.

DISCUSSION

In this study, LAB diversity in *xi-gua-mian* samples collected from different farmers markets and young watermelon fruits were studied. The final concentration of lactic acid and low pH values determined in the *xi-gua-mian* samples suggested that LAB contributed to the aroma and flavor development in *xi-gua-mian*.

The experimental data were treated according to critical values of Student's *t*-test. The viable acid-producing cell numbers between *xi-gua-mian* and fresh watermelon was significantly different ($p<0.0002$). We also found that the viable acid-producing cell numbers within geographical areas were different, but the statistical difference (standard deviation within *xi-gua-mian* group and fresh watermelon group was 0.61 and 0.93, respectively) was less than that between *xi-gua-mian* and fresh watermelon groups (4.56).

In addition, halotolerance of all isolates were assessed. All isolated strains grew well in MRS broth containing 3% and 6 % NaCl except *Leu. mesenteroides* isolates. Presence of NaCl in *xi-gua-mian* and isolation medium therefore might limited the growth of *Leu. mesenteroides*. Presence of *Leu. mesenteroides* was only observed in fresh watermelon fruits but not in *xi-gua-mian*. It is therefore considered that salt concentration has effect on diversity of LAB in the *xi-gua-mian*. Similar influence of NaCl concentrations on diversity of LAB in fermented foods has also been found in our previous studies (CHEN *et al.*, 2006a; 2006b).

Compared to the isolation results of *xi-gua-mian*, fewer viable acid-producing cell number were observed from the young watermelon fruit samples. It is presumably because the raw material always presents a lower number or microorganisms or the absence of salt in substratum used for the isolation from young watermelon fruit samples does not allow the selection of LAB. As in the case of *xi-gua-mian* samples, LAB stains are similar in samples collected in the same geographic region and diversities were observed between samples collected among different regions in the young watermelon fruit samples (Table 1). Different climate conditions were considered as the main factor, which may affect the distribution of LAB.

Although *xi-gua-mian* samples S1 and S2 were collected at different traditional farmers markets located in Tainan City, *L. plantarum* and *L. pentosus* were the most abundant LAB found in these two samples (Table 1). Different to the isolation results obtained in the Tainan City, *P. pentosaceus* was the most abundant LAB found in the sample collected in Taoyuan County (Table 1). Geographically, Tainan City is located in southern part of Taiwan that belongs to the tropics, while Taoyuan County is in northern subtropical regions. It is therefore considered that regional factors, such as climate conditions, raw materials for fermentation and fermentation methods, may affect the distribution of LAB.

Lactobacillus plantarum has been identified elsewhere as one of the most abundant LAB found in several Taiwanese fermented vegetables such as fermented bamboo shoots (*jiang-sun*), fermented cucumbers (*jiang-gua*), fermented broccoli stems (*yan-tsai-shin*) and fermented cummingcordia (*pobuzihi*) (CHEN *et al.*, 2010b, 2012, 2013a, 2013b). As well as *L. plantarum*, *P. pentosaceus* also have been previously found

as the most abundant LAB in the fermented mustard (*suan-tsai*) (CHEN *et al.*, 2006a). In addition, *L. plantarum* was found both in the partial samples of *xi-gua-mian* and the young watermelon fruits. It is therefore considered that *L. plantarum* found in *xi-gua-mian* may originate from the young watermelon fruits. To clarify these points, advanced analysis on more *xi-gua-mian* and young watermelon fruit samples will be necessary in the future.

The results of the antibacterial activity assay indicated that total 10 *Lc. lactis* subsp. *lactis* isolates showed inhibitory activities against *L. sakei* subsp. *sakei* JCM 1157^T. Complete inactivation of these BLIS produced by all 10 strains were observed after treating the cell-free supernatant with proteinase K, which indicates the proteinaceous nature of the active agents. When amplified with nisin-specific primers, the amplification band located at 320 bp indicated the existence of nisin-producing genes and BLIS from these 10 *Lc. lactis* subsp. *lactis* could be nisin-related variants (VILLANI *et al.*, 2001; ZENDO *et al.*, 2003). However, detailed information such as heat stability, their effect on enzymes, inhibition spectra, accurate molecular mass and amino acid sequences were not established in the current study.

Although LAB have been widely found in various fresh fruits, vegetables and various plant pickles, little information on the diversity of LAB associated with fermented watermelon or young watermelon fruits was obtained from previous studies. Future studies in our laboratory will characterize and identify the nisin-like BLIS, and we anticipate that the BLIS of LAB will be useful as food preservatives. The authors also hope that the results of this study can offer useful information for the improvement of *xi-gua-mian* production.

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REFERENCES

Chang S.Y., Chen Y.S., Pan S.F., Lee Y.S., Chang C.H., Chang C.H., Yu B. and Wu H.C. 2013. Enterocin TW21, a novel bacteriocin from *dochi*-isolated *Enterococcus faecium* D081821. J. Appl. Microbiol. 115: 673-678.

Chang C.H., Chen Y.S. and Yanagida F. 2011. Isolation and characterization of lactic acid bacteria from *Yan-jiang* (fermented ginger), a traditional fermented food in Taiwan. J. Sci. Food Agric. 91: 1746-1750.

Chen Y.S., Liou M.S., Ji S.H., Yu C.R., Pan S.F. and Yanagida F. 2013a. Isolation and characterization of lactic acid bacteria from *Yan-tsai-shin* (fermented broccoli stems), a traditional fermented food in Taiwan. J. Appl. Microbiol. 115: 125-132.

Chen Y.S., Wu H.C., Liu C.H., Chen H.C. and Yanagida F. 2010. Isolation and characterization of lactic acid bacteria from *Jiang-sun* (fermented bamboo shoots), a traditional fermented food in Taiwan. J. Sci. Food Agric. 90: 1977-1982.

Chen Y.S., Wu H.C., Lo H.Y., Lin W.C., Hsu W.H., Lin C.W., Lin P.Y. and Yanagida F. 2012. Isolation and characterisation of lactic acid bacteria from *jiang-gua* (fermented cucumbers), a traditional fermented food in Taiwan. J. Sci. Food Agric. 92: 2069–2075.

Chen Y.S., Wu H.C., Wang C.M., Lin C.C., Chen Y.T., Jhong Y.J. and Yanagida F. 2013b. Isolation and characterization of lactic acid bacteria from *pobuzihi* (fermented cummingcordia), a traditional fermented food in Taiwan. Folia Microbiol. 58: 103-109.

Chen Y.S., Yanagida F. and Hsu J.S. 2006a. Isolation and characterization of lactic acid bacteria from *suan-tsai* (fermented mustard), a traditional fermented food in Taiwan. J Appl Microbiol 101: 125-130.

Chen Y.S., Yanagida F. and Hsu J.S. 2006b. Isolation and characterization of lactic acid bacteria from *dochi* (fermented black beans), a traditional fermented food in Taiwan. Lett. Appl. Microbiol. 43: 229-235.

Chen Y.S., Yu C.R., Ji S.H., Liou M.S., Leong K.H., Pan S.F., Wu H.C., Lin Y.H., Yu B. and Yanagida F. 2013c. Enterocin T, a novel class IIa bacteriocin produced by *Enterococcus* sp. 812. Arch. Microbiol. 115: 125-132.

Jang J., Kim B., Lee J. and Han H. 2003. A rapid method for identification of typical *Leuconostoc* species by 16S rDNA PCR-RFLP analysis. J. Microbiol. Methods 55: 295-302.

Kozaki M., Uchimura T. and Okada S. 1992. *Experimental Manual of Lactic Acid Bacteria*. Asakurasyoten, Tokyo, Japan, pp. 29-72.

Lan W.T., Chen Y.S. and Yanagida F. 2009. Isolation and characterization of lactic acid bacteria from *Yan-dong-gua* (fermented wax gourd), a traditional fermented food in Taiwan. J. Biosci. Bioeng. 108: 484-487.

Leong K.H., Chen Y.S., Lin Y.H., Pan S.F., Yu B., Wu H.C. and Yanagida F. 2013. Weissellicin L, a novel bacteriocin from *sian-sianzih*-isolated *Weissella hellenica* 4-7. J. Appl. Microbiol. 115:70-76.

Lin Y.H., Chen K.S., Liou T.D., Huang J.W. and Chang P.F.L. 2009. Development of a molecular method for rapid differentiation of watermelon lines resistant to *Fusarium oxysporum* f. sp. *niveum*. Bot. Stu. 50: 273-280.

Sheu D.S., Wang Y.T. and Lee C.Y. 2000. Rapid detection of polyhydroxyalkanoate-accumulating bacteria isolated from the environment by colony PCR. Microbiology 146: 2019-2025.

Srionnual S., Yanagida F., Lin L.H., Hsiao K.N. and Chen Y.S. 2007. Weissellicin 110, a newly discovered bacteriocin from *Weissella cibaria* 110, isolated from *plaa-som*, a fermented fish product from Thailand. Appl. Environ. Microbiol. 73: 2247-2250.

Torriani S., Felis G.E. and Dellaglio F. 2001. Differentiation of *Lactobacillus plantarum*, *L. pentosus*, and *L. paraplantarum* by *recA* gene sequence analysis and multiplex PCR assay with *recA* gene-derived primers. Appl. Environ. Microbiol. 67: 3450-3454.

Villani F., Aponte M., Blaiotta G., Mauriello G., Pepe O. and Moschetti G. 2001. Detection and characterization of a bacteriocin, garviecin L1-5, produced by *Lactococcus garvieae* isolated from raw cow's milk. J. Appl. Microbiol. 90: 430-439.

Yanagida F., Chen Y.S., Srionnual S. and Shinohara T. 2006. Bacteriocin from *Lactococcus lactis* subsp. *lactis* C101910 isolated from lake water. Jpn. J. Lactic Acid Bact. 17: 51-56.

Zendo T., Fukao M., Ueda K., Higuchi T., Nakayama J. and Sonomoto K. 2003. Identification of the lantibiotic nisin Q, a new natural nisin variant produced by *Lactococcus lactis* 61-14 isolated from a river in Japan. Biosci. Biotechnol. Biochem. 67:1616-1619.

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