

Probiotics from Philippine dairy products are bactericidal for pathogens with transferable multiple drug resistance

Mary Catherine A. Mercado and Esperanza C. Cabrera*

Biology Department and Center for Natural Science and Ecological Research
De La Salle University, 2401 Taft Ave., Manila

Probiotic bacteria isolated from locally available commercial dairy products, namely, Nestle Chamyto Probiotic Drink, Yakult Probiotic Drink, Nestle Cerelac Infant Cereal, and Nido 3+ Powdered Milk were established to have antimicrobial activities using the agar spot assay. Tester strains used were extended-spectrum β -lactamase (ESBL)-producing strains of *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli*, metallo- β -lactamase (MBL)-producing strain of *Pseudomonas aeruginosa*, and three strains of methicillin resistant *Staphylococcus aureus* (MRSA). The probiotics inhibited the growth of all the tested multiple drug resistant strains. Further tests showed that the isolate from Chamyto displayed bactericidal activities on all the test strains. Yakult and Nestle Cerelac

probiotics were bactericidal for all strains except for ESBL-producing *E. coli*. Nido 3+ probiotic isolates were bactericidal for all strains except for ESBL-producing *K. pneumoniae*. The probiotic isolates were identified phenotypically and through 16S rDNA analysis to be *Lactobacillus casei* or *Lactobacillus paracasei*. The *in vitro* bactericidal activities of the isolated probiotics show great potential for further research in treatment and prophylaxis of infections from multiple drug resistant bacteria.

KEYWORDS

Probiotics, *Lactobacillus*, antibacterial, multiple drug resistant bacteria, extended-spectrum β -lactamase-producing *Enterobacteriaceae*, metallo- β -lactamase-producing *Pseudomonas aeruginosa*, methicillin resistant *Staphylococcus aureus*, ESBL, MRSA, MBL

INTRODUCTION

The rapid emergence of drug resistant strains of different microbial pathogens, especially those with multiple resistances, is a major health problem because of their high occurrence worldwide (Rouveix 2007). In the Philippines, the ready access of broad spectrum antibiotics to the general public who is mostly unaware of the proper antimicrobial usage, or on the issue of drug resistance poses a hindrance to the control of these strains.

*Corresponding author

Email Address: esperanza.cabrera@dlsu.edu.ph

Submitted: February 27, 2011

Revised: July 6, 2011

Accepted: July 19, 2011

Published: September 15, 2011

Editor-in-charge: Francis de los Reyes III

Among the drug resistant bacterial pathogens that are often implicated in various life-threatening diseases are the extended-spectrum β -lactamase (ESBL)-producing strains of Enterobacteriaceae such as *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Proteus mirabilis*, and *Escherichia coli*; metallo- β -lactamase (MBL)-producing

Pseudomonas aeruginosa and methicillin resistant *Staphylococcus aureus* (MRSA). ESBL producers are recalcitrant to treatment with all penicillins, cephalosporins and monobactams (CLSI 2010), while MBL producers are resistant to almost all broad spectrum β -lactams including carbapenems (Fujimura et al. 2007). MRSA is clinically resistant to all β -lactams (CLSI 2010). This complete resistance to β -lactams caused the switch to the use of glycopeptide antibiotic, specifically vancomycin. However, strains possessing high-level resistance, intermediate resistance, and tolerance to vancomycin have already been reported (Fridkin et al. 2003, Chang et al. 2003; Fridkin 2001; May et al. 1998). In addition, because the resistance genes of these three groups are found in plasmids (for ESBL and MBL) or in gene cassettes located in the chromosome (for MBL and MRSA), they are often also resistant to other classes of antimicrobials such as aminoglycosides and quinolones (Takashi 2007; Cabrera and Rodriguez 2009; Zhang et al. 2009). For the same reason, the genes coding for these are transferable to other bacteria, converting susceptible strains to resistance.

Probiotics are live microbial food supplements which improve the microbial balance in a host animal, thus providing beneficial health effects (Fuller 1989). Probiotics have likewise been found to have immunostimulatory properties (Trois et al. 2008), promoting the non-specific (Herich & Levkut 2002) and specific immune responses (Alvarez et al. 1998). In addition, they have been shown to competitively exclude other gut pathogens, preventing the adhesion of these pathogens to intestinal and urogenital epithelia (Chan et al. 1985). Probiotics have been reported to produce antimicrobial substances, which include organic acids such as lactic acid and acetic acid, diacetyl, hydrogen peroxide (produced by *Lactobacillus* upon transfer to an aerobic environment from an anaerobic one), and bacteriocins and microcins (Silva et al. 1987). Other metabolites of probiotic bacteria which can contribute to their effects include biosurfactants (Rodrigues et al. 2004), adhesion inhibitors (Meurman 2005), and co-aggregation molecules (Ekmekci et al. 2009; Gupta and Garg 2009).

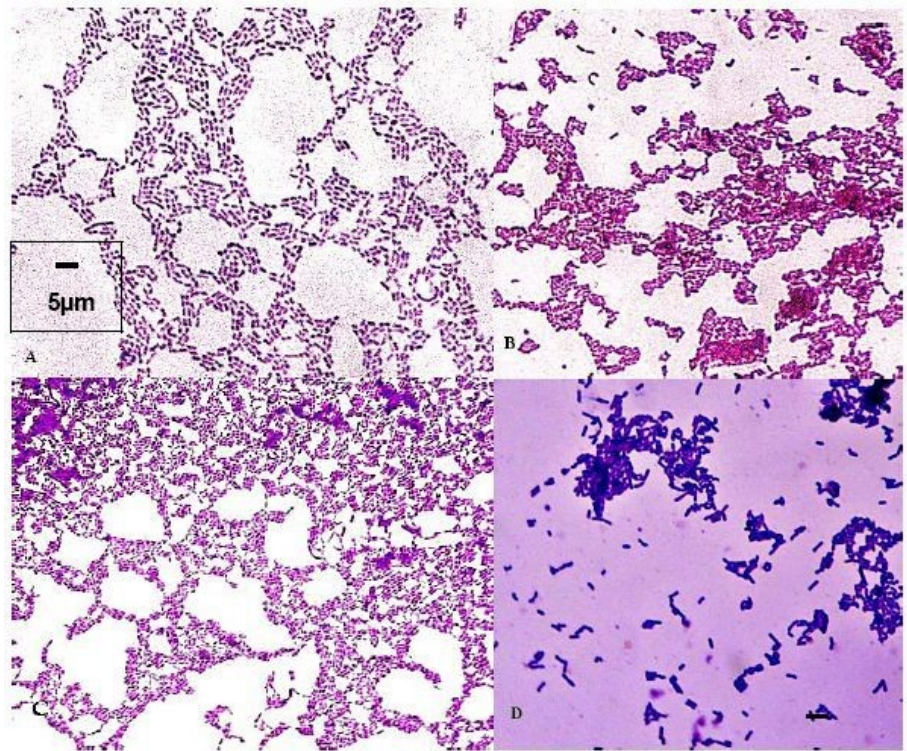


Figure 1. Gram stained morphology of the probiotic isolates from the commercial milk products showing Gram positive nonsporeforming rods. A. isolate from Yakult; B. isolate from Chamyto; C. isolate from Cerelac; D. isolate from Nido 3+ milk.

While there have been numerous studies on the antibacterial activities of different genera of probiotics (*Lactobacillus*, in particular), the significance and potential of probiotics in the prevention or reduction of emergent drug resistant strains and transmission of resistant genes has only been given minimal attention as of the moment. The increasing emergence of drug-resistant strains of pathogenic bacteria has become a great concern, as this may pose a problem for the control of nosocomial as well as community-acquired bacterial infections. Hence, this study aimed to assess the antimicrobial activities of probiotics from locally available dairy products on multiple drug resistant strains of bacterial pathogens. In addition, the identities of the isolated probiotics were determined using phenotypic characterization and 16S rRNA gene (or 16S rDNA) sequencing to ensure that these were the isolates from the dairy products, and that the antimicrobial activities were in fact from the probiotic isolates of interest.

MATERIALS AND METHODS

Test Microorganisms

The multiple drug resistant (MDR) bacterial strains and probiotic strains that were tested upon are listed in Tables 1 and 2, respectively. The MDR strains were obtained from the culture collection of the Microbiology Laboratory of De La Salle University Manila. Strains *Enterobacter aerogenes* MISC 253

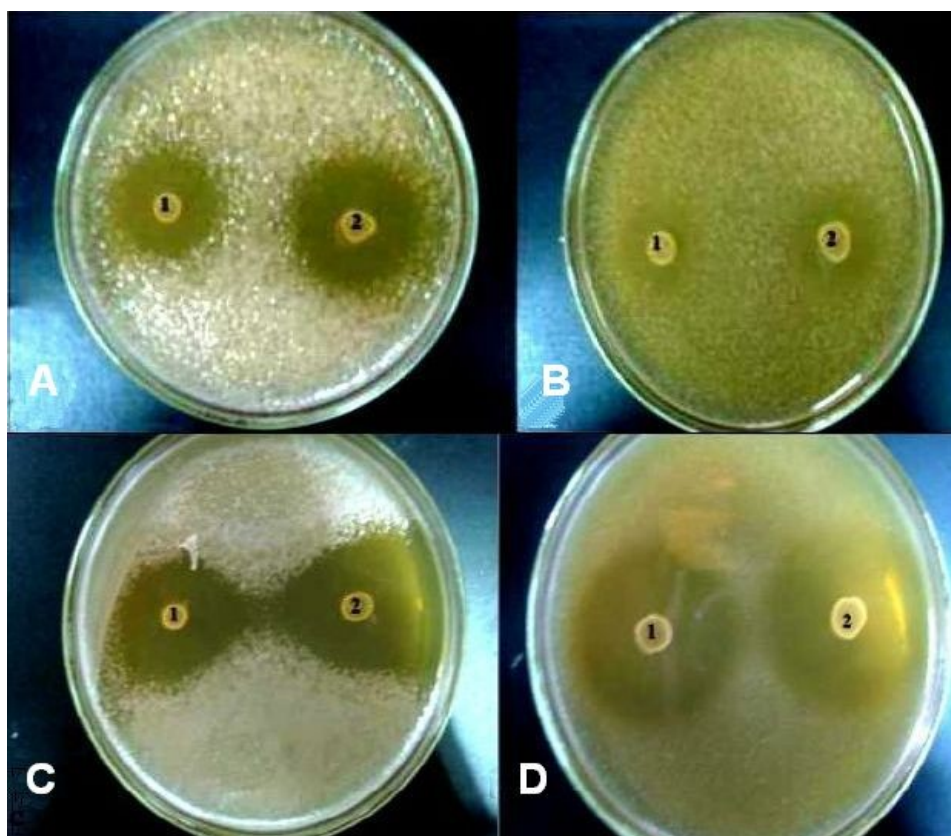


Figure 2. Zones of inhibition produced by probiotics from Nestle Cerelac (Spot1) and Yakult (Spot 2) on multiple drug resistant bacteria: A. *Klebsiella pneumoniae* 67 ESBL SHV12, B. *Staphylococcus aureus* MRSA NBP-9, C. *Pseudomonas aeruginosa* PA 97 MBL, D. *Proteus mirabilis* MISC 182 ESBL CTX M2

ESBL-CTX M3 and *Proteus mirabilis* MISC 182 ESBL CTX M2 were kind gifts from Dr. James H. Jorgensen of the University of Texas Health Center, San Antonio, Texas. The purity, identities and antimicrobial susceptibility patterns of all the tester strains were verified after they were revived from the -80°C glycerol stock, before they were used in the study. The antimicrobial susceptibility patterns were confirmed using the disc diffusion method (CLSI, 2010). All the tester strains showed the resistance phenotypes to the different antimicrobials reflected in Table 1. The probiotics were isolated from locally available commercial milk products.

Isolation and Phenotypic Identification of Probiotics from Dairy

The dairy samples were serially diluted from 10^{-1} to 10^{-3} and spread-plated in the amount of 100 μ l onto Elliker (Lactic) Agar (Elliker et al. 1956). The plates were incubated in a candle jar (5% CO_2) for 48-72 hours. Because the indicated probiotic Bifidus BL in Cerelac is reported to be *Bifidobacterium lactis* (Fisher 2007), the plates for these were anaerobically incubated in a jar with pyrogallol and potassium hydroxide. The colonies on the plates were observed for their morphology. White or cream-colored punctiform colonies that were convex and had

entire margins were selected from the plates for spore staining and Gram staining to check their microscopic morphology.

Non-sporeforming Gram positive bacilli in long or short chains were characterized using the following assays: catalase production, gelatin hydrolysis, sulfide and indole production, motility, and lactose fermentation. Catalase-negative, non-motile isolates, which did not hydrolyse gelatin, produce sulfide or indole, and which produced acid from lactose were further identified using 16srDNA sequencing.

Genotypic Identification using 16S rDNA

Chromosomal DNA was extracted using InstaGene Matrix™ (Bio-Rad Laboratories, USA) according to manufacturer's instructions. The colonies were washed twice with 500 μ l of TBE buffer. InstaGene Matrix resin in a volume of 100 μ l was added, and then mixed in a thermomixer at 56°C for 15-30 min. After vortexing at high speed for 10 s, the mixture was boiled for 8 min, mixed again for 10 s and centrifuged for 3 min at 14,000 x g. The supernatant served as the source of DNA

template for the polymerase chain reaction (PCR).

Universal primers were used to amplify the 1.5 kbp of 16S rDNA through PCR. The sequence for the forward primer is: 5'-CAGGCCTAACACATGCAAGTC-3', while the sequence for the 16S reverse primer is 5'-GGGCGGWTGTACAAGGC-3' (Kawai et al. 2002). The PCR mix was comprised of the following: 1X PCR buffer, 1.5 mM MgCl_2 , 2mM of the dNTPs, 0.5 μ M 16S forward primer, 0.5 μ M 16S reverse primer, and 0.05 U/ μ L Taq DNA polymerase, and 1 μ l of DNA extract. For the negative control, sterile deionized distilled water was utilized.

PCR was carried out in a PTC-100 Peltier Thermal Cycler (Bio-Rad Laboratories, USA) using the following conditions: 10 min at 94°C, 35 cycles of 94°C for 1 min, 55°C for 1 min, 72°C for 1 min, and final extension at 72°C for 5 min. The amplicons were viewed using a UV transilluminator after electrophoresis in 1.6% agar and staining with ethidium bromide.

The amplicons were sent to Macrogen Inc., Seoul, Korea for purification and sequencing. The sequences were analyzed using the Basic Local Alignment Search Tool (BLAST) Analysis in the

Testing for Antibacterial Activity of the Probiotics

The agar spot method was utilized for determining the antibacterial activity of the isolated probiotics. The probiotics were grown in Elliker broth (Elliker et al. 1956) at 37°C for 48-72 hr and standardized to the turbidity of MacFarland 1.0 (3.0×10^8 CFU/mL cell density). Two μ l of the probiotic isolates were spotted aseptically onto the Elliker agar medium, which was found to support good growth of all the four probiotic strains. The plates were then incubated in 5% CO₂ for 48-72 hours.

On the other hand, cultures of the MDR test bacteria were prepared in nutrient broth and incubated at 37°C for 16-18 hr. They were then transferred to fresh nutrient broth and incubated at 37°C for 4 hr. The turbidity of the bacteria was then standardized to that of MacFarland 0.5 (1.5×10^8 CFU/mL cell density). One ml of each of the MDR test strains was then inoculated into a flask with 100 ml molten nutrient agar. Approximately 7 ml of the agar were poured as an overlay on the plate with the grown probiotic, prepared as described above. This was allowed to solidify, before being incubated aerobically at 37°C for 16-18 hr. The diameters of the clear zones were measured to the nearest mm. The zones of inhibition were then swabbed, and tubes of nutrient broth were inoculated. After incubation at 37°C for 24 hr, the tubes were checked for turbidity. A clear tube indicated bactericidal activity of the probiotic, while a turbid tube indicated bacteriostasis (Chuayana et al. 2003). Each assay was done in triplicate.

RESULTS AND DISCUSSION

Identification of the Isolated Probiotics

The identities of the isolated bacteria were confirmed using phenotypic characterization and 16S rDNA sequencing to ascertain the reliability of the results of the antimicrobial assays to be those of the probiotics from the dairy products. All isolates from the dairy products were found to be nonmotile, Gram positive non-sporeforming bacilli in chains (Fig. 1), negative for catalase, hydrogen sulfide and indole production, and gelatin hydrolysis. Lactose was fermented with acid production but no gas. These are characteristics of lactobacilli.

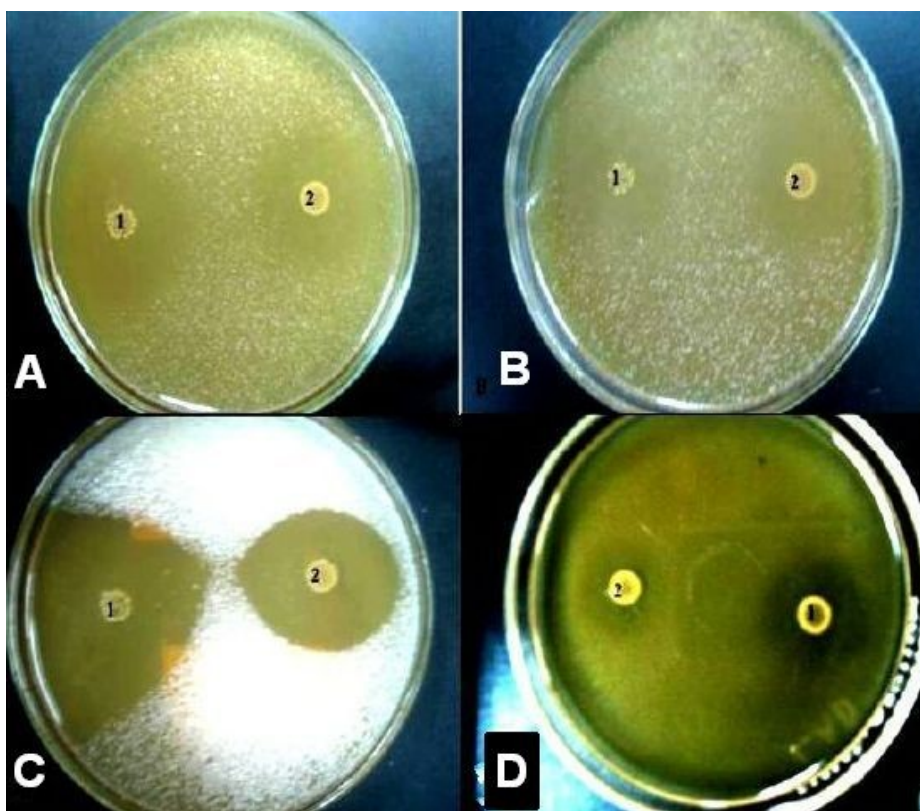


Figure 3. Zones of inhibition produced by probiotics from Chamyto (Spot 1) and Nido 3+ (Spot 2) on multiple drug resistant bacteria: A. *S. aureus* NBP-2, B. *S. aureus* NBP-12, C. *Enterobacter aerogenes* MISC 253 ESBL-CTX M3, and D. *Escherichia coli* 168 ESBL SHV12.

BLAST analyses of the 16S rDNA amplicons from the four probiotic isolates identified the probiotics as *Lactobacillus casei* or *Lactobacillus paracasei* with 100% homology and E value of 0.0, the same identities as those indicated on the product literature of Yakult, Chamyto and Nido 3+ (Table 2). *Lactobacillus* PROTECTUS (of Nido 3+) and *Lactobacillus* FORTIS (of Chamyto) are reported to be *L. paracasei* (Linantud et al. 2009). Although the Nido 3+ product claims to also contain a bifidus culture, it was not successfully isolated. Lastly, the isolate from Nestle Cerelac (advertised as Bifidus BL) was identified as *L. casei* or *L. paracasei*. The identity as such was further verified through the product specialists of Cerelac (personal communication, January 2011). It is noted that the 16S rDNA analyses were not able to differentiate between *L. casei* and *L. paracasei*. This limitation in the ability of the method to distinguish species has been observed in many studies, and other methods such as microarray-based technologies with 16S rDNA and other housekeeping genes have been used in such cases (Janda and Abbott 2007).

Table 1. Bacterial strains tested for response to probiotics from local dairy products.

Strain Name	Resistance Phenotype	Source
MRSA NBP-2	Resistant to all β -lactams and erythromycin	DLSU Manila culture collection
MRSA NBP-9	Resistant to all β -lactams	DLSU Manila culture collection
MRSA NBP-12	Resistant to all β -lactams	DLSU Manila culture collection
<i>Klebsiella pneumoniae</i> 67 ESBL SHV ₁₂	AZT, CAS, CTX, CRO, CFX, TE, NN, SXT	Cabrera & Rodriguez (2009)
<i>Escherichia coli</i> 168 ESBL SHV ₁₂	AZT, CAZ, CTX, CRO, CFX, CXT, TE, AK, CIP, NN	Cabrera & Rodriguez (2009)
<i>Enterobacter aerogenes</i> MISC 253 ESBL-CTX M3	Resistant to all penicillins, cephalosporins, AZT, TE, AK, CIP, NN, SXT	Lewis <i>et al.</i> (2007)
<i>Proteus mirabilis</i> MISC 182 ESBL CTX M2	Resistant to all penicillins, cephalosporins and AZT	Lewis <i>et al.</i> (2007)
<i>Pseudomonas aeruginosa</i> PA 97 MBL	AK, GM, CIP, CRO, NET, IMI, SXT, AZT, CAP, CTX	Balhon <i>et al.</i> (2006)

AK amikacin, AZT aztreonam, CAZ ceftazidime, CFX cefuroxime, CIP ciprofloxacin, CRO ceftriaxone, CTX cefotaxime, CXT cefoxitin, DLSU De La Salle University, GM gentamicin, IMI imipenem, MRSA methicillin resistant *Staphylococcus aureus*, NET nitrofurantoin, NN tobramycin, SXT sulfamethoxazole-trimethoprim, TE tetracycline.

Antimicrobial Activity of the Isolated Probiotics

All of the isolated probiotics from the four dairy products inhibited the growth of all the MDR bacterial strains as shown by zones of inhibition with mean diameters that ranged from 10.67mm to 38mm (Table 3, Fig. 2, 3). The *in vitro* inhibition of the multiple drug resistant strains by the probiotic strains may have been due to the secretion of organic acids, primarily lactic acid, thereby decreasing the pH of the medium to one that is unsuitable for growth (Marianelli *et al.* 2010). Other possibilities include the secretion of other antimicrobial compounds such as bacteriocins, low molecular weight peptides, and possibly hydrogen peroxide (Gupta and Garg 2009). Fayol-Messaoudi *et al.* (2005) noted that there may be a synergistic effect of the lactic acid secreted by *Lactobacillus* strains as well as the non-lactic acid molecules on Gram-negative bacteria. The lactic acid is said to increase the permeability of the outer membrane of the gram negative pathogens, making them more susceptible to the activity of antimicrobial molecules.

It is especially worth noting that most of the antimicrobial activities exhibited by the probiotics were proven to be bactericidal in nature on further subculture in nutrient broth, with the exception of the isolates from Yakult and Cerelac against *E. coli* ESBL 168, and the isolate from Nido 3+ against *K. pneumoniae* SHV₁₂ (Table 3). An earlier investigation by Chuayana *et al.* (2003) showed that the bacteria they isolated from Yakult (identified as *L. casei* strain Shirota in the product literature) was bacteriostatic against methicillin-susceptible *S. aureus* and antimicrobial susceptible *P. aeruginosa*. Since probiotics exert strain-specific effects against other bacteria, the indicator strains they tested may have been less susceptible. Linantud *et al.* (2009) also investigated the antimicrobial activity of bacteria from Yakult and Chamyto for *E. coli* and other Gram negative bacilli. However, they found that only the probiotics from Yakult displayed any

Table 2. Probiotic strains in dairy products and tested against multiple drug resistant bacterial strains

Strains Reported in Product Literature	Source	Manufacturer
<i>Lactobacillus casei</i> Shirota 610	Yakult Probiotic Drink	Yakult Philippines Inc.
<i>Lactobacillus Fortis</i> 6071	Chamyto Probiotic Drink	Nestle Philippines Inc.
<i>Lactobacillus Protectus</i> 6072	Nido 3+ Powdered Milk	Nestle Philippines Inc.
Bifidus culture	Nido 3+ Powdered Milk	Nestle Philippines Inc.
Bifidus BL	Nestle Cerelac Infant Cereal	Nestle Philippines Inc.

inhibitory activity, and only against *Vibrio cholerae*. To test the probiotics, they utilized a blank disk method where they immersed sterile blank disks in the dairy products for 5 minutes, then placed these onto the surface of Mueller Hinton plates previously streaked with the enteric bacteria. It is highly likely that the reason the inhibitory activity was scant, if present, was because the probiotics were not first cultured in appropriate media. It is only then that the organic acids and other antimicrobial compounds would be optimally secreted as the source of inhibitory activity. Secondly, lactobacilli grow poorly on Mueller Hinton (Ocaña et al. 2006). Robust growth is always recommended if one wants to test the probiotic, so that they may have an optimal release of metabolites.

Based on the results of the present study, the *in vitro* capacity of the probiotics isolated from the commercial milk products to inhibit the growth of and exert bactericidal effects on multiple drug resistant bacteria shows promise for future *in vivo* studies. *In vivo* studies are a necessary step in evaluating the potential efficacy of probiotics for use in the prophylaxis or treatment of infections caused by microbial pathogens. The MDR strains tested in the study were obtained from clinical specimens, and therefore are medically important as these strains are resistant to the actions of all β -lactams (MRSA) (CLSI 2010), to all penicillins, cephalosporins and monobactams (ESBL-producers)(CLSI 2010), and to almost all broad spectrum β -lactams including carbapenems (MBL producers) (Fujimura et al. 2007). In addition, strains have been found that are resistant to other classes of antimicrobials such as fluoroquinolones and aminoglycosides (Takashi 2007; Cabrera and Rodriguez 2009; Zhang et al. 2009). Aside from the multiple resistances, the genes coding for these are found in transferable plasmids or gene cassettes, and thus can convert susceptible bacterial strains to resistance.

This leaves behind very few options for physicians, so one cannot rely on antibiotics alone, as the pathogens will continue to develop resistance to newer drugs that will be developed. Probiotic supplements can help to provide an intervention due to the bactericidal effects of select strains, without worsening the problems caused by overuse of antibiotics. While each antibiotic has a specific mode of action (such as inhibiting transpeptidation), bacteriocins isolated from probiotic strains

Table 3. Effect of the probiotics on the multiple drug resistant test bacterial isolates and the mean diameters of zones of inhibition (in mm) in the agar spot assay (n=3).

Multiple drug resistant test Organisms	Probiotics from:			
	Chamyto	Yakult	Nido 3+	Cerelac
MRSA NBP-2	Bactericidal 26.67±1.16	Bactericidal 22.67±1.16	Bactericidal 30.67± 0.58	Bactericidal 26.00±2.00
MRSA NBP-9	Bactericidal 27.33±0.58	Bactericidal 21.33±0.58	Bactericidal 24.00±2.00	Bactericidal 24.67±1.15
MRSA NBP-12	Bactericidal 16.00±2.00	Bactericidal 16.67±1.16	Bactericidal 15.33±0.58	Bactericidal 14.67±2.08
<i>Klebsiella pneumoniae</i> 67 ESBL SHV ₁₂	Bactericidal 30.00±2.00	Bactericidal 29.33±0.58	Bacteriostatic 38.00±3.00	Bactericidal 22.00±2.00
<i>Enterobacter aerogenes</i> MISC253 ESBL CTXM3	Bactericidal 38.00±2.00	Bactericidal 31.33±1.53	Bactericidal 30.67±1.53	Bactericidal 27.33±1.15
<i>Proteus mirabilis</i> MISC182 ESBL CTX M2	Bactericidal 28.00±2.00	Bactericidal 34.67±1.16	Bactericidal 28.00±4.00	Bactericidal 34.00±2.00
<i>Pseudomonas aeruginosa</i> PA 97 MBL	Bactericidal 36.00±2.00	Bactericidal 35.33±2.52	Bactericidal 21.33±0.58	Bactericidal 30.00±1.00
<i>Escherichia coli</i> 168 ESBL SHV ₁₂	Bactericidal 11.33±0.58	Bacteriostatic 10.67±1.16	Bactericidal 11.33±0.58	Bacteriostatic 11.33±0.58

have several modes of action, reducing the probability of a resistance development (Sleator 2010). The results also support past studies which have demonstrated the benefits that consumption of probiotics can provide for our health.

CONCLUSION

It is inevitable for bacteria to continue developing resistance to antibiotics, not only because of their high rates of mutation and transferability of drug resistance genes, but also because antibiotics pose a selective pressure against these bacteria, prompting drug-resistant strains to outcompete the susceptible ones (Pray 2008). The rise of multi-drug resistant strains has therefore led researchers to look for alternative therapies such as probiotics that would decrease our reliance on antibiotic use.

The results of this *in vitro* study clearly demonstrated that probiotics have strong potential in this regard because of their capacity to kill the multiple drug resistant bacteria. The results also suggest that the consumption of products containing probiotics can help to protect an individual from developing infection caused by such bacteria. Thus, probiotics can be used

as a helpful tool for addressing the worldwide issue of antibiotic resistance.

ACKNOWLEDGMENT

The authors would like to thank Dr. James H. Jorgensen of the University of Texas Health Center, San Antonio, Texas for the *Enterobacter aerogenes* MISC 253 ESBL CTX M3 and *Proteus mirabilis* MISC 182 ESBL CTX M2 strains, Mr. Glenn Oyong for the valuable technical guidance, Dr. Anthony Lee for the PCR primers and Mr. Jeffrey Galvez for the Taq polymerase.

CONFLICTS OF INTEREST

We certify that there are no conflicts of interest related to the present study, nor in the preparation or submission of the manuscript.

CONTRIBUTION OF THE INDIVIDUAL AUTHORS

Mary Catherine A. Mercado: conduct of the entire study, preparation of manuscript.

Esperanza C. Cabrera: provision of the research problem and design, supervision of the conduct of the study, preparation of manuscript.

REFERENCES

Alvarez S, Gobbato N, Bru E, De Ruiz Holgado A, Perdigon G. Specific immunity induction at the mucosal level by viable *Lactobacillus casei*: A perspective for oral vaccine development. *Food Agric Immunol* 1998; 10: 79-87.

Balhon Z, Cabrera E, Rodriguez D. Prevalence of extended-spectrum- β -lactamase (ESBL) and metallo- β -lactamase (MBL) producing *Pseudomonas aeruginosa* isolates from the Philippine General Hospital. *Acta Manilana* 2006; 54: 1-6.

Cabrera E and Rodriguez R. First report on the occurrence of SHV-12 extended spectrum- β -lactamase Enterobacteriaceae in the Philippines. *J Microbiol Immunol Infect* 2009; 42:74-85

Chan R, Reid G., Irvin R, Bruce A, Costerton J. Competitive exclusion of uropathogens from human uroepithelial cells by *Lactobacillus* whole cells and cell wall fragments. *Infect Immun* 1985; 47(1): 84-89.

Chang S, Sievert D, Hageman J, Boulton M, Tenover F, Downes F, Shah S, Rudrik J, Pupp G, Brown W, Cardo D, Fridkin S. Infection with vancomycin-resistant *Staphylococcus aureus* containing the *vanA* resistance gene. *N Engl J Med* 2003; 348: 1342-1347.

Chuayana E, Ponce C, Rivera R, Cabrera E. Antimicrobial activity of probiotics from milk products. *Phil J Microbiol Infect Dis* 2003; 32(2): 71-74.

CLSI (Clinical Laboratory Standards Institute). Performance Standards for Antimicrobial Susceptibility Testing: Twentieth Informational Supplement, M100-S20 2010; 30:60-61.

Ekmekci H, Aslim B, Ozturk S. Characterization of vaginal lactobacilli coaggregation ability with *Escherichia coli*. *Microbiol Immunol* 2009; 53(2): 59-65.

Elliker P, Anderson A, Hannonson G. (1956). An agar culture medium for lactic acid streptococci and lactobacilli. *J Dairy Sci* 1956; 39: 1611-1612.

Fayol-Messaoudi D, Berger CN, Coconnier-Polter M, Lievin-Le Moal V, Servin AL. pH-, lactic acid-, and non-lactic acid-dependent activities of probiotic lactobacilli against *Salmonella enterica* serovar. Typhimurium.

Appl Environ Microbiol 2005; 71(10); 6008-6013.

Fisher R. Formulas and milks for infants and children. *Med Today* 2007; 8(10): 39-48.

Fridkin S. Vancomycin intermediate and resistant *Staphylococcus aureus*: What the infectious disease specialist needs to know. *Clin Infect Dis* 2001; 32(1): 108-115.

Fridkin S, Hageman J, McDougal L et al. Epidemiological and microbiological characterization of infections caused by *Staphylococcus aureus* with reduced susceptibility to vancomycin, United States, 1997–2001. *Clin Infect Dis* 2003; 36:429–439.

Fujimura S, Nakano Y, Sato T, Shiharata K, Watanabe A. Relationship between the usage of carbapenem antibiotics and the incidence of imipenem resistant *Pseudomonas aeruginosa*. *J Infect Chemother* 2007; 13(3): 147-150.

Fuller R. Probiotics in man and animals. *J Appl Bacteriol* 1989; 66: 365-378.

Gupta V, Garg R. Probiotics. *Ind J Med Microbiol* 2009; 27(3): 202-209.

Herich R, Levkut M. Lactic acid bacteria, probiotics and immune system. *Vet Med* 2002; (6): 169-180.

http://blast.ncbi.nlm.nih.gov/Blast.cgi?CMD=Web&PAGE_TYPE=BlastHome. Accessed 15 December 2010.

Janda J and Abbott S. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: Pluses, perils, and pitfalls. *J Clin Microbiol* 2007; 45: 2761-2764.

Kawai M, Matsutera E, Kanda H, Yamaguchi N, Tani K, Nasu M. 16S ribosomal DNA-based analysis of bacterial diversity in purified water used in pharmaceutical manufacturing processes by PCR and denaturing gradient gel electrophoresis. *Appl and Environ Microbiol* 2002; 68 (2): 699-704.

Lewis J, Herrera M, Wickes B, Patterson J, Jorgensen J. First report of the emergence of CTX-M-type extended-spectrum β -lactamases (ESBLs) as the predominant ESBL isolated in a US health care system. *Antimicrob Agents Chemother* 2007; 51(11): 4015-4021.

Linantud L, Gatcheco F, Ong C, Perez M. In vitro sensitivity of common bacterial pathogens causing acute gastroenteritis against *Lactobacillus* Shirota strain (Yakult) and *Lactobacillus* fortis (Chamyto). *Phil Sci J* 2009; 42(1): 14-19.

Marianelli C, Cifani N, Pasquali P. Evaluation of antimicrobial activity of probiotic bacteria against *Salmonella enterica* subsp. *enterica* serovar *typhimurium* 1344 in a common medium under different environmental conditions. *Res Microbiol* 2010; 161(8): 673-680.

May J, Shannon K, King A, French G. Glycopeptide tolerance in *Staphylococcus aureus*. *J Antimicrob Chemother* 1998; 42(2): 189-197.

Meurman J. Probiotics: Do they have a role in oral medicine and dentistry? *Eur J Oral Sci* 2005; 113(3): 188-196.

Ocaña V, Silva C, Nader-Macias M. Antibiotic susceptibility of potentially probiotic vaginal lactobacilli. *Infect Dis Obstet Gynecol* 2006; 1-6.

Pray L. Antibiotic resistance, mutation rates and MRSA. *Nature Education* 2008; 1(1).

Rodrigues L, van der Mei H, Teixeira J, Oliveira R. Influence of biosurfactants from probiotic bacteria on formation of biofilms on voice prostheses. *Appl Environ Microbiol* 2004; 70(7): 4408-4410.

Rouveix B. Implications of multiple drug resistant efflux pumps of pathogenic bacteria. *J Antimicrob Chemother* 2007; 59(6): 1208-1209.

Silva M, Jacobus N, Deneke C, Gorbach S. Antimicrobial substance from a human *Lactobacillus* strain. *Antimicrob Agent Chemother* 1987; 31(8): 1231-1233.

Sleator R. Probiotic therapy – recruiting old friends to fight new foes. *Gut Pathogens* 2010; 2:5.

Takashi I. Metallo-beta-lactamase producing *Pseudomonas aeruginosa*. *Antibiotics and Chemother* 2007; 23: 227-234.

Trois L, Cardoso E, Miura E. Use of probiotics in HIV-infected children: A randomized double-blind controlled study. *J Trop Pediatr* 2008; 54(1): 19-24.

Zhang K, McClure J, Elsayed S, Conly J. Novel staphylococcal cassette chromosome *mec* type, tentatively designated type VIII, harboring class A *mec* and type 4 *ccr* gene complexes in a Canadian epidemic strain of methicillin-resistant *Staphylococcus aureus*. *Antimicrob Agents Chemother* 2009; 53: 531-540.