QUALITY ANALYSIS OF COMMERCIAL PROBIOTIC PRODUCTS FOR FOOD ANIMALS

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Abstract. Thirteen commercial probiotic feed products were examined for microbiological content and the results were compared with the information available on the product labels. Antibiotic resistance of *Lactobacillus* and *Bacillus* was investigated. All the products were inaccurately labelled in either numbers or species of bacteria. Misnaming at the species level was the most common flaw. *Lactobacillus* exhibited higher-antibiotic resistance than *Bacillus* did. Plasmid was found in both *Lactobacillus* (22%) and *Bacillus* (2.5%). The *vanA* gene was present in one *L. plantarum* and one *B. subtilis* isolate. The *vanA*-containing *B. subtilis* also harbored the *tetW* gene. None of the genes detected appeared to be associated with a conjugative plasmid.

INTRODUCTION

Concerns that imprudent use of antibiotics in food animal production plays a role in widespread resistant of bacteria has been increasing. These resistant pathogens can be transmitted to humans through the food chain and contribute to a large pool of resistance genes that can be transferred to human pathogens. As a result, all antibiotic growth promoters have been banned and alternative feed ingredients, such as enzymes, organic acid, feed supplements and probiotics, have been researched and become commercially available. Together with the expanded market of organic farm animals, use of probiotics as animal feed additives has gained popularity.

Probiotics are considered feed additives and subject to regulations depending the policy of each country. While health benefits of probiotics depend on the number of viable bacteria, a minimal beneficial effect dose for probiotic bacteria has not been adequately established and most likely varies depending on the target animal and probiotic species. Most probiotic additives contain 10^{10} cfu/g and premixtures usually contain 10^{8} cfu/g (Coeuret *et al*, 2004). However, several studies have reported that a number of commercial probiotic products contained low viable counts, resulting in a loss of probiotic effect (Hamilton-Miller *et al*, 1999; Temmerman *et al*, 2001; Coeuret *et al*, 2004). As safety and functionality of probiotics are species and strain dependent, recent reports have identified probiotic products with inaccurate species/strain labeling (Weese, 2003; Coeuret *et al*, 2004). These raise particular concerns regarding beneficial effects and potential health risks of the probiotic products.

Lactobacillus and *Bacillus* have been formulated in several probiotic preparations sold commercially for veterinary use. *Lactobacillus* spp is an important part of the commensals of the animal body and has rarely

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been implicated in cases of infection. *Bacillus* spp is not an unusual component of microflora in gut and certain species are known to cause diseases: *B. cereus* may produce enterotoxin and *B. pumilus* has been associated with food poisoning and causes listeriosis like symptoms (Workowski and Flaherty, 1992). Recently, transmissible antibiotic resistance elements have been described in probiotic strains of *Lactobacillus* and *Bacillus* (Gevers *et al*, 2003; Hummel *et al*, 2007). A concern is these probiotic organisms may act as hosts for antibiotic resistance genes that are potentially transferred to commensal flora and pathogenic bacteria in the gut. In this case, widespread use of probiotic additives may result in increased distribution of potential sources for spread of antibiotic resistance genes.

The aim of this study was to evaluate the quality of commercial probiotics used for animal consumption. The study was performed to enumerate and determine species of *Lactobacillus* and *Bacillus*. The accuracy of labeling on the products was assessed. Antimicrobial susceptibilities and horizontal transfer of antibiotic resistance genes were determined.

MATERIALS AND METHODS

Commercially available probiotic products

Thirteen commercially available probiotics for food animals were evaluated in this study (Table 2). All products were freeze dried except product I which was in liquid form. Products II, III, IV, X, XI, XII and XIII were imported, the others were domestically manufactured. Five products, VII, IX, XI, XII and XIII, stated the expiration date on the product label and all of these products were tested at least 4 months before the expiration date. Two separate samples for each product were bought in bags or bottles. Each sample came from a different package. All products were stored at room temperature and analyzed within 7 days of being purchased.

Isolation and enumeration of bacteria

Isolation of *Lactobacillus* and *Bacillus* species was performed as described in ISO15214 and ISO7932, respectively. For dried products, a single 20 g portion from each sample was dissolved in 180 ml peptone saline diluting fluid (PSD; peptone 1.0 gm and NaCl 8.5 g in 1,000 ml distilled water). For liquid products, 1 ml of each product was diluted in 9 ml PSD. The samples were prepared in 10-fold dilutions and bacterial counts for each product were carried out in duplicate plates. The number of bacteria recorded for each sample was the mean of replicate counts. Counts of the total numbers of *Lactobacillus* and *Bacillus* were performed regardless of the species. Depending on the number of morphological types of colonies on an agar plate; 1-5 colonies of each type were randomly selected. All colonies were purified and subjected to Gram's stain and biochemical testing. All bacterial isolates were stored as 20% glycerol stock at -80ºC.

Identification of genus and species

Oligonucleotide primers used in this study are listed in Table 1. Genomic DNA was obtained from overnight cultures using QuickExtract™ (Epicentre® Biotechnologies, Madison, WI). Multiplex PCR assay was performed to verify genus and species of *Lactobacillus* (Nakagawa *et al*, 1994; Dubernet *et al*, 2002; Kwon *et al*, 2004). The *Bacillus* isolates were identified using amplified ribosomal DNA restriction analysis (ARDRA) (Wu *et al*, 2006). All PCR reactions were carried out using Eppendorf® MasterMix (Eppendorf, Hamburg, Germany) as described in the manufacturer's instructions. The representatives of PCR products were submitted for nucleotide sequencing for con-

Table 1
Oligonucleotide primers used in this study.

Primers	Sequence (5′-3′)	PCR type	Reference
Lactobacillus			
R16-1	CTTGTACACACCGCCCGTTCA	Genus-specificity	(Nakagawa *et al*, 1994; Dubernet *et al*, 2002)
LbLMA1-rev	CTCAAAACTAAACAAAGTTTC		
IDL03R	CCACCTTCCTCCGGTTTGTCA	All *Lactobacillus*	(Kwon *et al*, 2004)
IDL04L	AGGGTGAAGTCGTAACAAGTAGCC	All *Lactobacillus*	(Kwon *et al*, 2004)
IDL11F	TGGTCGGCAGAGTAACTGTTGTCG	*L. casei* group	(Kwon *et al*, 2004)
IDL22R	AACTATCGCTTACGCTACCACTTTGC	*L. acidophilus*	(Kwon *et al*, 2004)
IDL31F	CTGTGCTACACCTAGAGATAGGTGG	*L. delbrueckii*	(Kwon *et al*, 2004)
IDL42R	ATTTCAAGTTGAGTCTCTCTCTC	*L. gasseri*	(Kwon *et al*, 2004)
IDL52F	ACCTGATTGACGATGGATCACCAGT	*L. reuteri*	(Kwon *et al*, 2004)
IDL62R	CTAGTGGTAACAGTTGATTAAAACTGC	*L. plantarum*	(Kwon *et al*, 2004)
IDL73R	GCCAACAAGCTATGTGTTGCTTGC	*L. rhamnosus*	(Kwon *et al*, 2004)
Bacillus			
B-K1/F	TCACCAAGGCRACATGCG	All *Bacillus*	(Wu *et al*, 2006)
B-K1R	CGTATTCACCGCGGCATG		
Resistance genes			
aadEI	GCAGAACAGGATGAACGTATTCG	*aadE*	(Klare *et al*, 2007)
aadEII	ATCAGTCGGAACTATGTCCC		
tetKI	CAATACCTACGATATCTA	*tetK*	(Klare *et al*, 2007)
tetKII	TTGAGCTGTCTTGGTTCA		
tet(L)I	TGGTCCTATCTTCTACTCATTC	*tetL*	(Werner *et al*, 2003)
tet(L)II	TTCCGATTTCGGCAGTAC		
tet(M)I	GGTGAACATCATAGACACGC	*tetM*	(Werner *et al*, 2003)
tet(M)II	CTTGTTCGAGTTCCAATGC		
tet(O)I	AGCGTCAAAGGGGAATCACTATCC	*tetO*	(Klare *et al*, 2007)
tet(O)II	CGGCGGGGTTGGCAAATA		
tet(S)I	ATCAAGATATTAAGGAC	*tetS*	(Charpentier *et al*, 1993; Gevers *et al*, 2003)
tet(S)II	TTCTCTATGTGGTAATC		
tet(W)-I	GGMCAYRTGGATTTYWTIGC	*tetW*	(Aminov *et al*, 2001)
tet(W)-II	TCIGMIGGIGTRCTIRCIGGRC		
vanA1	GGGAAAACGACAATTGC	*vanA*	(Dutka-Malen *et al*, 1995)
vanA2	GTACAATGCGGCCGTTA		
vanB1	ATGGGAAGCCGATAGTC	*vanB*	(Dutka-Malen *et al*, 1995)
vanB2	GATTTCGTTCCTCGACC		
vanC1	GGTATCAAGGAAACCTC	*vanC*	(Dutka-Malen *et al*, 1995)
vanC2	CTTCCGCCATCATAGCT		
ermAI	TCTAAAAAGCATGTAAAAGAA	*ermA*	(Sutcliffe *et al*, 1996)
ermAII	CTTCGATAGTTTATTAATATTAGT		
ermBI	GAAAAGGTACTCAACCAAATA	*ermB*	(Sutcliffe *et al*, 1996)
ermBII	AGTAACGGTACTTAAATTGTTTAC		
ermCI	TCAAAACATAATATAGATAAA	*ermC*	(Sutcliffe *et al*, 1996)
ermCII	GCTAATATTGTTTAAATCGTCAAT		

Table 2
Analysis of probiotic products (n = 13).

Product	Information given on labels		Analysis of probiotic products		
	Strain	Number [a]	Strain	Number	Specific species
I	*B. subtilis*	$1x10^6$	*Bacillus* spp	$1x10^9$	*B. subtilis*, *B. licheniformis*,
			Lactobacillus spp	$6x10^8$	members of the *B.subtilis* cluster [b]
					L. plantarum, *L. gasseri*
II	*B. subtilis*	$1x10^6$	*Bacillus* spp	$1x10^6$	*B. subtilis*, *B. licheniformis*,
			Lactobacillus spp	80	members of the *B.subtilis* cluster
					L. plantarum, *L. delbrueckii*
III	*B. subtilis*	$5x10^7$ - $3x10^9$	*Bacillus* spp	$9.4x10^6$	Members of the *B.subtilis* cluster
IV	*B. licheniformis*	$1x10^7$	*Bacillus* spp	$3.8x10^7$	*B. subtilis*, *B. cereus*,
	B. subtilis	$1x10^7$	*Lactobacillus* spp	$1.9x10^7$	*B. licheniformis*, *L. delbrueckii*
V	*L. acidophilus*	$1x10^{10}$	*Bacillus* spp	$1.7x10^7$	*B. subtilis*, members of the
	L. plantarum	$1x10^{10}$	*Lactobacillus* spp	$2.5x10^7$	*B.subtilis* cluster, *B. cereus*,
	B. subtilis	$1x10^{10}$			*L. rhamnosus*, *L. plantarum*,
	B. licheniformis	$1x10^{10}$			*L. gasseri*
VI	*Bacillus* spp	$1x10^6$	*Bacillus* spp	$3.8x10^6$	*B. subtilis*, members of the
	L. plantarum	$1x10^6$	*Lactobacillus* spp	$1.2x10^7$	*B.subtilis* cluster, *L. rhamnosus*,
					L. plantarum, *L. delbrueckii*,
VII	*Bacillus* spp	$1x10^6$	*Bacillus* spp	$5.5x10^7$	*B. licheniformis*, members of the
	L. plantarum	$1x10^6$	*Lactobacillus* spp	$1.4x10^7$	*B.subtilis* cluster, *L. rhamnosus*,
					L. plantarum, *L. delbrueckii*,
					L. casei group
VIII	*L. acidophilus*	$1x10^6$	*Bacillus* spp	$3.1x10^6$	*B. subtilis*, members of the
	L. plantarum	$1x10^6$	*Lactobacillus* spp	$4.8x10^6$	*B.subtilis* cluster, *L. rhamnosus*,
	B. subtilis	$1x10^6$			*L. plantarum*, *L. delbrueckii*,
	B. licheniformis	$1x10^6$			*L. casei* group
IX	*B. subtilis*	$1x10^9$	*Bacillus* spp	$3.2x10^6$	Members of the *B.subtilis* cluster
	L. acidophilus	$1x10^4$	*Lactobacillus* spp	$8.0x10^6$	*L. rhamnosus*, *L. gasseri*
X	*L. acidophilus*	$1.67x10^8$	*Bacillus* spp	$3.0x10^5$	Members of the *B.subtilis* cluster
			Lactobacillus spp	$2.5x10^3$	*L. plantarum*
XI	*B. subtilis*	$1x10^9$	*Bacillus* spp	$1.4x10^8$	*B. subtilis*, members of the
	L. acidophilus	$1x10^4$	*Lactobacillus* spp	$4.2x10^8$	*B.subtilis* cluster, *L. plantarum*
XII	*B. subtilis*	$1x10^9$	*Bacillus* spp	$1.3x10^8$	*B. subtilis*, members of the
	B. licheniformis	$1x10^9$			*B.subtilis* cluster, *B. cereus*
XIII	*L. casei*	$1x10^9$	NF	NT	NT
	L. plantarum	$1x10^9$			
	L. brevis	$1x10^9$			
	S. faecium	$1x10^9$			

[a] Unit is cfu/g for all products except for product no.1 (cfu/ml).
[b] *B. pumilus*, *B. amyloliquefaciens* and *B. atrophaeus*
NF, not found; NT, not test

firmation of amplification specificity.

Antimicrobial susceptibility testing

Antimicrobial susceptibilities to ampicillin (AMP), chloramphenicol (CHP), ciprofloxacin (CIP), erythromycin (ERY), gentamicin (GEN), kanamycin (KAN), neomycin (NEO), rifampicin (RIF), streptomycin (STR), tetracycline (TET), trimethoprim (TRI) and vancomycin (VAN) were assessed by determining the minimum inhibitory concentration (MIC). For *Lactobacillus*, MICs were determined by microdilution using LAB susceptibility test medium as previously described (Klare *et al*, 2005, 2007). For *Bacillus*, MICs were determined in Muller Hinton agar (MHA) using a two-fold agar dilution technique according to Clinical and Laboratory Standards Institute guidelines (CLSI) formerly NCCLS (NCCLS, 2002). Breakpoints for clarifying *Lactobacillus* and *Bacillus* as resistant were those recommended by the Scientific Committee on Animal Nutrition (SCAN) (SCAN, 2003). *Pseudomonas aeruginosa* ATCC 27853 was used as a control organism. All antibiotics were purchased from Sigma-Aldrich (St Louis, MO).

PCR amplification of antibiotic resistance genes

PCR amplification of genes associated resistance to tetracycline (*tetK*, *tetL*, *tetM*, *tetO*, *tetS* and *tetW*), vancomycin (*vanA*, *vanB* and *vanC*), erythromycin (*ermA*, *ermB* and *ermC*) and streptomycin (*aadE*) was performed with template DNA from the isolates with corresponding resistance phenotypes. Template DNA of all isolates tested was prepared by the whole cell boiled lysate procedure (Leverstein-van Hall *et al*, 2002). The amplification conditions for all the *tet* genes were an initial 5 minutes of denaturation at 95ºC, followed by 25 cycles at 95ºC for 45 seconds, 52ºC for 45 seconds, 72ºC for 45 seconds, and a final extension step for 7 minutes at 72ºC. The *van*, *erm* and *aadE* genes were amplified using the same denaturation and extension conditions, except the annealing temperatures were changed to 53ºC, 48ºC and 52ºC for *van*, *erm* and *aadE*, respectively. *Campylobacter coli* CAC041 and CAC094 were used as positive controls for *tetO* and *aadE*, respectively (Ekkapobyotin *et al*, 2008). The *tetW* and *vanA* positive strains isolated in this study were used as positive controls for each gene. The positive control strains for *tetK*, *tetL*, *tetM* and *tetS* genes were *Escherichia coli* with the relevant gene obtained previously (Bryan *et al*, 2004).

Isolation of plasmid

Plasmid DNA was based on the alkaline lysis method as previously described (Kraft *et al*, 1988). Plasmid DNA was separated by electrophoresis on 0.8% agarose gel and visualized with ethidium bromide staining.

Conjugation experiments

The possibility of transfer of antibiotic resistance genes was tested by filter mating as described previously (Hummel *et al*, 2007). For *Lactobacillus*, spontaneous rifampicin-resistant mutants of *L. plantarum* L11.1 (MIC 64 µg/ml) and *L. plantarum* L11.5 (MIC 64 µg/ml) were used as recipients. For *Bacillus*, spontaneous tetracycline-resistant mutants *B. subtilis* B1.6 (MIC 64 µg/ml) and *B. licheniformis* B10.2 (MIC 64 µg/ml) were used as recipients. All the recipients were isolated in our laboratory and susceptible to other antibiotics tested. Transconjugants were selected onto MRS (*Lactobacillus*) or LB (*Bacillus*) containing the appropriate antibiotics at the following concentrations: rifampicin, 50 µg/ml; tetracycline, 10 µg/ml; chloramphenicol, 32 µg/ml; erythromycin, 20 µg/ml and vancomycin, 10 µg/ml. All mating experiments were repeated a minimum of two times.

RESULTS

Numbers and strains of probiotic bacteria

In seven products (II, III, IX, X XI, XII and XIII), the numbers of probiotic bacteria found were below the declared contents (Table 2). No viable *Lactobacillus* or *Bacillus* were found in product XIII, although high numbers of both bacteria were claimed on the label.

None of the products tested comprised all species mentioned on the contents and all products contained other species rather than those claimed on the label. In products I, II and IV, various *Lactobacillus* species were found but these bacteria were not listed on the label. Product X contained *Bacillus* spp which was not stated on the product label. Eight products (I, II, III, V, VIII, IX, XI and XII) labelled as containing *B. subtilis* contained members of the *B. subtilis* cluster (*B. pumilus*, *B. amyloliquefaciens* and *B. atrophaeus*). Three products (IV, V and XII) contained species in the *B. cereus* cluster (*B. cereus*, *B. thuringiensis* and *B. anthracis*). All colonies (n=9) classified as *B. cereus* were submitted for DNA sequencing. Nucleotide sequencing analysis revealed these colonies were *B. cereus*, though not stated on the product labels. Five products (V, VIII, IX, X and XI) claimed to possess *L. acidophilus*, but none was found.

Antibiotic resistance phenotypes

A total of 82 *Lactobacillus* and 119 *Bacillus* isolated were tested for antibiotic susceptibility. The MIC ranges for all antibiotics and the frequencies of antibiotic resistance are shown in Table 3. All isolates were resistant to at least one antibiotic. *Lactobacillus* had a higher prevalence of resistance than *Bacillus* to antibiotics examined, except erythromycin, rifampicin and tetracycline. All *Lactobacillus* isolates were resistant to gentamicin. Most *Lactobacillus* isolates exhibited resistance to ampicillin (91%), kanamycin (91%) and streptomycin (93%); none were resistant to chloramphenicol, erythromycin or rifampicin. Among *Bacillus* isolates, the highest frequency of resistance was to tetracycline (13%), none were resistant to ciprofloxacin or gentamicin.

Presence of plasmid and transfer of resistance determinants

All *Lactobacillus* and *Bacillus* isolates tested for antibiotic resistance were examined for the presence of a plasmid. Plasmid DNA was detected in 18 *Lactobacillus* spp and 3 *Bacillus* spp isolates. All strains harboring plasmids were used as donors for *in vitro* transfer experiments. Even though most *Bacillus* are intrinsically resistant to tetracycline (SCAN, 2003), all three *Bacillus* isolates used as donors were susceptible to tetracycline. Therefore, spontaneous tetracycline-resistant mutants were used as recipients. No transconjugants could be obtained with any donor-recipient combinations.

Antibiotic resistance genes

Twenty *Lactobacillus* spp isolates and 25 *Bacillus* spp isolates, including all the strains harboring plasmids were tested for the presence of various antibiotic resistance genes corresponding to their resistance phenotypes. Only one isolate, *L. plantarum*, harbored the *vanA* gene (vancomycin MIC >32 μg/ml). The *tetW* and *vanA* genes were present in a *B. subtilis* strain (tetracycline MIC >32 μg/ml; vancomycin MIC >32 μg/ml). Neither *L. plantarum* nor *B. subtilis* strains carrying resistance genes harbored plasmids (data not shown).

DISCUSSION

In this study, none of the probiotic products were satisfactory qualitatively or quantitatively. The low bacterial concentration in 7 products has negative implications on their

Table 3
MIC data of *Lactobacillus* (*n* = 82) and *Bacillus* (*n* = 119) species.

Strain (*n*)	MIC range (μg/ml)											
	AMP (%)[a]	CHP (%)	CIP (%)	ERY (%)	GEN (%)	KAN (%)	NEO (%)	RIF (%)	STR (%)	TET (%)	TRI (%)	VAN (%)
L. plantarum (39)	2->8 (90)	2-8 (0)	0.5->16 (46)	0.25->1 (0)	1->4 (100)	0.5-128 (90)	1-128 (62)	0.25-32 (0)	1-64 21(87)	0.25-32 (21)	1->64 (69)	0.25->32 (36)
L. rhamnosus (21)	1-4 (86)	4-8 (0)	0.5->16 (33)	0.25-0.5 (0)	1->4 (100)	0.5->128 (95)	4-64 (52)	0.25-16 (0)	16-64 (100)	0.5-8 (5)	16->64 (81)	0.25->32 (33)
L gasseri (5)	2->8 (100)	4-8 (0)	0.5->16 (60)	0.25-0.5 (0)	1->4 (100)	64->128 (100)	4-128 (80)	1-16 (0)	8-16 (80)	0.5-8 (0)	8->64 (60)	1->32 (60)
L. delbrueckii (11)	2->8 (100)	2-8 (0)	0.5->16 (82)	0.25-0.5 (0)	1->4 (100)	128->128 (100)	4-128 (27)	1-16 (0)	16-64 (100)	0.5-8 (0)	16->64 (82)	1->32 (73)
L. casei group (6)	2-4 (100)	4-8 (0)	1->16 (83)	0.25 (0)	1->4 (100)	0.5->128 (67)	1-128 (17)	0.25-16 (0)	16-64 (100)	4-8 (0)	8->64 (33)	0.25->32 (50)
Total (%)[b]	**91**	**0**	**51**	**0**	**100**	**91**	**52**	**0**	**93**	**11**	**71**	**43**
B. subtilis (31)	0.25-1 (0)	1-16 (6)	0.125 (0)	0.25-0.5 (0)	0.25-0.5 (0)	0.5-1 (0)	0.25-1 (0)	0.25-0.5 (0)	0.5-64 6()	0.25->64 (10)	0.25->64 (6)	0.25->32 (3)
B. licheniformis (6)	0.25-1 (0)	4-16 (17)	0.125 (0)	0.25->32 (17)	0.25-2 (0)	0.5-2 (0)	0.25-8 (0)	0.25-0.5 (33)	1-16 (0)	0.25-32 (33)	0.25-0.5 (0)	0.25-16 (33)
B. subtilis cluster (73)	0.25->8 (5)	1-8 (0)	0.125-0.5 (0)	0.25->32 (0)	0.25-4 (0)	0.5-2 (0)	0.25-128 (0)	0.25-32 (0)	0.5-32 (1)	0.5->32 (4)	0.25-8 (5)	0.25->32 (0)
B. cereus (9)	0.25->8 (89)	4-8 (0)	0.125-0.25 (0)	0.25 (33)	0.25-0.5 (0)	0.5-2 (0)	0.25-1 (33)	0.25-1 (44)	2-64 (0)	0.5-16 (89)	0.25->64 (89)	0.25 -2 (100)
Total (%)	**10**	**3**	**0**	**1**	**0**	**0**	**3**	**0.5**	**3**	**13**	**12**	**10**

[a] Resistance rate for each species is indicated in brackets.

[b] Total resistance rate for isolates is shown in bold.

AMP, ampicillin; CHP, chloramphenicol; CIP, ciprofloxacin, GEN, gentamicin; KAN, kanamycin; NEO, neomycin; RIF, rifampicin; STR, streptomycin, TET, tetracycline; TRI, trimethoprim; VAN, vancomycin

health benefits, in particular, product XIII which had an absence of living bacteria. This could be due to inadequate quality control in manufacturing, including freeze-drying, bacterial death during storage or short shelf life. The product label should state the minimal number of viable cells present at any time before the expiration date. Eight products, products I-VI, VIII and X, had no expiration date on the product label (data not shown).

The beneficial properties of probiotics can vary among strains. Since it cannot be expected different strains of the same species will produce similar beneficial effects, the product label should stipulate the specific strains of species included. None of the products fulfilled this criterion. The organisms were not identified to the strain level, and many were misidentified at the species level.

Several species of *Bacillus*, such as *clausii*, *licheniformis*, *cereus*, and *pumilus*, have been formulated in commercial probiotic products (Hong *et al*, 2005). Recent studies have shown that many products contain other *Bacillus* species mislabelled as *B. subtilis* (Hoa *et al*, 2000). This is in agreement with the results of the present study. The most common misidentified species was *B. subtilis*. *B. subtilis* cluster and *B. cereus* were mislabelled as *B. subtilis*. Such inaccurate labelling indicates poor identification techniques by the manufacturers.

The *Lactobacillus* and *Bacillus* isolates exhibited resistance to a broad range of antibiotics as previously observed (Danielsen and Wind, 2003; Klare *et al*, 2007). High resistance to aminoglycosides was observed with all *Lactobacillus* strains. This could be due to intrinsic resistance resulting from a lack of cytochrome-mediated electron transport in this bacterium (Charteris *et al*, 2001). Since lactic acid bacteria often harbor plasmids, they have the potential to serve as reservoirs for transferable resistance genes (Cataloluk and Gogebakan, 2004; Mathur and Singh, 2005). In this study, there were no transfers of resistance traits on plasmids observed. This does not mean resistance is not transferable but resistance transfer was not detected. The resistance determinants may be present in a small plasmid that is not transferred efficiently or be a part of a nonconjugative transposon.

In this study, only *tetW* and *vanA* were detected and not on a conjugative plasmid. The MIC values for the antibiotics for these isolates were not different from the strains without genes, indicating the presence of other resistance genes that were not investigated. The *tetW* gene has been previously found in several bacteria, including *Lactobacillus* (Kastner *et al*, 2006; Klare *et al*, 2007) and *Bifidobacterium* (Masco *et al*, 2006). The presence of an identical gene in different bacterial hosts indicates intra- and interspecies transfer of resistance determinants among bacteria. The *vanA* gene has been identified in *Enterococci* but it is rare for bacteria other than *Enterococci* to possess this gene. Transfer of the *vanA* cluster from *Enterococci* to *L. acidophilus* has been previously demonstrated *in vitro* and in the gut of mice (Mater *et al*, 2008) and raises concerns the transfer of vancomycin resistance genes may occur in the human digestive tract. A *vanA*-like gene cluster has been identified on the chromosome of a glycopeptide-resistant *B. cerculans* strain (Ligozzi *et al*, 1998). The *vanA* gene found in both the *Lactobacillus* and *Bacillus* in this study was borne by the chromosome. These data confirm transfer and genetic exchange of the *vanA* gene between *Enterococci* and nonenterococcal species *in vivo*.

The findings of this study suggest improvements are needed in product labelling and quality assurance procedures for

probiotic products used as animal feed additives. Only clearly identified, nontoxic, nonpathogenic strains that do not carry resistance to antimicrobials should be authorized for sale. Suppliers and producers should accurately declare the genus, species, strain and numbers of each probiotic organism included in a product. The expiration date needs to be clearly stated and manufacturers should guarantee the number of living microorganisms declared on the product label are present until expiration.

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