



Genome analysis of probiotic bacteria for antibiotic resistance genes

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Abstract To date, probiotic bacteria are used in the diet and have various clinical applications. There are reports of antibiotic resistance genes in these bacteria that can transfer to other commensal and pathogenic bacteria. The aim of this study was to use whole-genome sequence analysis to identify antibiotic resistance genes in a group of bacterial with probiotic properties. Also, this study followed existing issues about the importance and presence of antibiotic resistance genes in these bacteria and the dangers that may affect human health in the future. In the current study, a collection of 126 complete probiotic bacterial genomes was analyzed for antibiotic resistance genes. The results of the current study showed that there are various resistance genes in these bacteria that some of them are transferable to other bacteria. The *tet(W)* tetracycline resistance gene was more than other antibiotic resistance genes in these bacteria and this gene was found in *Bifidobacterium* and *Lactobacillus*.

In our study, the most numbers of antibiotic resistance genes were transferred with mobile genetic elements. We propose that probiotic companies before the use of a micro-organism as a probiotic, perform an antibiotic susceptibility testing for a large number of antibiotics. Also, they perform analysis of complete genome sequence for prediction of antibiotic resistance genes.

Keywords Antibiotic resistance gene · ARG-ANNOT · Probiotic · Resfinder · Whole genome sequencing

Introduction

The Food and Agriculture Organization of the United Nations (FAO), the World Health Organization (WHO) (FAO and WHO,) and the International Scientific Association for Probiotics and Prebiotics (ISAPP) (Fijan 2014) announce that microorganisms with probiotic properties are live microbes that their appropriate amounts are beneficial for the host health. The microbiota of the human gastrointestinal tract (GIT) is the most complex microbial ecosystems (Martín et al. 2013) and there are more than 1000 bacterial species in the adult human GIT (Frank et al. 2007; Qin et al. 2010). Nowadays, with the help of novel methods, the composition of microbiota in normal and diseased human populations has been

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described. Gut bacteria affect gastrointestinal function by altering gene expression in the intestinal mucosa of mammals (Hemarajata and Versalovic 2013). Van Baarlen et al. reported probiotics can affect gene expression patterns in the GIT (Van Baarlen et al. 2011). The human gut microbiota has an important role in the maintenance of immunological homeostasis, regulation of host energy metabolism and, function and mucosal integrity of the gastrointestinal tract (Pflughoeft and Versalovic 2012). Epithelial barrier fortification and differentiation, manipulation of gut microbial population, immunomodulation, suppression of pathogens, stimulation of intestinal epithelial cell proliferation (Thomas and Versalovic 2010) and, competition for nutrients (O'Toole and Cooney 2008) are of mechanisms of probiotics in the intestinal human. The health benefits of probiotic micro-organisms have been reported in the literature and some bacteria genera include: *Akkermansia* (Shin et al. 2019), *Bacillus* (Fijan 2014), *Bifidobacterium* (Fijan 2014), *Enterobacter* (Kuebutornye et al. 2019), *Enterococcus* (Fijan 2014), *Escherichia coli* (Fijan 2014), *Lactobacillus* (Fijan 2014), *Leuconostoc* (Fijan 2014), *Lactococcus* (Gao et al. 2011), *Paenibacillus* (Kim et al. 2010), *Pediococcus* (Fijan 2014), *Propionibacterium* (Falentini et al. 2010), *Streptococcus* (Fijan 2014), *Shewanella* (Kang et al. 2019) and, etc. have probiotic properties. Antibiotic resistance mechanisms in bacteria are: 1- intrinsic, that naturally occurs with four mechanisms, including beta-lactamases, efflux pumps, alteration in the antibacterial target site, metabolic pathway inhibition and 2- acquired, that occurs with mutations or acquisition of foreign DNA (Saarela et al. 2000; Sharma et al. 2014). One of the important ways to transfer the antibiotic resistance gene between bacteria is horizontal gene transfer (HGT) (Das et al. 2019) with different mechanisms, including conjugation, transduction or transformation (Korhonen 2010). Many antibiotic resistance genes have acquired by conjugation in lactic acid bacteria (LAB) and bifidobacteria (Ammor et al. 2007). Conjugative plasmids have been reported in *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Leuconostoc*, *Pediococcus*, and *Streptococcus thermophilus* (Sgorbati et al. 1982; Mercenier et al. 1994; Davidson et al. 1996; Ammor et al. 2007). One of the safety aspects for probiotics is that they do not have transferable antibiotic resistance genes (Saarela et al. 2000). Strains of bacteria harbouring jumping genes or

transposable elements (TEs) carrying antibiotic resistance genes are not suitable and acceptable as probiotics (Saarela et al. 2000). Antibiotic resistance has been widely reported in *Lactobacilli* (Charteris et al. 1998) that in many cases, they are not of the transmissible type (Saarela et al. 2000). Resistance to vancomycin has been reported in *Lactobacillus*, *Leuconostoc*, *Pediococcus* and, *Enterococcus* (Nicas et al. 1989; Swenson et al. 1990). Antibiotic resistance to glycopeptides, such as vancomycin may be encoded by genomic DNA or extra-genomic DNA. These resistant genes are often located within transposons (called jumping genes) such as Tn1546 or other TEs, which can serve as a reservoir for the transmission of resistance to other bacteria (Leavis et al. 2007; Werner et al. 2010; Faron et al. 2016). The important problem is that probiotic strains may naturally contain antibiotic resistance genes or harbor transferable antibiotic resistance genes that should not be used for animal, and human (Introduction of a Qualified Presumption of Safety 2007); therefore, due to the presence and transmission of such genes to other organisms, they should be carefully monitored for commercial use (Borriello et al. 2003). The aim of the current study was to survey the antibiotic resistance genes based on whole-genome sequences analysis in the probiotic bacteria and answer 3 questions about these bacteria, as follows: I- Are there antibiotic resistance genes in these bacteria? II- Which antibiotic resistance gene is the most common in these bacteria and III—How many of them are on transferable elements or chromosome?

Methods

Based on the literature search in Google Scholar, PubMed, Scopus, Embase and, Science Direct databases, a total number of 126 probiotic bacteria were found in articles in various genera, including *Akkermansia*, *Bacillus*, *Bifidobacterium*, *Lactobacillus*, *Carnobacterium*, *Enterococcus*, *Escherichia*, *Lactococcus*, *Leuconostoc*, *Paenibacillus*, *Pediococcus*, *Propionibacterium*, *Streptococcus*, *Shewanella* and, *Weissella* their genomes were downloaded from national center for biotechnology information (NCBI) microbial genomes repository. The whole-genome sequences of probiotic strains are listed in Table 1. Whole-genome sequences were analyzed for the

Table 1 The genus and species names of probiotic bacteria that surveyed in this study

<i>Akkermansia muciniphila</i> DSM 22959	<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i> DE111	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> Bi-07	<i>Bifidobacterium longum</i> DJO10A	<i>Lactobacillus acidophilus</i> La-14
<i>Bacillus amyloliquefaciens</i> DSM7	<i>Bacillus subtilis</i> subsp. <i>spizizenii</i> ATCC 6633	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BI-04	<i>bifidobacterium longum</i> GT15	<i>Lactobacillus acidophilus</i> NCFM
<i>Bacillus clausii</i> KSM-K16	<i>Bifidobacterium longum</i> subsp. <i>longum</i> 35624	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BLC1	<i>Bifidobacterium longum</i> NCC2705	<i>Lactobacillus acidophilus</i> LA1
<i>Bacillus coagulans</i> 36D1	<i>Bifidobacterium adolescentis</i> ATCC 15703	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> HN019	<i>Bifidobacterium longum</i> subsp. <i>infantis</i> ATCC15697	<i>Lactobacillus amylolyticus</i> L6
<i>Bacillus coagulans</i> strain DSM 2314	<i>Bifidobacterium animalis</i> BL3	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> V9	<i>Bifidobacterium longum</i> subsp. <i>longum</i> JDM301	<i>Lactobacillus brevis</i> ATCC 367
<i>Bacillus coagulans</i> S-lac	<i>Bifidobacterium animalis</i> RH	<i>Bifidobacterium bifidum</i> NCIMB 41171	<i>Carnobacterium</i> sp. 17-4	<i>Lactobacillus brevis</i> KB290
<i>Bacillus licheniformis</i> DSM 13 = ATCC 14580	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> I-2494	<i>Bifidobacterium breve</i> BR03	<i>Enterococcus durans</i> KLDS6.0930	<i>Lactobacillus casei</i> (Formerly <i>Lactobacillus paracasei</i>) LOCK919
<i>Bacillus</i> sp. DU-106	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> AD011	<i>Bifidobacterium breve</i> UCC2003	<i>Enterococcus faecalis</i> strain Symbioflor 1	<i>Lactobacillus casei</i> BL23
<i>Bacillus subtilis</i> PY79	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> B420	<i>Bifidobacterium longum</i> BBMN68	<i>Leuconostoc citreum</i> KM20	<i>Lactobacillus casei</i> LC2W
<i>Bacillus subtilis</i> TO-A JPC	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> BB-12	<i>Bifidobacterium longum</i> BORI	<i>Escherichia coli</i> strain Nissle 1917	<i>Lactobacillus casei</i> LC5
<i>Lactobacillus casei</i> LcA	<i>Lactobacillus fermentum</i> MTCC 5898	<i>Lactobacillus johnsonii</i> LBJ456 Contig01	<i>Lactobacillus plantarum</i> LZ95	<i>Lactobacillus reuteri</i> JCM 1112
<i>Lactobacillus casei</i> LcY	<i>Lactobacillus fermentum</i> VRI-003	<i>Lactobacillus paracasei</i> ATCC 334	<i>Lactobacillus plantarum</i> LZ206	<i>Lactobacillus reuteri</i> SD2112
<i>Lactobacillus casei</i> subsp. <i>casei</i> ATCC 393	<i>Lactobacillus gasseri</i> 4M13	<i>Lactobacillus paracasei</i> strain BD-II	<i>Lactobacillus plantarum</i> ST-III	<i>Lactobacillus reuteri</i> ZLR003
<i>Lactobacillus casei</i> W56	<i>Lactobacillus gasseri</i> ATCC 33323	<i>Lactobacillus paracasei</i> LC2W	<i>Lactobacillus plantarum</i> 5-2	<i>Lactobacillus rhamnosus</i> (Formerly <i>Lactobacillus casei</i>) LOCK908
<i>Lactobacillus casei</i> Zhang	<i>Lactobacillus heilongjiangensis</i> DSM 28069	<i>Lactococcus lactis</i> subsp. <i>lactis</i> CV56	<i>Lactobacillus plantarum</i> KLDS1	<i>Lactobacillus rhamnosus</i> 4B15
<i>Lactobacillus debrueckii</i> D7	<i>Lactobacillus helveticus</i> H10	<i>Lactobacillus pentosus</i> MP-10	<i>Lactobacillus plantarum</i> LZ227	<i>Lactobacillus rhamnosus</i> ATCC 53103
<i>Lactobacillus fermentum</i> 3872	<i>lactobacillus helveticus</i> KLDS1.8701	<i>Lactobacillus pentosus</i> SLC13	<i>Lactobacillus plantarum</i> subsp. <i>plantarum</i> P-8	<i>Lactobacillus rhamnosus</i> GG (ATCC 53103)
<i>Lactobacillus fermentum</i> CECT 5716	<i>Lactobacillus helveticus</i> MB2-1	<i>Lactobacillus plantarum</i> 10CH	<i>Lactobacillus plantarum</i> WCFS1	<i>Lactobacillus rhamnosus</i> Lc 705

Table 1 continued

<i>Lactobacillus fermentum</i> F-6	<i>Lactobacillus jensenii</i> SNUV360	<i>Lactobacillus plantarum</i> JDM1	<i>Lactobacillus plantarum</i> YW11	<i>Lactobacillus rhamnosus</i> SCT-10-10-60, ATCC 11443
<i>Lactobacillus fermentum</i> 47-7	<i>Lactobacillus johnsonii</i> NCC 533	<i>Lactobacillus plantarum</i> LPL-1	<i>Lactobacillus plantarum</i> ZJ316	<i>Lactobacillus salivarius</i> CECT 5713
<i>Lactobacillus salivarius</i> UCC118	<i>Lactococcus lactis</i> subsp. <i>lactis</i> NCDO 2118	<i>Leuconostoc kimchii</i> IMSNU 11154	<i>Propionibacterium freudenreichii</i> CIRM-BIA1	<i>Streptococcus thermophilus</i> CNRZ1066
<i>Lactobacillus</i> sp. HFC8	<i>Bifidobacterium animalis</i> subsp. <i>lactis</i> DSM 10140	<i>Paenibacillus polymyxa</i> E681	<i>Propionibacterium freudenreichii</i> DSM 20271	<i>Streptococcus thermophilus</i> KLDS 3.1003
<i>Pediococcus pentosaceus</i> LP28	<i>Streptococcus thermophilus</i> APC151	<i>Streptococcus thermophilus</i> LMD-9	<i>Bifidobacterium longum</i> LTBL16	<i>Lactobacillus gasseri</i> DSM 14869
<i>Lactobacillus plantarum</i> ATG-K8	<i>Lactobacillus plantarum</i> ATG-K2	<i>Lactobacillus plantarum</i> ATG-K6	<i>Lactobacillus rhamnosus</i> DSM 14870	<i>Weissella cibaria</i> CMU
<i>Weissella cibaria</i> CMS1	<i>Shewanella</i> sp. Pdp11	<i>Bacillus cereus</i> ATCC 14579	<i>Bacillus subtilis</i> subsp. <i>subtilis</i> NCIB 3610	<i>Bacillus toyonensis</i> BCT-7112
<i>Lactobacillus curvatus</i> WiKim38				

presence of antibiotic resistance genes using the antibiotic resistance gene-annotation (ARG-ANNOT) (Gupta et al. 2014) and Resfinder (Zankari et al. 2012). Expectation value (E) with 1.0E-100 and matrix with BLOSUM62 was used for ARG-ANNOT. The last update was July 2019 for ARG-ANNOT. The threshold for %ID, minimum length and, database update for Resfinder was 90%, 60%, and 01-Oct-2019 respectively.

Results

One hundred twenty-six whole-genome sequences of probiotic bacteria were analyzed for antibiotic resistance genes with ARG-ANNOT and Resfinder. Antibiotic resistance genes have been identified in the *Bacillus*: *bla-1*, *bla2*, *fosBx1*, *cfr(B)*, *ant4-Ib*, *erm(34)*, *cat*, *msr(D)*, *mef(A)*, *Lmr(B)*, *bsu-1*, *fosB1*, *aadK*, *tet(L)*, *mph(K)*/*Bifidobacterium*: *tetW*, *tetO*, *lnu(C)*/*Enterococcus*: *lsa(A)*, *mph(D)*, *dfrE*, *aac(6')*-*lih*/*Escherichia*: Penicillin_Binding_Protein, *ampC2*, *ampH*, *mdf(A)*/*Lactobacillus*: *vat(E)*, *tet(L)*, *tetW*, *dfrG*, *dfrK*/*Shewanella*: *blaOXA-548*. Analysis whole genome sequences with ARG-ANNOT and Resfinder

identify *erm(34)*, *ant4-Ib*, *cat*, *msr(D)*, *mef(A)*, *tetW*, *tetO*, *lnu(C)*, *lsa(A)*, *vat(E)*, *tetL* and, *dfrG*. *cfr(B)*, *bcl-1*, *Lmr(B)*, *bsu-1*, *bla-1*, *bla2*, *fosBx1*, *mph(D)*, *dfrE*, Penicillin_Binding_Protein, *ampC2*, *ampH* and, *dfrK* identified only by ARG-ANNOT. Also *aadK*, *blaOXA-548*, *mph(K)*, *fosB1*, *aac(6')*-*lih* and, *mdf(A)* identified only by Resfinder. Analysis of the results showed that the prevalence of *tetW* gene was more than other antibiotic resistance genes and this gene was found in *Bifidobacterium* and *Lactobacillus*. The data are shown in Tables 2, 3. Also, The percentage of each resistance genes with ARG-ANNOT and Resfinder are shown in Fig. 1 and 2. The genes, including *lsa(A)* and *mph(D)* were identified in *Enterococcus faecalis* Symbioflor 1 with ARG-ANNOT but Resfinder just identified *lsa(A)* gene. Resfinder identified one beta-lactamase gene (*blaOXA-548* gene) but ARG-ANNOT identified 6 beta-lactamase [*bcl-1*, *bsu-1*, *bla-1*, *bla2*, *ampC2*, and *ampH* genes]. The percentage of isolates in which the antibiotic resistance gene was encoded in a mobile element were 87% and 90% with ARG-ANNOT and Resfinder respectively.

Table 2 Details of analysis whole genome sequences of probiotic bacteria with ARG-ANNOT

Genus/Species	Strain	GenBank accession no	Antibiotic resistance gene	Transferable elements or chromosome	Transferable to other bacteria	Identity (%)
<i>Bacillus cereus</i>	ATCC 14579	NZ_CP034551	(Bla) <i>bla-1</i>	Chromosome	ND	92.31
			(Bla) <i>bla2</i>	Chromosome	ND	89.79
			(Fcyn) <i>fosBx1</i>	ND	ND	99.52
<i>Bacillus amyloliquefaciens</i>	DSM7	FN597644	(MLS) <i>cfr(B)</i>	Mobile genetic element	Yes	88.9
<i>Bacillus clausii</i>	KSM-K16	NC_006582	(AGly) <i>ant4-Ib</i> ¹	Chromosome	No	97.41
			(MLS) <i>erm(34)</i>	and Mobile genetic element	Yes	95.35
			(Bla) <i>bcl-1</i>			90.59
			(Phe) <i>cat</i>	Chromosome		96.76
				Mobile genetic element		
<i>Bacillus coagulans</i>	DSM 2314	NZ_CP033687	(MLS) <i>msr(D)</i>	Mobile genetic element	Yes	92.9
			(MLS) <i>mef(A)</i>			94.24
<i>Bacillus subtilis</i>	PY79	NC_022898	(MLS) <i>Lmr(B)</i>	Mobile genetic element	Yes	100
<i>Bacillus subtilis</i>	TO-A JPC	CP011882	(MLS) <i>Lmr(B)</i>	Mobile genetic element	Yes	97.99
<i>Bacillus subtilis</i> subsp. <i>inaquosorum</i>	DE111	CP013984	(MLS) <i>Lmr(B)</i>	Mobile genetic element	Yes	92.76
			(Bla) <i>bsu-1</i>			99.25
<i>Bacillus subtilis</i> subsp. <i>spizizenii</i>	ATCC 6633	NZ_CP034943	(MLS) <i>Lmr(B)</i>	Mobile genetic element	Yes	93.25
<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	NCIB 3610	CM000488	(MLS) <i>Lmr(B)</i>	Mobile genetic element	Yes	100
<i>Bacillus toyonensis</i>	BCT-7112	NC_022781	(Bla) <i>bla2</i>	Chromosome	ND	91.10
			(Fcyn) <i>fosBx1</i>	ND	ND	88.73
<i>Bacillus</i> sp.	DU-106	NZ_CP026607	(Bla) <i>bla-1</i>	Chromosome	ND	91.97
			(Bla) <i>bla2</i>	Chromosome	ND	89.79
			(Fcyn) <i>fosBx1</i>	ND	ND	99.04
<i>Bifidobacterium animalis</i>	BL3	NZ_CP017098	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i>	RH	NZ_CP007755	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	I-2494 <u>CNCM</u>	NC_017215	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	AD011	NC_011835	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.11
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	B420	CP003497	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BB-12	NC_017214	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16

Table 2 continued

Genus/Species	Strain	GenBank accession no	Antibiotic resistance gene	Transferable elements or chromosome	Transferable to other bacteria	Identity (%)
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Bi-07	CP003498	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BI-04	NC_012814	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BLC1	NC_017216	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	HN019	NZ_CP031154	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	DSM 10140	CP001606	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	V9	NC_017217	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.16
<i>Bifidobacterium breve</i>	BR03	NZ_CP034770	(Tet) <i>terO</i>	Mobile genetic element	Yes	99.69
<i>Bifidobacterium longum</i>	BORI	NZ_CP031133	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.54
<i>Bifidobacterium longum</i>	BBMN68	NC_014656	(Tet) <i>terW</i>	Mobile genetic element	Yes	99.95
			(MLS) <i>lnu(C)</i>	Mobile genetic element	Yes	98.99
<i>Bifidobacterium longum</i>	DJO10A	NC_010816	(Tet) <i>terW</i>	Mobile genetic element	Yes	99.9
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	JDM301	NC_014169	(Tet) <i>terW</i>	Mobile genetic element	Yes	98.18
<i>Enterococcus faecalis</i>	Symbioflor 1	NC_019770	(MLS) <i>lsa(A)</i>	Chromosome	Yes	99.8
			(MLS) <i>mph(D)</i>	Chromosome		99.4
			(Tmt) <i>dfrE</i>	Chromosome and Mobile genetic element		97.98
<i>Escherichia coli</i>	Nissle 1917	NZ_CP007799	(Bla)Penicillin_Binding_Protein	Chromosome		98.26
			(Bla) <i>ampC2</i>	Chromosome		98.15
			(Bla) <i>ampH</i>			97.5
<i>Lactobacillus heilongjiangensis</i>	DSM 28069	NZ_CP012559	(MLS) <i>vat(E)</i>	Mobile genetic element	Yes	96.28

Table 2 continued

Genus/Species	Strain	GenBank accession no	Antibiotic resistance gene	Transferable elements or chromosome	Transferable to other bacteria	Identity (%)
<i>Lactobacillus reuteri</i>	ZLR003	CP014786	(Tet) <i>tetW</i>	Mobile genetic element	Yes	98.07
			(Tet) <i>tetL</i>		No	99.56
			(Tmt) <i>dfpG</i>	Chromosome and Mobile genetic element	Yes	100
			(Tmt) <i>dfpK</i>		Yes	88.94
				Mobile genetic element		
				Mobile genetic element		

Synonym: *aadD2*, *ND* not determined

Discussion

To date, due to the emergence of antibiotic resistance, the safety of probiotic strains is essential for use in humans and animals (Sharma et al. 2014). Bacterial strains that are used in foods should not carry any transferable antibiotic resistance genes (Additives and Feed 2012). The hypothesis has been proposed that the antibiotic resistance genes reservoir in advantageous bacterial populations has been playing a role in the transfer of resistance genes to pathogenic and opportunistic bacteria presumably (Teuber et al. 1999; Salyers et al. 2004). Probiotic strains may have the potential to transmit antibiotic resistance genes into pathogenic or commensal bacteria (microbiota) (Sharma et al. 2014). There are concerns about antibiotic resistance in the LAB present in our diet. Also, LAB acquires antibiotic resistance genes from environmental bacteria and these resistance genes might be transferred to the endogenous microbiota (Sharma et al. 2014). Antibiotic resistance is not always transferable in LAB but genes that are carried on plasmid may transfer to other pathogenic bacteria (Ashraf and Shah, 2011). Some bacteria, such as *Carnobacterium* spp., *Enterococcus* spp., *Lactococcus* spp., *Leuconostoc* spp., and *S. thermophilus* found in various food products and supplements contain antibiotic resistance genes and are capable of transferring antibiotic resistance genes to others (Li and Wang 2008; Wang et al. 2006). One of the reasons that *Bacillus* strains are not suitable as probiotics is the ability to transfer ARG (Lee et al. 2019). In some

LAB, there is a conjugation system and this system is capable of transmission of antibiotic resistance genes presumably (Luo et al. 2005). Werner et al. reported that *vanA*-containing plasmids cannot transfer between *Enterococcus*, *Lactococcus*, and *Bifidobacterium* species (Werner et al. 2010). Chang et al. reported *vanX* gene in *Lactobacillus plantarum* in 2009 (Chang et al. 2009). In our study, vancomycin resistance genes were not identified in the strains. Mater et al. reported the transfer of *vanA* gene from enterococci to a probiotic *Lactobacillus acidophilus* strain in mice (Mater et al. 2008). The *ermB* resistance gene has been reported in *L. plantarum* DG507 (Gevers et al. 2003), *Lactobacillus lactis* (Devirgiliis et al. 2010), *Lactobacillus crispatus* (Rojo-Bezares et al. 2006), *Lactobacillus reuteri* (Zonenschain et al. 2009), *L. reuteri* CH2-2, *Lactobacillus curvatus*, *Lactobacillus sakei*, *Lactobacillus paracasei*, *Lactobacillus brevis*, *Lactobacillus salivarius* CHS1-E, CH7-1E (Zonenschain et al. 2009, Thumu and Halami, 2012), *Lactobacillus vaginalis* NWL35 (Nawaz et al. 2011), and *L. salivarius* BFE 7441 (Hummel et al. 2007) in commercial probiotic products. Tetracycline resistance genes, such as *tet*(O), *tet*(Q), *tet*(M), *tet*(S), *tet*(W), *tet*(36), *tet*(Z), *tet*(O/W/32/O/W/O), *tet*(W/O), *tet*(K), *tet*(L) and aminoglycoside resistance genes, such as *aph*(3)-IIIa, *aac*(6), *aph*(2), *ant*(6), *aaa*(60), *aph*(200), and *lnu*(A) have been reported in *Lactobacillus* (Lahtinen et al. 2009; Sharma et al. 2014). Toomey et al. in 2010 reported that *tet*(M) gene was transferred from *L. plantarum* to *Lactococcus lactis* BU-2–60 and to *Enterococcus*

Table 3 Details of analysis whole genome sequences of probiotic bacteria with Resfinder

Genus/Species	Strain	GenBank accession no	Antibiotic resistance gene	Transferable elements or chromosome	Transferable to other bacteria	Identity (%)
<i>Bacillus cereus</i>	ATCC 14579	NZ_CP034551	(Fcy)fosB1	Mobile genetic element	Yes	99.52
<i>Bacillus clausii</i>	KSM-K16	NC_006582	(AGly)ant(4')-Ib	Chromosome and Mobile genetic element	Yes	97.41
			(MLS)erm(34)	Mobile genetic element		95.35
			(Phe)cat	Mobile genetic element		96.64
<i>Bacillus coagulans</i>	DSM 2314	NZ_CP033687	(MLS)mef(A)	Mobile genetic element	Yes	94.24
			(MLS)msr(D)			92.9
<i>Bacillus subtilis</i>	PY79	NC_022898	(AGly)aadK	Chromosome and Mobile genetic element	Yes	100
			(Tet)tet(L)		No	100
			(MLS)mph(K)	Chromosome and Mobile genetic element		100
<i>Bacillus subtilis</i>	TO-A JPC	CP011882	(MLS)mph(K)	Chromosome and Mobile genetic element	Yes	97.72
			(AGly)aadK		No	100
			(Tet)tet(L)	Chromosome and Mobile genetic element		100
<i>Bacillus subtilis</i> subsp. <i>subtilis</i>	NCIB 3610	CM000488	(Tet)tet(L)	Chromosome and Mobile genetic element	No	100
			(MLS)mph(K)		Yes	100
			(AGly)aadK	Chromosome and Mobile genetic element		100
<i>Bacillus</i> sp.	DU-106	NZ_CP026607	(Fcy)fosB1	Mobile genetic element	Yes	99.04
<i>Bifidobacterium animalis</i>	BL3	NZ_CP017098	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i>	RH	NZ_CP007755	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	CNCM I-2494	NC_017215	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	AD011	NC_011835	(Tet)tet(W)	Mobile genetic element	Yes	98.8
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	B420	CP003497	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BB-12	NC_017214	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	Bi-07	CP003498	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BI-04	NC_012814	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	BLC1	NC_017216	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	HN019	NZ_CP031154	(Tet)tet(W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	V9	NC_017217	(Tet)tet(W)	Mobile genetic element	Yes	98.85

Table 3 continued

Genus/Species	Strain	GenBank accession no	Antibiotic resistance gene	Transferable elements or chromosome	Transferable to other bacteria	Identity (%)
<i>Bifidobacterium animalis</i> subsp. <i>lactis</i>	DSM 10140	CP001606	(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	98.85
<i>Bifidobacterium breve</i>	BR03	NZ_CP034770	(Tet) <i>tet</i> (O)	Mobile genetic element	Yes	99.69
<i>Bifidobacterium longum</i>	BORI	NZ_CP031133	(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	98.65
<i>Bifidobacterium longum</i>	BBMN68	NC_014656	(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	99.95
			(MLS) <i>lnu</i> (C)	Mobile genetic element	Yes	98.99
<i>Bifidobacterium longum</i>	DJO10A	NC_010816	(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	99.9
<i>Bifidobacterium longum</i> subsp. <i>longum</i>	JDM301	NC_014169	(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	99.11
<i>Enterococcus faecalis</i>	Symbioflor 1	NC_019770	(MLS) <i>lsa</i> (A)	Chromosome	Yes	99.8
<i>Enterococcus durans</i>	KLDS6.0930	NZ_CP012384	(AGly) <i>aac</i> (6′)- <i>Iih</i>	Chromosome		99.46
<i>Escherichia coli</i>	Nissle 1917	NZ_CP007799	(MLS) <i>mdf</i> (A)	Chromosome	ND	98.13
<i>Lactobacillus heilongjiangensis</i>	DSM 28069	NZ_CP012559	(MLS) <i>vat</i> (E)	Mobile genetic element	Yes	96.28
<i>Lactobacillus reuteri</i>	ZLR003	CP014786	(Tet) <i>tet</i> (L)	Chromosome and	No	99.64
			(Tet) <i>tet</i> (W)	Mobile genetic element	Yes	99.01
			(Tmt) <i>dfrG</i>	Mobile genetic element	Yes	100
<i>Shewanella</i> sp.	Pdp11	NZ_CP015194	(Bla) <i>bla</i> OXA-548	Mobile genetic element	ND	98.28

ND not determined

faecalis JH2-2 (Toomey et al. 2010). In addition, *tet*(M) gene is carried on the Tn916 family and Tn6086 (Clewett et al. 1995; Devirgiliis et al. 2013). HGT occurs in foodborne *Lactobacillus* and *Lactococcus* species, including *L. brevis*, *L. paracasei*, *Lactobacillus plantarum*, *Lactococcus lactis*, and *Lactococcus garviae* for *tet*(M) gene (Devirgiliis et al. 2013). HGT *erm*(B) gene has been reported in *Lactobacillus fermentum*, *Lactobacillus plantarum*, and *L. salivarius* (Devirgiliis et al. 2013). Rosander et al. reported *tet*(W) and *lnu*(A) [lincosamide resistance gene] genes carried on plasmids in *L. reuteri* ATCC 55,730 (Rosander et al. 2008). Jacobsen et al. reported horizontal transfer of *tet*(M) and *erm*(B) genes from *L. plantarum* to *E. faecalis* JH2-2 carried on the plasmid in mating experiments (Jacobsen et al. 2007).

In a laboratory study, it has been reported that *L. reuteri* 12,002 that harbors the *erm*(B) gene can be transferred to enterococci (Ashraf and Shah 2011). Some of the transposons, such as TnFO1 (This Tn was identified in *Enterococcus faecalis* strain FO1 that has been isolated from cheese) is transferred by the conjugation mechanism of *E. faecalis* into other organisms, including *Lactococcus lactis* subsp. *lactis* biovar. *diacetylactis*, *Leuconostoc mesenteroides*, etc. (Perreten et al. 1997a, b). Nawas et al. reported *erm*(B) and *tet*(S) genes in *Lactobacillus* and *Streptococcus thermophiles* in Chinese fermented foods (Nawaz et al. 2011). In another study, *tet*(M) and *erm*(B) genes were reported in *Lactobacillus gasseri*, *Lactobacillus casei*, *L. acidophilus* (Çataloluk and Gogebakan 2004), and *L. paracasei* (Comunian et al.

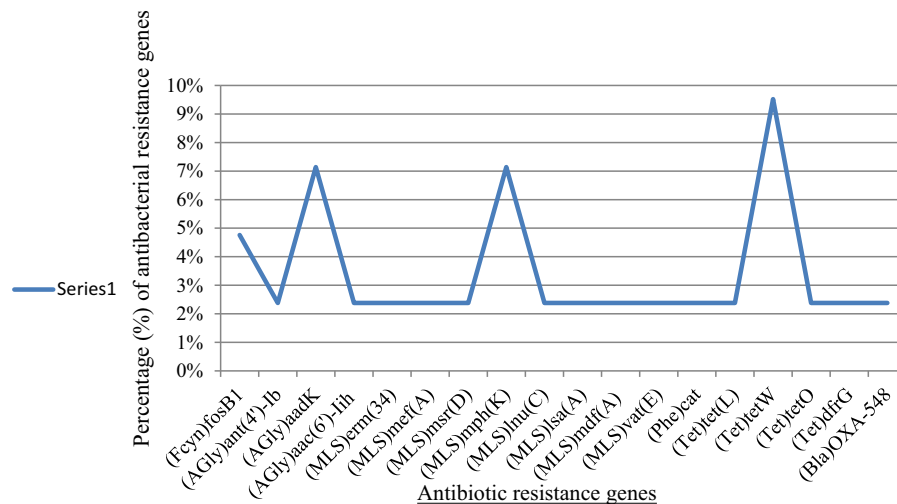


Fig. 1 The percentage of each resistance genes with Resfinder

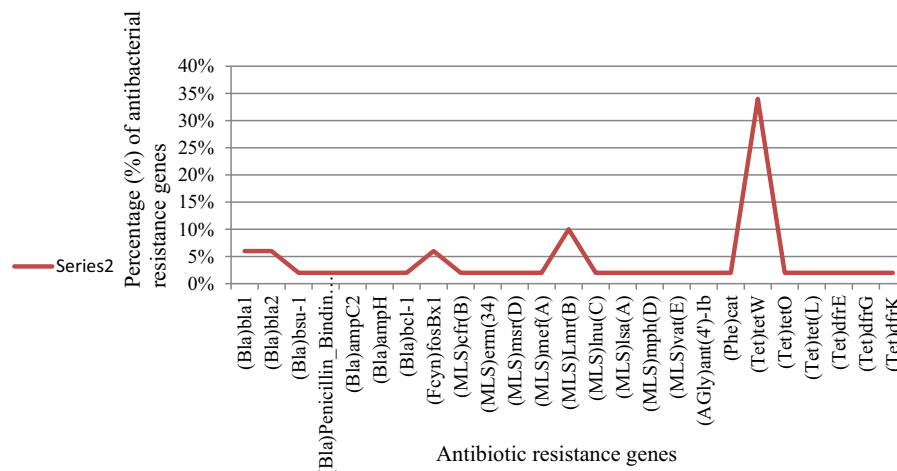


Fig. 2 The percentage of each resistance genes with ARG-ANNOT

2010). Pan et al. (2011) reported *tet*(M), *erm*(B), and *aphA3* genes in *L. plantarum*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, and *E. faecium* in Chinese fermented pickled foods and sausages (Pan et al. 2011). The presence of *tet*(L) gene has been reported in *Lactobacillus sakei* Rits 9 carried on a plasmid (Ammor et al. 2008). Ledina et al. reported *tet*(M) gene in *L. paracasei* isolated from raw Serbian milk cheeses (Ledina et al. 2018). Zonenschain et al. reported *erm*(B) and *erm*(C) genes in *L. plantarum* in fermented dry sausage (Zonenschain et al. 2009). Resistance genes *erm*(B), *tet*(M), and *tet*(S) were reported in *Lactobacillus fermentum* NWL24, *L. salivarius* NWL33, *L. sakei* NWL22, *L. brevis*

NWL59, *L. brevis*, and *Lactobacillus kefir* (Nawaz et al. 2011). In the study by Kastner et al. *tet*(W) gene was found in *L. reuteri* SD 2112 using PCR amplification (Kastner et al. 2006) but we did not identify this gene in the whole-genome sequence (NC_015697) of this bacterium with ARG-ANNOT and Resfinder. Tetracycline resistance genes of *tet*(M) and *tet*(L) were reported in *L. sakei* Rits 9 (Ammor et al. 2008) but only *tet*(L) gene was identified in *L. reuteri* ZLR003 in our study. Lincomycin resistance gene *luc*(A) was identified in *L. reuteri* ATCC 55730 (Rosander et al. 2008). Also, *dfrA* gene has been reported in *S. thermophilus* and *L. lactis* (Chang et al. 2009) but in our study, were identified *dfrG* and *dfrK*

genes in *L. reuteri* ZLR003 and *dfrE* gene in *E. faecalis* Symbioflor 1. In Resfinder, only *dfrG* gene was identified in *L. reuteri* ZLR003. Resistance genes *erm*(B), *tet*(W), and *tet*(M) were identified in *Lactobacillus*, *Pediococcus*, and *Lactococcus* (Klare et al. 2007) but these genes were not identified in *Lactococcus lactis* subsp. *lactis* NCDO 2118 and *Pediococcus pentosaceus* LP28 (Oliveira et al. 2014; Yasutake et al. 2016). The genes, such as *tet*(M), *ant6*, and *aph 3'*-IIIa were reported in *S. thermophilus* and *Lactobacillus delbrueckii* ssp. *Bulgaricus* in Chinese yoghurt (Zhou et al. 2012). In a study by Jaimee et al., the presence of aminoglycoside-resistance genes, including *aac*(6')Ie, *aph*(2'')Ia, and *aph*(3')IIIa was reported in *Lactobacillus plantarum* (Das et al. 2019). Ouoba et al. reported aminoglycoside resistance genes, including *aph*(3')-III and *aadA* in *Lactobacillus casei* and *L. paracasei* (Ouoba et al. 2008). Also, *erm*(B), *tet*(M), *erm*(LF), *vat*(E-1), *mdt*(A), *cat*, and *str* genes were reported in *Lactobacillus gasseri* dairy products (Ammor et al. 2007). In the current study, *vat*(E) gene was identified in *Lactobacillus heilongjiangensis* DSM 28069. Chloramphenicol acetyl transferases (*cat* genes) were reported in *L. acidophilus*, *Lactobacillus delbrueckii* subsp. *bulgaricus*, *Lactobacillus johnsonii*, *L. reuteri*, and *L. plantarum* (Sharma et al. 2014). Liu et al. reported *msrC* and *vanX* genes in *E. faecium* and *L. plantarum* isolated from marketed foods and drugs respectively. Also, they identified *dfrA* gene in *S. thermophilus* and *Lactococcus lactis* (Chang et al. 2009). Campedelli et al. assessed 182 whole-genome sequences for antibiotic resistance genes in *Lactobacillus* spp. using Comprehensive Antibiotic Resistance Database (CARD). They reported penicillin-binding proteins (PBPs) and D-alanine D-alanine ligase (Ddl) in all *Lactobacillus* genomes that were investigated. Also, other antibiotic resistance genes, such as *ant*(6), *ant*(9), *cmlA*, *cat*, *lsa*, *tet*(M), *tet*(S), *tet*(Q), and *tet*(W), *tet*(L) and *tet*(P), *erm*(B), *mef*(E), and *mef*(B) were identified in their study (Campedelli et al. 2019). In another study by Perreten et al., *str*, *tet*(S), and *cat* genes were reported in *Lactococcus lactis* strain K214 in raw milk soft cheese (Perreten et al. 1997a, b). In the current study, two whole-genome sequences of *Lactococcus lactis* subsp. *lactis* strains CV56 and NCDO 2118 were analyzed and there were no antibiotic resistance genes. These strains have been isolated from genital tract secretions of

healthy females (Gao et al. 2011) and frozen peas (Oliveira et al. 2014) respectively. *Bifidobacterium* species are resistant to gentamycin, kanamycin, metronidazole, nalidixic acid, neomycin, polymyxin B and, streptomycin intrinsically (Saarela et al. 2000). Tetracycline resistance gene *tet*(W) was reported in *B. longum* B36 (Moubareck et al. 2005) and *Bifidobacterium* spp. (Masco et al. 2006; Ammor et al. 2007). In our study, this gene was identified in *B. animalis* BL3, RH; *Bifidobacterium animalis* subsp. *lactis* I-2494, AD011, B420, BB-12, Bi-07, BI-04, BLC1, HN019, V9, DSM10140; *B. longum* BORI, BBMN68, DJO10A; *Bifidobacterium longum* subsp. *longum* JDM301, and *L. reuteri* ZLR003. Kazimierczak et al. reported *tet*(W) gene transfer at low frequencies between *Bifidobacterium longum* F8 and *Bifidobacterium adolescentis* L2-32R in laboratory tests (Kazimierczak et al. 2006). In the literature, have been reported *erm*(X), *tet*(L), *tet*(M), *tet*(O), *tet*(W), *tet*(O/W), and *tet*(W/32/O) genes in *Bifidobacterium* (Gueimonde et al., 2013). In our study, none of the strains possessed the *ermB*, *tet*(S), *aph A3*, *erm*(C), *tet*(S), and *tet*(M) genes but *tetO* and *msr*(D) genes were identified in *B. breve* BR03 and *B. coagulans* DSM 2314 respectively. In our study, *lnu*(C) gene was found in *B. longum* BBMN68; however, it was not identified with Resfinder. The gene *rpsL* was reported in *B. breve* in yakult (Kiwaki and Sato 2009) but this gene was not found in our study. The most common tetracycline resistance genes is *tet*(W) that has first been reported in *Butyrivibrio fibrisolvens* (Barbosa et al. 1999; Kazimierczak et al. 2006) and this gene is carried on a conjugative transposon TnB1230 (Melville et al. 2004) or *mob* (mobile genetic element) gene (Billington et al. 2002). Wilcks et al. that reported *tet*(L) gene is more readily transferred than *tet*(M) gene in *Enterococcus faecalis* isolated from raw foods (Wilcks et al. 2005). Also, *erm*(B) and *msr*(C) genes were reported in *E. faecium*, *Enterococcus durans*, and *P. pentosaceus* in traditional fermented foods and curd (Thumu and Halami, 2012). In our study, fosfomycin resistance gene (*fosBx1*) was identified in *Bacillus* sp. DU-106 and *Lmr*(B) gene was identified in *Bacillus subtilis* PY79, *B. subtilis* TO-A JPC, *Bacillus subtilis* subsp. *inaquosorum* DE111, and *Bacillus subtilis* subsp. *spizizenii* ATCC 6633. Murata et al. reported *Lmr*(B) gene in *B. subtilis* strain 168 (Murata et al. 2003). Bozdogan et al. and Girlich et al. reported *aad2*, *erm*(34), *bcl-1*, and *cat*(Bcl) genes in *Bacillus*

spp. (Gueimonde et al., 2013) and we identified *erm*(34), *cat*, and *bcl*-1 genes in *Bacillus clausii* KSM-K16. Girlich et al. (2007) reported *bcl*-1 gene in *B. clausii* NR (Girlich et al. 2007). However, *bcl*-1 gene was not identified using Resfinder in this bacterium. Also, in our study, *ant4*-Ib was identified in *B. clausii* KSM-K16. (Chang et al. 2009; Campedelli et al. 2019) The *bla*OXA-548 gene (this gene is in class D beta-lactamase) is the variant of the *bla*OXA-48 gene (this gene carried by plasmid) (<https://www.ncbi.nlm.nih.gov/nuccore/KY682750>, Poirel et al., 2012) and this variant has been reported in *Shewanella hafniensis* strain Sh29 (<https://www.ncbi.nlm.nih.gov/nuccore/KY682750>). In the literature, there is no information about its transmission to other bacteria; however, Ceccarelli et al. and Zou et al. reported variants of *bla*OXA-48-like gene in *Shewanella* species (Ceccarelli et al. 2017; Zou et al. 2019). In our study, Penicillin_Binding_Protein, *ampC2*, and *ampH* genes were identified in *E. coli* Nissle 1917 and there are any antibiotic resistance genes in *S. thermophilus* APC151, CNRZ1066, KLDS 3.1003, and LMD-9.

Conclusion

In many studies, LAB had acquired various antibiotic resistance genes especially tetracycline and erythromycin agents; therefore, fermented foods and dairy probiotic products may be a source of antibiotic resistance. For the use of a bacterium as a probiotic, we recommend that probiotic companies before the use of a micro-organism as a probiotic, perform an antibiotic susceptibility testing (AST) for a large number of antibiotics. Also, they perform analysis of complete genome sequence for prediction of antibiotic resistance genes. In the review of the literature, probiotic bacteria are considered as a pool of resistance genes and transfer them to microbiota and pathogenic bacteria. Our results showed that there are various antibiotic resistance genes in probiotic bacteria, such as *Lactobacillus*, *Bifidobacterium*, and *Bacillus*; therefore, designing new guidelines for the safety of probiotics is necessary.

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Data availability Not applicable.

Declarations

Conflict of interest The authors declare no conflicts of interest.

Research involving human and animal participants This article does not contain any studies with human participants or animals performed by any of the authors.

Informed Consent The article does not contain any studies in patients by any of the authors.

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