



Genus-Wide Assessment of Antibiotic Resistance in *Lactobacillus* spp.

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ABSTRACT *Lactobacillus* species are widely used as probiotics and starter cultures for a variety of foods, supported by a long history of safe usage. Although more than 35 species meet the European Food Safety Authority (EFSA) criteria for qualified presumption of safety status, the safety of *Lactobacillus* species and their carriage of antibiotic resistance (AR) genes is under continuing *ad hoc* review. To comprehensively update the identification of AR in the genus *Lactobacillus*, we determined the antibiotic susceptibility patterns of 182 *Lactobacillus* type strains and compared these phenotypes to their genotypes based on genome-wide annotations of AR genes. Resistances to trimethoprim, vancomycin, and kanamycin were the most common phenotypes. A combination of homology-based screening and manual annotation identified genes encoding resistance to aminoglycosides (20 sequences), tetracycline (18), erythromycin (6), clindamycin (60), and chloramphenicol (42). In particular, the genes *aac(3)* and *Isa*, involved in resistance to aminoglycosides and clindamycin, respectively, were found in *Lactobacillus* spp. Acquired determinants predicted to code for tetracycline and erythromycin resistance were detected in *Lactobacillus ingluviei*, *Lactobacillus amylophilus*, and *Lactobacillus amylophilus*, flanked in the genome by mobile genetic elements with potential for horizontal transfer.

IMPORTANCE *Lactobacillus* species are generally considered to be nonpathogenic and are used in a wide variety of foods and products for humans and animals. However, many of the species examined in this study have antibiotic resistance levels which exceed those recommended by the EFSA, suggesting that these cutoff values should be reexamined in light of the genetic basis for resistance discussed here. Our data provide evidence for rationally revising the regulatory guidelines for safety assessment of lactobacilli entering the food chain as starter cultures, food preservatives, or probiotics and will facilitate comprehensive genotype-based assessment of strains for safety screening.

KEYWORDS *Lactobacillus*, antimicrobial resistance, genomics

Antibiotics represent one of the most powerful therapeutic options in human and veterinary medicine for the treatment of infectious diseases caused by bacterial agents (1, 2). However, overprescribing and misuse of antibiotics in medicine, animal feed, aquaculture, and agriculture have led to the emergence and spread of antibiotic-resistant bacteria, which constitutes a serious problem for the health of both humans and animals (3). Today, the ever-increasing prevalence of antibiotic resistance (AR) in infectious microbes is a global public health emergency (4, 5).

Lactic acid bacteria (LAB) have been extensively used as probiotics and starter

Citation Campedelli I, Mathur H, Salvetti E, Clarke S, Rea MC, Torriani S, Ross RP, Hill C, O'Toole PW. 2019. Genus-wide assessment of antibiotic resistance in *Lactobacillus* spp. *Appl Environ Microbiol* 85:e01738-18. <https://doi.org/10.1128/AEM.01738-18>.

Editor Johanna Björkroth, University of Helsinki

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Received 17 July 2018

Accepted 10 October 2018

Accepted manuscript posted online 26 October 2018

Published 13 December 2018

cultures due to their long history of safe use, and many species have qualified presumption of safety (QPS) status (6, 7) including members of the genus *Lactobacillus*, *Lactococcus*, *Leuconostoc*, and *Pediococcus* (8). In Europe, the absence of acquired or transferable resistance factors must be established for a candidate probiotic or starter culture in order for them to be declared safe for human and animal consumption and to achieve QPS status from the European Food Safety Authority (EFSA) (9).

The significant economic and scientific impact of the members of the genus *Lactobacillus* includes many strains commonly used as probiotics and others marketed as probiotic cosmetics, drug supplements, or even medical devices (10). Furthermore, *Lactobacillus* species are probably those most widely used as starter cultures for industrial and agricultural applications (e.g., fermented foods and silage cultures), due to their long history of safe and technological use (11–13). Despite their safety status, many lactobacilli have been reported as being antibiotic resistant (12, 14, 15), where a vancomycin-resistant phenotype is perhaps the best-characterized intrinsic resistance mechanism (16). Most *Lactobacillus* species are intrinsically resistant to aminoglycosides (gentamicin, kanamycin, streptomycin, and neomycin), ciprofloxacin, and trimethoprim, and they are susceptible to penicillin and β -lactams, chloramphenicol, tetracycline, erythromycin, linezolid, and quinupristin-dalfopristin (14). However, acquired resistance to tetracycline, erythromycin, clindamycin, and chloramphenicol has been detected in lactobacilli isolated from fermented foods (17–20). Given the widespread use of some species of this genus in fermented food production and functional foods/probiotics, lactobacilli could act as donors or reservoirs for AR genes, with the potential risk of transferring these genes to pathogenic bacteria in food matrices as well as in the gastrointestinal tract (GIT) (21). Thus, even though more than 35 species meet the criteria of QPS status proposed by the EFSA (8), the safety of *Lactobacillus* species and their possible involvement in the spread of AR determinants along the food chain warrant investigation. QPS is an attribute of a species rather than a strain, and it is noteworthy that genome content often varies widely within species, including in lactobacilli (22–24).

The recent determination of the genome sequences of almost all *Lactobacillus* type strains (25, 26) allows the safety assessment of the genus *Lactobacillus* by surveillance of the presence of AR genes, as well as their potential for transfer to other microorganisms. Within the limits of database quality and annotation, whole-genome sequencing (WGS) potentially allows the identification of all possible genetic determinants of antimicrobial resistance in a microbial genome (27). WGS could revolutionize food safety assessments, resulting in a paradigm shift from phenotype-based to genotype-based assays of AR (28).

The aim of the current study was to determine the antibiotic susceptibility patterns of 197 type strains representing the whole *Lactobacillus* genus and to compare these phenotypes to their genotypes based on genome-wide annotation of AR genes. Parallel analysis of phenotype and genotype would provide the definitive knowledge base for the distribution, origins, and mechanisms of AR in the genus and would facilitate rational discussions about regulating strains or species harboring intrinsic, acquired, or transmissible AR mechanisms.

RESULTS

Determination of MICs. The MIC values of 16 antibiotics belonging to the most important antimicrobial classes used in human and veterinary medicine were tested using broth microdilution VetMIC plates for 197 *Lactobacillus* strains representing the whole *Lactobacillus* genus. The MIC profiles were obtained for 182 strains (because 15 strains were not capable of growth in the VetMIC medium) and were analyzed in the context of the *Lactobacillus* phylogroups described in references 25 and 29 and shown in Table 1.

A wide range of MIC values was exhibited by all phylogroups for most antibiotics analyzed, except for linezolid, quinupristin-dalfopristin, and chloramphenicol (see Table S1 in the supplemental material). In particular, a unimodal MIC distribution was

TABLE 1 Features of the 197 type strains of the genus *Lactobacillus* analyzed, including genome accession number and growth condition applied for the determination of MIC values

Species	Strain	Metabolism phenotype ^a	Phylogroup ^b	GenBank accession no. ^c	Source	Niche category	Growth conditions	Temp (°C)	Medium
<i>Lactobacillus acetotolerans</i>	DSM 20749T	FHE	<i>L. delbrueckii</i>	AYZC000000000	Fermented vinegar broth	Food	Anaerobic	30	MRS + 0.05% cysteine
<i>Lactobacillus acidifarinae</i>	DSM 19394T	OHE	<i>L. brevis</i>	AZDV000000000	Artisanal wheat sourdough	Food	Microaerophilic	30	MRS + 0.05% cysteine (pH 5.2)
<i>Lactobacillus acidipiscis</i>	DSM 15836T	FHE	<i>L. salivarius</i>	AZF100000000	Fermented fish	Food	Microaerophilic	37	MRS
<i>Lactobacillus acidophilus</i>	ATCC 4356 ^T	OHO	<i>L. delbrueckii</i>	AZCS000000000	Human	Animal	Microaerophilic	37	MRS
<i>Lactobacillus agilis</i>	DSM 20509T	FHE	<i>L. salivarius</i>	AYYP000000000	Municipal sewage	Environment	Microaerophilic	37	MRS + 0.05% cysteine
<i>Lactobacillus aligidus</i>	DSM 15638T	FHE	<i>L. salivarius</i>	AZDI000000000	Vacuum-packed beef	Food	Anaerobic	20	MRS (pH 5.7)
<i>Lactobacillus alimentarius</i>	DSM 20249T	FHE	<i>L. alimentarius</i>	AZDQ000000000	Marinated fish product	Food	Aerobic	30	MRS
<i>Lactobacillus amylolyticus</i>	DSM 11664T	OHO	<i>L. delbrueckii</i>	AZEP000000000	Acidified beer wort	Wine product	Preferably anaerobic	37	MRS
<i>Lactobacillus amylophilus</i>	DSM 20533T	OHO	<i>L. delbrueckii</i>	AYY500000000	Swine waste-corn fermentation	Animal	Aerobic	30	MRS + 1% glucose
<i>Lactobacillus amylotrophicus</i>	DSM 20534T	OHO	<i>L. delbrueckii</i>	AZCV000000000	Swine waste-corn fermentation	Animal	Aerobic	30	MRS
<i>Lactobacillus amylovorus</i>	DSM 20531T	OHO	<i>L. delbrueckii</i>	AZCM000000000	Cattle waste-corn fermentation	Animal	Microaerophilic-anaerobic	37	MRS
<i>Lactobacillus animalis</i>	DSM 20602T	OHO	<i>L. salivarius</i>	AYYW000000000	Dental plaque of baboon	Animal	Aerobic	37	MRS
<i>Lactobacillus antri</i>	LMG 22111T	OHE	<i>L. reuteri-L. vaccinostercus</i>	AZDK000000000	Gastric biopsy specimens, human stomach mucosa	Animal	Anaerobic	37	MRS
<i>Lactobacillus apinorum</i>	DSM 26257T	OHE	<i>L. fructivorans</i>	JXCT000000000	Honey stomach of honeybee	Animal	Anaerobic	37	MRS + 2% fructose
<i>Lactobacillus apis</i>	LMG 26964T	OHO	<i>L. delbrueckii</i>	JXLG000000000	Stomachs of honeybees	Animal	Anaerobic	37	MRS
<i>Lactobacillus apodemii</i>	DSM 16634T	OHE	<i>L. salivarius</i>	AZFT000000000	Feces of wild Japanese wood mouse	Animal	Microaerophilic	37	MRS
<i>Lactobacillus aquaticus</i>	DSM 21051T	OHO	<i>L. salivarius</i>	AYZD000000000	Surface of a eutrophic freshwater pond	Environment	Microaerophilic	37	MRS
<i>Lactobacillus avianus</i> subsp. <i>araffinosus</i>	DSM 20653T	OHO	<i>L. salivarius</i>	AYYZ000000000	Intestine of chicken	Animal	Microaerophilic	37	MRS + 0.05% cysteine-hydrochloride
<i>Lactobacillus avianus</i> subsp. <i>avianus</i>	DSM 20655T	OHO	<i>L. salivarius</i>	AYZA000000000	Chicken feces	Animal	Microaerophilic	37	MRS + 0.05% cysteine
<i>Lactobacillus backii</i>	DSM 18080T	OHO	<i>L. coryniformis</i>	NA	Orchardgrass silage	Plant	Preferably anaerobic	28	MRS
<i>Lactobacillus bifementans</i>	DSM 20003T	FHE	<i>L. coryniformis</i>	AZDA000000000	Blown cheese	Food	Anaerobic	30	MRS
<i>Lactobacillus bombi</i>	DSM 26517T	FHE	<i>L. alimentarius</i>	NA	Digestive tracts of bumblebee queens	Animal	Anaerobic	37	MRS
<i>Lactobacillus bombicola</i>	DSM 28793T	FHE	<i>L. delbrueckii</i>	NA	Bumble bee gut	Animal	Strictly anaerobic	37	MRS + 0.05% cysteine-hydrochloride
<i>Lactobacillus branitae</i>	DSM 23927T	FHE	<i>L. casei-L. manihotivorans</i>	AYZQ000000000	Feces of Canada goose	Animal	Microaerophilic	37	MRS
<i>Lactobacillus brevis</i>	DSM 20054T	OHE	<i>L. brevis</i>	AZCP000000000	Feces	Animal	Microaerophilic	30	MRS
<i>Lactobacillus buchneri</i>	DSM 20057T	OHE	<i>L. buchneri</i>	AZDM000000000	Tomato pulp	Plant	Microaerophilic	37	MRS
<i>Lactobacillus cacaonum</i>	DSM 21116T	FHE	<i>L. salivarius</i>	AYZE000000000	Cocoa bean heap fermentation	Plant	Microaerophilic	30	MRS
<i>Lactobacillus camelliae</i>	DSM 22697T	OHO	<i>L. casei-L. manihotivorans</i>	AYZJ000000000	Fermented tea leaves (miang)	Plant	Microaerophilic	37	MRS
<i>Lactobacillus capillatus</i>	DSM 19910T	FHE	<i>L. salivarius</i>	AZEF000000000	Fermented brine used for stinky tofu production	Food	Microaerophilic	30	MRS
<i>Lactobacillus casei</i>	DSM 20011T	FHE	<i>L. casei-L. manihotivorans</i>	AZCO000000000	Cheese	Food	Microaerophilic	30	MRS
<i>Lactobacillus certi</i>	DSM 22408T	FHE	<i>L. salivarius</i>	JQBZ000000000	Lungs of a beaked whale	Animal	Strictly anaerobic	37	MRS
<i>Lactobacillus coleohominis</i>	DSM 14060T	FHE	<i>L. reuteri-L. vaccinostercus</i>	AZEW000000000	Human vagina	Animal	Microaerophilic	37	MRS
<i>Lactobacillus collinoides</i>	DSM 20515T	OHE	<i>L. collinoides</i>	AYYR000000000	Fermenting apple juice	Food	Anaerobic	26	MRS
<i>Lactobacillus composti</i>	DSM 18527T	FHE	Other	AZGA000000000	Composting material of distilled shochu residue	Wine product	Microaerophilic	30	MRS
<i>Lactobacillus concavus</i>	DSM 17758T	OHO	Other	AZFX000000000	Walls of a distilled spirit fermenting cellar	Environment	Microaerophilic	30	MRS
<i>Lactobacillus coryniformis</i> subsp. <i>coryniformis</i>	LMG 9196 ^T	FHE	<i>L. coryniformis</i>	AZCN000000000	Silage	Plant	Microaerophilic	30	MRS
<i>Lactobacillus coryniformis</i> subsp. <i>torquens</i>	DSM 20004T	FHE	<i>L. coryniformis</i>	AZDC000000000	Air of cow shed	Environment	Microaerophilic	30	MRS
<i>Lactobacillus crispatus</i>	DSM 20584T	OHO	<i>L. delbrueckii</i>	AZCW000000000	Eye	Animal	Preferably anaerobic	37	MRS
<i>Lactobacillus crustorum</i>	LMG 23699T	OHO	<i>L. alimentarius</i>	JOCK000000000	Wheat sourdough	Food	Aerobic	30	MRS
<i>Lactobacillus curvatae</i>	JCM 18524 ^T	OHE	<i>L. buchneri</i>	CP018906	Tofu brine	Food	Facultatively anaerobic	30	MRS
<i>Lactobacillus curvatus</i>	DSM 20019T	FHE	<i>L. sakei</i>	AZDL000000000	Milk	Food	Microaerophilic	30	MRS
<i>Lactobacillus delbrueckii</i> subsp. <i>bulgaricus</i>	DSM 20081T	OHO	<i>L. delbrueckii</i>	JQAV000000000	Bulgarian yogurt	Food	Microaerophilic	37	MRS

(Continued on following page)

TABLE 1 (Continued)

Species	Strain	Metabolism phenotype ^a	Phylogroup ^b	GenBank accession no. ^c	Source	Niche category	Growth conditions	Temp (°C)	Medium
<i>Lactobacillus delbrueckii</i> subsp. <i>delbrueckii</i>	DSM 20074 ^T	OHO	<i>L. delbrueckii</i>	AZCR000000000	Sour grain mash	Food	Microaerophilic	37	MRS
<i>Lactobacillus delbrueckii</i> subsp. <i>indicus</i>	DSM 15996 ^T	OHO	<i>L. delbrueckii</i>	AZF100000000	Traditional dairy fermented product (Dahi type)	Food	Microaerophilic	37	MRS
<i>Lactobacillus delbrueckii</i> subsp. <i>jakobsenii</i>	DSM 26046 ^T	OHO	<i>L. delbrueckii</i>	JQCG000000000	Dolo wort (alcoholic fermented beverage)	Wine product	Anaerobic	37	MRS
<i>Lactobacillus delbrueckii</i> subsp. <i>lactis</i>	DSM 20072 ^T	OHO	<i>L. delbrueckii</i>	AZDE000000000	Emmental cheese	Food	Microaerophilic	37	MRS
<i>Lactobacillus dextrinicus</i>	DSM 20335 ^T	OHE	Other	AYYK000000000	Silage	Plant	Aerobic	30	MRS
<i>Lactobacillus dialivorans</i>	DSM 14421 ^T	OHE	<i>L. buchneri</i>	AZEY000000000	Maize silage	Plant	Aerobic	30	MRS
<i>Lactobacillus equi</i>	DSM 15883 ^T	OHO	<i>L. salivarius</i>	AZFH000000000	Horse feces	Animal	Microaerophilic	37	MRS
<i>Lactobacillus equicursoris</i>	DSM 19284 ^T	OHO	<i>L. delbrueckii</i>	AZDU000000000	Healthy thoroughbred racehorse	Animal	Microaerophilic	37	MRS
<i>Lactobacillus equigenerosi</i>	DSM 18793 ^T	OHE	<i>L. reuteri-L. vaccinosferus</i>	AZGC000000000	Feces of thoroughbred horse	Animal	Microaerophilic	37	MRS
<i>Lactobacillus tabifermentans</i>	DSM 21115 ^T	FHE	<i>L. plantarum</i>	AYGX000000000	Cocoa bean heap fermentation	Plant	Microaerophilic	30	MRS
<i>Lactobacillus faecis</i>	DSM 23956 ^T	OHO	<i>L. salivarius</i>	NA	Animal feces	Animal	Microaerophilic	37	MRS
<i>Lactobacillus farciminis</i>	LMG 9189	OHO	<i>L. alimentarius</i>	AZDR000000000	Sausage	Food	Microaerophilic	30	MRS
<i>Lactobacillus farraginis</i>	DSM 18382 ^T	FHE	<i>L. buchneri</i>	AZEY000000000	Composting material of distilled shochu residue	Wine product	Microaerophilic	30	MRS
<i>Lactobacillus fermentum</i>	DSM 20055 ^T	OHE	<i>L. reuteri-L. vaccinosferus</i>	JOAU000000000	Human saliva	Animal	Aerobic	30	MRS
<i>Lactobacillus floricola</i>	DSM 23037 ^T	OHO	<i>L. delbrueckii</i>	AYZL000000000	Flower of <i>Caltha palustris</i>	Plant	Aerobic	30	MRS
<i>Lactobacillus florum</i>	DSM 22689 ^T	OHE	<i>L. fructivorans</i>	AYZI000000000	Peony (<i>Paeonia suffruticosa</i>)	Plant	Anaerobic	28	MRS + 1% fructose
<i>Lactobacillus formosensis</i>	NBRC 109509 ^T	OHO	<i>L. alimentarius</i>	NA	Fermented soybean	Food	Anaerobic	37	MRS (pH 6.2)
<i>Lactobacillus formicalls</i>	JCM 12512 ^T	?	Other	NA	Human vagina	Animal	Aerobic	37	MRS
<i>Lactobacillus fructivorans</i>	DSM 20203 ^T	OHE	<i>L. fructivorans</i>	AZDS000000000	NA	Unknown	Microaerophilic	30	MRS
<i>Lactobacillus frumenti</i>	DSM 13145 ^T	OHE	<i>L. reuteri-L. vaccinosferus</i>	AZER000000000	Rye bran sourdough	Food	Anaerobic	40	MRS (pH 6.2)
<i>Lactobacillus fuchensis</i>	DSM 14340 ^T	FHE	<i>L. sakei</i>	AZEX000000000	Vacuum-packaged beef	Food	Aerobic	20	MRS
<i>Lactobacillus turturicola</i>	DSM 27174 ^T	OHO	<i>L. alimentarius</i>	NA	Rice bran paste	Plant	Microaerophilic	30	MRS
<i>Lactobacillus futsaii</i>	JCM 17355 ^T	OHO	<i>L. alimentarius</i>	AZDO000000000	Fu-t'ai, a traditional fermented mustard product	Food	Aerobic	30	MRS
<i>Lactobacillus gallinarum</i>	DSM 10532 ^T	OHO	<i>L. delbrueckii</i>	AZEL000000000	Chicken crop	Animal	Microaerophilic	37	MRS
<i>Lactobacillus gasseri</i>	LMG 9203 ^T	OHO	<i>L. delbrueckii</i>	NC_008530	Human	Animal	Preferably anaerobic	30	MRS
<i>Lactobacillus gastricus</i>	DSM 16045 ^T	OHE	<i>L. reuteri-L. vaccinosferus</i>	AZEN000000000	Gastric biopsy specimens, human stomach mucosa	Animal	Anaerobic	37	MRS
<i>Lactobacillus ghanensis</i>	DSM 18630 ^T	OHO	<i>L. salivarius</i>	AZGB000000000	Cocoa fermentation	Plant	Anaerobic	30	MRS
<i>Lactobacillus gigenorum</i>	DSM 23908 ^T	OHO	<i>L. delbrueckii</i>	AYZO000000000	Chicken crop	Animal	Microaerophilic	37	MRS
<i>Lactobacillus ginsenosidimutans</i>	DSM 24154 ^T	OHO	<i>L. alimentarius</i>	CP012034	Kimchi	Food	Anaerobic	30	MRS
<i>Lactobacillus gorillae</i>	DSM 28356 ^T	FHE	<i>L. reuteri-L. vaccinosferus</i>	NA	Gorilla feces	Animal	Microaerophilic	37	MRS
<i>Lactobacillus graminis</i>	DSM 20719 ^T	FHE	<i>L. sakei</i>	AYZB000000000	Grass silage	Plant	Microaerophilic	30	MRS
<i>Lactobacillus hammesii</i>	DSM 16381 ^T	FHE	<i>L. brevis</i>	AZEF000000000	Wheat sourdough	Food	Microaerophilic	30	MRS + 1% maltose + 0.5% yeast extract
<i>Lactobacillus hamsteri</i>	DSM 5661 ^T	FHE	<i>L. delbrueckii</i>	AZGI000000000	Hamster feces	Animal	Anaerobic	37	MRS
<i>Lactobacillus harbiniensis</i>	DSM 16991 ^T	FHE	<i>L. perolens</i>	AZEW000000000	Chinese traditional fermented vegetable	Food	Microaerophilic	37	MRS
<i>Lactobacillus hayakitensis</i>	DSM 18933 ^T	OHO	<i>L. salivarius</i>	AZGD000000000	Feces of thoroughbred horse	Animal	Microaerophilic	37	MRS
<i>Lactobacillus heliangiangensis</i>	LMG 26166 ^T	OHO	<i>L. alimentarius</i>	CP012559	Chinese pickle	Plant	Microaerophilic	28	MRS
<i>Lactobacillus helsingborgensis</i>	DSM 26265 ^T	OHO	<i>L. delbrueckii</i>	JXJR000000000	Honey stomach of honeybee	Animal	Anaerobic	35	MRS + fructose (20 g/liter)
<i>Lactobacillus helveticus</i>	LMG 22464	OHO	<i>L. delbrueckii</i>	JQCJ000000000	Malt whiskey fermentation	Wine product	Anaerobic	37	MRS
<i>Lactobacillus herbarum</i>	DSM 100358 ^T	OHE	<i>L. plantarum</i>	LFEE000000000	Fermented white radish	Food	Microaerophilic	25	MRS
<i>Lactobacillus hilgardii</i>	LMG 6895 ^T	OHE	<i>L. buchneri</i>	AZDF000000000	Wine	Wine product	Preferably anaerobic	37	MRS
<i>Lactobacillus hokkaidonensis</i>	DSM 26202 ^T	OHE	<i>L. reuteri-L. vaccinosferus</i>	JOCH000000000	Timothy grass silage	Plant	Microaerophilic	25	MRS
<i>Lactobacillus hominis</i>	DSM 23910 ^T	OHO	<i>L. delbrueckii</i>	AZVP000000000	Human intestine	Animal	Microaerophilic	37	MRS
<i>Lactobacillus homohiochii</i>	DSM 20571 ^T	FHE	<i>L. fructivorans</i>	JOBN000000000	Spoiled sake	Wine product	Microaerophilic	26	MRS
<i>Lactobacillus hordei</i>	DSM 19519 ^T	OHO	<i>L. salivarius</i>	AZDX000000000	Malted barley	Plant	Anaerobic	37	MRS
<i>Lactobacillus iners</i>	DSM 13335 ^T	OHO	<i>L. delbrueckii</i>	AZET000000000	Human urine	Animal	Anaerobic	37	MRS

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TABLE 1 (Continued)

Species	Strain	Metabolism phenotype ^a	Phylogroup ^b	GenBank accession no. ^c	Source	Niche category	Growth conditions	Temp (°C)	Medium
<i>Lactobacillus ingluviei</i>	DSM 15946 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZFK000000000	Pigeon, crop	Animal	Microaerophilic	37	MRS
<i>Lactobacillus insidii</i>	DSM 29801 ^T	OHO	<i>L. alimentarius</i>	NA	Fermented raw meat	Animal	Microaerophilic	30	MRS
<i>Lactobacillus intestinalis</i>	DSM 6629 ^T	FHE	<i>L. delbrueckii</i>	AZGN000000000	Intestine of rat	Animal	Aerobic	37	MRS
<i>Lactobacillus ivatensis</i>	DSM 26942 ^T	OHO	<i>L. coryniformis</i>	NA	Orchardgrass silage	Plant	Microaerophilic	30	MRS
<i>Lactobacillus jensenii</i>	DSM 20557 ^T	FHE	<i>L. delbrueckii</i>	AYYU000000000	Human vaginal discharge	Animal	Microaerophilic	37	MRS
<i>Lactobacillus johnsonii</i>	LMG 9436 ^T	OHO	<i>L. delbrueckii</i>	AZCY000000000	Human blood	Animal	Preferably anaerobic	30	MRS
<i>Lactobacillus kalixensis</i>	DSM 16043 ^T	OHO	<i>L. delbrueckii</i>	AZFM000000000	Gastric biopsy specimens, human stomach mucosa	Animal	Microaerophilic	37	MRS
<i>Lactobacillus kefiranoferiensis</i> subsp. <i>kefiranoferiensis</i>	LMG 19149 ^T	OHO	<i>L. delbrueckii</i>	AZGG000000000	Kefir grains	Plant	Anaerobic	30	MRS
<i>Lactobacillus kefiranoferiensis</i> subsp. <i>kefirgramum</i>	DSM 10550 ^T	OHO	<i>L. delbrueckii</i>	AZEM000000000	Kefir grains	Plant	Anaerobic	30	MRS
<i>Lactobacillus kefir</i>	DSM 20587 ^T	OHE	<i>L. buchneri</i>	AYYV000000000	Kefir grains	Plant	Aerobic	30	MRS
<i>Lactobacillus kimbladii</i>	DSM 26263 ^T	FHE	<i>L. delbrueckii</i>	JXLH000000000	Honey stomach of honeybee	Animal	Anaerobic	30	MRS
<i>Lactobacillus kimchicus</i>	JCM 15530 ^T	FHE	<i>L. collinoides</i>	AZCF000000000	Kimchi	Food	Aerobic	37	MRS
<i>Lactobacillus kimchiensis</i>	DSM 24716 ^T	OHO	<i>L. alimentarius</i>	JQCF000000000	Kimchi	Food	Microaerophilic	25	MRS
<i>Lactobacillus kisonensis</i>	DSM 19906 ^T	OHE	<i>L. buchneri</i>	AZEB000000000	Sunki, a Japanese traditional pickle	Food	Microaerophilic	30	MRS + maltose (10 g/liter) + L-arabinose (10 g/liter)
<i>Lactobacillus kitasatonis</i>	DSM 16761 ^T	OHO	<i>L. delbrueckii</i>	AZFU000000000	Chicken intestine	Animal	Microaerophilic	37	MRS
<i>Lactobacillus korensis</i>	JCM 16448 ^T	OHE	<i>L. brevis</i>	AZDP000000000	Cabbage kimchi	Food	Aerobic	30	MRS (pH 5.5)
<i>Lactobacillus kullabergensis</i>	DSM 26262 ^T	FHE	<i>L. delbrueckii</i>	JXBV000000000	Honey stomach of honeybee	Animal	Strictly anaerobic	30	MRS
<i>Lactobacillus kumkei</i>	DSM 12361 ^T	OHE	<i>L. fructivorans</i>	AZCK000000000	Commercial grape wine	Wine product	Aerobic	30	MRS + 0.05% cysteine (pH 5.2)
<i>Lactobacillus lindneri</i>	DSM 20690 ^T	OHE	<i>L. fructivorans</i>	JQBT000000000	Spoiled beer	Wine product	Aerobic	30	MRS + 0.05% cysteine (pH 5.2)
<i>Lactobacillus malefermentans</i>	LMG 11455 ^T	OHE	<i>L. collinoides</i>	AZGJ000000000	Sour beer	Wine product	Microaerophilic	30	MRS
<i>Lactobacillus mali</i>	ATCC 27304 ^T	OHO	<i>L. salivarius</i>	JQAR000000000	Wine must	Wine product	Aerobic	30	MRS
<i>Lactobacillus manihotivorans</i>	DSM 13343 ^T	OHO	<i>L. casei</i> -L. <i>manihotivorans</i>	AZEU000000000	Cassava sour starch fermentation	Plant	Aerobic	30	MRS
<i>Lactobacillus mellifer</i>	DSM 26254 ^T	FHE	<i>L. alimentarius</i>	JXJQ000000000	Honey stomach of honeybee	Animal	Anaerobic	35	MRS + fructose (20 g/liter)
<i>Lactobacillus mellis</i>	DSM 26255 ^T	FHE	<i>L. alimentarius</i>	JXBZ000000000	Honey stomach of honeybee	Animal	Strictly anaerobic	30	MRS + fructose (20 g/liter)
<i>Lactobacillus melliventris</i>	DSM 26256 ^T	FHE	<i>L. delbrueckii</i>	JXLI000000000	Honey stomach of honeybee	Animal	Strictly anaerobic	30	MRS + fructose (20 g/liter)
<i>Lactobacillus mindensis</i>	DSM 14500 ^T	OHO	<i>L. alimentarius</i>	AZEX000000000	Sourdough	Food	Microaerophilic	30	MRS + 0.05% cysteine-hydrochloride (pH 5.2)
<i>Lactobacillus mixtipabuli</i>	DSM 28580 ^T	OHE	<i>L. collinoides</i>	NA	Silage	Plant	Microaerophilic	30	MRS
<i>Lactobacillus modestisalitolerans</i>	NBRC 107235 ^T	OHO	<i>L. plantarum</i>	NA	Fermented fish	Food	Facultatively anaerobic	30	MRS
<i>Lactobacillus mucosae</i>	DSM 13345 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZEQ000000000	Pig small intestine	Animal	Preferably anaerobic	37	MRS
<i>Lactobacillus mudanjiangensis</i>	LMG 27194 ^T	OHO	<i>L. plantarum</i>	NA	Pickle	Plant	Microaerophilic	28	MRS
<i>Lactobacillus murinus</i>	DSM 20452 ^T	FHE	<i>L. salivarius</i>	AYYN000000000	Intestine of rat	Animal	Microaerophilic	37	MRS
<i>Lactobacillus nagelii</i>	DSM 13675 ^T	OHO	<i>L. salivarius</i>	AZEV000000000	Partially fermented wine	Wine product	Anaerobic	30	MRS
<i>Lactobacillus namurensis</i>	DSM 19117 ^T	OHE	<i>L. brevis</i>	AZDT000000000	Sourdough	Food	Microaerophilic	30	MRS + 0.05% cysteine + 0.7 % maltose (pH 5.2)
<i>Lactobacillus nantensis</i>	DSM 16982 ^T	FHE	<i>L. alimentarius</i>	AZFU000000000	Wheat sourdough	Food	Microaerophilic	30	MRS + 0.05% cysteine-hydrochloride + 1% maltose + 0.5% fresh yeast extract
<i>Lactobacillus nasuensis</i>	JCM 17158 ^T	OHO	<i>L. casei</i> -L. <i>manihotivorans</i>	AZDJ000000000	Sudangrass silage sample	Plant	Anaerobic	30	MRS
<i>Lactobacillus nenjiangensis</i>	LMG 27192 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	NA	Pickle	Plant	Microaerophilic	28	MRS
<i>Lactobacillus nodensis</i>	DSM 19682 ^T	FHE	<i>L. alimentarius</i>	AZDZ000000000	Japanese pickles	Food	Microaerophilic	30	MRS
<i>Lactobacillus odoratitofu</i>	DSM 19909 ^T	OHE	<i>L. collinoides</i>	AZEE000000000	Fermented brine used for stinky tofu production	Food	Microaerophilic	30	MRS
<i>Lactobacillus oeni</i>	DSM 19972 ^T	OHO	<i>L. salivarius</i>	AZEH000000000	Bobal wine	Wine product	Microaerophilic	30	MRS
<i>Lactobacillus oligofermentans</i>	DSM 15707 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZFE000000000	Broiler leg	Animal	Microaerophilic	25	MRS
<i>Lactobacillus oris</i>	DSM 4864 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZGE000000000	Human saliva	Animal	Anaerobic	37	MRS
<i>Lactobacillus oryzae</i>	DSM 26518 ^T	OHE	<i>L. collinoides</i>	BBJM000000000	Fermented rice grain	Food	Anaerobic	30	MRS
<i>Lactobacillus otakiensis</i>	DSM 19908 ^T	OHE	<i>L. buchneri</i>	AZED000000000	Sunki, a Japanese traditional pickle	Food	Microaerophilic	30	MRS
<i>Lactobacillus ozensis</i>	DSM 23829 ^T	OHE	<i>L. fructivorans</i>	AYYQ000000000	Chrysanthemum, Oze National Park	Plant	Microaerophilic	30	MRS

(Continued on following page)

TABLE 1 (Continued)

Species	Strain	Metabolism phenotype ^a	Phylogroup ^b	GenBank accession no. ^c	Source	Niche category	Growth conditions	Temp (°C)	Medium
<i>Lactobacillus panis</i>	DSM 6035 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZGM000000000	Sourdough	Food	Aerobic	37	MRS
<i>Lactobacillus pantheris</i>	DSM 15945 ^T	OHO	<i>L. casei</i> -L. <i>manihotivorans</i>	LJCF000000000	Jaguar feces	Animal	Microaerophilic	37	MRS
<i>Lactobacillus parabrevis</i>	LMG 11984 ^T	OHE	<i>L. brevis</i>	JOC100000000	Wheat	Food	Anaerobic	30	MRS
<i>Lactobacillus parabuchneri</i>	DSM 5707 ^T	OHE	<i>L. buchneri</i>	AZGK000000000	Human saliva	Animal	Aerobic	30	MRS
<i>Lactobacillus paracasei</i> subsp. <i>paracasei</i>	DSM 5622 ^T	FHE	<i>L. casei</i> -L. <i>manihotivorans</i>	AZGH000000000	NA	Unknown	Microaerophilic	30	MRS
<i>Lactobacillus paracasei</i> subsp. <i>tolerans</i>	DSM 20258 ^T	FHE	<i>L. casei</i> -L. <i>manihotivorans</i>	AYYJ000000000	Pasteurized milk	Food	Aerobic	30	MRS
<i>Lactobacillus paracollinoides</i>	DSM 15502 ^T	OHE	<i>L. collinoides</i>	AZFD000000000	Brewery environment	Environment	Anaerobic	25	MRS (pH 5.8)
<i>Lactobacillus parafarraginis</i>	LMG 24141 ^T	FHE	<i>L. buchneri</i>	AZFZ000000000	Composting material of distilled shochun residue	Wine product	Microaerophilic	28	MRS
<i>Lactobacillus parakefiri</i>	DSM 10551 ^T	OHE	<i>L. buchneri</i>	AZEN000000000	Kefir grain	Plant	Anaerobic	30	MRS
<i>Lactobacillus paralimentarius</i>	DSM 13238 ^T	FHE	<i>L. alimentarius</i>	AZE500000000	Sourdough	Food	Microaerophilic	30	MRS
<i>Lactobacillus paraplantarum</i>	DSM 10667 ^T	FHE	<i>L. plantarum</i>	AZE000000000	Beer contaminant	Wine product	Aerobic	30	MRS
<i>Lactobacillus pasteurii</i>	DSM 23907 ^T	FHE	<i>L. delbrueckii</i>	AYZN000000000	NA	Unknown	Microaerophilic	37	MRS
<i>Lactobacillus paucivorans</i>	DSM 22467 ^T	FHE	<i>L. brevis</i>	JQCA000000000	Yeast storage tank containing lager beer	Wine product	Anaerobic	28	MRS + 0.05% cysteine-hydrochloride + 1% fructose (pH 5.8)
<i>Lactobacillus pentosus</i>	DSM 20314 ^T	FHE	<i>L. plantarum</i>	AZCU000000000	NA	Unknown	Microaerophilic	30	MRS
<i>Lactobacillus perolens</i>	DSM 12744 ^T	FHE	<i>L. perolens</i>	AZEC000000000	Orange lemonade	Food	Anaerobic	30	MRS
<i>Lactobacillus plajomi</i>	NBRC 107333 ^T	FHE	<i>L. plantarum</i>	NA	Fermented fish	Food	Facultatively anaerobic	30	MRS
<i>Lactobacillus plantarum</i> (formerly <i>Lactobacillus arizonensis</i>)	DSM 20174 ^T	FHE	<i>L. plantarum</i>	AZEJ000000000	Pickled cabbage	Food	Aerobic	37	MRS
<i>Lactobacillus plantarum</i> subsp. <i>argenteatensis</i>	DSM 16365 ^T	FHE	<i>L. plantarum</i>	AZFR000000000	Fermented cassava roots (ufutu)	Plant	Microaerophilic	30	MRS
<i>Lactobacillus pobuzihii</i>	NBRC 103219 ^T	FHE	<i>L. salivarius</i>	JQCN000000000	Pobuzih (fermented cummingcordia), <i>Cordia dichotoma</i>	Plant	Microaerophilic	37	MRS + 5% NaCl
<i>Lactobacillus pontis</i>	DSM 8475 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZGO000000000	Rye sourdough	Food	Aerobic	30	MRS
<i>Lactobacillus porciniae</i>	LMG 26767 ^T	FHE	<i>L. casei</i> -L. <i>manihotivorans</i>	NA	Nem chua (fermented meat)	Food	Microaerophilic	28	MRS
<i>Lactobacillus psittaci</i>	DSM 15354 ^T	OHE	<i>L. delbrueckii</i>	AZFB000000000	Lung of parrot	Animal	Anaerobic	37	MRS
<i>Lactobacillus rapi</i>	DSM 19907 ^T	OHE	<i>L. buchneri</i>	AZEI000000000	Sunki, a Japanese traditional pickle	Food	Microaerophilic	30	MRS
<i>Lactobacillus rennini</i>	DSM 20253 ^T	FHE	<i>L. coryniformis</i>	AYYI000000000	Rennin	Animal	Microaerophilic	30	MRS
<i>Lactobacillus reuteri</i>	DSM 20016 ^T	OHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	AZDD000000000	Intestine of adult	Animal	Aerobic	37	MRS
<i>Lactobacillus rhamnosus</i>	DSM 20021 ^T	FHE	<i>L. casei</i> -L. <i>manihotivorans</i>	AZCQ000000000	NA	Unknown	Anaerobic	37	MRS
<i>Lactobacillus rodentium</i>	DSM 24759 ^T	OHO	<i>L. delbrueckii</i>	NA	Digestive tract of rodents	Animal	Microaerophilic	37	MRS
<i>Lactobacillus rossiae</i>	DSM 15814 ^T	OHE	Other	AZEF000000000	Wheat sourdough	Food	Microaerophilic	30	MRS + 1% maltose + 1% yeast extract (pH 5.6)
<i>Lactobacillus ruminis</i>	DSM 20403 ^T	OHO	<i>L. salivarius</i>	AYYL000000000	Bovine rumen	Animal	Anaerobic	37	MRS + 2% glucose
<i>Lactobacillus saerimneri</i>	DSM 16049 ^T	OHO	<i>L. salivarius</i>	AZFP000000000	Pig feces	Animal	Aerobic	37	MRS
<i>Lactobacillus sakei</i> subsp. <i>carnosus</i>	DSM 15831 ^T	FHE	<i>L. sakei</i>	AZFG000000000	Fermented meat product	Food	Microaerophilic	37	MRS
<i>Lactobacillus sakei</i> subsp. <i>sakei</i>	CECT 4591 ^T	FHE	<i>L. sakei</i>	AZDN000000000	"Moto" starter of sake	Wine product	Aerobic	30	MRS
<i>Lactobacillus salivarius</i>	DSM 20555 ^T	FHE	<i>L. salivarius</i>	AYYT000000000	Saliva	Animal	Microaerophilic	37	MRS
<i>Lactobacillus sanfranciscensis</i>	LMG 16002 ^T	OHE	<i>L. fructivorans</i>	AYYM000000000	Sourdough	Food	Anaerobic	28	MRS + 1% fructose (pH 5.5)
<i>Lactobacillus saniviri</i>	DSM 24301 ^T	FHE	<i>L. casei</i> -L. <i>manihotivorans</i>	JQCE000000000	Feces of a Japanese healthy adult male	Animal	Microaerophilic	37	MRS
<i>Lactobacillus satsumensis</i>	DSM 16230 ^T	OHO	<i>L. salivarius</i>	AZFQ000000000	Shochu mash	Wine product	Microaerophilic	30	MRS
<i>Lactobacillus secalliphilus</i>	DSM 17896 ^T	FHE	<i>L. reuteri</i> -L. <i>vaccinostercus</i>	JQBW000000000	Sourdough	Food	Microaerophilic	37	MRS
<i>Lactobacillus selangorensis</i>	ATCC BAA66 ^T	OHO	Other	JQAT000000000	Chili bo	Food	Aerobic	30	MRS
<i>Lactobacillus senioris</i>	DSM 24302 ^T	FHE	<i>L. buchneri</i>	AYZR000000000	Feces of a healthy 100-yr-old Japanese female	Animal	Microaerophilic	37	MRS
<i>Lactobacillus senmaizukei</i>	DSM 21775 ^T	FHE	<i>L. brevis</i>	AYZH000000000	Senmaizuke, a Japanese pickle	Food	Microaerophilic	30	MRS
<i>Lactobacillus sharpeae</i>	DSM 20505 ^T	OHO	<i>L. casei</i> -L. <i>manihotivorans</i>	AYYO000000000	Municipal sewage	Environment	Microaerophilic	30	MRS + 0.05% cysteine

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TABLE 1 (Continued)

Species	Strain	Metabolism phenotype ^a	Phylogroup ^b	GenBank accession no. ^c	Source	Niche category	Growth conditions	Temp (°C)	Medium
<i>Lactobacillus shenzhenensis</i>	DSM 28193T	OHE	<i>L. perolens</i>	AVAA000000000	Fermented dairy beverage	Food	Microaerophilic	37	MRS
<i>Lactobacillus sicerae</i>	KCTC 21012T	OHO	<i>L. salivarius</i>	NA	Spanish natural cider	Food	Anaerobic	37	MRS
<i>Lactobacillus silagei</i>	DSM 27022T	OHE	<i>L. collinoides</i>	NA	Orchardgrass silage	Plant	Microaerophilic	30	MRS
<i>Lactobacillus siliginis</i>	DSM 22696T	OHE	Other	JOCB000000000	Wheat sourdough	Food	Anaerobic	37	MRS
<i>Lactobacillus similis</i>	DSM 23365T	OHE	<i>L. collinoides</i>	AYZM000000000	Fermented cane molasses at alcohol plants	Wine product	Microaerophilic	37	MRS
<i>Lactobacillus songhuajiangensis</i>	DSM 28401T	OHO	<i>L. casei-L. manihotivorans</i>	NA	Sourdough	Food	Microaerophilic	28	MRS
<i>Lactobacillus spicheri</i>	DSM 15429T	FHE	<i>L. brevis</i>	AZFC000000000	Rice sourdough	Food	Microaerophilic	30	MRS (pH 5.8)
<i>Lactobacillus sucicola</i>	DSM 21376T	OHO	<i>L. salivarius</i>	AYZF000000000	Sap of an oak tree	Plant	Microaerophilic	30	MRS
<i>Lactobacillus suebicus</i>	DSM 5007T	OHE	<i>L. reuteri-L. vaccinostercus</i>	AZGF000000000	Apple mash	Food	Microaerophilic	30	MRS
<i>Lactobacillus sunkii</i>	DSM 19904T	OHE	<i>L. buchneri</i>	AZEA000000000	Sunki, a Japanese traditional pickle	Food	Microaerophilic	30	MRS
<i>Lactobacillus taiwanensis</i>	DSM 21401T	OHO	<i>L. delbrueckii</i>	AYZG000000000	Silage cattle feed	Plant	Microaerophilic	37	MRS
<i>Lactobacillus thailandensis</i>	DSM 22698T	OHO	<i>L. casei-L. manihotivorans</i>	AYZK000000000	Fermented tea leaves (miang)	Plant	Microaerophilic	30	MRS
<i>Lactobacillus tuccei</i>	DSM 20183T	OHO	<i>L. alimentarius</i>	AZDG000000000	Sausage	Food	Aerobic	30	MRS
<i>Lactobacillus ultunensis</i>	DSM 16047T	OHO	<i>L. delbrueckii</i>	AZFO000000000	Gastric biopsy specimens, human stomach mucosa	Animal	Anaerobic	37	MRS
<i>Lactobacillus uvarum</i>	DSM 19971T	OHO	<i>L. salivarius</i>	AZEG000000000	Must of Bobal grape variety	Plant	Microaerophilic	30	MRS
<i>Lactobacillus vaccinostercus</i>	DSM 20634T	OHE	<i>L. reuteri-L. vaccinostercus</i>	AYYY000000000	Cow dung	Animal	Microaerophilic	30	MRS
<i>Lactobacillus vaginalis</i>	LMG 12891T	OHE	<i>L. reuteri-L. vaccinostercus</i>	AZGL000000000	Vaginal swab	Animal	Anaerobic	28	MRS
<i>Lactobacillus versmoldensis</i>	DSM 14857T	OHO	<i>L. alimentarius</i>	AZEA000000000	Poultry salami	Food	Microaerophilic	30	MRS
<i>Lactobacillus vesputiae</i>	DSM 103408T	OHE	<i>L. fructivorans</i>	NA	Gut of queen wasp	Animal	Microaerophilic	30	MRS
<i>Lactobacillus vini</i>	DSM 20605T	FHE	<i>L. salivarius</i>	AYYX000000000	Must of grape	Plant	Aerobic	37	MRS
<i>Lactobacillus wasatchensis</i>	LMG 28678T	OHE	<i>L. reuteri-L. vaccinostercus</i>	AWTT000000000	Cheddar cheese	Food	Anaerobic	25	MRS
<i>Lactobacillus xiangfangensis</i>	LMG 26013T	FHE	<i>L. plantarum</i>	JQCL000000000	Pickles	Food	Aerobic	30	MRS
<i>Lactobacillus yonginensis</i>	DSM 29216T	FHE	<i>L. brevis</i>	NA	Kimchi	Food	Microaerophilic	30	MRS
<i>Lactobacillus zeae</i>	DSM 20178T	FHE	<i>L. brevis</i>	AZCT000000000	Corn steep liquor	Food	Microaerophilic	37	MRS
<i>Lactobacillus zymae</i>	DSM 19395T	OHE	<i>L. casei-L. manihotivorans</i>	AZDW000000000	Artisanal wheat sourdough	Wine product	Anaerobic	30	MRS + 0.05% cysteine (pH 5.2)

^aOHO, obligately homofermentative; FHE, facultatively heterofermentative; OHE, obligately heterofermentative.

^bThe phylogroups correspond to those described in references 25 and 29.

^cNA, not available.

generally observed for these antibiotics, which covered four 2-fold dilutions for most strains analyzed (89% of strains for quinupristin-dalfopristin and 96% for chloramphenicol and linezolid), ranging in actual MIC values from 2 to 16 $\mu\text{g/ml}$ for chloramphenicol, 1 to 8 $\mu\text{g/ml}$ for linezolid, and 0.5 to 4 $\mu\text{g/ml}$ for quinupristin-dalfopristin.

High MIC values were observed for trimethoprim and vancomycin, at ≥ 64 and >128 $\mu\text{g/ml}$, respectively, in the most strains analyzed (81 and 73%, respectively). Interestingly, strains belonging to the *Lactobacillus delbrueckii* group showed susceptibility to low concentrations of vancomycin (concentrations lower than 1 $\mu\text{g/ml}$ inhibited the growth of 92% of strains belonging to the *L. delbrueckii* group), despite the general consensus in the literature that *Lactobacillus* spp. are not inhibited by vancomycin.

The MICs of the remaining antibiotics showed variability across the genus. In particular, the MIC values for aminoglycosides covered more than nine 2-fold dilution steps, ranging from 2 to $>1,024$ $\mu\text{g/ml}$ for kanamycin, and from 0.2, 0.5, and 2 to >256 $\mu\text{g/ml}$ for gentamicin, neomycin, and streptomycin, respectively. Bimodal MIC distributions were observed for the *Lactobacillus alimentarius*, *Lactobacillus collinoides*, *Lactobacillus fructivorans*, *Lactobacillus plantarum*, and *Lactobacillus reuteri*-*L. vaccinostercus* groups for all aminoglycosides tested (Table S1). Moreover, strains of the *L. reuteri*-*L. vaccinostercus* phylogroup showed MIC values distributed across the whole concentration range tested for gentamicin (0.5 to 256 $\mu\text{g/ml}$), kanamycin (2 to 1,024 $\mu\text{g/ml}$), and neomycin (0.5 to 256 $\mu\text{g/ml}$).

The *L. alimentarius*, *L. collinoides*, *L. delbrueckii*, *L. fructivorans*, *L. plantarum*, and *L. reuteri*-*L. vaccinostercus* groups displayed bimodal MIC value distributions for tetracycline, erythromycin, and clindamycin, antibiotics which notably affect the function of the ribosome. In contrast, a unimodal MIC distribution was observed for β -lactams, including ampicillin and penicillin, except for some strains belonging to the *Lactobacillus brevis* group (*Lactobacillus spicheri* DSM 15429^T and *Lactobacillus zymae* DSM 19395^T), *L. collinoides* group (*Lactobacillus similis* DSM 23365^T), *L. plantarum* group (*Lactobacillus pentosus* DSM 20314^T), *Lactobacillus salivarius* group (*Lactobacillus ghanensis* DSM 18630^T), and other (*Lactobacillus selangorensis* ATCC BAA66^T) groups, which displayed MIC values of >16 $\mu\text{g/ml}$.

The distribution of rifampin and ciprofloxacin MIC values was broad; they trended toward the low-end concentration range tested for this antibiotic (0.12 to 16 $\mu\text{g/ml}$), except for *Lactobacillus jensenii* DSM 20557^T and *Lactobacillus oris* DSM 4864^T, belonging to the *L. delbrueckii* and *L. reuteri*-*L. vaccinostercus* groups, respectively, which showed MIC values higher than 64 $\mu\text{g/ml}$.

Identification of resistance phenotypes. Phenotypic resistance was interpreted based on the epidemiological cutoff (ECOFF) values reported in references 9, 30, and 31, classifying a strain as resistant when the MIC value for a specific antibiotic was higher than the corresponding ECOFF. EFSA specified the ECOFF value for the species *Lactobacillus casei*/*L. paracasei*, *Lactobacillus plantarum*/*L. pentosus*, and *Lactobacillus rhamnosus*. For other species of the genus *Lactobacillus*, EFSA refers to the fermentation metabolic categories, defining ECOFF values for *Lactobacillus* obligate heterofermentative (OHE), *Lactobacillus* obligate homofermentative (OHO), and *Lactobacillus* facultative heterofermentative (FHE) metabolic patterns (9). For comparative clarity, the fermentation metabolism for the strains analyzed are thus presented in Table 1.

Trimethoprim resistance was the most common phenotype observed (84% [152/182 strains]), and most of the *Lactobacillus* strains were not susceptible to vancomycin (77% [141/182 strains]) and kanamycin (61% [111/181 strains]) (Fig. 1). Multidrug resistance, defined as resistance to three or more different antimicrobials, was observed in 152 strains (84%). Interestingly, *Lactobacillus thailandensis* DSM 22698^T showed resistance to all 16 antibiotics tested (Fig. 1). In contrast, *Lactobacillus sanfranciscensis* LMG 16002^T and *Lactobacillus pobuzihii* NBRC 103219^T were identified as susceptible to all 16 antibiotics tested, including vancomycin. *Lactobacillus ozensis* DSM 23829^T, *Lactobacil-*

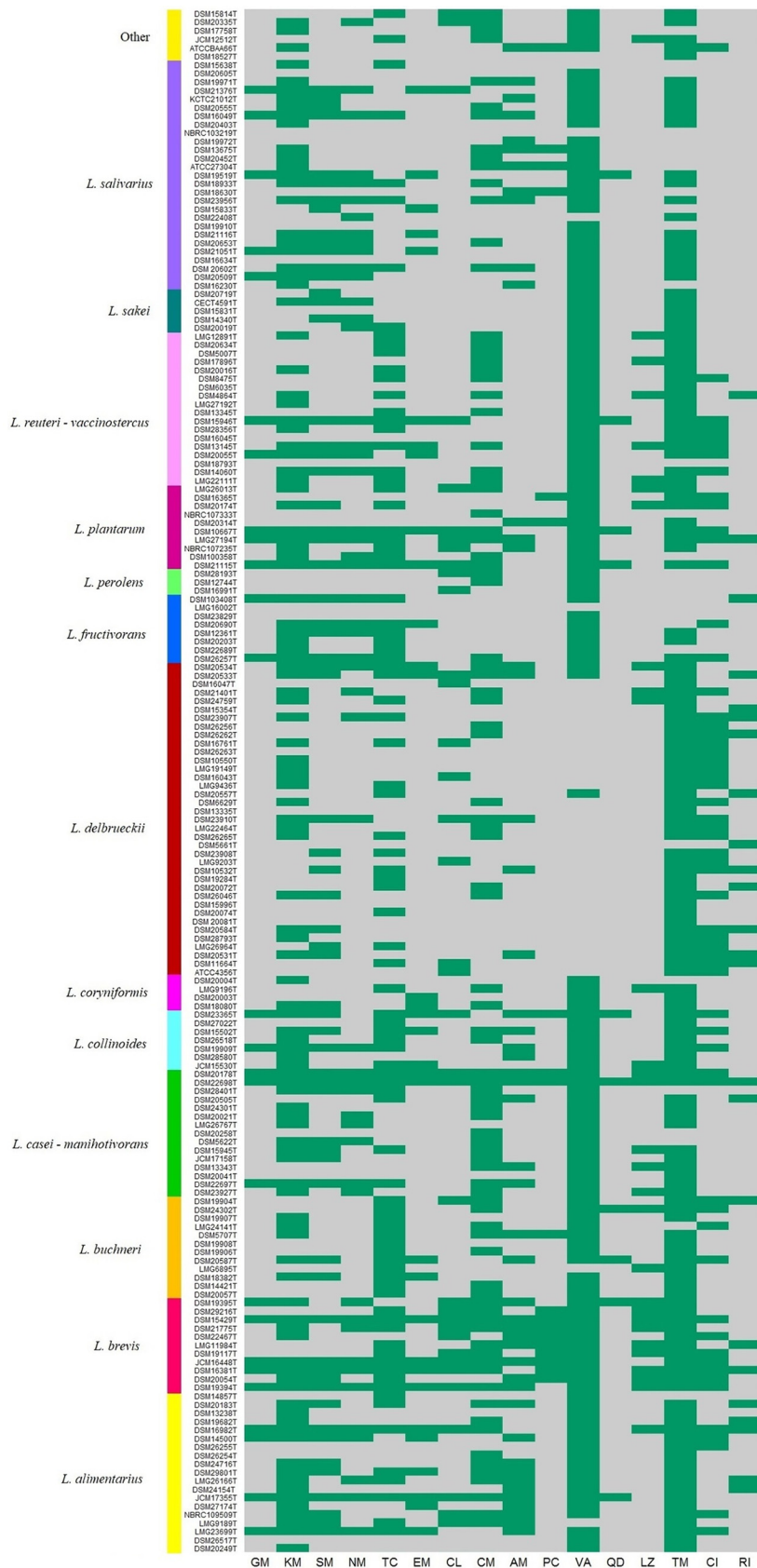


FIG 1 Resistance profiles of 182 type strains of the genus *Lactobacillus* compared with epidemiological cutoff values provided in references 9, 30, and 31. Resistant strains with MIC values higher than the (Continued on next page)

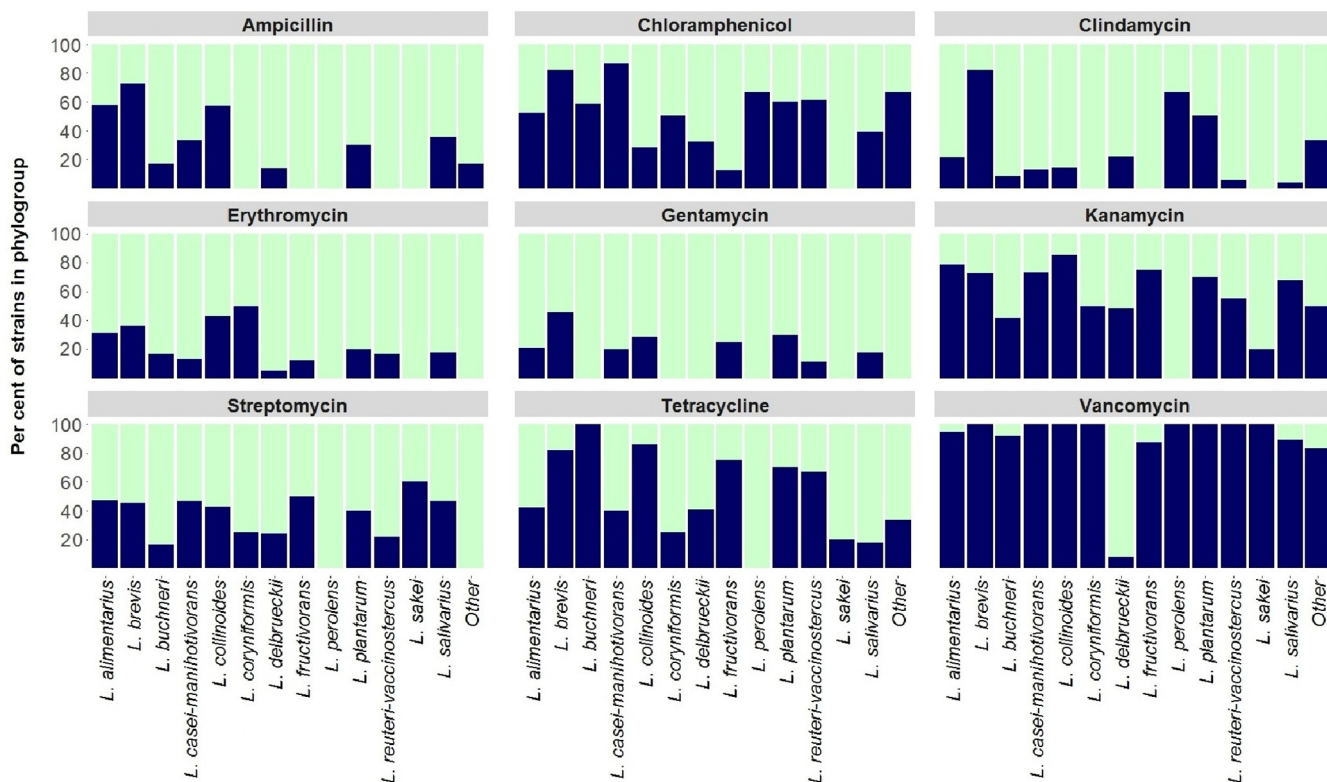


FIG 2 Prevalence of antibiotic-resistant (blue) and antibiotic-susceptible (green) strains within the *Lactobacillus* phylogroups tested for antimicrobial agents specified in reference 9, including inhibitors of cell wall synthesis (ampicillin and vancomycin), inhibitors of protein synthesis (erythromycin, clindamycin, chloramphenicol, and tetracycline), and aminoglycosides (gentamicin, kanamycin, and streptomycin).

L. equigenerosi DSM 18793^T, *Lactobacillus capillatus* DSM 19910^T, and *Lactobacillus vini* DSM 20605^T showed resistance only toward vancomycin.

Overall, the 182 type strains showed high susceptibility to gentamicin, erythromycin, penicillin, quinupristin-dalfopristin, linezolid, and rifampin (Fig. 1). In fact, only 5% of the strains investigated (10 out of 182) were resistant to quinupristin-dalfopristin. In contrast, the resistance levels toward ampicillin were higher than those identified for penicillin, and they were mainly detected in members of the phylogroups *L. brevis* (73%), *L. alimentarius* (58%), and *L. collinoides* (57%) (Fig. 2).

Notably, 50% and 49% of the type strains examined in this study were resistant to tetracycline and chloramphenicol, respectively, and 31% were resistant to both antibiotics. Tetracycline resistance phenotypes were mainly observed in species of the phylogroups *Lactobacillus buchneri*, *L. collinoides*, *L. plantarum*, *L. reuteri-L. vaccinostercus*, *L. fructivorans*, and *L. brevis*, while members of the phylogroups *L. brevis*, *Lactobacillus casei-L. manihotivorans*, and *Lactobacillus perolens* showed the highest prevalences of resistance to chloramphenicol (Fig. 2). For clindamycin and streptomycin, the resistance levels were low, at 20% and 18%, respectively, out of the 182 *Lactobacillus* strains analyzed. Finally, with regard to aminoglycosides, kanamycin resistance occurred in at least 50% of the members of all *Lactobacillus* phylogroups except for *L. perolens*, which was very susceptible to this antibiotic. Streptomycin resistance was observed in 35% of the strains examined.

FIG 1 Legend (Continued)

ECOFF are indicated in green, whereas sensitive strains are depicted in gray. Strains are clustered by the phylogroups reported by references 25 and 29 and are demarcated by the colored bar on the left of the heat plot. GM, gentamicin; KM, kanamycin; SM, streptomycin; NM, neomycin; TC, tetracycline; EM, erythromycin; CL, clindamycin; CM, chloramphenicol; AM, ampicillin; PC, penicillin; VA, vancomycin; QD, quinupristin-dalfopristin; LZ, linezolid; TM, trimethoprim; CI, ciprofloxacin; RI, rifampin.

Identification of AR genes. According to the EFSA guidelines, lactobacilli intended for human consumption should be tested for their resistance to gentamicin, kanamycin, streptomycin, tetracycline, erythromycin, clindamycin, chloramphenicol, ampicillin, and vancomycin, and they should be genetically investigated for the absence of acquired or transferable AR determinants (9). Thus, the genome sequences for 161 out of the 182 type strains (21 genome sequences were not available at the time of the study) tested for phenotypic resistance were aligned against the protein sequences of AR genes in the Comprehensive Antibiotic Resistance Database (CARD). Based on the selection criteria and manual annotation (see Materials and Methods), a total of 146 gene sequences were identified among the type strains analyzed, which are predicted to encode resistance to aminoglycosides (20 sequences), tetracycline (18), erythromycin (6), clindamycin (60), and chloramphenicol (42) (Fig. S1).

Genes encoding penicillin binding proteins (PBPs) and D-alanine D-alanine ligase (Ddl) involved in ampicillin and vancomycin resistance, respectively, were found in all genomes investigated. Furthermore, amino acid sequence analysis of the PBPs for the 161 type strains confirmed the presence of conserved amino acid residues in the known binding site motif for β -lactams. The Ddl enzyme of all vancomycin-resistant type strains exhibited a conserved phenylalanine (F) residue in the active site of the enzyme, while all members of the phylogroup *L. delbrueckii*, which were susceptible to vancomycin, were characterized by the presence of a tyrosine (Y) residue at this position, with the exception of strains *L. jensenii* DSM 20557^T, *Lactobacillus amylophilus* DSM 20533^T, and *Lactobacillus amylophilus* DSM 20534^T. Their respective proteins carried the Y-type motif, even though they were resistant to vancomycin. However, *Lactobacillus amylophilus* DSM 20533^T and *Lactobacillus amylophilus* DSM 20534^T harbored specific D-alanine-D-lactate ligase sequences in their genomes, which could explain their vancomycin resistance phenotypes. In addition, *L. sanfranciscensis* LMG 16002^T, *Lactobacillus hilgardii* LMG 6895^T, *Lactobacillus composti* DSM 18527^T, *L. pobuzihii* NBRC 103219^T, *Lactobacillus farciminis* LMG 9189, *Lactobacillus ceti* DSM 22408^T, and *Lactobacillus algidus* DSM 15638^T were characterized by the presence of Ddl of the F type, despite their susceptibility to vancomycin (Fig. S2).

Aminoglycoside resistance genes. Twenty different sequences predicted to encode aminoglycoside-modifying enzymes were identified among the 161 *Lactobacillus* genomes, which were mainly acetyltransferases (AACs) (7 sequences), nucleotidyltransferases (ANTs) (8 sequences), and phosphotransferases (APHs) (5 sequences) (Fig. S1). In particular, the AAC(3) family N-acetyltransferase was found in five *L. brevis* phylogroup genomes and two *L. delbrueckii* phylogroup members. All these strains showed resistance to kanamycin, and some of them were also resistant to streptomycin, such as *Lactobacillus acidifarinae* DSM 19394^T, *Lactobacillus koreensis* JCM 16448^T, *L. spicheri* DSM 15429^T, and *Lactobacillus hominis* DSM 23910^T. Moreover, *L. zymae* DSM 19395^T showed resistance to gentamicin, whereas *Lactobacillus namurensis* DSM 19117^T was susceptible to aminoglycosides despite harboring the *aac(3)* gene.

Gene sequences coding for nucleotidyltransferase enzymes, such as *ant(6)* and *ant(9)*, were identified in 7 type strains, as follows: *L. amylophilus* DSM 20533^T and *L. amylophilus* DSM 20534^T (*L. delbrueckii* phylogroup), *Lactobacillus fabifermentas* DSM 21115^T (*L. plantarum* phylogroup), *Lactobacillus animalis* DSM 20602^T, and *L. pobuzihii* NBRC 103219^T (*L. salivarius* phylogroup), *Lactobacillus sharpeae* DSM 20505^T (*L. casei-L. manihotivorans* phylogroup), and *Lactobacillus rossiae* DSM 15814^T ("other" phylogroup). In particular, the *ant(9)* gene was only found in the genome of *L. pobuzihii* NBRC 103219^T, despite its phenotypic susceptibility toward aminoglycosides. Similarly, the *ant(6)* gene was found in *L. sharpeae* DSM 20505^T and *L. rossiae* DSM 15814^T, which were susceptible to aminoglycosides. Conversely, the presence of this AR determinant in *L. amylophilus* DSM 20533^T, *L. amylophilus* DSM 20534^T, *L. fabifermentas* DSM 21115^T, and *L. animalis* DSM 20602^T might explain their resistance to kanamycin and streptomycin. Interestingly, in these strains, the predicted amino acid sequence encoded by the *ant(6)* gene found in the genomes of *L. animalis* DSM 20602^T and *L.*

amylophilus DSM 20533^T shared 99% similarity with the ANT6 aminoglycoside nucleotidyltransferases of *Streptococcus suis* (NCBI RefSeq accession no. [WP_044770667.1](#)) and *Clostridium difficile* (NCBI RefSeq accession no. [WP_077726164.1](#)). Regarding phosphotransferases, the *aph(3)* gene was found in five members of the *L. delbrueckii* phylogroup (*Lactobacillus acidophilus* ATCC 4356^T, *Lactobacillus gasseri* LMG 9203^T, *Lactobacillus johnsonii* LMG 9436^T, *Lactobacillus kalixensis* DSM 16043^T, and *Lactobacillus pasteurii* DSM 23907^T), which showed resistance to kanamycin, except for ATCC 4356^T and LMG 9203^T.

Clindamycin resistance genes. The *lsa* gene encoding a lincosamide efflux protein was found in 60 strains, 13 of which displayed resistance to clindamycin. Alignment of the amino acid sequence of the Lsa efflux protein revealed a truncated carboxy terminus for the predicted Lsa proteins in *L. hominis* DSM 23910^T, *Lactobacillus gallinarum* DSM 10532^T, and *Lactobacillus iners* DSM 13335^T. Four conserved amino acid domains were found in the remaining 57 sequences, corresponding to two copies of Walker A and B motifs, which play an important role in ATP binding and hydrolysis that energizes efflux (Fig. S3).

Chloramphenicol resistance genes. Among the 161 type strains of the genus *Lactobacillus*, 36 chloramphenicol resistance-related sequences were found coding for chloramphenicol acetyltransferase, like the *cat* gene, and for specific membrane-associated transporters, like *CmlA*. The *cat* gene was detected in 34 lactobacilli, two of which (*Lactobacillus kimchicus* JCM15530^T and *L. similis* DSM 23365^T) carried two copies of this gene. Moreover, *Lactobacillus hammesii* DSM 16381^T, *L. koreensis* JCM 16448^T, *L. namurensis* DSM 19117^T, and *L. zymae* DSM 19395^T (belonging to the phylogroup *L. brevis*) were characterized by the presence of either the *cmlA* or *cat* gene. Conversely, *L. acidifarinae* DSM 19394^T and *L. selangorensis* ATCC BAA66^T displayed only the presence of *cmlA*.

Tetracycline resistance genes. The 18 gene sequences found among *Lactobacillus* strains code for ribosomal protection proteins [*tet(M)*, *tet(S)*, *tet(Q)*, and *tet(W)*] and efflux pumps [*tet(L)* and *tet(P)*]. The *tet(L)* gene was found in the tetracycline-resistant strains *Lactobacillus suebicus* DSM 5007^T and *Lactobacillus ingluviei* DSM 15946^T. *L. ingluviei* DSM 15946^T was also characterized by the presence of *tet(W)* and *tet(M)*. Interestingly, *tet(M)* in DSM 15946^T exhibited 99% residue identity with the corresponding sequences of *Enterococcus faecalis* (NCBI RefSeq accession no. [WP_049098680.1](#)), *Enterococcus faecium* (NCBI RefSeq accession no. [WP_01077232.1](#)), *Streptococcus pneumoniae* PT814 (GenBank accession no. [HG799502.1](#)), and *Staphylococcus epidermidis* (NCBI RefSeq accession no. [WP_002403674.1](#)). Similarly, the sequence of *tet(L)* displayed 99% residue identity with those carried by *E. faecalis* (NCBI RefSeq accession no. [WP_002387933.1](#)), *E. faecium* (NCBI RefSeq accession no. [WP_096541192.1](#)), and *Streptococcus agalactiae* (NCBI RefSeq accession no. [WP_041974946.1](#)). *tet(W)* showed 99% residue identity with the sequences of *Trueperella pyogenes* OX9, *Bifidobacterium longum* subsp. *longum* F21, and *C. difficile* CI7. *tet(W)* was also identified in *L. pasteurii* DSM 23907^T, while *tet(M)* was found in *L. sharpeae* DSM 20505^T (*L. casei*-*L. manihottivorans* group), *L. acidophilus* ATCC 4356^T, *Lactobacillus crispatus* DSM 20584^T, *L. gallinarum* DSM 10532^T, *L. amylophilus* DSM 20533^T, *L. amylophilus* DSM 20534^T (*L. delbrueckii* group), and *L. equigenerosi* DSM 18793^T (*L. reuteri*-*L. vaccinostrercus* group). Strains DSM 20584^T, ATCC 4356^T, and DSM 18793^T showed susceptibility to tetracycline. Regarding other ribosomal protection proteins, the *tet(Q)* and *tet(S)* genes were found in *L. brevis* DSM 20054^T and *Lactobacillus heilongjiangensis* LMG 26166^T, respectively, both being resistant to tetracycline, while *tet(P)* was found in *L. gasseri* LMG 9203^T, *Lactobacillus taiwanensis* DSM 21401^T, *Lactobacillus ruminis* DSM 20403^T, and *L. johnsonii* LMG 9436^T. Only LMG 9436^T showed phenotypic resistance to tetracycline.

Erythromycin resistance genes. Across the 161 *Lactobacillus* genomes, six gene sequences predicted as contributing to erythromycin resistance were detected, which include the *erm(B)* gene coding for a predicted rRNA methylase, and two variants of the *mef*, *mef(E)*, and *mef(B)* genes, encoding macrolide efflux pumps. These genes were

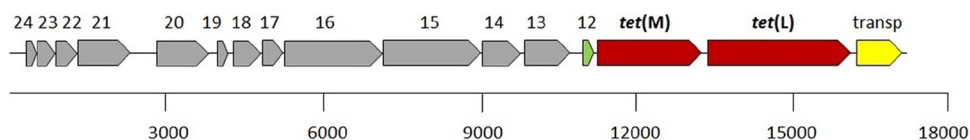


FIG 3 Diagram showing the genetic organization of the Tn916-like transposon (transp) identified in *L. ingluviei* DSM 15946^T. Red, AR genes; yellow, genes involved in genetic transfer; gray, ORFs involved in the conjugation process; green, regulatory sequences. Numbers above the diagram refer to loci in Tn916; numbers below are a base pair scale.

identified in three erythromycin-susceptible strains, namely *L. delbrueckii* subsp. *lactis* DSM 20072^T [*mef*(E)], *L. casei* DSM 20041^T, and *Lactobacillus paracasei* subsp. *paracasei* DSM 5622^T [*mef*(B)]. *erm*(B) was found in the erythromycin-resistant strains *L. amylophilus* DSM 20533^T, *L. amylotrophicus* DSM 20534^T, and *L. ingluviei* DSM 15946^T, which also showed the presence of tetracycline resistance determinants.

Analysis of ORFs in regions flanking tetracycline and erythromycin resistance genes. Tetracycline and erythromycin are two of the most widely used antimicrobials in human and animal therapy, and genetic determinants involved in the resistance to those antibiotics are usually associated with mobile genetic elements. Therefore, upstream and downstream regions of the tetracycline and erythromycin resistance genes were further investigated in *L. ingluviei* DSM 15946^T, *L. amylophilus* DSM 20533^T, and *L. amylotrophicus* DSM 20534^T, the only three strains that simultaneously harbored erythromycin and tetracycline resistance genes within the data set. Sequence analysis of the *L. ingluviei* DSM 15946^T genome revealed that *tet*(M) and *tet*(L) were closely located in the same genomic region, separated by 146 nucleotides. Upstream of *tet*(M), 12 open reading frames (ORFs) encoding conjugation transfer elements were identified that shared 99% residue identity with the corresponding sequences of the Tn5251 transposon carried by *S. pneumoniae* DP1322. Moreover, a sequence predicted to encode a transposase was found downstream of the *tet*(L) determinant (Fig. 3). This is a common configuration for a Tn916-like transposon. The analysis of the distribution of these genetic elements in the available genome sequences of *Lactobacillus* isolates revealed that the predicted transposon carried by *L. ingluviei* DSM 15946^T (GenBank accession no. CP016400.1) is essentially identical to that harbored by *L. johnsonii* BS15, except for the presence of a recombinase downstream of the *tet*(L) gene. In contrast, *L. salivarius* JCM 1046 (GenBank accession no. CP007650.1) and *L. salivarius* JCM 1047 (GenBank accession no. NBEF01000044.1) lack the *tet*(L) determinant, and the regulation region has been retained, similar to that of the Tn5251 transposon in *S. pneumoniae* DP1322. These two transposons are essentially identical except for the presence of ORF 23 in *L. salivarius* JCM 1047, which was not identified in the transposon harbored by *L. salivarius* JCM 1046. Interestingly, *L. iners* UMB1051 (GenBank accession no. PNGO01000001.1) was characterized by the presence of a Tn916-like transposon in which a Tn917 carrying an *erm*(B) gene was inserted, resulting in a Tn3872-like transposon (Fig. S4). The *tet*(W) and *erm*(B) genes were located in the same genomic region and shared 99% similarity with the corresponding sequences of the strain *S. suis* SsCA. Moreover, these AR determinants were flanked by regions with high similarity (99%) to replication proteins and to the integrase of the plasmid pLR581 of *Lactobacillus reuteri* SD2112 (GenBank accession no. CP002845.1) (Fig. 4).

L. amylophilus and *L. amylotrophicus* harbored the *tet*(M) and *erm*(B) genes in two different genomic regions, and they shared 99% residue identity with the corresponding sequences of *S. agalactiae* SG-M4 and *Staphylococcus hyicus* HW17, respectively. The flanking region structures of these AR determinants were identical at the amino acid level in the two type strains. In particular, the up- and downstream sequences surrounding *tet*(M) were characterized by the presence of several genes predicted to encode conjugation proteins and transposases (Fig. 5A). For *erm*(B), the upstream region showed an 83-bp sequence corresponding to a 27-amino-acid leader peptide. A gene encoding a mobilization protein was found upstream of the leader peptide

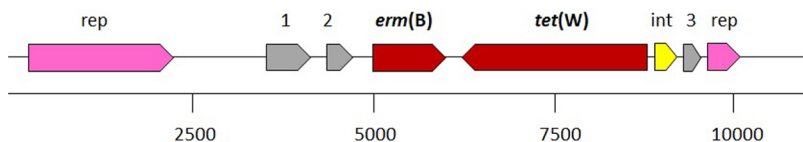


FIG 4 Genetic organization of sequences surrounding the *tet(W)* and *erm(B)* genes identified in *L. ingluviei* DSM 15946^T. Red, AR genes; yellow, genes involved in genetic transfer; pink, genes encoding plasmid-associated replication proteins; gray, gene coding for hypothetical proteins. Numbers above the diagram refer to loci; numbers below are a base pair scale. Rep, plasmid replication; int; plasmid integration.

sequence, sharing 99% residue identity with the corresponding sequence in the *S. hyicus* plasmid pSTE1 (GenBank accession no. [HE662694.1](https://www.ncbi.nlm.nih.gov/nuccore/HE662694.1)) (Fig. 5B). Unfortunately, the location of *erm(B)* at the 3' end of the contig did not allow for the characterization of the downstream region.

Phenotype-genotype correlation. Overall, phenotypic resistance correlated with genotypes for 67% of the cases examined with genomic data. In detail, the genotype was in accordance with the phenotype for 892 out of 1,449 phenotypic tests investigated, which included 782 cases representing a susceptible phenotype toward a specific antibiotic linked to the absence of AR determinants and 110 cases for which the resistance phenotype correlated with the presence of one or more AR genes. Most notably, the F-type Ddl enzyme was found in 99% of the vancomycin-resistant *Lactobacillus* strains, revealing the high relationship between genotype and phenotype for this antibiotic. For aminoglycosides, the 20 AR determinants identified in this study might explain the resistance phenotype for 13.7%, 14.2%, and 20.8% of the strains for kanamycin, streptomycin, and gentamicin, respectively. For chloramphenicol, the *cat* and *cmIA* genes were found in 20 strains out of 79 chloramphenicol-resistant lactobacilli and in 59 strains out of 82 lactobacilli susceptible to this antibiotic. However, the genetic basis of chloramphenicol resistance was not revealed for 74.7% of the resistant strains. The presence of the *Isa* gene positively correlated with the resistance phenotype for 40.6% of the strains. However, 47 strains harbored the *Isa* gene in their genomes even though they showed susceptibility to clindamycin. For tetracycline and erythromycin, the presence of AR genes explained the resistance phenotype for 12.7% and 10.3% of the strains, respectively. However, in a significant number of examples (364 cases), a genetic basis was not detected for a demonstrated resistance phenotype (Fig. 6).

DISCUSSION

The data reported in the present study provide evidence at phenotype and genotype levels for rationally revising the regulatory guidelines for safety assessment of

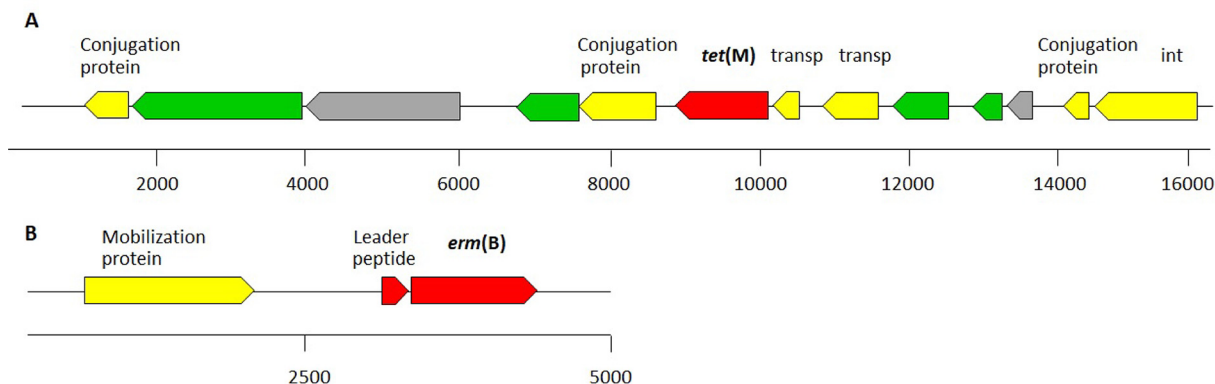


FIG 5 Genetic organization of sequences surrounding the *tet(M)* (A) and *erm(B)* (B) genes identified in *L. amylophilus* DSM 20533^T and *L. amylotrophicus* DSM 20534^T. Red, AR genes; yellow, genes involved in genetic transfer; green, genes encoding regulatory proteins; gray, gene coding for hypothetical proteins. int, integrase. Numbers below the diagram are a base pair scale.

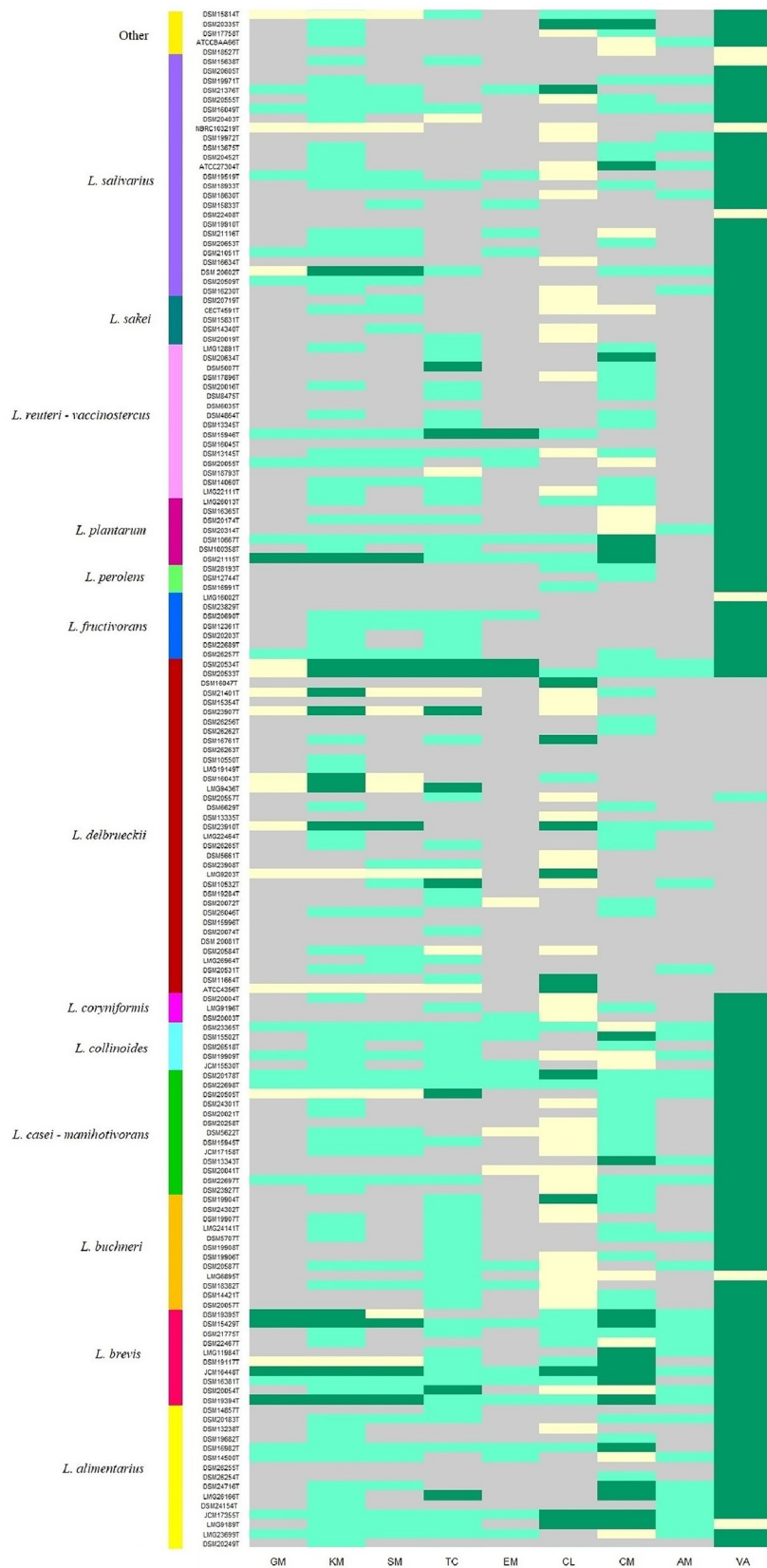


FIG 6 Phenotype-genotype correlation analysis for the 161 type strains of the genus *Lactobacillus*. Positive correlations between genomic data and phenotypes observed are presented in green and gray, whereas negative correlations are indicated in yellow and blue. GM, gentamicin; KM, kanamycin; SM, streptomycin; TC, tetracycline; EM, erythromycin; CL, clindamycin; CM, chloramphenicol; AM, ampicillin; VA, vancomycin.

lactobacilli, showing that certain resistances are widespread within the genus. For example, low-level resistance to linezolid, rifampin, and quinupristin-dalfopristin was generally observed for type strains, in line with previous reports (30–35). We also confirmed low-level resistance to rifampin, as previously reported for *Lactobacillus* species isolated from the human GIT (36), traditional dairy products (37), and in probiotic strains in marketed foods and drugs (38). Generally, we found *Lactobacillus* species to be susceptible to low concentrations of β -lactams, including penicillin and ampicillin (16), even though atypical insensitivity to higher concentrations of these cell wall inhibitors has been reported for some *L. crispatus* and *L. johnsonii* strains isolated from healthy chickens (39). Higher levels of resistance to ampicillin compared to penicillin were detected for the type strains analyzed, in line with some previous studies (40–42). Interestingly, ampicillin resistance in *L. reuteri* strains has been attributed to point mutations in the genes encoding the penicillin binding proteins (PBPs) (43), but the PBP sequences for the 161 type strains in this study all retained conserved amino acid residues (i.e., linked to sensitivity) in the binding sites for these antibiotics.

Conversely, the *Lactobacillus* strains showed high intrinsic (as opposed to acquired) resistance to trimethoprim and vancomycin. Folate auxotrophic lactobacilli have been reported as being intrinsically resistant to trimethoprim (44), including *L. johnsonii*, *L. acidophilus*, *L. salivarius*, *L. brevis*, *L. casei*, *L. gasseri*, *L. rhamnosus*, *L. delbrueckii*, *Lactobacillus fermentum*, *Lactobacillus helveticus*, *L. plantarum*, *L. reuteri*, *Lactobacillus sakei*, and *L. crispatus* (45), where the vancomycin-resistant phenotypes are perhaps the best-characterized resistance mechanisms described in lactobacilli (16), with many species being intrinsically resistant (30). Exceptions to this are *L. delbrueckii*, *L. acidophilus*, *L. johnsonii*, and *L. crispatus*, where the vancomycin-susceptible phenotype has been associated with the presence of a Y-type Ddl enzyme (46). In this genus-wide analysis, the vancomycin-susceptible strains were mainly represented by almost all members of the *L. delbrueckii* phylogroup, which were characterized by the presence of a Y-type Ddl. The substitution of tyrosine 261 in the Ddl enzyme by a phenylalanine residue (F-type enzyme) is associated with the synthesis of peptidoglycan precursors containing a D-Ala-D-Lac residue conferring vancomycin resistance (46). Notably, almost all vancomycin-resistant strains in this study carried a Ddl of the F type. However, we noted inconsistencies between phenotypes and genotypes for vancomycin in 8 of the species (*L. jensenii*, *L. sanfranciscensis*, *L. hilgardii*, *L. composti*, *L. pobuzihii*, *L. farciminis*, *L. ceti*, and *L. algidus*), which could be due to the presence of alternative resistance mechanisms or to alteration of gene expression.

Overall, *Lactobacillus* type strains displayed higher resistance toward kanamycin and streptomycin than to gentamicin and neomycin. Aminoglycoside resistance has been described as an intrinsic feature for some *Lactobacillus* species (i.e., *L. rhamnosus*, *L. acidophilus*, *L. delbrueckii* subsp. *bulgaricus*, and *L. helveticus*) (47, 48) due to the lack of cytochrome-mediated drug transport (15). In contrast, high-level susceptibility to gentamicin is most likely linked to the superior ability of this antibiotic to cross the membrane compared to other aminoglycosides (49).

We found genes for nucleotidyltransferases, such as *ant(6)* and *ant(9)*, in 7 type strains, 4 of which showed resistance to kanamycin and streptomycin (*L. amylophilus*, *L. amylophilus*, *L. fabifermentas*, and *L. animalis*), in accordance with the high affinity of ANT6 for streptomycin (50). Moreover, the nucleotide sequence of the *ant(6)* gene harbored by *L. animalis* DSM 20602^T and *L. amylophilus* DSM 20533^T was 99% identical to the sequences in *S. suis* and *C. difficile*, of which *S. suis* is usually associated with the animal GIT (51–53), from which *L. animalis* strains can be isolated (54). The GIT is characterized by a high cell density, which can lead to microbial interactions and facilitates horizontal gene transfer (HGT) events (55, 56). These findings suggest that the *ant(6)* gene is undergoing HGT between commensal/food bacteria and pathogenic species, which in and of itself is a particular concern. As such, these lactobacilli could be acting as vectors for AR genes in the food chain and the gut. Furthermore, the *ant(6)* and *aph(3)* genes are usually found on plasmids or transposons, increasing the risk of resistance dissemination between different bacteria (57). We found the *aph(3)* and

aac(3) genes in 9 *Lactobacillus* strains conferring resistance to kanamycin, in accordance with the substrate specificity of these AR determinants for aminoglycosides (46). Moreover, the *aph(3)* gene has been identified in *Lactobacillus* strains resistant to kanamycin, streptomycin, and gentamicin in a previous study (50).

Although the majority of *Lactobacillus* species are susceptible to antibiotics that inhibit protein synthesis, including erythromycin, tetracycline, clindamycin, and chloramphenicol (14), resistance to tetracycline and chloramphenicol was the most common phenotype detected. Even though this *Lactobacillus* type strain panel was characterized by low-level resistance to clindamycin, some strains carried the *Lsa* gene, which in *E. faecium* plays a key role in resistance to clindamycin and quinupristin-dalfopristin (58). The ABC transporter Walker A and B motifs (59) are conserved in the Lsa protein predicted for the strains in this study, suggesting that it is functional.

Chloramphenicol (*cat* genes) and tetracycline resistance determinants [*tet(M)*, *tet(S)*, *tet(W)*, *tet(O)*, and *tet(Q)*] are the most commonly acquired resistance genes found in lactobacilli (15, 19, 39, 60, 61). In particular, the most widespread resistance genes are *tet(M)* and *tet(S)* in foodborne and probiotic bacteria (12, 14, 62) due to the frequent association of *tet(M)* with conjugative transposons, such as Tn916 (63), which was corroborated by this study. Furthermore, our data revealed the association of *tet(M)* with a Tn916-like transposon in the genome of *L. ingluviei* DSM 15946^T. This transposon sequence is 99% identical to the Tn5251 of *S. pneumoniae* DP1322 (64), except for the presence of *tet(L)* in Tn916. Notably, this study reports a Tn916-like transposon in *L. ingluviei*, and the transferability of this element warrants further investigation. A Tn916-like transposon has been previously identified in *L. paracasei* and *L. sakei* isolated from Italian traditional cheese (65, 66) and in *L. salivarius* JCM 1046 (22).

Notably, we detected the simultaneous presence of *tet(M)* and *erm(B)* genes in *L. amylophilus* DSM 20533^T and *L. amylotrophicus* DSM 20534^T, which were flanked by transposases and conjugative proteins in both organisms. This is probably reflective of/arising from the close phylogenetic relatedness between *L. amylophilus* and *L. amylotrophicus* (67). The *erm(B)* gene (associated with erythromycin resistance) has been reported in *Lactobacillus* species in several studies (19, 30, 60, 68–70), where it is generally found in conjugative transposons located in chromosomes, in plasmids, and in nonconjugative transposons, such as Tn917 and Tn551 (15). These observations suggest that *L. ingluviei*, *L. amylophilus*, and *L. amylotrophicus* could act as dissemination vectors for tetracycline and erythromycin resistance. Strains of *L. ingluviei* and *L. amylophilus* are commonly isolated from animal GIT (71), whereas *L. amylotrophicus* has been found in swine waste (67); these are considered potential hotspots for promoting the dissemination of AR genes in the environment. Indeed, the transfer of *erm(B)* and *tet(M)* from lactobacilli to other microorganisms has been previously demonstrated *in vitro* (62, 68, 72). The present study reveals that in many cases, the genetic basis for AR remains unknown, as no known genes were identified which could explain the associated phenotype. This could be due to the inherent insensitivity of some of the strains to particular antibiotics due to intrinsic factors, such as cell envelope (wall/membrane) structure or the ability to produce polysaccharides. Indeed, it would be very difficult to predict these phenotypes based on just database comparisons alone, and as such, functional studies would be required to probe the basis of these resistances more deeply. Overall, this pangenomic study reveals that while AR is widespread in lactobacilli, the level of resistance to widely used antibiotics in human clinical medicine is low or not so common (with the possible exception of erythromycin and tetracycline). However, the vast majority (88%) of these strains were found to fail the EFSA guidelines for AR and as such might encounter problems in obtaining regulatory approval for food use unless the resistance is proven to be nontransferable. As such, opportunities to develop new applications for novel *Lactobacillus* strains could be delayed or lost, which highlights the urgent need to fundamentally understand AR and its spread within members of the genus. Only then can a proper framework to regulate their use for human and animal purposes be implemented.

MATERIALS AND METHODS

Bacterial strains and growth conditions. The 197 type strains of the *Lactobacillus* genus used in this study are listed in Table 1 and were obtained from the American Type Culture Collection (ATCC, Manassas, VA), BCCM/LMG Bacteria Collection (Ghent, Belgium), the Spanish Type Culture Collection (CECT, Valencia, Spain), the German Collection of Microorganisms and Cell Cultures (DSMZ, Braunschweig, Germany), the Korean Collection for Type Cultures (KCTC, Jeollabuk-do, South Korea), the Japan Collection of Microorganisms (JCM, Koyadai Tsukuba, Japan), and the NITE Biological Resource Centre (NBRC, Nishihara, Japan). *Lactobacillus* strains were grown in de Man-Rogosa-Sharpe (MRS) medium (Thermo Fisher Scientific, Waltham, MA, USA) under specific conditions reported in Table 1 and kept in liquid cultures with 20% (w/vol) glycerol at -80°C for long-term storage.

Antimicrobial susceptibility testing. The MICs of several antibiotics were determined using broth microdilution methods according to the Clinical and Laboratory Standards Institute (CLSI; www.clsi.org), the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org), and ISO standards. In particular, VetMIC plates (National Veterinary Institute, Uppsala, Sweden) for LAB were used containing serial 2-fold dilutions of 16 antibiotics (ampicillin, ciprofloxacin, clindamycin, chloramphenicol, erythromycin, gentamicin, kanamycin, linezolid, neomycin, penicillin, quinupristin-dalfopristin, rifampin, streptomycin, tetracycline, trimethoprim, and vancomycin). These antibiotics represent the main classes of antimicrobials employed in human and veterinary treatments and overlap the main antibiotics of interest to the EFSA.

MICs were evaluated in LAB susceptibility test medium (LSM) (73), a mixed formulation containing 90% Iso-Sensitest broth and 10% MRS Difco broth (Beckton, Dickinson and Company, Le Pont-de-Claix, France) supplemented with 0.05% (wt/vol) L-cysteine, as described in the ISO 10932 (IDF 223) document (74) and recommended by the EFSA (9). *L. paracasei* LMG 12586 was used as a control strain. Briefly, individual *Lactobacillus* strains were grown on MRS agar (with supplements as required for the specific strains and incubation for 24 to 48 h depending on the strain). One-microliter sterile loops with biomass from approximately 3 to 5 colonies were suspended in 4 ml maximum recovery diluent (MRD; Oxoid Ltd., Basingstoke, Hants, UK) sterile saline solution to obtain a concentration of approximately 3×10^8 CFU/ml in each case. This suspension was diluted 1:1,000 in LSM broth (final concentration, approximately 3×10^5 CFU/ml), and then 100 μl was added to each well of the VetMIC plate. This test was performed using a minimum of three biological replicates for each strain. Plates were incubated under anaerobic conditions at the recommended temperatures for 48 h. MICs were read as the lowest concentration of an antimicrobial agent at which visible growth was inhibited. Epidemiological cutoff (ECOFF) values were retrieved from reference 9. Breakpoints for antibiotics not covered by EFSA were adopted from references 30 and 31.

Identification of resistance genes. The annotated sequences of the available genomes for the type strains of the genus *Lactobacillus* (25) were downloaded from NCBI using the accession numbers reported in Table 1. These sequences were employed to query the Comprehensive Antibiotic Resistance Database (CARD, version 1.0.6; <http://arpcard.mcmaster.ca>) (75) through the Basic Local Alignment Search Tool (BLAST; <https://blast.ncbi.nlm.nih.gov>) in order to identify all AR genes involved in the resistance phenotypes observed. A gene was annotated as a putative AR determinant according to its best BLASTP hit in CARD, with a threshold of amino acid sequence identity of $>30\%$ and query coverage of $>70\%$. In addition, the amino acid sequences of all AR genes retrieved from CARD, resulting in a reference data set of 2,163 amino acid sequences, were aligned against the annotated genome sequences of the collection, and the best BLASTP hits were filtered as described above. In order to minimize putative false-negative or false-positive outputs, only the putative AR determinants obtained from both approaches were considered for subsequent analyses. Specifically, each putative AR determinant was manually annotated querying the NCBI nonredundant (NR) protein database to verify its putative function in the resistome and to determine its involvement in acquired phenotypes.

Phenotype-genotype correlation. By focusing on the nine antibiotics for which the EFSA defined reference ECOFFs (9) and the genome sequences available for 161 *Lactobacillus* strains, a total of 1,449 phenotypic tests were considered for the phenotype-genotype correlation. Each interpretation of a resistant or susceptible phenotype to a given antimicrobial agent was compared with the presence or absence of a known corresponding resistance gene(s) manually annotated and/or structural gene mutations identified through genome sequence analysis (76–78). The overall correlation between phenotype and genotype was classified as positive when genomic data agreed with phenotypic testing; thus, resistance and susceptible phenotypes correlated with the presence or absence of one or more AR genes, respectively. Otherwise, the correlation was considered negative.

Flanking regions of the AR genes. The genetic composition of upstream and downstream sequences flanking tetracycline and erythromycin resistance genes was characterized by performing a BLASTN and BLASTX alignment of the contigs carrying the AR genes against the NCBI NR database. This analysis was carried out for *L. ingluviei* DSM 15946^T, *L. amylophilus* DSM 20533^T, and *L. amylophilus* DSM 20534^T and allowed us to identify mobile genetic elements which might be involved in the spread of AR determinants.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AEM.01738-18>.

SUPPLEMENTAL FILE 1, PDF file, 2.9 MB.

ACKNOWLEDGMENTS

Work in the APC Microbiome Ireland was supported by a centre award from Science Foundation Ireland, grant SFI/12/RC/2273. E.S. has received funding from the European Union's Horizon 2020 research and innovation program under the Marie Skłodowska-Curie grant agreement no. 659801. I.C. has received funding from the University of Verona under the CooperInt Internalization Program.

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