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ORIGINAL ARTICLE

Comparative genomics analysis of *Lactobacillus* species associated with weight gain or weight protection

F Drissi[1], V Merhej[1], E Angelakis[1], A El Kaoutari[1], F Carrière[2], B Henrissat[3,4] and D Raoult[1]

BACKGROUND: Some *Lactobacillus* species are associated with obesity and weight gain while others are associated with weight loss. *Lactobacillus* spp. and bifidobacteria represent a major bacterial population of the small intestine where lipids and simple carbohydrates are absorbed, particularly in the duodenum and jejunum. The objective of this study was to identify *Lactobacillus* spp. proteins involved in carbohydrate and lipid metabolism associated with weight modifications.

METHODS: We examined a total of 13 complete genomes belonging to seven different *Lactobacillus* spp. previously associated with weight gain or weight protection. We combined the data obtained from the Rapid Annotation using Subsystem Technology, Batch CD-Search and Gene Ontology to classify gene function in each genome.

RESULTS: We observed major differences between the two groups of genomes. Weight gain-associated *Lactobacillus* spp. appear to lack enzymes involved in the catabolism of fructose, defense against oxidative stress and the synthesis of dextrin, L-rhamnose and acetate. Weight protection-associated *Lactobacillus* spp. encoded a significant gene amount of glucose permease. Regarding lipid metabolism, thiolases were only encoded in the genome of weight gain-associated *Lactobacillus* spp. In addition, we identified 18 different types of bacteriocins in the studied genomes, and weight gain-associated *Lactobacillus* spp. encoded more bacteriocins than weight protection-associated *Lactobacillus* spp.

CONCLUSIONS: The results of this study revealed that weight protection-associated *Lactobacillus* spp. have developed defense mechanisms for enhanced glycolysis and defense against oxidative stress. Weight gain-associated *Lactobacillus* spp. possess a limited ability to breakdown fructose or glucose and might reduce ileal brake effects.

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INTRODUCTION

Obesity is a major public health concern and reflects perturbations of the balance between food intake and energy expenditure.[1] We recently proposed a new area of research based on correlations between intestinal microbiota, weight change, antibiotic and probiotic therapies and malnutrition relief.[2,3] Although antibiotics have been used for decades as growth promoters in livestock, a correlation between the increasing global use of antibiotics and weight gain or acquired obesity in humans has only recently been proposed, as most studies of antibiotics or probiotics did not measure weight gain.[4] Evidence suggests that some antibiotics are associated with weight gain in malnourished children, neonates and adults.[5–8] The precise mechanisms by which antibiotics improve growth performance are not well characterized, and it has been suggested that antibiotics, such as avoparcin (vancomycin), exert selective pressures on Gram-positive bacteria, and *Lactobacillus* species are resistant to glycopeptides.[4,8]

In the 1940s, it was revealed that the administration of *Streptomyces aureofaciens* probiotics in food resulted in weight gain in animals. Since then, probiotics have commonly been used in agriculture to maintain or improve the health and feed efficiency of livestock.[9,10] Probiotics have also been used to treat acute malnutrition in humans.[11] Moreover, experiments with animal models have revealed that probiotic therapy might result in weight gain.[12–14] Evidence suggests that bacteriocins largely determine the effects of probiotics in gut microbiota.[2,3] The effects of probiotics are strain dependent, and related probiotic strains can significantly differ in genotype and phenotype; thus, the features of one bacterial strain or species are not necessarily present in a related bacterium.[15] The results of a recent meta-analysis revealed that *Lactobacillus acidophilus*, *Lactobacillus fermentum* and *Lactobacillus ingluviei* probiotic treatment was associated with weight gain, whereas *Lactobacillus plantarum* and *Lactobacillus gasseri* treatment was associated with weight loss.[16] In addition, *Lactobacillus sakei* has also been associated with weight gain.[17]

The gut environment markedly differs between different anatomical regions in terms of physiology, substrate availability, host secretions, pH and oxygen tension. The stomach and proximal small intestine, containing 10^5 colony-forming units (CFU) per ml of facultative anaerobic bacteria, are responsible for most nutrient digestion and absorption in humans, and ~66–95% of the proteins and all fats are absorbed before entering the large intestine[18,19] (Figure 1). By contrast, the proportion of carbohydrates digested and absorbed in the small intestine depends on the type of diet and the content of these compounds in the digested substrates. Thus, sucrose, lactose and starch in our diet are digested by human enzymes and absorbed before reaching the colon, whereas all other complex carbohydrates are

[1]Aix Marseille Université, URMITE, UM63, CNRS 7278, IRD 198, Inserm 1095, Marseille, France; [2]CNRS, Aix-Marseille Université, Enzymologie Interfaciale et Physiologie de la Lipolyse, UMR 7282, 31 chemin Joseph Aiguier, Marseille, France; [3]Architecture et Fonction des Macromolécules Biologiques, Aix-Marseille Université, Marseille, France and [4]Centre National de la Recherche Scientifique, CNRS UMR 7257, Marseille, France. Correspondence: Professor D Raoult, Aix Marseille Université, URMITE, Faculté de Médecine, UM63, CNRS 7278, IRD 198, Inserm 1095, 27 Boulevard Jean Moulin, Marseille CEDEX 05, 13385, France.
E-mail: didier.raoult@gmail.com
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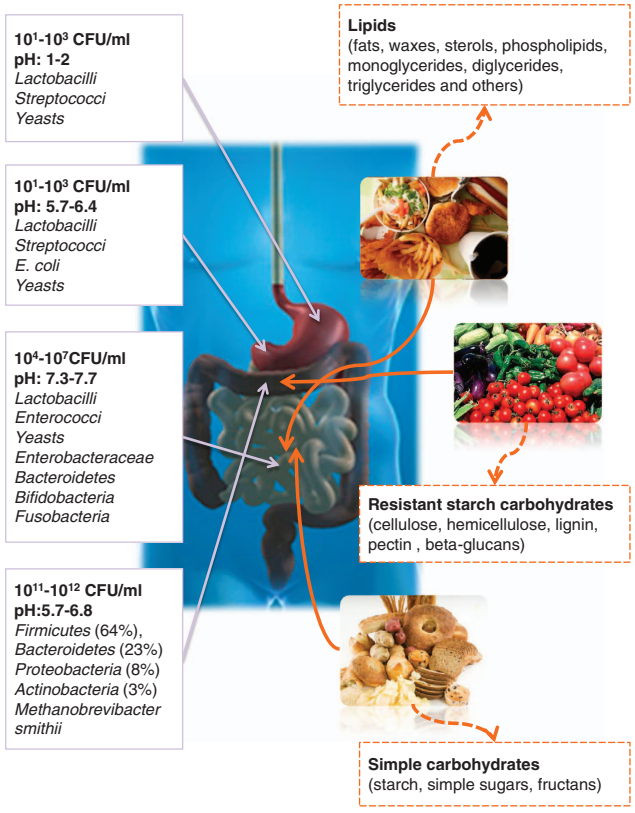


Figure 1. Microbial colonization of the human gastrointestinal tract and nutrients absorbed.

exclusively degraded and fermented by colonic bacteria.[20] Comparative genomics have revealed the unusual diversity of the genus *Lactobacillus* at both structural and functional levels.[21,22] The aim of this study was to examine the genomic content of *Lactobacillus* spp. associated with weight modification to identify the proteins associated with metabolism. Here, we analyzed 13 genomes of *Lactobacillus* spp. to identify the genes encoding bacteriocins and enzymes involved in carbohydrate and lipid metabolism.

MATERIALS AND METHODS

Search strategies

To identify the *Lactobacillus* spp. associated with weight modification, we searched PubMed database for peer-reviewed, English-language articles with no date restrictions. The search terms were combinations of '*Lactobacillus*', 'probiotics', 'microbiota' and 'weight', 'weight gain', 'weight protection', 'weight loss', 'weight change', 'weight modification', 'obesity', 'growth', 'body fat' or 'adipose tissue'. We retrieved the full text of the selected studies and searched the references cited in these articles. When necessary, we contacted the corresponding authors for further clarification or additional information.

Alignment and annotation of genomes using a combination of several search tools

The nucleotide sequences of strains belonging to the same species were subjected to genome alignment using Progressive Mauve software[23] using default parameters. All genomes were annotated using the Rapid Annotation using Subsystem Technology (RAST) (http://rast.nmpdr.org/).[24] The enzyme commission (EC) numbers were subsequently obtained from the RAST results (Figure 2). The conserved domains in protein sequences were identified using the Batch CD-Search tool. The Gene Ontology (GO) database was used for annotating and classifying gene function.[25] Using the GO data (http://www.geneontology.org), the correspondence between several identifiers obtained from CD-search and RAST were realized through manual curation between the data to obtain an accurate and non-redundant reference gene set. The resulting annotations of the different databases were retrieved and tabulated for each genome. We focused on the proteins for which the annotated function obtained from our analysis showed involvement in carbohydrate and lipid metabolism. The maps obtained from Kyoto Encyclopedia of Genes and Genomes were used to determine the metabolic pathways in which these enzymes were involved (http://www.genome.jp/kegg/pathway.html).[26] Moreover, Pfam HMM-profiles utilized with HMMER software package[27] facilitated the identification of genes encoding lipases.

Bacteriocins database

We established a bacteriocins database (Figure 2). The available data were obtained from Bactibase (http://bactibase.pfba-lab-tun.org/main.php), and the sequences of all bacteriocins reported in the literature were retrieved from the NCBI database. A multi-Fasta text file containing 247 retrieved protein sequences was subsequently generated. The bacteriocin sequences were aligned using the BioEdit program (http://www.mbio.ncsu.edu/BioEdit/bioedit.html)[28] and converted into a MEGA file. A bidirectional protein BLAST[29] of whole genome sequences against the bacteriocin database was performed. The manual examination of best BLAST hits (identity over 30% and coverage over 50%) facilitated the identification of bacteriocins in the *Lactobacillus* genomes. The bacteriocin sequences were compared using the Artemis Comparison Tool developed by the Sanger Institute. We constructed phylogenetic trees of the consensus sequences of the different types of bacteriocins in *Lactobacillus* spp. with MEGA5 software,[30] using the Neighbor-Joining method under the JTT model with 100 bootstrap sampling.

Clustering and statistical analysis of strains

A hierarchical clustering analysis based on *Pearson's* correlation was performed using TIGR Multi experiment Viewer (MeV) Version 2.2 (http://www.tigr.org) to identify the genes present in the different species. The gene content of the strains studied was described using a two-character matrix, with 0 for an enzyme not detected and 1 for the presence of an enzyme. Principal component analyses were performed using the PRINCOMP and BIPLOT functions of the R statistical package (Vienna, Austria, http://www.R-project.org/) to infer relationships between weight gain and weight protection-associated *Lactobacillus*. A *P*-value <0.05 was considered significant.

RESULTS

Strains selection

There are 120 validated *Lactobacillus* spp., and we identified 14 species associated with weight modification (Supplementary Figure 1). We were able to retrieve the genomes of 13 *Lactobacillus* strains, including the genomes of three strains of *L. plantarum*, three strains of *L. reuteri*, two strains of *L. acidophilus*, two strains of *L. fermentum*, one strain of *L. sakei* and one strain of *L. gasseri* available on the NCBI website in January 2013 when we initiated this analysis. We also retrieved the draft genome of *L. ingluviei*.[31] Based on literature analysis, we classified *L. reuteri*, *L. acidophilus*, *L. fermentum*, *L. sakei* and *L. ingluviei* as weight gain-associated *Lactobacillus* strains, whereas *L. plantarum* and *L. gasseri* were classified as weight protection-associated *Lactobacillus* strains.

General features of *Lactobacillus* genomes

The major features of the *Lactobacillus* genomes are summarized in Table 1. All of the genomes comprised a circular chromosome of 1.88–3.2 Mb in length. However, the weight protection-associated genomes were larger (2.9 Mb) than the weight gain-associated *Lactobacillus* genomes (2 Mb). Many *Lactobacillus* spp. harbor plasmids (*L. acidophilus* 30SC, *L. reuteri* SD2112, and *L. plantarum* strains ST-III and WCFS1), and some of these plasmids carry genes for bacteriocin production. The average guanine-cytosine (GC) content of each genome was 42.6%. The number of predicted proteins in *lactobacilli* ranges from 1051 to 3058. In addition, some *Lactobacillus* genomes harbor pseudogenes, with

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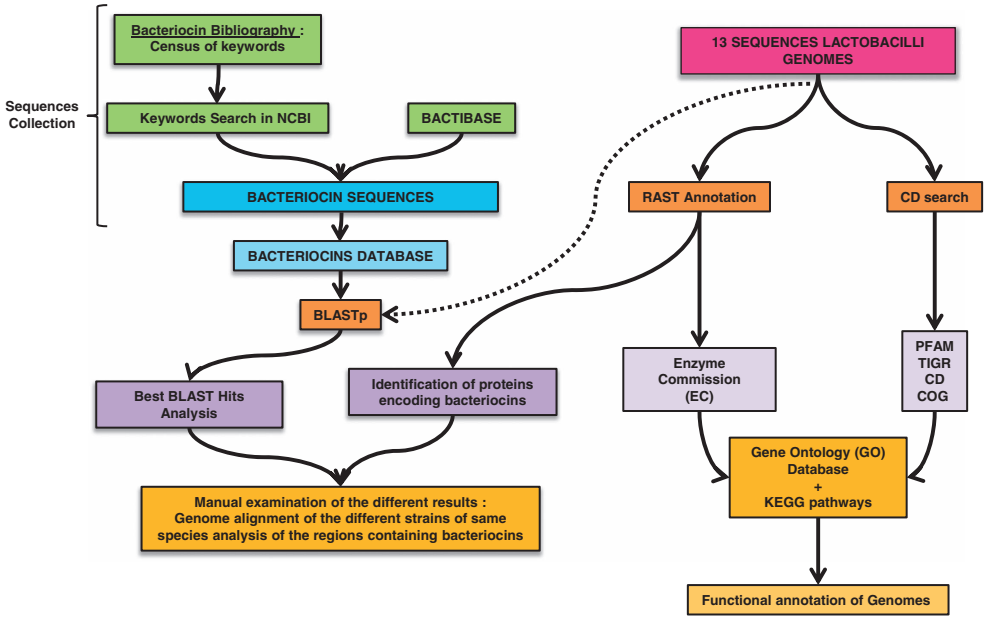


Figure 2. Strategy used for the genome annotation and bacteriocin gene analysis.

Table 1. General genome features

Species	*RefSeq*	*Genome size (Mb)*	*GC%*	*Protein*	*Pseudo-gene*	*rRNA*	*tRNA*	*Other RNA*	*Gene*	*Mean ORF size*
L. acidophilus 30SC	NC_015214.1	2.08	38.1	2037	—	12	63	—	2112	879
L. acidophilus 30SC pRKC30SC1	NC_015213.1	0.01	35.1	6	—	—	—	—	6	598
L. acidophilus 30SC pRKC30SC2	NC_015218.1	0.01	36.6	16	2	—	—	—	16	583
L. acidophilus NCFM	NC_006814.3	1.99	34.7	1864	—	13	61	—	1938	944
L. fermentum CECT 5716	NC_017465.1	2.1	51.5	1051	24	20	54	—	1149	1135
L. fermentum IFO 3956	NC_010610.1	2.1	51.5	1843	—	15	54	—	1912	916
L. gasseri ATCC 33323	NC_008530.1	1.89	35.3	1755	48	19	78	1	1898	955
L. ingluviei Autruche 4	CAKF00000000	1.97	50.90	1923	—	4	64	—	1927	921
L. plantarum JDM1	NC_012984.1	3.2	44.7	2948	3	16	62	—	3029	911
L. plantarum ST-III	NC_014554.1	3.25	44.6	2996	62	15	64	—	3137	893
L. plantarum ST-III Plsm	NC_014558.2	0.05	38.7	42	3	—	—	—	45	904
L. plantarum WCFS1	NC_004567.1	3.31	44.5	3058	42	5	62	—	3108	913
L. plantarum WCFS1 Pwcfs101	NC_006375.1	0	39.5	3	—	—	—	—	3	429
L. plantarum WCFS1 pWCFS102	NC_006376.1	0	34.3	4	—	—	—	—	4	292
L. plantarum WCFS1 pWCFS103	NC_006377.1	0.04	40.8	43	—	—	—	—	43	718
L. reuteri DSM 20016	NC_009513.1	2	38.9	1900	41	18	68	2	2027	898
L. reuteri JCM 1112	NC_010609.1	2.04	38.9	1820	—	18	63	—	1901	937
L. reuteri SD2112	NC_015697.1	2.32	39.0	2246	36	18	70	36	2425	879
L. reuteri SD2112 pLR580	NC_015699.1	0.01	39.2	7	1	—	—	—	7	696
L. reuteri SD2112 pLR581	NC_015700.1	0.01	40.0	16	—	—	—	—	16	609
L. reuteri SD2112 pLR584	NC_015701.1	0.02	36.9	17	—	—	—	1	18	851
L. reuteri SD2112 pLR585	NC_015698.1	0.01	41.1	14	2	—	—	—	14	821
L. sakei 23K	NC_007576	1.88	41.3	1306	30	21	63	—	1963	866

Abbreviations: GC%, percentage of guanine-cytosine; rRNA, ribosomal RNA; tRNA, transfer RNA; ORF, open reading frame.

up to 62 pseudogenes in *L. plantarum* ST-III. *Lactobacilli* also differ in the number of ribosomal RNA operons, ranging from 4 operons in *L. ingluviei* to 21 operons in *L. sakei* (Table 1). The number of transfer RNA (tRNA) ranges from 54 tRNA in *L. gasseri* to 78 tRNA in *L. fermentum*. The mean length of open reading frames for all studied *Lactobacillus* strains was 927 bp. Strikingly, *L. plantarum* WCFS1 displayed the largest variation in the length of the open reading frames, ranging from 36 bp for the gene encoding the protein for plantaricin biosynthesis to 15 870 bp for the gene encoding the non-ribosomal peptide synthetase NpsA. The global genomic alignment showed several variations in the gene order for *L. acidophilus, L. plantarum* and *L. reuteri* spp., potentially associated with bacterial virulence, whereas *L. fermentum* spp. showed strong collinearity (Supplementary Figure 2).

Gene content comparison

Using the previously described bioinformatics procedure in the Materials and methods section, we annotated the 13 *Lactobacillus* spp. genomes retrieved from NCBI. Altogether, 25 122 proteins were annotated. On average, the annotated proteins represented 88% of the genome. We identified a total of 2185 different

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functions in the *Lactobacillus* genus: 206 (9%) functions were shared with one or more weight protection-associated *Lactobacillus*, whereas 432 (20%) functions were specific to weight gain-associated *Lactobacillus*. The conserved core of genes present in all *Lactobacillus* spp. analyzed comprised 1546 functions (70%). However, these genes also included 283 genes for which the function is unknown and 303 genes with only a general prediction of biochemical activity. The functional distribution into gene families showed that the genes encoding the proteins involved in transcription (median ± intraquartile range, 178 ± 71 vs 118 ± 9, $P = 0.28$) and carbohydrate transport and metabolism (median ± intraquartile range, 253 ± 54 vs 158 ± 17, $P = 0.16$) were primarily identified in weight protection-associated *Lactobacillus* (Figure 3). In contrast, weight gain-associated *Lactobacillus* primarily contained genes involved in replication, recombination and repair (median ± intraquartile range, 195 ± 86 vs 106 ± 13, $P = 0.12$). In addition, a small number of genes involved in lipid transport and metabolism was observed in both groups (~53 genes per genome; Figure 3).

On the basis of the EC content observed in the *Lactobacillus* genomes and the number of gene copies, the principal component analyses revealed similar behaviors between the two weight protection-associated species *L. plantarum* and *L. gasseri* (Supplementary Figure 3). The genomes were projected on the first two principal component analyses axes, representing 75 and 14% of the total inertia. A significant difference was observed between weight protection and weight gain-associated *Lactobacillus*, particularly regarding EC 2.7.1.69, which represents the glucose permease involved in carbohydrate metabolism.[32] This sugar phosphotransferase, which mediates the transport of glucose across the membrane, was identified 48 times in weight protection-associated *Lactobacillus*, with a maximum occurrence in the *L. plantarum* strain JDM, whereas glucose permease was only identified an average of 8 times in weight gain-associated *Lactobacilli*.

Carbohydrate metabolism

An examination of the functional categories in genomes revealed that most of the genes present in *Lactobacillus* spp. are involved in carbohydrate metabolism. We therefore focused on the presence or absence of genes involved in the carbohydrate pathways of *lactobacilli* associated with weight gain and weight protection. A total of 31 genes involved in these functions were identified in weight protection-associated *Lactobacillus*, whereas these genes were absent in weight gain-associated *Lactobacillus* (Supplementary Table 1). These genes encoded proteins involved in the conversion of glycerone phosphate into L-rhamnose (L-rhamnose isomerase, rhamnulokinase and aldehyde-lyase rhamnulose-1-phosphate aldolase); the production of dextrin from α-D-glucose-1P (glucose-1-phosphate adenylyltransferase, starch synthase, 1,4-α-glucan branching enzyme, phosphorylase and α-amylase), which indicates the capacity of these bacteria to store carbohydrates in the form of glycogen; the pyruvate pathway (oxaloacetate decarboxylase, formate C-acetyltransferase and pyruvate oxidase); and the decomposition of hydrogen peroxide to water and oxygen (catalase; Figure 4). In contrast, six enzymes were identified in weight gain-associated *Lactobacillus* genomes (Supplementary Table 1). These enzymes were primarily involved in the conversion of fructose to sorbitol (sorbitol dehydrogenase); the production of 3-acetoacetyl-CoA from (S)-3-hydroxybutanoyl-CoA (3-hydroxybutyryl-CoA dehydrogenase); the formation of (R)-acetoin from (R,R)-butane-2,3-diol ((R,R)-butanediol dehydrogenase); the conversion of 2-deoxy-D-ribose 5-phosphate into D-glyceraldehyde 3-phosphate (deoxyribose-phosphate aldolase); the phosphorylation of D-xylulose (xylulokinase); and the conversion of sucrose into D-fructose and α-D-glucose-1-phosphate (sucrose phosphorylase) (Figure 4). The presence of enzymes involved in the conversion of sucrose into glucose and fructose suggests that weight gain-associated *Lactobacillus* genomes are adapted for foods rich in sucrose.

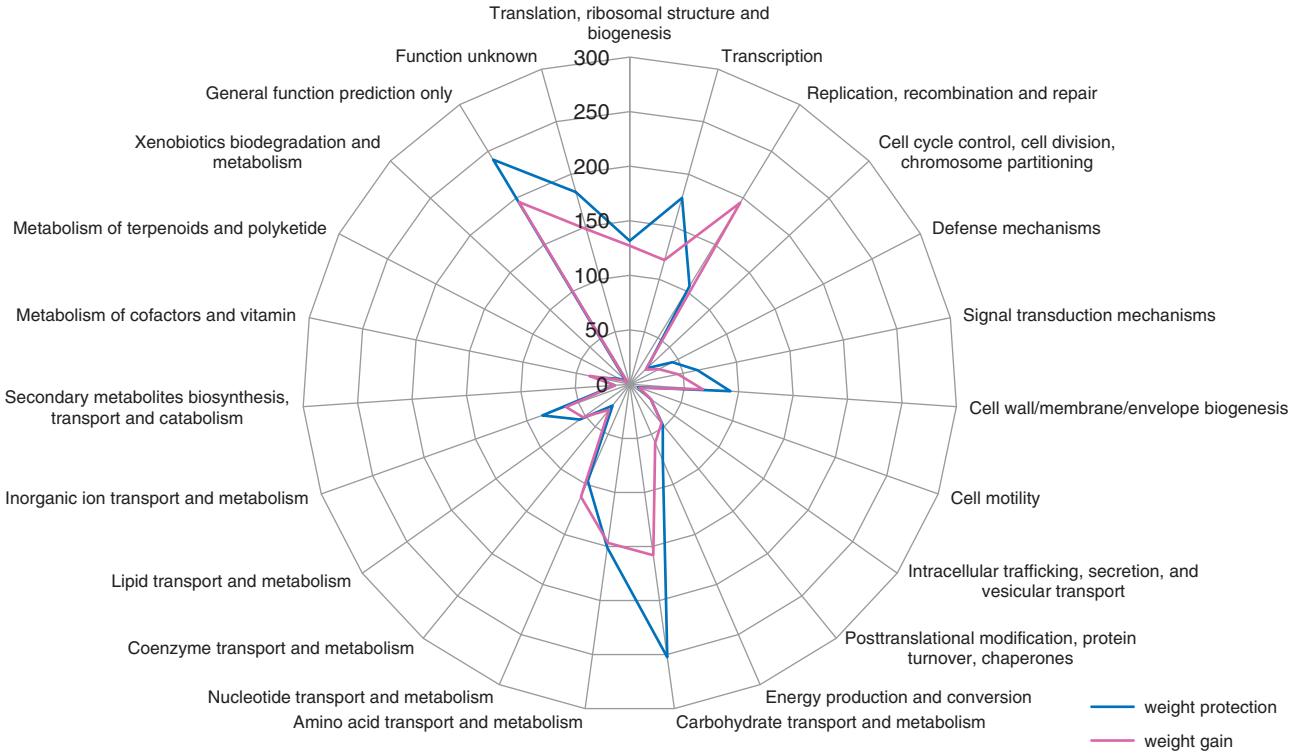


Figure 3. Comparison of the gene content profiles obtained for weight gain or weight protection-associated *Lactobacillus*, proportional to the size of the genomes (radar plot).

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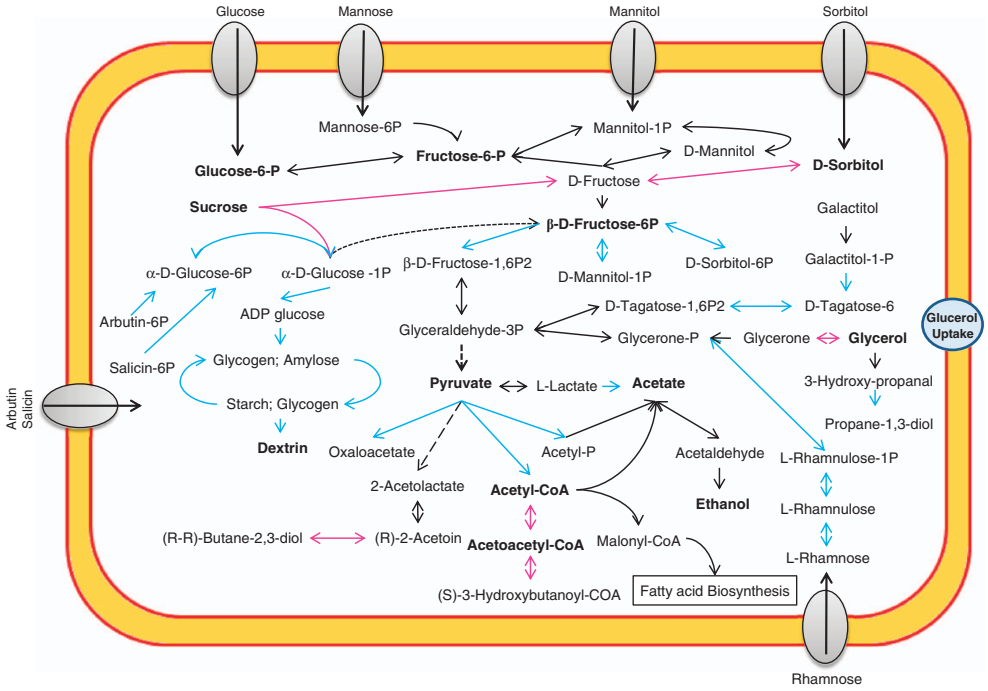


Figure 4. Schematic representation of the metabolic pathways associated with carbohydrate and lipid metabolism involving *Lactobacillus* genomes. The blue arrows show reactions present in weight protection-associated species. The pink arrows show reactions present in weight gain-associated species.

Lipid metabolism

The analysis of the genes involved in lipid metabolism revealed four proteins involved in lipid metabolism in weight protection-associated *Lactobacillus* (Supplementary Table 1). These enzymes were implicated in the production of acyl-CoA from carboxylate (acyl-CoA hydrolase); the conversion of glutathione to glutathione disulfide (glutathione peroxidase); the formation of 3-hydroxypropanal from propane-1,3-diol in glycerolipid metabolism (1,3-propanediol dehydrogenase); and the conversion of sn-glycerol 3-phosphate into CDP-glycerol in glycerophospholipid metabolism (glycerol-3-phosphate cytidylyltransferase). In contrast, glycerol dehydrogenase and acetyl-CoA C-acyltransferase (Thiolase I) were only present in weight gain-associated *Lactobacillus* (Figure 4). Glycerol dehydrogenase has been implicated in glycerolipid metabolism and the conversion of glycerone into glycerol, a molecule important in energy metabolism produced through the breakdown of dietary fats. Thiolase I intervenes in many pathways, including fatty acid metabolism, and functions in synthetic or degradative pathways. In addition, this enzyme catalyzes the final step of the β-oxidation using long-chain 3-ketoacyl-CoA as a substrate, generating acetyl-CoA. The gene encoding thiolase I also encodes thiolase II (EC 2.3.1.9), which intervenes in fatty acid degradation. Thiolase II catalyzes the reverse reaction that converts one molecule of acetoacetyl-CoA into two molecules of acetyl-CoA. However, the formation of acetoacetyl-CoA is favored when acetyl-CoA levels are too high, thereby promoting ketone body synthesis.

Lipases

Lipases have an essential role in the mobilization of fatty acids from dietary or storage fats. The HMM search revealed that among the 16 lipase families identified in the libraries in this study, 3 lipases were present in at least one *Lactobacillus* genome (Supplementary Table 2). Furthermore, all genomes analyzed contain many candidate genes encoding lipases from the families PF00561 (αβ hydrolase 1, including acid lipases and Pseudomonas-like lipases) and PF0785 (αβ hydrolase 3 or hormone-sensitive lipase family). PF00561 was the most abundant family in the genomes analyzed, and we identified 14 corresponding genes in *L. acidophilus* 30SC. The second most abundant lipase family was PF0785, with 12 lipases identified in *L. plantarum* JDM1. The PF00561 and PF0785 lipase families belong to a superfamily of proteins characterized by an α/β hydrolase fold, representing one of the largest group of enzymes with diverse catalytic functions, such as proteases, lipases, peroxidases, esterases, epoxide hydrolases and dehalogenases.[33] We calculated the number candidate lipases to determine the total lipase content in the genomes (Supplementary Table 2), but we did not observe significant differences between the genomes in weight gain-associated *Lactobacillus* and those associated with weight protection.

Bacteriocins

In the generated database, we observed that the sequences encoding bacteriocins differ greatly in size and composition: the sequences range from 7 amino acids (microcin C7) to 1585 amino acids (rhizobiocin), with an average length of 110 amino acids. Using BLASTp, we obtained 77 significant hits, which were subsequently compared with the annotations obtained using the RAST server. We identified 18 different types of bacteriocins in *Lactobacillus* spp., among which several sequences were previously annotated as hypothetical proteins in the NCBI database (Figure 5). Weight gain-associated *Lactobacillus* spp. encoded more bacteriocins (mean = 6) than weight protection-associated *Lactobacillus* spp. (mean = 4) (Supplementary Table 3). Moreover, *L. plantarum* encoded several bacteriocin precursors (from 3 to 5 per genome). *L. acidophilus* 30SC encoded the largest number of bacteriocins, with 15 putative genes. Plantaricins and colicins were the most commonly encoded bacteriocins. We identified bacteriocins annotated as colicin V proteins in *L. ingluviei* and *L. fermentum* genomes. In *L. fermentum* spp., the potential colicin V gene sequences were identified in regions with high collinearity, but no synteny with the region containing the gene encoding

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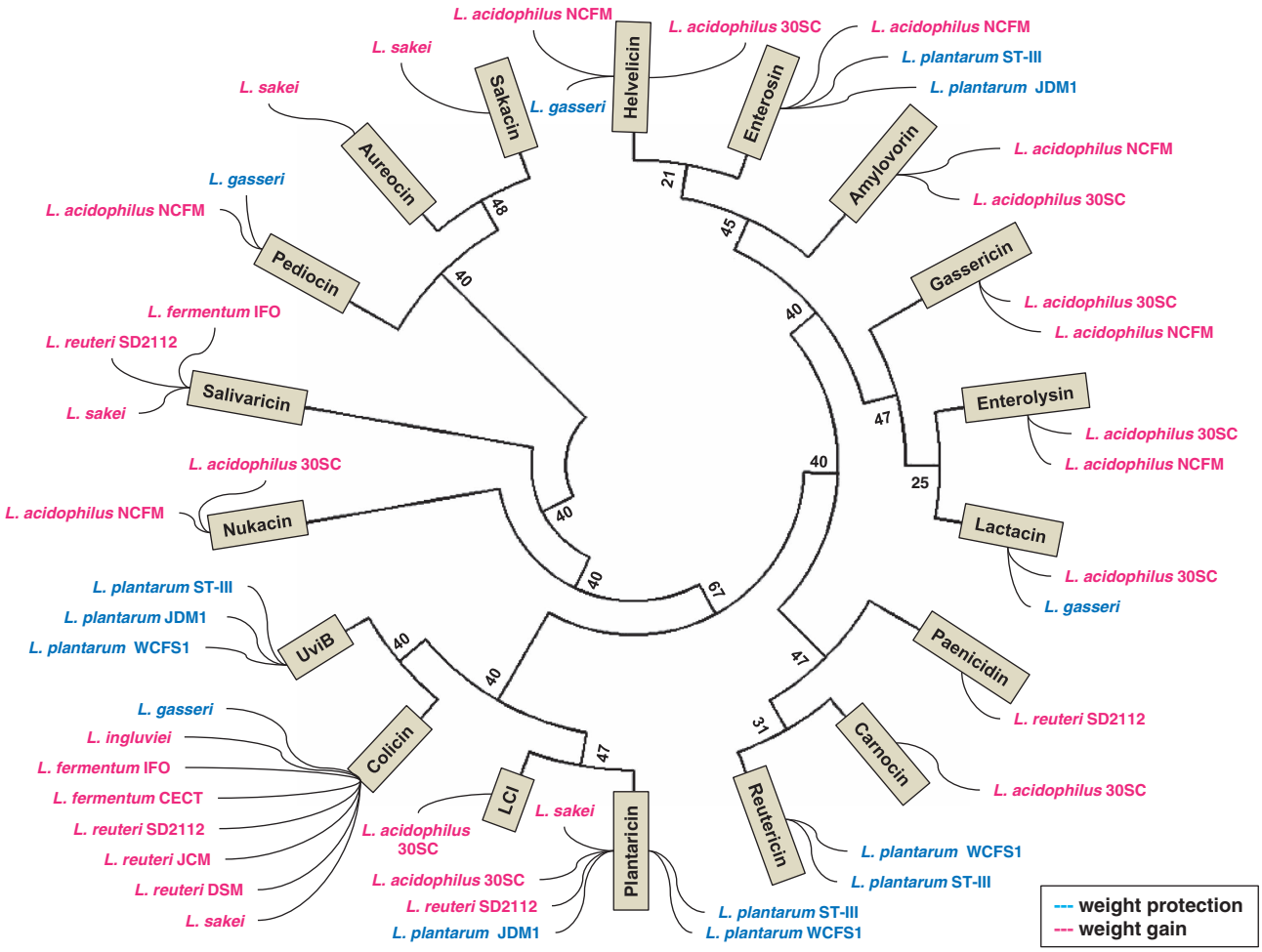


Figure 5. Overview of bacteriocin distribution in *Lactobacillus* spp. The phylogenetic tree of the bacteriocin sequences identified in the genomes studied was constructed using the Neighbor-Joining method, under the JTT model with 100 bootstrap sampling.

colicin V in *L. ingluviei* was observed (Supplementary Figure 4). However, the sequence alignment of the putative colicin V with colicin V obtained from the database showed a high degree of similarity, from 21 to 100%. The clustering analysis showed that *L. plantarum* strains associated with weight protection are separated from the other species based on the bacteriocin content, whereas *L. gasseri* are closely related to weight gain-associated *Lactobacillus* (Supplementary Figure 5).

DISCUSSION

In this study, we analyzed *Lactobacillus* spp. genomes, providing evidence of major differences between weight protection and weight gain-associated *lactobacilli*. The strains analysis was based on previously validated methods commonly used for genome comparisons.[34] A limitation of this study was that only a few *Lactobacillus* spp. genomes associated with weight modification were available at the time of analysis. The weight protection-associated *Lactobacillus* genomes were larger and encoded more bacteriocins and genes involved in transcription, carbohydrate transport and metabolism than the weight gain-associated *Lactobacillus* genomes. Moreover, the weight protection-associated *Lactobacillus* genomes encoded proteins implicated in fructose, mannose, starch and sucrose metabolism and contained a significant amount of glucose permease. Weight gain-associated *Lactobacillus* spp. harbored enzymes involved in lipid metabolism, which were not identified in weight protection-associated genomes.

Comparative genomics showed differences between *Lactobacillus* strains of the same species at the functional level. Different *L. acidophilus, L. reuteri, L. plantarum* and *L. fermentum* strains presented differences in the number of plasmids and the genes encoding lipases and bacteriocins. Moreover, alignment of these genomes showed wide variations in gene organization (Supplementary Figure 2). In a previous study, the genomes of 34 different *Lactobacillus paracasei* strains showed large variety and variability in the sugar utilization gene cassettes and other genetic variations, such as sugar metabolism, phages or plasmids.[35] In addition, the analysis of 100 different *Lactobacillus rhamnosus* strains showed two distinctive geno-phenotypes at the species level, associated with carbohydrate metabolism, including D-lactose, D-maltose and L-rhamnose,[36] and the comparison of 18 *L. plantarum* strains identified two distinctive glycerol- and ribitol-type wall teichoic acid structures.[37] Moreover, different *L. reuteri* strains showed different effects on weight.[14] Indeed, the administration of the ATCC strain in mice was associated with weight decrease, whereas the administration of the L6798 strain was associated with weight gain.[14]

Weight gain-associated *Lactobacillus* lack enzymes involved in the catabolism of fructose, but these strains encode several enzymes that participate in the conversion of sucrose into glucose and fructose and enzymes that promote fructose production. These observations suggest that these species are adapted for

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foods rich in sucrose, which contribute to obesity in these individuals. However, weight protection-associated *Lactobacillus* genomes actively participate in the degradation of fructose and promote the synthesis of dextrin, L-rhamnose and acetate. These three molecules respectively prevent obesity in animals by reducing blood glucose levels,[38] serum triacylglycerol levels[39] and body mass and fat accumulation.[40] In addition, the presence of glucose-1-phosphate adenylyltransferase, starch synthase, 1,4-α-glucan branching enzyme, phosphorylase and α-amylase in weight protection-associated *Lactobacillus* genomes suggest the storage of sugars in the form of bacterial glycogen. Weight protection-associated *Lactobacillus* spp. also encoded proteins involved in the metabolism of glutathione peroxidase and catalase, which have a major role in gut microbiota modulation, as both enzymes have been implicated in oxidative stress defense mechanisms.[41] Furthermore, these genomes produced a significant amount of glucose permease, which confers to the bacteria the ability to enhance glycolysis. Therefore, the degradation of sugars reduces storage in the body, thereby ensuring a weight protection effect. In humans, the amount of absorbable sugars before entering the colon varies depending on the diet composition. Carbohydrates like sucrose, lactose and starch are digested and absorbed in the small intestine, whereas the degradation of other complex carbohydrates from our diet involves a wide variety of enzymes produced almost exclusively by colonic bacteria.[20]

The data obtained in this study also showed that thiolases were only encoded in weight gain-associated *Lactobacillus*. Thiolases are ubiquitous enzymes that have key roles in the β-oxidation pathway of fatty acid degradation (Thiolase I; EC 2.3.1.16) and various biosynthetic pathways (Thiolase II; EC 2.3.1.9), such as poly β-hydroxybutyric acid synthesis or steroid biogenesis.[42] Thus, weight gain-associated *Lactobacillus* genomes mobilize the energy and carbon stored in fatty acids through β-oxidation. As a significant number of lipase genes have been identified in *Lactobacillus* spp., the fatty acids released through lipases from acylglycerols could be further degraded by weight gain-associated *Lactobacillus*. As a result, weight gain-associated *Lactobacillus* spp. could potentially participate in lipid digestion in the upper gastrointestinal tracts of humans through the degradation of dietary fats (acylglycerols). Although all fats are normally absorbed before entering the large intestine,[18,19] the rate at which these fats are degraded controls satiety mechanisms, such as the ileal brake.[43] This satiety phenomenon is primarily triggered through free fatty acids reaching the distal region of the small intestine before absorption. Weight gain-associated *Lactobacillus* spp. might accelerate fat digestion and fatty acid absorption/degradation, thereby reducing fatty acid levels in the lower small intestine and ileal brake effects.

The results of this study revealed that weight protection-associated genomes encoded more bacteriocins than weight gain-associated *Lactobacillus*. The antibacterial effects of bacteriocins largely determine the effect of probiotic strains on gut microbiota.[2,3] The antibacterial activity of bacteriocins has been extensively demonstrated *in vitro*.[44] *In vivo* experimental models have been used to determine the efficiency of *lactobacilli* to limit the dissemination of *Listeria monocytogenes*.[45] This effect is likely mediated through the antibiotic activities of *lactobacilli,* induced through bacteriocins.[2,3] In humans, obesity is associated with a significant decrease in the level of microbiota diversity and alterations in the representation of bacterial genes and metabolic pathways.[46] Animal models of obesity have also demonstrated an association between the alteration of the microbiota composition with the development of obesity,[47] leading to a reduction of *Bacteroidetes*.[48] Moreover, bacteriocin-producing *L. reuteri* and *L. gasseri* strains inhibited the growth and eliminated the presence of various enteropathogens, such as *Salmonella*, *Listeria* and *Campylobacter*.[11]

In conclusion, this genome analysis revealed large differences between weight gain and weight protection-associated *Lactobacillus* genomes with respect to the genes involved in transcription, replication, recombination and repair, lipid metabolism, carbohydrate transport and metabolism, and bacteriocin production. Significant differences were observed between the two groups of genomes, but the resulting hypotheses require further tests using experimental models to further confirm the implication of *lactobacilli* in weight modifications. To the best of our knowledge, this is the first study comparing the genomes of *Lactobacillus* strains associated with weight modifications. Functional foods and yogurts consumed by humans and children can contain large numbers of living bacteria, up to 10^9 CFU g^{-1}.[49] These probiotic amounts are considerable, compared with the concentration of 10^5 bacteria[18,19] observed in the upper intestinal tract, where the digestion and absorption of most nutrients occurs in humans. Obtaining a better understanding of the ability of specific probiotic bacteria that promote weight loss or weight gain could result in the specific use of *Lactobacillus* spp. as treatments against obesity or malnutrition.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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REFERENCES

1 Angelakis E, Armougom F, Million M, Raoult D. The relationship between gut microbiota and weight gain in humans. *Future Microbiol* 2012; **7**: 91–109.
2 Million M, Angelakis E, Drissi F, Raoult D. Occam's razor and probiotics activity on Listeria monocytogenes. *Proc Natl Acad Sci USA* 2013; **110**: E1.
3 Angelakis E, Merhej V, Raoult D. Related actions of probiotics and antibiotics on gut microbiota and weight modification. *Lancet Infect Dis* 2013; **13**: 889–899.
4 Raoult D. Human microbiome: take-home lesson on growth promoters? *Nature* 2008; **454**: 690–691.
5 Trehan I, Goldbach HS, LaGrone LN, Meuli GJ, Wang RJ, Maleta KM *et al.* Antibiotics as part of the management of severe acute malnutrition. *N Engl J Med* 2013; **368**: 425–435.
6 Ajslev TA, Andersen CS, Gamborg M, Sorensen TI, Jess T. Childhood overweight after establishment of the gut microbiota: the role of delivery mode, pre-pregnancy weight and early administration of antibiotics. *Int J Obes (Lond)* 2011; **35**: 522–529.
7 Ray K. Gut microbiota: adding weight to the microbiota's role in obesity-exposure to antibiotics early in life can lead to increased adiposity. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 615.
8 Thuny F, Richet H, Casalta JP, Angelakis E, Habib G, Raoult D. Vancomycin treatment of infective endocarditis is linked with recently acquired obesity. *PLoS One* 2010; **5**: e9074.
9 Cho I, Yamanishi S, Cox L, Methe BA, Zavadil J, Li K *et al.* Antibiotics in early life alter the murine colonic microbiome and adiposity. *Nature* 2012; **488**: 621–626.
10 Stokstad EL. Antibiotics in animal nutrition. *Physiol Rev* 1954; **34**: 25–51.
11 Smith MI, Yatsunenko T, Manary MJ, Trehan I, Mkakosya R, Cheng J *et al.* Gut microbiomes of Malawian twin pairs discordant for kwashiorkor. *Science* 2013; **339**: 548–554.
12 Angelakis E, Bastelica D, Ben AA, El FA, Dutour A, Mege JL *et al.* An evaluation of the effects of *Lactobacillus ingluviei* on body weight, the intestinal microbiome and metabolism in mice. *Microb Pathog* 2012; **52**: 61–68.
13 Miyoshi M, Ogawa A, Higurashi S, Kadooka Y. Anti-obesity effect of *Lactobacillus gasseri* SBT2055 accompanied by inhibition of pro-inflammatory gene expression in the visceral adipose tissue in diet-induced obese mice. *Eur J Nutr* 2013; e-pub ahead of print 6 August 2013.
14 Fak F, Backhed F. *Lactobacillus reuteri* prevents diet-induced obesity, but not atherosclerosis, in a strain dependent fashion in Apoe-/- mice. *PLoS One* 2012; **7**: e46837.

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15 Klaenhammer TR, Altermann E, Pfeiler E, Buck BL, Goh YJ, O'Flaherty S *et al.* Functional genomics of probiotic Lactobacilli. *J Clin Gastroenterol* 2008; **42**(Suppl 3 Pt 2): S160–S162.
16 Million M, Angelakis E, Paul M, Armougom F, Leibovici L, Raoult D. Comparative meta-analysis of the effect of *Lactobacillus* species on weight gain in humans and animals. *Microb Pathog* 2012; **53**: 100–108.
17 Stsepetova J, Sepp E, Kolk H, Loivukene K, Songisepp E, Mikelsaar M. Diversity and metabolic impact of intestinal *Lactobacillus* species in healthy adults and the elderly. *Br J Nutr* 2011; **105**: 1235–1244.
18 Krajmalnik-Brown R, Ilhan ZE, Kang DW, DiBaise JK. Effects of gut microbes on nutrient absorption and energy regulation. *Nutr Clin Pract* 2012; **27**: 201–214.
19 Flint HJ, Scott KP, Louis P, Duncan SH. The role of the gut microbiota in nutrition and health. *Nat Rev Gastroenterol Hepatol* 2012; **9**: 577–589.
20 El Kaoutari A, Armougom F, Gordon JI, Raoult D, Henrissat B. The abundance and variety of carbohydrate-active enzymes in the human gut microbiota. *Nat Rev Microbiol* 2013; **11**: 497–504.
21 Canchaya C, Claesson MJ, Fitzgerald GF, van Sinderen D, O'Toole PW. Diversity of the genus *Lactobacillus* revealed by comparative genomics of five species. *Microbiology* 2006; **152**: 3185–3196.
22 Lukjancenko O, Ussery DW, Wassenaar TM. Comparative genomics of Bifidobacterium, *Lactobacillus* and related probiotic genera. *Microb Ecol* 2012; **63**: 651–673.
23 Darling AE, Mau B, Perna NT. ProgressiveMauve: multiple genome alignment with gene gain, loss and rearrangement. *PLoS One* 2010; **5**: e11147.
24 Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA *et al.* The RAST Server: rapid annotations using subsystems technology. *BMC Genomics* 2008; **9**: 75.
25 Ashburner M, Ball CA, Blake JA, Botstein D, Butler H, Cherry JM *et al.* Gene ontology: tool for the unification of biology. The Gene Ontology Consortium. *Nat Genet* 2000; **25**: 25–29.
26 Ogata H, Goto S, Sato K, Fujibuchi W, Bono H, Kanehisa M. KEGG: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res* 1999; **27**: 29–34.
27 Johnson LS, Eddy SR, Portugaly E. Hidden Markov model speed heuristic and iterative HMM search procedure. *BMC Bioinformatics* 2010; **11**: 431.
28 Hall TA. BioEdit: a user-friendly biological sequence alignment editor and analysis program for Windows 95/98NT. *Nucleic Acids Symp Ser* 1999; **41**: 95–98.
29 Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J Mol Biol* 1990; **215**: 403–410.
30 Tamura K, Peterson D, Peterson N, Stecher G, Nei M, Kumar S. MEGA5: molecular evolutionary genetics analysis using maximum likelihood, evolutionary distance, and maximum parsimony methods. *Mol Biol Evol* 2011; **28**: 2731–2739.
31 Merhej V, Armougom F, Robert C, Raoult D. Genome sequence of *Lactobacillus ingluviei*, a bacterium associated with weight gain in animals. *J Bacteriol* 2012; **194**: 5697.
32 Hoffee P, Englesberg E. Effect of metabolic activity on the glucose permease of bacterial cells. *Proc Natl Acad Sci USA* 1962; **48**: 1759–1765.
33 Ollis DL, Cheah E, Cygler M, Dijkstra B, Frolow F, Franken SM *et al.* The alpha/beta hydrolase fold. *Protein Eng* 1992; **5**: 197–211.
34 Makarova K, Slesarev A, Wolf Y, Sorokin A, Mirkin B, Koonin E *et al.* Comparative genomics of the lactic acid bacteria. *Proc Natl Acad Sci USA* 2006; **103**: 15611–15616.
35 Smokvina T, Wels M, Polka J, Chervaux C, Brisse S, Boekhorst J *et al. Lactobacillus paracasei* comparative genomics: towards species pan-genome definition and exploitation of diversity. *PLoS One* 2013; **8**: e68731.
36 Douillard FP, Ribbera A, Kant R, Pietila TE, Jarvinen HM, Messing M *et al.* Comparative genomic and functional analysis of 100 *Lactobacillus rhamnosus* strains and their comparison with strain GG. *PLoS Genet* 2013; **9**: e1003683.
37 Tomita S, Irisawa T, Tanaka N, Nukada T, Satoh E, Uchimura T *et al.* Comparison of components and synthesis genes of cell wall teichoic acid among *Lactobacillus plantarum* strains. *Biosci Biotechnol Biochem* 2010; **74**: 928–933.
38 Wakabayashi S, Hashii Y. Food and sugar preparation containing indigestable dextrin. Japanese Patent EP0582518 A2, Matsutani Chemical Industries Co. Ltd., 1994.
39 Vogt JA, Ishii-Schrade KB, Pencharz PB, Jones PJ, Wolever TM. L-rhamnose and lactulose decrease serum triacylglycerols and their rates of synthesis, but do not affect serum cholesterol concentrations in men. *J Nutr* 2006; **136**: 2160–2166.
40 Yamashita H, Fujisawa K, Ito E, Idei S, Kawaguchi N, Kimoto M *et al.* Improvement of obesity and glucose tolerance by acetate in type 2 diabetic Otsuka Long-Evans Tokushima Fatty (OLETF) rats. *Biosci Biotechnol Biochem* 2007; **71**: 1236–1243.
41 Fernandez-Sanchez A, Madrigal-Santillan E, Bautista M, Esquivel-Soto J, Morales-Gonzalez A, Esquivel-Chirino C *et al.* Inflammation, oxidative stress, and obesity. *Int J Mol Sci* 2011; **12**: 3117–3132.
42 Haapalainen AM, Merilainen G, Wierenga RK. The thiolase superfamily: condensing enzymes with diverse reaction specificities. *Trends Biochem Sci* 2006; **31**: 64–71.
43 Maljaars PW, Peters HP, Mela DJ, Masclee AA. Ileal brake: a sensible food target for appetite control. A review. *Physiol Behav* 2008; **95**: 271–281.
44 Servin AL. Antagonistic activities of lactobacilli and bifidobacteria against microbial pathogens. *FEMS Microbiol Rev* 2004; **28**: 405–440.
45 Archambaud C, Nahori MA, Soubigou G, Becavin C, Laval L, Lechat P *et al.* Impact of lactobacilli on orally acquired listeriosis. *Proc Natl Acad Sci USA* 2012; **109**: 16684–16689.
46 Turnbaugh PJ, Hamady M, Yatsunenko T, Cantarel BL, Duncan A, Ley RE *et al.* A core gut microbiome in obese and lean twins. *Nature* 2009; **457**: 480–484.
47 Musso G, Gambino R, Cassader M. Obesity, diabetes, and gut microbiota: the hygiene hypothesis expanded? *Diabetes Care* 2010; **33**: 2277–2284.
48 Ley RE, Backhed F, Turnbaugh P, Lozupone CA, Knight RD, Gordon JI. Obesity alters gut microbial ecology. *Proc Natl Acad Sci USA* 2005; **102**: 11070–11075.
49 Angelakis E, Million M, Henry M, Raoult D. Rapid and accurate bacterial identification in probiotics and yoghurts by MALDI-TOF mass spectrometry. *J Food Sci* 2011; **76**: M568–M572.

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