INTERNATIONAL JOURNAL OF SYSTEMATIC AND EVOLUTIONARY MICROBIOLOGY

RESEARCH ARTICLE

Rios-Galicia *et al.*, *Int. J. Syst. Evol. Microbiol.* 2024;74:006210
DOI 10.1099/ijsem.0.006210

MICROBIOLOGY SOCIETY

OPEN ACCESS

Novel taxonomic and functional diversity of eight bacteria from the upper digestive tract of chicken

Bibiana Rios-Galicia[1,2], Johan S. Sáenz[1,2], Timur Yergaliyev[1,2], Christoph Roth[1,2], Amélia Camarinha-Silva[1,2,*] and Jana Seifert[1,2,*]

Abstract

Eight anaerobic strains obtained from crop, jejunum and ileum of chicken were isolated, characterized and genome analysed to observe their metabolic profiles, adaptive strategies and to serve as novel future references. The novel species *Ligilactobacillus hohenheimensis* sp. nov. (DSM 113870^T=LMG 32876^T), *Limosilactobacillus galli* sp. nov. (DSM 113833^T=LMG 32623^T), *Limosilactobacillus avium* sp. nov. (DSM 113849^T=LMG 32671^T), *Limosilactobacillus pulli* sp. nov. (DSM 115077^T=LMG 32877^T), *Limosilactobacillus viscerum* sp. nov. (DSM 113835^T=LMG 32625^T), *Limosilactobacillus difficilis* sp. nov. (DSM 114195^T=LMG 32875^T) and *Clostridium butanoliproducens* (DSM 115076^T=LMG 32878^T) are found in the upper gastrointestinal tract and present consistent adaptations that enable us to predict their ecological role. Molecular characterization using 16S rRNA gene analysis and long-read whole genome sequencing, confirmed the description of the novel genus *Faecalispora* gen. nov. with *Faecalispora anaeroviscerum* gen. nov. sp. nov. (DSM 113860^T=LMG 32675^T) as genus type species. After phylogenetic and taxonomic analysis, we recommend the reclassification of the species *Clostridium jeddahense* and *Clostridium sporosphaeroides* to the genus *Faecalispora*. Exploration of the microbiome from crop and small intestine of chicken expands our knowledge on the taxonomic diversity and adaptive functions of the inhabiting bacteria. The novel species identified in this project are part of a wider cultivation effort that represents the first repository of bacteria obtained from the crop and small intestine of chicken using culturomics, improving the potential handling of chicken microorganisms with biotechnological applications.

DATA SUMMARY

The authors confirm all supporting data, code and protocols have been provided within the article or through supplementary data files as a Figshare item. https://doi.org/10.6084/m9.figshare.24081663.v1[1]

All sequences generated in the present study are available at European Nucleotide Database (ENA) under the project number PRJEB56193, individual accession numbers are provided in File S1. Genome bioinformatics analyses were performed following the codes available at the project named chicken gut within the repository at https://github.com/Bibi888/chicken_gut.

Author affiliations: [1]Institute of Animal Science, University of Hohenheim, Emil-Wolff-Str. 6-10, 70593 Stuttgart, Germany; [2]HoLMiR - Hohenheim Center for Livestock Microbiome Research, University of Hohenheim, Leonore-Blosser-Reisen Weg 3, 70593 Stuttgart, Germany.
***Correspondence:** Amélia Camarinha-Silva, amelia.silva@uni-hohenheim.de; Jana Seifert, jseifert@uni-hohenheim.de
Keywords: chicken; crop; culture collection; ileum; jejunum; *Lactobacillaceae*; microbiome; novel taxa.
Abbreviation: GIT, gastrointestinal tract.
Clostridium butanoliproducens BCET17^T (DSM 115076^T=LMG 32878^T; WGS: GCA_947381515=ERS14260891; 16S rRNA gene: OQ831034), *Ligilactobacillus hohenheimensis* DJ3G5/DJ4M19^T (DSM 113870^T=LMG 32876^T; WGS: GCA_947381805=ERS14260917; 16S rRNA gene: OM760988), *Limosilactobacillus galli* BC6G17^T (DSM 113833^T=LMG 32623^T; WGS: GCA_947381495=ERS14260881; 16S rRNA gene: OM760982), *Limosilactobacillus avium* BIEM52^T (DSM 113849^T=LMG 32671^T; WGS: GCA_947381485=ERS14260884; 16S rRNA gene: OM760983), *Limosilactobacillus pulli* BIEP23/BCEP10^T (DSM 115077^T=LMG 32877^T; WGS assembly: GCA_947381555=ERS14260886; 16S rRNA gene: OQ831033), *Limosilactobacillus viscerum* CIEM62^T (tDSM 113835^T=LMG 32625^T; WGS: GCA_947387525=ERS14260897; 16S rRNA gene: OM760986), *Limosilactobacillus difficilis* BHUE2^T (type strain DSM 114195^T=LMG 32875^T; WGS: GCA_947387495=ERS14260907; 16S rRNA gene: OQ832131), and *Faecalispora anaeroviscerum* CIES53^T (DSM 113860^T=LMG 32675^T; WGS: GCA_947568225=ERS14260896; 16S rRNA gene: OM760984). Genomic sequences can be found under the Bioproject number PRJEB56193. The EBI accession URL is www.ebi.ac.uk/ena/browser/view/PRJEB56193.
Supplementary material is available with the online version of this article.

Impact Statement

The digestive system of chicken presents different physicochemical conditions along the gastrointestinal tract (GIT), shaping an individual microbial profile along sections with different metabolic capacities and divergence on the adaptations to the environment. Efforts to obtain cultivable bacteria originating from the upper region of chicken GIT enrich the reference genome database and provide information about the site-specific adaptations of bacteria colonizing such GIT sections allowing to understand the metabolic profile and adaptive strategies to the environment.

INTRODUCTION

Poultry production provides one of the best sources of quality protein and plays a crucial role in sustaining livelihoods in developing countries. The availability of organic carbon along the intestine, and the relatively short transition time of digesta (around 8–12 h), favours the colonization of heterotrophic fermentative microorganisms with a total or partial sensitivity to oxygen [2, 3]. In general, a healthy chicken microbiome profile will be dominated by the phyla *Bacillota*, *Bacteroidota*, *Actinomycetota* and *Pseudomonadota*, and its abundance will depend on the anatomical region, bird age, diet, geography and breeding conditions among others [2–8].

Molecular-based studies have improved our understanding of the diversity, composition and gene content of the gut microbiota in chickens. The recent description of metagenome assembled genomes representing more than 1000 novel species from chicken gut microbiome samples and caeca samples of two commercial bird genotypes highlights the unexplored nature of chicken microbial diversity [5, 9]. Although the majority of these studies have a high-throughput sequencing approach and good reproducibility, the lack of sufficient reference genomes and genes limits the interpretation of sequencing data and restrains deeper analysis of detailed functions and gene catalogues construction. Studies comparing bacteria along the different anatomical sections enhance how the anatomy of the gastrointestinal tract (GIT) and its physiology influence bacterial colonization [10–12]. The importance of handling colonizing species from crop, jejunum and ileum represents an advantage to observe the dynamics of nutrient digestibility, pathogen exclusion or diet additives interaction within microbiome of the upper digestive tract sections [11]. Despite the anatomical differences between digestive regions, and the key role of crop and small intestine in the digestive process, most studies of the chicken microbiome have been performed using samples from the lower digestive regions, namely caeca and faeces [13–16]. The important role of the crop and small intestine's microbiome within the whole digestive process has been missed. Therefore, studying cultivable bacteria originating from the upper digestive regions will enrich the reference genome database and provide information about the site-specific adaptations of bacteria colonizing such GIT sections. In this study, we successfully cultivated and characterized eight novel strains obtained from the upper digestive tract of chickens. The sequencing, annotation and taxonomic analysis of isolates originating from different regions allowed us to understand the metabolic profile and adaptive strategies of the species. Among them, seven represent new species and one represents a novel genus, expanding our knowledge of the specialized function of the chicken gut microbiome.

METHODS

Animal sampling

The study was approved by the Regierungspräsidium Tübingen, Germany (HOH50/17 TE). Animals were maintained and fed *ad libitum* with a commercial corn-based diet and housed at the Lindenhof experimental station of the University of Hohenheim. Ten 47–67-week-old Lohmann Brown [17] and classic laying hens (LSL) and six 21-day-old Ross 308 broilers were euthanized by hypoxia induction with CO_2 and immediately decapitated. The digestive tract was clamp-closed before being dissected and placed into a reductive solution of 0.5% cysteine for transportation.

Culture media and bacterial isolation

All culture media used in this work were prepared following Tanaka, Kawasaki and Kamagata recommendations (Supplementary material 1) [18]. Phosphate, protein, carbohydrate and agar solutions were prepared and autoclaved separately to avoid the formation of reactive oxygen species and ketosamines during sterilization [19]. Afterwards, solutions were equally mixed before solidification. Media with high sugar content such as de Man–Rogosa–Sharpe (MRS) medium and poultry-feed agar (PFA) were sterilized under different conditions than the rest (110°C for 30 min) to avoid sugar oxidation. Protein and peptone solutions were prepared using peptones from soybean plants. These measures were taken to improve the isolation of a greater diversity of organisms than when using traditional plate-dependent cultivation.

Samples were taken to an anaerobic station (Don Whitley Scientific) that contained a mixture of 80% N_2 (quality level 5.0), 15% CO_2 (quality level 3.0) and 5% H_2 (quality level 5.0), where digesta content was extracted from the digestive tube and

10-fold serially diluted with a sterile physiological solution (0.85% NaCl). Isolates were obtained from direct cultivation of the samples and enrichment cultures by plating 0.1 ml dilutions of 10^{-4} to 10^{-7} into tryptic soybean agar, gut microbiota medium, MRS (fructose 1% and maltose 1%), postgate standard medium and 2% PFA.

Dilution plates were monitored every 24 h and new colonies were collected into time batches at 48, 96, 144, 216 and 360 h. In parallel, enrichment cultures were obtained by inoculating 1 g sample into each culture media and plating 0.1 ml diluted enrichment broth on solid agar at the same time batches and dilutions used during the direct isolation. All isolates were serially cultured into new media plates to obtain axenic cultures. Cultures were incubated at 39°C under anaerobic conditions. All the isolates were conserved into the correspondent liquid media with 25% of glycerol solution at −80°C.

DNA extraction and isolate identification by 16S rRNA gene sequencing

DNA extraction was performed on fresh bacterial cultures, between 48 h and 96 h of incubation, following an enzymatic lysis protocol [20]. Isolates were identified by amplifying the 16S rRNA gene sequences using the primers 27F and 1492R [21]. Amplicons were compared using BLAST [22] and aligned with the closely related species at the non-redundant GenBank 16S ribosomal RNA database from the National Center for Biotechnology Information (NCBI). Phylogenetic trees were generated based on maximum-likelihood using FastTree and iTOL [23, 24]. Once identified, isolates with an identity percentage lower than 99.7% with type strains on the database were selected for whole genome sequencing.

Genome sequencing and processing

Genomes were sequenced using a 150 bp paired-end run on Illumina NovaSeq 6000 and PacBio Sequel II platforms (Pacific Biosciences) using P6 chemistry. Reads from Illumina were quality-controlled and decontaminated from sequencing artefacts and adapters, and merged using BBtools (version 37.62) [25]. Assembly was performed using SPAdes (version 3.15.0) [26] under a high-coverage isolate tag for trimmed reads. Long reads from PacBio were assembled using Trycycler (version 0.5.3) [27] utilizing post-filtered reads from PacBio and short-read corrected Illumina reads. All sequencing services were carried out by Novogene Company Ltd. (Cambridge, UK). The final genome assemblies were submitted to the ENA database under the BioProject number PRJEB56193. Individual genome accession numbers are given in File S1.

Annotation and gene prediction was done with Prokka (version 1.14.5) [28]. Further metabolic pathway analyses, motif validation and participation in individual biogeochemical transformations were annotated with METABOLIC (METabolic And BiogeOchemistry anaLyses In miCrobes; version 4.0) [29]. Both annotation strategies were used to predict the main metabolic pathways in the collection.

Metabolic characterization

Cellular fatty acid and biochemical profiling of the novel strains was carried out by DSMZ services, Leibniz-Institut DSMZ–Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). In total, 300 mg frozen wet-weight biomass was used for the Microbial Identification System (MIDI Inc., version 6.1). The composition of cellular fatty acids was identified by comparison with the TSBA40 naming table.

The Biolog GEN III MicroPlate system was used to perform 71 different carbon source utilization assays and 23 chemical sensitivity assays. In brief, the isolate was grown on an agar medium and then suspended in a special 'gelling' inoculating fluid (IF) at the recommended cell density. The cell suspension was inoculated (100 µl per well) into the GEN III MicroPlate and incubated to allow the phenotypic fingerprint to form. Increased respiration reduces the tetrazolium redox dye, giving a purple colour. Negative wells and the negative control (a well with no carbon source) remain colourless. After incubation, the phenotypic fingerprint of purple wells was compared to Biolog's species library to assign an identity.

Taxonomy analysis

Genomes were taxonomically classified with GTDB-Tk (version 2.1.0) [30] and GTDB (R214). Isolates that were not assigned to a reliable taxonomic identity were further analysed using genomic comparison of core genes using the concatenated-gene-alignment-fasta tool from Anvi'o version 7.1 [31]. Phylogenetic trees were inferred by maximum-likelihood using FastTree [23] and visualized in iTOL [24]. Identity parameters for taxonomic delineation, namely digital DNA–DNA hybridization (dDDH), average amino acid identity (AAI) and average nucleotide identity (ANI), were calculated using the Genome-to-Genome Distance Calculator from the DSMZ [32], EzAAI (version 1.2.1) [33] and FastANI (version 1.33; https://github.com/ParBLiSS/FastANI). Sequences for the type strains of the genera *Limosilactobacillus*, *Ligilactobacillus* and *Clostridium*, as well as the family *Oscillospiraceae*, were collected using the NCBI genome browser (www.ncbi.nlm.nih.gov/datasets/genome/) considering genus type strains for each taxonomic group according to the List of Prokaryotic Names with Standing in Nomenclature (LPSN). Trees were visualized and annotated in iTOL (version 6.5.8) [34]. Accession numbers of the reference genomes used in the trees are provided in File S1 (Supplementary Table 1.3).

RESULTS

In this work, bacterial cultivation from crop, jejunum and ileum of chicken provided eight novel species that were further characterized, sequenced and functionally annotated. These species represent novel taxa according to the 16S rRNA gene sequence analysis and genome-based taxonomic assignment. Among them, two species identified as *Limosilactobacillus galli* sp. nov. and *Clostridium butanoliproducens* sp. nov. were obtained from crop samples; one species characterized as *Ligilactobacillus hohenheimensis* sp. nov. was obtained from jejunum; four species of *Limosilactobacillus avium* sp. nov., *Limosilactobacillus viscerum* sp. nov., *Limosilactobacillus difficilis* sp. nov. and *Faecalispora anaeroviscerum* gen. nov. sp. nov., obtained from ileum, represent three novel species and one novel genus, respectively. Finally, two isolates obtained from samples of crop and ileum were identified and named as *Limosilactobacillus pulli* sp. nov. All species belong to the families *Clostridiaceae*, *Lactobacillaceae* and *Oscillospiraceae*.

Phylogenetic analysis of 16S rRNA gene and genomic sequences

The analysis of 16S rRNA genes revealed a similarity of below 99.7%, considered as a threshold for species delineation, for all the isolates when compared to closely related type strains Whole genome phylogenetic analysis was performed using a concatenated sequence of core genes and considering the pairwise indexes ANI (>95–96%), AAI (>96%) and cAAI (>97%) at intra-species level, and AAI (>65%), cAAI (>65%) and a G+C content difference of above 1% at intra-genus level. Annotation of trees reconstructed with 16S rRNA gene and genomic sequences depicts the species cluster in the first stripe (Figs 1–3). The genomes of *C. butanoliproducens* (Fig. 1), *Limosilactobacillus galli* (Fig. 2) and *Ligilactobacillus hohenheimensis* (Fig. 3) share a species cluster with accession numbers already available in the databases [13].

The taxonomic assignment and pairwise index calculation analysis of strain CIES53^T identified as *Faecalispora anaeroviscerum* sp. nov., revealed that the related species '*Clostridium merdae*', '*Clostridium minihomine*', *Clostridium sporosphaeroides* and *Clostridium jeddahense*, assigned to the genus *Clostridium*, family *Clostridiaceae* [35], actually belong to the family *Oscillospiraceae* and share a common ancestor. These species are related to the type species *Caproicibacter fermentans* EA1^T, *Caproiciproducens galactitolivorans* BS-1^T, *Neglectibacter timonensis* SN17^T, *Clostridium sporosphaeroides* ATCC 25781^T and *Clostridium jeddahense* JCD^T. The last two were previously classified as *Clostridium* but suggested to be reclassified according to [34], naming them *Faecalispora sporosphaeroides* DSM 1294^T and *Faecalispora jeddahensis* DSM 27834^T. The analysis in Fig. 4 depicts the phylogenetic and pairwise analysis that supported the description of the novel genus and the further combination of the species to the genus *Faecalispora*. A complete phylogenetic analysis including all members of the family *Oscillospiraceae* is available in the Fig. S1, available in the online version of this article.

Functional annotation

The main fermentation pathways detected on the novel species are summarized in Fig. 5. All novel *Lactobacillaceae* encode ethanol and lactic acid formation via lactate dehydrogenase, the latter being the most common fermentative pathway present among the novel species genomes, followed by the acetogenesis pathway, which was present in all novel species except for *Ligilactobacillus hohenheimensis*. Among the novel species, acetogenesis was detected solely by the acetate kinase. An enzyme that has been reported to stimulate the chemotaxis signal system, CheA-CheYBV [36], was detected in *F. anaeroviscerum* and *C. butanoliproducens*. The less common fermentative pathways were butyrate fermentation, detected only in *F. anaeroviscerum*, and butanol production via butanol dehydrogenase encoded by *C. butanoliproducens*.

Annotation revealed functional differences in the number and type of genes for carbohydrate-active enzymes (CAZyme database) and peptidases (MEROPS database). *C. butanoliproducens*, isolated from crop, encoded the highest amounts of genes assigned to glycosidases and peptidases (File S2). Such enzymes are specialized in the degradation of dextrins, furanosides, glucans, mannans and xyloglucans, which underlines the potential of the species to obtain carbon from complex carbohydrate hydrolysis. Genes encoding hydrogenase-processing endopeptidases were also found encoded in *C. butanoliproducens* and protease insulin-like degrading enzymes were found encoded in the novel species *F. anaeroviscerum*. Genes that encoded enzymes with possible interaction with glucuronic acid (K01195) were encoded in the genome of *Ligilactobacillus hohenheimensis*, these structures can potentially interact with glycosaminoglycans and structural polysaccharides found in connective tissue (Fig. 5).

Metabolic characterization

The species of *Limosilactobacillus* isolated in this work were able to assimilate glucose and raffinose, a trait that was confirmed by the presence of glucose and raffinose transporter genes. They all metabolized maltose, except for *Limosilactobacillus avium*, and fructose, except for *Limosilactobacillus viscerum*, despite maltose and fructose transporters being codified in all genomes. None of the novel species utilized ribose, unlike other *Limosilactobacillus* species; a phenotypic trait that matches the absence of the *rbs* gene responsible for ribose uptake and phosphorylation. All strains presented a high percentage of the cellular fatty acids $C_{16:0}$ and $C_{18:0}$. *Limosilactobacillus avium* had a different membrane lipid profile, presenting additional

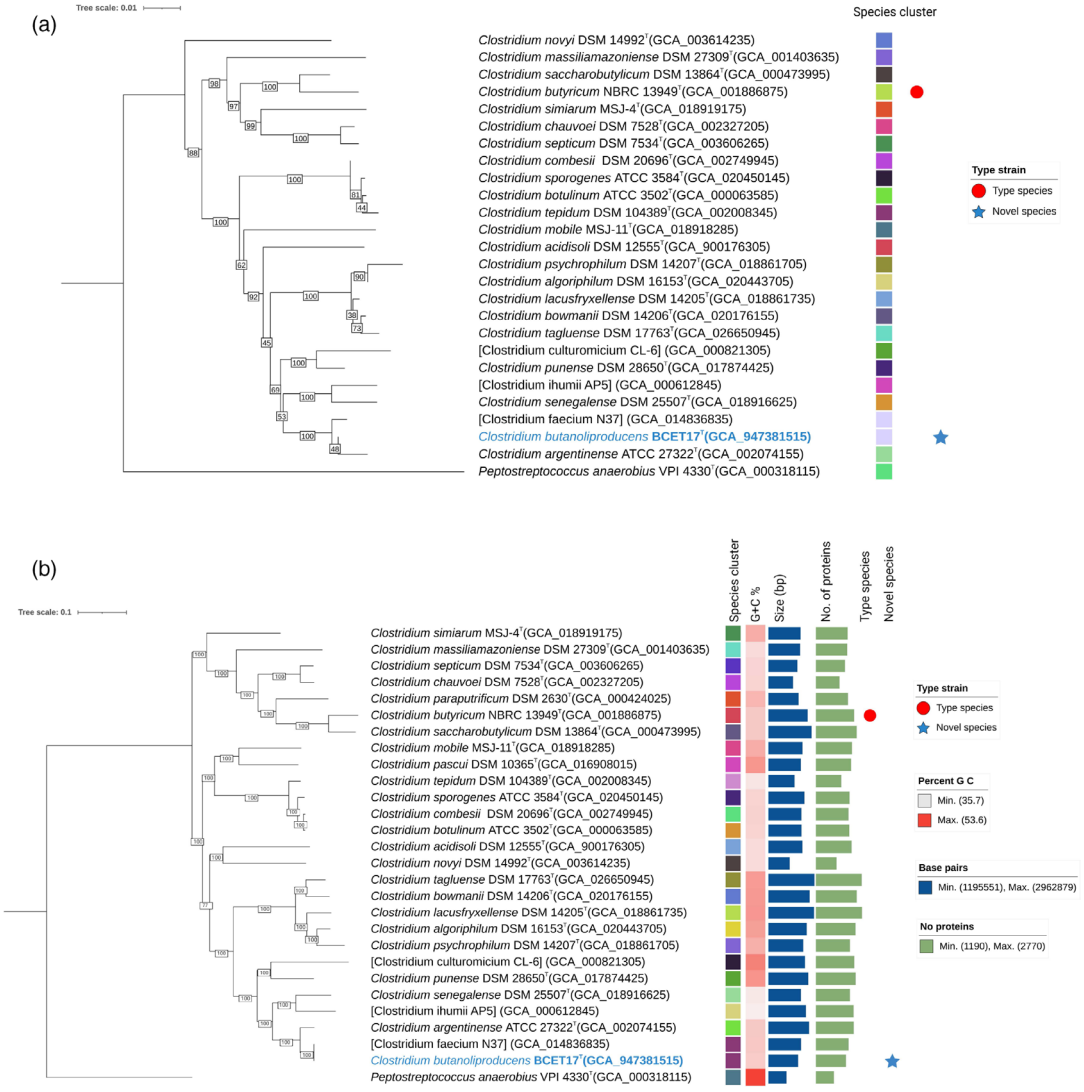

Fig. 1. Phylogenetic trees of species from the genus *Clostridium* including the novel species Clostridium *butanoliproducens* and annotation of the related species. Novel species are highlighted in blue. (a) Phylogenetic tree based on the 16S rRNA gene sequences. (b) Phylogenetic tree based on 87 concatenated core genes obtained from each genome. Both trees were inferred by the maximum-likelihood method and visualized with iTol using *Clostridium butyricum* NBRC 13949^T as the genus type species and *Peptostreptococcus anaerobius* VPI 4330^T as an outgroup. Bootstrap values (1000 replicates) are depicted above branches and branch lengths correspond to sequence differences indicated by the scale bar above.

unsaturated lipids $C_{19:0}$ cyclo ω10*c*/19 ω6 and $C_{19:1}$ ω6*c* and no $C_{18:1}$ ω7*c*, as compared to the *Limosilactobacillus* reference strains (*L. reuteri* DSM 20016^T and *L. fermentum* ATCC 14931^T) (File S3).

Ligilactobacillus hohenheimensis presented a narrower carbohydrate utilization profile under the tested conditions, despite it encoding the phosphotransferase transporter system for sucrose, fructose, mannitol, mannose and sorbitol, and Leloir pathway genes for D-galactose catabolism. Additionally, its growth with acetic acid as a source of carbon might be assimilated via the reductive acetyl-CoA pathway encoded in its genome. Compared to reference strains, *Ligilactobacillus hohenheimensis* presented the highest percentage of $C_{18:0}$, which was not present in the genus type strain *L. salivarius* DSM 20555^T and was the third most common fatty acid in cells of *L. pabuli* JCM 34518^T (File S3).

Faecalispora anaeroviscerum is a Gram positive, spore forming rod according to Gram stain and fluorescence microscopy. It expressed a wide carbohydrate and fatty acid utilization spectrum that included glucose, galactose, fructose, mannose, turanose, glucuronic acid, cellobiose, fucose, gentiobiose, maltose, melibiose, raffinose and rhamnose, as well as butyrate, hydroxy-butyrate, acetate, propionate, formate and sodium butyrate. The wide range of carbon source utilization from this species and other members of the family *Oscillospiraceae* does not match the narrow profile of carbohydrate utilization of

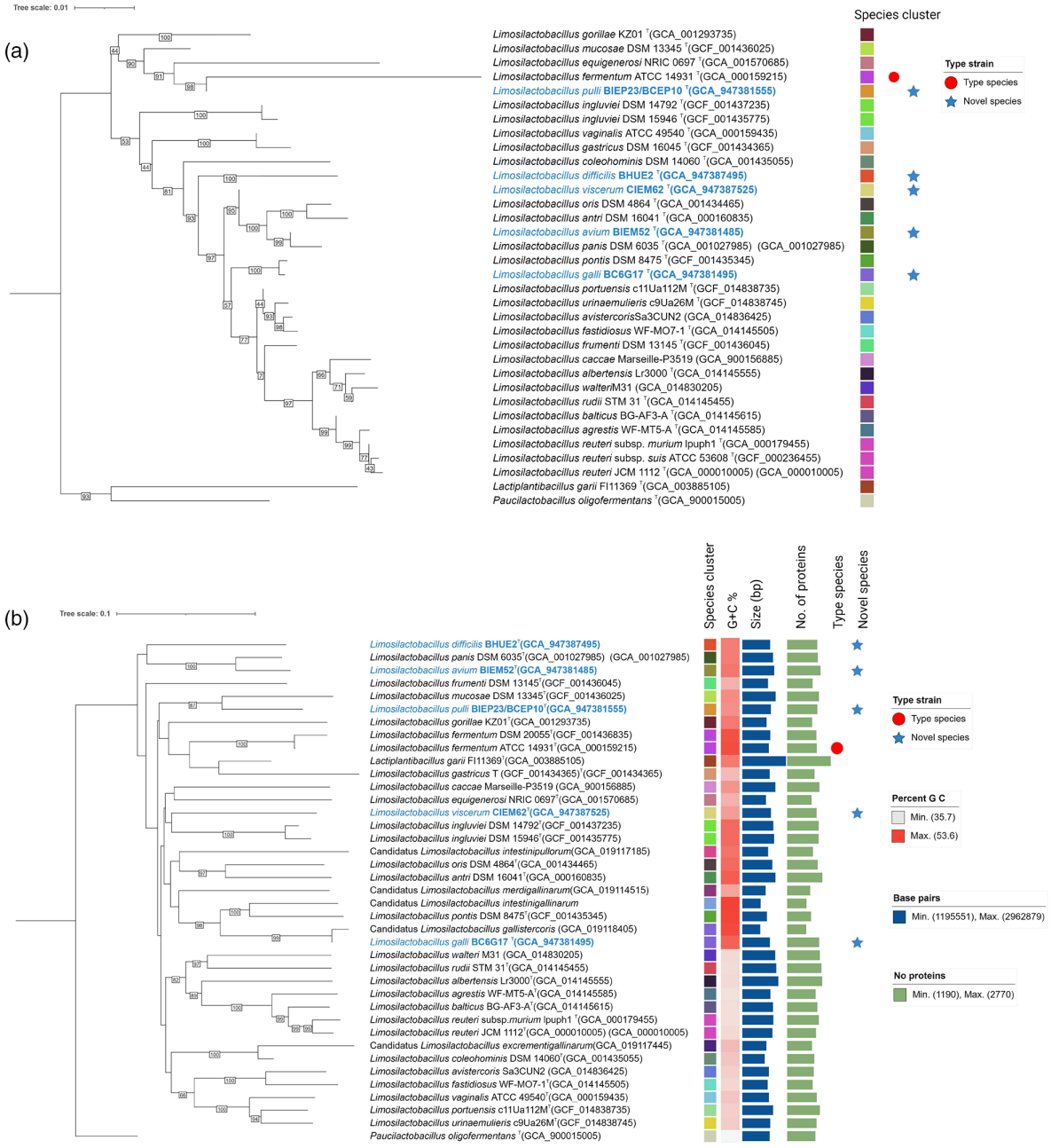

Fig. 2. Phylogenetic trees of species from the genus *Limosilactobacillus* and annotation of the related species. Novel species are highlighted in blue. (a) Tree based on the 16S rRNA gene sequences. (b) Tree based on 71 concatenated core genes obtained from each genome. Both trees were inferred by the maximum-likelihood method and visualized with iTol using *Limosilactobacillus fermentum* ATCC 14931^{T} as the genus type species and *Paucilactobacillus oligofermentans* AMKR18^{T} as an outgroup. Bootstrap values (1000 replicates) are depicted above branches and branch lengths correspond to sequence differences indicated by the scale bar above.

species of *Clostridium* from the family *Clostridiaceae* where this group was formerly assigned. This highlights the ecological and taxonomic separation within these two groups and supports the need for re-classification. The cellular fatty acid profile matches the high percentage of $C_{14:0}$ and $C_{16:0}$ N alcohol of *Caproicibacter fermentans* DSM 107079^{T}, but additionally expressed more than 10% of $C_{17:1}$ iso and $C_{18:1}$ ω7*c*, which is unique within the compared strains. Metabolic capabilities and a description of the genus are detailed in Table 1, and the rest of the species comparisons are listed in File S3.

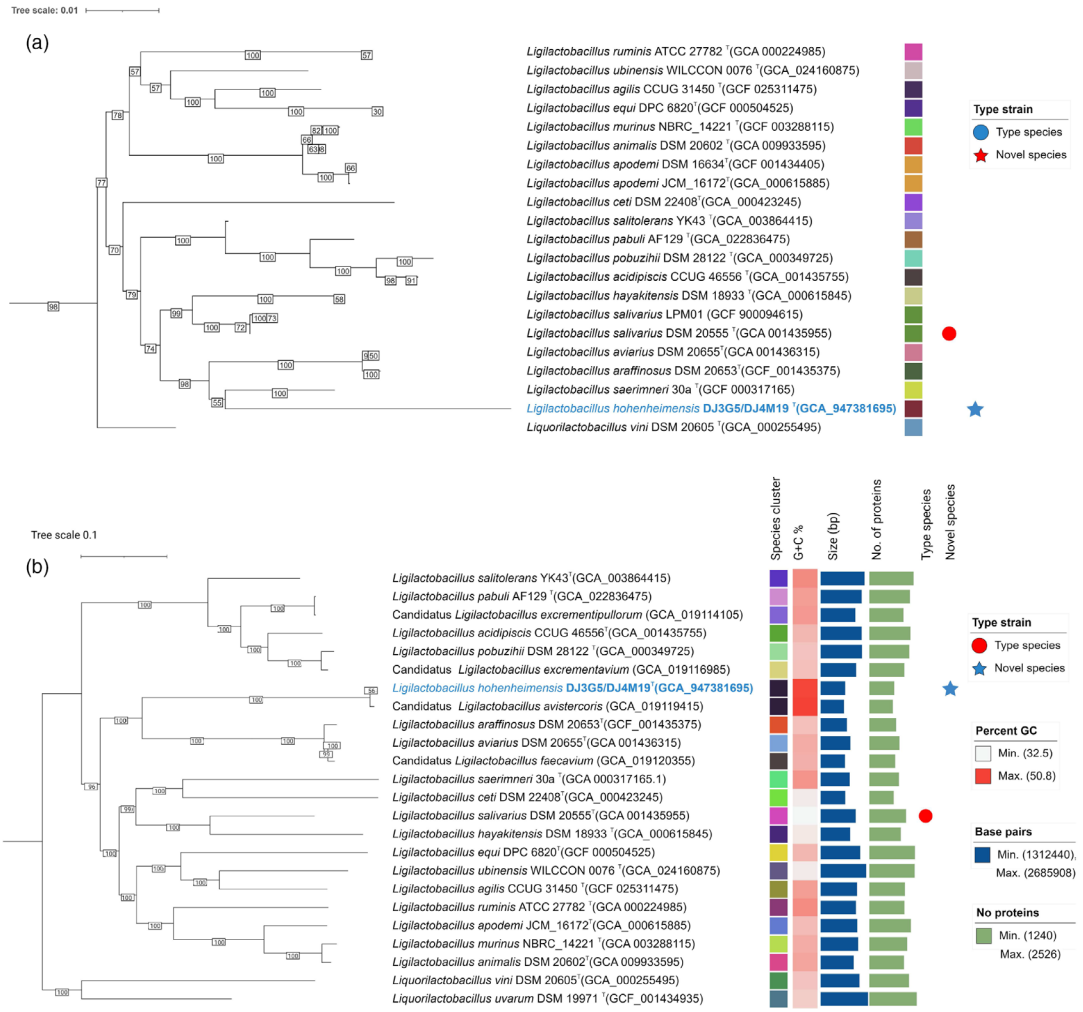

Fig. 3. Phylogenetic tree of species from the genus *Ligilactobacillus* including the novel species Ligilactobacillus *hohenheimensis* and annotation of the related species. Novel species is highlighted in blue. (a) Tree based on 16S rRNA gene sequences. (b) Tree based on 91 concatenated core genes obtained from each genome. Both trees were inferred by the maximum-likelihood method and visualized with iTol using *Ligilactobacillus salivarius* DSM 20555^T as the genus type species and *Liquorilactobacillus vini* DSM 20605^T as an outgroup. Bootstrap values (1000 replicates) are depicted above branches and branch lengths correspond to sequence differences indicated by the scale bar above.

Taxonomic descriptions

The classification of the isolates was done based on 16S rRNA gene and genome sequence analysis using the GTDB database (R214) with the tool GTDB-Tk and reconstructing phylogenomic trees. Additionally, differences within species in terms of DNA G+C content (>1%) also supported the status of distinct species (data S3).

DESCRIPTION OF *LIMOSILACTOBACILLUS GALLI* SP. NOV.

Limosilactobacillus galli (gal'li. L. gen. n. *galli*, of a chicken).

Cells are anaerobic facultative, Gram-stain-positive and have a short rod shape. The genome encodes aminotransferase class I and II, complex V (ATPsynthase) and genes predicting the utilization of lactate, cellulose and oligosaccharides as possible carbon sources. Formation of ethanol and lactic acid after fermentation of sugars and glutaredoxin are predicted. Sugar phosphatases *ybiV*, *ybjI* and *yidA*, dITP/XTP pyrophosphatase, oligoribonuclease and PAP phosphatases, pyrophosphatase *ppaX*, phosphoserine phosphatase, tyrosine phosphatase, phosphoglycolate phosphatase, threonine phosphatase, and exopolyphosphatase are encoded. On Biolog agar media with 5% sheep blood incubated at 37°C under microaerophilic conditions, the species assimilates maltose, sucrose, raffinose, fructose, lactic acid, serine, α-keto-glutaric acid, acetic acid,

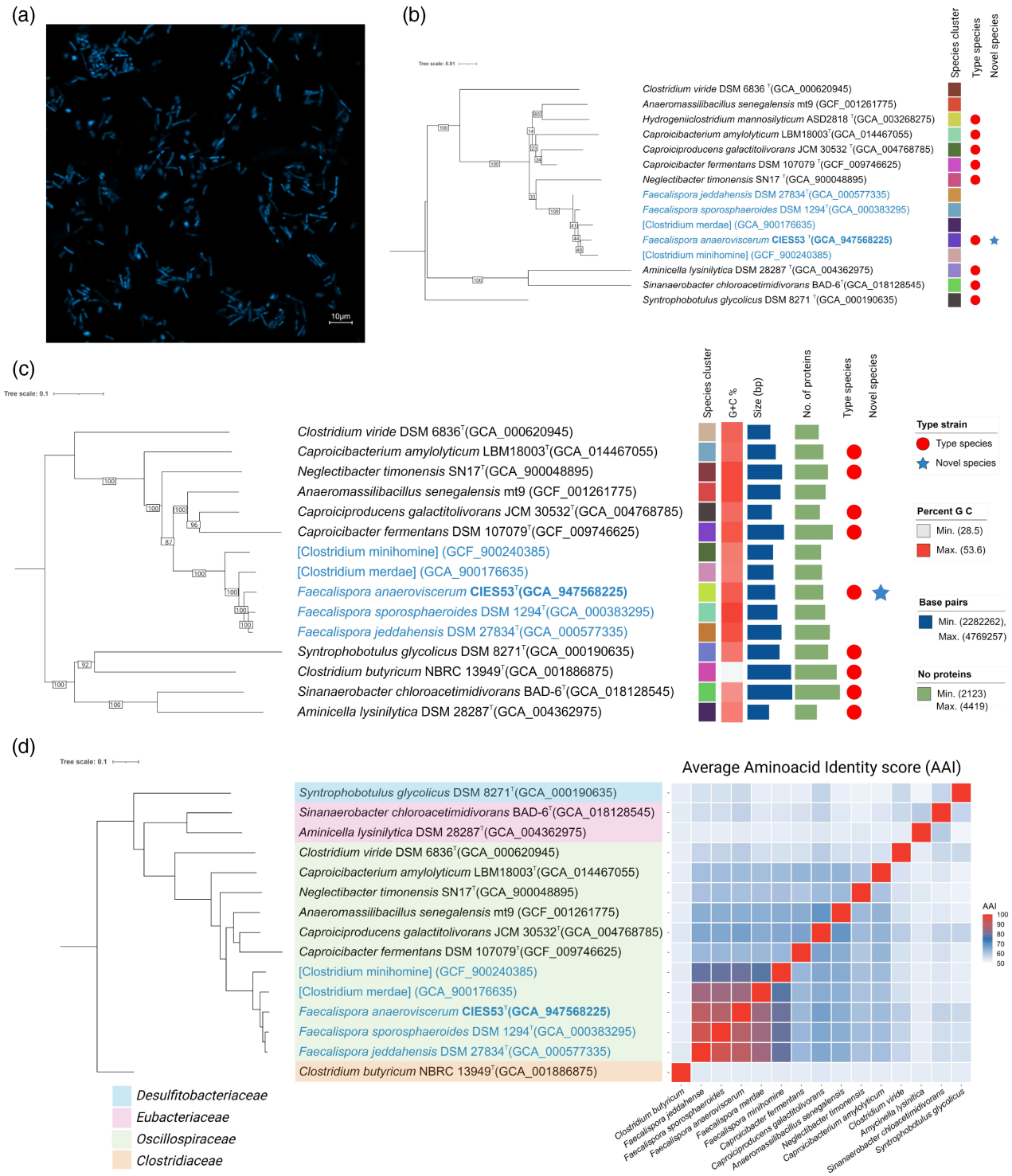

Fig. 4. Phylogenetic analysis of species closely related to the novel genus description *Faecalispora* gen. nov. (a) The type species of the genus, *F. anaeroviscerum* CIES53^T, at 48 h of incubation, stained with DAPI and detected by fluorescent microscopy (Zeiss cLSM900). (b) Tree based on 16S rRNA gene sequences. (c) Tree based on a concatenated sequence of 49 core genes. Both trees were inferred by the maximum-likelihood method, annotated according to the genome features of each strain and visualized with iTol. The type species of each genus are marked with a red dot and the clade of *Faecalispora* gen. nov. is marked in blue letters. *Clostridium butyricum* NBRC 13949^T, *Syntrophobotulus glycolicus* DSM 8271^T, *Sinanaerobacter chloroacetimidivorans* BAD-6^T and *Aminicella lysinilytica* DSM 28287^T were used as external groups from the families *Clostridiaceae*, *Desulfitobacteriaceae* and *Eubacteriaceae*, respectively. Both figures show monophyly and high similarity among species of *Faecalispora* gen. nov. (d) Correlation matrix of average amino acid identity scores calculated among reference species closely related to *F. anaeroviscerum* CIES53^T. Branch lengths correspond to sequence differences indicated by the scale bar above each tree.

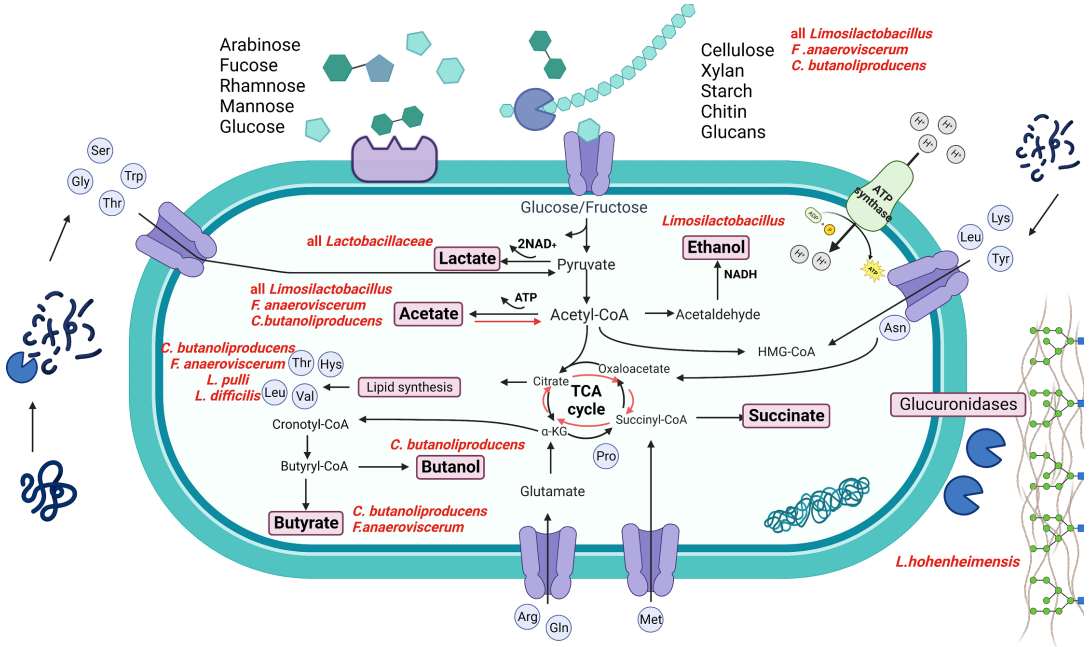

Fig. 5. Outline of the main fermentation pathways encoded by novel species obtained from the upper digestive system of chicken. The taxonomic identity is highlighted in red next to the encoded pathway.

butyric acid, sodium butyrate and acetoacetic acid, and tolerates minocycline, tetrazolium violet, tetrazolium blue potassium tellurite, Tween 40, 8% NaCl and sodium bromate. The predominant cellular fatty acids are $C_{16:0}$, $C_{18:0}$ and $C_{19:0}$.

The type strain, BC6G17^T (=DSM 113833^T=LMG 32623^T), was isolated on gut microbiota medium from crop of Brown Lohmann laying hens. The genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947381495 and OM760982, respectively.

DESCRIPTION OF *LIMOSILACTOBACILLUS AVIUM* SP. NOV.

Limosilactobacillus avium: (a'vi.um. L. gen. pl. n. *avium*, of birds).

Cells are anaerobic facultative, Gram-stain-positive and have a short rod shape. The genome encodes flavin prenyltransferase, aminotransferase class I and II, complex V (ATPsynthase) and subunit I of cytochrome ubiquinol oxidase. Utilization of lactate, cellulose, rhamnosides and xylosides from hemicellulose and oligosaccharides as possible carbon substrates and formation of ethanol, acetate and lactic acid after fermentation of sugars is encoded. Genes for sugar phosphatases *ybi*V, *ybjI* and *yidA*, dITP/XTP pyrophosphatase, oligoribonuclease and PAP phosphatases, pyrophosphatase PpaX, phosphoserine phosphatase, tyrosine phosphatase, phosphoglycolate phosphatase, threonine phosphatase, and exopolyphosphatase are identified. On Biolog agar media with 5% sheep blood incubated at 37°C under microaerophilic conditions, the species assimilates trehalose, gentiobiose, glucose, raffinose, fructose, lactic acid, serine, sorbitol, glucoronamide, α-keto-glutaric acid, α-keto-butyric acid, acetic acid, butyric acid, propionic acid, formic acid, acetoacetic acid, sodium butyrate and sodium lactate, and tolerates rifamycin, minocycline, potassium tellurite, guanidine HCl, vancomycin, tetrazolium, nalidixic acid, 8% NaCl and sodium bromate. The predominant cellular fatty acids are $C_{16:0}$ and summed feature 7 ($C_{19:0}$ cyclo ω10*c*/19 ω6 and $C_{19:1}$ ω6*c*).

The type strain, BIEM52^T (=DSM 113849^T=LMG 32671^T), was isolated on MRS medium from ileum of Brown Lohmann laying hens. The genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947381485 and OM760983, respectively.

DESCRIPTION OF *LIMOSILACTOBACILLUS PULLI* SP. NOV.

Limosilactobacillus pulli (pul'li. L. gen. n. *pulli*, of a chicken).

Cells are anaerobic facultative, Gram-stain-positive and have a small rod shape. The genome encodes for phosphoserine and the aromatic aminotransferases for histidinol, aspartate and tyrosine aromatic aminotransferase, aminotransferase class I and II,

Table 1. Comparison of phenotypic traits of type species related to *Faecalispora* gen. nov

Trait	*Faecalispora anaeroviscerum* sp. nov. CIES53^T	*Faecalispora sporosphaeroides* DSM 1294^T	*Faecalispora jeddahensis* DSM 27834^T	*Caproicibacter fermentans* DSM 107079^T	*Caproiciproducens galactitolivorans* JCM 30532^T	*Neglectibacter timonensis* DSM 102082^T	*Clostridium butyricum* CCUG 34471^T
Spore formation	+	+	+	NA	NA	NA	+
L-Arabinose	NA	NA	NA	+	+	NA	NA
D-Ribose	NA	NA	NA	NA	+	+	NA
D-Xylose	NA	NA	NA	+	+	+	NA
D-Galactose	+	NA	NA	+	+	NA	+
D-Glucose	+	NA	NA	+	+	+	+
D-Fructose	+	NA	NA	+	+	+	NA
D-Mannose	+	NA	na	+	+	NA	NA
Turanose	+	NA	NA	NA	NA	NA	NA
D-Glucuronic acid	+	NA	NA	NA	NA	NA	NA
Cellobiose	+	NA	NA	+	+	NA	NA
D-Ribose	NA	NA	NA	NA	NA	+	NA
Aesculin	NA	NA	NA	NA	NA	+	NA
Cellulose	NA	NA	NA	NA	NA	NA	+
Fucose	+	NA	NA	NA	+	NA	NA
Dulcitol	NA	NA	NA	NA	+	NA	NA
Glycerol	NA	NA	NA	NA	+	+	NA
Galactitol	NA	NA	NA	+	NA	NA	NA
Gentobiose	+	NA	NA	NA	NA	NA	NA
Maltose	+	NA	NA	NA	NA	+	NA
Mannitol	NA	NA	NA	+	NA	NA	NA
Melibiose	+	NA	NA	NA	NA	NA	NA
Sucrose	NA	NA	NA	+	NA	+	NA
Starch	NA	NA	NA	+	+	NA	NA
Sorbitol	NA	NA	NA	+	NA	NA	NA
Raffinose	+	NA	NA	NA	NA	NA	NA
Rhamnose	+	NA	NA	NA	NA	NA	NA
Fatty acids (%):	NA	NA	NA	NA	NA	NA	NA
$C_{12:0}$	15.1	4.3	NA	NA	NA	NA	0.7
$C_{13:0}$ iso	0.9	1	NA	NA	NA	5	0.6
$C_{13:1}$ at 12–13	4.3	NA	NA	NA	NA	NA	NA
$C_{14:0}$	17.4	4.5	NA	23.1	3.2	10	13.7
$C_{14:0}$ iso	NA	NA	NA	NA	NA	4.8	NA
$C_{14:0}$ DMA	NA	8.9	NA	8.2	5.4	NA	NA
$C_{15:0}$ iso	NA	NA	NA	NA	NA	12.5	NA
$C_{15:0}$ anteiso	NA	NA	NA	NA	NA	18.3	NA
$C_{16:0}$	8.9	1.9	NA	13.2	3.1	31.3	27.1
$C_{16:0}$ aldehyde	NA	22.6	NA	NA	14.1	NA	NA
$C_{16:0}$ DMA	NA	35.9	NA	17	22.1	NA	NA

Continued

Table 1. Continued

Trait	*Faecalispora anaerovisceru*m sp. nov. CIES53^T	*Faecalispora sporosphaeroides* DSM 1294^T	*Faecalispora jeddahensis* DSM 27834^T	*Caproicibacter fermentans* DSM 107079^T	*Caproiciproducens galactitolivorans* JCM 30532^T	*Neglectibacter timonensis* DSM 102082^T	*Clostridium butyricum* CCUG 34471^T
$C_{16:0}$ N alcohol	16.9	NA	NA	NA	NA	NA	5.4
$C_{16:1}$ ω5*c*	NA	NA	NA	8	NA	NA	0.8
C16:1 ω7*c*	0.8	NA	NA	11.7	NA	NA	5.2
$C_{16:1}$ ω7*c* DMA	NA	NA	NA	5	NA	NA	3.8
$C_{16:1}$ ω9*c*	1	1.2	NA	NA	NA	NA	7.7
$C_{16:1}$ ω9*c* DMA	NA	NA	NA	NA	NA	NA	5.7
$C_{17:0}$ anteiso	NA	NA	NA	NA	5.9	1.4	1.9
$C_{17:0}$ iso 3OH	NA	NA	NA	NA	NA	NA	1.5
$C_{18:0}$	0.5	NA	NA	NA	NA	9.4	2.2
$C_{18:0}$ aldehyde	NA	1.8	NA	NA	3.1	NA	NA
$C_{18:0}$ DMA	NA	2.5	NA	NA	4	NA	NA
$C_{18:1}$ ω11*c* DMA	NA	NA	NA	NA	4.6	NA	NA
$C_{18:1}$ ω7*c*/12*t*/9*t*	NA	NA	NA	NA	1.1	NA	6.9
$C_{18:1}$ ω7*c* DMA	NA	NA	NA	1.6	NA	NA	2.8
$C_{18:1}$ ω9*c*	1.3	NA	NA	NA	7.6	2.4	3
$C_{18:1}$ ω9*c* DMA	NA	NA	NA	NA	NA	NA	1.8
$C_{18:2}$ ω9*c*/ω12*c*	NA	1.4	NA	NA	1.1	1.9	NA
$C_{19:0}$ cyclo ω8*c*	NA	NA	NA	NA	NA	NA	4.2
$C_{19:0}$ cyclo ω9*c*	NA	NA	NA	NA	NA	NA	1.1
Summed feature 1	9.9	4.3	NA	1.3	2.5	NA	NA
Summed feature 2	NA	NA	NA	NA	NA	NA	NA
Summed feature 3	5.79	NA	NA	NA	NA	NA	NA
Summed feature 4	16	NA	NA	3	NA	NA	NA
Summed feature 5	NA	4.7	NA	NA	2.4	NA	NA
Summed feature 7	NA	NA	NA	NA	5.6	NA	NA
Summed feature 8	1.1	NA	NA	NA	3.5	NA	NA
Summed feature 9	NA	NA	NA	NA	NA	NA	NA
Summed feature 10	NA	NA	NA	1.1	NA	NA	NA
Summed feature 11	NA	1.1	NA	NA	10	NA	NA
Unidentified	NA	NA	NA	NA	NA	NA	3.1

NA mean information was not available for the type strain.

complex V (ATPsynthase), and subunit I of cytochrome ubiquinol oxidase. Utilization of lactate, cellulose and oligosaccharides through β-glucosidases and β-galactosidases as possible carbon substrates and formation of ethanol acetate and lactic acid after fermentation of sugars is encoded. Genes encoding acylphosphatases, alkaline phosphatase, sugar phosphatases *YbiV*, *YbjI* and *YidA*, dITP/XTP pyrophosphatase, oligoribonucleases, pyrophosphatase PpaX, phosphoserine phosphatase, tyrosine phosphatase, phosphoglycolate phosphatase, threonine phosphatase and exopolyphosphatase are identified. On Biolog agar media with 5% sheep blood incubated at 37°C for 48 h under anaerobic conditions, the species assimilates dextrin, D-glucose, D-fructose, D-galactose, D-glucuronic acid, maltose, turanose, fucose, gentiobiose, glucuronamide, inosine, pectin, sucrose, stachyose, sodium lactate, sodium butyrate, D-serine and L-arginine, and tolerates fusidic acid, lincomycin, minocycline, nalidixic acid, potassium

tellurite, rifamycin, tetrazolium, troleandomycin, Tween 40, vancomycin and 1–4% NaCl. The predominant cellular fatty acids are $C_{16:0}$, $C_{18:0}$ and summed feature 7 ($C_{18:1}$ ω7*c* and $C_{18:1}$ ω6*c*).

The type strain, BIEP23/BCEP10^T (=DSM 115077^T=LMG 32877^T), was isolated in PFA medium from the ileum of Brown Lohmann laying hens. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947381555 and OQ831033, respectively.

DESCRIPTION OF *LIMOSILACTOBACILLUS VISCERUM* SP. NOV.

Limosilactobacillus viscerum (vis'ce.rum. L. gen. pl. n. *viscerum*, from the internal organs).

Cells are anaerobic facultative,Gram-stain-positive and have a short rod shape. The genome encodes for aminotransferase class I and II, and the ATPase proton pump type F (complex V). Utilization of lactate, cellulose and xylose from hemicellulose as possible carbon substrates and formation of formate, acetate, ethanol and lactic acid from the fermentation of sugars is encoded. Genes for exopolyphosphatases, nucleosidases, tyrosine phosphatases, uracil phosphatase, threonine and serine phosphatase, sugar phosphatases *YbiV* and *YbjI*, dITP/XTP pyrophosphatase, and acylphosphatase were identified. On Biolog agar media with 5% sheep blood incubated at 37°C under microaerophilic conditions, the species assimilates maltose, raffinose, lactose, D-glucose, D-mannitol, D-serine, α-keto-glutaric acid, acetoacetic acid and sodium butyrate. Tolerates minocycline, tetrazolium violet, tetrazolium blue, Tween 40, potassium tellurite and sodium bromate. The predominant cellular fatty acids are $C_{16:0}$, $C_{18:0}$ and cyclopropane acid $C_{19:0}$ ω8*c*.

The type strain, CIEM62^T (=DSM 113835^T=LMG 32625^T), was isolated in MRS medium from the ileum of Brown Lohmann laying hens. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947387525 and OM760985, respectively.

DESCRIPTION OF *LIMOSILACTOBACILLUS DIFFICILIS* SP. NOV.

Limosilactobacillus difficilis (dif.fi'ci.lis. L. masc. adj. *difficilis*, difficult).

Cells are anaerobic facultative, Gram-stain-positive and have a short rod shape. The genome encodes for phosphoserine aminotransferase, aminotransferase class I and II, branched-chain amino acid aminotransferase, histidinol aminotransferase, the ATPase proton pump type F (complex V), subunit I of cytochrome ubiquinol oxidase, and chlorite dismutase. Utilizes lactate, cellulose, rhamnose and xylose from hemicellulose debranching and oligosaccharides as possible carbon substrates and forms acetate, ethanol and lactic acid from the fermentation of sugars. Genes encoding tyrosine phosphatases, sugar phosphatases YidA, exopolyphosphatases, uracil phosphatase, tyrosine-phosphatase, dITP/XTP pyrophosphatase, histidinol phosphatase, phosphoglycolate phosphatase, acylphosphatase, threonine and serine phosphatase, pyrophosphatase PpaX, and nucleosidase are identified. On Biolog agar media with 5% sheep blood incubated at 37°C for 18 h under microaerophilic conditions this species can assimilate cellobiose, dextrin, D-fructose, D-fucose, D-galactose, D-galacturonic acid, D-glucose, D-glucuronic acid, lactose, maltose, D-mannose, melibiose, raffinose, S-salicin, D-serine, D-sorbitol, trehalose, turanose, glucuronamide, inosine, L-fucose, L-galactonic acid lactone, L-rhamnose, *N*-acetyl neuraminic acid, *N*-acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, *N*-acetyl-D-mannosamine, pectin, stachyose, sucrose, acetic acid, acetoacetic acid, hydroxy-butyric acid, keto-butyric acid, keto-glutaric acid, methyl-D-glucoside, formic acid, propionic acid, sodium butyrate andsodium lactate. Tolerates aztreonam, fusidic acid, gentiobiose, guanidine HCl, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof 4, potassium tellurite, rifamycin, tetrazolium, troleandomycin, Tween 40 and vancomycin. The predominant cellular fatty acids are $C_{16:0}$, cyclopropane acid $C_{19:0}$ ω8*c* and summed feature 8 ($C_{18:1}$ ω7*c*).

The type strain, BHUE2^T (=DSM 114195^T=LMG 32875^T), was isolated in MRS medium from the ileum of broilers Ross 308. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947387495 and OQ832131, respectively.

DESCRIPTION OF *LIGILACTOBACILLUS HOHENHEIMENSIS* SP. NOV.

Ligilactobacillus hohenheimensis (ho.hen.heim.en'sis. N.L. masc. adj. *hohenheimensis*, pertaining to Hohenheim, Germany; the place of isolation).

Cells are anaerobic facultative, Gram-stain-positive and have a short rod shape. The genome encodes β-glucoronidases, β-galactosidases and hexosaminidases, and the ATPase proton pump type F (Complex V). Utilization of lactate and cellulose, and formation of acetate, ethanol and L-lactic acid from the fermentation of sugars is predicted by the genes. Genes encoding acylphosphatase, nucleosidase, dITP/XTP pyrophosphatase, threonine and serine phosphatase, exopolyphosphatases, pyrophosphatase *ppa*X, tyrosine phosphatases, tyrosine-phosphatase, and phosphoglycolate phosphatase are identified. On Biolog agar media with 5% sheep blood incubated at 37°C for 48 h under anaerobic conditions, the species assimilates maltose, D-serine,

D-fructose-6-PO_4, L-histidine, glucuronamide, sodium lactate, sodium butyrate, quinic and mucic acid. Tolerates 8% NaCl, minocycline, guanidine HCl, lithium chloride, potassium tellurite, Tween 40, tetrazolium violet and tetrazolium blue. The predominant cellular fatty acids are $C_{18:0}$ and $C_{16:0}$.

The type strain, DJ3G5/DJ4M19^T (=DSM 113870^T=LMG 32876^T), was isolated in MRS medium from the jejunum of broilers Ross 308. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947381805 and OM760988, respectively.

DESCRIPTION OF *CLOSTRIDIUM BUTANOLIPRODUCENS* SP. NOV.

Clostridium butanoliproducens (bu.ta.no.li.pro.du'cens. L. neut. n. *butanol*, butanol; L. pres. part. *producens*, producing; N.L. part. adj. *butanoliproducens*, butanol producing).

Cells are strictly anaerobic, Gram-stain-positive, rod-shaped and produce endospores. The genome encodes branched-chain amino acid aminotransferases, aspartate and tyrosine aminotransferases, acyl-CoA dehydrogenase, formate C-acetyltransferase, and the Wood–Ljungdahl pathway. It presents gene clusters for nitrogen fixation (*nifDK*), sulphur oxidation (*dsrAB*), sulphite reduction (*asrABC*), a cytoplasmic Fe-Fe hydrogenase, and the ATPase proton pump types V and F (complex V). Degrades chitin and produces ethanol butanol and acetate. Encodes nucleoside triphosphatases, phosphoglycolate phosphatase, dTTP/UTP pyrophosphatases, phosphosulfolactate phosphatase, arginine-phosphatases, histidinol phosphatases, CheY-P phosphatase (chemotaxis), sugar phosphatases *ycdXY* and *yidA*, dITP/XTP pyrophosphatase, and pyrophosphatases *ppaX*. On Biolog agar media with 5% sheep blood incubated at 37°C for 47.25 h under anaerobic conditions, the species assimilates dextrin, D-galacturonic acid, D-glucose, D-glucuronic acid, D-fructose, fucose, glucuronamide, L-galactonic acid lactone, L-rhamnose, pectin, acetoacetic acid, aminobutyric acid, hydroxy-butyric acid, keto-butyric acid, keto-glutaric acid, *p*-hydroxy-phenylacetic acid, sodium lactate, sodium butyrate, butyric acid and D-serine. Tolerates aztreonam, fusidic acid, gentiobiose, guanidine HCl, lincomycin, lithium chloride, minocycline, nalidixic acid, niaproof 4, potassium tellurite, rifamycin, tetrazolium, troleandomycin, Tween 40, vancomycin and NaCl at 1, 4 and 8% concentration. The predominant cellular fatty acids are $C_{16:0}$ and the summed feature 4 ($C_{17:1}$ iso I/anteiso B).

The type strain, BCET17^T (DSM 115076^T=LMG 32878^T), was isolated in tryptic soybean agar medium from the crop of Brown Lohmann laying hens. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947381515 and OQ831034, respectively.

DESCRIPTION OF *FAECALISPORA* GEN. NOV.

Faecalispora (Fae.ca.li.spo'ra. N.L. masc./fem. adj. *faecalis*, pertaining to faeces; Gr. fem. n. *spora*, a seed, in biology, a spore; N.L. fem. n. *Faecalispora*, a spore-forming organism from faeces).

A bacterial genus identified by genomic analysis of the species *F. anaeroviscerum*, assigned to the family *Oscillospiraceae*. The species *Clostridium jeddahense* and *Clostridium sporosphaeroides*, formerly assigned to *Clostridium* [35, 37], are closely related species that cluster together as members of the novel genus *Faecalispora* and share a common ancestor according to the phylogenetic analysis (Fig. 4).

DESCRIPTION OF *FAECALISPORA ANAEROVISCERUM* SP. NOV.

Faecalispora anaeroviscerum (an.a.e.ro.vis'ce.rum. Gr. pref. *an*, not; Gr. masc. n. *aer*, air; L. neut. pl. n. *viscera*, internal organs of the body; N.L. gen. pl. n. *anaeroviscerum*, anaerobic from the internal organs).

Cells are strictly anaerobic, Gram-stain-positive, rod-shaped and do not produce endospores. The genome encodes for aminotransferase class I and II, phosphoserine aminotransferase, ornithine aminotransferase branched-chain amino acid aminotransferase, aspartate and tyrosine aromatic aminotransferase, histidinol aminotransferase, and acyl-CoA dehydrogenase. Presents gene clusters for sulphur oxidation (*dsrAB*), sulphite reduction (*asrABC*), a cytoplasmic Fe-Fe hydrogenase, and the ATPase proton pump type V and F (complex V). Degrades cellulose and chitin and utilizes ethanol, butanol and lactate as carbon sources. Transforms pyruvate to formate. Genes for histidinol phosphatases, dITP/XTP pyrophosphatase, *cheY-P* phosphatase (chemotaxis), Undecaprenyl-diphosphatase, pyrophosphatases *ppaX*, phosphoglycolate phosphatase, tyrosine-phosphatase, phosphoserine phosphatase, arginine phosphatases, phosphoglycolate phosphatase and nucleoside phosphatases are encoded. On Biolog agar media with 5% sheep blood incubated at 37°C for 22 h under anaerobic conditions, the species assimilates maltose, cellobiose, gentiobiose, turanose, raffinose, melibiose, D-glucose, D-mannose, D-fructose, D-galactose, 3-methyl glucose, D-fucose, L-fucose, L-rhamnose, sodium lactate, D-serine, D-glucose-6-PO_4, D-fructose-6-PO_4, L-histidine, L-serine, pectin, D-galacturonic acid, L-galactonic acid lactone, D-glucuronic acid, glucuronamide, mucic acid, amino-butyric acid, β-hydroxy-D,L-butyric acid, α-keto-butyric acid, acetoacetic acid, propionic acid, acetic acid, formic acid and sodium butyrate. Tolerates troleandomycin, rifamycin, minocycline, lincomycin, guanidine HCl, vancomycin, tetrazolium violet, tetrazolium blue, *p*-hydroxy-phenylacetic acid, methyl

pyruvate, nalidixic acid, lithium chloride, potassium tellurite, aztreonam and sodium bromate. The predominant cellular fatty acids are $C_{14:0}$, $C_{16:0}$ N OH and summed feature 4 ($C_{17:1}$ iso I/anteiso B).

The type strain, CIES53^T (=DSM 113860^T=LMG 32675^T), was isolated in postgate standard medium from the ileum of Brown Lohmann laying hens. Genome and 16S rRNA gene sequences are available from the NCBI database under the accessions GCA_947568225 and OM760984, respectively.

DESCRIPTION OF *FAECALISPORA JEDDAHENSIS* COMB. NOV.

Faecalispora jeddahensis (jed.dah.en'sis. N.L. fem. adj. *jeddahensis*, pertaining to Jeddah).

Basonym: *Clostridium jeddahense* Lagier *et al*. 2016.

The nomenclature of '*jeddahense*' is malformed as it contravenes Rule 12 c(1) of the International Code of Nomenclature of Prokaryotes; therefore, *jeddahensis* is suggested. The description of *Faecalispora jeddahensis* comb. nov. is identical to that given for the species *Clostridium jeddahense* by Lagier *et el*. [37]. The genome size is 3613503 bp and the DNA G+C content of the type strain is 51.95mol%.

The type strain is JCD^T (=CSUR P693^T=DSM 27834^T). The 16S rRNA gene and genome sequence accession numbers of *Faecalispora jeddahensis* are HG726040 and CBYL00000000, respectively.

DESCRIPTION OF *FAECALISPORA SPOROSPHAEROIDES* COMB. NOV.

Faecalispora sporosphaeroides (spo.ro.sphae.ro'i.des. Gr. fem. n. *spora*, seed; Gr. fem. n. *sphaira*, sphere; Gr. neut. adj. suff. *-eides*, resembling, similar; from Gr. neut. n. *eidos*, that which is seen, form, shape, figure; N.L. neut. adj. *sporosphaeroides*, having spherical spores).

Basonym: *Clostridium sporosphaeroides* Soriano and Soriano 1948 (Approved Lists 1980).

The description of *Faecalispora sporosphaeroides* comb. nov. is identical to that given for the species *Clostridium sporosphaeroides* by [35]. The genome size is 2942092 bp and the DNA G+C content of the type strain is 54mol%.

The type strain is VPI 4527^T (=ATCC 25781^T=DSM 1294^T). The 16S rRNA gene and genome sequence accession numbers of *Faecalispora sporosphaeroides* are X66002 and GCA_000383295, respectively.

DISCUSSION

Cultivation of bacteria from the chicken intestine has focused on caeca and faeces samples and the search for pathogenic strains, leaving the upper regions less explored. Although cultivation methods carry a selection bias to micro-organisms that adapt better to the culture conditions, and their prevalence and abundance do not always reflect the ecology and dynamics of the community, efforts in cultivating chicken bacteria help to broaden the pool of chicken gut micro-organisms, studying closely their metabolism and handling strains formerly represented only by metagenomic sequencing. In this work, we succeeded in isolating eight novel species. Former studies have described *Bacillota* as a major representative of dominant groups at the crop, gizzard and small intestine [6, 38–40]. All the species described in this work represent members of the phylum *Bacillota*. This information improves the cultivation approaches by unveiling the community composition, adaptations encoded and dynamics, providing a reliable perspective of niche roles within the community.

The ecology of the novel species

The novel taxa are heterotrophic, facultative anaerobic or strict anaerobic bacteria, that obtain energy and carbon from the oxidation of carbohydrates (mainly from glucose, pentose, maltose, cellobiose and cellulose), aliphatic amino acids (glutamine, lysine, arginine, serine) and organic acids (acetate, lactate, butyrate and long-chain fatty acids). Niches might differ along the intestine depending on the availability and complexity of nutrients at the digestion stage. Still, the primary energy and carbon fluxes lead to the formation of lactate, acetate, butyrate, succinate, ethanol and butanol.

The species *C. butanoliproducens* was isolated from crop at relatively high redundancy. Interestingly, this species has already been detected in faeces of chicken reported under a non-valid published name; highlighting the fact that its presence is not unique to a digestive section and that it is a common species found in chicken. The novel species of *Limosilactobacillus* presented a high count of genes encoding sugar and protein phosphatases including phosphoserine phosphatases, tyrosine-phosphatases and exopolyphosphatase (File S2), a linear polymer of residues of orthophosphate involved in energy storage, that might compete with the host for phosphate scavenging but can also be a colonization advantage involved in bacterial motility or biofilm formation [41].

The cultivation of *Ligilactobacillus hohenheimensis* was exclusively from jejunal samples and was isolated with low redundancy in samples of broilers (three isolates). This novel species presented a very reduced genome (1.3 Mbp) compared to the rest of the members of the genus *Ligilactobacillus*. Functionally, it has a very narrow potential of partially degrading cellulose, lactose and lactate and a complete absence of amino acid utilization genes. The limited range of carbon utilization suggests an adaptation as a commensal member of the community that depends on nutrient supplies from the host or the environment. Therefore, the success of its isolation may have been a consequence of the enrichment of the samples that preceded the isolation.

The GIT section with the highest cultivation diversity and metabolic functionality in this work was the ileum. Species obtained in this section presented the highest diversity of genes encoding peptidases and glycosidases. The highest number of novel taxa were found in this section, including *F. anaeroviscerum*, isolated eight times under different isolation strategies; *Limosilactobacillus avium*, *Limosilactobacillus difficilis*, *Limosilactobacillus viscerum* and *Limosilactobacillus pulli*, isolated with a redundancy of two to four times. The diversity of fermentation products detected in the strains isolated from this region might be due to the increase of glucose absorption in the small intestine that influence substrate intake diversification to other sources of carbon where these bacteria might find an advantage [42].

Finally, the presence of different groups of phosphatases included with all the novel species descriptions (File S2) provides insights into the potential contributions of these species to the supply of available phosphate from insoluble sources such as proteins, nucleotides, inulins, phytates and other organic sources of unavailable phosphate present in the plant-based diet given to chickens, which is not accessible to its metabolism [2, 38].

The isolation success of novel taxa within crop and small intestine of chicken lies in the exploration of these sections. The newly obtained strains encode adaptations to the host-lifestyle environment and represent reference members of the chicken microbiome of crop jejunum or ileum. These results are part of the first deep cultivation approach of bacteria from the chicken's upper digestive system and describe the unexplored taxonomic and functional diversity of bacteria inhabiting these sections, improving the potential handling of chicken microbiota with biotechnological applications.

Funding information
This work was in part funded by the Rehovot-Hohenheim partnership program.

Acknowledgements
The authors acknowledge support by the High Performance and Cloud Computing Group at the Zentrum für Datenverarbeitung of the University of Tübingen, the state of Baden-Württemberg through bwHPC and the German Research Foundation (DFG) through grant no INST 37/935–1 FUGG. We acknowledge the kind collaboration with the culture collections at DSMZ, Braunschweig and BCCM/LMG, Gent. We are thankful for the critical comments and helpful suggestions of the reviewers and editors to improve the manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

Ethical statement
The use of animals in this study was reviewed and approved by the Regierungspräsidium Tübingen, Germany (approval number HOH50/17 TE).

References

1. Rios-Galicia B, Sáenz JS, Yergaliyev T, Roth C, Camarinha-Silva A, *et al*. Novel taxonomic and functional diversity of eight bacteria from the upper digestive tract of chicken. *Figshare*. 2024.
2. Witzig M, Camarinha-Silva A, Green-Engert R, Hoelzle K, Zeller E, *et al*. Spatial variation of the gut microbiota in broiler chickens as affected by dietary available phosphorus and assessed by T-RFLP analysis and 454 pyrosequencing. *PLoS One* 2015;10:e0143442.
3. Xiao Y, Xiang Y, Zhou W, Chen J, Li K, *et al*. Microbial community mapping in intestinal tract of broiler chicken. *Poult Sci* 2017;96:1387–1393.
4. Borda-Molina D, Seifert J, Camarinha-Silva A. Current perspectives of the chicken gastrointestinal tract and its microbiome. *Comput Struct Biotechnol J* 2018;16:131–139.
5. Glendinning L, Stewart RD, Pallen MJ, Watson KA, Watson M. Assembly of hundreds of novel bacterial genomes from the chicken caecum. *Genome Biol* 2020;21:34.
6. Gong J, Si W, Forster RJ, Huang R, Yu H, *et al*. 16S rRNA gene-based analysis of mucosa-associated bacterial community and phylogeny in the chicken gastrointestinal tracts: from crops to ceca. *FEMS Microbiol Ecol* 2007;59:147–157.
7. Kers JG, Velkers FC, Fischer EAJ, Hermes GDA, Lamot DM, *et al*. Take care of the environment: housing conditions affect the interplay of nutritional interventions and intestinal microbiota in broiler chickens. *Anim Microbiome* 2019;1:10.
8. Videnska P, Sedlar K, Lukac M, Faldynova M, Gerzova L, *et al*. Succession and replacement of bacterial populations in the caecum of egg laying hens over their whole life. *PLoS One* 2014;9:e115142.
9. Feng Y, Wang Y, Zhu B, Gao GF, Guo Y, *et al*. Metagenome-assembled genomes and gene catalog from the chicken gut microbiome aid in deciphering antibiotic resistomes. *Commun Biol* 2021;4:1305.
10. Künzel S, Borda-Molina D, Zuber T, Hartung J, Siegert W, *et al*. Relative phytase efficacy values as affected by response traits, including ileal microbiota composition. *Poult Sci* 2021;100:101133.
11. Classen HL, Apajalahti J, Svihus B, Choct M. The role of the crop in poultry production. *Worlds Poult Sci J* 2016;72:459–472.
12. Glendinning L, Watson KA, Watson M. Development of the duodenal, ileal, jejunal and caecal microbiota in chickens. *Anim Microbiome* 2019;1:17.
13. Gilroy R, Ravi A, Getino M, Pursley I, Horton DL, *et al*. Extensive microbial diversity within the chicken gut microbiome revealed by metagenomics and culture. *PeerJ* 2021;9.
14. Medvecky M, Cejkova D, Polansky O, Karasova D, Kubasova T, *et al*. Whole genome sequencing and function prediction of 133 gut anaerobes isolated from chicken caecum in pure cultures. *BMC Genomics* 2018;19:561.

15. Crhanova M, Karasova D, Juricova H, Matiasovicova J, Jahodarova E, *et al*. Systematic culturomics shows that half of chicken caecal microbiota members can be grown in vitro except for two lineages of *Clostridiales* and a single lineage of *Bacteroidetes*. *Microorganisms* 2019;7:11.
16. Zenner C, Hitch TCA, Riedel T, Wortmann E, Tiede S, *et al*. Early-life immune system maturation in chickens using a synthetic community of cultured gut bacteria. *mSystems* 2021;6:e01300-20.
17. Kobayashi K, Ehrlich SD, Albertini A, Amati G, Andersen KK, *et al*. Essential *Bacillus subtilis* genes. *Proc Natl Acad Sci U S A* 2003;100:4678–4683.
18. Tanaka T, Kawasaki K, Daimon S, Kitagawa W, Yamamoto K, *et al*. A hidden pitfall in the preparation of agar media undermines microorganism cultivability. *Appl Environ Microbiol* 2014;80:7659–7666.
19. Einarsson H, Snygg BG, Eriksson C. Inhibition of bacterial growth by Maillard reaction products. *J Agric Food Chem* 1983;31:1043–1047.
20. Ulrich RL, Hughes TA. A rapid procedure for isolating chromosomal DNA from *Lactobacillus* species and other gram-positive bacteria. *Lett Appl Microbiol* 2008;32:52–56.
21. Relman DA. Universal bacterial 16S rRNA amplification and sequencing. *Diag Mol Microbiol* 1993:489–495.
22. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, *et al*. Gapped BLAST and PSI-BLAST: a new generation of protein database search programs. *Nucleic Acids Res* 1997;25:3389–3402.
23. Price MN, Dehal PS, Arkin AP. FastTree 2--approximately maximum-likelihood trees for large alignments. *PLoS One* 2010;5:e9490.
24. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res* 2019;47:W256–W259.
25. Bushnell B, Rood J, Singer E. BBMerge - accurate paired shotgun read merging via overlap. *PLoS One* 2017;12:e0185056.
26. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, *et al*. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 2012;19:455–477.
27. Wick RR, Judd LM, Cerdeira LT, Hawkey J, Méric G, *et al*. Trycycler: consensus long-read assemblies for bacterial genomes. *Genome Biol* 2021;22:266.
28. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 2014;30:2068–2069.
29. Zhou Z, Tran PQ, Breister AM, Liu Y, Kieft K, *et al*. METABOLIC: high-throughput profiling of microbial genomes for functional traits, metabolism, biogeochemistry, and community-scale functional networks. *Microbiome* 2022;10:33.
30. Chaumeil P-A, Mussig AJ, Hugenholtz P, Parks DH, Hancock J. GTDB-Tk: a toolkit to classify genomes with the Genome Taxonomy Database. *Bioinformatics* 2019;36:1925–1927.
31. Eren AM, Kiefl E, Shaiber A, Veseli I, Miller SE, *et al*. Community-led, integrated, reproducible multi-omics with anvi'o. *Nat Microbiol* 2021;6:3–6.
32. Meier-Kolthoff JP, Carbasse JS, Peinado-Olarte RL, Göker M. TYGS and LPSN: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. *Nucleic Acids Res* 2022;50:D801–D807.
33. Kim D, Park S, Chun J. Introducing EzAAI: a pipeline for high throughput calculations of prokaryotic average amino acid identity. *J Microbiol* 2021;59:476–480.
34. Ludwig W, Viver T, Westram R, Francisco Gago J, Bustos-Caparros E, *et al*. Release LTP_12_2020, featuring a new ARB alignment and improved 16S rRNA tree for prokaryotic type strains. *Syst Appl Microbiol* 2021;44:126218.
35. Soriano S, Soriano A. Nueva bacteria anaerobia productora de una alteracion en sordinas envasadas. *Rev Asoc Argent Dietol* 1948;6:36–41.
36. Afanzar O, Di Paolo D, Eisenstein M, Levi K, Plochowietz A, *et al*. The switching mechanism of the bacterial rotary motor combines tight regulation with inherent flexibility. *EMBO J* 2021;40:e104683.
37. Lagier J-C, Bibi F, Ramasamy D, Azhar EI, Robert C, *et al*. Non contiguous-finished genome sequence and description of *Clostridium jeddahense* sp. nov. *Stand Genomic Sci* 2014;9:1003–1019.
38. Borda-Molina D, Vital M, Sommerfeld V, Rodehutscord M, Camarinha-Silva A. Insights into broilers' gut microbiota fed with phosphorus, calcium, and phytase supplemented diets. *Front Microbiol* 2016;7:2033.
39. Rehman HU, Vahjen W, Awad WA, Zentek J. Indigenous bacteria and bacterial metabolic products in the gastrointestinal tract of broiler chickens. *Arch Anim Nutr* 2007;61:319–335.
40. Stanley D, Geier MS, Hughes RJ, Denman SE, Moore RJ. Highly variable microbiota development in the chicken gastrointestinal tract. *PLoS One* 2013;8:e84290.
41. Denoncourt A, Downey M. Model systems for studying polyphosphate biology: a focus on microorganisms. *Curr Genet* 2021;67:331–346.
42. Riesenfeld G, Sklan D, Bar A, Eisner U, Hurwitz S. Glucose absorption and starch digestion in the intestine of the chicken. *J Nutr* 1980;110:117–121.