



Metagenomes and Metagenome-Assembled Genomes from Microbial Communities Fermenting Ultrafiltered Milk Permeate

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ABSTRACT Fermentative microbial communities can be utilized for the conversion of various agroindustrial residues into valuable chemicals. Here, we report 34 metagenomes from anaerobic bioreactors fed lactose-rich ultrafiltered milk permeate and 278 metagenome-assembled genomes (MAGs). These MAGs can inform future studies aimed at generating renewable chemicals from dairy and other agroindustrial residues.

The metagenomes reported here originate from two anaerobic bioreactors, i.e., a continuously stirred tank reactor (CSTR) and an upflow sludge blanket reactor (USB), operated to investigate the valorization of agroindustrial residues via fermentation. Both bioreactors were inoculated with acid-phase anaerobic digester sludge from the Nine Springs Wastewater Treatment Plant (Madison, WI, USA) and fed ultrafiltered milk permeate amended with ammonium chloride as a nitrogen source. The CSTR was operated at pH 5.5 and 35°C, whereas the USB was operated at pH 5.5 and 21°C. DNA was periodically extracted from the bioreactors using a phenol-chloroform extraction procedure described by Scarborough et al. (1) but omitting the bead-beating step. DNA aliquots of 500 ng (27 samples) and 3,000 ng (7 samples) were submitted to the Joint Genome Institute (JGI) (Berkeley, CA, USA) for paired-end 2 × 150-bp NovaSeq S4 sequencing (Illumina, Inc., San Diego, CA, USA) and single-molecule real-time (SMRT), long-read sequencing using a Sequel II platform (Pacific Biosciences, Inc. [PacBio], Menlo Park, CA, USA), respectively. Illumina libraries were end repaired, A tailed, and ligated with Illumina adapters using the KAPA HyperPrep kit (Roche, USA) as described (2). PacBio library construction included shearing of genomic DNA to 6 to 10 kb (size selection with BluePippin; Sage Science, USA) and ligation using the SMRTbell Express template preparation v2.0 kit following the standard protocol (PacBio). All software used default parameters unless otherwise noted. Illumina reads were filtered and error corrected using *bbcms* (v38.86) (*mincount*=2, *highcountfraction*=0.6) (3), assembled with *metaSPAdes* (v3.14.1) (4), and mapped with *BBMap* (v38.86) (*ambiguous*=random) (3) following the JGI Metagenome Workflow (2). PacBio reads were filtered using *BBtools* (v38.87/38.88, *rqc.filter2.sh*) (3), and CCS reads were assembled using *metaFlye* (v2.8.1-b1676) (5), polished with subreads using *GCpp* (v1.0.0-SL-release-8.0.0) (<https://github.com/PacificBiosciences/gcpp>), and mapped using *minimap2* (v2.17-r941) (6). For all libraries, contigs were binned with *MetaBAT* (v2:2.15) (7). The resulting Illumina libraries contained between 71 and 126 million

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TABLE 1 (Continued)

Table with columns: Strain name, Code, Reactor, Day, ANIm, dRep, GTDB-Tk classification, Reference genome, Sequencing platform, Completeness, Contamination, MAG size, No. of contigs, M50, GC content, No. of rRNAs, No. of tRNAs, No. of rNS rRNAs, GenBank accession no., SRA accession no., No. of raw reads aligned to MAG (x1,000). Rows list various bacterial strains like UW_MP_ATO2_1, UW_MP_ATO3_2, etc., with their corresponding genomic data.

(Continued on next page)

TABLE 1 (Continued)

Strain name ^a	Code ^b	Reactor	Day	ANI ^d	dRep ^e	GTDB-Tk classification	Reference genome ^f	Sequencing platform	Completeness (%)	Contamination (%)	MAG size (Mbp)	No. of contigs	M ₅₀ (Mbp)	GC content (%)	No. of rRNAs	No. of tRNAs	No. of rS rRNAs	No. of rZS rRNAs	GenBank accession no.	SRA accession no.	No. of raw reads aligned to MAG (x1,000)
UW_MP_SPHING1_2	SP01	CSTR	0	0.9999	121	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus	GCA_900543345.1	Illumina NovaSeq 54	97.69	1.64	3.059	62	0.0623	63.2	49	1	2	0	JALCR000000000	SRAI_2655481	405
UW_MP_SP01_1	SP01	CSTR	192	0.9997	120	s__Sporolactobacillus; Sporolactobacillus; Sporolactobacillaceae; Sporolactobacillus	GCF_90099623.1	Illumina NovaSeq 54	96.74	1.94	3.636	39	0.1143	49.1	41	5	2	0	JALCNT000000000	SRAI_2655976	510
UW_MP_STREP1_1	STREP1	CSTR	78		122	d__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus		Illumina NovaSeq 54	98.71	0.57	2.274	67	0.0391	35.0	51	3	0	0	JALCTW000000000	SRAI_2655143	206
UW_MP_STREP1_2	STREP1_2	CSTR	49	0.9999	120	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus		Illumina NovaSeq 54	97.16	0.57	2.402	84	0.0436	34.9	39	1	0	0	JALCSL000000000	SRAI_2654484	220
UW_MP_STREP1_3	STREP1_3	CSTR	72	0.9997	109	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus	NA	Illumina NovaSeq 54	87.08	0.82	2.106	95	0.0273	35.0	43	2	0	0	JALCTO000000000	SRAI_2655142	216
UW_MP_STREP2_1	STREP2	USB	28		118	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus		Illumina NovaSeq 54	94.04	0.66	2.517	41	0.0783	35.1	42	1	0	0	JAKVKK000000000	SRAI_2656283	238
UW_MP_STREP2_2	STREP2_2	USB	21	0.9999	104	g__MMGLO5-15__		Illumina NovaSeq 54	78.81	0.66	2.101	30	0.1097	35.2	4	1	0	0	JAKVKS000000000	SRAI_2655989	491
UW_MP_STREP3_1	STREP3	CSTR	192		115	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus	GCF_002070765.2	Illumina NovaSeq 54	91.32	0	2.102	39	0.0881	35.7	43	2	0	0	JALCNS000000000	SRAI_2655976	250
UW_MP_STREP4_1	STREP4	USB	21		114	d__Bacteriophyta; Fimicitutes; C__Bacillia; Bacillales; G__Lactobacillus; Lactococcus; Lactococcaceae; Lactococcus		Illumina NovaSeq 54	90.57	0.5	1.911	37	0.0827	40.4	29	1	0	0	JAKVKT000000000	SRAI_2655989	579
UW_MP_TREP1_1	TREP1	USB	105		107	d__Bacteriophyta; Spirochaetes; Spirochaetia; Spirochaetiales; Spirochaetaceae; Spirochaeta; Spirochaeta	NA	Illumina NovaSeq 54	84.62	0.35	2.692	95	0.0361	44.8	43	0	0	0	JALCCR000000000	SRAI_2656339	367

^a Strain name assigned to each MAG. The UW_MP prefix stands for University of Wisconsin Milk Permeate bioreactor. MAGs are clustered during dereplication using dRep (11). Strains with a numerical suffix of _1 are the highest quality, dRep representative MAGs for a given cluster; nonrepresentative MAGs in each cluster are assigned the same strain name with sequential numerical suffixes (e.g., _2 and _3), assigned in order of decreasing quality, according to the dRep score.

^b ACET, *Acetobacter*; ACID, *Acidaminococcaceae*; ACT, *Actinomycetaceae*; ACUT, *Acutalibacteraceae*; AGR, *Agrobacteriaceae*; ANA, *Anaerovoracaceae*; ATO, *Atopobacteraceae*; BACIL, *Bacilli*; BACTE, *Bacteroidales*; BIF, *Bifidobacterium*; BUL, *Bulleidia*; BURK, *Burkholderiaceae*; CARN, *Carnobacteriaceae*; CAUL, *Caulobacteriaceae*; CLOS, *Clostridium*; EGG, *Eggerthellaceae*; ENTER, *Enterobacteriaceae*; LAC, *Lactobacillus*; LCO, *Lachnospiraceae*; LENLAC, *Lentilactobacillus*; LEUC, *Lecunostoc*; LIQLAC, *Liquorilactobacillus*; MEG, *Megasphaera*; METH, *Methanomethylophilus*; MIC, *Microbacteriaceae*; MORAX, *Moraxellaceae*; MUR, *Muribaculaceae*; MYC, *Mycobacteriaceae*; OSC, *Oscillospiraceae*; PREV, *Prevotella*; PROP, *Propionibacteriaceae*; RUM, *Ruminococcaceae*; SACCH, *Saccharofermentans*; SCHLAC, *Schleiferilactobacillus*; SELEN, *Selenomonadaceae*; SPH, *Sphaerochaetaceae*; SPHING, *Sphingobium*; SPOR, *Sporolactobacillaceae*; STREP, *Streptococcaceae*; TREP, *Treponema*.

^c Sample from which a given MAG was derived, described as the bioreactor of origin and the sampling day, where day 0 is designated as the day the bioreactor was inoculated. Each bioreactor was fed ultrafiltered milk permeate amended with 400 mg/L of N, in the form of NH₄Cl. The CSTR was operated at pH 5.5 and 35°C, with a 6-day solids/hydraulic retention time. The USB was operated at pH 5.5 and 21°C, with a 1.4-day hydraulic retention time and with dynamic solids removal.

^d Average nucleotide identity (ANI) between representative MAG and other MAGs included in the same cluster by dRep.

^e dRep scoring calculation: $(A \times \text{completeness}) - (B \times \text{contamination}) + [C \times (\text{contamination} \times (\text{strain heterogeneity}/100))] + [D \times \log(M_{50})] + [E \times \log(\text{genome size})] + [F \times (\text{centrality} - \text{ANI})]$, where A to F were weighted with values of 1, 0.5, 1, 5, 0, and 1, respectively.

^f NCBI GenBank accession number of the reference genome in GTDB-Tk (13) that is closest to the representative MAG. NA, not applicable, i.e., MAGs without a closely matched reference genome when using the default minimum alignment fraction of 0.65.

^g NCBI GenBank accession number for each reported MAG.

^h NCBI SRA accession number for the raw reads of the metagenome for each reported MAG. For PacBio samples that utilized multiple runs, the NCBI Biosample for the experiment is provided.

ⁱ The number of raw reads that aligned to each reported MAG with BBMap or minimap2 for Illumina and PacBio reads, respectively. These reads originated from the metagenome in which the MAG was assembled.

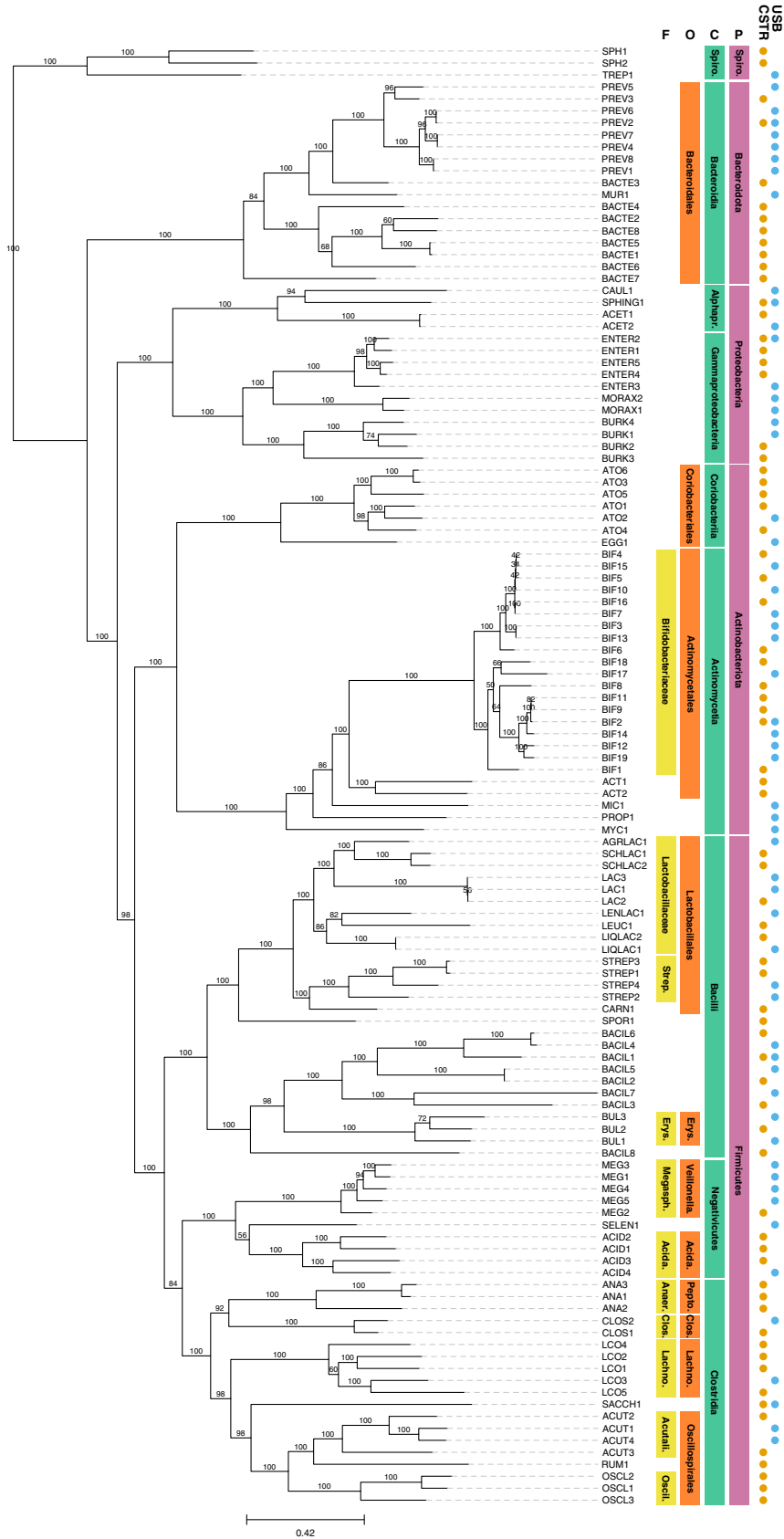


FIG 1 Phylogenetic tree of dRep representative bacterial MAGs and their presence in two bioreactors (CSTR and USB) fermenting ultrafiltered milk permeate. ACET, *Acetobacter*; ACID, *Acidaminococcaceae*; (Continued on next page)

FIG 1 Legend (Continued)

ACT, *Actinomycetaceae*; ACUT, *Acutalibacteraceae*; AGRAC, *Agrilactobacillus*; ANA, *Anaerovoracaceae*; ATO, *Atopobiaceae*; BACIL, *Bacilli*; BACTE, *Bacteroidales*; BIF, *Bifidobacterium*; BUL, *Bulleidia*; BURK, *Burkholderiaceae*; CARN, *Carnobacteriaceae*; CAUL, *Caulobacteraceae*; CLOS, *Clostridium*; EGG, *Eggerthellaceae*; ENTER, *Enterobacteriaceae*; LAC, *Lactobacillus*; LCO, *Lachnospiraceae*; LENLAC, *Lentilactobacillus*; LEUC, *Lecunostoc*; LIQLAC, *Liquorilactobacillus*; MEG, *Megasphaera*; MIC, *Microbacteriaceae*; MORAX, *Moraxellaceae*; MUR, *Muribaculaceae*; MYC, *Mycobacteriaceae*; OSCL, *Oscillospiraceae*; PREV, *Prevotella*; PROP, *Propionibacteriaceae*; RUM, *Ruminococcaceae*; SACCH, *Saccharofermentans*; SCHLAC, *Schleiferilactobacillus*; SELEN, *Selenomonadaceae*; SPH, *Sphaerochaetaceae*; SPHING, *Sphingobium*; SPOR, *Sporolactobacillaceae*; STREP, *Streptococcaceae*; TREP, *Treponema*. Higher taxonomic levels are labeled, from left to right, family (F), order (O), class (C), and phylum (P). *Spiro.*, *Spirochaetota*; *Alphapr.*, *Alphaproteobacteria*; *Spiro.*, *Spirochaetia*; *Acida.*, *Acidaminococcales*; *Clos.*, *Clostridiales*; *Erys.*, *Erysipelotrichales*; *Lachno.*, *Lachnospirales*; *Pepto.*, *Peptostreptococcales*; *Veillonella.*, *Veillonellales*; *Acida.*, *Acidaminococaceae*; *Acutali.*, *Acutalibacteraceae*; *Anaer.*, *Anaerovoracaceae*; *Clos.*, *Clostridiaceae*; *Erys.*, *Erysipelotrichaceae*; *Lachno.*, *Lachnospiraceae*; *Megasph.*, *Megasphaeraceae*; *Oscil.*, *Oscillospiraceae*; *Strep.*, *Streptococcaceae*. The phylogenetic tree was generated in RAxML-ng (14) with 500 bootstraps using the concatenation of 120 bacterial single-copy marker genes (Bac120) identified by GTDB-Tk (13). Bootstrap values greater than 50 are shown. The scale bar represents evolutionary distance and indicates the number of nucleotide substitutions per sequence site.

150-bp reads, and the PacBio libraries contained between 21,000 and 1,122,000 reads, 5 to 8 kb in average length. The resulting metagenome-assembled genomes (MAGs) were annotated using the JGI Metagenome Annotation Pipeline (MAP) (v5.0.23) (8) and the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (v6.0) (9). To improve MAG quality, contaminant contigs from all MAGs were identified and removed using ProDeGe (v2.3) (10) and custom tetranucleotide frequency analysis scripts (run.GC.sh and Calculating_TF_Correlations.R [https://github.com/GLBRC/metagenome_analysis]). All refined MAGs were dereplicated using dRep (v3.2.2) (dereplicate command with `-conW 0.5` and `-N50W 5` custom parameters) (11) by clustering MAGs by identity and selecting the highest-quality MAG as a representative for each cluster (Table 1). Quality statistics were obtained using CheckM (v1.0.11) (12), and MAGs with over 75% completeness were retained for further analysis. Taxonomy was assigned for dRep representative MAGs using GTDB-Tk (v1.5.1, database release 202) (13). RAxML-NG (v0.9.0) (14) and TreeViewer (v2.0.1) were used to generate and visualize a phylogenetic tree containing dRep representative MAGs (Fig. 1). We report 278 annotated MAGs from 34 samples, grouped into 123 dereplicated clusters that describe the microbial community composition of the two bioreactors (Table 1). These data contribute to the knowledgebase of microbial communities bioconverting agroindustrial residues (1, 15–25).

Data availability. Raw metagenomic sequence data and MAGs for each sample are available at NCBI GenBank under BioProject accession number [PRJNA768492](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA768492). NCBI genome accession numbers for all 278 MAGs are displayed in Table 1. All information on library construction and sequencing can be found at <https://img.jgi.doe.gov> using JGI GOLD Study identification number [Gs0150020](https://www.jgi.doe.gov/gold/Gs0150020). All custom scripts are available on GitHub (https://github.com/GLBRC/metagenome_analysis).

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