Draft genome sequences of 21 *Bacillus* sp. isolates from raw bovine milk

Gauri Khullar,[1,2] Zhangbin Cai,[1] Bridget O'Brien,[1] Jinha Suh,[1] Jennifer Ronholm[1,3,4]

AUTHOR AFFILIATIONS See affiliation list on p. 3.

ABSTRACT Members of the *Bacillus* genus are commonly used as probiotics in livestock production. We isolated several *Bacillus* strains from healthy dairy cattle. The role of these strains in mammary health is of interest. Here, we present 21 draft genome assemblies and annotations of *Bacillus* sp. isolated from fresh raw milk.

Members from the *Bacillus* genus, such as *Bacillus subtilis, B. coagulans*, *B. cereus*, and *B. licheniformis*, are commonly used as probiotics in livestock production (1–3). *Bacillus* probiotics can improve digestion, increase nutrient absorption, and reduce gas emissions (4–6). Some may also prevent bacterial infections. Members of the *Bacillus* genus can produce lipopeptides, surfactins, bacteriocins, and organic acids, each of which has antimicrobial activity (1).

Intramammary infections (IMIs) are common in dairy cattle (7). Evidence indicates that *Bacillus* sp. may have antagonistic effects against IMI pathogens and may be used to prevent IMIs. For example, feeding cattle *B. subtilis* decreased the incidence of IMIs (8). *Bacillus* sp. isolated from healthy cows can inhibit the growth of several gram-positive IMI pathogens in co-culture (9), and exopolysaccharides synthesized by *Bacillus velezensis* have anti-biofilm activity against *Staphylococcus aureus* (10). Although cross-species use of *Bacillus* probiotics is common (11), it is understood that host colonization is more successful if the probiotic strain was isolated from the species and host niche the probiotic aims to colonize. To develop bovine anti-IMI probiotics, we collected 21 *Bacillus* isolates from healthy Holstein dairy cows. Here, we present draft genome sequences of these isolates.

Raw milk was collected directly from a single healthy cow on the Macdonald campus farm of McGill University (45.41168, -73.94355). The milk was transferred to an Eppendorf tube and was centrifuged at $3,800 \times g$ for 20 min (12). The supernatant was discarded, and the pellet was spread on modified plate count agar (Hardy Diagnostics, USA). Plates were incubated overnight at 37°C. Isolated colonies were differentiated based on phenotypic properties. Unique colonies were grown in Brain Heart Infusion (BHI) broth for 48 h at 37°C. Aliquots of 1 mL of bacterial culture were preserved by adding 15% glycerol (vol/vol) and were stored at −80°C. Each isolate was tentatively identified by Sanger Sequencing of the full-length 16S rRNA gene using the 8F (5′-AGAGTTTGATCCTG GCTCAG-3′) and 1492R (5′-ACCTTGTTACGACTT-3′) primers.

Each −80°C stock solution was streaked onto a BHI agar plate and was incubated overnight at 37°C. A single colony was inoculated into 20 mL of BHI broth and was grown overnight at 37°C and 200 rpm. The DNeasy UltraClean Microbial Kit DNA was used to extract bacterial DNA from liquid cultures (Qiagen, USA). The Quant-IT dsDNA High-Sensitivity assay (Invitrogen, USA) was used to assess the final DNA quantity. Sequencing libraries were made using the Nextera DNA Flex Prep kit and were sequenced using the MiSeq benchtop sequencer and the MiSeq Reagent Kit v3 (2 × 300 bp)

TABLE 1 Genome quality and prediction of *Bacillus* sp. isolated from bovine milk in Canada[a]

Isolate identification no.	Species	Assembly accession no.	BioSample accession no.	SRA accession no.	GenBank accession no.	Coverage (X)	No. of contigs	Draft genome size (bp)	GC content (%)	No. of CDS	No. of RNAs	N50 (bp)
1	B. licheniformis	GCF_039704985.1	SAMN39861007	SRR28833364	JBDICK000000000	23.9734	53	4075575	46.189556	4439	69	161839
7	B. licheniformis	GCF_039704965.1	SAMN39861008	SRR28833363	JBDICJ000000000	56.1865	27	4075098	46.189	4432	68	402185
23b	B. licheniformis	GCF_039704945.1	SAMN39861009	SRR28833352	JBDICI000000000	73.6856	27	4121131	46.152622	4502	69	298889
21_C	B. licheniformis	GCF_039704925.1	SAMN39861010	SRR28833348	JBDICH000000000	31.8968	31	4410271	45.68565	4925	81	287648
27	B. licheniformis	GCF_039704865.1	SAMN39861011	SRR28833347	JBDICG000000000	18.6028	107	4273296	45.819504	4793	80	66976
33	B. licheniformis	GCF_039704875.1	SAMN39861012	SRR28833346	JBDICF000000000	62.6018	28	4410102	45.686153	4937	79	346695
35	B. licheniformis	GCF_039704845.1	SAMN39861013	SRR28833345	JBDICE000000000	57.1802	40	4429233	45.64856	4957	71	296898
39	B. licheniformis	GCF_039704855.1	SAMN39861014	SRR28833344	JBDICD000000000	65.0995	29	4283372	45.843018	4726	71	296898
2	B. licheniformis	GCA_039704785.1	SAMN39861015	SRR28833343	JBDICC000000000	38.0009	32	4423964	45.74099	4944	70	284038
8	B. licheniformis	GCF_039704805.1	SAMN39861016	SRR28833342	JBDICB000000000	76.2563	31	4425022	45.74045	4934	70	296898
11	B. licheniformis	GCF_039704745.1	SAMN39861017	SRR28833362	JBDICA000000000	28.0249	35	4285446	45.831657	4731	81	296898
17a	B. licheniformis	GCF_039704765.1	SAMN39861018	SRR28833361	JBDIBZ000000000	116.5275	31	4361801	45.669575	4893	72	424554
5	B. licheniformis	GCF_039704755.1	SAMN39861019	SRR28833360	JBDIBY000000000	37.4583	32	4345927	45.808407	4811	67	228812
28	B. licheniformis	GCF_039704725.1	SAMN39861020	SRR28833359	JBDIBX000000000	65.022	29	4284751	45.8404	4725	71	296898
32	B. licheniformis	GCF_039704565.1	SAMN39861021	SRR28833358	JBDIBW000000000	25.8095	29	4396779	45.70391	4920	69	296898
38	B. licheniformis	GCF_039704625.1	SAMN39861022	SRR28833357	JBDIBV000000000	16.7513	196	4382965	45.650055	5040	69	46449
4	B. pumilus	GCF_039704575.1	SAMN39861023	SRR28833356	JBDIBU000000000	47.1156	16	3685821	41.56838	3841	62	932876
7_C	B. pumilus	GCF_039704555.1	SAMN39861024	SRR28833355	JBDIBT000000000	43.1238	18	3684661	41.562927	3839	62	932849
1_C	B. safensis	GCF_039704545.1	SAMN39861025	SRR28833354	JBDIBS000000000	22.8362	26	3638373	41.65884	3793	58	256236
3	B. aerius	GCF_039581685.1	SAMN41074328	SRR28833353	JBCPSH000000000	81.0985	16	3718211	41.24785	3869	64	540547
21	B. subtilis	GCF_039581705.1	SAMN41074331	SRR28833349	JBCPSF000000000	104.0174	9	4145080	43.58461	4347	70	2210931

[a]CDS, coding sequence; RNAs, sum of rRNA and tRNA counts.

(Illumina, USA). All software used default parameters unless otherwise noted. Reads were assembled using ProkaryoteAssembly (v. 0.1.6) (https://github.com/BFSSI-Bioinformatics-Lab/ProkaryoteAssembly), filtering low-quality sequences with a Q-score <20. Following assembly, contigs with fewer than 1,000 bp were removed (Table 1). Quality of final assemblies was assessed with QualiMap (v. 2.2.2) (13). Gene annotations and predictions were performed using NCBI Prokaryotic Genome Annotation Pipeline (v. 6.7) (14). The annotation and functional assignment were validated using Rapid Annotations Subsystems Technology (RAST) kit (15). The completeness of the genome assemblies was assessed using Benchmarking Universal Single-Copy Orthologs (BUSCO v5) (16).

AUTHOR AFFILIATIONS

[1]Faculty of Agricultural and Environmental Sciences, Macdonald Campus, McGill University, Montréal, Québec, Canada
[2]Department of Food Technology, Faculty of Science, Chulalongkorn University, Patumwan, Bangkok, Thailand
[3]Mastitis Network, Saint-Hyacinthe, Québec, Canada
[4]Regroupement FRQNT Op+Lait, Saint-Hyacinthe, Québec, Canada

AUTHOR ORCIDs

Zhangbin Cai http://orcid.org/0009-0006-9890-2679
Bridget O'Brien http://orcid.org/0000-0002-3050-0108
Jennifer Ronholm http://orcid.org/0000-0001-7902-3368

DATA AVAILABILITY

The raw reads have been submitted to SRA, and the genome sequences have been deposited in DDBJ/ENA/GenBank under the BioProject accession numbers PRJNA1074428 and PRJNA1104282, as provided in Table 1.

REFERENCES

1. Mingmongkolchai S, Panbangred W. 2018. *Bacillus* probiotics: an alternative to antibiotics for livestock production. J Appl Microbiol 124:1334–1346. https://doi.org/10.1111/jam.13690
2. Cheng G, Hao H, Xie S, Wang X, Dai M, Huang L, Yuan Z. 2014. Antibiotic alternatives: the substitution of antibiotics in animal husbandry? Front Microbiol 5:217. https://doi.org/10.3389/fmicb.2014.00217
3. Fijan S. 2014. Microorganisms with claimed probiotic properties: an overview of recent literature. Int J Environ Res Public Health 11:4745–4767. https://doi.org/10.3390/ijerph110504745
4. Jeong JS, Kim IH. 2014. Effect of *Bacillus subtilis* C-3102 spores as a probiotic feed supplement on growth performance, noxious gas emission, and intestinal microflora in broilers. Poult Sci 93:3097–3103. https://doi.org/10.3382/ps.2014-04086
5. Latorre JD, Hernandez-Velasco X, Wolfenden RE, Vicente JL, Wolfenden AD, Menconi A, Bielke LR, Hargis BM, Tellez G. 2016. Evaluation and selection of *Bacillus* species based on enzyme production, antimicrobial activity, and biofilm synthesis as direct-fed microbial candidates for poultry. Front Vet Sci 3:95. https://doi.org/10.3389/fvets.2016.00095
6. Shobharani P, Halami PM. 2016. *In vitro* evaluation of the cholesterol-reducing ability of a potential probiotic *Bacillus* spp. Ann Microbiol 66:643–651. https://doi.org/10.1007/s13213-015-1146-6
7. Aghamohammadi M, Haine D, Kelton DF, Barkema HW, Hogeveen H, Keefe GP, Dufour S. 2018. Herd-level mastitis-associated costs on Canadian dairy farms. Front Vet Sci 5:100. https://doi.org/10.3389/fvets.2018.00100
8. Urakawa M, Zhuang T, Sato H, Takanashi S, Yoshimura K, Endo Y, Katsura T, Umino T, Tanaka K, Watanabe H, Kobayashi H, Takada N, Kozutsumi T, Kumagai H, Asano T, Sazawa K, Ashida N, Zhao G, Rose MT, Kitazawa H, Shirakawa H, Watanabe K, Nochi T, Nakamura T, Aso H. 2022. Prevention of mastitis in multiparous dairy cows with a previous history of mastitis

by oral feeding with probiotic *Bacillus subtilis*. Anim Sci J 93:e13764. https://doi.org/10.1111/asj.13764

9. Al-Qumber M, Tagg JR. 2006. Commensal bacilli inhibitory to mastitis pathogens isolated from the udder microbiota of healthy cows. J Appl Microbiol 101:1152–1160. https://doi.org/10.1111/j.1365-2672.2006.03004.x

10. Sabino YNV, Araújo Domingues K de, Mathur H, Gómez-Mascaraque LG, Drouin G, Martínez-Abad A, Tótola MR, Abreu LM, Cotter PD, Mantovani HC. 2023. Exopolysaccharides produced by *Bacillus spp*. inhibit biofilm formation by *Staphylococcus aureus* strains associated with bovine mastitis. Int J Biol Macromol 253:126689. https://doi.org/10.1016/j.ijbiomac.2023.126689

11. Hong HA, Duc LH, Cutting SM. 2005. The use of bacterial spore formers as probiotics. FEMS Microbiol Rev 29:813–835. https://doi.org/10.1016/j.femsre.2004.12.001

12. Raynal-Ljutovac K, Gaborit P, Lauret A. 2005. The relationship between quality criteria of goat milk, its technological properties and the quality of the final products. Small Ruminant Res 60:167–177. https://doi.org/10.1016/j.smallrumres.2005.06.010

13. Okonechnikov K, Conesa A, García-Alcalde F. 2016. Qualimap 2: advanced multi-sample quality control for high-throughput sequencing data. Bioinformatics 32:292–294. https://doi.org/10.1093/bioinformatics/btv566

14. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaslavsky L, Lomsadze A, Pruitt KD, Borodovsky M, Ostell J. 2016. NCBI prokaryotic genome annotation pipeline. Nucleic Acids Res 44:6614–6624. https://doi.org/10.1093/nar/gkw569

15. Brettin T, Davis JJ, Disz T, Edwards RA, Gerdes S, Olsen GJ, Olson R, Overbeek R, Parrello B, Pusch GD, Shukla M, Thomason JA, Stevens R, Vonstein V, Wattam AR, Xia F. 2015. RASTtk: a modular and extensible implementation of the RAST algorithm for building custom annotation pipelines and annotating batches of genomes. Sci Rep 5:8365. https://doi.org/10.1038/srep08365

16. Manni M, Berkeley MR, Seppey M, Zdobnov EM. 2021. BUSCO: assessing genomic data quality and beyond. Curr Protoc 1:e323. https://doi.org/10.1002/cpz1.323