Draft Genome Sequences of 11 Bacterial Strains Isolated from Commercial Corn-Based Poultry Feed

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ABSTRACT Here, we report 11 bacterial strains isolated from commercial corn-based poultry feed to determine their potential as hygienic indicator microorganisms through a comparison of genome sizes and distribution patterns of unique genes. These isolates belonged to the genera Klebsiella, Kosakonia, Pantoea, Stenotrophomonas, and Enterococcus.

The microbial composition of poultry feed could be a critical factor for the development and growth of broilers and the establishment of their gastrointestinal tract (GIT) microbiome, particularly for those given feeds treated with antimicrobials, such as formaldehyde (1). Poultry feed harbors a wide array of microorganisms, including some potential pathogens (2). However, little is known about the taxonomy of nonpathogenic bacteria associated with commercial feeds. Since the presence of pathogens is relatively infrequent, it is important to gain a better understanding of the distribution and prevalence of nonpathogens (1, 2). Hygienic indicator organisms, represented by total aerobic colony count and coliform count, as well as Enterobacteriaceae, function in a way similar to that of particular foodborne pathogens and thus offer a permanent method for assessing and predicting the efficiency of sanitization agents against consequent pathogens that are difficult to detect (2).

Based on previous findings, next-generation sequencing based on 16S rRNA gene amplification has been proposed for use in characterizing microbial populations in poultry feeds (2, 3). Therefore, application of whole-genome sequencing (WGS) has been widely accepted for predicting possible microbial threat or preventing premature product spoilage under the food safety purview (4). WGS analysis performed on nonpathogenic and potentially pathogenic poultry feed isolates in this study will facilitate understanding of the distribution of candidate genes encoding proteins/enzymes related to virulence, toxins, stress, antimicrobial resistance, porins, monoxygenases, oxidoreductases, dioxygenases, and catabolism of heavy metals (lead, arsenic), among others, and will eventually determine the most suitable hygienic indicator bacteria in the poultry processing pipeline.

Bacterial isolates from corn-based chicken feed were recovered on aerobic plate count (APC) agar (5). Initially, 10 g of feed was shaken in 100 ml tryptic soy broth (TSB) (BD Difco, Franklin Lakes, NJ) for 2 minutes; the mixtures were serially diluted and plated onto APC agar. Isolates were grown overnight at 37°C in an incubator. Unique colonies were isolated and purified repeatedly (3 times). Finally, 11 morphologically different colonies were selected for WGS analysis. Genomic DNA was extracted from pure cultures grown overnight in TSB at 37°C in an incubator using a DNase blood and tissue kit for bacteria (Sigma-Aldrich Corporation, Natick, MA) following the manufacturer’s protocol (http://www.bea.ki.se/documents/EN-DNeasy%20handbook.pdf). A Nanodrop lite (Thermo Fisher Scientific, Waltham, MA) analysis was per-
formed on each isolated DNA sample for quantification purposes and to ensure sample purity (5).

WGS was performed at the Hubbard Center for Genome Studies (University of New Hampshire, Durham, NH). A paired-end library was constructed using a Nextera DNA library preparation kit (Illumina, San Diego, CA) and sequenced with an Illumina HiSeq 2500 instrument to produce 250-bp paired-end reads. The total number of reads for each of the 11 strains is listed in Table 1. Fastq files were trimmed for Nextera adapters and low-quality bases using Trimmomatic version 0.32 (6). For read trimming, trailing and leading bases were removed if the quality score was below 3. In addition, the reads were scanned using a 4-base sliding window and trimmed if the average quality dropped below 15. Trimmed sequencing reads were then assembled using the SPAdes pipeline version 3.5 (7) with default settings. QUAST version 4.6.0 (8) was used to assess the contiguity of the assemblies, and coverage statistics were calculated by mapping fastq reads to the assembled contigs with BWA-MEM (default settings) (9). The assembled genomes were annotated via the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (10). The taxonomic identity of each isolate was further confirmed by performing a BLASTn (11) search on 16S rRNA (1,500 bp) and translation initiation factor 2 (IF-2; 2,750 bp) gene sequences against the NCBI nucleotide database. For all isolates, the species identities ranged between 99% and 100%. The assembly metrics and annotated features are given in Table 1.

**Data availability.** Draft genome sequences and raw sequencing reads have been deposited at DDBJ/ENA/GenBank under the BioProject accession number PRJNA543860, and the described accession numbers in this publication are listed in Table 1.

**ACKNOWLEDGMENTS**

This project was partly funded by Pittsburg State University Graduate and Continuing Studies and the Kansas IDeA Network of Biomedical Research Excellence (K-INBRE) (grant number P20 GM103418, National Institute of General Medical Sciences). The whole-genome sequencing was supported by the New Hampshire IDeA Network of Biomedical Research Excellence (NH-INBRE) (grant number P20 GM103506, National Institute of General Medical Sciences).

We declare no conflicts of interest.

**REFERENCES**

1. Ricke SC, Richardson K, Dittoe DK. 2019. Formaldehydes in feeds and interaction with the poultry gastrointestinal tract microbial community—a review. Front Vet Sci 6:188. [https://doi.org/10.3389/fvets.2019.00188](https://doi.org/10.3389/fvets.2019.00188).
2. Ricke SC. 2018. Feed hygiene, p 177–209. In Dewulf J, Immersel FV (ed), Biosecurity in animal production and veterinary medicine—from principles to practice. ACCO, Leuven, Belgium.
3. Feye KM, Thompson DR, Rothrock MJ, Koquot MH, Ricke SC. 2020. Poultry processing and the application of microbiome mapping. Poult Sci 99:678–688. [https://doi.org/10.1016/j.psj.2019.12.019](https://doi.org/10.1016/j.psj.2019.12.019).

**TABLE 1** Accession numbers, assembly metrics, and annotated features of the sequenced strains isolated from commercial corn-based chicken feed

| Bacterial species | Strain | GenBank accession no. | SRA accession no. | Avg coverage (%) | No. of contigs | Total no. of reads | Genome assembly size (bp) | N50 (bp) | G+C content (%) | No. of coding genes | No. of rRNAs | No. of tRNAs | No. of ncRNAs |
|-------------------|--------|-----------------------|-------------------|-----------------|----------------|-------------------|--------------------------|---------|----------------|------------------|-------------|-------------|-------------|
| Enterococcus sp.  | PF-2   | VFLR00000000          | SRS4994585        | 425             | 29             | 6,481,210         | 3,667,502                | 436,646 | 53.30          | 6                | 51          | 4           |
| Enterococcus sp.  | PF-3   | VFLT00000000          | SRS4994583        | 1,003           | 29             | 15,261,978        | 3,667,758                | 516,576 | 43.74          | 8                | 51          | 4           |
| Klebsiella variicola | PF-5 | VFLW00000000         | SRS4994578        | 348             | 30             | 8,094,380         | 5,548,017                | 466,212 | 57.35          | 15               | 78          | 9           |
| Klebsiella variicola | PF-1 | VFLS00000000         | SRS4994582        | 405             | 32             | 10,377,912        | 5,548,808                | 409,857 | 56.66          | 52               | 78          | 9           |
| Kosakonia cowanii | PF-6   | VFLU00000000          | SRS49945579       | 340             | 24             | 7,202,962         | 4,806,877                | 532,407 | 56.11          | 3,350           | 9           | 74          | 11          |
| Kosakonia cowanii | PF-9   | VFLQ00000000          | SRS49945586       | 409             | 25             | 8,787,018         | 4,807,035                | 532,407 | 56.06          | 4,381           | 9           | 76          | 11          |
| Pantoea vagans   | PF-104 | VFLR00000000          | SRS49945580       | 435             | 25             | 9,139,632         | 4,807,035                | 532,407 | 54.78          | 4,384           | 9           | 76          | 11          |
| Pantoea vagans   | PF-103 | VFLQ00000000          | SRS49945584       | 510             | 19             | 9,486,610         | 4,573,523                | 527,489 | 53.64          | 4,180           | 15          | 70          | 6           |
| Pantoea vagans   | PF-7   | VFLY00000000          | SRS49945576       | 420             | 19             | 8,753,260         | 4,696,349                | 584,032 | 53.22          | 4,253           | 16          | 69          | 13          |
| Stenotrophomonas maltophilia | PF-8 | VFLQ00000000          | SRS49945577       | 530             | 28             | 11,071,680        | 4,807,035                | 405,440 | 65.89          | 4,053           | 7           | 64          | 4           |
| Stenotrophomonas maltophilia | PF-4 | VFLV00000000          | SRS49945581       | 485             | 26             | 9,405,034         | 4,377,836                | 351,312 | 66.15          | 3,788           | 7           | 64          | 4           |

aSRA, Sequence Read Archive.
bncRNAs, noncoding RNAs.
4. Ronholm J, Nasheri N, Petronella N, Pagotto F. 2016. Navigating microbiological food safety in the era of whole-genome sequencing. Clin Microbiol Rev 29:837–857. https://doi.org/10.1128/CMR.00056-16.

5. Micciche AC, Meyer LM, Ricke SC. 2018. 16S rDNA-based sequencing as a rapid method to identify bacteria cultivated from nonselective aerobic plate bacterial colonies isolated from commercial poultry feed, abstr 516P, p 114. Abstr PSA 2018. Annual Meeting of the Poultry Science Association, San Antonio, TX.

6. Bolger AM, Lohse M, Usadel B. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics 30:2114–2120. https://doi.org/10.1093/bioinformatics/btu170.

7. Nurk S, Bankevich A, Antipov D, Gurevich AA, Korobeynikov A, Lapidus A, Prijibelski AD, Pyshkin A, Sirotkin A, Sirotkin Y, Stepansauskas R, Clingenpeel SR, Woyke T, McLean JS, Lasken R, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling single-cell genomes and metagenomes from chimeric MDA products. J Comput Biol 20:714–737. https://doi.org/10.1089/cmb.2013.0084.

8. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. Bioinformatics 29:1072–1075. https://doi.org/10.1093/bioinformatics/btt086.

9. Li H. 2013. Aligning sequence reads, clone sequences and assembly contigs with BWA-MEM. arXiv 1303.3997v2 [q-bio.GN]. https://arxiv.org/abs/1303.3997.

10. Tatusova T, DiCuccio M, Badretdin A, Chetvernin V, Nawrocki EP, Zaaslavsky 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.

11. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. 1990. Basic local alignment search tool. J Mol Biol 215:403–410. https://doi.org/10.1016/0022-2836(90)90360-2.