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Draft genome sequences of four lactic acid bacteria from fermented chicken meat unveil biosynthetic gene clusters for antimicrobial compounds

Mahabub Alam[a], Tamanna Hassan[b], Tanvir Ahmad Nizami[a], Lipi Akter[c], Tofazzal Md Rakib[a#]

a. Faculty of Veterinary Medicine, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

b. Faculty of Food Science and Technology, Chattogram Veterinary and Animal Sciences University, Chattogram, Bangladesh.

c. Transboundary Animal Diseases Research Center, Faculty of Veterinary Medicine, Kagoshima University, Kagoshima, Japan.

Running title: Biosynthetic gene clusters of lactic acid bacteria

#Address correspondence to Tofazzal Md Rakib, rakibtofazzal@cvasu.ac.bd.

Abstract

Lactic acid bacteria play a crucial role in fermented food production and serve as important sources of antimicrobial peptides. This study reports four lactic acid bacteria strains, isolated from fermented chicken meat, which harbor biosynthetic gene clusters encoding antimicrobial compounds. These strains are classified within the genera *Pediococcus* and *Lactiplantibacillus*.

Announcement

Fermented foods have long carried probiotics, prebiotics, and postbiotics. Lactic acid bacteria (LAB) enhance food safety, inhibit spoilage, and support gut health. LAB, from the phylum *Firmicutes*, include *Lactobacillus*, *Pediococcus*, *Leuconostoc*, *Lactococcus*, and *Enterococcus* (1, 2). With rising antibiotic resistance, bacteriocins are gaining attention as potential alternatives (3). Genome mining and high-throughput screening of biosynthetic gene clusters present a promising pathway for discovering novel bacteriocins, addressing resistance challenges, and advancing sustainable food preservation (4, 5). Three broiler chickens from a live bird market (22.336218 N, 91.830187 E) in Chattogram, Bangladesh, were slaughtered under standard procedures. Breast meat (100 g) was stored anaerobically at 4°C for three days to propagate probiotic bacteria. One gram of meat was mixed with 5 mL MRS broth (Oxoid, Hampshire, England) and incubated anaerobically at 37°C for 48 hours, promoting LAB growth. Single colonies were isolated on MRS agar, purified, and confirmed by genus-specific 16S rRNA amplification. Genomic DNA was extracted from 1.5 mL of 24-hour cultured broth using a AllPrep Bacterial DNA/RNA/Protein Kit (Qiagen, Hilden, Germany), and purity was assessed with a Nanodrop One (Thermo Fisher Scientific, MA, USA). DNA libraries were prepared using the Nextera XT DNA Library Preparation Kit (Illumina, CA, USA). Genome sequencing was conducted on an Illumina NextSeq2000 platform at the Poultry Research and Training Center, Chattogram Veterinary and Animal Sciences University, Bangladesh,

35 generating 2×150 bp paired-end reads. Raw data quality, including read quality, GC content, and adapter
36 contamination, was assessed using FastQC v0.12.1 (6). Trim Galore v0.6.5dev (7) was employed to trim
37 low-quality bases and adapter sequences, followed by normalization of read coverage with bbnorm
38 v39.08 (8) to reduce redundancy. De novo genome assembly was performed using Unicycler v0.4.8 (9),
39 with subsequent polishing using Pilon v1.24 (10) to enhance accuracy. Genome assembly quality was
40 assessed using QUAST v5.2.0 (11), while Samtools v1.13 (12) was used for alignment file manipulation and
41 indexing. The de novo genome assembly, conducted through the BV-BRC (13) comprising above tools,
42 yielded coverage ranging from 65.4× to 145.6×, with raw reads between 1,452,975 and 1,662,521. The
43 assembled genomes were annotated using NCBI PGAP v6.9 (14) and PATRIC (15) with the in-built RASTtk
44 pipeline to identify the species, with transport proteins identified via TCDB (16), drug targets predicted
45 using DrugBank v6.0 (17), and additional analyses performed for CRISPR arrays and prophages using
46 CRISPRCasFinder (18), and Integrative and Conjugative Elements using ICEFinder (19). All software
47 parameters were set to default unless specified otherwise. Detailed genomic characteristics are provided
48 in Table 1.

49 The genomes of four LAB isolates were mined for biosynthetic gene clusters (BGCs) associated with
50 antimicrobial production using the software antiSMASH v7.0 (20) and BAGEL4 (21). The genomes were
51 found to contain multiple BGCs encoding ribosomally synthesized and posttranslationally modified
52 peptides (RiPP like compounds), Cyclic Lactone Autoinducer, Type III Polyketide Synthase (T3PKS),
53 Terpene Precursors, Terpene, and Class IIa (Pediocin) and IIb (Plantaricin_J) bacteriocins. The isolates or
54 their purified bacteriocins may be used in the future for food preservation and commercial probiotics to
55 manage gut health and related diseases.

56 **Data availability statement**

57 The accession numbers for the genomes of the four LAB strains are shown in Table 1. All genomes are
58 available under BioProject accession number PRJNA1189463.

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66 **Author ORCIDs**

67 Mahabub Alam https://orcid.org/0000-0001-9837-2598
68 Tamanna Hassan https://orcid.org/0009-0009-4875-9386
69 Tanvir Ahmad Nizami https://orcid.org/0000-0002-7024-4970
70 Lipi Akter https://orcid.org/0000-0002-6317-9410
71 Tofazzal Md Rakib https://orcid.org/0000-0003-2642-9908

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Table 1: Genomic features of the sequenced four LAB strains isolated from fermented chicken meat in Chattogram, Bangladesh

Information Title \ Strain Name	CVASU1	CVASU3	CVASU2	CVASU4
LAB Species	*Pediococcus pentosaceus*	*Pediococcus pentosaceus*	*Lactiplantibacillus plantarum*	*Lactiplantibacillus argentoratensis*
Genome Submission ID	SUB14898186	SUB14898186	SUB14898218	SUB14898234
Bioproject ID	PRJNA1189463	PRJNA1189463	PRJNA1189463	PRJNA1189463
BioSample accession number	SAMN44971012	SAMN44971014	SAMN44971013	SAMN44971015
SRA accession number	SRR31442436	SRR31442434	SRR31442435	SRR31442433
Genome assembly accession number	GCA_046736495.1	GCA_046736455.1	GCA_046736505.1	GCA_046736475.1
GenBank accession number	JBJPFK000000000	JBJPFJ000000000	JBJPFL000000000	JBJPFM000000000
FastQC Phred score	36	35	37	35
Raw Reads	1452975	1925165	1600310	1662521
Coverage	113.9×	145.6×	65.4×	70.9×
Contig count	24	26	62	99
Coarse consistency (%)	99.1	99.1	97.9	97.9
Fine consistency (%)	98.2	98.2	96	96.3

Completeness (%)	100	100	100	100
Contamination (%)	1.5	1.5	0	0.6
Genome size (bp)	1775093	1775155	3346512	3162126
Contigs N50 (bp)	190398	174289	197047	118879
Contigs L50	4	4	6	9
Largest contig (bp)	297937	297937	471909	305211
GC Content (%)	37.153152	37.153095	44.325077	45.074894
Genes (Total), PGAP	1800	1,799	3247	3008
CDS (PGAP; PATRIC)	1749; 1783	1748; 1783	3175; 3353	2947; 3108
tRNA	45	45	66	56
rRNA	3	3	3	2
CDS Ratio	1.0044544	1.0044193	1.0019387	0.98288304
Hypothetical CDS	350	350	1507	1333
Hypothetical CDS Ratio	0.29220414	0.29220414	0.50611395	0.49131274
PLFAM CDS	1700	1701	0	0
PLFAM CDS Ratio	0.95344925	0.95401007	-	-
Hypothetical proteins	351	351	1508	1334

Proteins with functional assignments	1432	1432	1845	1774
Proteins with EC number assignments	491	491	687	669
Proteins with GO assignments	407	407	584	561
Proteins with Pathway assignments	336	336	488	470
Proteins with Subsystem assignments	570	570	716	716
Proteins with PATRIC genus-specific family (PLfam) assignments	1700	1701	0	0
Proteins with PATRIC cross-genus family (PGfam) assignments	1727	1728	3233	2990
Proteins with FIGfam assignments	0	0	0	0
Transporter (TCDB)	2	2	15	8
Drug Target (DrugBank)	1	1	1	0
Antibiotic Resistance Genes (PATRIC)	21	21	25	27
Integrative and Conjugative Elements (ICEs)	2 (819699..838639, 18941 bp; 1645942..1683899, 37958 bp)	3 (819699..838639, 18941 bp; 1174724..1193031, 18308 bp; 1662631..1699235, 36605 bp)	2 (2983152..3034384, 51233 bp; 3313868..3325625, 11758 bp)	1 (3096716..3139121, 42406 bp)
No. of Clustered Interspaced Short Repeats (CRISPR)	6 (4) (cas9_TypeII, cas1_TypeII, cas2_TypeI-II-III, csn2_TypeIIA)	6 (4) (cas9_TypeII, cas1_TypeII, cas2_TypeI-II-III, csn2_TypeIIA)	2 (1) (cas3_TypeI)	22 (8) (cas6_TypeIE, cas5_TypeIE, cas7_TypeIE, cse2_TypeIE, cse1_TypeIE,

(number of Cas clusters) (name of cas genes)				cas3_TypeI, cas2_TypeIE, cas1_TypeIE)
Bacteriocins and biosynthetic gene clusters (BGCs)	Pediocin, terpene-precursor, T3PKS	Pediocin, T3PKS	Plantaricin_J, cyclic-lactone-autoinducer, RiPP like, terpene, T3PKS	terpene-precursor (3 loci), T3PKS, terpene