Complete Genome Sequence of *Chryseobacterium mulctrae* KACC 21234<sup>T</sup>: A Potential Proteolytic and Lipolytic Bacteria Isolated from Bovine Raw Milk

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**Abstract**

*Chryseobacterium mulctrae* KACC 21234<sup>T</sup> is a novel species isolated from raw bovine milk. Psychrotrophic bacteria are considered contaminants and are hypothesized to originate from the environment. In this investigation, the *C. mulctrae* KACC 21234<sup>T</sup> genome was determined to be 4,868,651 bp long and assembled into four contigs with a G+C ratio of 33.8%. *In silico* genomic analyses revealed the presence of genes encoding proteases (endopeptidase C1p, oligopeptidase b, carboxypeptidase) and lipases (phospholipase A2, phospholipase C, acylglycerol lipase) that can catalyze the degradation of the proteins and lipids in milk, causing its quality to deteriorate. Additionally, antimicrobial resistance and putative bacteriocin genes were detected, potentially intensifying the pathogenicity of the strain. The genomic evidence presented highlights the need for improved screening protocols to minimize the potential contamination of milk by proteolytic and lipolytic psychrotrophic bacteria.

**Keywords**

*Chryseobacterium mulctrae*, complete genome, milk spoilage, proteolytic, lipolytic

The genus *Chryseobacterium* is composed of Gram-negative, aerobic, non-fermentative, psychrotrophic bacilli with characteristic yellow pigmentation. The genus is considered a part of the normal environmental microflora and has been associated with human infections [1,2]. Recently, several novel species have been isolated from raw milk samples, including *C. mulctrae* KACC 21234<sup>T</sup> [3-6]. And although *Chryseobacterium* is not part of the normal microflora of milk, contamination can occur during the milking process (i.e., from cow udder, human handling, and immediate environment) or transport and processing [7]. Psychrotrophs can take advantage of the high nutritional content of milk and grow at low temperatures (7°C and below). The activity of these microorganisms may lead to different types of milk spoilage - souring, gas production, proteolysis, ropiness, change in milk fat, flavor defect, and color defect. In addition, some contaminating microorganisms are potentially pathogenic [8].

The presence of lipolytic and proteolytic microorganisms in milk may lead to a variety of defects and shortened shelf-life through the production of extracellular enzymes that can hydrolyze proteins (b-casein and a<sub>1</sub>-casein) and triglycerides, ultimately causing spoilage of the product. Proteases, particularly plasmin, are linked with gelation of UHT sterilized milk, development of bitterness in milk, and reduction in yield of soft cheese while the action of lipases affects the flavor profile of dairy products [8]. Moreover, the extracellular enzymes synthesized by psychrotrophic bacteria are heat stable, withstand-
$132^\circ$C for 2 sec) processes, presenting a challenge in securing the quality and safety of milk and dairy products [8,9].

*C. mulctrae* KACC 21234$^\top$ was isolated based on its proteolytic activity on skim milk agar plate (SMA: 5% skim milk), incubated at $10^\circ$C for 10 days. Strain KACC 21234$^\top$ was routinely cultured in tryptic soy agar (TSA, BD Difco, USA) at $30^\circ$C [6]. The genomic DNA was extracted using QIAamp PowerFecal DNA kit (Qiagen, Germany) and sent to ChunLab (Korea) for sequencing using PacBio RSII Single Molecule Real-Time (SMRT) platform with 20 kb SMRTbell$^{\text{TM}}$ template library. De novo assembly of the PacBio reads was performed using the PacBio SMRT analysis software ver. 2.3.0.

Genome annotation was performed using the Rapid Annotation using Subsystem Technology (RAST) using default parameters [10]. Transfer RNAs and ribosomal RNAs were identified using tRNAscan-SE ver. 1.3.1 [11] and INFERNAL ver. 1.1.3 software using the Rfam 12.0 database [12], respectively (Fig. 1, 2). The genome features of *C. mulctrae* KACC 21234$^\top$ are listed in Table 1.

![Circular genome map of *Chryseobacterium mulctrae* KACC 21234$^\top$. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio. G, guanine; C, cytosine.](https://www.ejmsb.org/)

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**Table 1.** Genomic features of *Chryseobacterium mulctrae* KACC 21234$^\top$.
Functional genome annotation revealed several genes encoding for proteases and lipases (Table 2). Several studies have reported that members of *Chryseobacterium* showed greater spoilage ability than *Pseudomonas* spp. based on its proteolytic and lipolytic activity [7,13]. Additionally, although less frequently reported, the production of phos-

**Table 1. Genome features of *Chryseobacterium multrae* KACC 21234<sup>T</sup>**

| Attribute                  | Value          |
|----------------------------|----------------|
| Genome size (bp)           | 4,868,651      |
| G+C content (%)            | 33.8           |
| No. of contigs             | 4              |
| Protein-coding genes       | 4,610          |
| tRNA                       | 75             |
| rRNA                       | 18             |
| Plasmids                   | 0              |
| GenBank Accession No.       | VAJL00000000   |

G, guanine; C, cytosine.

**Fig. 2.** Subsystem category distribution by (A) KEGG annotation and (B) Cluster of Orthologous Groups. KEGG, Kyoto Encyclopedia of Genes and Genomes.
Table 2. Predicted proteases and lipases from C. multrae KACC 21234\textsuperscript{T} genome

| Gene name     | Length (bp) | Product                      | Predicted function                                                                 | Reference |
|---------------|-------------|------------------------------|--------------------------------------------------------------------------------------|-----------|
| CS110_00311   | 1,581       | Carboxypeptidase             | Aminopeptidase, metallopeptidase                                                    | [14]      |
| CS110_00621   | 687         | Endopeptidase Clp            | Chymotrypsin-like activity                                                          | [15]      |
| CS110_00616   | 2,133       | Oligopeptidase B             | Serine-type endopeptidase, hydrolysis of carboxyl side of basic amino acids        | [16]      |
| CS110_01328   | 1,539       | Peptidyl-Asp metalloendopeptidase | Cleave Xaa-Cya or Xaa-Asp at N-terminus                                           | [17]      |
| CS110_02075   | 1,797       | Prolyl oligopeptidase        | Selective cleavage of peptide bonds at the carboxyl group of internal proline residue | [18]      |
| CS110_01857   | 1,038       | Phospholipase A(2)           | Cleaves fatty acid in position 2 of phospholipids                                 | [19]      |
| CS110_02528   | 528         | Phospholipase C              | Lipid catabolism, phospholipolysis                                                 | [20]      |
| CS110_03091   | 939         | Acylglycerol lipase          | Hydrolysis of monoacylglycerol                                                     | [21]      |

Xaa-Cya, cysteic acid residue; Xaa-Asp, aspartic acid residue.

Phospholipases (CS110_01857 and CS110_02528) was associated with sweet curdling and bitter cream in milk due to the aggregation of fat globules. C. multrae KACC 21234\textsuperscript{T} also has genes for \( \beta \)-galactosidase (CS110_00343), which may cause unwanted hydrolysis of \( \beta \)-galactosidic bonds in lactose.

Antimicrobial resistance genes were also detected using the Resistance Gene Identifier with The Comprehensive Antibiotic Resistance Database (https://card.mcmaster.ca/analyze/rgi). Specifically, CPS-1, adeF, and qacG, which confers resistance to carbenem, fluoroquinolone and tetracycline, and antiseptics, respectively, were identified. Furthermore, two open reading frames (ORF) encoding a putative bacteriocin (Linocin M18 and Carocin D) were identified via BAGEL4 (http://bagel4.molgenrug.nl/). However, there were no immunity and transport proteins associated with the bacteriocin genes.

The \textit{in-silico} analyses of \textit{C. multrae} KACC 21234\textsuperscript{T} genome revealed the presence of various proteolytic and lipolytic enzymes, bacteriocins, and antimicrobial resistance genes which highlights the risks involved in microbial contamination of milk. Thus, it is imperative to develop effective screening methods for the detection of contaminating microorganisms and their enzymes to improve the quality and safety of milk and related products.

### Nucleotide Sequence Accession Number

The Whole Genome Shogun project has been deposited at GenBank under the accession number VAJL00000000. The version described in this paper has the accession number VAJL01000000, consisting of sequences VAJL01000001 – VAJL01000004.

### Conflict of Interest

The authors declare no potential conflict of interest.
References

1. Alfouzan W, Dhar R, Al-Hashemi H, Al-Sweih N, Albert MJ. Clinical and microbiological characteristics of Chryseobacterium spp. isolated from neonates in Kuwait. JMM Case Rep. 2014;1:e001008.
2. Mwanza EP, Hugo A, Charimba G, Hugo CJ. Pathogenic potential and control of Chryseobacterium species from clinical, fish, food and environmental sources. Microorganisms. 2022;10:895.
3. Hantsis-Zacharov E, Halpern M. Chryseobacterium halfense sp. nov., a psychrotolerant bacterium isolated from raw milk. Int J Syst Evol Microbiol. 2007;57:2344-2348.
4. Hantsis-Zacharov E, Shakéd T, Senderovich Y, Halpern M. Chryseobacterium oranimense sp. nov., a psychrotolerant, proteolytic and lipolytic bacterium isolated from raw cow’s milk. Int J Syst Evol Microbiol. 2008;58:2635-2639.
5. Lee JE, Yoon SH, Lee GY, Lee DH, Huh CS, Kim GB. Chryseobacterium vaccae sp. nov., isolated from raw cow’s milk. Int J Syst Evol Microbiol. 2020;70:4859-4866.
6. Yoon SH, Lee JE, Han RH, Kwon M, Kim GB. Chryseobacterium mulctrae sp. nov., isolated from raw cow’s milk. Int J Syst Evol Microbiol. 2019;69:3478-3484.
7. Yuan L, Sadiq FA, Liu TJ, Li Y, Gu JS, Yang HY, et al. Spoilage potential of psychrotrophic bacteria isolated from raw milk and the thermo-stability of their enzymes. J Zhejiang Univ Sci B. 2018;19:630-642.
8. Bekker A, Jooste P, Steyn L, Bothma C, Hugo A, Hugo C. Lipid breakdown and sensory analysis of milk inoculated with Chryseobacterium joostei or Pseudomonas fluorescens. Int Dairy J. 2016;52:101-106.
9. Baur C, Krewinkel M, Kranz B, von Neubeck M, Wenning M, Scherer S, et al. Quantification of the proteolytic and lipolytic activity of microorganisms isolated from raw milk. Int Dairy J. 2015;49:23-29.
10. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, et al. The RAST Server: rapid annotations using subsystems technology. BMC Genomics. 2008;9:75.
11. Chan PP, Lowe TM. tRNAscan-SE: searching for tRNA genes in genomic sequences. Methods Mol Biol. 2019;1962:1-14.
12. Nawrocki EP, Eddy SR. Infernal 1.1: 100-fold faster RNA homology searches. Bioinformatics 2013;29:2933-2935.
13. Odeyemi OA, Alegbeleye OO, Strateva M, Stratev D. Understanding spoilage microbial community and spoilage mechanisms in foods of animal origin. Compr Rev Food Sci Food Saf. 2020;19:311-331.
14. Deitener K, Hendriks D, Scharpé S, Lambeir AM. Carboxypeptidase M: multiple alliances and unknown partners. Clin Chim Acta. 2009;399:24-39.
15. Bhandari V, Wong KS, Zhou JL, Mabanglo MF, Batey RA, Houry WA. The role of ClpP protease in bacterial pathogenesis and human diseases. ACS Chem Biol. 2018;13:1413-1425.
16. Coetzer TH, Goldring JP, Huson LE. Oligopeptidase B: a processing peptidase involved in pathogenesis. Biochimie. 2008;90:336-344.
17. Hagmann ML, Geuss U, Fischer S, Kresse G. Peptidyl-Asp metalloendopeptidase. Methods Enzymol. 1995;248:782-787.
18. Svarcbahs R, Julku UH, Norrbacka S, Myöhänen TT. Removal of prolyl oligopeptidase reduces alpha-synuclein toxicity in cells and in vivo. Sci Rep. 2018;8:1552.
19. Burke JE, Dennis EA. Phospholipase A2 structure/function, mechanism, and signaling. J Lipid Res. 2009;50 Suppl:S237-S242.
20. Munsch-Alatossava P, Kakela R, Ibarra D, Youbi-Idrissi M, Alatossava T. Phospholipolyis caused by different types of bacterial phospholipases during cold storage of bovine raw milk is prevented by N₂ gas flushing. Front Microbiol. 2018;9:1307.
21. Chahinian H, Vanot G, Ibrik A, Rugani N, Sarda L, Comeau LC. Production of extracellular lipases by Penicillium cyclopium purification and characterization of a partial acylglycerol lipase. Biosci Biotechnol Biochem. 2000;64:215-222.