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ARTICLE

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

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Abstract

Chryseobacterium mulctrae KACC 21234^T 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^T 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 Clp, oligopeptidase b, carboxypeptidase) and lipases (phospholipase A(2), 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^T [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℃ 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_s-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, withstanding pasteurization conditions (72℃ for 15 sec) and even ultrahigh temperature (UHT,

132℃ for 2 sec) processes, presenting a challenge in securing the quality and safety of milk and dairy products [8,9].

C. mulctrae KACC 21234^{T} was isolated based on its proteolytic activity on skim milk agar plate (SMA; 5% skim milk), incubated at 10℃ for 10 days. Strain KACC 21234^{T} was routinely cultured in tryptic soy agar (TSA, BD Difco, USA) at 30℃ [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^{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 perdformed 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^{T} are listed in Table 1.

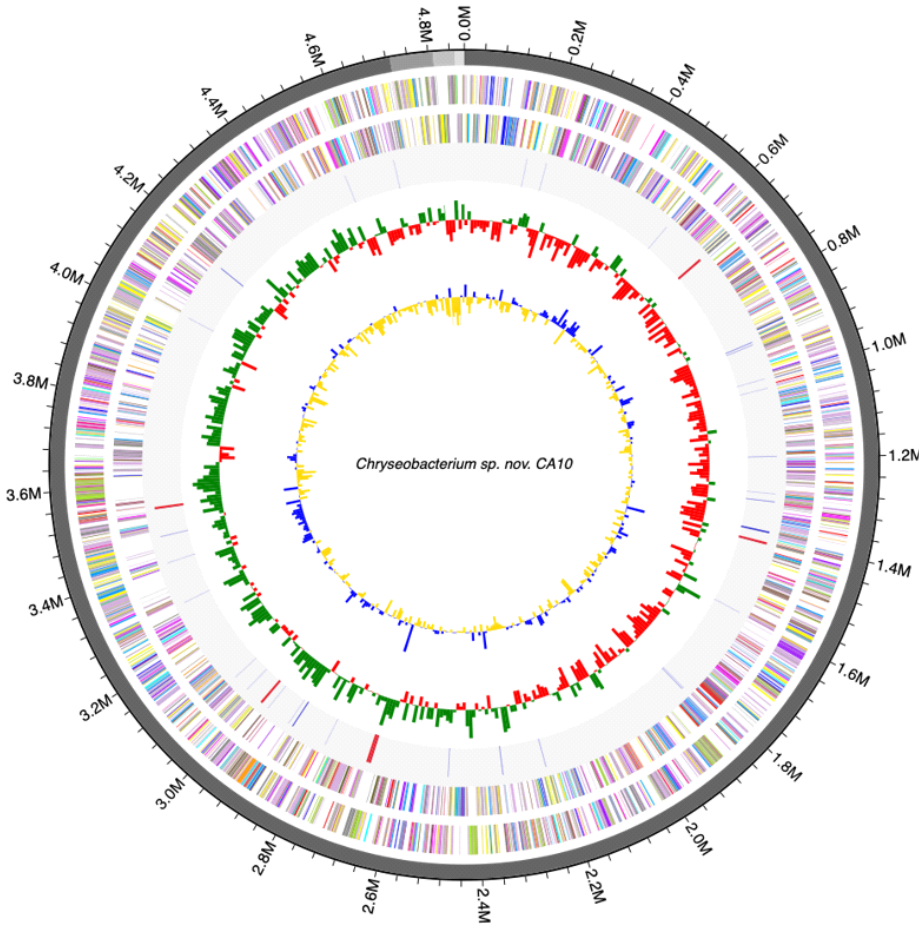

Fig. 1. Circular genome map of *Chryseobacterium mulctrae* KACC 21234^{T}. Circles represent the following characteristics from the outermost circle to the center: (1) contig information, (2) coding sequences on forward strand, (3) coding sequences on reverse strand, (4) transfer RNAs (tRNAs) and ribosomal RNAs (rRNAs), (5) GC skew, and (6) GC ratio. G, guanine; C, cytosine.

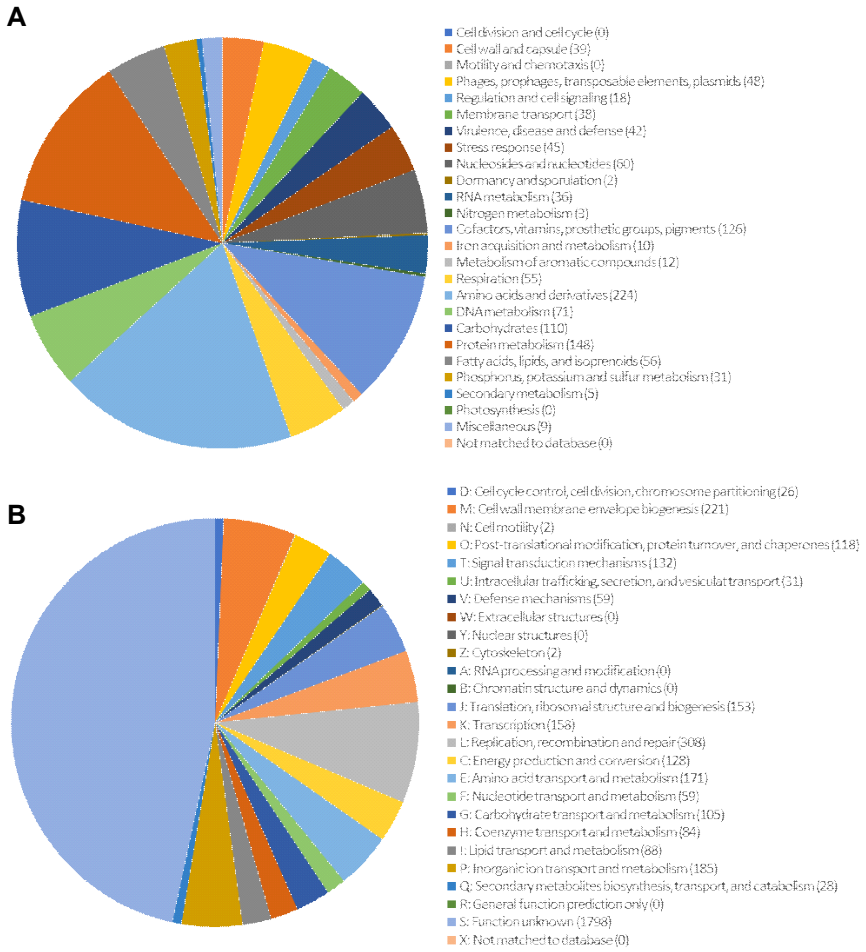

Fig. 2. Subsystem category distribution by (A) KEGG annotation and (B) Cluster of Orthologous Groups. KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 1. Genome features of *Chryseobacterium mulctrae* KACC 21234^T

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.

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 2. Predicted proteases and lipases from *Chryseobacterium mulctrae* KACC 21234^T genome

Gene name	Length (bp)	Product	Predicted function	Reference
Protease				
CS110_00311	1,581	Carboxypeptidase	Aminopeptidase, metallodipeptidase	[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]
Lipase				
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.

pholipases (CS110_01857 and CS110_02528) was associated with sweet curdling and bitter cream in milk due to the aggregation of fat globules. *C. mulctrae* KACC 21234^T also has genes for b-galactosidase (CS110_00343), which may cause unwanted hydrolysis of β-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 carbapenem, 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 *in-silico* analyses of *C. mulctrae* KACC 21234^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.

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