Phenotypic and genomic characterizations of *Klebsiella pneumoniae* ssp. *pneumoniae* and *Rahnella inusitata* strains reveal no clear association between genetic content and ropy phenotype

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**ABSTRACT**

Ropy defect of pasteurized fluid milk is a type of spoilage which manifests itself by an increased viscosity, slimy body, and string-like flow during pouring. This defect has, among other causes, been attributed to the growth, proliferation and exopolysaccharide production by coliform bacteria, which are most commonly introduced in milk as post-pasteurization contaminants. As we identified both *Klebsiella pneumoniae* ssp. *pneumoniae* and *Rahnella inusitata* that were linked to a ropy defect, the goal of this study was to characterize 3 *K. pneumoniae* ssp. *pneumoniae* strains and 2 *R. inusitata* for (1) their ability to grow and cause ropy defect in milk at 6°C and 21°C and to (2) probe the genetic basis for observed ropy phenotype. Although all *K. pneumoniae* ssp. *pneumoniae* and *R. inusitata* strains showed net growth of >4 log10 over 48 h in UHT milk at 21°C, only *R. inusitata* strains displayed growth during 28-d incubation period at 6°C (>6 log10). Two out of 3 *K. pneumoniae* ssp. *pneumoniae* strains were capable of causing the ropy defect in milk at 21°C, as supported by an increase in the viscosity of milk and string-like flow during pouring; these 2 strains were originally isolated from raw milk. Only one *R. inusitata* strains was able to cause the ropy defect in milk; this strain was able to cause the defect at both 6°C and 21°C, and was originally isolated from a pasteurized milk. These findings suggest that the potential of *K. pneumoniae* ssp. *pneumoniae* and *R. inusitata* to cause ropy defect in milk is a strain-dependent characteristic. Comparative genomics provided no definitive answer on genetic basis for the ropy phenotype. However, for *K. pneumoniae* ssp. *pneumoniae*, genes *rffG, rffH, rfbD*, and *rfbC* involved in biosynthesis and secretion of enterobacterial common antigen (ECA) could only be found in the 2 strains that produced ropy defect, and for *R. inusitata* a set of 2 glycosyltransferase- and flippase genes involved in nucleotide sugar biosynthesis and export could only be identified in the ropy strain. Although these results provide some initial information for potential markers for strains that can cause ropy milk, the relationship between genetic content and ropiness in milk remains poorly understood and merits further investigation.

**Key words:** dairy spoilage, ropy milk, *Klebsiella*, *Rahnella*, genomics

**INTRODUCTION**

Microbial spoilage of milk is of serious concern because it contributes to overall food loss and waste, and is responsible for economic losses for both the dairy industry and consumers (FAO, 2019). Approximately half of all fluid milk spoilage can be attributed to heat-sensitive gram-negative bacteria that are introduced into milk as post-pasteurization contamination (PPC; Martin et al., 2012). Although PPC contamination is possible at any stage of fluid milk processing after pasteurization until the point of container filling, it is predominantly associated with filling machines as the primary source of entry (Ralyea et al., 1998, Eneroth et al., 2000). Gram-negative bacteria associated with PPC are typically represented by *Pseudomonas* sp. and representatives of the family *Enterobacteriaceae* including coliform bacteria (Alles et al., 2018, Reichler et al., 2018). These bacteria can grow quickly in milk at refrigeration temperatures, reaching levels above regulatory limits relatively early in the shelf life (e.g., 7–10 d after pasteurization; Martin et al., 2012). Along with flavor and odor defects that they can cause by producing a variety of enzymes and other compounds (Harwalkar et al., 1989, Hayes et al., 2002, Dogan and Boor, 2003, Fromm and Boor, 2004), some of these spoilage organisms can also cause severe body defects. One type of body defect associated with microbial spoilage is ropiness which is characterized by a higher viscosity and a slimy body and string-like flow dur-
ing pouring (Mader, 1940, Morton and Barrett, 1982, Cheung and Westhoff, 1983). These slimy and viscous properties are mostly associated with the extracellular polysaccharides, high molecular weight sugar-based polymers, produced and secreted by these spoilage microorganisms. These bacterial polysaccharides can be classified into 2 different types based on their location relative to the cell: capsular polysaccharide (CPS) are found bound, often covalently, to phospholipid or lipid A molecules forming a cohesive layer (Whitfield and Valvano, 1993, Whitfield et al., 2020), while exopolysaccharides or EPS are excreted into the environment (Bouzar et al., 1996, Tytgat and Lebeer, 2014, Ryan et al., 2015). It should be noted that the CPS can also be released into the environment and that EPS may be closely associated with the cell surface (Taylor and Roberts, 2005). CPS and EPS play an important role in (1) providing protection from stress (e.g., high salinity, high and low temperature, and acidity; Kawahara et al., 1996, Nguyen et al., 2014, Dertli et al., 2015, Mukhtar et al., 2020, Bhagat et al., 2021), (2) the interaction between bacteria and their environment (Ruas-Madiedo et al., 2006), and (3) contributing to surface adhesion and biofilm formation (Schembri et al., 2004, Boks et al., 2008, Ly et al., 2008). Bacterial EPS in some beneficial microbes (e.g., lactic acid bacteria) may exhibit functional properties, representing a great interest for the food industry, particularly for the production of yogurt, cheese, fermented cream, and milk-based dessert (Bouzar et al., 1999, Boks et al., 2008, Ly et al., 2008). Bacterial EPS and resultingropy defect are typically not associated with any food hazard, fluid milk that shows the so-called “ropy” defect is rejected by consumers leading to both short-term and long-term economic losses for the dairy industry.

First reports of bacteria responsible for ropy defect date back to 1840 when *Pseudomonas syringanthas* was isolated fromropy milk by Ehrenberg (Ehrenberg, 1840). Later, several studies reported *Alcaligenes viscosus* as the most prominent bacterial species causing ropy defect in milk (Punch et al., 1965, Morton and Barrett, 1982). Other reports attributed bacterial ropiness in milk to several species including *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Flavobacterium multivorum*, *Yersinia pestis*, *Enterobacter agglomerans*, and *Pseudomonas* spp. (Buchanan and Hammer, 1915, Gainor and Wegener, 1954, Cheung and Westhoff, 1983, Trmčić et al., 2015). However, limited information is available about these bacterial species and their ability to cause ropy defect in milk. Therefore, collecting further data are pivotal to assess and address the risk of spoilage by bacteria that can cause ropy defect in milk.

In this study, 3 *Klebsiella* sp. strains and 2 *Rahnella inusitata* strains were analyzed together to get an insight into the species and strains that are involved in development of the ropy defect, their phenotypic properties and ultimately genetic constitution that may be attributed to ropy defect incidents. Our specific objectives were (1) to investigate the ability of monocultures of *K. pneumoniae* ssp. *pneumoniae* and *R. inusitata* to grow in milk at 6°C and 21°C, (2) to investigate the ability of those monocultures to change the viscosity of milk during growth at 6°C and 21°C, and (3) to characterize and compare the genomes of strains from the same species to identify a genetic marker(s) associated with the ability to cause ropy defect.

**MATERIALS AND METHODS**

**Bacterial Strain Selection and Culture Conditions**

No human or animal subjects were used, so this analysis did not require approval by an Institutional Animal Care and Use Committee or Institutional Review Board.

Five bacterial strains, which were initially identified as *Klebsiella* sp. (Food Safety Lab [FSL] A6–0140, FSL R5–0773, and FSL W5–0628) and *R. inusitata* (FSL A6–0213 and FSL J3–0059), belonging to the FSL culture collection, Department of Food Science, Cornell University, Ithaca, New York, were used in this study (Supplemental Table S1; https://ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881bf898a; Princic et al., 2023). Two strains (FSL W5–0628 and FSL A6–0213) were isolated from different milk samples showing ropy defect (obtained as part of several dairy-quality research and surveillance projects conducted by the Milk Quality Improvement Program [MQIP], Department of Food Science, Cornell University, Ithaca, New York), while 3 strains (FSL R5–0773, FSL J3–0059 and FSL A6–0140) were isolated from fluid milk and yogurt without reported ropy defects (Trmčić et al., 2015, Hervet et al., 2016, Masiello et al., 2016). The 3 strains obtained from unspoiled fluid milk and yogurt were selected for their close partial 16S rDNA sequence similarity with the 2 strains associated with ropy defect. The 3 *Klebsiella* sp. strains were identified as *K. pneumoniae* ssp. *pneumoniae* in this current study. Specific strain information can be found in the Food Microbe Tracker Database (www.foodmicrobetracker.com; Vangay et al., 2013).

The strains were maintained at ~80°C in Brain Heart Infusion (BHI) broth (Difco, BD) with 15% (vol/vol) glycerol. To obtain standardized cultures, each strain was streaked out from frozen stocks onto BHI agar and incubated aerobically at 32°C for 20 to 24 h. The
As the inoculated milk was used to inoculate a fresh BHI broth and incubated at 32°C for 20 to 24 h. These cultures were used to inoculate fresh BHI, which was further incubated for 20 to 24 h under the same conditions before being used for growth experiments or extraction of genomic DNA.

**Characterization of Growth and Development of the Ropy Defect in Milk**

Growth and development of the ropy defect in milk were determined at 6°C and 21°C to separately simulate storage of milk at refrigeration and temperature abuse at room temperature, respectively. Three separate biological replicates were performed at each of the 2 temperatures and each replicate included inoculation of 2 technical milk replicates. The inoculum of each strain was prepared from standardized liquid cultures as described in the section “Bacterial strains selection and culture conditions.” Standardized liquid culture was inoculated 1:5 into fresh BHI broth and incubated aerobically at 32°C for 20 to 24 h. Subsequently, suspension was adjusted to an Optical Density at 600 nm (OD600) of ~0.3, representing ~6 to 7 log10 cfu/mL, and then diluted to reach a starting concentration of ~2 to 3 log10, cfu/mL of milk. Aliquots of 100 µL of appropriate dilution were used for inoculation of 2 separate sterile glass bottles containing 450 mL of UHT milk. To determine the initial population in inoculated UHT milk (d = 0), a 1-mL aliquot of each inoculated UHT milk sample was serially diluted in PBS, if necessary, and appropriate dilutions were spiral plated onto BHI agar using 50-µL exponential setting (Autoplate 5000; Advanced Instruments, Inc.). Colonies were enumerated following a 24 h incubation on BHI agar at 32°C using the Q-Count colony counter and expressed as cfu/mL.

**Microbiological Analysis**

To determine the total bacteria count in milk samples, 1 mL of milk was taken from the initial 36 mL of milk sampled at each time point, serially diluted in PBS, if necessary, and appropriate dilutions were spiral plated (50 µL), as described above, in technical duplicate onto BHI agar. Colonies were enumerated following a 24 h incubation on BHI agar at 32°C using the Q-Count colony counter and expressed as cfu/mL.

**Physicochemical Characterization**

The pH values of collected milk samples were determined at room temperature in duplicate using a calibrated pH meter and pH probe (SevenGo, Mettler Toledo). The development of the ropy defect in milk during the experiments was quantified at 6°C using 2 methods; (1) viscosity and (2) flow through serological pipette. Viscosity of each milk sample was determined using viscometer ViscoQC 300-L (Anton Paar) equipped with an L1 spindle. The speed of the spindle was 100 rpm, and the shear rate was kept as 1 s⁻¹. The viscosity measured was reported in mPas. The flow through a serological pipette was determined by measuring time required for 10 mL of each milk sample to flow through a serological pipette (i.e., 10 mL) results were expressed in mL/s (Supplemental Table S2; https: //ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881b8898a; Prinčič et al., 2023).

**DNA Extraction, Whole-Genome Sequencing**

Genomic DNA of one *K. pneumoniae* ssp. *pneumoniae* strain (FSL A6–0140 and FSL W5–0628), the genomic DNA was extracted from individual colonies grown on plate count milk agar (PCMA, BCP plate count skim milk agar with 0.2% mass fraction starch; Standards, 2013). The genomic DNA of all 5 strains was extracted by using QIAamp DNA Mini Kit (Qiagen) according to the manufacturer’s instruction, which was modified to include an additional 45 min lysis step. Briefly, cultures of strains grown overnight in BHI broth and single colonies from PCMA agar resuspended in Buffer ALT (supplied in QIAamp Mini Kit) were pelleted by centrifugation and subsequently lysed with 20 µL of proteinase K (20 mg/mL) by incubation for 45 min at 56°C. DNA was eluted twice in 50 µL volumes of 10 mM Tris-HCl (pH 8.0), and DNA purity was assessed using a Nanodrop spectrophotometer (Thermo Fischer Scientific).

**DNA Extraction, Whole-Genome Sequencing**

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Scientific). The DNA samples were submitted to the Cornell University Institute of Biotechnology Genomics Faculty (Ithaca, NY) for sequencing library preparation using Nextera XT DNA Library Preparation Kit (Illumina) and sequencing. Pooled DNA samples were sequenced using Illumina MiSeq (Illumina) with 2 × 150-bp pair-end reads or NextSeq 550 instrument with 2 × 150-bp pair-end reads.

Read Processing, Quality Control, Genome Assembly, and Annotation

Raw sequencing reads were trimmed based on the quality of the bases sequenced and adapters were trimmed using Trimmomatic version 0.39 (Bolger et al., 2014). Short read quality of the remaining raw sequencing reads was assessed using FastQC version 0.11.8 (Andrews, 2015). Trimmed reads were assembled de novo using SPAdes version 3.15.4 (Bankevich et al., 2012). Contigs with less than 200 bases were removed before creating final assemblies (draft genomes). The quality metrics of the final assembled genomes was assessed using QUAST version 5.1.0rc1 (Gurevich et al., 2013). Final draft assemblies were annotated using Prokka version 1.14.5 (Seemann, 2014). Subsequently, InterProScan v5.44–79.0 (Jones et al., 2014) was used to retrieve additional annotations for the genes annotated by Prokka as encoding hypothetical proteins. The gene annotations were manually inspected for functions that may be associated with production of exopolysaccharides. Functional annotation was carried out using the Kyoto Encyclopedia of Genes and Genomes (KEGG: http://www.genome.jp/kegg/) and the Carbohydrate-Active Enzymes Database (CAZy). Sequence reads and assembled draft genomes were deposited into the National Center for Biotechnology Information (NCBI) Sequence Read Archive and GenBank (https://www.ncbi.nlm.nih.gov/genbank/) databases, respectively, under BioProject PRJNA907796. Accession IDs for individual strains can be found in Supplemental Table S1.

Phylogenetic Analysis

To confirm the species identity of each strain, an Average Nucleotide Identity (ANI) by BLAST (ANIB) distance matrix was constructed using the pyani suite python script average_nucleotide_identity.py (Pritchard et al., 2016), which calculated the ANI according to Richter and Rossello-Mora (2009). For K. pneumoniae ssp. pneumoniae, the ANIb matrix was constructed using the 3 K. pneumoniae ssp. pneumoniae whole-genome assemblies generated in this study along with 15 type strain assemblies downloaded from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) representing the Klebsiella species K. aerogenes, K. africana, K. grimontii, K. huaxiensis, K. indica, K. michiganensis, K. oxytoca, K. pasteurii, K. pneumoniae ssp. ozaenae, K. pneumoniae ssp. pneumoniae, K. pneumoniae ssp. rhinoscleromatis, K. quasipneumoniae, K. quasivariicola, K. spallanzani, and K. variicola. For R. inusitata, the 2 R. inusitata whole-genome assemblies generated in this study and 5 available assemblies downloaded from NCBI GenBank representing R. inusitata were included.

Comparative Genomic Analyses

Roary version 3.12.0 (Page et al., 2015) was used to identify core and accessory orthologous gene clusters among (1) the 3 K. pneumoniae ssp. pneumoniae genomes and (2) the 2 R. inusitata genomes using the default parameters.

The annotation of genes associated with ropy phenotype were manually inspected for functions that may contribute to the biosynthesis of exopolysaccharides. The presence of elements involved in horizontal transfer events such as transposase genes and prophage sequences, that may contribute to different phenotypes among bacteria, was also compared between strains showing ropy and nonropy phenotype.

Statistical Analysis

Statistical analyses of data were performed in RStudio (Yang et al., 2013). Shapiro–Wilk’s test was used to verify normality and Levene’s test was used to verify homogeneity of variances. Spearman’s correlation coefficients were obtained to investigate potential linear correlations among pH and bacterial counts. Confidence intervals for the mean values and mean value differences were used to show significant differences within experimental results and were calculated at a confidence level of 95% using the “groupwiseMean” function of the “rcompanion” package (Mangiafico and Mangiafico, 2017). The figures summarizing the growth dynamics, pH, and viscosity change were generated using packages “ggplot2” (Wickham, 2016) and “ggpubr” (Kassambara, 2020).

RESULTS

Klebsiella pneumoniae ssp. pneumoniae Showed Growth at 21°C and No Growth at 6°C

At a storage temperature of 6°C, bacterial counts of the tested K. pneumoniae ssp. pneumoniae strains in milk did not change substantially over 28 d storage period. The maximum mean bacterial counts for milk
samples inoculated with FSL A6–0140, FSL R5–0773, and FSL W5–0628 at ~2 to 3 log₁₀ cfu/mL and stored at 6°C for 28 d were 3.75, 4.42, and 3.12 log₁₀ cfu/mL, respectively (Figure 1A). The mean bacterial counts were reduced after reaching the maximum growth and on d 28 were determined to be 1.22 log₁₀ cfu/mL and 2.56 log₁₀ cfu/mL for milk samples inoculated with FSL R5–0773 and FSL W5–0628, respectively; the bacterial counts on d 28 for milk inoculated with FSL A6–0140 were below the detection limit (<0.1 cfu/mL). The correlation analysis indicated a weak negative linear association between the increase in bacterial counts and the pH in milk (r = −0.316, Figure 1B).

At 21°C, all 3 strains of *Klebsiella pneumoniae* ssp. *pneumoniae* showed net bacterial growth, defined as difference between mean log₁₀ cfu/mL at the end of storage period (48 h) and mean log₁₀ cfu/mL at start of storage period (0 h), of more than 4.25 log₁₀ units over 48 h (Figure 1A). Accordingly, the pH values of milk decreased from initial 6.67 to 5.66 at the end of the storage period (Figure 1B); the calculated correlation value between concentration and pH was −0.884.

Both *Rahnella inusitata* strains were able to grow in milk at both 6°C and 21°C.

Both *R. inusitata* strains grow in milk stored at 6°C. Milk inoculated with *R. inusitata* FSL A6–0213 and milk inoculated with *R. inusitata* FSL J3–0059 stored at 6°C showed net bacterial growth of 6.76 and 8.60 log₁₀ units, respectively (Figure 2A); net bacterial growth here represents the difference in mean log₁₀ cfu/mL at d 28 and mean log₁₀ cfu/mL at d 0. The correlation analysis indicated a negative correlation between the increase in bacterial counts and the pH (r = −0.836; Figure 2B).

At 21°C, *R. inusitata* FSL J3–0059 in milk showed net bacterial growth of 5.44 log₁₀ units over 48 h storage period (Figure 2A). Similarly, *R. inusitata* FSL A6–0213 in milk showed net growth of 4.98 log₁₀ units.

![Figure 1](image_url)

**Figure 1.** Growth of *Klebsiella pneumoniae* ssp. *pneumoniae* strains FSL A6–0140 (KP+), FSL R5–0773 (KP−), and FSL W5–0628 (KP+) in milk at 6°C and 21°C. (A) Concentration (log₁₀ cfu/mL), (B) pH of UHT milk, and (C) viscosity of milk. Each data point represents an average from 3 biological replicates with 2 technical replicates, with error bars representing 95% CI. KP+ represents positive ropy phenotype. KP− represents negative ropy phenotype.

over 48 h. The pH values of inoculated milk samples remained relatively stable throughout the first 12 h of storage at 21°C ranging from 6.55 to 6.71; after 12 h pH decreased gradually until the end of the experiment (48 h) reaching pH values between 5.64 and 5.81 (Figure 2B). The correlation analysis indicated a negative correlation between the increase in bacterial counts and the pH ($r = -0.846$).

**Tested Strains Revealed Large Range in Ability to Cause Viscosity Changes During Growth in Milk**

Milk samples inoculated with *K. pneumoniae* ssp. *pneumoniae* strains (i.e., FSL A6–0140, FSL R5–0773, and FSL W5–0628) did not show relevant change of viscosity during storage at 6°C (Figure 1C). At 21°C, the viscosity of milk inoculated with FSL R5–0773 remained stable with values ranging from 3.94 to 5.57 mPas over the 48-h storage period. For milk samples inoculated with FSL A6–0140 or FSL W5–0628, the viscosity did not change substantially over the first 12 h (range: 3.69–4.54 mPas). We observed an increase of viscosity after 12 h with mean viscosity values for milk samples inoculated with FSL A6–0140 or FSL W5–0628 reaching 13.37 mPas (range: 9.17–17.57 mPas) and 16.62 mPas (range: 16.19–17.05 mPas), respectively after 48 h of incubation at 21°C. A significant difference in the viscosity between milk samples inoculated with FSL R5–0773 (5.57 mPas, 95% CI: 4.48–6.66 mPas) and milk samples inoculated with FSL A6–0140 (8.44 mPas; 95% CI, 6.81–10.07 mPas) or FSL W5–0628 (11.85 mPas; 95% CI, 9.31–14.39 mPas) was seen at 24 h at 21°C. At 48 h of incubation the viscosity of milk samples inoculated with FSL W5–0628 (16.62 mPas, 95% CI: 11.16–22.08 mPas) remained significantly different from milk samples inoculated with FSL R5–0773 (4.89 mPas; 95% CI, 2.71–7.06 mPas). *R. inusitata* FSL A6–0213 significantly increased the viscosity of milk from initial mean viscosity of 5.78 mPas (95% CI: 5.61–5.96 mPas, $d = 0$) to 24.6 mPas (95% CI: 18.92–30.32 mPas) on d 10 of storage at 6°C, followed by additional significant increase after d 14.

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**Figure 2.** Growth of *Rahnella inusitata* strains FSL A6–0213 (RP+) and FSL J3–0059 (RP−) in milk at 6°C and 21°C. (A) Concentration (log₁₀ cfu/mL), (B) pH of UHT milk, and (C) viscosity of milk. Each data point represents an average from 3 biological replicates with 2 technical replicates, with error bars representing 95% CI. RP+ represents positive ropy phenotype. RP− represents negative ropy phenotype.
reaching a level of 77.36 mPas (95% CI: 6.38–88.16 mPas) at the end of storage period (d = 28; Figure 2C). Consistent with that, the milk displayed low flow through serological pipette on d 10, 14, 21 and 28 post-inoculations (Supplemental Table S2). For milk inoculated with the same strain and stored at 21°C, the viscosity remained relatively stable over 24 h (range: 3.09–5.57 mPas), and minimally increased from 5.39 mPas (range: 3.22–7.58 mPas) at 24 h to 11.40 mPas (range: 5.99–16.80 mPas) after 48 h of storage. Conversely, the viscosity of milk inoculated with \( R. \) inusitata FSL J3–0059 remained stable (range: 4.5–8.3 mPas) throughout all sampling points of all experiments at the 2 incubation temperatures (i.e., 6°C and 21°C). The mean viscosity of milk samples inoculated with this strain was significantly lower on d 10 of storage at 6°C compared with milk samples inoculated with FSL A6–0213 (mean difference, 19.02 mPas, 95% CI: 18.92–30.32 mPas).

On the basis of viscosity assessment results, 3 strains, namely \( K. \) pneumoniae ssp. pneumoniae FSL A6–0140, \( K. \) pneumoniae ssp. pneumoniae FSL W5–0628, and \( R. \) inusitata FSL A6–0213, presented ropy positive phenotype (hereafter referred to as “P+”) and the remaining strains, including \( K. \) pneumoniae ssp. pneumoniae FSL R5–0773 and \( R. \) inusitata FSL J3–0059, displayed a ropy negative phenotype (hereafter referred to as “P−”). To improve readability, KP+/KP− indicates \( K. \) pneumoniae subsp. pneumoniae strains, and RP+/RP− represents \( R. \) inusitata strains.

**Taxonomic Identification**

Before this study, FSL A6–0140 (KP+), FSL R5–0773 (KP−), and FSL W5–0628 (KP+) had been identified as \( Klebsiella \) species based on 16S rRNA gene sequences (Trmčić et al., 2015, Hervert et al., 2016, Hervert et al., 2017). To confirm the taxonomic position of these 3 strains we compared their genomes to 15 publicly available type strains of \( Klebsiella \) species using the ANIb approach. FSL A6–0140 (KP+), FSL R5–0773 (KP−), and FSL W5–0628 (KP+) showed ANI values between 99.1% and 99.2% with \( K. \) pneumoniae ssp. pneumoniae ATCC 13883\( ^{T} \) strain, indicating that FSL A6–0140 (KP+), FSL R5–0773 (KP−), and FSL W5–0628 (KP+) belong to \( K. \) pneumoniae ssp. pneumoniae (Supplemental Table S3; https://ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881bf898a; Princic et al., 2023).

The ANIb analysis of the 2 \( R. \) inusitata strains confirmed their previous taxonomic classification (Trmčić et al., 2015, Masiello et al., 2016) as the ANI between the 2 genomes and publicly available genome assemblies of \( R. \) inusitata was 98.3% to 99.1% (Supplemental Table S4; https://ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881bf898a; Princic et al., 2023).

Comparative genomics revealed substantial differences in the number of genes shared between \( K. \) pneumoniae ssp. pneumoniae strains with ropy and nonropy phenotypes. The genome size of the 3 \( K. \) pneumoniae ssp. pneumoniae assembled genomes varied between 5.63 and 5.84 Mb, with an average GC-content of 56.8% for all strains (Supplemental Table S1), which are consistent with previously sequenced \( K. \) pneumoniae genomes (Holt et al., 2015).

The total pangenome size of the 3 \( K. \) pneumoniae ssp. pneumoniae strains amounted to 6,769 protein-coding genes, which included 4,410 core genes and 2,358 accessory genes (i.e., genes present in all 3 strains and genes absent in at least one of the strains) respectively (Figure 3; Supplemental Table S5A; https://ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881bf898a; Princic et al., 2023). Further analysis of the differences between pairs of 2 strains showed that FSL A6–0140 (KP+) and FSL W5–0628 (KP+) shared more genes (n = 627), than FSL A6–0140 (KP+) and FSL R5–0773 (KP−), or FSL W5–0628 (KP+) and FSL R5–0773 (KP−; n = 65 and 78, respectively).

**Figure 3.** Venn diagram of core, accessory, and unique gene families identified in 3 \( Klebsiella \) pneumoniae subsp. pneumoniae strains. The number of core genes shared by all strains is in the center (4,410). The number of accessory gene families is represented in the overlapping portions of each oval. The number in nonoverlapping portions of each oval represents the number of unique genes. KP+ represents positive ropy phenotype. KP− represents negative ropy phenotype.
Interestingly, FSL A6–0140 (KP+) has multiple genes encoding heat-shock and cold-shock proteins, and several urease system-related genes, which were not found in FSL W5–0628 (KP+) and FSL R5–0773 (KP−; Supplemental Table S5C). These included chaperone protein DnaJ, cold shock-like protein CspH, DNA adenine methylase, DNA primase TraC, urease. These FSL A6–0140 (KP+) genes may play a role in adapting the cells to stressful conditions such as heat, cold, and acid stress. Notably, FSL A6–0140 was obtained from a yogurt sample, which probably presented the strain to heat, cold and acid stress, whereas FSL R5–0773 was obtained from raw fluid milk.

Nonropy strain R. inusitata FSL J3–0059 has a higher number of strain-specific genes than the ropy strain R. inusitata FSL A6–0213.

The genomes of FSL A6–0213 (RP+) and FSL J3–0059 (RP−) possess an average GC-content of 52.9% and a total genome length of 5.12 Mb and 5.29 Mb, respectively (Supplemental Table S1), which are consistent with previously sequenced Rahnella genomes (Xu et al., 2022).

Genome annotation of FSL A6–0213 (RP+) identified 4,769 coding sequences, a total of 74 tRNA-coding genes, and 3 rRNA-coding genes. A putative function was assigned to 3,337 genes, while 1,354 genes had no assigned function, and are described as hypothetical proteins.

For FSL J3–0059 (RP−), 4,741 coding sequences, 71 tRNA-coding genes, and 3 rRNA-coding genes were identified. A total of 3,374 and 1,442 genes were identified as functional and hypothetical proteins, respectively. The characteristics of the 2 genomes are shown in Supplemental Table S1.

The 2 R. inusitata genomes shared 4,141 protein-coding sequences (Supplemental Figure S1; https://ecommons.cornell.edu/items/099542b5-55ff-44f6-88e0-630881b898a; Prinčič et al., 2023), while 549 and 677 protein-coding genes were unique to FSL A6–0213 (RP+) and FSL J3–0059 (RP−), respectively; most of these unique proteins cannot be classified according to KEGG or show no significant BLAST hits to proteins currently in GenBank (Supplemental Table S5B).

**Genes with Sequence Homology to Genes Involved in Metabolism of Polysaccharides Can Be Found in All Tested Strains Regardless of the Ropy Phenotype**

The K. pneumoniae ssp. pneumoniae and R. inusitata genomes contained several genes with sequence homology to genes involved in sugar uptake, synthesis, regulation, polymerization, and export of polysaccharides (Supplemental Table S5A and S5B). The phosphoenolpyruvate phosphotransferase system (PEP-PTS) is the most common sugar transport system in bacteria and consists of histidine-containing phosphoprotein, phosphoeneolpyruvate-dependent phosphotransferase, and sugar-specific permease enzymes (Laws et al., 2001). The genomes of K. pneumoniae ssp. pneumoniae and R. inusitata harbored ptsl encoding phosphoeneolpyruvate-protein phosphotransferase, and sizA, encoding phospholhisitidine phosphatase. In addition, K. pneumoniae ssp. pneumoniae harbored genes responsible for glucose, N-acetyl-d-glucosamine, maltose/glucose, β- and α-glucoside, trehalose, sucrose, fructose, N-acetylmuramic mannnitol, cellbiose, mannnose, fructoselysine/glucoselysine, sorbose, sorbitol, and L-ascorbate-specific PTS. K. pneumoniae ssp. pneumoniae R5–0773 (KP−) did not possess genes responsible for sorbose-specific PTS system, while they were present in FSL A6–0140 (KP+) and FSL W5–0628 (KP+). In case of R. inusitata, genes encoding for glucose, N-acetyl-d-glucosamine, maltose/glucose, sucrose, β- and α-glucoside, trehalose, mannnitol, cellbiose, mannnose, fructose, galactitol, and L-ascorbate-specific PTS were identified in both FSL A6–0213 (RP+) and FSL J3–0059 (RP−). This finding suggests that these sugars may be transported by the PEP-PTS in these strains.

The ATP-binding cassette (ABC) transporter pathway is another sugar transport system mainly known for the production of CPS (Whitney and Howell, 2013). Therefore, we sought to identify genes coding for enzymes and regulatory proteins involved in ABC-transporter dependent pathway within K. pneumoniae ssp. pneumoniae and R. inusitata to characterize potential genes associated with the ropy phenotype. All 3 K. pneumoniae ssp. pneumoniae strains encoded enzymes involved in transport of oligosaccharides (i.e., maltose/maltodextrin, galactose oligomers) and monosaccharides (i.e., ribose, L-arabinose, galactofuranose, methylgalactose, D-xyllose, rhamnose, erythritol, and glycerol 3-phosphate). The genomes of R. inusitata also harbor genes encoding these enzymes, except the genes for enzyme responsible for ABC-transport of erythritol.

Our analyses also indicated that K. pneumoniae ssp. pneumoniae and R. inusitata genomes studied were equipped with several genes involved in non-PEP-PTS systems such as primary and secondary transport systems (Cui et al., 2016). Primary transport systems couples sugar to ATP hydrolysis (Fath and Kolter, 1993), whereas in secondary transport systems the import of sugar is coupled to transport of ions or other solutes (Poolman, 1993). Genes involved in secondary transport system included lacY gene encoding for lactose permease (a secondary transport system responsible for the import and export of lactose and galactose), and lacZ, the gene that encodes β-galactosidase for hydrolyzing lactose.
lactose into glucose and galactose. The presence of these gene indicates that *K. pneumoniae* ssp. *pneumoniae* and *R. inusitata* can transport lactose and galactose from the environment and metabolize it. In addition, the genomes of studied strains also harbored genes that encode several enzymes involved in the Leloir pathway, in which galactose is converted to uridine diphosphate (UDP)-glucose and glucose-1-phosphate. These genes include enzyme glucokinase (*gkl*) which phosphorylates glucose to glucose-6-phosphate, and phosphoglucosemutase (*pgm*) that catalyzes the production of glucose-1-phosphate. In addition, UDP-glucose-1-phosphate uridylyltransferase gene (*galU*) which catalyzes the conversion of glucose-1-phosphate to UDP-glucose, was present in all studied genomes.

**Identification of Gene Clusters Potentially Involved in CPS Biosynthesis in *K. pneumoniae* ssp. *pneumoniae***

Three gene clusters (cluster K-1, K-2, and K-3) have been identified in the genomes of the 3 *K. pneumoniae* ssp. *pneumoniae* strains and they have been shown to contain genes involved in CPS biosynthesis (Figure 4A, Supplemental Table S5A).

Although all 3 *K. pneumoniae* ssp. *pneumoniae* strains contained cluster K-1, there are some differences in gene composition between these strains. For instance, in FSL A6–0140 (KP+) and FSL W5–0628 (KP+), cluster K-1 extended from *gnd* through *rffG*, *rffH*, *rfbD*, *rfbC*, *ugd* to *wbgU*. In FSL R5–0773 (KP−), the *gnd-wbgU* region is composed of *manC1* (protein involved in GDP-D-mannose synthesis), *algC* (encoding phosphomannomutase) and a gene encoding hypothetical protein (group_1910). This observation suggests that the presence of *rffG*, *rffH*, *rfbD*, and *rfbC* genes in cluster K-1 may be linked to ropy phenotype for *K. pneumoniae* ssp. *pneumoniae*. The proteins encoded by *rffH*, *rffG*, *rfbC*, and *rfbD* are known to be involved in catalysis of the steps in the biosynthesis of enterobacterial common antigen (ECA). This gene cluster may not represent the complete pathway for ECA biosynthesis; several other CPS biosynthesis genes were found elsewhere in the genome, including (1) *rcsA*, encoding an activator of capsule synthesis genes, (2) genes involved in lipopolysaccharide transport system (*rfaC*, *lptA*, and *lptC*), and (3) genes encoding capsule regulators (*argR*, *mpR*, *ompR*, *slyA*, and *wzyY*). Additionally, regulator of the capsule synthesis genes A and B (*rcsA* and *rcsB*) were also found in the genomes of all 3 *K. pneumoniae* ssp. *pneumoniae* strains. In addition, in the FSL R5–0773 (KP−) genome, genes unique to this strain are found upstream *gnd*, including (1) *wcaJ*, which is regarded as an initial glycosyltransferase for capsule synthesis, (2) 2 genes encoding hypothetical proteins, and (3) 3 genes involved in mannose synthesis (i.e., *gmm*, *fcl*, and *gmd*).

Cluster K-2 was found in all 3 *K. pneumoniae* ssp. *pneumoniae* strains and included *udk*, *dcd*, *galF*, 5 genes encoding hypothetical proteins, and a putative enzyme tyrosine-protein kinase gene. *udk* encodes a uridine/cytidine kinase, *dcd* encodes dCTP deaminase, and *galF* encodes for protein homologous to GalU. By NCBI BLASTP searches, 2 hypothetical proteins within cluster K-2 of FSL A6–0140 (KP+) and FSL W5–0628 (KP+) showed high identity match (>92.34) with CPS synthesis gene cluster of *Klebsiella* sp. 2005/49 (GenBank: AB924557.1). The same 2 hypothetical proteins within cluster K-2 of FSL R5–0773 (KP−) showed 100% and >99.16% identity to those of *Klebsiella* sp. 2005/49 and *Escherichia coli* SCU-308 (GenBank: CP053281.1), respectively.

Cluster K-3, found in genomes of all 3 *K. pneumoniae* ssp. *pneumonia* strains, showed conserved genetic organization extending from a gene encoding for a transporter protein (*yfK*) to a gene encoding the transcriptional termination factor (*rho*), and included *wecE*, *wzyE*, *wecF*, *wzzE*, *wecE*, *wecD*, a second copy of *rffH*, a second copy of *rffG*, *wecC*, *wecB*, *wzzE*, and *wecA*.

Moreover, we found that the genomes of the 3 *K. pneumoniae* ssp. *pneumonia* strains contained a small gene cluster consisting of 4 genes, *pgaA*, *pgaB*, *pgaC*, and *pgaD*. These genes are involved in synthesis and secretion of poly-β-linked N-acetylglucosamine (PNAG), an extracellular polysaccharide that has been reported to be associated with microbial biofilms in a limited number of bacterial species including *K. pneumoniae*, *Yersinia pestis*, *Staphylococcus epidermidis* and *E. coli* (Mack et al., 1996, Jarrett et al., 2004, Wang et al., 2004, Cywes-Bentley et al., 2013).

**Identification of Gene Clusters Potentially Involved in Extracellular Polysaccharide Biosynthesis in *R. inusitata***

The *R. inusitata* genomes included 2 gene clusters likely involved in EPS biosynthesis (cluster R-1 and R-2) and one cluster (cluster R-3) potentially responsible for CPS biosynthesis (Figure 4B, Supplemental Table S5B). Although both *R. inusitata* strains presented each the 2 putative EPS clusters, there was a variability in gene content between the 2 strains.

Cluster R-1 in both *R. inusitata* genomes comprised (1) *mshA* for glycosyltransferase, which is involved in steps of the polysaccharide biosynthesis route (i.e., linking the first sugar of the repeating unit to the lipid carrier), (2) 4 genes (i.e., *gmd*, *manC1*, *algC*, and *wcaJ*) involved in the synthesis of sugar nucleotides, and (3)
Figure 4. Comparison of gene clusters potentially involved in exopolysaccharide and capsule biosynthesis in *Klebsiella pneumoniae* ssp. *pneumoniae* strains FSL A6-0140 (KP+), FSL R5-0773 (KP−), and FSL W5-0628 (KP+), and *Rahnella inusitata* strains FSL A6-0213 (RP+) and FSL J3-0059 (RP−). (A) Schematic representation of the K-1, K-2, and K-3 gene clusters of *K. pneumoniae* ssp. *pneumoniae* strains, while RP+/RP− refers to *R. inusitata* strains.
one gene encoding for putative tyrosine-protein kinase, which regulates the exopolysaccharide biosynthetic enzymes through phosphorylation. Additionally, cluster R-1 in strain FSL A6–0213 (RP+) harbored (1) a second copy of mshA, (2) a flippase gene (murJ), associated with the export of repeating sugar nucleotide units, (3) wzb for putative tyrosine-protein kinase, (4) a gene encoding for acetyltransferase (dapH), and (5) 4 genes encoding hypothetical proteins. In FSL J3–0059 (RP−), cluster R-1 also included (1) a gene encoding for glycosyltransferase (pglJ), and (2) 8 genes encoding hypothetical proteins with unknown function.

Cluster R-2, found in genomes of both R. inusitata strains, included (1) wzzB for chain length determinant protein, (2) a glucose-6-phosphate dehydrogenase encoding gene (gnd), (3) a transferase-encoding gene (rmlA), and (4) 3 genes (i.e., rfbB, rfbC, and rfbD) encoding enzymes involved in dTDP-L-rhamnose biosynthesis pathway. Cluster R-2 found in FSL A6–0213 (RP+) also includes (1) 2 genes coding for different glycosyltransferases (epsD and gylG), involved in the linking of the sugars of the repeating units to the lipid core, and (2) 5 genes (i.e., group_569, group_572, group_573, group_574, and group_575) identified as encoding hypothetical proteins. Cluster R-2 found in FSL J3–0059 (RP−) also harbored 5 genes (i.e., group_1188, group_1189, group_1190, group_1191, and group_1192) encoding hypothetical proteins located between gnd and rfbC genes.

Finally, cluster R-3 within genomes of both R. inusitata strains contained yifK, 12 genes responsible for polysaccharide synthesis (weeG, weeF, weeE, weeD, weeC, and weeB), polymerization (wzyE), genes related to export of sugars (wzx and wzzE), and rho.

**DISCUSSION**

The phenotypic characteristics of the species and strains involved in ropy defect of milk and their genetic makeup that may be associated with incidences of this defect have not be thoroughly examined so far. Therefore, in this study, we explored the ability of 3 K. pneumoniae ssp. pneumoniae and 2 R. inusitata to grow and develop the ropy defect in milk at 2 different temperatures and the interplay between ropy spoilage phenotype and the strains’ accessory genomes. We found that the tested K. pneumoniae ssp. pneumoniae and R. inusitata strains have variable ability to cause ropy defect in milk. Although we found putative EPS and CPS biosynthesis gene clusters in the genomes of K. pneumoniae ssp. pneumoniae strains, we found no clear association between any of the genes and the ability to cause a ropy defect. In addition, the genomic comparison of R. inusitata allowed us to identify one gene cluster that may potentially be involved in the observed ropy phenotype of strain R. inusitata FSL A6–0213 (RP+). Importantly, our data indicate a complex interplay between genetics and ropy phenotype, suggesting a need for further research of genes and genetic mechanisms responsible for ropy phenotype.

The spoilage potential of bacteria is generally determined by their ability to grow and proliferate during storage. Therefore, we analyzed the growth ability and development of ropy defect in milk inoculated with K. pneumoniae ssp. pneumoniae and R. inusitata strains at 2 different temperatures.

In the present study, the 3 K. pneumoniae ssp. pneumoniae strains showed growth capabilities in milk and only 2 out of 3 strains caused ropiness at 21°C, whereas at 6°C, none of the strains had the ability to grow and cause ropy defect in milk. Several studies of the minimum growth temperatures of Klebsiella spp. reported that certain strains are able to grow in milk and on culture media at temperature <7°C (Michener and Elliott, 1964, Wessels et al., 1989). Another study reported spoilage activity for K. oxytoca and K. pneumoniae isolates in steamed whole milk at 10°C within 24 h and 7 d, respectively (Cheung and Westhoff, 1983). Because several K. pneumoniae ssp. pneumoniae have been isolated from fluid milk (Cheung and Westhoff, 1983, Trmčić et al., 2015), it should be noted that even K. pneumoniae ssp. pneumoniae that cannot grow or show reduced growth in milk at refrigeration temperatures (e.g., 6°C) may negatively affect shelf life, for example, if products are subjected to temperature abuse (e.g., poor temperature control during transport and storage).

The finding of R. inusitata strains being able to grow in milk at both 6°C and 21°C is consistent with a previous study that reported R. inusitata growth to levels of >4 log_{10} cfu/mL in milk at both 6°C and 21°C within 21 d and 48 h, respectively (Trmčić et al., 2015). This bacterial species has previously been described to grow at refrigeration conditions and consequently cause spoilage of refrigerated foods (Jensen et al., 2001). Our findings are also consistent with other studies where Rahnella isolates were isolated from pasteurized fluid milk and shown to be able to grow at refrigeration temperatures (i.e., 6 and 7°C; Masiello et al., 2016; Wessels et al., 1989). However, the ability to cause the ropy defect in milk at either 6 or 21°C was only observed for R. inusitata FSL A6–0213.

Moreover, we noticed differences in the degree to which ropy strains altered the viscosity of milk under different conditions (i.e., temperature and time). More specifically, we found that in milk samples inoculated with R. inusitata FSL A6–0213 (RP+) the mean viscosity was 11.40 mPas after 48 h at 21°C, whereas in
milk samples inoculated with *K. pneumoniae* ssp. *pneumoniae* FSL A6–0140 (RP+) and *K. pneumoniae* ssp. *pneumoniae* FSL W5–0628 (RP+) the mean viscosity values were 13.37 and 16.62 mPas after 48 h, respectively. Even higher was the viscosity of milk samples inoculated with *R. inusitata* FSL A6–0213 (RP+) and incubated at 6°C, where viscosity on d 28 was 77.36 mPas. Previous studies also indicated varied capability of bacteria isolated from raw milk to causeropy defect in milk (Wegener and Gainor, 1954, Carini et al., 1978, Gennari et al., 1992). For example, one study showed that among 160 *Acinetobacter* sp. isolates from dairy products tested for spoilage capability, only 3.7% of isolates showed ability to cause ropiness in raw milk (Gennari et al., 1992). Additionally, variedropy spoilage activity has been reported for type strain *Acinetobacter johnsonii* ATCC 9036 (Baumann et al., 1968, Morton and Barrett, 1982, Yang et al., 2013), which may be due to different temperatures used in those studies or loss of the trait during subculturing. Possible reasons for the observed heterogeneity may be distinct quantity, type and properties of EPS produced (Sebastiani and Zelger, 1998, Ruas-Madiedo et al., 2002). For example, EPS with identical chemical structure was shown to change viscosity of fermented milk to different degrees as result of differences in EPS molecular mass (Faber et al., 1998). Overall, we conclude that the ability of *K. pneumoniae* ssp. *pneumoniae* and *R. inusitata* to cause ropy defect in milk is a strain-specific trait.

In silico analysis reveals the presence of putative EPS and CPS biosynthesis clusters within *K. pneumoniae* ssp. *pneumoniae* strains, but reveals no clear association with the ability to cause ropy defect in milk.

The ability of ropy strains to cause ropy defect in milk is said to be a result of the production of EPS and their release into the growth medium during bacterial growth (Cheung and Westhoff, 1983, Hassan, 2008, Mende et al., 2016). Genomic analysis of the 3 *K. pneumoniae* ssp. *pneumoniae* genomes revealed the presence of gene clusters putatively involved in capsule formation in all 3 strains. Capsule is a polysaccharide matrix found on the cell surface and is generally composed of acidic polysaccharides, including uronic acid repeats, mannose, rhamnose, galactose, pyruvate, and fructose residues (Sahly et al., 2008). Indeed, the 3 *K. pneumoniae* ssp. *pneumoniae* strains possess genes involved in the biosynthesis, transport and assembly of capsule. This observation is in agreement with reports on *K. pneumoniae* and *E. coli*, where CPS clusters are also consistently conserved (Arakawa et al., 1991, Arakawa et al., 1995, Shu et al., 2009, Pan et al., 2013).

Beside CPS genes, many other genes involved in cell-surface proteins including poly-1,6-N-acetyl-D-glucosamine, colonic acid and ECA genes could potentially play a role in the ropy phenotype. In fact, clusters K-1 and K-3 contain rffG, rffH, rfbD, and r fbC genes involved in biosynthesis and secretion of ECA (Marolda and Valvano, 1995). ECA is a nonimmunogenic surface carbohydrate antigen built of repeating units of the 3 amino sugars found in various forms in *Enterobacteriaceae*, and plays role in motility, protection from a hostile environment, interaction with the environment and increasing the ability of the outer membrane to provide structural support to the cell (Whitfield and Roberts, 1999, Lerouge and Vanderleyden, 2002, Robins-Browne and Hartland, 2002, Rojjas et al., 2018). Moreover, we observed the presence of rffG, rffH, rfbD, and rfbC genes in K-1 cluster in 2 ropy *K. pneumoniae* ssp. *pneumoniae* strains (i.e., FSL A6–0140 and FSL W5–0628) but not in the nonropy strain FLS R5–0773 (KP−). This observation suggests, that the gene products of this cluster may be responsible for ropy defect caused by the 2 strains. It would be therefore interesting to further investigate these genes by gene expression analysis and mutant characterization to further assess the genetic mechanisms involved in ropy defect in *Klebsiella*. Previous studies have reported that genes found in ECA clusters of *K. pneumoniae* can mediate the increasing production of capsular polysaccharides, hence enhancing the viscosity of *K. pneumoniae* (Yu et al., 2006, Cheng et al., 2010, Hsu et al., 2011, Russo et al., 2018). Further studies using more isolates associated with ropy defect from *Rahnella* and *Klebsiella* genera are needed to clarify the precise role of the predicted cps gene cluster in ropy defect phenotype.

Although ropiness by the test strains could have been a consequence of higher number of genes involved in EPS biosynthesis, the revelation that some strains could also cause ropiness but at a lower degree, did not fully support this hypothesis.

The putative EPS and CPS gene clusters identified in *R. inusitata* FSL A6–0213 may be responsible for the ability to cause ropy defect in milk.

Comparative analysis of the 2 *R. inusitata* strains revealed differences in genetic content of 2 strains with different phenotypes. For instance, the cluster R-1 in the *R. inusitata* FSL A6–0213 (RP+) genome contained the set of genes that share homology with other proteins described as being implicated in the synthesis of EPS by limited number of bacterial species (Hidalgo-Cantabrana et al., 2014, Deo et al., 2019, Li et al., 2022). Specifically, 2 glycosyltransferase-encoding genes and a gene encoding a flippase involved in nucleotide sugar biosynthesis and export were observed within the cluster of this strain, whereas the strain FSL J3–0059 (RP−) only encoded one glycosyltransferase gene. Thus, these genes may be important targets for future studies to confirm their role in ropy defect ability of...


CONCLUSIONS

In this study, phenotypic characterization of K. pneumoniae ssp. pneumoniae and R. inusitata strains revealed that the tested strains have variable ability to cause ropy defect in milk. The one ropy strain of R. inusitata was able to cause the ropy defect in milk both at 6°C and 21°C, while the 2 ropy strains of K. pneumoniae ssp. pneumoniae were capable of causing the ropy defect in milk only at 21°C. The in silico genomic analysis allowed us to conclude that there is no consensus structural organization of genes corresponding to the detected ropy phenotype. However, the genomic comparison of R. inusitata allowed us to identify the one gene cluster that may be potentially involved in the observed ropy phenotype of strain FSL A6–0213. To our knowledge, this is the first study, using strains capable of causing ropy defect, to systematically analyze the genes associated with extracellular polysaccharides biosynthesis, and to correlate genotypes with observed phenotype. However, future studies using more strains associated with ropy defect from Rahnella, Klebsiella and other genera are needed for further exploration of the gene modules responsible for the ropy defect of milk, what exopolysaccharides are expressed in situ, and how expression is regulated in response to process and environmental factors.

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