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# *Kaistella rhinocerotis* sp. nov., isolated from the faeces of rhinoceros and reclassification of *Chryseobacterium faecale* as *Kaistella faecalis* comb. nov.

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**Abstract**

Strain Ran72$^T$, a novel Gram-stain-negative, obligately aerobic, non-motile, and rod-shaped bacterium, was isolated from the faeces of the rhinoceros species *Ceratotherium simum*. The novel bacterial strain grew optimally in Reasoner's 2A medium under the following conditions: 0% (w/v) NaCl, pH 7.5, and 30°C. Based on phylogenetic analysis using 16S rRNA gene sequencing, strain Ran72$^T$ was found to be most closely related to *Chryseobacterium faecale* F4$^T$ (98.4%), *Kaistella soli* DKR-2$^T$ (98.0%), and *Kaistella haifensis* H38$^T$ (97.4%). A comprehensive genome-level comparison between strain Ran72$^T$ with *C. faecale* F4$^T$, *K. soli* DKR-2$^T$, and *K. haifensis* H38$^T$ revealed average nucleotide identity, digital DNA–DNA hybridization, and average amino acid identity values of ≤74.9, ≤19.3, and ≤78.7%, respectively. The major fatty acids were anteiso-$C_{15:0}$ (22.3%), with MK-6 being the predominant respiratory quinone. The major polar lipids of strain Ran72$^T$ were phosphatidylethanolamine, four unidentified aminolipids, and two unidentified lipids. Based on our chemotaxonomic, genotypic, and phenotype characterizations, strain Ran72$^T$ was identified as representing a novel species in the genus *Kaistella*, for which the name *Kaistella rhinocerotis* sp. nov. is proposed, with the type strain Ran72$^T$ (=KACC 23136$^T$=JCM 36038$^T$). Based on the outcomes of our phylogenomic study, *Chryseobacterium faecale* should be reclassified under the genus *Kaistella* as *Kaistella faecalis* comb. nov.

## INTRODUCTION

The genus *Kaistella* was first described by Kim *et al*. as a novel member of the *Chryseobacterium–Bergeyella–Riemerella* branch, with the type species being *Kaistella koreensis* [1]. Nicholson *et al*. subsequently distinguished the genus *Kaistella* from the genus *Chryseobacterium*, thus transferring 11 species from genus *Chryseobacterium* (*C. anthropi*, *C. antarcticum*, *C. carnis*, *C. chaponense*, *C. haifense*, *C. jeonii*, *C. montanum*, *C. palustre*, *C. solincola*, *C. treverense*, and *C. yonginense*) to the genus *Kaistella* [2]. The genus *Chryseobacterium* has been identified not only in the natural environments (e.g., Arctic tundra soil, eutrophic lake, and industrially contaminated sediments), but also in samples from a variety of animal hosts and clinical sources (e.g., fish, frogs, human blood, lung biopsy, and urethra) [3–8]. This highlights the significance of studying *Kaistella* species found in rhinoceroses. *Kaistella* species, categorized within the phylum Bacteroidota, constitute a fundamental microbial community within the gastrointestinal tracts of large herbivores, such as rhinoceros [9]. *Chryseobacterium faecale* F4$^T$, the type strain most closely related to Ran72$^T$ based on 16S rRNA sequences, has also been isolated from herbivores such as camels [10]. Therefore, further characterization of *Kaistella* strains isolated from rhinoceroses could provide valuable insights into the broader microbiota of herbivores. Moreover, members of the genus *Kaistella* have primarily been isolated from various environments, including freshwater, Antarctic soil, and oil-contaminated experimental soil [1, 11–13]. *Kaistella*

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**Abbreviations:** AAI, average amino acid identity; AL, unidentified aminolipid; ANI, average nucleotide identity; ARG, antibiotic resistance gene; CDS, coding sequence; dDDH, digital DNA–DNA hybridization; DW, deionized water; LB, Luria–Bertani; ME, minimum-evolution; ML, maximum-likelihood; NB, nutrient broth; NJ, neighbor-joining; PE, phosphatidylethanolamine; R2A, Reasoner's 2A; REALPHY, Reference Sequence Alignment Based Phylogeny Builder; TSB, tryptic soy broth.
The genomic and 16S rRNA sequences of the *Kaistella* strain Ran72$^T$ have been deposited in the GenBank database under the accession numbers JASSYY000000000 and OQ408149, respectively.
Three supplementary figures are available with the online version of this article.

species derived from aquatic habitats (e.g., *Kaistella jeonii*) are known to possess the potential to degrade polyethylene terephthalate by colonizing its surfaces and harbouring esterases (e.g., serine hydrolases) [14]. According to the List of Prokaryotic names with Standing in Nomenclature taxonomy browser database, the genus *Kaistella* comprised 17 recognized species with validly published names at the time of writing (https://lpsn.dsmz.de/genus/kaistella). The *Kaistella* genomes deposited in the NCBI databases exhibit various genome sizes from 2.4 Mb (e.g., *K. montana* WG4$^T$) to 3.7 Mb (e.g., *K. flava* 7-3A$^T$) among the type strains. In this study, we obtained a *Kaistella* species (strain Ran72$^T$) from the faeces of *Ceratotherium simum* at Seoul Grand Park, the local zoo, and reclassify *Chryseobacterium faecale* as *Kaistella faecale*.

## ISOLATION

Strain Ran72$^T$ was isolated from the faeces of *Ceratotherium simum* housed at Seoul Grand Park (37° 25′ 39.1″ N 127° 1′ 1.3″ E). The strain was isolated using the same methods as in previous studies [15, 16]. In brief, the faeces of rhinoceros were collected to isolate the faecal micro-organisms within 12 h after excretion during the summer month of August 2020. The faecal sample underwent immediate bacterial screening through serial dilution in PBS, followed by plating on Reasoner's 2A (R2A) agar plates (0.5 g l$^{-1}$ yeast extract, proteose peptone no. 3, casamino acid, D-glucose, and soluble starch; 0.3 g l$^{-1}$ sodium pyruvate, $K_2HPO_4$; 0.05 g l$^{-1}$ $MgSO_4$; 15 g l$^{-1}$ agar powder). Colonies with different pigments were randomly selected, and single colonies were obtained through repeated streaking. The samples were incubated at 30°C for 3 days. The 16S rRNA sequences of the isolated colonies were analysed by PCR amplification for strain identification, confirming the possibility that yellow colonies, specifically strain Ran72$^T$, represent a novel species. For bacterial long-term preservation, strain Ran72$^T$ was cultured in R2A liquid medium and stored at −80°C after being mixed with 100% (v/v) glycerol at a 1:1 (v/v) ratio. For comparative taxonomic analysis, *C. faecale* F4$^T$, *K. soli* DKR-2$^T$, and *K. haifensis* H38$^T$ were selected as the type strains. *C. faecale* F4$^T$ and *K. soli* DKR-2$^T$ were obtained from the Korean Agricultural Culture Collection, whereas *K. haifensis* H38$^T$ was purchased from the DSMZ-German Collection of Microorganisms. For the comparative physiology and chemotaxonomy experiments, the strains were cultured in R2A medium at 30°C.

## 16S rRNA GENE PHYLOGENY

The 16S rRNA gene sequence of strain Ran72$^T$ was amplified via PCR with the 27F (5′-AGAGTTTGATCMTGGCTCAG-3′) and 1492R (5′-TACGGYTACCTTGTTACGACTT-3′) universal primers, after which the obtained amplicons were sequenced [17]. The PCR product sequencing for the 16S rRNA gene was conducted at Macrogen (Seoul, Republic of Korea). The resulting 16S rRNA gene sequence (1338 bp) of strain Ran72$^T$ was compared with those of *Kaistella* species obtained from the NCBI (www.ncbi.nlm.nih.gov/nuccore) and the EzBioCloud database (www.ezbiocloud.net/identify) to identify the phylogenetic lineage of strain Ran72$^T$. The 16S rRNA gene sequence of strain Ran72$^T$ exhibited similarity to species within the genera *Chryseobacterium* or *Kaistella* such as *C. faecale* F4$^T$ (=KACC 22401$^T$, 98.4% similarity), *K. soli* DKR-2$^T$ (=KACC 22070$^T$, 98.0%), and *K. haifensis* H38$^T$ (=DSM 19056$^T$, 97.4%), as identified in the NCBI and EzBioCloud databases. Multiple sequence alignments and reconstruction of phylogenetic trees based on 16S rRNA gene sequences were performed using the maximum-likelihood (ML), neighbour-joining (NJ), and minimum-evolution (ME) algorithms of the Molecular Evolutionary Genetics Analysis (MEGA) software version X (www.megasoftware.net) and a bootstrap test was evaluated with 1000 bootstrap replications using the Kimura two-parameter model (Figs 1 and S1, available in the online Supplementary Material) [18]. A total of 19 type strains were examined for their 16S rRNA sequences, and *Ornithobacterium rhinotracheale* DSM 15997$^T$ was employed as an outgroup. The 16S rRNA sequence similarity between strain Ran72$^T$ and other type strains was estimated using the EzBioCloud and NCBI databases. The top four strains with the highest similarity to strain Ran72$^T$ were *C. faecale* F4$^T$ (98.4%), *K. soli* DKR-2$^T$ (98.0%), *K. haifensis* H38$^T$ (97.4%), and *K. treverensis* DSM 22251$^T$ (97.4%) (Table 1). The strains *C. faecale* F4$^T$, *K. soli* DKR-2$^T$, and *K. haifensis* H38$^T$ were selected for further analysis based on the similarity of their 16S rRNA gene sequences and the phylogenetic trees.

## GENOME FEATURES

The draft genome sequences of strain Ran72$^T$ were analysed using an Illumina NovaSeq 6000 sequencer coupled with the Unicycler version 0.4.7 assembler. The sequencing analysis and assembly procedures were performed by DNA Link (Seoul, Republic of Korea). The absence of genome contamination was confirmed using CheckM vrsion 1.0.18 [19]. The draft genome of strain Ran72$^T$ was deposited in the GenBank database under the accession number JASSYY000000000. The draft genome of strain Ran72$^T$ was estimated to be 2513287 bp long, consisting of four contigs. The contigs have an N50 size of 953775 bp, indicating that half of the genome is contained within contigs of at least this size. The strain also exhibits a relatively high G+C content of 43.2 mol%, which is higher than the G+C content observed in other *Kaistella* species (ranging from 34.5 to 42.9 mol%). The gene prediction and annotation results were analysed using the DFAST web-based tool (available at https://dfast.ddbj.nig.ac.jp/dfc/). After performing gene prediction and annotation, a total of 2267 protein-encoding genes, three rRNA genes, and 39 tRNA genes were identified in the genome of strain Ran72$^T$.

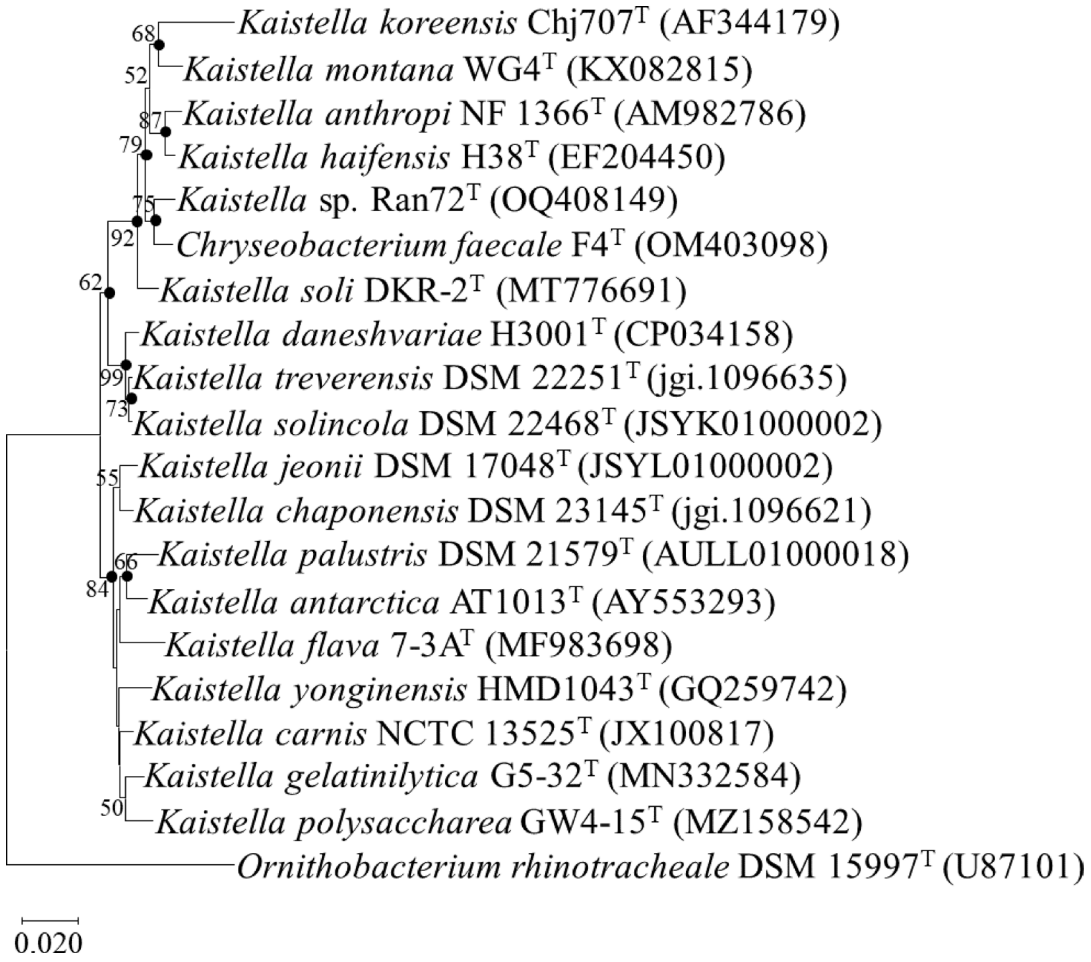


**Fig. 1.** Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the relationships between strain Ran72$^T$ (bold) and the strains of closely related species. Bootstrap percentages (>60%) based on 1000 replicates are indicated at branch nodes by filled circles (●). *Ornithobacterium rhinotracheale* DSM15997$^T$ (U87101) was used as an outgroup. Scale bar: 0.020 changes per nucleotide position.

## GENOMIC RELATEDNESS AND PHYLOGENOMIC ANALYSIS

To determine genome similarities between strain Ran72$^T$ and 13 other publicly available type strains with whole-genome sequences, the average nucleotide identity (ANI, average amino acid identity (AAI), and digital DNA–DNA hybridization (dDDH) values were assessed using the EzBioCloud ANI tool (www.ezbiocloud.net/tools/ani), the Enveomics AAI tool (http://enve-omics.ce.gatech.edu/aai/), and the Genome-to-Genome Distance Calculator tool (https://ggdc.dsmz.de/ggdc.php), respectively [20–22]. The genomic relatedness between the novel Ran72$^T$ strain and closely related type strains are detailed in Table 1. Strain Ran72$^T$ was identified as representing a novel species within the genus *Kaistella*, meeting the ANI, AAI, and dDDH cut-off values of 95, 95 and 70% respectively, which indicated its distinctiveness from other analysed type strains. The ANI values for the four type strains (*C. faecale* F4$^T$, *K. soli* DKR-2$^T$, *K. haifensis* H38$^T$, and *K. treverensis* DSM 22251$^T$) with the closest 16S rRNA sequences to strain Ran72$^T$ were 74.9, 74.4, 74.0, and 73%, respectively, all below the 95% threshold, confirming its distinctiveness. The highest AAI value, 78.7%, was observed between strain Ran72$^T$ and *C. faecale* F4$^T$, followed by AAI values between *K. soli* DKR-2$^T$, *K. haifensis* H38$^T$, and *K. treverensis* DSM 22251$^T$ at 77.7, 76.7, and 74.6%, respectively. This indicates that the AAI values are below the 95% threshold. Moreover, the dDDH values between Ran72$^T$ and (*C. faecale* F4$^T$, *K. soli* DKR-2$^T$, *K. haifensis* H38$^T$, and *K. treverensis* DSM 22251$^T$ were found to be below the aforementioned 70% threshold with values of 19.3, 16.7, 15.7, and 15.3%, respectively. To further support the ANI, AAI, and dDDH results indicating the novelty of strain Ran72$^T$ within the genus *Kaistella*, a phylogenomic tree based on whole-genome sequences was reconstructed using the Reference sequence Alignment based Phylogeny builder (REALPHY) tool (https://realphy.unibas.ch/realphy/) (Fig. 2) [23]. The REALPHY algorithm was used for multiple sequence alignments. The phylogenies were then reconstructed using the maximum-likelihood method PhyML, employing the general time-reversible substitution matrix and a gamma-distributed rate heterogeneity model. Taken together, the genomic relatedness and whole-genome-based

**Table 1.** 16S rRNA similarity, average nucleotide identity (ANI), and average amino acid identity (AAI), digital DNA–DNA hybridization (dDDH) values (%) between strain Ran72$^T$ and phylogenetically related strains

| | 16S rRNA similarity (%) | ANI (%) | AAI (%) | dDDH (%) |
|---|---|---|---|---|
| ***Chryseobacterium faecale* F4$^T$** | 98.4 | 74.9 | 78.7 | 19.3 |
| ***Kaistella soli* DKR-2$^T$** | 98.0 | 74.4 | 77.7 | 16.7 |
| ***Kaistella haifensis* H38$^T$** | 97.4 | 74.0 | 76.7 | 15.7 |
| ***Kaistella treverensis* DSM 22251$^T$** | 97.4 | 73.0 | 74.6 | 15.3 |
| ***Kaistella montana* WG4$^T$** | 97.1 | 74.1 | 77.1 | 16.2 |
| ***Kaistella anthropi* NF 1366$^T$** | 97.1 | 73.9 | 65.4 | 15.8 |
| ***Kaistella solincola* DSM 22468$^T$** | 97.1 | 73.2 | 75.0 | 15.2 |
| ***Kaistella daneshvariae* H3001$^T$** | 97.0 | 72.9 | 74.4 | 15.2 |
| ***Kaistella carnis* NCTC 13525$^T$** | 96.0 | 72.3 | 74.0 | 14.1 |
| ***Kaistella koreensis* Chj707$^T$** | 95.9 | 72.5 | 72.9 | 13.9 |
| ***Kaistella jeonii* DSM 17048$^T$** | 95.6 | 72.6 | 73.9 | 14.3 |
| ***Kaistella palustris* DSM 21579$^T$** | 95.4 | 72.7 | 74.0 | 14.6 |
| ***Kaistella chaponensis* DSM 23145$^T$** | 95.4 | 72.6 | 73.9 | 14.3 |

phylogenetic tree analysis strongly suggest that strain Ran72$^T$ represents a novel species within the genus *Kaistella*, consistent with the findings presented in Table 1. Strain Ran72$^T$, obtained from a faecal sample of rhinoceros exposed to various antibiotics during captivity, highlights concern about potential antibiotic-resistance genes (ARGs) in animal microbiomes, urging a comprehensive analysis due to the implications for multidrug-resistant bacteria and human health surveillance [16, 24–26]. To examine the antibiotic resistance genes present in strain Ran72$^T$, we utilized the Microbial Genomics modules within CLC Genomics Workbench version 21 (Qiagen). Four databases, namely the ResFinder nucleotide database version 4.1, the CARD nucleotide database (2021-updated), the VFDB nucleotide database (2019-updated), and the QMI-AR nucleotide database (2021-updated), were employed for the ARG search. Interestingly, even when using a low minimum identity and

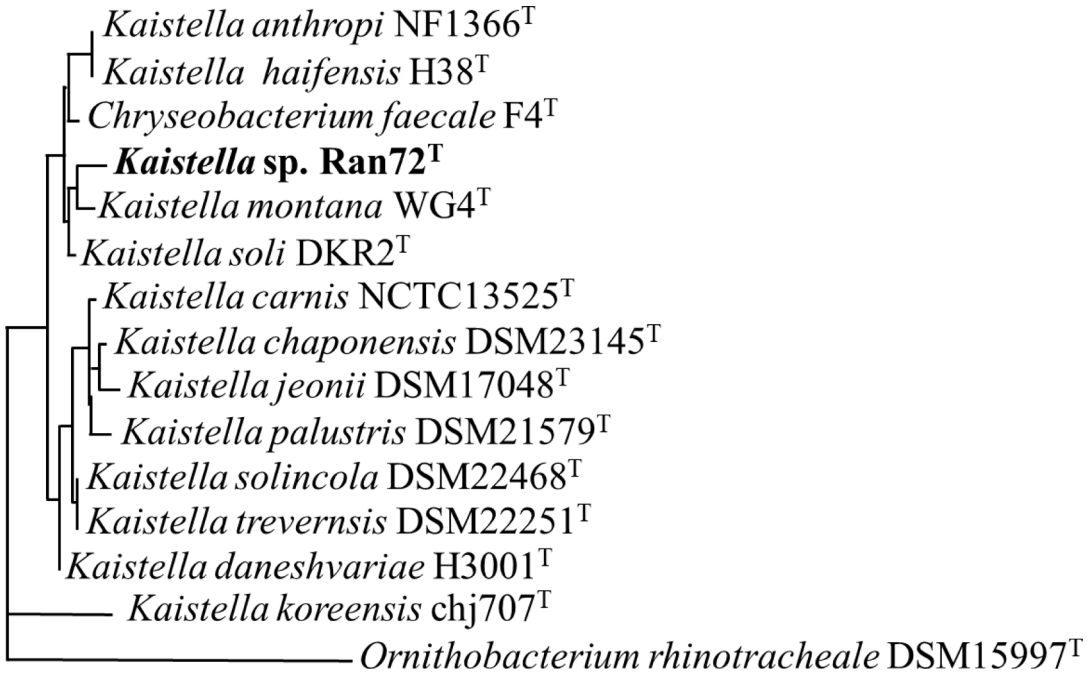


**Fig. 2.** Phylogenomic tree of strain Ran72$^T$ (bold) and the type strains of closely related *Kaistella* species. *Ornithobacterium rhinotracheale* DSM 15997$^T$ (GCA_000265465) was used as an out-group.

minimum length cut-off (both set as 30%) during analysis, no ARG was identified in the genome of the Ran72$^T$ strain. The herbivore-hosted strain Ran72$^T$ might exhibit a gene cluster responsible for polysaccharide degradation [27]. Analyses using the BlastKOALA web tool version 3.0 (www.kegg.jp/blastkoala/) confirmed the presence of beta-glucosidase, an enzyme capable of degrading cellobiose and cellodextrin into glucose. Furthermore, the metabolic pathways of strain Ran72$^T$ unveiled that a notable 28% are dedicated to carbohydrate metabolism.

## PHYSIOLOGY

The Gram type of strain Ran72$^T$ was analysed by using a Gram-staining kit (bioMérieux) according to the manufacturer's instructions. Cell size (0.4–0.8×1.4–2.5 µm) was measured by phase-contrast microscopy (Carl Zeiss). The rod-shaped Ran72$^T$ strain was observed at the exponential phase after the cells were cultured in R2A medium at 30°C and the cells were confirmed to be rod-shaped using a Quanta 250 FEG (FEI) field-emission scanning electron microscope (Fig. S2). To test the optimum growth conditions of strain Ran72$^T$, the growth of the cells were monitored with different temperature, pH, and NaCl concentrations for 3 days on R2A medium (Table 2). To determine the optimal growth temperature for strain Ran72$^T$, the photobiobox was employed to cultivate the strains with 2°C increments within the range of 4–50°C. Periodic measurements of cell density ($OD_{600}$) were conducted using a spectrophotometer (Tecan) to validate that 30°C was identified as the ideal temperature. The pH of the media was adjusted with 1 M filtered HCl or KOH after sterilizing the medium at 121°C for 20 min. The growth across the pH range of 4–11 in 0.5 pH unit intervals was measured and determined that the optimal pH for the growth of strain Ran72$^T$ was pH 7.5. Investigating the optimal NaCl concentration for strain Ran72$^T$ involved incrementally adding NaCl to the medium in 1% increments within the 0–5% range, demonstrating optimal bacterial growth at 0% NaCl. The growth of strain Ran72$^T$ was assessed on R2A agar, Luria–Bertani (LB) agar (BioShop), nutrient broth (NB;BD Difco), tryptic soy agar (TSA; BD Difco), and in McConkey medium (BD Difco) at 30°C for 5 days to ensure sufficient growth. Strain Ran72$^T$ grew on R2A, LB, and TSB agar, but not in NB and McConkey media. The anaerobic growth of strain Ran72$^T$ was tested using R2A liquid medium ($CO_2$:$N_2$ 8:2) at 30°C for 2 weeks. Anaerobic growth tests were conducted simultaneously using *Pseudomonas putida* KT2440 and *Escherichia coli* ATCC 25922 as negative and positive controls, respectively. Anaerobic growth was not detected for strain Ran72$^T$, similar to the other members of the previously reported genus *Kaistella*. The swimming and swarming motilities of the bacterial cells were tested on R2A agar plates (0.3%, 0.5% agar) at 30°C for 2 days, resulting in no motility in strain Ran72$^T$. Catalase and oxidase activity assays were performed by adding 10 µl of 3% hydrogen peroxide solution and 1% Wurster's reagent, respectively, to cultures of strain Ran72$^T$ and other type strains, aiming to evaluate the presence of catalase and oxidase enzymes [28, 29]. Strain Ran72$^T$ and the tested type strains (*C. faecale* F4$^T$, *K. soli* DKR-2$^T$, and *K. haifensis* H38$^T$) showed positive oxidase and catalase activities, as indicated by bubble formation and a violet disc coloration, respectively. Moreover, the ability of the strain to hydrolyse starch, cellulose, and 1% skimmed milk was assessed based on R2A medium. The strains (*C. faecale* F4$^T$, *K. soli* DKR-2$^T$, and *K. haifensis* H38$^T$) exhibited degradation of Tween 60 based on R2A medium, whereas strain Ran72$^T$ was unable to hydrolyse Tween 60 (Table 2). The enzymatic and biochemical characteristics of strain Ran72$^T$ and the other type strains were identified using the API 20NE and API ZYM kits (bioMérieux) with cells suspended in a 0.85% NaCl medium to confirm the physiological properties. Incubation times were based on the manufacturer's instructions, with API 20NE confirming a reaction after 24 and 48 h and API ZYM confirming a reaction after 4 h. The phenotypic characteristics of strain Ran72$^T$ and the three type strains are summarized in Table 2.

## CHEMOTAXONOMY

Strain Ran72$^T$ was cultured in R2A broth at 30°C for 24 h with aeration (180 r.p.m) to analyse quinones and polar lipids. For quinone analysis, a high-performance liquid chromatography system equipped with a Nova-Pack C18 reverse-phase column (4 µm particle size, Waters) was utilized. The quinones were eluted using a mobile phase consisting of a methanol–isopropyl ether solution (3:1, v/v) at a flow rate of 0.7 ml min$^{-1}$ [30]. Menaquinone-6 (MK-6) was identified as the major respiratory quinone of Ran72$^T$, which is typical of the genus *Kaistella* [1, 11–13]. Strain Ran72$^T$ and the other species were cultured under identical conditions to analyse the fatty acid methyl ester profiles during exponential growth. Fatty acid samples from strain Ran72$^T$ and the other type strains were prepared following standard protocols and subjected to analysis using the MIDI/Hewlett Packard Microbial Identification System (Agilent) [31]. The fatty acid profile of Ran72$^T$ was mainly comprised anteiso-$C_{15:0}$ (22.3%), followed by iso-$C_{15:0}$ (18.7%), $C_{16:0}$ (8.9%), $C_{18:0}$ (8.0%), iso-$C_{17:0}$ 3OH (6.8%), and summed feature 9 (iso-$C_{17:1}$ ω9*c*, 6.6%) (Table 3). Particularly, strain Ran72$^T$ and the closest type strain *C. faecale* F4$^T$ exhibited anteiso-$C_{15:0}$ as the primary fatty acid, whereas the remaining strains displayed distinct major fatty acids, such as iso-$C_{15:0}$. Modifications in the composition and remodelling of membrane fatty acids could potentially change the permeability of membranes, leading to varied susceptibilities to stress [32]. Polar lipids were analysed using a two-dimensional TLC technique. The analysis involved the separation of polar lipid samples on TLC Kiesel gel 60 F254 plates (10×10 cm). In the first direction, a mobile phase consisting of a chloroform, methanol, and deionized water (DW) solution at a 65:25:3.8 (v/v) ratio was used to separate the samples. Afterward, in the second direction, the samples were further separated using a mobile phase consisting of chloroform, acetic acid, methanol, and DW at a 80:12:15:3.6 ratio (v/v) [33]. The results revealed that the major polar lipids in strain Ran72$^T$ were phosphatidylethanolamine, unidentified

**Table 2.** Comparison of the phenotypic properties of strain Ran72$^T$ and the type strains of closely related species

Strains: 1, Ran72$^T$ (this study); 2, *Chryseobacterium faecale* F4$^T$; 3, *Kaistella soli* DKR-2$^T$; 4, *Kaistella haifensis* H38$^T$; 5, *Kaistella koreensis* Chj707$^T$; 6, *Chryseobacterium gleum* F93$^T$. All strains analysed in this study are positive for the following characteristics: hydrolysis of Tween 20, Tween 40, aesculin, skimmed milk, and cellulose; enzyme activity of alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, crystine arylamidase, α-chymotrypsin, acid phospatase, and naphthol-AS-BI-phosphohydrolase. All strains are negative for the following characteristics: fermentation of D-glucose; hydrolysis of L-arginine, urea, and *p*-nitrophenyl-*β*-D-galactopyranoside; assimilation of L-arabinose and *N*-acetyl-glucosamine; enzyme activity of lipase (C14), α-galactosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, α-mannosidase, and α-fucosidase. +, Positive; –, negative; w, weakly positive; ND, not determined.

| **Characteristic†** | **1** | **2** | **3** | **4** | **5[d]** | **6[e, f, h]** |
|---|---|---|---|---|---|---|
| Isolation source | Rhinoceros faeces | Camel faeces | Oil-contaminated experimental soil | Raw milk | Freshwater stream | High vaginal swab |
| Cell length (μm) | 1.4–2.5* | 1.4–2.4* | 1.2–3.4* | 1.0–1.9* | ND | 2.0–3.0 |
| Cell width (μm) | 0.4–0.8* | 0.4–1.2* | 0.5–1.0* | 0.4–0.8* | ND | 0.7 |
| Catalase | +* | +* | +* | +* | + | + |
| Oxidase | +* | +* | +* | +* | + | + |
| Reduction of nitrate to nitrite | –* | –* | –* | –* | – | + |
| Temperature range for growth (°C) | 7–36* | 18–40[a] | 10–35[b] | 4–41[c] | 6–42 | 5–42 |
| pH range for growth | 6.0–9.0* | 7.0–8.0[a] | 6.0–9.5[b] | 6.5–10.5[c] | ND | 6.0–8.5 |
| NaCl tolerance for growth (%, w/v) | 0.0–2.0* | 0.0-2.0[a] | 0.0–1.5[b] | 0.0–2.5[c] | ND | 0.0–2.5 |
| Hydrolysis of: | | | | | | |
| Tween 60 | –* | +* | +* | +* | ND | + |
| Starch | +* | +* | +* | –* | – | + |
| Gelatin (API 20NE) | +* | –* | +* | +* | + | ND |
| Assimilation (API 20NE) of: | | | | | | |
| D-Glucose | + | + | + | + | – | ND |
| D-Mannose | +* | –* | –* | w* | – | ND |
| Maltose | +* | +* | +* | –* | + | ND |
| Potassium gluconate | +* | –* | +* | –* | – | + |
| Adipic acid | +* | +* | –* | –* | ND | ND |
| Malic acid | +* | –* | –* | –* | + | – |
| Trisodium citrate | –* | w* | –* | +* | ND | ND |
| D-Mannitol | –* | –* | –* | –* | + | – |
| Phenyl-acetic acid | –* | –* | –* | –* | + | ND |
| Enzyme activities (API ZYM): | | | | | | |
| Trypsin | +* | +* | –* | w* | ND | + |
| *β*-Glucuronidase | –* | –* | –* | +* | ND | ND |
| *α*-Glucosidase | w* | –* | +* | –* | ND | ND |

*Indicates the parameters analysed in this study.
†Data from: a, Son *et al.* [10]; b, Chaudhary *et al.* [13]; c, Hantsis-Zacharov *et al.* [34]; d, Kim *et al.* [1]; e, Holmes *et al.* [35]; f, Heidler von Heilborn *et al.* [36]; g, Meng *et al.* [37]; h, Zhao *et al.* [38].

aminolipid, and unidentified lipids (Fig. S3). These polar lipid distributions of strain Ran72$^T$ were similar to those of other *Kaistella* species [10, 13]. Based on our data, strain Ran72$^T$ represents a novel species of the genus *Kaistella*, for which the name *Kaistella rhinocerotis* sp. nov. is proposed.

## DESCRIPTION OF *KAISTELLA RHINOCEROTIS* SP. NOV.

*Kaistella rhinocerotis* (rhi.no.ce.ro'tis. L. gen. n. *rhinocerotis*, referring to the isolation of the organism from *Rhinoceros* faeces).

**Table 3.** Fatty acid composition (%) comparison between Ran72$^T$ and closely related strains

Strains: 1, Ran72$^T$ (this study); 2, *Chryseobacterium faecale* F4$^T$; 3, *Kaistella soli* DKR-2$^T$; 4, *Kaistella haifensis* H38$^T$. All data reported below were obtained from this study. The data are presented as percentages of total fatty acids. Percentages in bold indicate major fatty acid components (>5.0%). TR, Trace (<1%); –, not detected.

| Fatty acids | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Saturated straight-chain: | | | | |
| $C_{11:0}$ | 1.1 | 1.5 | TR | TR |
| $C_{16:0}$ | 8.9 | 8.9 | 5.8 | 3.3 |
| $C_{18:0}$ | 8.0 | 8.6 | 4.8 | 2.8 |
| Saturated branched: | | | | |
| iso-$C_{13:0}$ | TR | TR | 2.1 | TR |
| iso-$C_{14:0}$ | 1.7 | 1.9 | 2.3 | TR |
| iso-$C_{15:0}$ | 18.7 | 15.5 | 27.1 | 18.2 |
| iso-$C_{16:0}$ | 2.7 | 1.4 | 4.5 | 1.7 |
| iso-$C_{16:1}$ H | TR | 1.7 | – | TR |
| anteiso-$C_{15:0}$ | 22.3 | 15.7 | 21.3 | 15.3 |
| anteiso-$C_{17:0}$ | TR | TR | TR | 1.2 |
| Unsaturated: | | | | |
| $C_{14:1}$ ω5*c* | 1.6 | 2.1 | 1.0 | TR |
| anteiso-$C_{17:1}$ ω9*c* | TR | TR | – | 1.9 |
| Hydroxy: | | | | |
| $C_{8:0}$ 3-OH | 1.9 | 2.2 | 1.2 | TR |
| $C_{15:0}$ 2-OH | 2.2 | 3.8 | 2.1 | 3.1 |
| $C_{17:0}$ 2-OH | 3.4 | 2.4 | 1.6 | 4.7 |
| iso-$C_{15:0}$ 3OH | 2.0 | 4.7 | 4.0 | 4.8 |
| iso-$C_{16:0}$ 3OH | 2.4 | 4.4 | 3.7 | 2.3 |
| iso-$C_{17:0}$ 3OH | 6.8 | 7.0 | 7.4 | 10.9 |
| Summed features:* | | | | |
| 3 | 3.1 | 3.2 | 2.1 | 2.0 |
| 9 | 6.6 | 9.0 | 4.8 | 22.3 |

*Summed features are fatty acids that cannot be resolved reliably from another fatty acid using the chromatographic conditions chosen. The MIDI system groups these fatty acids together as one feature with a single percentage of the total. Summed feature 3, $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; summed feature 9, iso-$C_{17:1}$ *ω9c*.

The bacterial cells were obligate aerobic, Gram-stain-negative, and rod-shaped (0.4–0.8×1.4–2.5 µm) with positive catalase and oxidase activity. Cellular growth in R2A medium was observed at 7–36°C (optimum, 30°C) and pH 6.0–9.0 (optimum, pH 7.5) with 0–2.0% (w/v) NaCl (optimum, 0%). The cells can hydrolyse starch, 1% skimmed milk, cellulose, gelatin, and aesculin. However, they cannot hydrolyse Tween 60, L-arginine, urea, and *p*-nitrophenyl-*β*-D-galactopyranoside. The strain could assimilate D-glucose, D-mannose, maltose, potassium gluconate, adipic acid, and malic acid but not L-arabinose, D-mannitol, *N*-acetyl-glucosamine, or phenyl-acetic acid. Furthermore, enzyme activities of trypsin, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, crystine arylamidase, *α*-chymotrypsin, acid phospatase, and naphthol-AS-BI-phosphohydrolase were detected in strain Ran72$^T$. In contrast, *α*-glucosidase activity was weak, and the activities of lipase (C14), *α*-galactosidase, *β*-glucosidase, *N*-acetyl-*β*-glucosaminidase, *α*-mannosidase, and *α*-fucosidase were not identified. The strain was not able to reduce nitrate to nitrite and could not ferment D-glucose. Menaquinone-6 (MK-6) was identified as the main respiratory quinone of strain Ran72$^T$, and its predominant polar lipids were phosphatidylethanolamine, four unidentified aminolipids, and two unidentified lipids. Anteiso-$C_{15:0}$ and iso-$C_{15:0}$ were identified as the primary fatty acids of strain Ran72$^T$.

The type strain Ran72$^T$ (=KACC 23136$^T$=JCM 36038$^T$) was isolated from a rhinoceros faecal sample obtained from a zoo in Seoul, Republic of Korea. The Ran72$^T$ strain has a genome size of 2.51 Mbp with a G+C content of 43.2 mol%. The GenBank accession numbers of the 16S rRNA and genome sequences of the Ran72$^T$ strain are OQ408149 and JASSYY000000000, respectively.

## DESCRIPTION OF *KAISTELLA FAECALIS* COMB. NOV.

*Kaistella faecalis* (fae.ca'lis. N.L. fem. adj. *faecalis*, derived from faeces).

Basonym: *Chryseobacterium faecale* Son *et al*. 2022.

The phenotypic characteristics are those described by Son *et al*. [10] with the following amendments, based on the study of the type strains. *Kaistella faecalis* is a 0.4–0.8 µm wide and 1.4–2.4 µm long with anteiso-$C_{15:0}$ and iso-$C_{15:0}$ as the primary fatty acids. The type strain F4$^T$ (KACC 22401$^T$=JCM 34836$^T$) was isolated from camel faecal samples. The GenBank accession numbers for the 16S rRNA and whole genome sequences of the F4$^T$ strain are OK513273.1 and CP087583.1, respectively. Based on our obtained data, a proposal is made to reclassify *Chryseobacterium faecale* under the genus *Kaistella*. The phylogenetic tree reconstructed based on the 16S rRNA sequence and whole genome sequence showed that *Chryseobacterium faecale* F4$^T$ is closely related to *Kaistella* (Figs 1, 2 and S1). The 16S rRNA gene sequences demonstrated a similarity of ≤95% with the members of *Kaistella*, which is below the taxonomic threshold for the definition of a species (Table 1) [34]. Based on our study, reclassification of *C. faecale* as *Kaistella faecalis* comb. nov. is proposed.

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Author contributions
Y.P. and W.P. designed the study. Y.P. performed all experiments and analysis. Y.P., J.M., and W.K. isolated the bacterium. Y.P. and W.P. drafted the manuscript. All authors contributed to and approved the final version of this manuscript.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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