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ORIGINAL PAPER

# *Gramella crocea* sp. nov., isolated from activated sludge of a seafood processing plant

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**Abstract** A novel aerobic Gram-negative strain, designated as YB25$^T$, was isolated from an activated sludge sample collected from a seafood processing plant in Zhoushan, Zhejiang Province, China, and characterized by using a polyphasic taxonomic approach in this study. Strain YB25$^T$ was motile by gliding, and short-rod-shaped. The isolate grew at 4–37 °C (optimum 28 °C), pH 6.0–9.0 (optimum pH 7.0) and 0.0–10.0% NaCl (optimum 2.0%, w/v). Phylogenetic analysis based on 16S rRNA gene indicated that strain YB25$^T$ belonged to the genus *Gramella,* and showed the highest sequence similarity of 97.59% to *Gramella lutea* YJ019$^T$. The DNA G+C content was 39.5%. In silico DNA-DNA hybridization (DDH) and average nucleotide identity (ANI) values between strain YB25$^T$ with most closely strains were below the threshold, which is considered to the phylogenetic definition of a novel species. Chemotaxonomic analysis indicated that the only respiratory quinone was menaquinone-6 and the major fatty acids were iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 9 (iso-$C_{17:1}\omega 9c$ and $C_{16:0}$ 10 methyl). The polar lipid profile was composed of phosphatidylethanolamine, an unidentified phospholipid, two unidentified amino lipids, three unidentified glycolipids, and four unidentified lipids. Compared with the reference strains, strain YB25$^T$ contained higher abundance of genes for carbohydrates metabolism,nitrogen metabolism, sulfur metabolism and respiration based on its genomic metabolic pathways and had been found a certain potential in the degradation of pectin. On the basis of the taxonomic evidence, strain YB25$^T$ represents a novel species of the genus *Gramella*, for which the name *Gramella crocea* sp. nov. is proposed. The type strain is YB25$^T$ (=KCTC 82680 $^T$=MCCC 1K05761$^T$).

**Keywords** *Gramella* · *Gramella crocea* · Activated sludge · Seafood processing plant · Polyphasic taxonomy

**Abbreviations**

| | |
|---|---|
| MA | Marine 2216 agar |
| MB | Marine 2216 broth |
| PE | Phosphatidylethanolamine |

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G+C Guanine (G) plus cytosine (C) (nitrogenous bases)
ORF Open reading frames

## Introduction

Members of phylum *Bacteroidetes*, formerly known as the *Cytophaga-Flavobacteria-Bacteroides* phylum, are known for degrading a wide variety of complex carbohydrates, making them dominant in many different environments such as marine ecosystems and activated sludge systems (wastewater treatment systems) (Glöckner et al. 1999; Alonso et al.2007; McKee et al.2021). *Flavobacteriaceae* is often the predominant bacterial family decomposing derived polysaccharides. The genus *Gramella* belongs to the family *Flavobacteriaceae*, and the type species is *Gramella echinicola* KCTC 12278 $^{T}$, which was obtained from sea urchins and proposed in 2005 (Nedashkovskaya et al. 2005). The marine-derived bacterium *Gramella forsetii* uses macromolecular polysaccharides in a mechanism similar to bacteria in the intestinal tract (Kabisch et al. 2014). Another species *Gramella flava* JLT2011 was discovered to have potential polysaccharide utilization systems based on the catabolic pathway models for xylan and pectin (Tang et al. 2017).

At the time of writing, the genus *Gramella* has 18 valid names according to LPSN (https://lpsn.dsmz.de/genus/gramella). The members of the genus *Gramella* are isolated from the marine environments, including marine organisms (Nedashkovskaya et al. 2005; Liu et al. 2020), seawater (Liu et al. 2014), marine sediments (Li et al. 2018; Yoon et al. 2015), tidal flats (Jeong et al. 2013; Park et al. 2015), and eutrophic surface water (Panschin et al. 2017). Most members of the genus *Gramella* are characterized as Gram-stain-negative, aerobic respiration, immobile (or gliding motion), yellow or orange pigmentation, rod-shaped morphology, requiring sea water or sodium ions to grow, and containing menaquinone-6 (MK-6) as major isoprenoid quinone.

In 2020, during our resource investigation study on the screening and isolation of culturable bacteria from activated sludge, a *Bacteroidetes*, designated as strain YB25$^{T}$, was isolated from a seafood processing plant in P.R. China. In the present study, the isolate was considered to represent a novel species of the genus *Gramella*, for which the name *Gramella crocea* sp. nov. was proposed by using the polyphasic taxonomical approaches, including phenotypic, chemotaxonomic, genomic and genotypic analysis.

## Materials and methods

### Isolation and cultivation

In summer 2020, we collected the activated sludge samples from the sewage secondary sedimentation tank of Dayang seafood processing plant, Zhoushan City, Zhejiang Province, P.R. China. Samples were collected with a filter press, transported to the laboratory in an icebox and stored at 4 °C. Approximately 3 g sediment was added to 27 mL seawater for enrichment, spread on MA plates by the traditional dilution-plating method, and finally incubated at 28 °C. After 5 days of incubation, a yellow-orange colony was picked and named as YB25$^{T}$. After repeated plate streaking on the same medium, pure strain was obtained from individual colonies and preserved at -80 °C in 25% (v/v) glycerol for further study. Meanwhile, strain YB25$^{T}$ has been deposited at the Korean Collection for Type Cultures (KCTC) and the Marine Culture Collection of China (MCCC). Unless otherwise stated, all strains in this experiment were cultured on MA or MB medium under the same conditions.

### 16S rRNA gene sequencing and phylogenetic analysis

After extracting and purifying genomic DNA, the 16S rRNA gene of strain YB25$^{T}$ was amplified with universal primers 27F (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-TACGGYTACCTTGTTACGACTT-3') as described by Okai (2015). The PCR product was cloned into *E. coli* DH5α using the pMD® 19-T vector kit (TaKaRa) for sequencing by thermal shock transduction, and the almost-complete 16S rRNA gene sequence was obtained. To determine the phylogenetic position of the novel isolate, the 16S rRNA gene sequence (1494 bp) of strain was submitted to NCBI GenBank database (www.ncbi.nlm.nih.gov/) (Accession No: OL853706) and compared by EzTaxon-e database for pairwise sequence alignment (https://eztaxon-e.ezbiocloud.net) (Yoon et al. 2017). The 16S rRNA gene sequences of relevant strains

were obtained from EzBioCloud database and the multiple alignments of the sequences were performed by Clustal W(Thompson et al. 1994). Then phylogenetic trees were constructed with neighbour-joining method (Saitou et al. 1987), maximum-likelihood (Felsenstein et al. 1981), and maximum-evolution (Rzhetsky and Nei 1993) algorithms using MEGA X software (Kumar et al. 2018), with *Capnocytophaga ochracea* DSM 7271 $^{T}$ (ABTH01000001) as an outgroup. Bootstrap values of the three phylogenetic trees were evaluated by performing 1000 resamplings and the Kimura's two-parameter model (Xu et al. 2019) was set to calculate the genetic distance in NJ method.

## Morphology, physiology and biochemistry

For phenotypic tests, the strain was grown in MA medium for 72 h at 28 °C before use. The Gram reaction was performed with a Gram stain kit (Hangzhou Tianhe Micro-organism Reagent) according to the manufacturer's instructions (Chen et al., 2019) after using optical microscope (BX40; Olympus). Transmission electron microscopy (JEM-1230; JEOL) was used for observing flagella and cell morphology. The motility of the strain YB25$^{T}$ was tested using hanging-drop method and semi-solid agar method as described by Bernardet et al. (1996). To determine temperature ranges for growth, broth cultures of the MB medium was incubated at 4, 10, 15, 20, 25, 28, 30, 35, 37, 40, 42, and 45 °C. Modified MB was used for NaCl tolerance tests, in which NaCl was omitted (0%) or added at 0.5–12.0% (w/v) (at intervals of 0.5%). The growth range of pH was tested at pH 5.0–10.0 at intervals of 0.5 using the medium and buffer system described by Ye et al. (2020). All the growth results were monitored by optical density (OD) at 590 nm using a spectrophotometer (Ultrospec 6300 pro; Amersham Biosciences) after 2 days of incubation. Anaerobic growth was investigated by using the anaerobic system (AnaeroPack-MicroAero, 2.5 l, MGC, Japan) and 20 mM sodium thiosulfate, 5 mM sodium sulfite, 20 mM sodium sulfate, 5 mM sodium nitrite or 20 mM sodium nitrate were added as potential electron acceptors (Zhang et al. 2020). Oxidase activity was determined using oxidase reagent (bioMérieux) and catalase activity was determined by dripping 3% (v/v) $H_2O_2$ to observe bubbles (Zhang et al. 2021). Production of indole and $H_2S$ production, methyl red and Voges-Proskauer tests, and hydrolysis of CM-Cellulose, Tweens (20, 40, 60, and 80), gelatin, and filter paper were evaluated as described by Chen et al. (2019). Other physiological and biochemical characteristics were processed using API 20NE, API ZYM, API 50CH systems (bioMérieux) and GENIII MicroPlates (Biolog) according to the manufacturers' instructions.

## Chemotaxonomic characterization

Whole-cell fatty acids of strain YB25$^{T}$ were extracted and analyzed according to the standard protocol of Microbial Identification System (Sherlock Version 6.0; MIDI database: ANAER6). Isoprenoid quinones were extracted and purified by TLC as described by Minnikin et al. (1984) and then identified by an HPLC–MS system (Agilent) and Thermo Finnigan LCQ DECA XP MAX mass spectrometer (Yu et al. 2018). Polar lipids were extracted from 4.0 g freeze-dried cells and examined by two-dimensional TLC on silica gel 60 F254 plates (Merck) after drying for 30 min at 55 °C, and then identified as previously described (Tindall et al. 1990).

## Genome sequence assembly, annotation and analysis

Several *Gramella* strains have been found to have absolute advantages in the utilization and degradation of polysaccharides and play an important role in the decomposition of polysaccharides in the carbon cycle of marine ecosystems, thus the draft genome of strain YB25$^{T}$ was sequenced using the Illumina HiSeq 2000 platform (Beijing Genomics Institute). The de novo assembly reads were assembled using ABySS 1.5.2 software (Simpson et al. 2009). The contigs shorter than 2000 bp were removed, and the quality of the assembled genome sequence was assessed using CheckM version 1.0.7 (Parks et al. 2015). Rapid Annotation by Subsystem Technology (RAST) server online (Overbeek et al. 2014) was used for calculating the DNA G + C content and predicting the open reading frames (ORFs). The Comprehensive Antibiotic

Resistance Database (CARD) (https://card.mcmaster.ca/analyze/blast) was used for identifying resistance genes. CGView Server (http://cgview.ca/) was used to make a genome circle map, and TYGS (https://tygs.dsmz.de/) server was applied to reconstruct a phylogenetic tree based on the whole genome. The online tool Kyoto Encyclopedia of Genes and Genomes (KEGG) Mapper (Gerlich et al. 2000) was used to reveal the metabolic pathways. The genomes of reference type strains of were downloaded from the NCBI database. The in silico DNA-DNA hybridization (isDDH) values and the average nucleotide identity (ANI) values were calculated by genome to genome distance calculator (GGDC) with recommended BLAST + alignment and Formula 2 (Meier-Kolthoff et al. 2013) and the OrthoANIu algorithm (Lee et al. 2016).

## Results and discussion

### 16S rRNA gene sequencing and phylogenetic analysis

Uploading the almost complete sequence (1494 bp) to EzBioCloud showed that strain YB25$^T$ had the highest 16S rRNA sequence similarity with *Gramella lutea* YJ019$^T$ (97.59%). In the phylogenetic trees with three different algorithms described above, strain YB25$^T$ was located in the *Gramella* genus, and formed a distinct branch, adjacent to *G. lutea* YJ019$^T$ (97.59%) and *Gramella forsetii* KT0803$^T$ (97.31%) (Fig. 1, S1 and S2). The similarity between strain YB25$^T$ and *Gramella echinicola* KCTC 12278 $^T$ was 97.15%, which was the type species in the *Gramella* genus, and the similarities with other strains were very low. Accordingly, *G. lutea* YJ019$^T$, *G. forsetii* KT0803$^T$ and *G. echinicola* KCTC 12278 $^T$ were

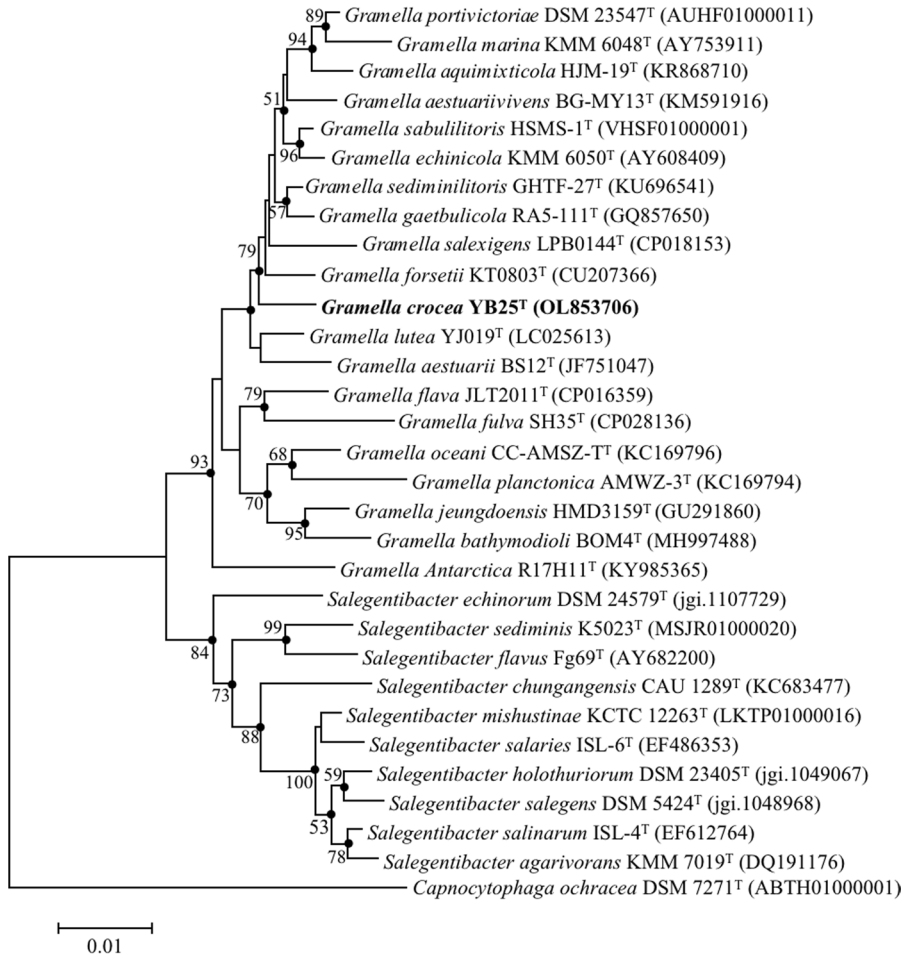


**Fig. 1** Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain YB25$^T$ and the related species. Bootstrap values based on 1000 replicates are listed as percentages at branching points. Only bootstrap values above 50% are shown. Bar, 0.01 substitutions per nucleotide position. Filled circles indicate that the corresponding nodes were also recovered in both maximum-likelihood and maximum-evolution algorithms. *Capnocytophaga ochracea* DSM 7271.$^T$ (ABTH01000001) was used as an outgroup

selected as reference strains and ordered from various collection centers. All strains in this experiment were cultured on MA or MB medium under the same conditions.

## Morphology, physiology and biochemistry

After incubation on MA medium at 28 °C for 5 days, the colony of strain YB25$^T$ formed was 1–2 mm in diameter, round, convex, smooth, and bright yellow-orange color. The cells were Gram-negative, aerobic, and motile by gliding. The transmission electron microscope showed that it was short-rod-shaped (0.6–0.8 × 1.8–2.0 μm) without flagella (Fig. S3). The isolates grew at 4–37 °C (optimum 28 °C), pH 6.0–9.0 (optimum 7.0), and 0–10.0% (w/v) NaCl (optimum 2.0%).

Strain YB25$^T$ and reference strains were positive for oxidase and catalase, alkaline phosphatase, esterase (C4), esterase lipase (C8), α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, hydrolysis of starch, esculin, Tweens (40 and 60), D-fructose-6-phosphate, glucuronamide and not sensitive to tetrazolium violet and tetrazolium blue. Simultaneously, they were negative for reducing nitrates to nitrites, production of $H_2S$ and indole, Voges-Proskauer test, arginine dihydrolase, hydrolysis of gelatin, CM-cellulose and filter paper, assimilation of N-acetylglucosamine, D-maltose, capric acid, adipic acid, phenylacetic acid, and utilization of gluconate and 2-keto-gluconic acid. Consequently, the strain YB25$^T$ maintained commonality with related strains in many respects and had some characteristics common to others within this genus. Nevertheless, there were also some differences. The physiological and biochemical characteristics can be used to distinguish strain YB25$^T$ and reference strains are shown in Table 1. Unlike the reference strains, the strain YB25$^T$ could use pectin as a sole carbon source, but could not use D-glucuronic acid. In contrast to the closest phylogenetic neighbor *G. lutea* YJ019$^T$, strain YB25$^T$ hydrolyze starch, gelatin, casein, Tween 80, and positive for leucine arylamidase, valine arylamidase, trypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, and N-acetyl-β-glucosaminidase. Strain YB25$^T$ could not use pineulose and amidon to produce acid, and could not utilize L-aspartic acid, D-glucuronic acid, and acetoacetic acid as a sole carbon source, which were different from *G. forsetii* KT0803$^T$. Compared with *G. echinicola* KCTC 12278 $^T$, strain YB25$^T$ could utilize D-mannose, xylose, cellobiose, and lactose.

## Chemotaxonomic characterization

The fatty acid profiles of strain YB25$^T$and reference strains are shown in Table 2. The predominant fatty acids (relative account > 10%) of the novel isolate were iso-$C_{15:0}$ (14.9%), anteiso-$C_{15:0}$ (10.5%), iso-$C_{17:0}$ 3-OH (10.3%), and summed feature 9 (iso-$C_{17:1}\omega 9c$ and $C_{16:0}$ 10 methyl) (12.7%). According to the content of anteiso-$C_{15:0}$ and summed feature 9, strain YB25$^T$ could be separated from the closest phylogenetic taxa *G. lutea* KCTC 42382 $^T$. In addition, all reference strains contained the typical main fatty acid of this genus (iso-$C_{15:0}$), but YB25$^T$ had anteiso-$C_{15:0}$ which could be clearly distinguished. The polar lipids of the novel isolate included phosphatidylethanolamine (PE), an unidentified phospholipid, two unidentified amino lipids, three unidentified glycolipids, and four unidentified lipids (Fig. S4), which agrees with previous data for other species of the genus *Gramella.* The measured molecular weight of respiratory quinone indicating that the only respiratory quinone of strain YB25$^T$ was menaquinone-6 (MK-6) which was also a common feature of the *Flavobacteriaceae* family.

## Genome sequence assembly, annotation and analysis

The draft genome sequence of strain YB25$^T$ generated 1314 Mb of clean data and the total genome size of the sequence was 3.90 Mb with 32 contigs after assembly. The genome completeness of strain YB25$^T$ was 99.62% with 1.0% contamination. N50 value of strain YB25$^T$ was 183,208 bp, and the value of L50 was 7. As a result, the quality of the genome was high enough for further analysis. The circular map of the chromosome of strain YB25$^T$ was shown in Figure S6. The DNA G + C content of strain YB25$^T$ calculated from the genome sequence was 39.5%, close to *G. lutea* YJ019$^T$(38.3%), *G. forsetii* KT0803$^T$(36.6%) and *G.* echinicola KCTC 12278 $^T$(36.9%). The reconstructed phylogenomic tree showed that strain YB25$^T$ formed the closest relatives to *G. echinicola* KCTC 12278 $^T$, *G. forsetii* KT0803$^T$ and *G. lutea* YJ019$^T$, which located in the clade of genus *Gramella* (Fig. S7). The DDH value between strain YB25$^T$ and its

**Table 1** Differential phenotypic and biochemical characteristics of strain YB25.$^{T}$ and its most closely related species

| Characteristic | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Cell shape | Short-rods | Straight-rods* | Rods† | Rods¶ |
| Motility | + | –* | +† | +¶ |
| Temperature (°C) | 4–37 | 20–37* | 2–30† | 4–37¶ |
| Optimal growth temperature (°C) | 28 | 30* | 22–25† | 23–25¶ |
| NaCl (%) | 0.0–10.0 | 0.0–8.0* | 0.13–6.0† | 1.0–15.0¶ |
| Optimal Nacl for growth (w/v) | 2.0 | ND | 2.0–3.0† | ND |
| pH | 6.0–9.0 | 6.0–9.0* | 6.0–8.3† | ND |
| Optimal pH for growth | 7.0 | 7.0* | 7.5† | |
| *Hydrolysis of* | | | | |
| Starch | + | – | + | + |
| Gelatin | + | – | + | + |
| Tween 20 | + | – | – | – |
| Tween 80 | + | – | w | + |
| *Enzyme activites* | | | | |
| Lipase(C14) | – | – | + | + |
| Leucine arylamidase | + | – | + | + |
| Valine arylamidase | + | – | + | + |
| Trypsin | + | – | – | + |
| α-galactosidase | + | – | – | – |
| β-galactosidase | + | – | – | + |
| β-glucuronidase | + | – | – | – |
| α-glucosidase | + | – | – | + |
| β-glucosidase | + | – | – | + |
| N-acetyl-β-glucosaminidase | + | – | + | – |
| Urease | – | + | + | – |
| *Acid production from* | | | | |
| Melizitose | – | – | + | – |
| Amidon | – | w | + | + |
| D-mannose | + | – | + | – |
| Xylose | + | + | – | – |
| Cellobiose | + | – | + | – |
| Lactose | + | + | + | – |
| *Utilization of* | | | | |
| Pectin | + | – | - | – |
| L-Aspartic acid | – | + | + | – |
| D-Glucuronic acid | – | + | + | + |
| L-Histidine | – | – | – | + |
| Acetoacetic acid | – | – | + | + |

Strains: 1, strain YB25$^{T}$; 2, *G. lutea* YJ019$^{T}$; 3, *G. forsetii* KT0803$^{T}$; 4, *G. echinicola* KCTC 12278.$^{T}$. Unless stated otherwise, all data were obtained from this study under identical growth conditions. +, positive; –, negative; w, weakly positive

*Data from Yoon et al.

†Data from Panschin et al.

¶Data from Nedashkovskaya et al.

closely related strains was 17.5–19.8%. In addition, the ANI values between YB25$^{T}$ and references genomes was 75.9–76.2%. These results were lower than the 70% threshold value for GGDC and 95% for ANI proposed for the delineation of bacterial species (Goris et al 2007; Richter 2009).

The different genomic features of the novel isolate and reference strains based on the RAST result were listed in Table 3. Genome annotation and KEGG analysis was shown in Figure S8 and S9. On the basis of the genome annotation and the KEGG analysis, many genes related to biological metabolisms and environmental adaptations were found in the strain YB25$^{T}$, of which 38 genes related to sulfur metabolism, 18 genes related to phosphorus metabolism, and 24 genes related to nitrogen metabolism. As for stress

**Table 2** Cellular fatty acid profiles (%) of strain YB25.$^{T}$ and reference strains

| Peak name | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| *Staturated* | | | | |
| $C_{10:0}$ | 1.1 | Tr | – | Tr |
| $C_{14:0}$ | Tr | 1.3 | – | Tr |
| $C_{16:0}$ | Tr | 2.4 | Tr | 3.9 |
| Anteiso-$C_{15:0}$ | **10.5** | 4.6 | 5.7 | 9.0 |
| *Branched staturated* | | | | |
| iso-$C_{10:0}$ | – | 1.4 | – | Tr |
| iso-$C_{14:0}$ | 1.2 | 1.2 | Tr | 1.9 |
| iso-$C_{15:0}$ | **14.9** | **23.3** | **26.8** | **26.4** |
| iso-$C_{16:0}$ | 3.1 | 2.6 | 2.1 | 6.6 |
| iso-$C_{17:0}$ | 1.0 | Tr | Tr | Tr |
| iso-$C_{18:0}$ | 1.4 | 2.00 | 2.3 | Tr |
| *Unsaturated* | | | | |
| iso-$C_{16:1}$H | 1.7 | 1.1 | 1.50 | 1.4 |
| $C_{14:1}$ *ω5c* | Tr | 1.2 | – | Tr |
| $C_{15:1}$ *ω6c* | Tr | 1.3 | 2.8 | 1.9 |
| $C_{17:1}$ *ω6c* | 2.3 | 1.3 | 3.4 | 1.5 |
| $C_{17:1}$ *ω8c* | 1.4 | Tr | 1.5 | Tr |
| $C_{18:1}$ *ω9c* | Tr | – | – | Tr |
| Anteiso-$C_{17:1}$ *ω9c* | 5.9 | Tr | 6.3 | Tr |
| iso-$C_{15:1}$ G | – | 5.6 | Tr | 1.2 |
| *Hydroxy* | | | | |
| $C_{8:0}$ 3-OH | 1.0 | Tr | – | Tr |
| $C_{15:0}$ 2-OH | 2.8 | 1.1 | 3.5 | 2.2 |
| $C_{16:0}$ 3-OH | Tr | 1.1 | Tr | 2.6 |
| $C_{17:0}$ 2-OH | 6.5 | – | 4.8 | 2.5 |
| iso-$C_{15:0}$ 3-OH | 2.1 | 3.2 | 2.9 | 2.2 |
| iso-$C_{16:0}$ 3-OH | 2.3 | 1.9 | 4.5 | 4.4 |
| iso-$C_{17:0}$ 3-OH | **10.3** | **12.0** | **13.1** | 9.4 |
| Summed feature 3* | 8.5 | **15.7** | 8.2 | **14.5** |
| Summed feature 4* | 1.9 | 1.4 | 1.2 | – |
| Summed feature 9* | **12.7** | 8.1 | 1.7 | 1.4 |

Strains: 1, strain YB25$^{T}$; 2, *G. lutea* YJ019$^{T}$; 3, *G. forsetii* KT0803$^{T}$; 4, *G. echinicola* KCTC 12278.$^{T}$. The major fatty acids (> 10%) in the table are bolded. -, not detected; Tr, trace amount (< 1%)

*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 includes $C_{16:1}$ *ω7c* and $C_{16:1}$ *ω6c*, Summed feature 4 includes iso $C_{17:1}$I and Anteiso B and Summed feature 9 includes iso-$C_{17:1}$*ω9c* and $C_{16:0}$ 10 methyl

responses, 28 genes were also found in the genome of strain YB25$^{T}$, including 20 genes associated with oxidative stress and 5 genes associated with periplasmic stress. There were 31 genes involved in virulence, disease and defense, of which the most are resistant genes to antibiotics and toxic compounds, including 8 genes related to copper homeostasis and tolerance, 3 genes related to cobalt-zinc-cadmium resistance, 2 genes related to the resistance of fluoroquinolone drugs, and 2 genes were related to the multidrug-resistant efflux pump.

Compared with the reference strains, strain YB25$^{T}$ contained 174 genes responsible for carbohydrates metabolism, which was more than that of the reference strains. It was found in physiological and biochemical experiments that YB25$^{T}$ could use pectin as a single carbon source in physiological and biochemical experiments. Research on its genomic metabolic pathways found that it had pectin esterase [EC:3.1.1.11] and could undergo a hydrolysis reaction: $\mathrm{Pectin} + \mathrm{nH2O} <=> \mathrm{nMethanol} + \mathrm{Pectate}$. Accordingly, YB25$^{T}$ had a certain potential in the degradation of polysaccharides. In addition, strain YB25 $^{T}$ had higher abundance of genes in nitrogen metabolism, sulfur metabolism and respiration than the reference strains, which was worth exploring and applying.

## Taxonomic conclusion

In conclusion, strain YB25$^{T}$ represents a novel species in the genus *Gramella* based on the phenotypic, phylogenetic, chemotaxonomic and genotypic properties presented in this study, for which the name *Gramella crocea* sp. nov. is proposed.

## Description of Gramella crocea sp. nov.

*Gramella crocea (cro.ce'a. L. fem. adj. crocea yellow)*

Cells are Gram-stain-negative, aerobic, non-spore-forming, motile by gliding, and rod-shaped with a size of 0.6–0.8 × 1.8–2.0 μm. Colonies on MA agar are yellow-orange, circular (approximately 1–2 mm

**Table 3** Genomic features of strain YB25.$^{T}$ and the reference strains

| Genomic characteristics | 1 | 2 | 3 | 4 |
|---|---|---|---|---|
| Size (bp) | 3,902,243 | 3,466,166 | 3,558,816 | 3,513,826 |
| DNA G + C contents (%) | 39.5 | 38.3 | 36.5 | 36.9 |
| N50 | 183,208 | 639,985 | 3,759,264 | 927,778 |
| L50 | 7 | 2 | 3 | 2 |
| Number of contigs | 32 | 31 | 35 | 18 |
| Number of coding sequences | 3552 | 3254 | 3525 | 3232 |
| Number of RNAs | 34 | 46 | 42 | 44 |
| *Subsystem feature counts* | | | | |
| Cofactors, vitamins, prosthetic groups, pigments | 154 | 142 | 149 | 145 |
| Virulence, disease and defense | 31 | 22 | 37 | 29 |
| Membrane transport | 40 | 35 | 39 | 39 |
| Potassium metabolism | 9 | 13 | 12 | 10 |
| RNA metabolism | 39 | 38 | 41 | 38 |
| DNA metabolism | 62 | 52 | 67 | 71 |
| Protein metabolism | 118 | 117 | 112 | 112 |
| Regulation and cell signaling | 8 | 8 | 10 | 5 |
| Secondary metabolism | 6 | 5 | 5 | 5 |
| Fatty acids, lipids, and isoprenoids | 23 | 23 | 35 | 23 |
| Nitrogen metabolism | 24 | 7 | 13 | 8 |
| Respiration | 55 | 33 | 29 | 29 |
| Stress response | 28 | 23 | 25 | 22 |
| Metabolism of aromatic compounds | 11 | 10 | 10 | 10 |
| Amino acids and derivatives | 239 | 236 | 245 | 234 |
| Sulfur metabolism | 38 | 13 | 9 | 9 |
| Phosphorus metabolism | 18 | 16 | 19 | 19 |
| Carbohydrates | 174 | 88 | 105 | 94 |

Strains: 1, strain YB25$^{T}$; 2, *G. lutea* YJ019$^{T}$; 3, *G. forsetii* KT0803$^{T}$; 4, *G. echinicola* KCTC 12278.$^{T}$

in diameter after 5 days incubation at 28 °C) with entire edges, convex and smooth. Growth occurs at 4–37 °C (optimum 28 °C), pH 6.0–9.0 (optimum 7.0), and 0–10% (w/v) NaCl [optimum 2.0% (w/v)]. It is positive for both catalase and oxidase. The polar lipids profile is composed of phosphatidylethanolamine (PE), an unidentified phospholipid, two unidentified amino lipids, three unidentified glycolipids, and four unidentified lipids. The major respiratory quinone is MK-6. The major cellular fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH, and summed feature 9 (iso-$C_{17:1}\omega 9c$ and $C_{16:0}$ 10 methyl). The DNA G + C content of the type strain is 39.5%.

The type strain, YB25$^{T}$ (= KCTC 82680$^{T}$ = MCCC 1K05761$^{T}$), was isolated from activated sludge samples collected from the sewage secondary sedimentation tank of Dayang seafood processing plant, Zhoushan City, Zhejiang Province, P.R. China. The GenBank accession numbers for the 16S rRNA gene sequence and the whole genome sequence of strain YB25$^{T}$ are OL853706 and JAJSON000000000, respectively.

**Authors' contributions** MW and CC designed the experiments and guided the manuscript writing. XYZ was responsible for the major experiments, data analysis and preparation of manuscripts. LHZ assisted in enzymatic experiments. MX and JYW assisted in the determination of fatty acids and polar lipids experiment. ZCW and TW were involved in purchasing reference strains and revising manuscript. All authors read and approved the manuscript.

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**Data availability** The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence and whole genome sequence of strain YB25T are OL853706 and JAJSON000000000 respectively. The whole genome sequence project of *G. lutea* YJ019$^T$ has been deposited under the accession JAKVTV000000000.

**Declarations**

**Conflict of interest** The authors declare that there are no conflicts of interest.

**Ethical approval** This article does not contain any studies with human participants or animals performed by any of the authors.

**Consent for publication** All authors are consent for publication in *Antonie van Leeuwenhoek*.

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