Deefgea salmonis sp. nov., isolated from gills of rainbow trout (Oncorhynchus mykiss)

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Abstract
A Gram-stain-negative, milky white, aerobic, rod-shaped bacterium named strain H3-26\textsuperscript{T} was isolated from gills of Oncorhynchus mykiss in Lhasa, Tibet Autonomous Region, PR China. Strain H3-26\textsuperscript{T} grew at 4–30 °C and pH 5.0–11.0 (optimum, 25 °C and pH 7.0) with 0–1% (w/v) NaCl (optimum, 0%). The 16S rRNA gene sequence of strain H3-26\textsuperscript{T} showed the highest similarity to Deefgea rivuli WB 3.4-79\textsuperscript{T} (98.42%), followed by Deefgea chitinilytica Nsw-4\textsuperscript{T} (96.91%). Phylogenetic analysis based on 16S rRNA genes indicated that strain H3-26\textsuperscript{T} was a new member of the genus Deefgea. The digital DNA–DNA hybridization and average nucleotide identity values between the genome sequence of strain H3-26\textsuperscript{T} and Deefgea spp. were 21.2–21.9% and 76.3–77.4%, respectively. The genomic DNA G+C content of strain H3-26\textsuperscript{T} was 48.74%. The predominant fatty acids were C\textsubscript{12:0}, C\textsubscript{14:0}, C\textsubscript{16:0} and C\textsubscript{16:1} ω7c. Based on phenotypic, phylogenetic, and genotypic data, strain H3-26\textsuperscript{T} is considered to represent a novel species of the genus Deefgea, for which the name Deefgea salmonis sp. nov. is proposed. The type strain is H3-26\textsuperscript{T} (= JCM 35050\textsuperscript{T} = CICC 25103\textsuperscript{T}).

Keywords Deefgea · Oncorhynchus mykiss · Comparative genomics · Tibet

Introduction
The genus Deefgea, a member of the family Neisseriaceae, which were mainly isolated from water and fish. Deefgea spp. may be related to fish’s diseases and are a component of intestinal microflora in fish (Jeon et al. 2017; Shtykova et al. 2018; Terova et al. 2021). Up until now, only two validly species (Deefgea rivuli WB 3.4-79\textsuperscript{T} and Deefgea chitinilytica Nsw-4\textsuperscript{T}) and one draft genome sequence (Deefgea sp. CFH1-16) were published (Stackebrandt et al. 2007; Chen et al. 2010; Han 2021). The physiological and biochemical characteristics, phylogenetic and genotypic data of genus Deefgea were lack of systematic understanding (Jung and Jung-Schroers. 2011). During the investigation of pathogenic microorganism of rainbow trout in Lhasa, a milky white bacterium, named strain H3-26, was isolated from the gills of Oncorhynchus mykiss. Genomic, phylogenetic and phenotypic data obtained from strain H3-26 support the definition of a new Deefgea species, for which the name Deefgea salmonis sp. nov. is proposed.

Materials and methods
Isolation and cultivation of strain H3-26\textsuperscript{T}
In June 2020, a study of pathogenic microorganism of farmed rainbow trout in Lhasa led to the isolation of strain of Deefgea. The sample site was located in the Lhasa, Tibet Autonomous Region, China (29° 36.4’ N, 91° 15.3’ E, Altitude: 3657 m). The water temperature range of sample site is 10.0–18.0 °C. Scraping mucus from the gills of Oncorhynchus mykiss with a sterile scalpel, then the mucus sample...
was diluted and spread on R2A agar medium at 15 °C. The R2A agar medium (g/L) contained: yeast extract 0.5 g, peptone 0.5 g, casein hydrolysate 0.5 g, dextrose 0.5 g, soluble starch 0.5 g, dipotassium phosphate 0.3 g, magnesium sulfate 0.024 g, sodium pyruvate 0.3 g, agar 18.0 g, pH value 7.2 ± 0.2. After 10 days of incubation, a milky white-colored colony was collected and named as H3-26T. Strain H3-26T was routinely cultured on R2A agar medium at 15 °C after repeated purifying. The purified strain was preserved at –80 °C with 25% (v/v) glycerol. Strain H3-26T has been deposited at CICC (China Center of Industrial Culture Collection) and JCM (Japan Collection of Microorganisms).

Phylogenetic analysis based on 16S rRNA gene

Genomic DNA of strain H3-26T was extracted using Min-iBEST Bacterial Genomic DNA Extraction Kit Version 2.0 (TaKaRa Biotechnology Co., Tokyo, Japan). Amplification of the 16S rRNA gene was performed using the extracted highly purified genomic DNA as a template under the following conditions: 95 °C for 10 min, followed by 94 °C for 45 s, 56 °C for 45 s, and 72 °C for 90 s for 30 cycles with a final 10 min extension at 72 °C. The PCR products were detected by agarose gel electrophoresis and then sent to GENEWIZ.lnc for sequencing. Primers used for amplification and sequencing of 16S rRNA were 27F/1492R (Lane 1991). The 16S rRNA gene was aligned in EzBioCloud (Yoon et al. 2017a, b). Maximum-likelihood, neighbor-joining, and maximum-evolution trees were constructed using Type (Strain) Genome Server (Meier-Kolthoff et al. 2022).

Genome sequencing and comparative genomic analysis

Deefgea chitinilytica LMG 24817T was obtained from Laboratory of Microbiology, Ghent University (LMG) for genome sequencing. The genomic DNA of strain H3-26T and Deefgea chitinilytica LMG 24817T was sequenced with BGISEQ-500 platform in China Center of Industrial Culture Collection. The genomic sequence information of H3-26T was deposited in EzBioCloud, the draft genome sequences of Deefgea rivuli WB 3.4-79T (JHVM00000000) were obtained from NCBI database. The digital DNA–DNA hybridization (dDDH) values and confidence intervals were calculated using the recommended settings of Genome-to-Genome Distance Calculator (Meier-Kolthof et al. 2013). The average nucleotide identity (ANI) was determined between strains H3-26T and closely related strains of the genus Deefgea using OrthoANIu (Yoon et al. 2017a, b). The whole-genome evolution trees were constructed using Type (Strain) Genome Server (Meier-Kolthoff et al. 2022).

Phenotypic characterization

The phenotypic characteristics of H3-26T were tested on R2A agar in parallel after incubation for 24 h at 25 °C. Cell morphology of strain H3-26T cultured at 25 °C for 24 h was observed by both light microscopy (CX31, Olympus) and scanning electron microscopy (Hitachi FE-SEM SU8010). The temperature for optimal growth was tested at 4–45 °C (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C). The pH range for growth was determined by measuring the OD600 of the culture grown in R2A broth, which was adjusted prior to sterilization to various pH values (pH 3.0–12.0 with an interval of 1.0 units) using appropriate biological buffers (Chung et al. 1995). The salt tolerance was determined with various NaCl concentrations (0, 1, 2, 3, 4, 5, 6, 7, 8, 9 and 10%, w/v). Gram-staining reaction was carried out according to Claus (1992). Oxidase activity was tested by oxidase test strips with 1% (w/v) tetra-methyl-p-phenylenediamine. Catalase activity was determined by bubble production after mixing a loopful of cells with 3% (v/v) H2O2. Hydrolyses of starch and Tween 80 were tested on R2A agar with starch (1%, w/v) and Tween 80 (1%, v/v), respectively. Cell motility was tested by the hanging drop method with 0.2% agar. Anaerobic growth was checked using the Oxoid AnaeroGen system. Other tests to determine the biochemical characteristics were carried out using API 50CH, API 20E, API 20NE, and API ZYM strips according to the manufacturer’s instructions (BioMérieux).

Determination of fatty acid profiles

After incubation on R2A at 25 °C for 48 h, cells were collected for fatty acids test. Fatty acids were saponified, methylated and extracted according to the standard protocol of the Sherlock Microbial Identification System (MIDI), analyzed via gas chromatography and identified using the Sherlock Aerobic Bacterial Database (RTSBA 6.2B) (Miller 1982).

Results and discussion

Phylogenetic analysis

Compared to the sequences deposited in EzBioCloud, the 16S rRNA gene sequence of strain H3-26T shared highest similarity with Deefgea rivuli WB 3.4-79T (98.42%).
followed by *Deefgea chitinilytica* Nsw-4ⁿ (96.91%). Phylogenetic analysis of H3-26ⁿ based on 16S rRNA genes confirmed its placement within the *Deefgea* genus, to form a separate branch of evolution with a very high bootstrap support (98–100%). A neighbor-joining tree derived from full 16S rRNA alignments is shown in Fig. 1, similar results were obtained using maximum-likelihood and maximum-evolution methods (Figs. S1, S2).

### Genomic characteristics and comparative genomics analysis

The draft genome of strain H3-26ⁿ contained 26 contigs with an N₅₀ value of 387,129 bp and an N₉₀ value of 69,269 bp. The genome size of strain H3-26ⁿ is 3.26 Mb. A total of 2995 genes were predicted in the draft genome of strain H3-26ⁿ. The genomic DNA G+C content of H3-26ⁿ is 48.74 mol%, which is similarity with *Deefgea rivuli* WB 3.4-79ⁿ (48.50%) and *Deefgea chitinilytica* Nsw-4ⁿ (48.29%). The whole-genome evolution tree of H3-26ⁿ and 19 related bacteria shown that *Deefgea salmonis* H3-26ⁿ, *Deefgea chitinilytica* Nsw-4ⁿ and *Deefgea rivuli* WB 3.4-79ⁿ formed a stable evolutionary branch (Fig. 2). Furthermore, the dDDH (d₄) and ANI values between H3-26ⁿ and other related strains were 18.1–22.1% and 68.3–77.4%, which were lower than the threshold values of 70% and 95–96% for species discrimination. The homologous genes analyses of strain H3-26ⁿ, *Deefgea chitinilytica* Nsw-4ⁿ and *Deefgea rivuli* WB 3.4-79ⁿ are shown in a Venn diagram (Fig. S3). 2432, 2604 and 2656 genes were identified as unique genes with no detectable homologs in each other. Both 16S rRNA gene and whole genome in the phylogenetic trees demonstrated that strain H3-26ⁿ had the closest phylogenetic relationship with members of the genus of *Deefgea*.

### Morphological, cultural, physiological and biochemical characteristics

Colonies of strain H3-26ⁿ were milky white, round, moist, translucent, neat edges on R2A solid medium (Fig. S4A). Strain H3-26ⁿ was Gram-stain-negative, aerobic, motile, rod-shaped, single or paired, 0.6–0.9 μm × 0.9–2.7 μm (Fig. S4B–D). Strain H3-26ⁿ grew at 4–30 °C and pH 5.0–11.0.
(optimum, 25 °C and pH 7.0) with 0–1% (w/v) NaCl (optimum, 0%). Strain H3-26T showed many similar phenotypic characteristics with reference strains of Deefgea, but there were a few of differences. Detailed results of the phenotypic and biochemical characterization of strain H3-26T and related species are provided in Table 1. The negative properties of strain H3-26T to API 50CH, API 20E, API ZYM and API 20NE are listed in Table S1.

Fatty acid profiles analysis

The predominant fatty acids of strain H3-26T (>5.0% of the total amounts) were composed of C16:1 ω7c (40.77%), C16:0 (23.07%), C12:0 (5.89%) and C14:0 (5.88%). The major differences between strain H3-26T and its close relatives were shown in Table S2. The presence of C15:0 3-OH, C17:1 anteiso ω9c and C18:3 ω6c could be used to distinguish strain H3-26T from Deefgea rivuli WB 3.4-79T and Deefgea chitinilytica Nsw-4T.

Description of Deefgea salmonis sp. nov

Deefgea salmonis sp. nov. (sal.mo’nis. L.gen.masc.n. salmonis, of salmon)

Cells are Gram-negative, aerobic, rod-shaped, single or paired, 0.6–0.9 μm in width, and 0.9–2.7 μm in length. Colonies grown on R2A at 25 °C for 2 days are milky white, round, moist, translucent, neat edges and 0.8–1.6 mm in diameter. Growth occurs in the presence of 0–1% (w/v) NaCl (optimum, 0% NaCl), pH 5.0–11.0 (optimum, 7.0), and 4–30 °C (optimum, 25 °C). In API
20NE tests, it is positive for reduction of nitrate to nitrite, aesculin hydrolysis and utilization of glucose, mannose, N-acetyl-glucosamine and potassium gluconate. In API ZYM kit, it is positive for esterase (C4), Lipidase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetylglucosaminidase. In API 50CH tests, it is positive for d-ribose, d-glucose, d-fructose, d-mannose, N-acetylglucosamine and potassium gluconate.

The predominant fatty acids of strain H3-26\(^{T}\) (> 5.0% of the total amounts) were composed of C\(_{16:1}\) \(\omega7c\) (40.77%), C\(_{16:0}\) (23.07%), C\(_{12:0}\) (5.89%) and C\(_{14:0}\) (5.88%). The genome size of strain H3-26\(^{T}\) is 3.26 Mb with a low genomic DNA G+C content of 48.74 mol%.

The type strain H3-26\(^{T}\) (=JCM 35050\(^{T}\) = CICC 25103\(^{T}\)) was isolated from gills of rainbow trout in Lhasa, Tibet Autonomous Region, PR China.

The GenBank accession numbers of 16S rRNA gene sequences and whole genome sequence of strain H3-26\(^{T}\) are OK077561 and JAJAWG0000000000, respectively.

Table 1 Differential phenotypic characteristics of strain H3-26\(^{T}\) and its closely related species of the genus Deefgea

| Characteristic                  | 1           | 2           | 3           |
|--------------------------------|-------------|-------------|-------------|
| Cell size (width x length; μm) | 0.6–0.9 x 0.9–2.7 | 0.7–0.9 x 1.9–3.7 | 0.7–0.9 x 1.7–3.2 |
| Source                         | Gills       | Hard-water  | Wetland     |
| Temperature range (optimum) (°C) | 4–30 (25)   | 4–32 (23–28) | 15–37 (25–30) |
| pH range (optimum)             | 5.0–11.0 (7.0) | 5.8–8.5 (7.3–7.6) | 6.0–8.0 (7.0) |
| Anaerobic growth               | –           | +           | –           |
| Glucose acidification          | –           | –           | +           |
| Glucose assimilation           | +           | –           | +           |
| Gluconate assimilation         | +           | +\(^{w}\)   | +           |
| N-acetylglucosamine assimilation | +           | +\(^{w}\)   | +           |
| Nitrate reduction              | +           | +\(^{w}\)   | +           |
| Mannose assimilation           | +           | –           | +           |
| Tryptophan deaminase           | –           | –           | +           |
| Alkaline phosphatase           | –           | ND          | +           |
| Trypsin                        | –           | ND          | +           |
| d-Mannose                      | +           | –           | +           |
| d-Sucrose                      | –           | +           | –           |
| Salicin                        | –           | –           | +           |
| d-Cellobiose                   | –           | –           | +\(^{w}\)   |
| d-Maltose                      | –           | –           | +\(^{w}\)   |
| d-Fucose                       | –           | –           | +\(^{w}\)   |
| Potassium 5-ketogluconate      | –           | –           | +           |
| DNA G+C content (mol%)         | 48.74       | 48.50       | 48.29       |

Strains: 1, H3-26\(^{T}\); 2, Deefgea rivuli WB 3.4-79\(^{T}\); 3, Deefgea chitinilytica Nsw-4\(^{T}\)
+ , Positive; – , negative; +\(^{w}\), weakly positive; ND, not determined. The data of strain H3-26\(^{T}\) were obtained in this study. Data of Deefgea rivuli WB 3.4-79\(^{T}\) and Deefgea chitinilytica Nsw-4\(^{T}\) were taken from Stackebrandt et al. (2007) and Chen et al. (2010)

**Supplementary Information** The online version contains supplementary material available at https://doi.org/10.1007/s00203-022-02980-0.

**Author contributions** YT and HP conceived the project. MC, CZ, JZ, LT and WW performed the experiments. MC and HP analyzed the data, and MC, HP and YT drafted and revised the manuscript. All authors have read and approved the final version of the manuscript.

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**Data availability** All data have been made fully available to the public.

**Declarations**

**Conflict of interest** All the authors declared that they have no conflict of interest.

**Consent to participate** All authors gave their consent to participate in this study.
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