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

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Research Article

Keywords: Deefgea, Oncorhynchus mykiss, Comparative genomics, Tibet

Posted Date: April 5th, 2022

DOI: https://doi.org/10.21203/rs.3.rs-1352369/v2

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Abstract

A Gram-stain-negative, milky-white, aerobic, motile, rod-shaped bacterium named strain H3-26^T was isolated from gills of *Oncorhynchus mykiss* in Lhasa, Tibet Autonomous Region, PR China. Strain H3-26^T grew at 4–30℃ and pH 5.0–11.0 (optimum, 25℃ and pH 7.0) with 0–1% (w/v) NaCl (optimum, 0%). The 16S rRNA gene sequence of strain H3-26^T showed the highest similarity to *Deefgea rivuli* WB 3.4-79^T (98.42%), followed by *Deefgea chitinilytica* Nsw-4^T (96.91%). Phylogenetic analysis based on 16S rRNA genes indicated that strain H3-26^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^T and related strains were 21.20-21.90% and 76.29-77.43%, respectively. The genomic DNA G+C content of strain H3-26^T was 48.29%. The predominant fatty acids were $C_{12:0}$, $C_{14:0}$, $C_{16:0}$ and $C_{16:1}$ ω7c. Based on phenotypic, phylogenetic, and genotypic datas, strain H3-26^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^T (=JCM 35050^T=CICC 25103^T).

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^T and *Deefgea chitinilytica* Nsw-4^T) and one draft genome sequence (*Deefgea* sp. CFH1-16) were published (Stackebrandt et al. 2007, Chen et al. 2010, Han et al. 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 *Oncorhynchus mykiss* in Lhasa, a milky-white bacterium, named strain H3-26, was isolated from the gills of *Oncorhynchus mykiss*. Genomic, phylogenetic and phenotypic datas 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^T

In June 2020, a study of pathogenic microorganism of *Oncorhynchus mykiss* in Lhasa were led to the isolation of a novel species 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℃. 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℃. 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 d of incubation, a milky white-coloured colony was collected and named as H3-26^T. Strain H3-26^T was routinely cultured on R2A agar medium at 15℃ after repeated purifying. The purified strain was preserved at -80℃ with 25% (v/v) glycerol. Strain H3-26^T 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 H3-26^T was extracted using MiniBEST 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℃ for 10 min, followed by 94℃ for 45 s, 56℃ for 45 s, and 72℃ for 90 s for 30 cycles with a final 10 min extension at 72℃, the PCR products were detected by agarose gel electrophoresis and then sent to GENEWIZ.Inc for sequencing. Primers used for amplification and sequencing of 16S rRNA was 27F/1492R (Lane 1991). The 16S rRNA gene was aligned in EzBioCloud (Yoon et al., 2017). Maximum-likelihood, neighbour-joining and maximum evolution trees were constructed using MEGA7.0 software with bootstrap values of 1000 replicates (Felsenstein 1985, Kumar et al. 2016).

Genome sequencing and comparative genomic analysis

Deefgea chitinilytica LMG 24817^T was obtained from Laboratory of Microbiology, Ghent University (LMG) for genome sequencing. The genomic DNA of strain H3-26^T and *Deefgea chitinilytica* LMG 24817^T was sequenced with BGISEQ-500 platform in China Center of Industrial Culture Collection. The genomic sequence information of H3-26^T and *Deefgea chitinilytica* Nsw-4^T had been submitted to the National Centre for Biotechnology Information (NCBI) database under the accession number JAJAWG000000000 and WOFE00000000. Draft genome assemblies were prepared from the ONT reads using Apades v3.11.0, gene prediction using Glimmer 3.02 software.

Based upon the close relationship with the test strain in phylogenetic analyses, the draft genome sequence of *Deefgea rivuli* WB 3.4-79^T (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 H3-26^T and closely related strains of the genus *Deefgea* using OrthANIu (Yoon et al.2017). The whole-genome evolution trees were constructed using Type (Strain) Genome Server (Meier-Kolthoff et al., 2022).

Phenotypic characterization

The phenotypic characteristics of H3-26^T were tested on R2A agar in parallel after incubation for 24 h at 25℃. Cell morphology of strain H3-26^T cultured at 25℃ 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℃ (4, 10, 15, 20, 25, 30 ,37, 40 and 45℃). The pH range for growth was determined by measuring the OD_{600} 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-straining reaction was carried out according to Claus (1992). Oxdase activity was tested by oxidase test strips with 1% (w/v) tetramethyl-p-phenylenediamine. Catalase activity was determined by bubble production after mixing a loopful of cells with 3% (v/v) H_2O_2. 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 by 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℃ 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), analysed via gas chromatography and identified using the Sherlock Aerobic Bacterial Database (RTSBA 6.2B) (Miller 1982).

Results And Discussion

Phylogenetic analysis

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

Genomic characteristics and comparative genomics analysis

The draft genome of strain H3-26^T contained 26 contigs with an N_{50} value of 387129 bp and an N_{90} value of 69269 bp. The genome size of strain H3-26^T is 3.26 Mb. A total of 2995 genes were predicted in the draft genome of strain H3-26^T. The genomic DNA G + C content of H3-26^T is 48.74 mol%, which is similarity with *Deefgea rivuli* WB 3.4-79^T (48.50%) and *Deefgea chitinilytica* Nsw-4^T (48.29%). The whole-genome evolution trees of H3-26^T and 19 related bacteria shown that *Deefgea salmonis* H3-26^T, *Deefgea chitinilytica* Nsw-4^T and *Deefgea rivuli* WB 3.4-79^T formed a stable evolutionary branch (Fig. 2). Furthermore, the dDDH (d4,%) values between H3-26^T and other related strains were 18.1–22.1%, which were lower than the threshold values of 70% for species discrimination. The homologous genes analysis

of strain H3-26^T, *Deefgea chitinilytica* Nsw-4^T and *Deefgea rivuli* WB 3.4-79^T were shown in a Venn diagram (Fig. S3). 2432, 2604 and 2656 genes were identified in the genomes of strain H3-26^T, *Deefgea chitinilytica* Nsw-4^T and *Deefgea rivuli* WB 3.4-79^T, respectively, with 2221 genes shared in all of them. In the genomes of strain H3-26^T, *Deefgea chitinilytica* Nsw-4^T and *Deefgea rivuli* WB 3.4-79^T, 10, 16 and 15 genes were identified as unique genes with no detectable homologous in each other. Both 16S rRNA gene and whole-genome in the phylogenrtic trees demonstrated that strain H3-26^T had the closest phylogenetic relationships with members of the genus of *Deefgea.*

Morphological, cultural, physiological and biochemical characteristics

Colonies of strain H3-26^T were milky-white, round, moist, translucent, neat edges on R2A solid medium (Fig. S4A). Strain H3-26^T was Gram-stain-negative, aerobic, motile, rod-shaped, single or paired, 0.6–0.9 μm×0.9–2.7 μm (Fig. S4B, C and D). Strain H3-26^T grew at 4–30℃ and pH 5.0–11.0 (optimum, 25℃ and pH 7.0) with 0–1% (w/v) NaCl (optimum, 0%). Strain H3-26^T 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 H3-26^T are provided in Table 1 and in the species description.

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×length; μm)	0.6–0.9×0.9–2.7	0.7–0.9×1.9–3.7	0.7–0.9×1.7–3.2
Source	gills	hard-water	wetland
Temperature range (optimum) (℃)	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).

Fatty acid profiles analysis

The predominant fatty acids of strain H3-26^T (≥5.0% of the total amounts) were comprised of $C_{16:1}$ ω7c (40.77%), $C_{16:0}$ (23.07%), $C_{12:0}$ (5.89%) and $C_{14:0}$ (5.88%). The major differences between strain H3-26^T and its closely relatives were shown in Table 2. The presence of $C_{13:0}$ anteiso, $C_{14:0}$ anteiso, $C_{15:0}$ 3-OH, $C_{17:1}$ anteiso ω9c and $C_{18:3}$ ω6c could be used to distinguish strain H3-26^T from *Deefgea rivuli* WB 3.4-79^T and *Deefgea chitinilytica* Nsw-4^T.

Table 2
Comparison of fatty acid profiles of strain H3-26^T and its closely related species

Fatty acid	**1**	**2**	**3**
$C_{12:0}$	5.89	3.2	3.6
$C_{12:0}$ 3-OH	2.95	2.6	3.2
$C_{13:0}$ anteiso	2.00	–	–
$C_{14:0}$ anteiso	1.57	–	–
$C_{14:0}$	5.88	2.1	3.9
$C_{15:0}$ 3-OH	3.34	/	–
$C_{16:0}$	23.07	22.5	24.5
$C_{16:0}$ 3-OH	1.18	0.5	1.4
$C_{16:1}$ ω7c	40.77	51.0	50.9
$C_{17:1}$ anteiso ω9c	–	5.1	3.7
$C_{18:0}$	2.08	2.7	1.1
$C_{18:1}$ ω7c	0.97	3.0	4.1
$C_{18:3}$ ω6c	1.62	–	–

Strains: 1, strain H3-26^T; 2, *Deefgea rivuli* WB 3.4-79^T; 3, *Deefgea chitinilytica* Nsw−4^T. Values are percentages of the total fatty acids. Fatty acids that make up≥0.5% of the total are not shown. "– "Not detected or ≥0.5%. 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).

Description of Deefgea salmonis **sp. nov.**

Deefgea salmonis sp. nov. (sal.mo'nis. L.gen.masc.n. *salmonis*, of *salmon*, since it was first discovered in *salmon*)

Cells are gram-negative, aerobic, motile, 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℃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℃ (optimum, 25℃). Positive for oxidase, catalase. Negative for hydrolysis of starch, motility, tweens 80, β-galactosidases, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, citric acid utilization, Voges-Prokauer test, gelatin, urease, hydrogen sulfide and indole production. Positive reactions (API 20NE) for nitrate reduction, eaculin hydrolysis, assimilation of glucose, mannose, N-acetylglucosamine and potassium gluconate. Positive enzyme reactions (API ZYM) for C4 esterase, C8 esterase, leucine aromatase, acid phosphatase, naphthol-AS-BI-phosphohydrolase and N-acetylglucosaminidase. The following compounds are utilized in the API 50CH test system: 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 comprised of $C_{16:1}$ ω7c (40.77%), $C_{16:0}$ (23.07%), $C_{12:0}$ (5.89%) and $C_{14:0}$ (5.88%).

The type strain H3-26^T (= JCM 35050^T = CICC 25103^T) was isolated from gills of *Oncorhynchus mykiss* in Lhasa, Tibet Autonomous Region, PR China. 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 GenBank accession numbers of 16S rRNA gene sequences and whole genome sequence of strain H3-26^T are OK077561 and JAJAWG000000000, respectively.

Declarations

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.

Funding

Natural Science Foundation of Tibet (XZ2019ZRG-80(Z)) and the "Double First-Class" construction project of Hunan Agricultural University (SYL201802002) funded this work.

Data availability

All data have been made fully available to the public.

Conflict of interest

All the authors have declared no conflict of interest.

Consent to participate

All authors gave their consent to participate in this study.

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Figures

Figure 1

Neighbor-joining tree based on 16S rRNA gene sequences revealing the relationship between strain H3-26^T and other species of the genus *Deefgea*. *Cupriavidus taiwanensis* LMG 19424^T was used as an out-group. Bootstrap values ≥ 50% are shown. Bar, 0.01 substitutions per nucleotide position.

Figure 2

The whole-genome evolution trees of H3-26^T and related bacteria with dDDH (d4,%) values

Supplementary Files

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