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Lautropia dentalis sp. nov., Isolated from Human Dental Plaque of a Gingivitis Lesion

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Abstract

A novel Gram-stain-negative, motile, and facultative anaerobic coccus, strain ChDC F240^T was isolated from human subgingival dental plaque of a gingivitis lesion. The phylogenetic analysis based on the 16S ribosomal RNA gene (16S rDNA) sequence showed that the strain belonged to the genus *Lautropia*. 16S rDNA of strain ChDC F240^T had the highest similarity to that of *Lautropia mirabilis* ATCC 51599^T (98.8%). Major cellular fatty acids of strain ChDC F240^T were $C_{16:0}$ (43.9%) and $C_{16:1\omega6C}/C_{16:1\omega7C}$ (38.1%). Draft genome of the strain was 3,834,139 bp in length and the G+C content was 65.0 mol%. Average nucleotide identity and genome-to-genome distance values between strain ChDC F240^T and *L. mirabilis* ATCC 51599^T were 81.99% and 28.50% (26.1–30.9%), respectively. These results reveal that strain ChDC F240^T is a novel species within the genus *Lautropia*, for which the name *Lautropia dentalis* sp. nov. is proposed; type strain is ChDC F240^T (= KCOM 2505^T = JCM 33297^T).

Introduction

The genus *Lautropia* is Gram-stain-negative and facultative anaerobic coccus. It was isolated from a human oral cavity [6]. *Lautropia mirabilis* has been the only *Lautropia* sp. till date (https://www.bacterio.net/lautropia.html). Six strains of *L. mirabilis* were isolated from human saliva sampled from the back of the tongue [6]. This species was also isolated from the sputum of a patient with cystic fibrosis [2] and from the oral cavities of human immunodeficiency virus-infected children [13].

Strain ChDC F240^T was isolated from the human subgingival dental plaque of a gingivitis lesion in right maxillary first molar of a male from the Republic of Korea in 2003. According to polyphasic taxonomic characterization, strain ChDC F240^T represents a novel species of the genus *Lautropia*.

Materials and Methods

Bacterial Strain and Culture Conditions

Strain ChDC F240^T was grown on tryptic soy agar (TSA; BD Difco Laboratories, Franklin Lakes, NJ, USA) plate supplemented with 0.5% yeast extract, 0.05% cysteine HCl–H_2O,

YK Lim and S-N Park contributed equally to this study.

DPD number: TA00934. GenBank accession number of 16S rRNA gene for strain ChDC F240^T: MK748163. GenBank accession number of genome for strain ChDC F240^T: RRUE01000000.

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0.5 mg/ml hemin, and 2 µg/ml vitamin K_1 (TSA-YCHVk) [4] at 37 °C in an anaerobic chamber (Bactron I, Sheldon Manufacturing Inc., Cornelius, OR, USA) under 10% H_2, 5% CO_2, and 85% N_2.

Phylogenetic Analysis

The 16S ribosomal RNA gene (16S rDNA) strain ChDC F240^T was cloned and sequenced using PCR and the dideoxy chain termination method as described previously [8]. 16S rDNA sequences of type strains of *L. mirabilis* and other bacterial species were obtained from GenBank (Fig. 1). Multiple sequences were aligned using the CLUSTAL W algorithm and sequence similarities were calculated using the MegAlign program (DNAStar Lasergene™ 8.0, DNAStar Inc., Madison, WI, USA) [4]. Evolutionary distance was calculated according to the Kimura two-parameter model [9] and phylogenetic trees were constructed by the neighbor-joining method [14] in MEGA 6.06 software [15]. Stability of the phylogenetic trees was assessed by bootstrap analysis [5] of 1000 replicates.

Genome Sequence

Genomic DNA of strain ChDC F240^T was prepared using the phenol–chloroform extraction method as described previously [4]. The genomic DNA sequencing of the strain was carried out using the Illumina Hiseq 2500 platform by the next-generation sequencing service of the Macrogen Inc. (Seoul, Korea). Two libraries of 350 bp paired-end and 8 kb mate-pair were constructed and sequenced which generated approximately 1127 Mb (294.0×) with 11,160,068 read and 321 Mb (83.9×) with which 3,834,139 reads, respectively. The de novo assembly was performed by SPAdes (https://bioinf.spbau.ru/spades) [1]. All gaps among the scaffolds were filled by GapCloser (https://soap.genomics.org.cn/soapdenovo.html) [11]. Error correction was performed by Pilon (https://platanus.bio.titech.ac.jp/platanus-assembler) [7]. Genome annotation was conducted using the NCBI Prokaryotic Genome Annotation Pipeline through the NCBI Genome Submission Portal (GenomeSubmit at https://ncbi.nlm.nih.gov) [16]. The genome sequence of strain ChDC F240^T was deposited at GenBank (Accession Number: RRUE01000000).

Pairwise Genome Comparisons

Average nucleotide identity (ANI) and genome-to-genome (GGD) analyses were performed to discriminate strains ChDC F240^T from *L. mirabilis* at the species level as previously described [10, 12]. GenBank accession number of *L. mirabilis* ATCC 51599^T was NZ_AEQP00000000.1, which was downloaded from GenBank (https://www.ncbi.nlm.nih.gov/genome).

Morphological, Physiological, and Chemotaxonomic Characterization and Biochemical Analysis

Cell shape and size were determined under a field-emission scanning electron microscope (S-4800, Hitachi, Tokyo, Japan) as previously described [4].

Flagella were stained using Leifson stain solution (1.2% basic fuchsin, 0.75% sodium chloride, and 1.5% tannic acid) and methylene blue as previously described [3]. Light microscope image was taken using a CX43 Biological Microscope (Olympus, Tokyo, Japan).

Optimal growth conditions of strain ChDC F240^T that depended on the temperature, pH, and NaCl concentration were investigated as previously described with minor modifications [4]. Briefly, growth at different temperatures (25–55 °C at intervals of 5 °C and 37 °C) and various pH values (5–9.5 at intervals of 0.5) were determined on TSB-YCHVk

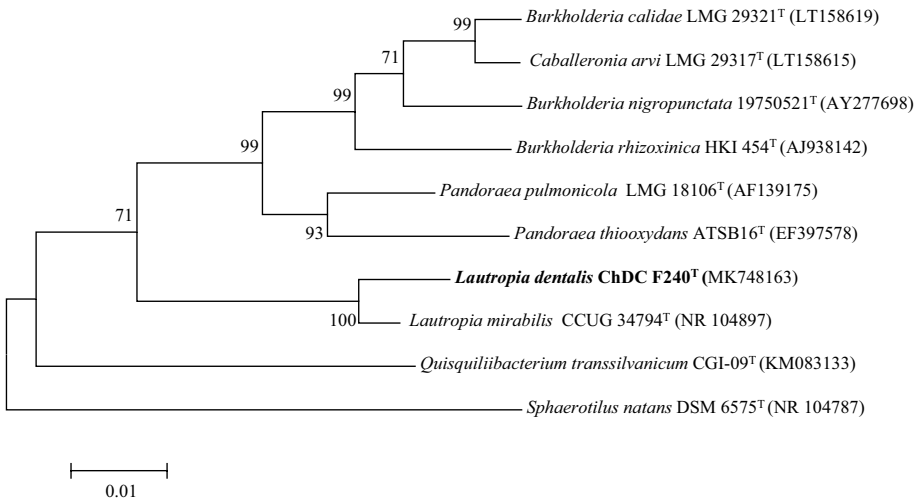

Fig. 1 Neighbor-joining phylogenetic tree based on 16S rDNA sequences of strain ChDC F240^T and type strains of related species. Stability of the tree was assessed using bootstrap analysis of 1000 replicates with MEGA version 6.06 [15]. Bar = 0.01 changes per nucleotide position. GenBank accession numbers of 16S rDNA sequences of strains were given in parenthesis

Table 1 Cellular fatty acid compositions of strain ChDC F240^T and *Lautropia mirabilis*

Characteristic	ChDC F240^T	*Lautropia mirabilis*	
		Mean ($n = 10$)	Range
10:0	–	T	T
10:0 3OH	2.3	2	1–3
12:0	2.4	3	2–6
12:0 3OH	1.3	–	–
14:0	2.8	3	2–4
15:0	–	1	T–3
16:0	**43.9**	**44**	**35–48**
15:0 iso 3OH	2.5	–	–
18:1 ω9c	0.3	–	–
18:0	0.7	–	–
20:4 ω6,9,12,15c	0.6	–	–
16:1 ω7c	–	**41**	**34–48**
Summed feature 3 (16:1 ω7c/16:1 ω6c)	**38.1**	–	–
Summed feature 5 (18:0 ante/18:2 ω6,9c)	0.5	–	–
18:1 ω7c	–	4	2–6
Summed feature 8 (18:1 ω7c/18:1 ω6c)	4.5	–	–

Values are expressed as percentages of fatty acids. The data of *L. mirabis* were obtained from Gerner-Smidt et al. [6]. Symbol: – not detected, *T* trace amount (0.4–0.8%). Number in bold is the major fatty acid components of each strain

agar medium for three days in an anaerobic condition. Growth at various NaCl concentrations was assessed in the TP-YCHVk agar medium containing 0, 1, 2, 3, 4, or 5% (w/v) NaCl (pH 7 at 37 °C) for three days in an anaerobic condition.

The cellular fatty acids composition was determined using the MIDI/Hewlett Packard Microbial Identification System (MIDI, Microbial ID, Newark, DE, USA) as previously described [4].

API 20NE and GNI systems (bioMerieux, Marcy-l'Etoile, France) were used to analyze biochemical traits of strain ChDC F240^T according to the manufacturer's instructions.

Results and Discussion

Phylogenetic analysis based on 16S rDNA revealed that strain ChDC F240^T belonged to the genus *Lautropia* (Fig. 1). Stability of the resulting tree was confirmed by maximum likelihood and minimum evolution methods (Supplementary Fig S1). 16S rDNA sequence of strain ChDC F240^T had the highest similarity to that of *L. mirabilis* ATCC 51599^T (98.8%, Supplementary Table S1). Draft genome size of strain ChDC F240^T was 3,834,139 bp in length, which was longer than that of *L. mirabilis* ATCC 51599^T (3,137,198 bp). The average G+C content of the strain was 65.0 mol%, similar to those of the strains of *L. mirabilis* (65.4–65.3 mol%) [6]. ANI and GGD values between strain ChDC F240^T and *L. mirabilis* ATCC 51599^T were 81.99% and 28.50% (26.1–30.9%), respectively. Considering that threshold values of ANI and GGD for bacterial species classification were 95–96% and 70%, respectively [10, 12], these finding reveal that strain ChDC F240^T is a novel species belonging to the genus *Lautropia*.

The cellular fatty acid profile of strain ChDC F240^T revealed that $C_{16:0}$ (43.9%) and $C_{16:1\omega6C}/C_{16:1\omega7C}$ (38.1%) were predominant (Table 1). Cellular fatty acid composition of strain ChDC F240^T was similar to those of the strains of *L. mirabilis* (Table 1).

Flagella staining result revealed that strain ChDC F240^T possessed lophotrichous flagella (Supplementary Fig. S2).

API 20NE and GNI test results were summarized in Supplementary Tables S2 and S3, respectively. Strain ChDC F240^T could be discriminated by the urease activity and acid production from D-glucose from *L. mirabilis* (Table 2).

Based on these results, strain ChDC F240^T represented a novel species of the genus *Lautropia*, for which the name *Lautropia dentalis* is proposed.

Description of *Lautropia dentalis* sp. nov.

Lautropia dentalis de'n.ta.lis. L. n. *dens dentis*, a tooth; L. fem. suff. *-alis*, suffix denoting pertaining to; N.L. fem. adj. *dentalis*, pertaining to teeth.

Colonies grown on TSA-YCHVk agar are opaque, crisp, and convex with irregular margins. They spread to diameters of sizes that ranged from 0.04 to 0.8 mm after three days at 37C. Cells are Gram-stain-negative, facultative

Table 2 Biochemical characteristics of strain ChDC B114^T and *Lautropia mirabilis*

Characteristics	ChDC F240^T	AB2188^T
Reduction of nitrates to nitrites	+	+
H_2S production	–	–
Enzyme activity		
Urease	–	+
β-Galactosidase	–	–
β-Glucuronidase	–	–
Ornithine decarboxylase	–	–
Lysine decarboxylase	–	–
Acidification		
Adonitol	–	–
D-Glucose	–	+
D-Maltose	+	+
D-Mannitol	+	+
D-Sorbitol	–	–
Sucrose	+	+
D-Trehalose	–	–

The data of *L. mirabis* AB2188^T were obtained from Gerner-Smidt et al. [6]. Symbols: + positive, *w* weakly positive, – negative

anaerobic, motile, and spherical bacteria with a typical cell size of 2.09 ± 0.75 µm in diameter. Temperature range for their growth was 25–37 °C (optimum: 30–37 °C). The pH range for their growth was 6.5–8.5 (optimum; 7.5). Growth occurred in the presence of 0–1% (w/v) NaCl (optimum: 0.05%). In the API 20NE system, positive reaction is reduction of nitrates to nitrites. From the GNI test results, glutamyl arylamidase and γ-glutamyl-transferase are present. Acid is produced from D-mannitol, D-maltose, and sucrose. The Ellman test is positive. Major cellular fatty acids are $C_{16:0}$ and $C_{16:1\omega6C}/C_{16:1\omega7C}$.

Type strain ChDC F240^T (= KCOM 2505^T = JCM 33297^T) was isolated from human subgingival dental plaque of a gingivitis lesion in the Republic of Korea. The G+C content of type strain is 65.0 mol%.

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Compliance with Ethical Standards

Conflict of interest The authors declare that they have no conflicts of interest.

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