Aureispira anguillae sp. nov., isolated from Japanese eel Anguilla japonica leptocephali

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ORIGINAL PAPER

Aureispira anguillae sp. nov., isolated from Japanese eel *Anguilla japonica* leptocephali

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Abstract
A novel filamentous eel-leptocephalus pathogenic marine bacterium, designated strain EL160426^T, was isolated from Japanese eel, *Anguilla japonica*, leptocephali reared at a laboratory in Mie, Japan. In experimental infection studies on eel larvae, the strain EL160426^T caused massive larval mortality and was reisolated from moribund leptocephali. Characteristically, observations of infected larvae found that EL160426^T forms columnar colonies on the cranial surface of larvae. The novel isolate exhibited growth at 15–30 °C, pH 7–9, and seawater concentrations of 60–150% (W/V). Phylogenetic analysis based on 16S rRNA gene sequences indicated that strain EL160426^T was most closely related to *Aureispira maritima* 59SA^T with 97.7% sequence similarity. The whole genome sequence analysis of the strain EL160426^T showed that the strain maintained a circular chromosome with a size of approximately 7.58 Mbp and the DNA G + C content was 36.2%. The major respiratory quinone was MK-7 and the predominant cellular fatty acids were 16:0, 20:4 w6c (arachidonic acid), 17:0 iso and 16:0 N alcohol. DNA relatedness between the closest phylogenetic neighbor strain EL160426^T and *A. maritima* (JCM23207^T) was less than 13%. On the basis of the polyphasic taxonomic data, the strain represents a novel species of the genus *Aureispira*, for which the name *Aureispira anguillae* sp. nov. is proposed. The type strain is EL160426^T (= JCM 35024^T = TSD-286^T).

Keywords *Aureispira anguillae* sp. nov · Arachidonic acid · Pathogenic marine bacteria · Leptocephali · Japanese eel · *Anguilla japonica*

Abbreviations

MK	Menaquinone
JCM	Japan Collection of Microorganisms
TSD	Type Strain Deposit number in the American Type Culture Collection (ATCC)

Introduction

The Japan Fisheries Research and Education Agency succeeded in the completion of the life cycle of Japanese eel, *Anguilla japonica* in captivity, for the first time in the world in 2010 (Tanaka 2015). Since 2014, the Agency has been researching the seed production techniques of Japanese eels to develop a mass-production system of glass eels. A variety of aquaculture research was conducted to learn how to spawn the artificially matured adults and rear the leptocephalus larvae of the Japanese eel (Okamura et al. 2014; Tanaka 2015), which are unusual compared to other types of fish larvae (Miller 2009), in part due to their fragile gelatinous bodies and unusual feeding preferences (Tsukamoto and Miller 2020). Various problems were encountered during

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the refinement of culturing techniques included notochord deformities (Okamura et al. 2011) and open lower jaw angle problems (Okamura et al. 2007). It was also found that sterile conditions in the rearing tanks were important and that the reared larvae were susceptible to some types of bacteria (Nakase et al. 2015).

In 2016, the first case of mortality from infection by filamentous bacteria was observed in leptocephali of Japanese eels that were spawned and reared at the Nansei branch of the Fisheries Technology Institute, Fisheries Research and Education Agency in Mie, Japan. The bacteria infecting the body surface of the affected fish were larger in morphology than known piscine filamentous or gliding bacteria including the genera *Flavobacterium* and *Tenacibaculum* in the order Flavobacteriales, and were up to 100 μm in length. Numerous cells of the filamentous bacterium were observed on the body surfaces of moribund or deceased leptocephali when mortality occurred during seed production. Diseases of eel leptocephali are generally less understood than those of adult eels, because trial cases of rearing of eel leptocephali have been limited. To know the properties of fish disease bacteria is important for implementing epidemic-prevention measures in an aquaculture site. Therefore, we isolated the pathogen affecting the Japanese eel leptocephali and then identified the bacterium as *Aureispira* sp. using 16S rRNA gene sequence analysis.

The small genus *Aureispira* is known as being arachidonic acid-producing marine bacteria in the family Saprospiraceae and has two species, *A. marina* (Hosoya et al. 2006), and *A. maritima* (Hosoya et al. 2007). Both species were isolated from marine materials including a sponge, algae and barnacle debris along the southern coastline of Thailand. Except for these species, a strain of genus *Aureispira*, CCB-QB1 was isolated from seaweed obtained from the Queens Bay coastal region of Penang, Malaysia (Furusawa et al. 2015a). The genus *Aureispira* does not include any piscine parasitic bacteria, although species of *Aureispira* have previously been suggested to be bacteria-predatory filamentous bacteria (Yeoh et al. 2021) by using ixotrophy (Furusawa et al. 2015b). In this study, we attempted to characterize a novel piscine parasitic filamentous bacterium in comparison with the genus *Aureispira* including *A. marina* and *A. maritima*, because the sequences of 16S rRNA of both species were close to that of this species.

Materials and methods

Isolation, culture and preservation

The novel bacterial strain EL160426^T was isolated from moribund leptocephalus larvae of the Japanese eel using modified cytophaga agar supplemented with seawater (mCySW agar) [0.7 × natural seawater, 0.2% tryptone, 0.05% yeast extract, 0.02% beef extract, 0.02% KCl, 0.02% sodium acetate and 1.5% agar]. The samples of Japanese eel leptocephali were collected from the seed production aquaria in the institute (34°33′46″ N 136°69′18″ E) on 26 April 2016, when mortality from the bacterial infection occurred. The rearing water temperature was approximately 23 °C. A leptocephalus that showed typical symptoms of the disease is shown in Fig. 1. Bacterial cells were observed to form columnar colonies on the cranial surface of leptocephali in infected larvae (Fig. 2). To isolate pathogenic bacteria, the whitish head of the larvae where the infection with the filamentous bacteria was observed by light microscope was smeared on the agar and incubated at 25 °C for a week. To purify the bacteria, a fingerprint-like colony that may have been formed due to the filamentous structure of the bacteria was taken and spread on another mCySW agar using a sterilized cotton swab. Subcultures of the purified bacteria were made 6 times on the mCySW agar, and then the bacteria were preserved in mCySW broth supplemented with 20% glycerol at – 80 °C until using in the experimental infection tests. Strain EL160426^T was then subcultured on the mCySW agar at 25 °C once a week for each taxonomic test. The EL160426^T strain was deposited in the American Type Culture Collection (ATCC) under reference TSD-286^T and the Japan Collection of Microorganisms (JCM) under reference JCM 35024^T. As a reference strain for the DNA-DNA hybridization experiment, *A. maritima* (JCM23207^T) was purchased from the JCM and grown on mCySW agar at 25 °C.

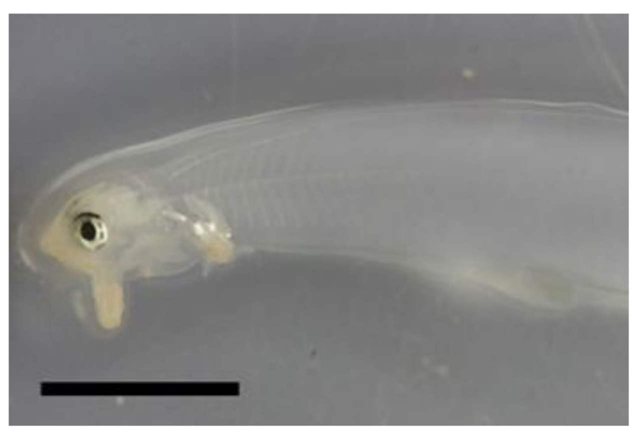

Fig. 1 Visible signs of infection by the filamentous bacterium on an affected Japanese eel leptocephalus, shown by whitish or opaque head color. This specimen shows a morphological abnormality of the lower jaw, which keeps the mouth open. Bar indicates 5 mm

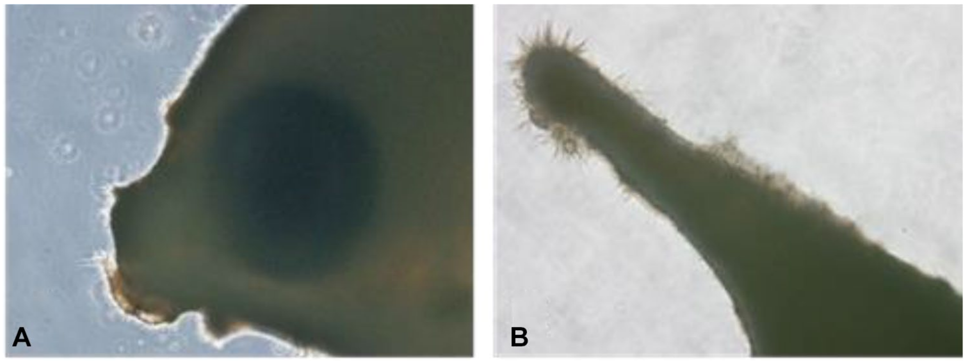

Fig. 2 Microscopic images of the lesions with infections of the filamentous bacteria in affected leptocephali showing **A** the rostrum and the maxillary (×40), and **B** the mandibular of the lower jaw (×100). Several columns of bacteria are seen on the surface of each area

Histopathology

Six moribund leptocephali with whitish head (3.0–4.5 cm in total length) after natural infection were fixed in Davidson's solution for 24 h. Paraffin sections were made by the usual method and stained with hematoxylin and eosin (HE) or May-Grünwald-Gimsa (MG) stain. Additionally, the leptocephali in the experimental infection described below were also used for histopathology. Each of the 3 fish that were exposed or non-exposed to the bacteria were sampled at 12- and 24-h post exposure to bacteria (hpe). Procedures for preparing the sections and staining in the experimental infection tests were the same as for natural infection.

Pathogeny of the new isolate in experimental infection

The isolate of filamentous bacteria EL160426^{T} was used for experimental infections to confirm the pathogenicity of the isolate to leptocephali. The isolate was cultured in 250 mL mCySW broth with stirring at 25 °C for 3 d and the bacterial cells were collected by centrifuging at 3,000*g* for 5 min. The amount of the cells collected was approximately 0.8 g in wet weight. All the collected cells were then added to a 10 L tank containing 16 leptocephali (approximately 3 cm in total length) in 5 L of seawater. Then, the leptocephali were kept in the bacterial suspension for 1 h. After the immersion, the tank was supplied by free-flowing sand-filtered seawater at 25 °C. As a control, 16 larvae were reared in another 5 L of seawater in the same type of tank that was supplied with the same water. At 12 and 24 hpe, 3 larvae were sampled from the infected aquarium. At the same time, 3 larvae were sampled from the control aquarium. The mortality of the 10 larvae in each aquarium was monitored from 12 and 120 hpe and moribund or dead individuals were sampled for bacterial isolation. To confirm the state of bacterial infection, the heads of moribund or dead leptocephali were histologically observed.

16S rRNA gene amplification and phylogeny

The 16S rRNA gene sequence of the isolated EL160426^{T} bacteria was analyzed to characterize its taxonomic position. DNA was extracted from a single bacterial colony using a Maxwell® 16 benchtop instrument (Promega, Madison, WI, USA). The 16S rRNA gene fragments (Approx. 1500 bp) were amplified by PCR using a KOD -Plus- Ver. 2 (Toyobo, Japan) with universal primers (27F: 5′- AGA GTT TGA TCM TGG CTC AG -3′ and 1492R: 5′- GGY TAC CTT GTT ACG ACT T -3′) (Weisburg et al. 1991). The reaction condition was as follows: 94 °C for 2 min, 53 °C for 30 s, and 68 °C for 90 s for 35 cycles. The PCR products were treated using an illustra ExoProStar (GE Healthcare Life Sciences, Pittsburgh, PA, USA) and sequenced directly with 27F/1492R and additional two universal primers (341F: 5′-CCT ACG GGA GGC AGC AG -3′ and 800R: 5′-TAC CAG GGT ATC TAA TCC-3′) using the ABI PRISM 3130xl sequencer (Applied Biosystems, Foster City, CA, USA). The sequence was analyzed using the homology search of the blast server of the National Center for Biotechnology Information (Bethesda, Maryland, USA). The 1.3 kbp nucleotide sequences of the 16S rRNA genes (covering base positions 98–1427, *E. coli* numbering) were used for phylogenetic analysis. Nucleotide substitution rates were calculated using the maximum composite likelihood method (Tamura et al. 2004). Alignment gaps and primer regions for PCR amplification were not taken into consideration for the calculations. The phylogenetic tree was drawn using the MEGA X program v10.1.8 (Kumar et al. 2018) by the neighbor-joining (NJ) method (Saitou and Nei 1987) and the maximum likelihood (ML) method (Felsenstein 1981). The topology of the phylogenetic trees was evaluated by the bootstrap resampling method with 1000 replicates (Felsenstein 1985).

Genome sequencing and analysis

Whole genome sequencing (WGS) was performed on a flow cell (R9.4) with a MinION instrument (Oxford Nanopore Technologies). In total, 773,893 reads with an average length of 1656 bp were obtained, and the assembly was performed using the Long Read Support plugin of CLC Genomics Workbench v21.0.3. The pair-end sequencing with an illumina HiSeq platform for genome polishing was outsourced to Novogene Co. Ltd., China. A total of 10,974,474 reads were mapped to the draft genome and were polished using CLC Genomics Workbench. Gene annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Tatusova et al. 2016). The genomic relatedness was estimated based on the average nucleotide identity (ANI) that was calculated using the ANI calculator (Goris et al. 2007).

DNA-DNA hybridization and G + C content

To analyse the genetic relationship between the EL160426^T strain and *A. maritima* (JCM23207^T) that is the closest phylogenetic neighbor, DNA-DNA hybridization with DNA extracted by NucleoSpin Plant II (MACHEREY–NAGEL, DE) was conducted according to the methods of Ezaki et al. (1989).

The DNA G + C content of strain EL160426^T was calculated from the whole-genome sequence. In addition, the DNA G + C content was evaluated by the method of Katayama-Fujimura et al. (1984) with the DNA of the strain extracted using the same kit as for DNA-DNA hybridization, since the data of the DNA G + C contents by Hosoya et al. (2006) and Hosoya et al. (2007) were not based on whole genome sequences.

Morphological, physiological and biochemical characteristics

To determine cell morphology and motility of the isolated bacteria, cells were grown in mCySW broth at 25 °C for 2 days and observed using light microscopy. Gram reaction was examined using a Gram stain kit (Nissui, Tokyo). The phenotypic characteristics of the isolate were performed as follows. Salt tolerance was examined using Sap2 agar (containing 0.1% tryptone, 0.1% yeast extract, and 1.5% agar) supplemented with different concentrations of artificial sea water (ASW; 0, 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 110, 120, 130, 140, 150, 160, 170, 180, 190, and 200% v/v, Marine Art SF-1, Tomita Pharmaceutical Ltd.). Growth at different temperatures (5, 10, 15, 20, 25, 30, 35, and 37 °C) were examined using Sap2 agar supplemented with ASW since the isolate required high salinity for growth. Growth at different pH values was examined using Sap2 agar supplemented with ASW at pH5, 6, 7, 8, 9 and 10. Oxidase and catalase activities, the degradation of casein, DNA, starch, Tweens 20, 40, 60 and 80 and L-tyrosine, hydrolysis of agar and carboxymethyl cellulose, and acid production from carbon sources were tested by the method of Hosoya et al. (2006) that was mostly based on Barrow and Feltham (1993) and Smibert and Krieg (1994), except all media used were supplemented with ASW. For the nitrate reduction test, KNO_3 broth (0.1% peptone, 0.05% beef extract, and 0.1% KNO_3) supplemented with ASW was prepared. Biochemical characteristics in the new isolate were studied using the commercial systems API ZYM and API 20E (bioMe´rieux) according to the manufacturer's instructions. The incubation temperature and time were the same as the method of Hosoya et al. (2006).

Chemotaxonomic characterization

For analysis of the cellular fatty acids, cells were grown for 48 h at 25 °C in mCySW broth, washed by distilled water three times, freeze-dried and used for the analysis according to the manual of Sherlock Microbial Identification System (Version 6.0) (MIDI, USA; Sasser 1990).

Respiratory quinones were extracted by the method of Bligh and Dyer (1959) and were purified by a solid-phase extraction column (Sep-Pak plus silica, Waters, USA). The purified respiratory quinones were analyzed by HPLC (ACQUTTY UPLC-Class system, Waters, USA) according to the method of Komagata and Suzuki (1987).

Sequence deposition

The 16S rRNA gene and complete genome sequences of the EL160426^T strain are available in GenBank under the accession numbers LC645360 and AP026867 to AP026869, respectively (AP026867 for chromosome, AP026868 for plasmid pAUEa and AP026869 for plasmid pAUEb).

Results and discussion

Morphological characteristics of the new isolate

The novel isolate formed yellowish colonies on agar plates and the cells were Gram-negative (Fig. 3A), aerobic, nonsporulating, and 0.4–0.6 by 1.4–2.4 µm in size. Helical cells were 1.5–2.0 µm in width and 30–110 µm in length with a twist occurring every 5–6 µm (Fig. 3B). Cells showed gliding or pivot movement in broth.

Pathogeny of the new isolate in experimental infection

Affected leptocephali from the natural infection were characterized by a whitish coloration of the head, especially on the rostrum area including the maxillary and mandibular of the upper and lower jaws, and the larvae exhibited sluggish behavior. The infection was often observed in the

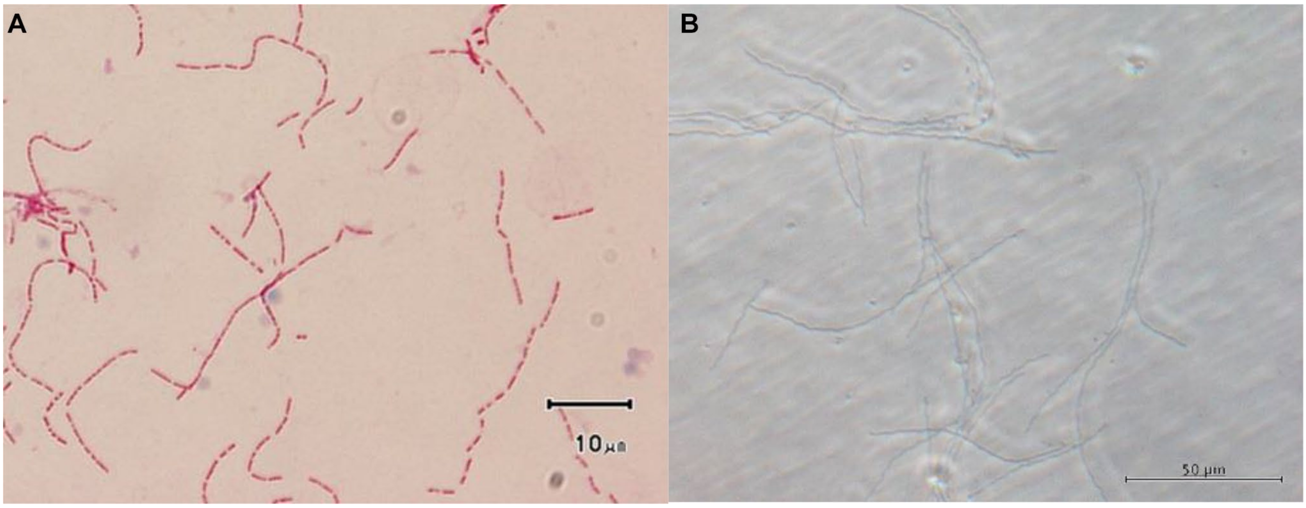

Fig. 3 Micrographs of cells of strain EL160426^T showing **A** an image of strain EL160426^T with gram stain. The bacterium shows gram negative and long filamentous morphology that are likely composed of short rods, 0.8–1.5 µm in length. Bar indicates 10 µm. **B** Image of strain EL160426^T in bright field, which was grown in mCySW broth at 25 °C for 48 h. Bar, 50 µm

leptocephali with a morphological deformity of the mandibular, suggesting that the deformity may be a trigger of the infection. This deformity of the lower jaw being locked in a downward position has frequently been seen in cultured Japanese eel leptocephali (Okamura et al. 2011), but the relationship between the jaw abnormality and the bacterial infection has not been previously reported. Histopathological findings indicated that the tissue damage was limited to the area from the rostrum to the nasal part of the head in individuals with mild natural infection. The epidermis of the head was detached from the connective tissue at the rostrum part of the maxillary and/or mandibular, and the epithelium of the olfactory organ was disintegrated. Degenerated tissue fragments (debris) adhered to the rostrum (Fig. 4A). Various forms of bacterial cells were found in the debris, but long rod-shaped bacilli or filamentous bacteria were dominant in the connective tissue (Fig. 4B). The size of the long bacilli or filamentous cells reached 40 µm and their width was 0.4–0.6 µm, which is consistent with the isolate in morphology. The filamentous cells in the tissues were likely composed of short rods, which was the same feature as the isolate (Fig. 3A). Neither significant tissue degeneration nor bacterial infection was observed in the brain, eyes, gills, or most of the tissues of the head other than the rostrum. No abnormalities were observed in the tissues of the trunk and tail. In individuals with heavy natural infection, a wide area of epidermis of the head including the mandibular was detached from the connective tissues. The long bacilli or filamentous bacteria spread over the surface of the connective tissues where the epidermis was detached (data not shown). The gill tissue was disintegrated with infection by the filamentous bacteria. The brain and retina were degenerated, but the bacteria were rarely observed. The histological abnormalities were observed mainly in the head and the anterior trunk, but did not extend to the posterior trunk or tail. From these histopathological findings, it was considered that affected fish were infected by a unique kind of bacteria, suggesting that the cause of mortality of Japanese eel leptocephali was an infection with the filamentous bacteria.

In the experimental infection tests, mortality in the infected aquarium containing 16 leptocephali was 4 at 48 hpe and 6 at 72 hpe ($n = 10$). No mortality was observed in the control aquarium. The affected larvae showed the same gross signs as during the natural infections and filamentous bacteria column formation on the rostrum was observed. The filamentous bacteria were reisolated from moribund fish in the infected aquarium and the sequence of re-isolated bacteria was identical with the isolate used for the experimental infection. In histology, the filamentous bacteria were also detected on the surface of the rostrum of the head in three specimens sampled at 12 hpe (data not shown). More severe infection with the bacteria was detected over a wide area of the head in three specimens sampled at 24 hpe (data not shown). No pathogen except the filamentous bacteria was observed in these six specimens. In the three specimens of the control, all tissues were normal without infection with the bacteria. Histological images of specimens with the experimental infection were the same as those of the natural infection, which indicated that the infection reappeared during the experimental infection with the isolate.

These results indicate that the isolate is a causative agent of the cases of mortalities in the leptocephali observed

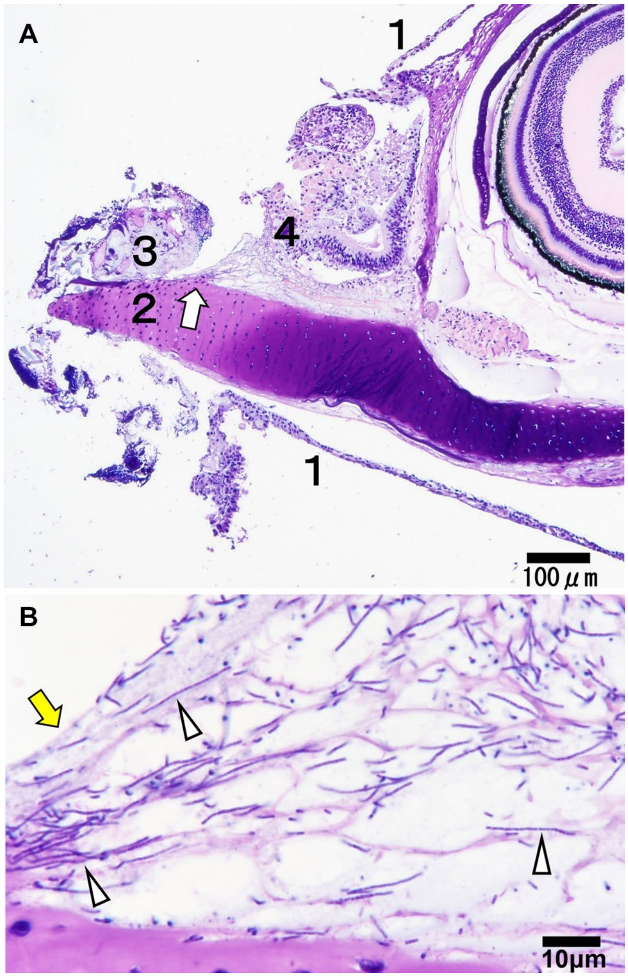

Fig. 4 Histopathological image of a cross-section of the head mildly infected with *Aureispira* sp. after MG staining, **A** a part of the head with low magnification and **B** a high magnification image of the area indicated by the white arrow in **A**. In **A** (1) indicates epidermis, which is missing at the rostrum part in section (2). The debris of the epidermis (3) is observed on the surface of the cartilage tissues. (4) shows the epithelium of the disintegrated olfactory organ. In **B**, the yellow arrow shows the degenerated connective tissue with the epidermis being missing. Numerous filamentous bacteria are observed inside the tissue. The arrowheads show parts of bacterial cells

during the seed production of Japanese eels in this particular case observed at the laboratory in Mie. This is the first report we are aware of in which a serious pathogenic bacteria of eel leptocephali was successfully isolated and identified and seems to be the first example of *Aureispira* sp. being pathogenic to fish.

16S rRNA gene sequence and phylogenetic analysis

The result of the phylogenetic analysis based on 16S rRNA gene sequences showed that the isolate was placed in the genus *Aureispira* belonging to the family Saprospiraceae, and it clustered with the known *Aureispira* species with a 100% bootstrap value (Fig. 5). The clustering of nodes related to the genus *Aureispira* in the two phylogenetic tree construction methods, NJ (Fig. 5) and ML (data not shown), was consistent. However, strain EL160426^T showed 94.6 and 97.7% similarity with *A. marina* strain 24 ^{T} and *A. maritima* strain 59SA^T (comparison using 1447 bp corresponding to *E. coli* number 28–1444), respectively, indicating that the 16S rRNA similarity values were all below the threshold (98.65%) for prokaryotic species delineation (Kim et al. 2014). Furthermore, the 16S rRNA gene sequence of strain EL160426^T obtained by PCR amplification showed sequence similarities of 99.8, 99.9, 100, and 100%, respectively, with the four copies of this gene obtained by genome sequencing of strain EL160426^T. It strongly supports that the new isolate EL160426^T is a new species of the genus *Aureispira*.

Genome analysis

WGS of strain EL160426^T revealed the presence of a circular chromosome (7.58 Mbp) and two small plasmids, pAUEa (122 Kbp) and pAUEb (87 Kbp). The genome contained four rRNAs and a total of 68 tRNAs were predicted. The genomic DNA G + C content of strain EL160426^T was 36.2%, which was slightly different from the related species *Saprospira grandis* strain WH^T (46.6%), which has its complete genome sequenced (Heng et al. 2023). The ANI values between strain EL160426^T and *S. grandis* strain WH^T was 78.7%, which was sufficiently lower to separate it as a different genus, though the cutoff value for prokaryotic genus definitions have been discussed (Richter and Rosselló-Móra 2009; Rodríguez and Konstantinidis 2014; Qin et al. 2014). In addition, the DNA G + C content between the genus *Aureispira* and *Saprospira* was different by 36.2–39.4% and 49.8% (Hosoya et al. 2006), respectively. It supports the results of the 16S rRNA-based phylogenetic analysis showing that the genus *Aureispira* has a significant phylogenetic distance from *S. grandis* WH^T. Since no genomic information is available for *A. maritima* strain 59SA^T, which is the most closely related to the strain EL160426^T, it is necessary to compare genomic similarity by genomic DNA-DNA hybridization among both strains for evaluating the taxonomic position of the new isolate.

DNA-DNA hybridization and G + C contents

The DNA-DNA relatedness between strain EL160426^T and *A. maritima* 59SA^T was less than 13%, which indicates that each isolate is a distinct species (Wayne et al. 1987). The DNA G + C content of strain EL160426^T was 36.2% by whole genome sequence analysis and 36.9% by the method of Katayama-Fujimura et al. (1984). Although both values were very slightly different, this may have been due to the measurement methods, and the value of *A. maritima* 59SA^T,

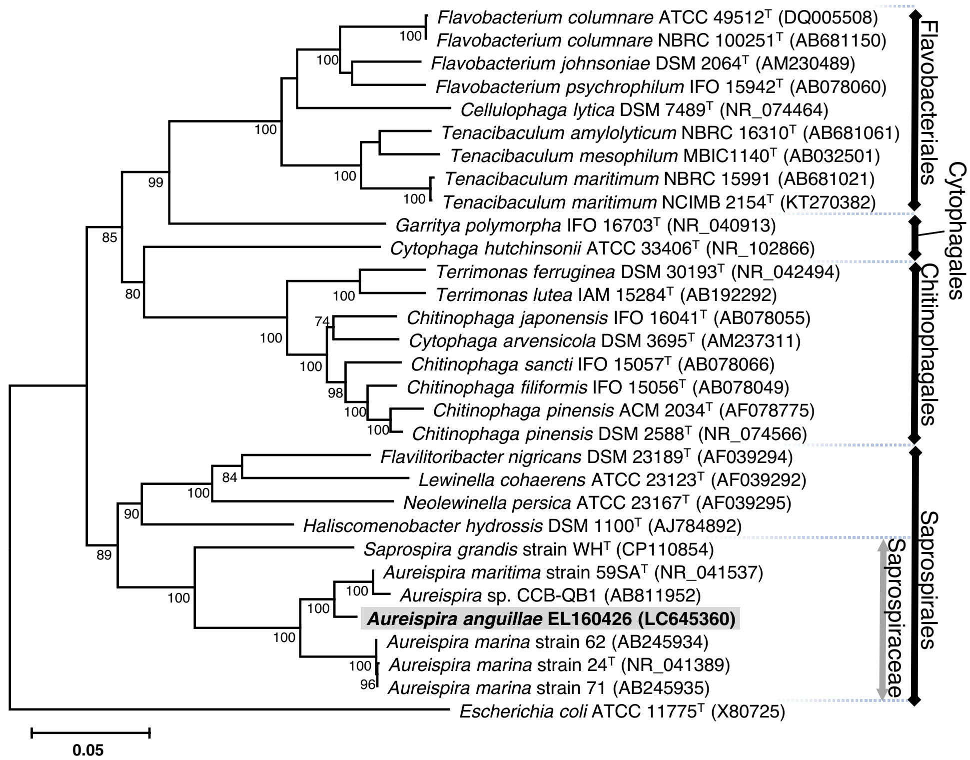

Fig. 5 Neighbor-joining phylogenetic tree of novel strains EL160426^{T} and related members of the phylum Bacteroidetes, based on 16S rRNA gene sequence analysis. The numbers at the nodes indicate the percentages of occurrence in 1000 bootstrapped trees. Only values greater than 70% are shown. Bar = 0.05 K_{nuc}

38.9% in DNA G + C content (Hosoya et al. 2007), was obviously distinguishable.

Biochemical characterization

Details of the phenotypic characteristics are shown in the species description below. The major phenotypic characteristics of the genus *Aureispira*, i.e., aerobic, helical shape, gram-negative, catalase and oxidase positive, gliding or pivot movement, and others, coincide with the description of the genus *Aureispira* (Hosoya et al. 2006, 2007). The biochemical characteristics of strain EL160426^{T} and *A. marina* or *A. maritima* were different in the tests of esterase (C4), lipase (C14), valine arylamidase, cystine arylamidase, trypsin and degradation of carboxymethylcellulose, DNA and tyrosine, and reduction of nitrate (Table 1), indicating

Table 1 Differential characteristics of the present isolate, *Aureispira maritima* and *A. marina*

Characteristics	EL160426^{T}	*A. maritima* 59SA^{T} (Hosoya et al. 2007)	*A. marina* 24^{T}, 62 and 71 (Hosoya et al. 2006)
Colony colour	Yellow	Yellow	Yellowish orange
Esterase (C4)	+	−	+
Lipase (C14)	+	−	−
Valine arylamidase	+	+	−
Cystine arylamidase	+	+	−
Trypsin	+	+	−
Degradation of Carboxymethyl-cellulose	−	+	−
DNA	−	+	−
Tyrosine	−	+	+
Reduce of Nitrate	+	−	−
DNA G + C contents (%)	36.2 /36.9	38.9	39.4

Data for *A. maritima* and *A. marina* ($n = 3$) are from Hosoya et al. (2007) and Hosoya et al. (2006), respectively. The 36.2% and 36.9% in DNA G + C contents of the present isolate were evaluated by the whole genome sequence and the method of Katayama-Fujimura et al. (1984), respectively that the phenotypic characteristics of the present isolate are not consistent with either *A. marina* nor *A. maritima*.

Chemotaxonomic characterization

The prevalent cellular fatty acids (> 10% of the total fatty acid) of strain EL160426^T were determined to be 16:0, 20:4 w6c, 17: 0 iso, and 16:0 N alcohol (Table 2). A high level of 20:4 w6c (arachidonic acid) was found (24.3% of total fatty acids), which was a lower percentage than that of *A. marina* and *A. maritima*. However, the 16:0 N alcohol and 16:0 of the present strain were higher in percentage than that of *A. marina* and *A. maritima*. Recent studies have pointed out that the fatty acids of this genus contain molecular species that cannot be accurately identified by the MIDI fatty acid identification method using GLC (Kawahara et al. 2023). However, since the fatty acid composition of the new isolate was measured under almost the same conditions as those of the previously known species, it is a reasonable comparative analysis for bacterial identification. The fatty acid composition of the isolate, especially the presence of arachidonic acid, is similar to that of the known *Aureispira* spp., and it is appropriate to classify it as the same genus. This is clearly different from the genus *Saprospira*, which does not secrete arachidonic acid (Hosoya et al. 2006). The major isoprenoid quinone of the strains was MK-7, which is consistent with that in both *A. maritima* 59SA^T (Hosoya et al. 2007) and *A. marina* 24^T (Hosoya et al. 2006).

Taxonomic conclusion

On the basis of the results of DNA-DNA hybridization and phylogenetic and phenotypic analyses, strain EL160426^T, which was found to be a pathogenic agent of Japanese eel leptocephali, is shown to be a novel species of the genus *Aureispira* and the name of *Aureispira anguillae* sp. nov. is proposed.

Description of *Aureispira anguillae* sp. nov.

Aureispira anguillae (an.guil'lae. L. gen. n. *anguillae*, of the Japanese eel *Anguilla japonica*)

Cells are Gram-reaction-negative, aerobic, oxidase- and catalase-positive, which are rods (0.4–0.6 by 1.4–2.4 µm), which form helical cells in broth that are 1.5–2.0 µm in width and 30–110 µm in length, with a twist occurring every 5–6 µm. Colonies are light yellow with swarming formations on agar. The optimal growth temperature is 25–30 °C; no growth occurred at 10 or 35 °C. The pH range for growth is 7.0–9.0. Growth occurs at seawater concentrations of 60–150% (w/v). In the API ZYM test, they are positive for esterase (C4), esterase lipase (C8), lipase (C14), leucine arylamidase, valine arylamidase, cystine arylamidase, trypsin and naphthol-AS-BI-phosphohydrolase and for the degradation of casein, gelatin and Tweens 20, 40, 60 and 80. Carboxymethylcellulose, DNA and tyrosine is not degraded. Agar, citrate and starch are not decomposed. Nitrate is reduced. Acetoin, H_2S and indole are not produced. Negative in tests for chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, N-acetyl-β-glucosamidase, α-mannosidase and α-fucosidase. Does not produce acid from arabinose, cellobiose, dulcitol, fructose, galactose, glucose, glycerol, inositol, lactose, maltose, mannitol, mannose, raffinose, rhamnose, sorbitol, sucrose, trehalose or xylose. The DNA G + C content of the strain is 36.9 mol%. The type strain, EL160426^T (= JCM 35024^T = TSD-286^T), was isolated from a leptocephalus of the Japanese eel *Anguilla japonica* reared at the laboratory located by Gokasyo Bay in Mie, Japan.

Table 2 Fatty acid profiles of the present isolate, *A. maritima* and *A. marina*

Fatty acid	EL160426^T	*A. maritima* 59SA^T (Hosoya et al. 2007)	*A. marina* 24^T, 62 and 71 (Hosoya et al. 2006)
iso-15:0	3.0	3.0	7.0–12.2
16:0 N alcohol	12.5	5.7	0–3.3
iso-16:0	2.7	3.7	2.4–8.8
16:0	35.5	25.6	9.6–16.9
iso-17:0	12.8	7.1	13.3–25.8
16:0 3-OH	0.6	Trace	0–1.5
iso-18:0	0.7	2.1	0–2.9
iso-17:0 3-OH	1.4	1.5	2.3–4.3
20:4ω6c (arachidonic acid)	24.3	43.6	33.4–46.3
18:0 3-OH	2.8	Not detected	0–2.1

Data for *A. maritima* and *A. marina* ($n = 3$) are from Hosoya et al. (2007) and Hosoya et al. (2006), respectively. Values are percentages (w/w) of total fatty acids

The GenBank accession numbers for the 16S rRNA gene and complete genome sequences of the EL160426^{T} strain are LC645360 and AP026867 to AP026869, respectively (AP026867 for chromosome, AP026868 for plasmid pAUEa and AP026869 for plasmid pAUEb).

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Author contributions Yuasa performed the isolation, deposition and polyphasic taxonomy. Mekata performed genome analysis. Kiryu performed histological observations and revised the manuscript. Nomura and Sudo collected infected fish from a rearing tank and performed an experimental infection test. Satomi revised the manuscript. All authors read and approved the final manuscript.

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Data availability The 16S rRNA gene and complete genome sequences have been deposited in GenBank under the accession numbers LC645360 and AP026867 to AP026869 (AP026867 for chromosome, AP026868 for plasmid pAUEa and AP026869 for plasmid pAUEb), respectively.

Declarations

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

Ethical approval The care and use of experimental animals was conducted in accordance with the guidelines of the Japan Fisheries Research and Education Agency and the guidelines set by the Japanese Ministry of Environment regarding standards for the care and use of laboratory animals, including the minimization of pain.

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