**Fibrivirga algicola** gen. nov., sp. nov., an algicidal bacterium isolated from a freshwater river

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**Abstract** An aerobic, gram-stain-negative, pink-colored, non-motile and rod-shaped algicidal bacterium, designated as JA-25<sup>T</sup> was isolated from freshwater in Geumgang River, Republic of Korea. Strain JA-25<sup>T</sup> grew at 15–30 °C and pH 6–9, and did not require NaCl. Phylogenetic analysis based on 16S rRNA gene sequences revealed that strain JA-25<sup>T</sup> belongs to the family ‘Spirosomaceae’ and is most closely related to *Fibrella aestuarina* BUZ 2<sup>T</sup> (93.6%). Strain JA-25<sup>T</sup> showed <90% sequence similarity to other members of the family ‘Spirosomaceae’. The average nucleotide identity (ANI), in silico DNA-DNA hybridization and average amino acid identity (AAI) values based on the genomic sequences of JA-25<sup>T</sup> and *F. aestuarina* BUZ 2<sup>T</sup> were 74.4, 20.5, and 73.6%, respectively. Strain JA-25<sup>T</sup> showed an algicidal effect on the marine flagellate alga *Hetero- capsia triquetra*, but no effect on fresh water cyanobacterium (*Nostoc*). In genome analysis, RIPP-like peptides were detected and predicted to resemble the indolmycin biosynthetic gene cluster, which possibly influence its algicidal effect. Furthermore, a bacteriorhodopsin gene with photoheterotrophic characteristics was detected. The genomic DNA G+C content was 52.5 mol%. The major cellular fatty acids were summed feature 3 (C16:1 ω6c/C16:1 ω7c), C16:1 ω5c, C16:0 (>10%). The major respiratory quinone was menaquinone 7 and major polar lipids were phosphatidylethanolamine, two unidentified aminolipids, two phospholipids, and five unidentified lipids. Considering the phylogenetic inference, phenotypic, and chemotaxonomic data, strain JA-25<sup>T</sup> should be classified as a novel species in the novel genus *Fibrivirga*,
with the proposed name *Fibrivirga algicola* sp. nov. The type strain is JA-25\(^T\) (=KCCM 43334\(^T\) = NBRC 114259\(^T\)).

**Keywords**  
*Fibrivirga algicola* · Taxonomic · Freshwater · Algicidal bacterium

**Introduction**

Marine harmful algal blooms occur when toxin-producing algae grow rapidly. These blooms occur worldwide and greatly impact aquatic ecosystems and human health (Patel et al. 2020). Over the last 30 years, harmful algal bloom caused financial losses of over 0.87 billion US dollars because of the massive fish and shellfish mortalities and negative impacts on tourism in China (Yan et al. 2022). The dinoflagellate genus *Heterocapsa* can cause harmful algal blooms (HABs) and produce toxins that affect the marine ecosystem (Patin et al. 2020). Additionally, several *Heterocapsa* strains have been found in Korea (Choi et al. 2021; Sakamoto et al. 2021). Thus, to control harmful algal blooms while maintaining the marine ecosystem, specific algicides for *Heterocapsa* sp. may be useful. The family ‘Spirosomaceae’ is a member of the order *Cytophagales* within the phylum *Bacteroidetes* (García-López et al. 2019). Currently, this family comprises 25 genera including *Fibrella* on the List of Prokaryotic names with Standing in Nomenclature (https://lpsn.dsmz.de/family/spirosomaceae). Cells in this family are gram-negative, aerobic, or facultative anaerobic, and non-spore forming, with variable motility. The rods have various degrees of curvature, sometimes resulting in the formation of rings, coils, and undulating filaments. Colonies contain a pink or yellow, non-water-soluble pigment. The major quinone is menaquinone 7 and major polar lipid is phosphatidylethanolamine. The G+C content calculated from genome sequences is 35.1–56.4%. The genus *Fibrella* was proposed by Filippini et al. (2011) and was reported to participate in antagonistic interactions against the cyanobacterium *Nostoc muscorum* (Svercel et al. 2011). The freshwater cyanobacterium *Nostoc* is different from the marine eukaryotic flagellum *Heterocapsa* in terms of cell envelope and physiology. Unlike strain *Fibrella aestuaria* BUZ 2\(^T\), strain JA-25\(^T\) did not negatively affect *Nostoc* sp.

In the present study, the novel algicidal strain JA-25\(^T\), which was isolated from freshwater, was evaluated using genotypic, chemotaxonomic, and phenotypic approaches and found to be a representative novel species in the new genus *Fibrivirga*.

**Materials and methods**

**Isolation and culture conditions**

Freshwater was collected from the Geumgang River in South Korea (35° 50’ 38.1” N, 127° 24’ 51.8” E). The freshwater was diluted in 50 mM phosphate buffer and spread on Reasoner’s 2A agar (R2A; MBcell, Seoul, Korea) plates. The plates were incubated at 25 °C for 2 weeks, after which the colonies were streaked onto R2A agar at least three times to obtain pure colonies. The identity of the colonies was determined using 16S rRNA sequencing. Based on the 16S rRNA sequencing results, one colony designated as JA-25\(^T\) was selected for polyphasic characterization. Strain JA-25\(^T\) was routinely cultured on R2A agar at 25 °C for 3 days for phenotypic, physiological, and chemotaxonomic analyses and maintained in glycerol suspensions (20%, v/v) at -80 °C. For comparative analyses, *F. aestuaria* DSM 22563\(^T\) was purchased from the Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH (DSMZ, Braunschweig, Germany) and cultured under the same conditions as used for strain JA-25\(^T\).

**Harmful algae and algal culture**

We used the harmful algae strain *Heterocapsa triquetra* (LIMS-PS-1783), which was obtained from the Library of Marine Samples Korea (Korean Institute of Ocean Science and Technology, Busan, Korea). This strain was cultured in f/2-Si medium (Sigma-Aldrich, St. Louis, MO, USA) at 20 °C with a light intensity of 40 μmol/m²/s and 14:10 h light:dark cycle.

**16S rRNA gene phylogenetic analyses**

Chromosomal DNA was extracted from strain JA-25\(^T\) using a DNA extraction kit (iNTRON Biotechnology, Gyeonggi-do, South Korea). The 16S rRNA gene was
amplified using AccuPower PCR PreMix (Bioneer, Daejeon, Korea) and the bacteria-specific primers 27F (5′-AGAGTTTGTATCMTGGCTCAG-3′) and 1492R (5′-AAGGAGGTGATCCAGCCGC-3′). 16S rRNA gene sequencing was performed as previously described (Roh et al. 2008). The almost full-length 16S rRNA gene sequence (1,451 bp) was assembled and curated using SeqMan Pro 14 (DNASTAR, Madison, WI, USA). Phylogenetic neighbors were identified, and pairwise sequence similarities were calculated using EzBioCloud (Yoon et al. 2017). Phylogenetic relationships between closely related species were determined using the MEGA6 program (Tamura et al. 2013). Phylogenetic trees were constructed using the neighbor-joining (Saitou and Nei 1987), maximum-parsimony (Fitch 1971), and maximum-likelihood methods (Felsenstein 1981), and evolutionary distances were calculated using Kimura’s two-parameter model. To evaluate the stability of the phylogenetic trees, bootstrap analysis was performed by obtaining a consensus tree based on 1,000 randomly generated trees.

Whole-genome sequencing analyses

The genomic DNA of strain JA-25T was extracted and purified using a QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The purified genomic DNA was quantified using a Qubit 3.0 fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). A genome library was prepared using the Nextera DNA Flex Library Prep Kit (Illumina, San Diego, CA, USA). The average library size was 550 bp, and draft genome sequencing was performed on a Novaseq 6000 platform (Illumina) with 101 bp paired-end reads according to the manufacturer’s protocols. Low-quality reads were trimmed using a quality threshold of Q20. The raw data were assembled using Unicycler 0.4.7 (Wick et al. 2017). The average nucleotide identity was calculated using JSpecies (Richter and Rosselló-Móra 2009), and the distance (1-similarity) was visualized in the R environment ver. 4.0.4 (https://www.r-project.org/). The average amino acid identity (AAI) values were calculated with a 20% identity cut-off using the AAI Calculator (http://enve-omics.ce.gatech.edu/aai/index). The digital DNA–DNA hybridization (dDDH) was calculated using Genome-to-Genome Distance Calculator 2.1 (https://ggdc.dsmz.de/ggdc.php) using formula 2. The genome BLAST distance phylogeny (GBDP) tree was inferred from the draft genome of strain JA-25T (https://ggdc.dsmz.de/ggdc.php). The GenBank/EMBL/DBJ accession numbers for the 16S rRNA gene and draft genome sequences of strain JA-25T are MN559427 and WAEL01000000, respectively. The known-biosynthetic gene clusters were predicted using anti-SMASH version 5.1.2 (https://antismash.secondarymetabolites.org). OrthoVenn2 (https://orthovenn2.bioinfokits.net/) was used to analyze shared or independent gene clusters between strain JA-25T and closely related type strains.

Phenotypic and chemotaxonomic characteristics

To investigate its morphological and physiological properties, strain JA-25T was routinely cultivated on R2A agar at 25 °C. Gram staining was performed using the non-staining method described by Buck with a Gram staining kit (bioMérieux, Marcy-l’Etoile, France). Growth under anaerobic conditions was determined after incubation for 1 week in a GasPak EZ Anaerobe Pouch System (BD, Franklin Lakes, NJ, USA) on R2A. Cell morphology was observed using a transmission electron microscope (H-7650, Hitachi, Tokyo, Japan). Gliding motility was investigated as described by Bernardet et al. (2002) using a modified R2A medium. Growth was assessed under various conditions, including different temperature (10 °C, 15 °C, 20 °C, 37 °C, 40 °C, and 45 °C) and pH (pH 5, 6, 7, 8, 9, 10, and 11) values, on R2A agar. The pH values were adjusted by adding the following buffers as needed: 10 mM MES for pH 5.0 and 6.0; 10 mM bis–Tris propane for pH 7.0, 8.0, and 9.0; and 10 mM CAPS for pH 10.0 and 11.0. NaCl tolerance (0, 1, 2, 3, and 5%, w/v) was tested on R2A agar. Production of flexirubin-type pigments was determined according to the reversible color shift to red, purple, or brown when yellow or orange colonies were covered with aqueous 20% KOH solution (Siddiqi et al. 2016). Hydrolysis of CM-cellulose and starch was tested by adding 0.5% of the respective polysaccharides to R2A agar medium. API ZYM, API 20NE, and 50CH strips (bioMérieux) were used to determine the physiological and biochemical characteristics of the strain. The API 20NE and 50CH results were recorded after 3 days, and those of API ZYM were recorded after 1 day at 25 °C. Catalase and oxidase activities were measured using a 3% (v/v) hydrogen
peroxide solution and 1% (w/v) tetramethyl-p-phenylenediamine (bioMérieux), respectively. Quinones in strain JA-25T grown on R2A medium were analyzed using high-performance liquid chromatography (Waters, Milford, MA, USA) as described by Hirashi et al. (1996). The cellular fatty acid composition was analyzed according to the instructions provided in the Sherlock Microbial Identification System (Miller 1982), using strain JA-25T and F. aestuarina DSM 22563T cultivated under the same conditions on R2A agar at 25 °C for 3 days. Fatty acids were analyzed using gas chromatography (Hewlett Packard 6890; Agilent Technologies, Santa Clara, CA, USA) and identified using the Microbial Identification software package (Sasser 1990) based on the TSBA6 database. Polar lipids of strain JA-25T were extracted and separated using two-dimensional thin-layer chromatography on a silica gel glass plate (Merck, Darmstadt, Germany) as described by Xin et al. (2000). The separated polar lipid spots were detected by spraying the plate with 5% ethanolic molybdophosphoric acid (for total polar lipids) and ninhydrin (aminolipids) as described by Minnikin et al. (1984). Phospholipids were detected by spraying the plate with Zinzadze reagent (Minnikin et al. 1984).

Algicidal activity assay

The algicidal activity assay was performed as described previously by Cho and Kim (2018) with slight modifications. Strain JA-25T was cultured in R2A broth medium for 3 days, after which the bacterial cells were collected at ~3000 ×g. The bacterial cell pellets were washed three times with algal medium and then diluted with algal medium. Bacteria at concentrations J1 (6 mg/mL), J2 (3 mg/mL), J3 (1.5 mg/mL), and J4 (0.75 mg/mL) were added to 24-well plates containing harmful algae in exponential growth phase. Equal amounts of algal medium were used as controls. The plates were cultivated under the culture conditions of harmful algae. Algicidal activity was calculated every day using the following equation, and the assay was performed in triplicate.

\[
\text{Algicidal rate (\%)} = \left( \frac{N_c - N_t}{N_c} \right) \times 100
\]

where \(N_c\) and \(N_t\) are the numbers of algal cells in the control and treatment groups, respectively.

Results and discussion

Phylogenetic and phylogenomic analyses

Strain JA-25T showed the highest 16S rRNA gene sequence similarity to the type strain of F. aestuarina BUZ 2T (93.6%), followed by Spirosoma migulaei 15J9-8T (89.4%). The pairwise 16S rRNA gene sequence similarity values between strain JA-25T and the other members of the family ‘Spirosomaceae’ were below 90.0%. Strain JA-25T clustered with F. aestuarina BUZ 2T in the neighbor-joining tree (Fig. 1), and this cluster was independent from other related genera in the family ‘Spirosomaceae’. The maximum likelihood tree, neighbor-joining, and maximum-parsimony trees all showed the same topology. For 16S rRNA signature nucleotide analysis, approximately 25 species were selected based on their 16 s rRNA sequence similarity and phylogenetic tree topology (Fig. 1). Analysis of the 16S rRNA signature nucleotide patterns revealed shared patterns between strain JA-25T and related species in the family, with family-specific signature nucleotide patterns at positions 158 (C), 290–310 (G-C), 291–298 (G-C), 298–305 (G-C), 371–390 (G-C), 420 (C), 530–881 (U-A), 571–881 (U-A), 682–708 (C-G), 930–1387 (U-A), 1075–1082 (C-G), 992 (A), 1168 (C), 1189 (C), 1224 (G), and 1335 (G). Signature nucleotides of Fibrivirga algicola strain JA-25T were identified at positions 173 (T), 207 (C), 658 (C), 659 (A), 670–736 (U-A), and 1007–1022 (U-A). The draft genome assembly of strain JA-25T contained 60 contigs (N50, 670,798 bp) with 1377.57 × coverage. The total estimated combined length of the genome was 6,718,605 bp with 52.5 mol% G+C. The completeness of the genome was very high at 97.2%, and the probability of contamination was very low at 0.9%. A whole genome-based phylogeny of strain JA-25T was determined using MIGA (Rodriguez-R et al. 2018) and core gene based phylogenetic analysis was conducted using Bacterial Pan Genome Analysis (BPGA) (Chaudhari et al. 2016) for related 5 strains (Figure S1, S2). The topology of the two trees was similar to that of the 16S rRNA-based tree, indicating that strain JA-25T forms a novel genus within
the family Spirosomaceae. This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under accession number WAEL01000000. The ANI, AAI, and dDDH values of strain JA-25\textsuperscript{T} against the most closely related \textit{F. aestuarina} BUZ \textsuperscript{2T} were 74.4%, 73.6%, and 20.5% respectively, and against closely related genera within the family were 70.7–74.4%, 61.8–73.6%, and 19.5–20.5%, respectively (Table S1). These values are below the defined thresholds for species delineation of 95–96% for ANI and 70% for dDDH (Chun et al. 2018; Rosselló-Móra and Amann 2015; Goris et al. 2007), supporting that strain JA-25\textsuperscript{T} is a novel genus in the family ‘Spirosomaceae’. In addition, the AAI values based

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**Fig. 1** Neighbor-joining tree showing strain JA-25\textsuperscript{T} among related taxa. Branch points and bootstrap values (> 80%, NJ/ML/MP) calculated by the neighbor-joining(NJ), maximum likelihood(ML) and maximum parsimony(MP) methods. \textit{Verrucomicrobium spinosum} DSM 4136\textsuperscript{T} (NR 026266.1) was used as an outgroup. The scale bar represents 0.05 substitutions per nucleotide position.
on protein sequences showed that relatedness values ranged from 61.8 to 73.6% (Table S1), satisfying the threshold range of 60–80% for separating different genera as proposed by Luo et al. (2014) and Rodriguez and Konstantinidis (2014); these results supported that the strain is a novel genus in the family ‘Spirosomaceae’. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP). A total of 5,440 genes, including 5,393 coding sequences, 66 pseudogenes, and 47 RNA genes were annotated in Fibrella sp. JA-25T. The assembly contained 1, 3, and 1 of full-length 5S, 16S, and 23S rRNA genes, respectively; 40 tRNA genes; and two non-coding RNAs. OrthoVenn2 (https://orthovenn2.bioinforookitks.net/) was used to analyze shared or independent gene clusters between JA-25T and the closest relative F. aestuarina BUZ 2T. Both strains shared 3,840 gene clusters. Strain JA-25T and BUZ 2T had 66 and 77 independent gene clusters, respectively. Interestingly, the bacteriorhodopsin (NZ_WAEL0100002.1; 312,795–313,550) and Brp/Blh family beta-carotene dioxygenase (NZ_WAEL0100002.1; 314,744–313,797) genes were only found in JA-25T and not in F. aestuarina BUZ 2T is absent (Table 1).

In the genome of F. algicola JA-25T, three terpene biosynthetic gene clusters (cartenoids) were found at 62,764–83,918 bp (NZ_WAEL01000011, showing 100% and 45% gene similarity with Fibrella sp. ES10-3-2-2 and F. aestuarina BUZ 2, respectively), 301,447–322,694 bp (NZ_WAEL01000002, 88% of genes similarity with Fibrella sp. ES10-3-2-2), and 204,981–225,817 bp (NZ_WAEL01000008, 100% and 30% of genes similarity with Fibrella sp. ES10-3-2-2 and F. aestuarina BUZ 2, respectively) (Table 2). In addition, two non-ribosomal peptide

### Table 1 Differential characteristics between strain JA-25T and closely related genera in the in the family ‘Spirosomaceae’

| Characteristics                          | 1                      | 2                      | 3                      | 4                      |
|------------------------------------------|------------------------|------------------------|------------------------|------------------------|
| Isolation source                         | Fresh water            | Costal mud             | Soil                   | Air                    |
| Colony colour                            | Pink                   | Pink                   | Pale Yellow            | Orange                 |
| Cell morphology                          | Rods/Filaments         | Rods/Filaments         | Rods                   | Rods/Filaments         |
| Growth range for temperature (°C)        | 15 – 30                | 15 – 37                | 10 – 30                | 10–40                  |
| Growth range for pH                       | 6 – 9                  | 6 – 8                  | 6.5 – 8.5              | 6 – 11                 |
| Oxidase                                  | –                      | –                      | +                      | +                      |
| Catalase                                  | +                      | +                      | –                      | +                      |
| Hydrolysis of gelatin                    | –                      | +                      | ND                     | –                      |
| CM-Cellulose                             | –                      | +                      | ND                     | –                      |
| Agar                                     | –                      | +                      | ND                     | –                      |
| Enzyme activity(API zyme)                |                        |                        |                        |                        |
| Trypsine                                 | +                      | +                      | –                      | –                      |
| α-chymotrypsin                           | +                      | –                      | +                      | –                      |
| α- Manocidase                            | –                      | +                      | +                      | +                      |
| α-Fucosidase                             | –                      | +                      | –                      | –                      |
| Genotypic features                       |                        |                        |                        |                        |
| Genome size(Mb)                          | 6.72                   | 6.94                   | ND                     | 6.55                   |
| Protein                                  | 5.316                  | 5.693                  | ND                     | 5.163                  |
| 16S rRNA gens                            | 3                      | 3                      | ND                     | 3                      |
| tRNA genes                               | 40                     | 71                     | ND                     | 39                     |
| DNA G+C content (%)                      | 52.5                   | 56.5                   | 47                     | 54.3                   |

Strains: 1, Fibrivirga algicola JA-25T (this study); 2, Fibrella aestuarina BUZ 2T (this study); 3, Spirosoma migulaei 15J9-8T (Okiria et al., 2017); 4, Rudanella lutea 5715S-11T (Weon et al., 2008) +, Positive; −, negative; ND, not available

Data of F. algicola JA-25T and F. aestuarina BUZ 2 T were obtained in this study. In API ZYM strips, two strains were positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase, and N-acetyl-b-glucosaminidase activities.
synthetase cluster biosynthetic gene clusters were detected at 69,444–113,589 bp (function unknown, NZ_WAEL01000005, 100% and 26% of genes similarity with *Fibrella* sp. ES10-3-2-2 and *F. aestuarina* BUZ 2, respectively) and 263,722–310,420 bp (putative cylindrospermopsin, NZ_WAEL01000007, 100% of genes similarity with *Fibrella* sp. ES10-3-2-2). Type III polyketide synthase clusters (325,872–367,062 bp, NZ_WAEL01000008, 100% gene similarity with *Fibrella* sp. ES10-3-2-2) were detected, which were expected because hierridin B or C (antitumor or antiproliferative agents) was found. In addition, a bacteriocin or other unspecified ribosomally synthesized and post-translationally modified peptide product (RiPP) cluster was found at 624,756–635,901 bp (NZ_WAEL01000004, 100% of genes similarity with *Fibrella* sp. ES10-3-2-2). This RiPP-like peptide was predicted to resemble the indolmycin biosynthetic gene cluster from *Streptomyces griseus* subsp. *griseus*. Indolmycin is a natural tryptophan analog that competes with tryptophanyl-tRNA synthetase (RtpRS) enzymes (Williams et al. 2016). Indolmycin of *F. algicola* JA-25T was preliminarily predicted to affect its algicidal effect. The RiPP region of strain JA-25T was detected in other closely related type strains but was not in the form of gene clusters and was only partially present. The RiPP region of strain JA-25T was partially present in *F. aestuarina* BUZ 2 around at 1,727,515–1,726,044, 3,647,488–3,649,270, 3,907,379–3,910,150, and 4,432,318–4,433,213 bp (query cover and percent identity were 70% and 73.4%, respectively) and partially present in the genome of *Rudanella lutea* DSM 19,387 (67%/79.01%), *Spirosoma telluris* HMF3257 (56%/71.65%), *Spirosoma lacussanchae* CPCC 100,624 (55%/73.92%), and *Rudanella paleauranthibacter* HX-22-17 (34%/79.19%). The function of RiPP of these strains, including *F. algicola* JA-25T should be confirmed in biochemical studies (Table 2).

### Phenotypic and chemotaxonomic characteristics

Strain JA-25T was a gram-negative, catalase-positive, catalase-positive, and oxidase-negative. Colonies of strain JA-25T grown on R2A were circular, smooth, and pink. The cells were rod-shaped (0.8–1.2 μm wide and 1.5–3.5 μm long), as shown in Figure S3. Gliding motility was absent, and the production of flexirubin pigments was negative. Optimal growth of strain JA-25T was observed at 25 °C and pH 7.0 in medium containing 0% (w/v) NaCl. Strain JA-25T produced

| Contigs                  | Position (bp) | Gene clusters                                      | Compound                                      | Similarity                                      |
|-------------------------|---------------|---------------------------------------------------|-----------------------------------------------|------------------------------------------------|
| NZ_WAEL01000011         | 62,764–83,918 | Terpene biosynthetic gene clusters                 | Carotenoids                                   | 100% (*Fibrella* sp. ES10-3-2-2) 45% (*F. aestuarina* BUZ 2) |
| NZ_WAEL01000002         | 301,447–322,694 | Terpene biosynthetic gene clusters                 | Carotenoids                                   | 88% (*Fibrella* sp. ES10-3-2-2) 45% (*F. aestuarina* BUZ 2) |
| NZ_WAEL01000008         | 204,981–225,817 | Terpene biosynthetic gene clusters                 | Carotenoids                                   | 100% (*Fibrella* sp. ES10-3-2-2) 45% (*F. aestuarina* BUZ 2) |
| NZ_WAEL01000005         | 69,444–113,589 | Non-ribosomal peptide synthetase cluster (NRPS) biosynthetic gene clusters | Unknown                                       | 100% (*Fibrella* sp. ES10-3-2-2) 26% (*F. aestuarina* BUZ 2) |
| NZ_WAEL01000007         | 263,722–310,420 | Non-ribosomal peptide synthetase cluster (NRPS) biosynthetic gene clusters | Putative cylindrospermopsin                   | 100% (*Fibrella* sp. ES10-3-2-2) |
| NZ_WAEL01000008         | 325,872–367,062 | Type III Polyketide synthase clusters              | Hierridin B or C (antitumor or antiproliferative agents) | 100% (*Fibrella* sp. ES10-3-2-2) |
| NZ_WAEL01000004         | 624,756–635,901 | Bacteriocin or other unspecified ribosomally synthesized and post-translationally modified peptide product (RiPP) cluster | Indolmycin                                    | 100% (*Fibrella* sp. ES10-3-2-2) |
A pink pigment with absorbance peaks at 480 and 510 nm, with a major peak at 482 nm, which is similar to F. aestuarina BUZ 2T. The isolation source, colony color, and growth temperature and pH ranges were used to distinguish between closely related genera and strain JA-25T. A detailed comparison of the characteristics of JA-25T with those of its close relatives in the family ‘Spirosomaceae’ is provided in Table 1. The cellular fatty acid profiles of strain JA-25T and Fibrella aestuarina BUZ 2T are shown in Table 3. The dominant fatty acids of strain JA-25T were summed feature 3 (comprising C16:1 ω6c and/or C16:1 ω9c; 40.8%), C16:1 ω5c (21.4%) and C16:0 (10%). Although the overall fatty acid composition of the strain JA-25T was similar to that of strain BUZ 2T, it can be distinguished from its closest phylogenetic neighbors by its smaller proportion of summed feature 3 and larger proportion of C16:0. Menaquione 7 was the predominant respiratory quinone in strain JA-25T. The major polar lipids of strain JA-25T were phosphatidylethanolamine, two unidentified aminolipids, two phospholipids, and five unidentified lipids (Figure S4). Catalase was positive but oxidase was negative. Nitrate reduction was negative. In API ZYM tests, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, valine arylamidase, cysteine arylamidase, trypsin, α-chymotrypsin, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-galactosidase, β-galactosidase, α-glucosidase, β-glucosidase and N-acetyl-β-glucosaminidase were positive. In API 50CH assays, acid was produced from d-xylene, d-galactose, d-glucose, d-fructose, mannose, methyl-d-mannoside, methyl-d-glucopyranoside, N-acetyl-glucomamine, amygdalin, arbutin, salicin, cellobiose, maltose, sucrose, trehalose, inulin, melizitose, d-raffinose, turanose, and gentibiose.

### Algicidal activity

Strain JA-25T showed algicidal activity after 4 days of co-culture. The concentrations of J1 (6 mg/mL) and J2 (3 mg/mL) showed the highest algicidal activity toward the harmful algae H. triquetra (Fig. 2). Meaningful algicidal activity was not observed in the bacterial culture supernatant or bacterial cell extracts against harmful algae (data not shown). Algicidal activity was more effective in bacterial co-culture. Further studies are needed to understand these results, such as by changing the extraction methods and bacterial culture conditions. The amount of bacterial inoculum is an important factor affecting algicidal activity, as demonstrated by the differences between treatment groups (Hu et al. 2019). Under a scanning electron microscope, a large number of algal cells surrounded by microorganisms was observed, and burst algal cells to which microorganisms were attached were observed. Although additional studies are necessary, the mechanisms of the algicidal effects may be based on direct interaction (Hu et al. 2019).

### Conclusion

A strain phylogenetically related to algicidal strain JA-25T was characterized using a polyphasic approach. The DNA GC contents, menaquinone, and major polar lipids of strain JA-25T were showed that this strain belongs to the family ‘Spirosomaceae’ description. Genome analysis based on the ANI and AAI values suggested that strain JA-25T is a novel species in a new genus. Thus, based on the phylogenetic inference, phenotypic, and chemotaxonomic

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**Table 3** Cellular fatty acid composition (%) of strain JA-25T and most related genus in the family ‘Spirosomaceae’

| Saturated          | 1     | 2     |
|--------------------|-------|-------|
| C14:0              | 1.3   | 1.5   |
| C16:0              | 10    | 4.6   |
| C18:0              | –     | 1.2   |
| Unsaturated:       |       |       |
| C16:1 ω5c          | 21.4  | 19.2  |
| Branched:          |       |       |
| iso-C14:0          | 1.3   | TR    |
| iso-C15:0          | 7.4   | 7.3   |
| iso-C16:0          | 1.4   | 1.1   |
| anteiso-C15:0      | 2.9   | 4.7   |
| Hydroxy:           |       |       |
| C15:0 3-OH         | 3     | 5.6   |
| iso-C15:0 3-OH     | 2.5   | 4.4   |
| iso-C16:0 3-OH     | 2.1   | 1.1   |
| iso-C17:0 3-OH     | 3.4   | 3.3   |
| Summed feature:    | 40.8  | 44.5  |

| Strains: 1, Fibrivirga algicola JA-25T (this study); 2, Fibrella aestuarina BUZ 2T (this study); ND, not detected; TR, trace amount (< 1.0%) |
data, strain JA-25\textsuperscript{T} should be classified as a novel species in the novel genus \textit{Fibrivirga} within the family ‘Spirosomaceae’ for which the name \textit{Fibrivirga algicola} sp. nov. is proposed. The type strain is JA-25\textsuperscript{T} (=KCCM 43334\textsuperscript{T} = NBRC 114259\textsuperscript{T}).

Description of \textit{Fibrivirga} gen. nov.

\textit{Fibrivirga} (Fi.bri.vir’ga, L. fem. n. fibra, a fibre or filament; N.L fem. n. virga, a rod; N.L. fem. n. Fibrivirga, a filamentous rod).

Cells are pink-pigmented, rod-shaped, non-motile, gram-negative, and obligately aerobic. The major respiratory quinone is menaquinone 7. This genus is part of the family \textit{Spirosomaceae}. The type species is \textit{Fibrivirga algicola}.

Description of \textit{Fibrivirga algicola} sp. nov.

(Al.gi.co’la. L. fem. n. alga (gen. algae), a seaweed; L. masc./fem. suff.—cola, dweller.; and L. masc./fem. n. incola, an inhabitant, dweller; N.L. fem. n. Algiocola, inhabitant of algae).

Cells are Gram-stain-negative, aerobic. The cells are rod-shaped (0.8–1.2 μm wide and 1.5–3.5 μm long). Gliding motility is absent, and the production of flexirubin pigments is negative. Growth occurs at 15–30 °C (optimum 25 °C), and pH 6–9 (optimum 7.0) and does not require NaCl. The predominant cellular fatty acids are summed feature 3 (comprising C16:1 ω6c and/or C16:1 ω6c; 40.8%), C16:1 ω5c (21.4%) and C16:0 (10%). The major polar lipids are phosphatidylethanolamine, unidentified aminolipids, phospholipids and five unidentified lipids.

The type strain is JA-25\textsuperscript{T} (=KCCM 43334\textsuperscript{T} = NBRC 114259\textsuperscript{T}). The DDBJ/ENA/GenBank accession numbers for the 16S rRNA gene and genome sequences of \textit{Fibrivirga algicola} JA-25\textsuperscript{T} are MN559427 and WAEL01000000, respectively.

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Data availability Because of confidentiality agreements, supporting data can only be made available to bona fide researchers subject to a non-disclosure agreement.

Declarations

Conflict of interest The authors declare no conflict of interest.

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