Aggregatimonas Sangjinii Gen. Nov., Sp. Nov., A Novel Silver Nanoparticle Synthesizing Bacterium Belonging to the Family *Flavobacteriaceaee*

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Research Article

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Abstract

A gram-negative, orange-pigmented, non-flagellated, gliding, rod-shaped, and aerobic bacterium, designated strain F202Z8^T, was isolated from a rusty iron plate found in the intertidal region of Taean, South Korea. Notably, this strain synthesized silver nanoparticles (AgNPs), and 17 putative genes responsible for the synthesis of AgNPs were found in its genome. The complete genome sequence of strain F202Z8^T is 4,723,614 bp, with 43.26% G + C content. Phylogenetic analysis based on 16S rRNA gene sequence revealed that strain F202Z8^T forms a distinct lineage with closely related genera *Maribacter*, *Pelagihabitans*, *Pseudozobellia*, *Zobellia*, *Pricia*, and *Costertonia* belonging to the family *Flavobacteriaceae*. The 16S rRNA sequence similarity was < 94.5%. The digital DNA–DNA hybridization and average nucleotide identity values calculated from the whole genome-sequence comparison between strain F202Z8^T and other members of the family *Flavobacteriaceae* were in the ranges of 12.7–16.9% and 70.3–74.4%, respectively. Growth was observed at 15–33°C (optimally at 30°C), at pH 6.5–7.5 (optimally at pH 7.0), and with the addition of 2.5–4.5% (w/v) NaCl to the media (optimally at 4.0%). The predominant cellular fatty acids were iso-$C_{15:0}$, iso-$C_{15:1}$ G, and iso-$C_{17:0}$ 3-OH; the major respiratory quinone was MK-6. Polar lipids included phosphatidylethanolamine, five unidentified lipids, and two unidentified aminolipids. Our polyphasic taxonomic results suggested that this strain represents a novel species of a novel genus in the family *Flavobacteriaceae*, for which the name *Aggregatimonas sangjinii* gen. nov., sp. nov. is proposed. The type strain of *Aggregatimonas sangjinii* is F202Z8^T (= KCCM 43411^T = LMG 31494^T).

Introduction

The family *Flavobacteriaceae* (Reichenbach 1992; Bernardet et al. 2002) is one of the major branches of the phylum *Bacteroidetes* (Garrity and Holt 2001). Currently, this family consists of 159 genera with validly published names (http://www.bacterio.net), and the members are widely distributed in global ecosystems such as soil, sediment, freshwater, and seawater. According to its high diversity, this family is divided into five phylogenetic clades based on 16S rRNA gene sequence analysis, namely, Marine clade, *Capnocytophaga* clade, *Flavobacterium* clade, *Tenacibaculum - Polaribacter* clade, and *Chryseobacterium* clade (McBride 2014). The members of the Marine clade contain a larger and more diverse genera than other clades of the family *Flavobacteriaceae*. The species within the Marine clade are gram-negative, rod-shaped cells that are non-motile or motile by gliding and grow aerobically. Colonies are pigmented yellow to a deep orange by carotenoid and/or flexirubin pigments, and MK-6 is the major respiratory quinone. The DNA G + C content ranges from 27 to 43 mol% except for the genus *Robiginitalea*, which has 50.1 and 55 mol% DNA G + C content (Bowman 2006; McBride 2014).

In the process of isolating aerobic heterotrophic bacteria from intertidal habitats in Korea, one isolate that formed orange colonies on ZoBell medium was obtained. Notably, a novel bacterial strain (designated F202Z8^T) was found to biologically synthesize silver nanoparticles (AgNPs) without additional physical conditions or chemical agents. AgNPs have been widely used in various fields such as food, medicine, agriculture, and bio-sensing (Kumar et al 2008; Lü et al 2009; Sastry et al 2010; Yu et al 2019). To facilitate the application of AgNPs produced by this strain, F202Z8^T was taxonomically assigned using a polyphasic approach. Moreover, we manually searched genes potentially associated with AgNP production in the genome of this strain. Based on the collective findings, we propose this strain as a novel species of a novel genus in the family *Flavobacteriaceae*.

Materials And Methods

Isolation of the bacterial strain and culture conditions

The strain F202Z8^T was isolated from a rusty iron plate found in the intertidal region of Taean, Korea (36° 35' 25"N, 126° 17' 17"E) on June 1, 2018. For strain isolation, the collected samples were serially diluted with sterile seawater and spread on 1/10 diluted ZoBell medium (Kwon et al. 2012). The inoculated plates were incubated at 20°C for five days; then, individual colonies were isolated from marine agar 2216 (MA; Difco) on the basis of morphological differences. After primary isolation and purification, the strain was routinely cultured on MA at 25°C and preserved with 20% (v/v) glycerol at -80°C. *Costertonia aggregata* KCCM 42265^T and *Pricia antarctica* JCM 17291^T were used as reference strains for the analyses of fatty acids and polar lipids, and other phenotypic characterization. These strains were obtained from the Korean Culture Collection of Microorganisms (KCCM) and the Japan Collection of Microorganisms (JCM), respectively.

Biological synthesis of AgNPs

The synthesis of AgNPs by strain F202Z8^T was examined as previously described (Srivastava and Constanti 2012). F202Z8^T was cultured in marine broth at 27 °C for three days and then the cells were harvested by centrifugation at 10,000 × *g* for 10 min. Pellets were thoroughly washed three times with phosphate-buffered saline and sterilized water, and 0.2 g wet weight pellets were mixed with an equal volume of $AgNO_3$ solution (final concentration of 1 mmol/L). After 36 h incubation, the cells were removed by centrifugation at 5,000 × *g* for 10 min, and the biosynthesized AgNPs from the supernatant were confirmed using ultraviolet-visible, energy dispersive X-ray spectroscopy, and transmission electron microscope (TEM) analysis.

Phenotypic and biochemical characterization

Cell morphology and the presence of flagella were examined using TEM (Tecnai G^2 Spirit Twin, FEI, Hillsboro, OR, USA) after negative staining of cells grown on MA for two days. Gram staining was performed using the Gram stain kits (BD, Franklin Lakes, NJ, USA), according to the manufacturer's instructions. Gliding motility was determined using the hanging drop technique as described by Bernardet et al. (2002). The growth temperature was tested on MA incubated at 5, 10, and 15°C (seven days) and 20, 23, 26, 30, 33, 36, 39, 42, and 45°C (48 h). Growth with 0–5% NaCl (at intervals of 0.5%, w/v) and 6–15% (at intervals of 1%, w/v) was performed in NP broth (containing 0.5% tryptone, 0.1% yeast extract, 0.5% $MgCl_2$, 0.2% $MgSO_4$, 0.1% $CaCl_2$, 0.1% KCl, and distilled water, pH 7) (Kwon et al. 2018). Growth rates were determined for pH values 4–12 in NP broth with 4% NaCl adjusted by HCl (pH 4–6), phosphate buffer (pH 7 and 8), glycine/NaOH (pH 9 and 10), and Tris/HCl (pH 11 and 12). Anaerobic growth was examined on MA at 30°C for 15 days with a GasPak EZ anaerobic container system (BD) according to the manufacturer's instructions. Ionic requirements were examined according to the method described by Kwon et al. (2018).

Oxidase and catalase activities were determined using oxidase reagent (bioMérieux, Marcy-l'Étoile, France) and the production of bubbles after the addition of a drop of 3% H_2O_2 solution, respectively. Hydrolysis of starch (amylase), casein and skim milk (protease), CM-cellulose (cellulose), L-asparagine (asparaginase), and Tweens 80 (lipase) were examined after five days of incubation on MA-based media. Carbon utilization was determined using Biolog GN2 plate, according to the manufacturer's instructions, with artificial sea water (ASW, containing 4% NaCl, 0.5% $MgCl_2$, 0.2% $MgSO_4$, 0.1% $CaCl_2$, 0.1% KCl, 0.0001% $FeSO_4$, and distilled water) (Smibert and Krieg 1994) as the cell suspension solution at 30°C for two days. Acid production from carbohydrates was determined using API 50 CH strips (bioMérieux), according to the manufacturer's instructions, except that API 50 CHB/E medium for the cell suspension was supplemented with 4% NaCl, 1% $MgCl_2{\cdot}6H_2O$, and 0.1% $CaCl_2$. The enzymatic activities and biochemical properties were investigated by using API 20NE and API ZYM strips (bioMérieux), according to the manufacturer's instructions. The inoculum was the cell suspension in ASW solution, and it was incubated at 30°C for two days. Antibiotic susceptibility was performed using the disc-diffusion method (Liofilchem, Roseto degli Abruzzi, Italy) on MA, and the growth inhibition zones were observed after three days of incubation at 30°C.

Chemotaxonomic characterization

For analysis of cellular fatty acids, quinones, and polar lipids, strain F202Z8^T and two reference strains were cultivated in MA at their optimal temperatures. Cellular fatty acids of microbial cells were saponified, methylated, and analyzed using GC (7890B, Agilent Technologies, Santa Clara, CA, USA), according to the instructions of the Microbial Identification System (MIDI; version 6.3), with the RTSBA6 database. Isoprenoid quinones were extracted according to the method of Minnikin et al. (1984) and analyzed using a model SP930D HPLC system (Youngin Chromass, Seoul, South Korea) equipped with a diode array detector (UV730D; Youngin Chromass) and a reversed-phase column (250×4.6 mm, Waters Spherisorb) (Collins 1985). Polar lipids were examined using two-dimensional TLC and identified using the procedures described by Minnikin et al. (1984).

Phylogenetic and genotypic analysis

Extraction of genomic DNA was performed using Exgene DNA extraction kit (Gene All, Seoul, South Korea), and the amplified 16S rRNA gene sequencing was performed with an Applied Biosystems automated sequencer (ABI 3730XL) at Macrogen (Seoul, South Korea). To ascertain the phylogenetic position of strain F202Z8^T, the 16S rRNA gene sequence was compared with that of published species from the EzBioCloud server (ezbiocloud.net/identify) (Yoon et al. 2017). A total of 1,385 unambiguously aligned sequences were compared, and phylogenetic trees were reconstructed using the neighbor-joining (Saitou and Nei 1987), maximum-likelihood (Felsenstein 1981), and maximum-parsimony (Kluge and Farris 1969) algorithms. Genetic distances for these analyses were calculated using Kimura's two-parameter model (Kimura 1980), and in all three analyses the 1000 bootstrap resampled datasets were used to estimate node robustness, using MEGA7 software (Kumar et al. 2016).

Genomic sequencing of strain F202Z8^T and *Costertonia aggregata* KCCM 42265^T were obtained using a combination of PacBio RS II single-molecule real-time (SMRT) and Illumina Novaseq 6000 sequencers at DNA Link (Seoul, South Korea). A 20-kb insert library was constructed for both F202Z8^T and KCCM 42265^T and sequenced, yielding > 129.3× and > 86.3× average genome coverage, respectively. *De novo* assembly of the 80,294 reads (1,134,945,802 bp in total) for F202Z8^T and 64,233 reads (519,541,435 bp total) for 42265^T was conducted using the hierarchical genome-assembly process pipeline of the SMRT Analysis v2.3.0. Open reading frames were predicted using the National Centre for Biotechnology Information Prokaryotic Genomes Annotation Pipeline. The qualities of the resulting genomes of strain F202Z8^T and C. *aggregata* KCCM 42265^T were assessed based on their completeness and contamination rates using CheckM software v1.0.4 (Parks et al. 2015). The average nucleotide identity (ANI) and *in silico* DNA–DNA hybridization (DDH) values between strain F202Z8^T and other related taxa were calculated using the Orthologous Average Nucleotide Identity Tool (OAT) software available in the EzBioCloud server (www.ezbiocloud.net/sw/oat) (Lee et al. 2016) and the Genome-to-Genome Distance Calculator v2.1 (http://ggdc.dsmz.de/distcalc2.php) (Meier-Kolthoff et al. 2013), respectively. In addition, the functional annotation of different databases for a whole genome BLAST search was performed, including Cluster of Orthologous Groups of proteins (COG) (Michael et al. 2015), Gene Ontology (GO) (Ashburner et al. 2000), Kyoto Encyclopedia of Genes and Genomes (KEGG) (Kanehisa et al. 2016), Non-Redundant Protein (NR) (Li et al. 2002), and Swiss-Prot databases (Bairoch and Apweiler 2000). Putative secondary biosynthetic metabolites of the genome were predicted using antiSMASH 5.0 (Blin et al. 2019). The genome sequences of strain F202Z8^T and *C. aggregata* KCCM 42265^T were deposited in GenBank under the accession numbers CP040710 and CP058595, respectively.

Results And Discussion

Phylogenetic and genotypic characteristics

Strain F202Z8^T was found to share 16S rRNA gene sequence similarities with other genera within the family *Flavobacteriaceae*, such as *Maribacter* (94.48% –92.40%), *Pelagihabitans* (94.25%), *Pseudozobellia* (93.72%), *Zobellia* (93.28% –92.53%), *Pricia* (93.03%), and *Costertonia* (93.01%). In the phylogenetic analysis based on 16S rRNA gene sequences of related taxa within the family *Flavobacteriaceae*, strain F202Z8^T formed a distinct, independent clade between both genus *Pricia* and *Costertoni*, representing a genus position, which suggested that strain F202Z8^T should be assigned to a novel genus in the family *Flavobacteriaceae* (Fig. 1).

The complete genome sequences of F202Z8^T and KCCM 42265^T consisted of 4,723,614 bp (G + C content of 43.26%) in one contig and 3,910,334 bp (G + C content of 39.44%) in one contig within one scaffold chromosome without plasmids, respectively. The genome of F202Z8^T contained a total of 3,942 predicted genes and 3,856 protein coding sequences, while the genome of KCCM 42265^T contained a total of 3,598 predicted genes and 3,527 protein coding sequences. The general genomic features of strain F202Z8^T and KCCM 42265^T were compared with those of other related reference strains (Table 1). The completeness and contamination rates of the genomes of strain F202Z8^T and KCCM 42265^T were 99.6% and 0.7% and 99.3% and 0.3%, respectively, which clearly satisfied the criteria (≥ 90 and ≤ 10%, respectively) to be considered as a high-quality genome (Parks et al. 2015). The ANI and *in silico* DDH values between strain F202Z8^T and other related genera were clearly lower than the thresholds (< 95% ANI and < 70% DDH) for prokaryotic species delineation (Table 2) (Stackebrandt et al. 2002; Richter and Rosselló-Móra 2009), suggesting that strain F202Z8^T represents a new genus of the family *Flavobacteriaceae*.

Table 1
General genomic features of strain F202Z8T and other members of the family Flavobacteriaceae.

Characteristic	1[a]	2[a]	3[b]	4	5	6	7
Genome status	Complete	Complete	Draft	Draft	Draft	Draft	Complete
Contigs	1	1	52	23	7	44	1
Size (bp)	4,723,614	3,910,334	4,851,997	4,560,827	4,050,606	5,036,817	5,521,712
G + C contents(mol%)	43.26	39.44	43.78	44.50	38.90	47.10	42.80
Genes	3,942	3,598	4,227	3,998	3,499	4,192	4,485
Protein coding genes	3,856	3,527	4,176	3,935	3,425	4,104	4,403
rRNA genes (5S, 16S, 23S)	6 (2, 2, 2)	9 (3, 3, 3)	5 (2, 1, 2)	3 (1, 1, 1)	6 (2, 2, 2)	8 (2, 4, 2)	6 (2, 2, 2)
tRNA genes	39	42	37	36	40	38	40
ncRNA genes	4	4	9	4	4	4	4
Pseudogenes	37	16	–	20	24	38	32
GenBank accession number	CP040710	CP058595	FNAO00000000	VIKU00000000	LDAS00000000	FQYU00000000	FP476056

Strains: 1, F202Z8^T; 2, *Costertonia aggregata* KCCM 42265^T; 3, *Pricia antarctica* DSM 23421^T; 4, *Pelagihabitans pacificus* TP-CH-4^T; 5, *Maribacter thermophilus* HT7-2^T; 6, *Pseudozobellia thermophila* DSM 19858^T; 7, *Zobellia galactanivorans* DsiJ^T

[a] Genomes sequenced in this study.

[b] Data from the Integrated Microbial Genomes (IMG) server under IMG-taxon 2684622891.

Table 2
Pair-wise average nucleotide identity (ANI) and in silico DNA-DNA hybridization (DDH) values among strain F202Z8T and other members of the family Flavobacteriaceae. The upper matrix represents ANI values, while the lower matrix represents in silico DDH values.

Strains	1	2	3	4	5	6	7
1	–	71.4	71.1	71.8	70.3	70.9	71.0
2	13.0	–	71.2	71.2	71.5	70.8	71.4
3	12.8	12.7	–	71.1	70.3	72.1	72.3
4	13.5	13.1	12.9	–	70.3	71.0	71.0
5	12.8	13.6	12.7	13.1	–	73.0	71.4
6	13.0	13.2	13.0	13.2	14.8	–	74.4
7	13.0	13.3	13.2	13.1	13.9	16.9	–

Strains: 1, F202Z8^T (CP040710); 2, *Costertonia aggregata* KCCM 42265^T (CP058595); 3, *Pricia antarctica* JCM 17291^T (FNAO00000000); 4, *Pelagihabitans pacificus* TP-CH-4^T (VIKU00000000); 5, *Maribacter thermophilus* HT7-2^T (LDAS00000000); 6, *Pseudozobellia thermophila* DSM 19858^T (FQYU00000000); 7, *Zobellia galactanivorans* DsiJ^T (FP476056)

Based on the COG, GO, KEGG, NR, and Swiss-Prot databases, the genomic analysis of F202Z8^T revealed the presence of 17 genes participating in the synthesis of AgNPs, including laccase, catalase, alkaline phosphatase, alkaline phosphatase isozyme, thioredoxin reductase, thioredoxin, NADPH dehydrogenase, glutathione synthase, glutathione s-transferase, glutathione-disulfide reductase, protein-disulfide isomerase, c-type cytochrome, riboflavin, cytochrome c reductase, cytochrome c oxidase, nitroreductase, and nitrate reductase (Li et al. 2020; Lis et al. 2020).

To identify gene clusters involved in secondary metabolite biosynthesis, the F202Z8^T genome was analyzed with the antiSMASH. The analysis revealed the presence of four predicted gene clusters for potential secondary metabolites, such as alkylpyrone involved in

inhibiting spore development (Grubbs et al. 2017), carotenoid, atratumycin involved in anti-tuberculosis (Sun et al. 2019), and flexirubin (Supplementary Table S1).

Phenotypic characteristics

The cells of strain F202Z8^T were gram negative, aerobic, and non-flagellated (Supplementary Fig. S1) but showed gliding motility. In addition, this strain was found to produce naturally occurring AgNPs. The biosynthesized AgNPs were spherical and polygonal in shape, with size ranging from 5.5 to 74.5 nm (unpublished data). Microbial NPs have received attention owing to their potential application in catalysis, optics, medicine, and energy development (Mandal et al. 2006).

The novel isolate was resistant to gentamicin (10 µg), kanamycin (30 µg), nalidixan (30 µg), and streptomycin (10 µg), and susceptible to ampicillin (10 µg), chloramphenicol (30 µg), cefazolin (30 µg), cephalothin (30 µg), erythromycin (15 µg), ofloxacin (5 µg), penicillin (10 IU), rifampicin (5 µg), tetracyclines (30 µg), and vancomycin (30 µg). Strain F202Z8^T did not grow in the absence of NaCl in the medium or in NP broth supplemented with only NaCl, $MgCl_2$, or $CaCl_2$. Growth of F202Z8^T was slightly improved by the addition of both NaCl and $MgCl_2$ to NP broth. Moreover, addition of NaCl, $MgCl_2$, and $CaCl_2$ led to drastically increased growth of this strain. Further morphological, physiological, and biochemical characteristics of the isolate are presented in the description of strain F202Z8^T and other related genera of the family *Flavobacteriaceae* in Table 3.

Table 3

Differential characteristics of strain F202Z8^T and other members of the family *Flavobacteriaceae*.

Characteristics	1	2	3	4[c]	5[d]	6[e]	7[f]
Isolation source	Rusty iron plate	Marine biofilm	Sandy sediment	Marine sponge	Seaweed	Green alga	Red alga
Gliding motility	+	–	–	–	+	+	+
Colony color	Deep orange	Light orange	Yellow	Orange	Yellow	Dark orange	Yellow
Growth at							
Temperature (°C) (optimum)	15–33 (30)	10–35 (26–32)[a]	0–25 (17–19)[b]	4–37 (25–30)	4–50 (40–42)	4–49 (ND)	13–45 (35)
pH (optimum)	6.5–7.5 (7.0)	6.5-9.0 (7.5-8.0)[a]	6.0-9.5 (7.5-8.0)[b]	7–9 (7.0–8.0)	5.5–8.5 (7.0)	ND	6.0-8.5 (7.0)
NaCl (%) (optimum)	2.5–7.5 (4.0)	1.5–12.0 (3.0)[a]	0.5-6 (2–3)[b]	1.0–4.0 (2.0)	0–8.0 (2.0–3.0)	0.5-8.0 (4–5)	0.5-6.0 (2.5)
Hydrolysis of							
Casein	+	+	–	–	–	–	+
CM-Cellulose	–	+	–	–	–	–	–
L-aspaginase	–	–	+	ND	ND	ND	ND
Starch	+	–	+	+	–	–	+
Tween 80	W	–	–	–	+	+	–
Acid production (API 50CH)							
L-rhamnose assimilation	–	+	–	ND	ND	ND	+
D-saccharose assimilation	+	–	–	ND	ND	ND	ND
D-melezitose assimilation	–	+	–	ND	ND	ND	ND
Biochemical activity (API 20NE)							
Gelatin hydrolysis	–	+	–	+	+	+	+
Capric acid assimilation	+	+	–	–	–	–	ND
Enzyme activity (API ZYM)							
Acid phosphatase	+	+	–	+	+	+	ND
Naphtol-AS-BI-phosphohydrolase	+	W	–	+	+	+	+
α-galactosidase	–	W	–	+	+	+	+

Strains: 1, F202Z8^T; 2, *Costertonia aggregata* KCCM 42265^T; 3, *Pricia antarctica* JCM 17291^T; 4, *Pelagihabitans pacificus* TP-CH-4^T; 5, *Maribacter thermophilus* HT7-2^T; 6, *Pseudozobellia thermophila* DSM 19858^T; 7, *Zobellia galactanivorans* DsiJ^T. All data are from this study except where indicated. +, Positive reaction; –, negative reaction; W, weak positive reaction; ND, no data available. The strains (1, 2, and 3) from this study were positive for oxidase, catalase and aesculin, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, and *N*-acetyl-*β*-glucosaminidase; utilization of D-cellobiose, *α*-D-glucose, maltose, and D-trehalose.

[a]Data from Kwon et al. (2006); [b]Data from Yu et al. (2012); [c]Data from Wang et al. (2020); [d]Data from Hu et al. (2015); [e]Data from Nedashkovskaya et al. (2009) and Wang et al. (2020); [f]Data from Barbeyron et al. (2001) and Nedashkovskaya et al. (2004).

Characteristics	1	2	3	4[c]	5[d]	6[e]	7[f]
β-galactosidase	–	–	+	+	+	+	+
β-glucosidase	–	+	+	W	+	+	ND
α-mannosidase	–	+	+	–	+	+	ND
Major fatty acids (> 10%)	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH	iso-$C_{15:0}$, iso-$C_{15:1}$ G	iso-$C_{15:0}$	iso-$C_{15:0}$, unknown ECL 13.565	iso-$C_{15:0}$, iso-$C_{15:1}$ G	iso-$C_{15:0}$, iso-$C_{17:0}$ 3-OH

Strains: 1, F202Z8^T; 2, *Costertonia aggregata* KCCM 42265^T; 3, *Pricia antarctica* JCM 17291^T; 4, *Pelagihabitans pacificus* TP-CH-4^T; 5, *Maribacter thermophilus* HT7-2^T; 6, *Pseudozobellia thermophila* DSM 19858^T; 7, *Zobellia galactanivorans* DsiJ^T. All data are from this study except where indicated. +, Positive reaction; –, negative reaction; W, weak positive reaction; ND, no data available. The strains (1, 2, and 3) from this study were positive for oxidase, catalase and aesculin, alkaline phosphatase, leucine arylamidase, valine arylamidase, cystine arylamidase, and *N*-acetyl-*β*-glucosaminidase; utilization of D-cellobiose, *α*-D-glucose, maltose, and D-trehalose.

[a]Data from Kwon et al. (2006); [b]Data from Yu et al. (2012); [c]Data from Wang et al. (2020); [d]Data from Hu et al. (2015); [e]Data from Nedashkovskaya et al. (2009) and Wang et al. (2020); [f]Data from Barbeyron et al. (2001) and Nedashkovskaya et al. (2004).

Chemotaxonomic characteristics

The major respiratory quinone was shown to be MK-6, in line with all other members of the family *Flavobacteriaceae* (Kimura 1980; Kumar et al. 2016). The dominant fatty acids (> 5%) were determined to be iso-$C_{15:0}$ (27.5%), iso-$C_{15:1}$ G (20.5%), iso-$C_{17:0}$ 3-OH (12.6%), iso-$C_{15:0}$ 3-OH (7.3%), summed feature 3 (comprising $C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*; 11.0%), and summed feature 9 ($C_{16:0}$ 10-methyl and iso-$C_{17:1}$ *ω9c*; 5.3%). Although these compositions and relative contents were similar to those of related members in the family *Flavobacteriaceae*, differences were observed in the proportions of major and minor components (Supplementary Table S2). The predominant polar lipids were phosphatidylethanolamine, five unidentified lipids, and two unidentified aminolipids (Supplementary Fig. S2).

Based on the data from phenotypic tests and genotypic differences between strain F202Z8^T and its close phylogenetic relatives, strain F202Z8^T was concluded to represent a novel genus belonging to the family *Flavobacteriaceae*, for which the name *Aggregatimonas sangjinii* gen. nov., sp. nov. is proposed.

Description of Aggregatimonas gen. nov.

Aggregatimonas (Ag.gre.ga.ti.mo'nas. L. v. *aggregare*, to aggregate, form clumps; L. fem. n. *monas* a unit, monad; N.L. fem. n. *Aggregatimonas*, an aggregating monad).

The cells are strictly aerobic, gliding, gram-negative rods. They are oxidase- and catalase-positive and require Na^+, Mg^{2+} and Ca^{2+} ions for growth. The major respiratory quinone is MK-6. The major cellular fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G and iso-$C_{17:0}$ 3-OH. The analysis of 16S rRNA gene sequences showed that the genus *Aggregatimonas* should be considered a member of the family *Flavobacteriaceae*, phylum *Bacteroidetes*. The type species is *Aggregatimonas sangjinii*.

Description of Aggregatimonas sangjinii sp. nov.

Aggregatimonas sangjinii (sang.jin'i.i. N.L. gen. n. *sangjinii* of Sangjin), was named to honor the Korean microbiologist Sang-Jin Kim, for his contribution to the knowledge of marine microbiology.

The cells are rods with rounded ends approximately 0.3–0.5 (width) × 0.9–2.7 (length) μm. The colonies grown on MA are deep orange colored, slightly convex, and smooth with entire margins. Gliding motility is present. It grows under aerobic but not under anaerobic conditions. Growth occurs at 15–33°C (optimally at 30°C), at pH 6.5–7.5 (optimally at pH 7.0), and in the presence of 2.5–4.5% (w/v) NaCl (optimally at 4.0%). Magnesium and calcium ions are required for growth. It is positive for oxidase, catalase, and hydrolysis of starch, casein, and Tweens 80, and negative for hydrolysis of CM-cellulose, L-asparaginase, and skimmed milk. In API 20NE test, it is positive for aesculin hydrolysis and assimilation of capric acid but negative for nitrate reduction to nitrite, indole production, glucose fermentation, arginine dihydrolase, urease, gelatin hydrolysis, and β-galactosidase activity, and assimilation of glucose, arabinose, mannose, mannitol, *N*-acetyl-glucosamine, maltose, gluconate, adipate, malate, citrate, and phenyl-acetate. In API ZYM test, it is positive for alkaline phosphatase, esterase (C4), esterase lipase (C8), valine arylamidase, trypsin, *α*-chymotrypsin, acid phosphatase, naphthol-AS-

Bl-phosphohydrolase, and *N*-acetyl-*β*-glucosaminidase, and weakly positive for lipase (C14), leucine arylamidase, and cystine arylamidase, but negative for *α*-galactosidase, β-galactosidase, *β*-glucuronidase, *β*-glucosidase, *α*-glucosidase, *α*-mannosidase, and *α*-fucosidase. In API 50 CH test, the following carbohydrates are acidified: L-arabinose, D-xylose, D-galactose, D-glucose, D-fructose, D-mannose, L-rhamnose, methyl *α*-D-mannopyranoside, methyl *α*-D-glucopyranoside, *N*-acetyl-glucosamine, amygdalin, arbutin, aesculin, salicin, cellobiose, maltose, lactose, sucrose, raffinose, starch, turanose, and D-lyxose. The following carbohydrates are slightly acidified: D-arabinose, methyl *β*-D-xylopyranoside, melibiose, trehalose, gentiobiose, D-tagatose, D-fucose, and L-fucose. In Microlog GN2 microplates, it utilizes *α*-cyclodextrin, dextrin, *N*-acetyl-D-glucosamine, D-cellobiose, D-fructose, D-galactose, gentiobiose, *α*-D-glucose, *α*-D-lactose, lactulose, maltose, D-mannose, D-melibiose, methyl *β*-D-glucoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, turanose, D-galacturonic acid, D-glucuronic acid, L-asparagine, L-aspartic acid, L-glutamic acid, glycyl L-aspartic acid, and glucose 1-phosphate, and weakly utilizes glycogen, *γ*-hydroxybutyric acid, succinic acid, glycyl L-glutamic acid, hydroxy L-proline, and L-ornithine. The main fatty acids are iso-$C_{15:0}$, iso-$C_{15:1}$ G, iso-$C_{17:0}$ 3-OH, and summed feature 3 ($C_{16:1}$ *ω7c* and/or $C_{16:1}$ *ω6c*). The predominant polar lipids are phosphatidylethanolamine, five unidentified lipids and two unidentified aminolipids. The DNA G + C content of the type strain is 43.26 mol%.

The type strain is F202Z8^T (KCCM 43411^T = LMG 31494^T), isolated from a rusty iron plate found in the intertidal region of Taean, Korea.

Declarations

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Conflicts of interest/Competing interests

The authors declare that there are no conflicts of interest.

Availability of data and material

All data generated or analyzed during this study are included in this manuscript and its supplementary information files.

Code availability

Not applicable

Authors' contributions

DC and YMK performed all experiments, data analysis, and manuscript finalization. JYHK and KWK participated in the review of the manuscript and isolation of this strain. All authors have read and approved the manuscript.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Consent to participate

Not applicable

Consent for publication

Not applicable

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Figures

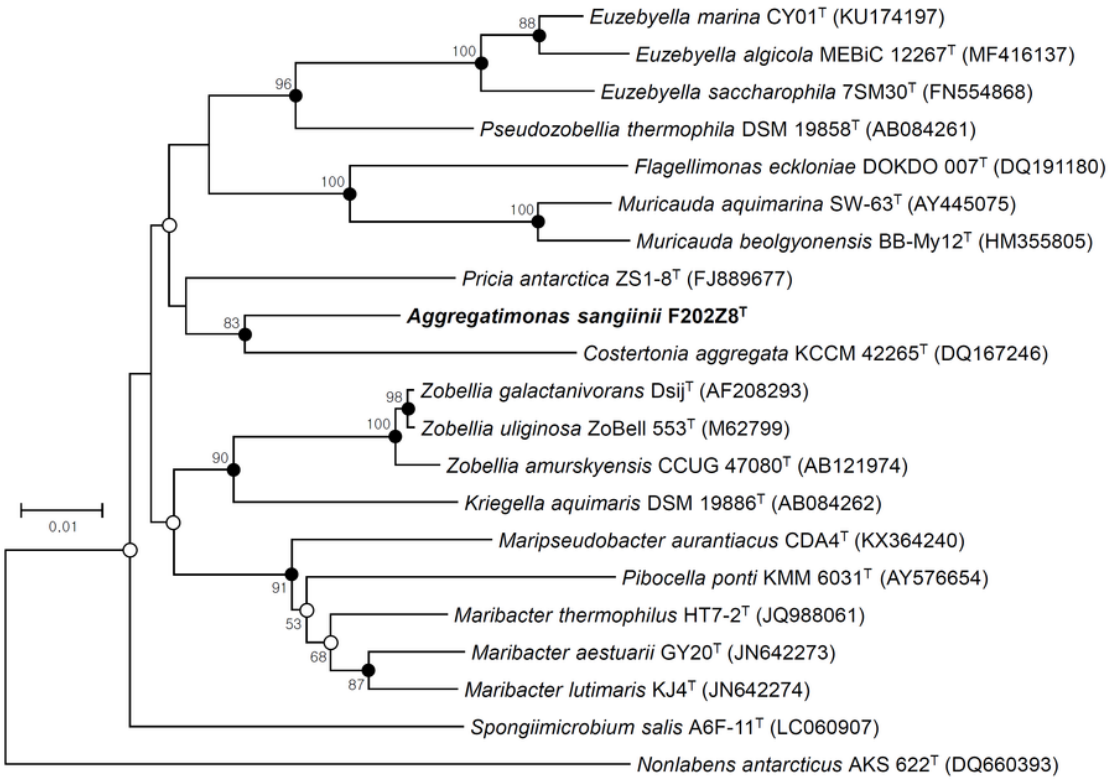

Figure 1

Neighbor-joining tree based on 16S rRNA gene sequences showing the phylogenetic relationships of strain F202Z8T (in bold type) and other members of the family Flavobacteriaceae. GenBank accession numbers are given in parentheses. Bootstrap values (>50%) based on 1,000 replicates are indicated at nodes. Closed and open circles indicate nodes recovered from the three treeing methods (neighbor-joining, maximum-likelihood, and maximum-parsimony) or two treeing methods, respectively. Nonlabens antarcticus AKS622T was used as an outgroup. Bar, 0.01 changes per nucleotide position.

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