Ferrimonas futtsuensis sp. nov. and *Ferrimonas kyonanensis* sp. nov., selenate-reducing bacteria belonging to the *Gammaproteobacteria* isolated from Tokyo Bay

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Two novel mesophilic, facultatively anaerobic, selenate-reducing bacteria, designated strains FUT3661^T and Asr22-7^T, were isolated from a sediment sample and the alimentary tract of littleneck clams, respectively. Both sources of the samples were collected from the coast of Tokyo Bay, Japan. Cells were Gram-negative rods and motile by means of a polar flagellum. The strains reduced selenate to elemental selenium (Se⁰) and also reduced iron(III) oxyhydroxide, iron(III) citrate, arsenate, manganese(IV) oxide, elemental sulfur and oxygen and used lactate, pyruvate, yeast extract, tryptone and Casamino acids as electron donors and carbon sources. The strains contained both menaquinone (MK-7) and ubiquinones (Q-7 and Q-8) as isoprenoid quinones. The major fatty acids were C_{16:0} and C_{16:1ω9c}. The G + C content of the genomic DNA was 58.1 mol% for strain FUT3661^T and 57.2 mol% for strain Asr22-7^T. Phylogenetic analysis based on 16S rRNA gene sequences revealed that the strains were related to members of the genus *Ferrimonas* (<94.0% similarities), although the two novel strains formed a separate lineage. 16S rRNA gene sequence similarity between strains FUT3661^T and Asr22-7^T was 96%. On the basis of this polyphasic analysis, it was concluded that strains FUT3661^T and Asr22-7^T represent two novel species within the genus *Ferrimonas*, for which the names *Ferrimonas futtsuensis* sp. nov. (type strain FUT3661^T = NBRC 101558^T = DSM 18154^T) and *Ferrimonas kyonanensis* sp. nov. (type strain Asr22-7^T = NBRC 101286^T = DSM 18153^T) are proposed.

Selenium (Se) is a required element for the synthesis of the essential amino acid selenocysteine, but it is highly toxic at micromolar concentrations. Selenate [SeO₄²⁻, Se(VI)] and selenite [SeO₃²⁻, Se(IV)] are distributed in oxic environments, while insoluble elemental selenium (Se⁰) is distributed in anaerobic environments (Conde & Sanz Alaejos, 1997). The reduction of selenium oxyanions occurs primarily via microbial dissimilatory reduction (Stolz & Oremland, 1999). Moreover, the microbial reduction of

soluble Se(VI) to insoluble Se⁰ is an important process in the removal of Se(VI) from Se-contaminated water. In recent years, several bacteria capable of reducing selenate to elemental selenium have been isolated from different environments. These isolates include *Bacillus* sp. SF-1 from a selenium-polluted sediment (Fujita *et al.*, 1997), *Bacillus selenitireducens* MLS10^T from alkaline lake sediments (Switzer Blum *et al.*, 1998), *Sulfurospirillum barnesii* SES-3^T from freshwater sediments (Stolz *et al.*, 1999), *Selenihalanaerobacter shriftii* DSSe-1^T from deep-sea sediments (Switzer Blum *et al.*, 2001), *Salana multivorans* Se-3111^T from an aerobic bioreactor (von Wintzingerode *et al.*, 2001) and *Citrobacter freundii* Iso-Z7 from selenium-contaminated sediment (Zhang *et al.*, 2004). Recently, a dissimilatory metal-ion-reducing bacterium, *Shewanella oneidensis* MR-1^T, within the *Gammaproteobacteria* was found to reduce selenite to elemental selenium (Klonowska *et al.*, 2005). Here we report the characterization of novel mesophilic, facultatively anaerobic bacteria belonging to the *Gammaproteobacteria*, which are related to the genus *Ferrimonas* and are able to reduce selenate to elemental selenium. Given that the type strain of the type species of the

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Abbreviation: DAPI, 4',6-diamidino-2-phenylindole.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of *Ferrimonas futtsuensis* FUT3661^T and *Ferrimonas kyonanensis* Asr22-7^T are AB245515 and AB245514, respectively.

Transmission electron micrographs of cells of strains FUT3661^T and Asr22-7^T, figures showing the effects of temperature, NaCl concentration and selenate concentration on growth of these two strains and a table detailing their fatty acid contents are available as supplementary material in IJSEM Online.

genus *Ferrimonas*, *Ferrimonas balearica* PAT^T, is known to be an iron(III)- and manganese(IV)-reducing bacterium (Rosselló-Mora *et al.*, 1995), the reduction of selenate, selenite, iron(III) and manganese(IV) was investigated in the novel isolates and in the type strains of recognized members of the genus *Ferrimonas*.

Sediment samples were collected with an Eckman grabber from a mudflat at Futtsu beach on the coast of Tokyo Bay, Japan. Black-coloured sediments were recovered from cores by means of plastic syringes, immediately put into 150 ml sterilized serum bottles containing 100 ml modified bicarbonate-buffered (MBB) medium without electron donor and acceptor and then sealed with a butyl rubber cap under a gas phase of 100% N₂. The samples were transferred to our laboratory within a few hours. A sample of the slurry (1 ml) was used to inoculate 30 ml MBB medium that was prepared anaerobically under N₂/CO₂ (80:20, v/v; Widdel & Bak, 1992), the gas mixture being supplied through a deoxygenized gas pressure injector (IP-8; Sanshin Industrial). The basal medium was composed of 20 g NaCl l⁻¹, 3 g MgCl₂·6H₂O l⁻¹, 0.15 g CaCl₂·2H₂O l⁻¹, 0.25 g NH₄Cl l⁻¹, 0.2 g KH₂PO₄ l⁻¹ and 0.5 g KCl l⁻¹. The following were added per litre of the basal medium: 1 ml non-chelated trace element mixture (Widdel *et al.*, 1983), 1 ml selenite/tungstate solution (0.4 g NaOH l⁻¹, 6 mg Na₂SeO₃·5H₂O l⁻¹, 8 mg Na₂WO₄·2H₂O l⁻¹), 30 ml bicarbonate solution (84 g NaHCO₃ l⁻¹), 1 ml vitamin mixture [40 mg 4-aminobenzoic acid l⁻¹, 10 mg D-biotin, 100 mg nicotinic acid l⁻¹, 50 mg calcium D-pantothenate l⁻¹ and 150 mg pyridoxine dihydrochloride l⁻¹ dissolved in 10 mM sodium phosphate buffer (pH 7.1)], 1 ml thiamine solution [100 mg thiamine chloride dihydrochloride l⁻¹ dissolved in 25 mM sodium phosphate buffer (pH 3.4)] and 1 ml vitamin B₁₂ solution (50 mg cyanocobalamin l⁻¹ dissolved in distilled water). The pH of the medium was adjusted with 1 M HCl or 1 M CaCO₃ to 7.0. Selenate (1 M) and lactate (2 M) were separately autoclaved and added to the medium to a final concentration of 5 mM as an electron acceptor and electron donor, respectively. Serum bottles (70 ml) sealed with butyl rubber stoppers under a headspace of N₂/CO₂ (80:20, v/v) were used for cultivation. The inoculated cultures were incubated at 25 °C in the dark and shaken by hand for a few seconds every week. An orange- to red-coloured precipitate formed in the culture as a result of the formation of insoluble elemental selenium. Several elemental selenium-forming enrichment cultures were established by subculturing twice in the same medium. The enrichment cultures were diluted in anaerobic molten agar (1.1%, w/v; Bacto) of the same medium, and orange- to red-coloured colonies were obtained in the agar shake tubes. Purification of the colonies by the agar shake tube method was repeated twice before aerobic plating on a marine agar 2216 (MA; Difco) plate. A single colony on the MA plate was incubated back into MBB medium containing 5 mM selenate and 5 mM lactate and, after several days, red-coloured precipitate developed in the culture due to the formation of insoluble elemental selenium. This isolate was

designated strain FUT3661^T. The purity of the final culture was confirmed by microscopic examination and partial sequencing of the 16S rRNA gene using appropriate PCR primers.

Littleneck clams, *Ruditapes philippinarum*, were collected at Kyonan beach on the coast of Tokyo Bay and maintained in an atmosphere of 100% CO₂ during transfer to our laboratory. The clams were dissected, and approximately 1 g alimentary tract homogenate was used for the isolation of bacteria. Serial decimal dilutions (10⁻¹–10⁻¹⁰) of the alimentary tract extracts were made with saline; 0.1 ml dilutions were spread on LYPm agar plates and cultivated at room temperature (approximately 23 °C) in an atmosphere that contained 100% CO₂ for 1 month or more. LYPm medium was composed of 10 g α-lactose, 10 g yeast extract (Difco), 5 g polypeptone (Nihon Seiyaku), 20 g NaCl, 0.025 g Tween 80, 5 ml of salt solution and 1 ml distilled water; the initial pH of the medium was adjusted to 6.0. The salt solution contained (per litre of distilled water) 40 mg MgSO₄·7H₂O, 2 mg MnSO₄·4H₂O, 2 mg FeSO₄·7H₂O and 2 mg NaCl. Visible colonies grown on LYPm agar medium were collected and the purification procedure was repeated several times until the cultures were deemed to be pure. The first pure culture was designated strain Asr22-7^T. The purity of the final culture was confirmed by microscopic examination and partial sequencing of the 16S rRNA gene using appropriate PCR primers. Strain Asr22-7^T was maintained on MA plates. Several experiments for selenate reduction by strain Asr22-7^T were performed anaerobically with MBB medium. An orange-coloured precipitate developed in the culture bottle during incubation at 25 °C as a result of the formation of insoluble elemental selenium.

Cells were observed under a phase-contrast microscope (AX70; Olympus). Gram staining was carried out using a standard procedure (Hucker & Conn, 1923) with *Enterococcus faecalis* NBRC 100481^T as a positive control for the staining. Cells of strain FUT3661^T grown to late-exponential phase at 30 °C in marine broth 2216 (MB, pH 7.0; Difco) supplemented with NaCl to 3.0% (w/v), and those of strain Asr22-7^T grown to the same growth phase in MB at 25 °C, were negatively stained with 1% (w/v) phosphotungstic acid and observed under a Hitachi transmission electron microscope at an accelerating voltage of 80 kV. Cells of strain FUT3661^T were Gram-negative, motile rods (0.7–0.9 × 0.4–0.7 µm), as were those of strain Asr22-7^T (0.7–1.1 × 0.5–0.8 µm). Cells of both strains possessed a polar monotrichous flagellum (see Supplementary Fig. S1 in IJSEM Online). In most cases, strains FUT3661^T and Asr22-7^T appeared as single cells. No endospore production was observed.

Strains FUT3661^T and Asr22-7^T grew rapidly both in MB and on MA under aerobic conditions. The doubling time of strain FUT3661^T incubated aerobically in MB (pH 7.0; NaCl 3.0%) at 30 °C was 48 min, whereas that of strain Asr22-7^T incubated aerobically in MB (pH 7.0; NaCl 2.0%) at 25 °C was 78 min. To determine the optimum growth

temperature and NaCl concentration under selenate-reducing conditions, tubes with MBB medium containing 5 mM selenate and 5 mM lactate were inoculated with the two novel strains and cultivated anaerobically without shaking in the dark for 2 weeks (Table 1). Growth of the strains was determined according to the optical density of the tubes at 600 nm. All experiments were conducted in duplicate. Strain FUT3661^T grew at temperatures between 15.0 and 30.0 °C, with optimal growth at 30.0 °C (see Supplementary Fig. S2 in IJSEM Online). Strains FUT3661^T and Asr22-7^T required NaCl for growth (see Supplementary Fig. S3 in IJSEM Online). The ability to grow at pH 5.5–9.0 was tested in MBB medium containing 5 mM selenate and 5 mM lactate.

To clarify the potential utilization of electron donors and carbon sources under selenate-reducing conditions, the two strains were cultivated anaerobically in pressure-proof culture tubes (Sanshin Industrial) containing MBB medium supplemented with 5 mM selenate and one of the following potential carbon sources: 0.1 % (w/v) yeast extract, 0.1 % (w/v) tryptone, 0.1 % (w/v) Casamino acids, 5 mM D-glucose, 5 mM D-fructose, 5 mM D-maltose, 5 mM D-xylose, 5 mM D-galactose, 5 mM citrate, 5 mM fumarate, 5 mM malate, 5 mM pyruvate, 5 mM succinate,

5 mM propionate, 5 mM lactate, 5 mM acetate, 5 mM ethanol, 5 mM methanol, 0.1 % (w/v) L-glutamate, 0.1 % (w/v) glycine, 0.1 % (w/v) L-serine, 0.1 % (w/v) L-phenylalanine and 0.1 % (w/v) L-proline. Each tube was cultivated anaerobically without shaking in the dark for 2 weeks. All experiments were conducted in duplicate. The concentration of selenate in the liquid medium was analysed by HPLC (2695; Waters). Strains FUT3661^T and Asr22-7^T could not utilize acetate or glutamate, in contrast to the type strains of *F. balearica* and *Ferrimonas marina* (Table 1). All potential electron acceptors, e.g. 5 mM selenate (Na₂SeO₄), 5 mM selenite (Na₂SeO₃), 0.01 g iron(III) oxyhydroxide [Fe(OH)₃] ml⁻¹ (Lovley & Phillips, 1986), 5 mM iron(III) citrate (FeC₆H₅O₇), 5 mM arsenate (Na₂HAsO₄), 0.03 g manganese(IV) oxide (MnO₂) ml⁻¹, 0.01 g elemental sulfur (S⁰) ml⁻¹, 5 mM thiosulfate (Na₂S₂O₃), 5 mM sulfate (Na₂SO₄), 5 mM nitrate (NaNO₃) and 20 % (in the head space) oxygen (O₂), were tested in MBB medium supplemented with 5 mM lactate. All experiments were conducted in duplicate. The concentrations of selenate, arsenate and nitrate in the cultivated tube were analysed via HPLC (2695; Waters). The amounts of total arsenic [As(V) and As(III)], reduced manganese and nitrite in the cultures were analysed with Pack Test kits (WAK-As, -Mn and -NO₂; Kyoritsu Chemical-Check Lab). The amount of iron(II) in

Table 1. Phenotypic characteristics that differentiate strains FUT3661^T and Asr22-7^T from other strains within the genus *Ferrimonas*

Strains: 1, FUT3661^T; 2, Asr22-7^T; 3, *F. balearica* PAT^T (Rosselló-Mora *et al.*, 1995; Katsuta *et al.*, 2005); 4, *F. marina* A4D-4^T (Katsuta *et al.*, 2005); 5, *F. balearica* A2A-18 (Katsuta *et al.*, 2005); 6, *Ferrimonas* sp. A3B-58 (Katsuta *et al.*, 2005); 7, *Ferrimonas* sp. A3B-57-2 (Katsuta *et al.*, 2005). +, Positive reaction; –, negative reaction; ND, no data. Production of H₂S was investigated by using TSI plates. Fe(OH)₃, HAsO₄²⁻, MnO₂ and S⁰ were utilized by strains 1–4. Fe(III) citrate, O₂ and lactate were utilized by all strains. NO₃⁻ was utilized by strains 1–3. SO₄²⁻ was not utilized by strains 1–4.

Characteristic	1	2	3	4	5	6	7
Optimum growth temperature (°C)	30	25–28	28	25–30	25	25	30
Growth at 42 °C	–	–	+	–	+	ND	ND
pH for growth	6.0–9.0	6.0–9.0	6.0–9.0	6.0–11.0	6.0–11.0	6.0–11.0	6.0–11.0
NaCl concentration for growth (%)	0.7–5.0	2.0–5.0	0.5–7.5	1.5–5.5	0.5–7.5	ND	ND
Production of H ₂ S	+	+	+	–	+	ND	ND
Gelatinase	+	+	–	ND	ND	ND	ND
Arginine dihydrolase	–	+	ND	ND	ND	ND	ND
Electron acceptor:							
SeO ₄ ²⁻	+	+	+	–*	+	+	+
SeO ₃ ²⁻	–	–	+	–*	+	+	+
S ₂ O ₃ ²⁻	+	–	+	–*	ND	ND	ND
Utilization of:							
D-Glucose	–	–	–	+	ND	ND	ND
Maltose	–	–	–	+	ND	ND	ND
Acetate	–	–	+	+	ND	ND	ND
L-Glutamate	–	–	+	+	ND	ND	ND
DNA G+C content (mol%)	58	57	60	61	60	ND	ND
Major fatty acids	C _{16:0} ; C _{18:1ω9c}	C _{16:0} ; C _{18:1ω9c}	C _{16:0} ; C _{18:1ω9c}	C _{16:0} ; C _{18:1ω9c}	C _{18:1ω9c}	C _{16:0} ; C _{18:1ω9c}	C _{16:0} ; C _{18:1ω9c}

*Also investigated in the present study.

the cultures was measured with the phenanthroline method (Tamura *et al.*, 1974). Hydrogen sulfides produced from elemental sulfur, thiosulfate and sulfate in the incubated media were measured with the methylene blue formation reaction method (Cline, 1969). The production of nitrite from nitrate was also tested via the API 20 NE system (bioMérieux). Cell density in the cultures was determined by counting cells using epifluorescence microscopy after filtration of paraformaldehyde-fixed cells stained with 4',6-diamidino-2-phenylindole (DAPI) through polycarbonate Nucleopore (Millipore) membranes (0.2 µm pore size) (Porter & Feig, 1980). Strain FUT3661^T was able to utilize selenate, iron(III) oxyhydroxide, iron(III) citrate, arsenate, manganese(IV) oxide, elemental sulfur, thiosulfate, nitrate and oxygen (Table 1), the yield with each electron acceptor being 6×10^7 , 1×10^8 , 6×10^7 , 6×10^7 , 6×10^7 , 1×10^8 , 2×10^8 , 6×10^7 and 6×10^7 cells ml⁻¹, respectively. Nitrate was reduced to nitrite. No growth of strain FUT3661^T was observed in the culture with selenite (1×10^6 cells ml⁻¹). Strain Asr22-7^T was able to utilize selenate, iron(III) oxyhydroxide, iron(III) citrate, arsenate, manganese(IV) oxide, elemental sulfur, nitrate and oxygen (Table 1), the yield with each electron acceptor being 6×10^7 , 6×10^7 , 1×10^8 , 9×10^7 , 5×10^7 , 1×10^8 , 8×10^7 and 2×10^8 cells ml⁻¹, respectively. Nitrate was reduced to nitrite. No growth of strain Asr22-7^T was observed in the culture with selenite.

The optimal selenate concentration for growth in test tubes containing MBB medium supplemented with several concentrations of selenate and 5 mM lactate was determined at 30 °C for strain FUT3661^T and at 25 °C for strain Asr22-7^T without shaking in the dark for 2 weeks. Growth was assessed based on the optical density of the tubes at 600 nm. All experiments were conducted in duplicate. The optimal selenate concentrations for growth of strains FUT3661^T and Asr22-7^T were 12.5 and 5.0 mM, respectively (see Supplementary Fig. S4 in IJSEM Online). The time courses of the reduction of selenate and concomitant bacterial growth of strains FUT3661^T and Asr22-7^T were examined in 150 ml serum bottles containing 120 ml of anaerobic MBB medium supplemented with 12.5 mM selenate, 10 mM lactate and 0.01 % (w/v) yeast extract for strain FUT3661^T, and 5 mM selenate, 5 mM lactate and 0.01 % (w/v) yeast extract for strain Asr22-7^T (Fig. 1). Cultures were sampled periodically and analysed for cell density by staining paraformaldehyde-fixed cells with DAPI. The experiments were conducted in triplicate. The concentrations of selenate decreased, accompanied by precipitation of elemental selenium, during growth of the two strains. Thus, strains FUT3661^T and Asr22-7^T were found to be facultatively anaerobic, selenate-reducing chemo-organotrophs.

The abilities of cultured strains of the genus *Ferrimonas* to reduce selenate and selenite were also examined. *F. balearica* PAT^T (=DSM 9799^T), *F. balearica* A2A-18 (=MBIC 06164), *Ferrimonas* sp. A3B-58 (=MBIC 06350) and

Fig. 1. Time course of selenate reduction (filled squares) and concomitant bacterial growth (filled circles) of strains FUT3661^T (a) and Asr22-7^T (b).

Ferrimonas sp. A3B-57-2 (=MBIC 06481) were able to utilize both selenate and selenite, whereas no growth of *F. marina* A4D-4^T (=MBIC 06480^T) incubated with selenate or selenite was observed.

Strains FUT3661^T and Asr22-7^T produced H₂S on triple-sugar iron (TSI) plates (Difco), and hydrolysed gelatin (API 20 NE system; bioMérieux). Strain Asr22-7^T showed a positive reaction for arginine dehydrolase on the same API system.

For quinone analyses, strain FUT3661^T was grown in MB containing 3 % (w/v) NaCl at pH 7.0 at 30 °C, while strain Asr22-7^T was grown in MB at pH 7.0 at 25 °C. Isoprenoid quinones were extracted from freeze-dried cells with chloroform/methanol (2:1, v/v) and fractionated by TLC (Collins *et al.*, 1977). Menaquinone and ubiquinone for the LC-MS (LCMS-8000 α , Shimadzu) analysis were excised from the chromatographs. The LC-MS analysis indicated that strains FUT3661^T and Asr22-7^T contained menaquinones (MK-7) and ubiquinones (Q-7 and Q-8) as the predominant isoprenoid quinones. Similarly, strains of the genus *Ferrimonas* also contained MK-7, Q-7 and Q-8 (Katsuta *et al.*, 2005). The major cellular fatty acid contents of strains FUT3661^T and Asr22-7^T were analysed by GC (Agilent GC 6890N; MIDI Inc.). The two novel strains were grown to late-exponential growth phase in MB containing 3 % NaCl at pH 7.0 at 30 °C, and in MB at pH 7.0 at 25 °C and the cellular fatty acids were extracted from the cells and converted to methyl esters according to the manufacturer's

recommendations. The major cellular fatty acids of strains FUT3661^T and Asr22-7^T were C_{16:1ω9c} and C_{16:0} (see Supplementary Table S1 in IJSEM Online), contrasting with the results for members of the genus *Ferrimonas* grown in MB (Katsuta *et al.*, 2005) (Table 1).

Genomic DNA was extracted from cells grown in MB. The G + C content of the DNA of strains FUT3661^T and Asr22-7^T was 58.1 and 57.2 mol%, respectively, as determined by direct analysis of the deoxyribonucleosides using HPLC (Tamaoka & Komagata, 1984; Mesbah *et al.*, 1989). The 16S rRNA gene was amplified by PCR using Eubac27F and 1492R primers (DeLong, 1992). The sequence of the PCR product was directly determined in both strands by the dideoxynucleotide chain-termination method with a BigDye v3.1 sequencing kit (PE Applied Biosystems) and a DNA sequencer (model 3100; PE Applied Biosystems) according to the manufacturers' recommendations. We searched for sequences similar to the 16S rRNA gene sequences of these strains in the databases of the National Center for Biotechnology Information and DNA Database of Japan using the BLAST (Altschul *et al.*, 1997) and FASTA programs (Lipman & Pearson, 1985). The 16S rRNA gene sequences of strains FUT3661^T and Asr22-7^T were most

closely related to the sequence of a marine, facultative iron(III)-reducing bacterium, *F. balearica* PAT^T, isolated from the surface sediment of a harbour (Rosselló-Mora *et al.*, 1995) with 93.8 and 93.7% similarity, respectively. Phylogenetic analysis was performed using MEGA3 software (Kumar *et al.*, 2004). The Kimura two-parameter model was used to estimate pairwise distances. Phylogenetic trees were inferred by the neighbour-joining and maximum-parsimony methods. Bootstrap values were determined from 1000 replications. The phylogenetic tree constructed from the data indicated that strains FUT3661^T and Asr22-7^T clustered within the *Gammaproteobacteria* encompassing the genus *Ferrimonas*, including *F. balearica* PAT^T (Rosselló-Mora *et al.*, 1995) and *F. marina* A4D-4^T (Katsuta *et al.*, 2005), but represented a novel lineage (Fig. 2). DNA–DNA hybridization experiments were performed using the fluorometric microdilution plate method (Ezaki *et al.*, 1988) to determine the genetic relatedness between strains FUT3661^T and Asr22-7^T, and between the two novel strains and *F. balearica* PAT^T and *F. marina* A4D-4^T. Strains FUT3661^T and Asr22-7^T showed a mean DNA–DNA relatedness of 2.9% when their DNAs were used individually as labelled DNA probes for cross-hybridization. These data demonstrate that strains FUT3661^T and

Fig. 2. Phylogenetic tree of representative strains within the order *Alteromonadales* and strains FUT3661^T and Asr22-7^T inferred from 16S rRNA gene sequences based on the neighbour-joining method. Numbers beside branch points indicate bootstrap percentages determined from 1000 replications; numbers in italics indicate bootstrap percentages determined by the maximum-parsimony method. *Bacillus subtilis* was used as the outgroup (not shown). Bar, 2 substitutions per 100 nt.

Asr22-7^T represent members of different genomic species. Strain FUT3661^T showed mean DNA–DNA relatedness of 0.4 % to *F. balearica* PAT^T and 0.2 % to *F. marina* A4D-4^T. Strain Asr22-7^T showed mean DNA–DNA relatedness of 5.9 % to *F. balearica* PAT^T and 9.2 % to *F. marina* A4D-4^T.

Strains FUT3661^T and Asr22-7^T can be separated from their nearest relatives, i.e. members of the genus *Ferrimonas*, based on their genotypic and phenotypic characteristics (Table 1 and Fig. 1). Moreover, strains FUT3661^T and Asr22-7^T can each be considered to represent a different species based on DNA–DNA hybridization analysis together with differences in DNA G+C content and in their phenotypic properties (Table 1 and Fig. 2; see also Supplementary Figs S2, S3 and S4 in IJSEM Online). We propose the names *Ferrimonas futtsuensis* sp. nov. and *Ferrimonas kyonanensis* sp. nov. to accommodate these two novel strains.

Description of *Ferrimonas futtsuensis* sp. nov.

Ferrimonas futtsuensis (fut.tsu.en'sis. N.L. fem. adj. *futtsuensis* from Futtsu, the place of isolation).

Cells are Gram-negative rods (0.7–0.9 × 0.4–0.7 µm) and motile by a polar flagellum. No spores are observed. Mesophilic, facultatively anaerobic, chemo-organotroph. Circular, opaque, beige colonies are formed after 2 days on MA plates at 30 °C. Growth occurs at 15.0–30.0 °C, with an optimum at 30.0 °C. The pH range for growth is 6.0–9.0. NaCl is required for growth; growth occurs at 0.7–5.0 % (w/v), with an optimum at 3.0 %. Major isoprenoid quinones are MK-7, Q-7 and Q-8. Major cellular fatty acids are C_{16:0} (19.4 %), C_{16:1ω9c} (20.4 %) and C_{18:1ω9c} (15.9 %). Growth is observed with yeast extract, tryptone, Casamino acids, pyruvate, fumarate, propionate and lactate as the electron donor and carbon source in the presence of selenate. Gelatin is hydrolysed. Utilizes selenate, iron(III) oxyhydroxide, iron(III) citrate, arsenate, manganese(IV) oxide, elemental sulfur, thiosulfate and oxygen as an electron acceptor. Can reduce selenate to elemental selenium. The G+C content of the genomic DNA is 58.1 mol% (as determined by HPLC).

The type strain, FUT3661^T (=NBRC 101558^T=DSM 18154^T), was isolated from Futtsu beach on the coast of Tokyo Bay, Japan.

Description of *Ferrimonas kyonanensis* sp. nov.

Ferrimonas kyonanensis (ky.o.nan.en'sis. N.L. fem. adj. *kyonanensis* from Kyonan, the place of isolation).

Cells are Gram-negative rods (0.7–1.1 × 0.5–0.8 µm) and motile by a polar flagellum. No spores are observed. Mesophilic, facultatively anaerobic, chemo-organotroph. Circular, opaque, beige colonies are formed after 2 days on MA at 25 °C. Growth occurs at 15.0–32.5 °C, with an optimum at 25.0–28.0 °C. The pH range for growth is 6.0–9.0. NaCl is required for growth; growth occurs at

2.0–5.0 % (w/v), with an optimum at 2.0 %. Major isoprenoid quinones are MK-7, Q-7 and Q-8. Major cellular fatty acids are C_{16:0} (15.6 %), C_{16:1ω9c} (28.5 %) and C_{18:1ω9c} (10.9 %). Growth is observed with yeast extract, tryptone, Casamino acids, pyruvate and lactate as the electron donor and carbon source in the presence of selenate. Gelatin and arginine are hydrolysed. Utilizes selenate, iron(III) oxyhydroxide, iron(III) citrate, arsenate, manganese(IV) oxide, elemental sulfur, nitrate and oxygen as an electron acceptor. Reduces selenate to elemental selenium. The G+C content of the genomic DNA is 57.2 mol% (as determined by HPLC).

The type strain, Asr22-7^T (=NBRC 101286^T=DSM 18153^T), was isolated from the alimentary tract of littleneck clams collected from Kyonan beach on the coast of Tokyo Bay, Japan.

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