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TAXONOMIC DESCRIPTION
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Grimontia sedimenti sp. nov., isolated from benthic sediments near coral reefs south of Kuwait

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Abstract

A Gram-stain-negative, rod and rod-curved shaped motile bacterium designated strain S25^T was obtained from benthic sediment collected near the Kubbar Island coral reefs south of Kuwait. Phenotypic analysis revealed that strain S25^T was slightly halophilic, mesophilic and facultative anaerobic, fermenting D-glucose, D-ribose, D-mannose, D-mannitol, maltose, fructose, gentiobiose, cellobiose, melibiose, trehalose and sucrose. It was positive for oxidase and indole production and negative for arginine dihydrolase and lysine and ornithine decarboxylases. It contained $C_{16:1}\omega7c/C_{16:1}\omega6c$ (summed feature 3), $C_{18:1}\omega7c$ (summed feature 8) and $C_{16:0}$ as the major fatty acids. Strain S25^T grew optimally at 30 °C and pH 8 in the presence of 3% (w/v) NaCl. Phylogenetic analysis based on 16S rRNA sequences revealed that strain S25^T is related to species of the genus *Grimontia*, having 99.15% similarity to '*Grimontia indica*' AK16^T, 99.08% to *Grimontia celer* 96-237^T and 98.66% to *Grimontia marina* IMCC 5001^T. The DNA G+C content was 48.8mol% and the full genome analysis for the strain S25^T showed that the bacterium has a genome size of 5158621 bp and contains 4730 predicted protein-encoding genes. The average nucleotide identity values between the S25^T genome and the genomes of its nearest matches ranged between 81.39 and 94.16%. The strain was distinguishable from the phylogenetically related genera through differences in several phenotypic properties. On the basis of the phenotypic, phylogenetic and genetic data, strain S25^T represents a novel species in the genus *Grimontia*, for which the name *Grimontia sedimenti* sp. nov. is proposed. The type strain of *Grimontia sedimenti* is S25^T (=DSM 28878^T=LMG 28315^T).

INTRODUCTION

The sediment around coral reefs is important in the reef biogeochemistry and few papers have been produced investigating the diversity, function and dynamics of coral reef sediment microbes as well as the factors shaping and controlling the microbial assemblage in the reef sediments [1–3]. The heterotrophic bacteria within the sediments have been proved to be key players in both remineralizing organic matter in the reefs and in recycling nutrients that maintain high ecosystem productivity in otherwise nutrient-poor surrounding waters. Vibrionales members are a major part of the sediment microbial assemblage and the majority belongs to the genus *Vibrio* [2]. In the current study, culturing the bacterial assemblage of the sediment surrounding the Kubbar coral reefs south of Kuwait revealed the presence of various bacterial members including Vibrionales. Isolate S25^T was of special interest since it belongs to the genus *Grimontia*, a relatively newly described genus. A study done by Ruimy *et al.* [4] to determine the phylogenetic position of *Vibrio hollisae* with other members of the families Vibrionaceae, Aeromonadaceae and Plesiomonadaceae suggested the novelty of this genus, a matter that was confirmed later on by Thompson *et al.* [5], who renamed the genus as *Grimontia*. To date, four species have been described for this genus. The first was *Grimontia hollisae* [5] isolated from patient stool and it shared a pathogenic gene cluster with *Vibrio* [6]. The other three species, *Grimontia marina* [7], '*Grimontia indica*' [6] and *Grimontia celer* [8] are all isolated from seawater. Among them, '*Grimontia indica*' has pathogenesis genes, suggesting that this species has a life cycle involving pathogenic interaction with marine animals.

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Keywords: *Grimontia sedimenti*; Kuwait; coral reefs; sediments; Arabian Gulf.
Abbreviations: ANI, average nucleotide identity; ARG, antibiotic resistance gene; DDH, DNA–DNA hybridization; GGDC, Genome-to-Genome Distance Calculator; MA, marine agar; ML, maximum-likelihood; MP, maximum-parsimony; NA, nutrient agar; NJ, neighbour-joining; TCBS, thiosulfate citrate bile sucrose; WGS, whole genome sequence; ZMA, Zobell marine agar.
The GenBank accession number for the 16SrRNA gene sequence of strain S25^T is MW404042.1. The GenBank/DDBJ/ENA accession number of the whole genome sequence of strain S25^T is JAALDL000000000.
Ten supplementary figures and one table are available with the online version of this article.
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Table 1. Key characteristics that differentiate strain S25^T from the reference strains

Strains: 1, S25^T; 2, *Grimontia celer* 96-237^T [current study]; 2, *'Grimontia indica'* AK16^T [6]; 3, *Grimontia marina* IMCC 5001^T [7]; 4, *Grimontia hollisae* ATCC 33564^T [5]. +, Positive; w, weak positive; –, negative; ND, not determined.

Characteristic	1	2	3	4	5
Growth range with NaCl (%)	2–8	1–9	2–6	1–8	1–6
Urease	–	+	–	–	+
Aesculin hydrolysis	w	–	–	+	–
Citrate assimilation	–	+	–	ND	–
Nitrate reduction	+	w	+	+	+
Gluconate assimilation	w	+	–	–	+
Growth with sole carbon sources:					
D-Galactose	w	+	–	–	+
Trehalose	+	–	–	–	–
Cellobiose	+	–	–	ND	–
G+C content (mol%)	48.8*	48.4*	48.7*	52.6†	51†

*G+C content determined from whole genome sequence.
†G+C content determined by reverse-phase HPLC [14].

ISOLATION AND ECOLOGY

The taxonomic position of strain S25^T, isolated from benthic sediment collected near Kubbar coral reefs on 5 July 2011 (south of Kuwait, North West of the Arabian Gulf; (29° 02′ 53.6″ N, 48° 17′ 23.3″ E) was determined. The seawater salinity, pH, temperature and conductivity were recorded near the sediment at the time of sampling using a Horiba Water Quality Checker. The seawater salinity was 4%, pH was 8.3, temperature was 30 °C and conductivity reached 60.7 ms cm $^{-1}$. Culturable bacteria were originally isolated from benthic sediment collected in sterile 15 ml tubes by scuba divers from the upper 10 cm of the seafloor and at 10 m depth. One gram of the homogenized sediment samples was serially diluted with 3% sterile saline (NaCl) and diluents of 10^{-1}, 10^{-3} and 10^{-5} were used in plating. One hundred microlitres of each diluent was inoculated into Zobell marine agar 2216 (ZMA; Himedia) with pH adjusted to 7.6. All plates were incubated at 30 °C for 24 h. The novel isolate S25^T was purified and maintained at 30 °C on ZMA. Stocks of the purified culture were preserved as suspensions at Microbank (Prolab Diagnostic) and stored at −80 °C.

Several morphologically distinct single colonies were individually recultured into new media and purified. Isolate S25^T developed small colonies that were 1 mm in diameter and circular, entire, raised and non-pigmented after 24 h but after 48 h the colonies were 2 mm in diameter, small, circular, convex, semi-opaque, dull, beige and had entire margins (Fig. S1, available in the online version of this article). In addition, strain S25^T was grown on thiosulfate citrate bile sucrose (TCBS; BD Difco) medium and it formed green colonies after 48 h (Fig. S2). For comparison, some of the investigations were performed with *G. celer* 96-237^T (from the BCCM/LMG bacteria collection) as a reference strain, which was cultured and maintained under the same conditions as that used for isolate S25^T (Table 1). *G. celer* 96-237^T produced colonies with similar cultural characteristics on marine agar (MA) and TCBS media to that reported for the strain S25^T colonies.

PHYSIOLOGY AND CHEMOTAXONOMY

Cell morphology was determined by phase contrast microscopy (Zeiss Axio) and cell motility was determined by using both the hanging drop technique and flagella staining following the methods of Leifson [9] and Murray *et al.* [10]. Cells of strain S25^T were monotrichous flagellated, rod and curved-rod shaped (Fig. S3). Cells were [average (minimum-maximum) SD] 1 µm (0.7–1.3 µm) 0.22 µm long and 0.33 µm (0.3–0.4 µm) 0.03 µm wide.

Growth of strain S25^T was tested under different NaCl concentrations in the range of 0–15% (w/v), in increments of 1% using MA [11] and nutrient agar (NA; BD Difco). Growth of the strain was also tested under various temperatures ranging from 5 to 55°C and pH values ranging from pH 4 to 11. The isolate grow successfully at various NaCl concentrations from 2 to 8 % (w/v), temperature ranges between 15 and 40 °C and pH ranges between pH 6 and 10, with optimal growth with 3% (w/v) NaCl, at 30 °C and at pH 8. The optimal growth conditions for the reference strain *G. celer* 96-237^T using MA were similar to that reported for strain S25^T. It is worth

mentioning that the reference strain *G. celer* 96-237^T grew with NaCl ranging from 1 to 9%.

The detailed biochemical characteristics of strain S25^T are given in Tables 1 and S1, and in the species description. A series of physiological and biochemical characteristics could distinguish strain S25^T from the reference as well as other related genera. The phenotypic characterization was carried out using GEN III MicroPlates (Biolog) and API 20E, API 20NE, API ZYM and API 50CH strips (bioMérieux) following the manufacturers' instructions. Also, strain S25^T was inoculated on MA and incubated at 30 °C inside the Mitsubishi Gas Chemical Anaerobic System for 2 weeks to determine the strain's anaerobic growth ability. In addition, catalase and oxidase activities were determined according to Smibert and Krieg [12].

The major differences between strain S25^T and the reference strain *G. celer* 96-237^T and other *Grimontoa* species are reported in Table 1. Strain S25^T showed the ability to grow on various substrates as sole carbon source including cellobiose and trehalose while *G. celer* 96-237^T failed to do so. In addition, strain S25^T was urease negative, showing similarity to both *G. marina* IMCC 5001^T and '*Grimontia indica*' AK16^T but opposing the results of the reference strain *G. celer* 96-237^T and *G. holliase* ATCC 33564^T. Also strain S25^T was negative for citrate assimilation while the reference strain *G. celer* 96-237^T was positive.

The cellular fatty acid composition of strain S25^T was determined by the identification services of Deutche Sammlung von Mikroorganismen und Zelkulturen (DSMZ) in Germany using the Sherlock Microbial Identification System (MIDI) as described by Sasser [13]. The cellular fatty acid of S25^T was analysed in culture at the late exponential stage of growth after growing the isolate on MA with 3% (w/v) NaCl and incubating at 30 °C for 24 h. The major fatty acids were $C_{16:1}\,\omega7c$/$C_{16:1}\,\omega6c$ (summed feature 3; 36.2%), $C_{18:1}\,\omega7c$ (summed feature 8; 21%) and $C_{16:0}$ (17.4%) (Table S1). Likewise, analysis of respiratory quinones for the strain S25^T was also carried out by the DSMZ. Two ubiquinone were detected: Q7 (1.7%) and Q8 (98.3%). The dominant fatty acids and respiratory quinones of strain S25^T were similar to those reported by Choi *et al.* [7] for *G. marina* IMCC 5001^T and *G. hollisae* KCCM 41680^T grown on MA at 30 °C for 48 h, i.e. the same conditions used to analyse the S25^T sample, suggesting that this strain indeed belongs to the genus *Grimontia*.

The G+C content of the strain S25^T DNA was 46.5 mol% as determined by reverse-phase HPLC of nucleosides [14], yet the whole genome sequencing results showed that the G+C content of S25^T is higher at 48.8 mol%, which is near the G+C content of *G. celer* 96-237^T (48.4 mol%) and *'Grimontia indica*' AK16^T (48.7%) mentioned elsewhere [6, 8].

16S rRNA GENE SEQUENCED BASED PHYLOGENY

The complete 16S rRNA gene sequence of S25^T was determined by direct sequencing of PCR-amplified 16S rRNA. Genomic DNA extraction was carried out using the MasterPure Gram Positive DNA Purification Kit (Epicentre Biotechnologies) according to the manufacturer's instructions. PCR-mediated amplification of the 16S rRNA and purification of the PCR product was carried out as described in Rainey *et al.* [15]. Purified PCR products were sequenced using the CEQ DTCS-Quick Start Kit (Beckmann Coulter) as directed in the manufacturer's protocol. Sequence reactions were electrophoresed using the CEO 8000 Genetic analysis system.

The length of the 16S rRNA gene sequence of strain S25^T was a continuous stretch of 1451 bp. Comparing the 16S rRNA of strain S25^T with corresponding sequences in the GenBank, EMBL and RDP II databases indicated that strain S25^T is a member of the phylum Proteobacteria, class Gammaproteobacteria, order Vibrionales, family Vibrionaceae, genus *Grimontia*. A comparative analysis was undertaken between the strain S25^T 16S rRNA gene sequence and its closely related strains with the following results (similarity %): '*G. indica*' AK16^T (99.15%), *G. celer* 96-237^T (99.08%), *G. marina* IMCC 5001^T (98.66%) and *G. hollisae* ATCC 33564^T (96.03%).

A phylogenetic tree was reconstructed for strain S25^T with its closest relatives. The analysis was performed on the Phylogeny.fr platform [16, 17] and comprised the following steps. All sequences were aligned with ClustalW (version 2.1) [18]. After alignment, ambiguous regions (i.e. containing gaps and/or poorly aligned) were removed with Gblocks (version 0.91b) [19]. The neighbour-joining (NJ) method including bootstrap (1000 times) analysis [20] was implemented in the BioNJ program [21]. Graphical representation and editing of the phylogenetic tree were performed with TreeDyn (version 198.3) [22]. Furthermore, pairwise sequence similarities were calculated using the method recommended by Meier-Kolthoff *et al.* [23] for the 16S rRNA gene available via the Genome-to-Genome Distance Calculator (GGDC) web server [24] available at http://ggdc.dsmz.de/. Phylogenies were inferred by the GGDC web server available at http://ggdc.dsmz.de/ using the DSMZ phylogenomics pipeline [25] adapted to single genes. A multiple sequence alignment was created with MUSCLE [26]. Maximum-likelihood (ML) and maximum-parsimony (MP) trees were inferred from the alignment with RAxML [27] and TNT [28], respectively. For the ML tree, rapid bootstrapping in conjunction with the autoMRE bootstopping criterion [29] and subsequent search for the best tree was used; for the MP tree, 1000 bootstrapping replicates were used in conjunction with tree-bisection-and-reconnection branch swapping and 10 random sequence addition replicates. The sequences were checked for a compositional bias using the X^2 test as implemented in PAUP* [30]. The input nucleotide matrix comprised 22 operational taxonomic units and 1577 characters, 250 of which were variable and 181 of which were parsimony-informative. The base-frequency check indicated no compositional bias (P=1.00, α=0.05). ML analysis under the GTR+GAMMA model yielded a highest log likelihood of −5493.54, whereas the estimated alpha parameter was 0.06. The ML bootstrapping converged after 700 replicates; the average support was 70.37%. MP analysis yielded a best score of 653 (consistency index 0.53, retention index 0.58)

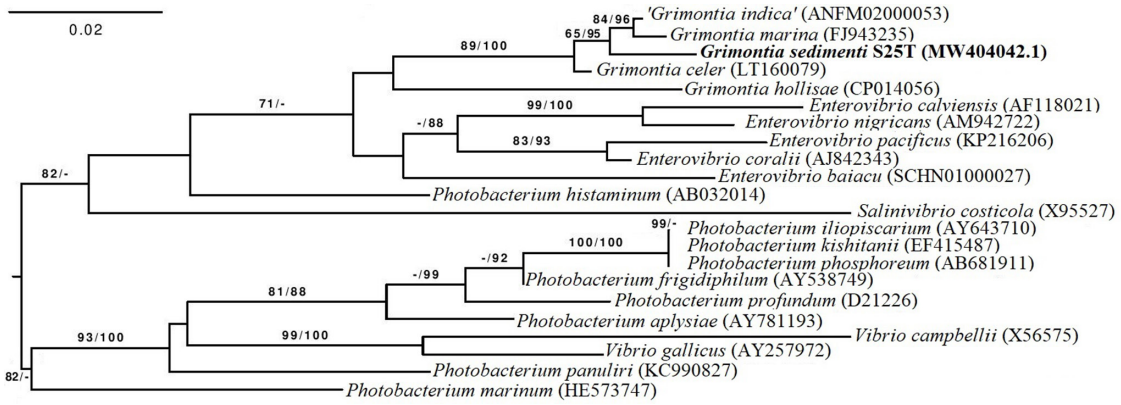

Fig. 1. 16S rRNA gene sequences maximum-likelihood (ML) and maximum-parsimony(MP) tree inferred under the GTR+GAMMA model and rooted by midpoint-rooting. The branches are scaled in terms of the expected number of substitutions per site. The numbers above the branches are support values when larger than 60% from ML (left) and MP (right) bootstrapping. Sequence length was 1451 bp. The accession numbers of the 16S rRNA genes in the GenBank are shown between brackets. The scale bar denotes the number of substitutions per site.

and four best trees. The MP bootstrapping average support was 76.32%.

The phylogenetic analysis of the 16S rRNA gene showed that strain S25^T is part of the clade formed by *Grimontia species* (Figs 1 and S4) and closer to the marine species than to *G. hollisae*. The NJ and ML/MP phylogenetic trees suggested that the ancestor of isolate S25^T existed before the ancestors of *G. marina* and '*G. indica*'.

GENOME FEATURES AND PHYLOGENOMIC ANALYSIS

The whole genome sequence (WGS) of S25^T was determined. The DNA was extracted using the PrepMan Ultra Sample Preparation Reagents (ThermoFisher) according to the manufacturer's instructions and the sample quality check was performed using Qubit DNA BR assay kit and E0gen NGS 0.8% agarose. The NEBNEXT Fast DNA Library Prep Set for Ion Torrent (Biolabs Inc.) and the KAPA Library Quantification Kit were used. The whole genome was sequenced using Ion Torrent, the Ion PI Hi-Q sequencing 200 kit and the Ion PI chip kit version 3 (ThermoFisher Scientific). In total, 6.8 million single reads were produced with an average length of 190 bases. The SPAdes genome assembler version 3.1.0 was used to perform a *de novo* genome assembly, which yielded 185 contigs where 59 contigs had length greater than 500 bp and an N50 value of 208501 bp. The longest contig measured 622561 bp. The full genome analysis for strain S25^T showed that the bacterium had a genome size of 5126793 bp and contained 4730 predicted protein-encoding genes and 120 RNAs. The authenticity and contamination of the WGS of the strain S25^T were checked as indicated by Chun *et al.* [31] and executed using EZBioCloud Contamination Estimator by 16S (ContEst16S) [32] where the sequences of the 16S rRNA gene from the genome and that of the PCR-determined sequence were compared and found to be identical and the S25^T genome was confirmed to be free of contamination. In addition, the S25^T genome was analysed using Check M from the Kbase database [33] and the data showed that the percentage of genome contamination was 0.13% with 100% gene completeness (Fig. S5).

The genomic relatedness among the type strains of the four published *Grimontia* species and S25^T was determined using *in silico* DNA–DNA hybridization (DDH). The GGDC provided by DSMZ [24] was used to estimate the DDH values between S25^T and '*G. indica*' AK16^T (ANFM02; 55.6%), *G. celer* 96-237^T (FIZX01; 39.5%), *G. marina* IMCC 5001^T (FIYY01; 36.1%) and *G. hollisae* ATCC 33564^T (CP014055/6; 21.65%). The DDH values produced were below the borderline (threshold 70%) for same species delimination, which confirmed the novelty of strain S25^T as representing a new species under the genus *Grimontia*. The DDH results were confirmed by the average nucleotide identity (ANI) value calculated using an ANI calculator (EZBioCloud) and following the OrthoANIu algorithm [34] as well as using the Kostas lab ANI calculator to generate ANI figures. The OrthoANIu values (%) between S25^T and '*G. indica*' AK16^T (94.1%), *G. celer* 96-237^T (90.3%), *G. marina* IMCC 5001^T (88.7%) and *G. hollisae* ATCC 33564^T (80.8%) all are less than 95%, which was confirmed by the Kostas lab ANI calculator results (Fig. S6). Knowing that ANI values of ≥95% correspond to the transitional 70% DDH threshold, the current study results shows the novelty of strain S25^T [35]. Therefore, the DDH and ANI values support the recognition of strain S25^T as representing a new species.

The relatedness of strain S25^T to its nearest match from GenBank was also compared at a whole-genome level. The genome sequence data were uploaded to the Type (Strain) Genome Server (TYGS), a free bioinformatics platform available under https://tygs.dsmz.de, for a whole genome-based taxonomic analysis [36]. Determination of closest type strain genomes was done in two complementary ways: First, all user

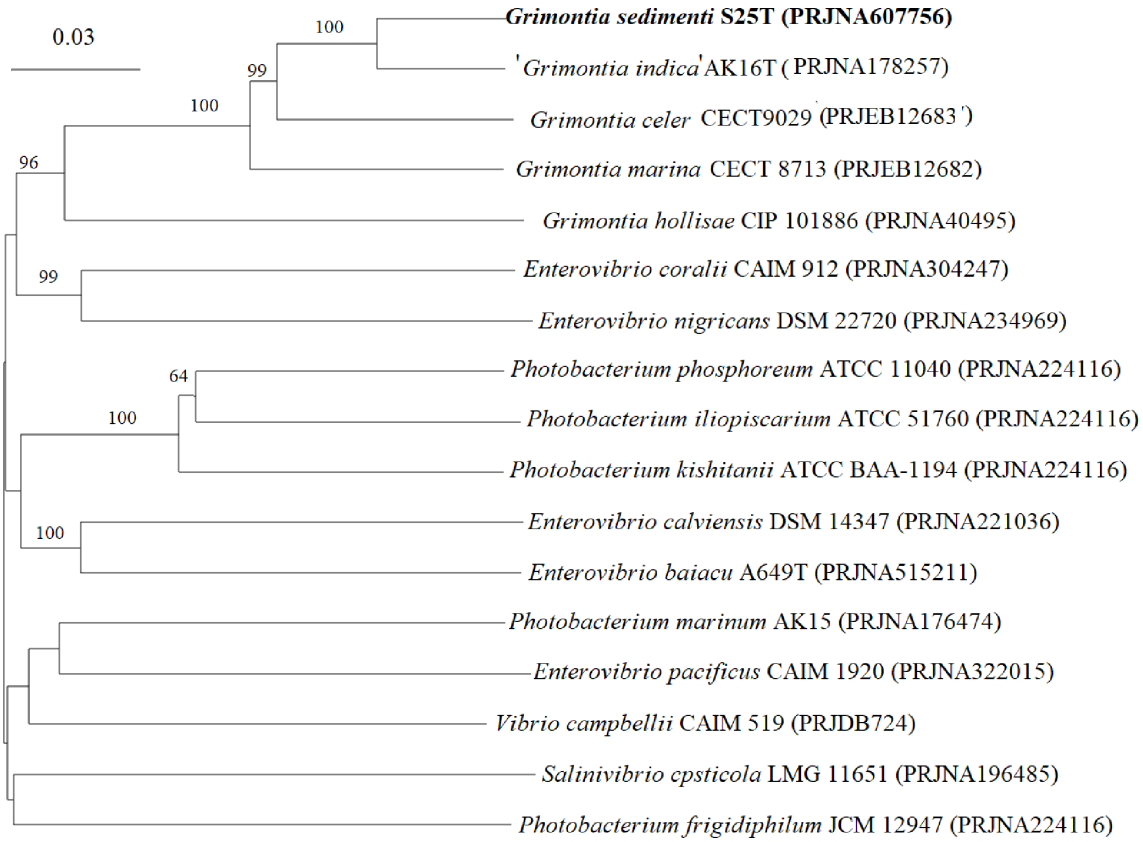

Fig. 2. Tree inferred with FastME 2.1.6.1 from GBDP distances calculated from whole genome sequences of the strain S25^T and 17 related genomes. The branch lengths are scaled in terms of GBDP distance formula *d5*. The numbers above branches are GBDP pseudo-bootstrap support values >60% from 100 replications, with an average branch support of 70.1%. The tree was rooted at the midpoint. The bar equal to 0.03 of the phylogenetic distance. The corresponding genome project numbers are included between brackets.

genomes were compared against all type strain genomes available in the TYGS database via the MASH algorithm, a fast approximation of intergenomic relatedness [37], and, the ten type strains with the smallest MASH distances chosen per user genome. Second, an additional set of 10 closely related type strains was determined via the 16S rRNA gene sequences. These were extracted from the user genomes using RNAmmer [38] and each sequence was subsequently BLASTed [39] against the 16S rRNA gene sequence of each of the currently 13787 type strains available in the TYGS database. This was used as a proxy to find the best 50 matching type strains (according to the bitscore) for each user genome and to subsequently calculate precise distances using the Genome BLAST Distance Phylogeny approach (GBDP) under the algorithm 'coverage' and distance formula *d5* [24]. These distances were finally used to determine the 10 closest type strain genomes for each of the user genomes. The resulting intergenomic distances were used to infer a balanced minimum-evolution tree with branch support via FastME 2.1.4 including SPR postprocessing [40]. Branch support was inferred from 100 pseudobootstrap replicates each. The trees were rooted at the midpoint [41] and visualized with PhyD3 [42]. The result of the phylogenomic tree clustered strain S25^T with the rest of the *Grimontia* species and showed that it shared a common ancestor with '*G. indica*' AK16^T (Fig. 2).

A more conservative comparative analysis based on *RecA*, *FtsZ* and *MreB* gene sequences extracted from the full genome of strain S25^T was executed using the National Center for Biotechnology Information (NCBI) database analysis software. NJ phylogenetic trees for each of the compared gene proteins were reconstructed using the Saitou and Nei method [43]. The maximum sequence difference was set at 0.85 the Grishin method was used to calculate distances [44]. Comparing the S25^T *RecA*, *FtsZ* and *MreB* housekeeping genes with its nearest match in the GenBank resulted in phylogenetic trees clustering strain S25^T with *Grimontia* species. However, they did not show 100% similarity to any of the published *Grimontia* species equivalent housekeeping genes, neither did they show a consistent relationship to the same *Grimontia* species, as can be concluded from Figs S7–S9.

The functional annotation for the strain S25^T genome was achieved by the Rapid Annotation using Subsystem Technology (RAST) server [45–47]. Among the coding sequences, the dominant sequences belonged to conserved regions such as those of metabolic, cellular and regulatory processes. Also

sequences belong to stress responses, secondary metabolism and virulence, disease, and defence were detected (Fig. S10). The majority of the genes belonged to carbohydrates>amino acids and derivatives>proteins metabolism>cofactors, vitamins, prosthetic groups and pigments subsystems. The majority of the carbohydrate genes belonged to central carbohydrate metabolism including TCA cycle, pyruvate metabolism and glycolysis followed by fermentation and monosaccharides genes, especially acetyl-COA fermentation to butyrate and monosaccharides catabolism genes. On the other hand, the majority of the amino acid related genes belonged to arginine metabolism, urea cycle and polyamines metabolism, followed by lysine, threonine, methionine, and cysteine biosynthesis and degradation genes. The protein metabolism subsystem was dominated by protein synthesis genes, while the cofactors, vitamins, prosthetic groups and pigments subsystems were dominated by folate and pterines biosynthesis genes, followed by riboflavin genes and biotin genes. The WGS also showed that the strain S25^T genome harboured resistance to antibiotic and toxic compounds genes as well as metabolism of aromatic compounds genes in addition to genes related to oxidative, osmotic and heat shock stress. The presence of antibiotic resistance genes (ARG) in marine microbes was documented by Hatosy and Martiny [48], who described the ocean as a global reservoir for clinically relevant and potentially novel ARG. The Kuwait sea is largely affected by the input of untreated or partially treated sewage, which participates in depositing antibiotics and other pharmaceutical compounds in addition to heavy metals into it [49, 50]. Therefore, it is important to study and investigate the ARG in bacterial isolates thriving in the environment. Kuwait, as an oil-exporting country, releases oil from refineries, ports and oil tankers either accidently or deliberately, which also enriches coral reef systems with aromatic compounds. A previous study by Al-Dahash and Mahmoud [51] suggested that corals in Kuwait can adapt to oil contamination by harbouring oil-degrading bacteria. Also, the heat shock protein-encoding genes may reflect the S25^T isolate's potential ability to survive the fluctuation in temperature between the summer and winter [52]; however, more experimental work is needed to be conducted in order to confirm this assumption.

On the basis of the comprehensive analysis of the phenotypic, chemotaxonomic and phylogenetic characteristics, as well as the nearly full genome sequencing, strain S25^T should be considered as representing a new species for which we propose the name *Grimontia sedimenti* sp. nov. The major differences between strain S25^T and the reference strain *G. celer* 96-237^T include the range of NaCl tolerance, where *G. celer* 96-237^T is able to grow at wider range of NaCl concentration than S25^T. In addition, strain S25^T is able to grow on trehalose and cellobiose as a sole carbon source, while *G. celer* 96-237^T is not. Variation was also detected between strain S25^T and *G. celer* 96-237^T in urease, gluconate and citrate assimilation, nitrate reduction, and aesculin hydrolysis. Furthermore, DDH and ANI analyses for the WGS clearly differentiated strain S25^T from *G. celer* 96-237^T as well as from all other previously described *Grimontia* species.

DESCRIPTION OF *GRIMONTIA SEDIMENTI* SP. NOV.

Grimontia sedimenti (se.di.men'ti. L. gen. n. *sedimenti* of sediment, from where the type strain was isolated).

Cells are Gram-stain-negative, motile by monotrichous flagella, facultative anaerobic and rod to curved-rod shape. Cells are 0.7–1.3 µm long and 0.3–0.4 µm wide. Colonies on MA are small (1 mm in diameter after 24 h), circular, entire, raised in shape and non-pigmented. After 48 h, colonies are 2 mm in diameter, small, circular, convex, semi-opaque, dull, beige and have entire margins. Growth occurs at 15–40 °C with optimum growth at 30 °C. Growth is observed at pH 6–10 with optimal growth at pH 8. Furthermore, the NaCl range for growth is 2–8% (optimum, 3%). The major fatty acids are $C_{16:1}$ *ω7c*/$C_{16:1}$ *ω6c* (summed feature 3), $C_{18:1}$ ω7*c* (summed feature 3) and $C_{16:0}$. Strain S25^T is oxidase-, catalase- and aminopeptidase-positive. Strain S25^T is able to reduce nitrate and hydrolyse gelatin but unable to hydrolyse starch and Tween 40. Strain S25^T is able to hydrolyse aesculin weakly. In the API 20E and 20NE strips, there is a positive result for β-galactosidase. Positive results are recorded for the assimilation of glucose, mannose, mannitol, *N*-acetylglucoseamine, maltose, malate and citrate as well as a weak positive result for gluconate assimilation. Strain S25^T shows a positive reaction for the Voges–Proskauer test but negative results for arginine dihydrolase, lysine and ornithine decarboxylases, urease and H_2S production. Indole is produced from tryptophan. Glucose is fermented with production of acid, as are mannitol and sucrose. In addition to those three carbohydrates, the following carbohydrates are fermented as determined by the API 50CHE kit: D-ribose, D-fructose, D-mannose, *N*-acetyl-D-glucosamine, maltose, cellobiose, melibiose, trehalose and gentiobiose, as well as D-galactose and inositol weakly. In the API ZYM strip, alkaline phosphatase, leucin arylamidase and *N*-acetyl-β-glucoseaminidase show strong positive activity (≥40 nmol of hydrolysed substrate), esterase, esterase lipase, acid phosphatase and naphthol-AS-BI-phosphohydrolase show medium activity (10–20 nmol), while β-galactosidase, α-glucosidase and valine arylamidase are weak positive with 5 nmol hyrolysed substrate .

Isolate S25^T is resistant to the following antibiotics (µg disc^{-1}): oxacillin (5), aztreonam (30), vacomycin (30), doxycycline (30), teicoplanin (3 0), bacitracin (10 IU disk^{-1}) and nystatin (100 IU disc^{-1}).

The type strain, S25^T, was isolated from benthic sediment collected near the Kubbar Island coral reefs, south of Kuwait, at a depth of 10 m. The DNA G+C content of the strain is 48.8 mol% and the draft genome showed that the bacterium has a genome size of 5126793 bp and contains 4730 predicted protein-encoding genes.

The full length 16S rRNA gene sequence of strain S25^T is available fromGenBank with accession number MW404042.1. The whole genome sequencing draft genome of strain S25^T is available from the GenBank/

DDBJ/ENA databases with the accession number JAALDL000000000. The type strain is S25^T (=DSM 28878^T=LMG 28315^T).

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Author contributions
Writing–original draft, review and editing: H. M. Investigation: H. M., L. J. and S. E. Formal analysis: H. M. Molecular analysis: H. M. Resources: H. M.

Conflicts of interest
The authors declare that there are no conflicts of interest.

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