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Vibrio comitans sp. nov., *Vibrio rarus* sp. nov. and *Vibrio inusitatus* sp. nov., from the gut of the abalones *Haliotis discus discus*, *H. gigantea*, *H. madaka* and *H. rufescens*Tomoo Sawabe,¹ Yusuke Fujimura,¹ Kentaro Niwa² and Hideaki Aono²¹Laboratory of Microbiology, Faculty of Fisheries Sciences, Hokkaido University, 3-1-1 Minato-cho, Hakodate 041, Japan²National Research Institute of Fisheries Science, 6-31-1 Nagai, Yokosuka, Kanagawa 238-0316, Japan

Nine alginolytic, facultatively anaerobic, non-motile bacteria were isolated from the guts of the abalones *Haliotis discus discus*, *H. gigantea*, *H. madaka* and *H. rufescens*. Phylogenetic analyses based on 16S rRNA gene sequences indicated that these bacteria were closely related to *Vibrio superstes* G3-29^T (98.6–99.3% sequence similarity). DNA–DNA hybridization and phylogenetic analysis based on the *gapA* gene demonstrated that six strains constituted one bacterial species, two strains represented a second species and one strain represented a third species. The three novel bacterial species were different from all currently known vibrios. The names *Vibrio comitans* sp. nov. (type strain GHG2-1^T=LMG 23416^T=NBRC 102076^T; DNA G+C content 45.0–48.0 mol%), *Vibrio inusitatus* sp. nov. (type strain RW14^T=LMG 23434^T=NBRC 102082^T; DNA G+C content 43.1–43.7 mol%) and *Vibrio rarus* sp. nov. (type strain RW22^T=LMG 23674^T=NBRC 102084^T; DNA G+C content 43.8 mol%) are proposed to encompass these new taxa. Several phenotypic features were revealed that discriminate *V. comitans*, *V. rarus* and *V. inusitatus* from other *Vibrio* species.

Vibrio haliotocoli and four genetically related species (*Vibrio neonatus*, *V. ezurae*, *V. gallicus* and *V. superstes*) have been reported by our group (Hayashi *et al.*, 2003; Sawabe *et al.*, 1998, 2004a, b; Sawabe, 2006). The unique characteristics of the *V. haliotocoli*-related species are that they are alginolytic, non-motile, fermentative marine bacteria. In addition, these bacteria shared similar ecological niches in the gut of *Haliotis* abalones all over the world. *V. haliotocoli*, *V. neonatus* and *V. ezurae* are abundant in Japanese (Sawabe *et al.*, 1995, 2002, 2003, 2004b) and South African (Sawabe *et al.*, 2003) abalone, whereas *V. gallicus* and *V. superstes* are found in French (Sawabe *et al.*, 2004a) and Australian (Hayashi *et al.*, 2003) abalone. High genetic diversity of *V. haliotocoli* was observed in several abalone species (Sawabe *et al.*, 2002; Sawabe, 2006). These bacteria are thought to be involved in digestion of the polysaccharide of kelps ingested by the

abalone. These bacteria are also active in making volatile, short-chain fatty acids, mainly acetic acid and formic acid, via fermentation (Sawabe *et al.*, 2003).

The observed genetic diversity of *V. haliotocoli*-related species is attributed to the 'co-evolution concept', in which a long symbiotic relationship has been established between the *V. haliotocoli*-related species and the host abalones (Sawabe, 2006). To understand the driving force of the genetic diversity and speciation of *V. haliotocoli*-related species, it is important to attempt to isolate novel *V. haliotocoli*-related species. In this study, we isolated nine strains that were phylogenetically most similar to *V. superstes* from the gut of warm-water-adapted wild Japanese abalones (*Haliotis discus discus*, *H. gigantea* and *H. madaka*) and the Californian red abalone (*Haliotis rufescens*). DNA–DNA hybridization experiments, phenotypic characterizations and phylogenetic and genetic analyses demonstrated that these strains represent three as-yet unknown species of *Vibrio*.

Six strains, GHD1-9 (=LMG 23413=NBRC 102078), GHG2-1^T (=LMG 23416^T=NBRC 102076^T), GHG2-4 (=LMG 23417=NBRC 102077), NHG1-3 (=LMG 23421=NBRC 102080), NHG1-11 (=LMG 23422=NBRC 102079) and NHM1-4 (=LMG 23425=NBRC

Abbreviations: ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences of six *V. comitans* strains, two *V. inusitatus* strains and *V. rarus* RW22^T are respectively DQ922914–DQ922919, DQ922920–DQ922921 and DQ914239. Those of the *gapA* gene sequences of five *V. comitans* strains, *V. inusitatus* RW14^T and *V. rarus* RW22^T are respectively DQ922906–DQ922910, DQ922911 and DQ922913.

102081), were isolated from the guts of wild-caught Japanese abalones *H. discus discus*, *H. gigantea* and *H. madaka*. These animals were collected on the coast of Goto Island (Nagasaki Prefecture, Japan) and Nagai (Kanagawa Prefecture, Japan) by scuba-diving in May and July 2005, respectively, with the permission of the local fishery management. From two, three and three individual animals, heterotrophic bacteria were grown on ZoBell 2216E agar containing 0.5 % sodium alginate at 20 °C according to Sawabe *et al.* (1995). Among 30 randomly selected bacterial colonies from each animal sample, *V. haliotocoli*-like strains showing facultatively anaerobic, non-motile, alginolytic and Gram-negative rods (Sawabe *et al.*, 1998) were retained for this experiment. Except for strain pairs GHG2-1^T and GHG2-4 and NHG1-3 and NHG1-11, strains were not from same individual.

Strains RW22^T (=LMG 23674^T=NBRC 102084^T), RW14^T (=LMG 23434^T=NBRC 102082^T) and RW21 (=LMG 23673=NBRC 102083) were isolated and selected from the gut of one individual of the Californian red abalone, *H. rufescens*. This animal was purchased from The Abalone Farm (Cayucos, CA, USA) in July 2005. Strains were cultured on ZoBell 2216E agar containing 0.5 % alginate (Oppenheimer & ZoBell, 1952) and stored at -80 °C in 10 % glycerol.

16S rRNA gene sequences (1400 bp) of the nine strains were determined according to Sawabe *et al.* (1998) using four sequencing primers (24F, 1100F, 920R and 1540R) by means of a RISA384 DNA sequencer (Shimadzu). The 16S rRNA gene sequences of the novel strains were subjected to a BLAST search against the latest release of GenBank and related sequences were retained. Finally, 16S rRNA gene sequences of *V. superstes* G3-29^T, *V. haliotocoli* IAM 14596^T, *V. neonatus* HDD3-1^T, *V. ezuræ* HDS1-1^T, *V. gallicus* CIP 107863^T, *Vibrio agarivorans* 289^T, *Vibrio wodanis* NVI 88/441^T, *Vibrio logei* ATCC 15832^T and *Vibrio salmonicida* NCMB 2262^T were included in the phylogenetic analysis as related sequences (Fig. 1a). Phylogenetic trees were constructed using three different methods [neighbour joining (NJ), maximum likelihood (ML) and maximum parsimony (MP)] according to Sawabe *et al.* (2004b) (details of the phylogenetic analysis are available at <http://bioinfo.unice.fr/>). For the NJ analysis, distance matrices were calculated using Kimura's two-parameter correction in MEGA version 3.0 (Kumar *et al.*, 2004). ML and MP analysis were conducted by using PHYLIP version 3.573c (Felsenstein, 1993). Because of the close relationships, almost the entire sequence corresponding to positions 4–1474 of strain GHG2-1^T was used for the analysis (a short insertion in the sequences of the novel strains between positions 27–31 and 42–48 of strain GHG2-1^T was excluded from the analysis). The trees in Fig. 1 correspond to subsets of the final trees obtained using 100 bootstrap replications and the NJ method. Nodes supported by ML and MP analysis are also displayed in Fig. 1.

The glyceraldehyde-3-phosphate dehydrogenase gene (*gapA*) is an informative gene to delineate *V. haliotocoli*

Fig. 1. Unrooted phylogenetic trees based on sequence data from the 16S rRNA gene (a) and glyceraldehyde-3-phosphate dehydrogenase gene (*gapA*) (b). These figures combine the results of three analyses, NJ, MP and ML. The topology shown was obtained using the NJ method and 100 bootstrap replications (values at branches). Branches also obtained in the ML analysis are indicated by * ($P < 0.01$). Monophyletic units also obtained in the MP tree are indicated by +.

and related species (Sawabe *et al.*, 2004b). The *gapA* genes of the nine strains were sequenced according to Sawabe *et al.* (2004b). A phylogenetic tree was constructed in the same way as the 16S rRNA gene tree. Positions 9–703 of the *gapA* gene of strain GHG2-1^T were used for the phylogenetic analysis. Sequences of *V. haliotocoli* IAM 14596^T, *V. neonatus* HDD3-1^T, *V. ezuræ* HDS1-1^T, *V. agarivorans* LMG 21448 and *V. superstes* G3-29^T were included (Fig. 1b).

The results of our phylogenetic analysis based on the 16S rRNA gene clearly showed that the strains belong to the gamma-3 subgroup of the phylum *Proteobacteria* (Garrity & Holt, 2001). The closest phylogenetic neighbour of the nine abalone strains is *V. superstes* G3-29^T (Fig. 1a). Strains GHG1-9, GHG2-1^T, GHG2-4, NHG1-3, NHG1-11 and NHM1-4 (*Vibrio comitans* sp. nov.) had high levels of 16S rRNA gene sequence similarity to each other, above 99.9 %, and related species (Sawabe *et al.*, 2004b).

and 99.3–99.5 % similarity towards *V. superstes* G3-29^T. Strain RW22^T (*Vibrio rarus* sp. nov.) had 99.6–99.7 % similarity towards the six strains of the first group, 98.9–99.0 % similarity to strains RW14^T and RW21 and 98.6 % similarity to *V. superstes* G3-29^T. Finally, strains RW14^T and RW21 (*Vibrio inusitatus* sp. nov.) shared over 99.9 % similarity and showed 99.0 % similarity towards the six strains of the first group and 98.7 % similarity to *V. superstes* G3-29^T. Similarity levels below 98.5 % were found with other *Vibrio* species.

Phylogenetic analysis of the *gapA* gene revealed robust clades of the six strains of *V. comitans* sp. nov. and the two strains of *V. inusitatus* sp. nov. (Fig. 1b). Both clades were supported by all three phylogenetic analysis methods with 100 % bootstrap values in NJ. The closest phylogenetic neighbour of the two clades was *V. superstes* G3-29^T. *V. rarus* sp. nov. was clustered with *V. haliotocoli*, *V. neonatus* and *V. ezurae* (Fig. 1b). The six strains of *V. comitans* sp. nov. had 99.4–99.9 % intraspecies *gapA* gene sequence similarity and 94.6–94.8 % similarity towards the strains of *V. inusitatus* sp. nov. *V. rarus* RW22^T had 93.3 % similarity towards *V. neonatus* HDD3-1^T and *V. ezurae* HDS1-1^T and 93.4 % similarity towards *V. haliotocoli* IAM 14596^T. Similarity levels below 93 % were found with other *Vibrio* species.

DNAs of bacterial strains were prepared by the procedures of Marmur (1961) with minor modifications. G + C content of the DNA was determined by HPLC (Tamaoka & Komagata, 1984). DNA–DNA hybridization experiments were performed in microdilution wells using a fluorometric direct-binding method described previously by Ezaki *et al.*

(1988, 1989). DNA–DNA relatedness data are shown as mean values of triplicate experiments.

Mutual DNA–DNA hybridization experiments showed that three representative strains of *V. comitans* sp. nov., GHG2-1^T, NHG1-11 and NHM1-4, were conspecific strains clearly apart from the strains of *V. inusitatus* sp. nov. and *V. rarus* sp. nov., *V. superstes* and the other phylogenetic neighbours (Table 1). Strain RW22^T and the two strains RW14^T and RW21 also represented distinct species separate from *V. superstes* and *V. haliotocoli* (Table 1).

A total of 81 phenotypic characteristics, including alginate activity, were determined by standard manual characterization established in our laboratory (Baumann *et al.*, 1984; Hidaka & Sakai, 1968; Holt *et al.*, 1994; Leifson, 1963; Ostle & Holt, 1982; West *et al.*, 1977). Carbon assimilation tests were conducted by using basal seawater medium (Baumann *et al.*, 1984) using a method reported previously (Sawabe *et al.*, 1998, 2004b). These phenotypic characterizations were done at 20 °C.

The nine abalone strains have the main phenotypic features of the genus *Vibrio* (except for the absence of flagella). The strains are non-motile, Gram-negative and fermentative (Sawabe *et al.*, 1998). No flagellated cells were observed. The strains required salt for growth and were oxidase-positive (Table 2). No peritrichous cells were observed when the strains were cultivated on solid media. Other phenotypic features of the novel strains are shown in Table 2. The six abalone isolates of *V. comitans* sp. nov. and two isolates of *V. inusitatus* sp. nov. were phenotypically most similar to each

Table 1. DNA–DNA relatedness among representative novel strains and related type strains

Strain	G + C content (mol%)*	Reassociation (%) with biotinylated DNA from:			
		GHG2-1 ^T	RW22 ^T	RW14 ^T	<i>V. superstes</i> LMG 21323 ^T
<i>Vibrio comitans</i> sp. nov.					
GHG2-1 ^T	45.0	100	26	63	52
NHG1-11	46.7	85	24	57	53
NHM1-4	48.0	81	25	59	51
<i>Vibrio rarus</i> sp. nov.					
RW22 ^T	43.8	26	100	37	29
<i>Vibrio inusitatus</i> sp. nov.					
RW14 ^T	43.1	50	24	100	39
RW21	43.7	46	23	76	37
<i>V. superstes</i> LMG 21323 ^T	48.6 ^a	48	23	53	100
<i>V. haliotocoli</i> LMG 18542 ^T	43.1 ^a	22	30	32	24
<i>V. neonatus</i> LMG 19973 ^T	43.9 ^a	26	33	29	25
<i>V. ezurae</i> LMG 19970 ^T	43.6 ^a	26	33	32	27
<i>V. gallicus</i> CIP 107863 ^T	44.3 ^b	18	20	22	19
<i>V. agarivorans</i> LMG 21448	44.8 ^c	13	16	16	14

*Data from other studies indicated as follows: a, Sawabe *et al.* (2004b); b, Hayashi *et al.* (2003); c, Macián *et al.* (2001).

Table 2. Phenotypic characteristics for distinguishing the novel species *V. comitans*, *V. rarus* and *V. inusitatus* from phenotypically and phylogenetically related *Vibrio* species

Species/strains: 1, *V. comitans* sp. nov. (six strains); 2, *V. rarus* sp. nov. RW22^T; 3, *V. inusitatus* sp. nov. (two strains); 4, *V. neonatus* LMG 19973^T; 5, *V. ezurae* LMG 19970^T; 6, *V. haliotocoli* LMG 18542^T; 7, *V. gallicus* CIP 107863^T; 8, *V. superstes* LMG 21323^T (data in columns 4–8 from Sawabe *et al.*, 2004b); 9, *V. pelagius* ATCC 25916^T; 10, *V. harveyi* LMG 4044^T; 11, *V. splendidus* HUPF 9117^T (data in columns 9–11 from Sawabe *et al.*, 1998). +, Positive; –, negative; d+, variable, type strain positive (percentage of strains testing positive in parentheses); d–, variable, type strain negative (percentage of strains testing positive in parentheses). All taxa are negative for pigmentation, swarming, growth above 37 °C, hydrolysis of agar, gas production from D-glucose, acetoin production, acid production from L-arabinose, inositol and L-rhamnose, arginine dihydrolase and utilization of L-tyrosine, meso-erythritol, DL-malate and aconitate. All taxa are positive for Na⁺ requirement, growth at 15–30 °C, oxidase, catalase, methyl red test, growth in 3% NaCl, nitrate reduction, growth on TCBS, O/129 (150 µg) sensitivity, acid production from D-glucose, D-mannitol and maltose and utilization of D-glucose, maltose, D-mannitol and N-acetylglucosamine. All taxa are fermentative.

Characteristic	1	2	3	4	5	6	7	8	9	10	11
Motility	–	–	–	–	–	–	–	–	+	+	+
Luminescence	–	–	–	–	–	–	–	–	–	+	+
Growth at/in:											
4 °C	d+ (83 %)	–	+	–	–	–	–	–	–	–	–
37 °C	–	–	–	–	–	–	–	–	+	+	+
1 % NaCl broth	+	–	+	–	–	–	–	–	+	+	+
6 % NaCl broth	+	+	+	–	–	–	–	–	+	+	+
Production of											
Amylase, gelatinase, chitinase	–	–	–	–	–	–	–	–	–	+	+
Alginase	+	+	+	+	+	+	+	+	+	+	–
Lipase	–	–	–	–	–	–	–	–	+	+	+
DNase	d+ (67 %)	–	d– (50 %)	–	–	–	–	–	+	+	+
Lysine decarboxylase, ornithine decarboxylase	–	–	–	–	–	–	–	–	–	+	–
Indole production	–	+	–	+	+	+	+	–	–	+	+
ONPG	d+ (83 %)	–	–	–	–	+	–	–	+	–	+
Acid production from:											
Sucrose	–	–	–	–	–	–	–	–	+	+	+
D-Sorbitol	–	–	–	–	–	–	–	–	+	–	+
Requirement for organic growth factors	–	+	–	–	–	–	–	–	–	–	–
Utilization of:											
D-Mannose	–	+	+	–	+	–	–	+	+	+	+
Sucrose	–	+	–	–	–	–	–	+	+	+	+
D-Gluconate	+	+	+	–	+	–	–	+	+	+	+
D-Sorbitol	–	+	+	–	–	–	–	–	–	–	–
Glycerol	d– (16 %)	–	–	–	–	+	–	–	+	–	+
2-Oxoglutarate	–	–	–	–	–	–	–	–	+	+	+
D-Galactose	+	–	–	–	–	–	–	+	+	+	+
Cellobiose	+	+	+	–	–	–	–	+	–	+	+
Melibiose, lactose	–	–	–	–	–	–	–	+	–	–	+
D-Glucuronate	+	–	–	–	–	–	–	+	–	+	+
Trehalose, propionate	–	–	–	–	–	–	–	+	+	+	+
Putrescine	–	–	–	–	–	–	–	–	+	–	+
γ-Aminobutyrate	d+ (50 %)	–	d+ (50 %)	–	–	–	–	+	+	–	–
Acetate	+	+	+	–	–	–	–	+	+	–	+
Pyruvate	+	+	d+ (50 %)	–	–	–	–	–	+	+	+
L-Glutamate	+	+	+	+	+	–	–	+	+	+	+
D-Fructose	+	–	+	+	+	+	+	+	+	+	+
D-Glucosamine	+	+	d– (50 %)	+	+	+	–	+	+	+	+
Fumarate, succinate	+	+	+	+	+	+	–	+	+	+	+
D-Xylose	+	+	+	–	–	–	–	+	–	–	–
Citrate	d– (67 %)	–	–	–	–	–	–	–	–	–	–

other, although the strains differed in four traits (assimilation of D-mannose, D-sorbitol, D-galactose and D-glucuronate) out of 81 tested (Table 2). *V. rarus* sp. nov. was phenotypically most similar to *V. inusitatus* sp. nov. *V. rarus* and *V. inusitatus* differed in six traits (growth at 4 °C, indole production, growth in 1 % NaCl, requirement for organic growth factors and assimilation of sucrose and D-fructose) (Table 2). Growth at 4 °C, indole production and a combination of carbon sources are required to differentiate the *V. haliotocoli*-related species (Table 2).

Only three strains of *V. haliotocoli*-like bacteria were found among 30 randomly selected gut isolates from the gut of the red abalone. As we could use only one individual of the Californian red abalone because of conservation of the species, *V. rarus* sp. nov. and *V. inusitatus* sp. nov. should be proposed with limited numbers of strains. The *V. haliotocoli* group now comprises eight species, including *V. comitans*, *V. rarus* and *V. inusitatus*, which are phylogenetically more related to *V. superstes* than to *V. haliotocoli* based on 16S rRNA gene sequences (Fig. 1a). Phenotypic traits of *V. comitans*, *V. rarus* and *V. inusitatus* are likely to be similar (Table 2).

Recently, the presence of a radula was reported in the soft-bodied mollusc fossil *Odontogrophus omalus* found in Middle-Cambrian Burgess shale (Caron *et al.*, 2006). It is speculated that scraping feeding behaviour on cyanobacterial encrustations using the radula might have started 500 million years ago (Caron *et al.*, 2006). Scraping feeding behaviour is one of the physiological characteristics of abalone, especially in newly hatched juveniles. It is interesting to trace back the evolution of the gut vibrios of scraping feeding abalone with respect to the 'co-evolution concept'. Further genetic analysis by multilocus sequence analysis or genome analysis among the abalone gut vibrios might reveal unique evolutionary histories of vibrios associated with these molluscs.

In conclusion, our polyphasic study clearly demonstrated that the nine abalone isolates represent three novel species of the genus *Vibrio*, for which we propose the names *Vibrio comitans* sp. nov., *Vibrio rarus* sp. nov. and *Vibrio inusitatus* sp. nov. These novel *Vibrio* species are described from limited resources of abalone from various parts of the world.

Description of *Vibrio comitans* sp. nov.

Vibrio comitans (co'mi.tans. L. part. adj. *comitans* accompanying).

Gram-negative, facultatively anaerobic, non-motile and non-flagellated. Cells in ZoBell 2216E broth are rod-shaped, with rounded ends (0.5–1.0 × 1.2–2.0 µm). No endospores or capsules are formed. Flagellation is not observed when the organism is cultivated on solidified medium or in liquid medium. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex with an entire edge. Sodium ions are essential for growth. Mesophilic and

neutrophilic chemo-organotroph that grows at 4–30 °C. No growth above 37 °C. Growth occurs on thiosulfate/citrate/bile salts/sucrose (TCBS) agar (green colonies). Positive for acid production from D-glucose, D-mannitol and maltose, nitrate reduction, hydrolysis of alginate, oxidase, catalase and assimilation of D-gluconate, D-galactose, cellobiose, D-glucuronate, pyruvate, L-glutamate, D-fructose, D-glucose, maltose, D-xylose, D-mannitol, D-glucosamine, *N*-acetylglucosamine, fumarate and succinate. The following tests are negative: gas production from glucose, acetoin production, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, indole production, luminescence, pigmentation, requirement for organic growth factors, β -galactosidase test, hydrolysis of starch, gelatin, chitin, Tween 80 and agar, acid production from L-arabinose, inositol, L-rhamnose, sucrose and D-sorbitol and assimilation of D-mannose, sucrose, D-sorbitol, 2-oxoglutarate, melibiose, lactose, trehalose, putrescine, acetate, L-tyrosine, propionate, L-proline, *meso*-erythritol, DL-malate and aconitate. The G + C content of the DNA is 45.0–48.0 mol%.

The type strain is GHG2-1^T (=LMG 23416^T=NBRC 102076^T). The type strain and five reference strains [GHD1-9 (=LMG 23413=NBRC 102078), GHG2-4 (=LMG 23417=NBRC 102077), NHG1-3 (=LMG 23421=NBRC 102080), NHG1-11 (=LMG 23422=NBRC 102079) and NHM1-4 (=LMG 23425=NBRC 102081)] were isolated from the guts of wild-caught abalone (*H. discus discus*, *H. gigantea* and *H. madaka*).

Description of *Vibrio rarus* sp. nov.

Vibrio rarus (ra'rus. L. masc. adj. *rarus* few, scarce, rare).

Gram-negative, facultatively anaerobic, non-motile and non-flagellated. Cells in ZoBell 2216E broth are rod-shaped, with rounded ends (0.5–1.0 × 1.0–2.0 µm). No endospores or capsules are formed. Flagellation is not observed when the organism is cultivated on solidified medium or in liquid medium. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex with an entire edge. Sodium ions are essential for growth. Mesophilic and neutrophilic chemo-organotroph that grows at 15–30 °C. No growth above 37 °C. Growth occurs on TCBS agar (green colonies). Positive for acid production from D-glucose, D-mannitol and maltose, nitrate reduction, indole production, hydrolysis of alginate, oxidase, catalase, requirement for organic growth factors and assimilation of D-mannose, sucrose, D-gluconate, cellobiose, D-sorbitol, acetate, pyruvate, L-glutamate, L-proline, D-glucose, maltose, D-xylose, D-mannitol, D-glucosamine, *N*-acetylglucosamine, fumarate and succinate. The following tests are negative: gas production from glucose, acetoin production, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, luminescence, pigmentation, β -galactosidase test, hydrolysis of starch, gelatin, chitin, Tween 80, agar and DNA, acid production from L-arabinose, inositol, L-rhamnose, sucrose and L-sorbitol and assimilation of glycerol, 2-oxoglutarate, D-galactose, melibiose, lactose, D-glucuronate, trehalose,

putrescine, γ -aminobutyrate, L-tyrosine, propionate, D-fructose, citrate, *meso*-erythritol, DL-malate and aconitate. The G + C content of the DNA is 43.8 mol%.

The type strain RW22^T (=LMG 23674^T=NBRC 102084^T) was isolated from the gut of the Californian red abalone, *H. rufescens*.

Description of *Vibrio inusitatus* sp. nov.

Vibrio inusitatus (i.nu.si.ta'tus. L. masc. adj. *inusitatus* unusual, uncommon).

Gram-negative, facultatively anaerobic, non-motile and non-flagellated. Cells in ZoBell 2216E broth are rod-shaped, with rounded ends (0.5–1.0 $\times$ 1.0–2.0 μ m). No endospores or capsules are formed. Flagellation is not observed when the organism is cultivated on solidified medium or in liquid medium. Colonies on ZoBell 2216E agar are beige, circular, smooth and convex with an entire edge. Sodium ions are essential for growth. Mesophilic and neutrophilic chemo-organotroph that grows at 4–30 °C. No growth above 37 °C. Growth occurs on TCBS agar (green colonies). Positive for acid production from D-glucose, D-mannitol and maltose, nitrate reduction, hydrolysis of alginate, oxidase, catalase and assimilation of D-mannose, cellobiose, D-fructose, D-glucose, maltose, D-mannitol, N-acetylglucosamine, fumarate, succinate, D-xylose and L-glutamate. The following tests are negative: gas production from glucose, acetoin production, lysine decarboxylase, arginine dihydrolase, ornithine decarboxylase, indole production, luminescence, pigmentation, requirement for organic growth factors, β -galactosidase test, hydrolysis of starch, gelatin, chitin, Tween 80 and agar, acid production from L-arabinose, inositol, L-rhamnose, sucrose and D-sorbitol and assimilation of sucrose, D-sorbitol, glycerol, 2-oxoglutarate, D-galactose, melibiose, lactose, D-glucuronate, trehalose, putrescine, acetate, L-tyrosine, L-proline, propionate, *meso*-erythritol, citrate, DL-malate and aconitate. The G + C content of the DNA is 43.1–43.7 mol%.

The type strain RW14^T (=LMG 23434^T=NBRC 102082^T) and reference strain RW21 (=LMG 23673=NBC 102083) were isolated from the gut of the Californian red abalone, *H. rufescens*.

Acknowledgements

We are grateful to Toji Konishi, President of Ojika Fisheries Cooperative Association, and Dr Toyomitsu Horii, National Research Institute of Fisheries Science, for providing Japanese abalone. We thank Dr Jean Euzéby for helpful comments on the nomenclature of the *Vibrio* species. This work was supported by the Institute of Fermentation Osaka.

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