**Hippea jasoniae** sp. nov. and **Hippea alviniae** sp. nov., thermoacidophilic members of the class **Deltaproteobacteria** isolated from deep-sea hydrothermal vent deposits

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Thirteen novel, obligately anaerobic, thermoacidophilic bacteria were isolated from deep-sea hydrothermal vent sites. Four of the strains, designated EP5-r\(^T\), KM1, Mar08-272r\(^T\) and Mar08-368r, were selected for metabolic and physiological characterization. With the exception of strain EP5-r\(^T\), all strains were short rods that grew between 40 and 72 °C, with optimal growth at 60–65 °C. Strain EP5-r\(^T\) was more ovoid in shape and grew between 45 and 75 °C, with optimum growth at 60 °C. The pH range for growth of all the isolates was between pH 3.5 and 5.5 (optimum pH 4.5 to 5.0). Strain Mar08-272r\(^T\) could only grow up to pH 5.0. Elemental sulfur was required for heterotrophic growth on acetate, succinate, Casamino acids and yeast extract. Strains EP5-r\(^T\), Mar08-272r\(^T\) and Mar08-368r could also use fumarate, while strains EP5-r\(^T\), KM1 and Mar08-272r\(^T\) could also use propionate. All isolates were able to grow chemolithotrophically on \(\text{H}_2\), \(\text{CO}_2\), sulfur and vitamins. Phylogenetic analysis of 16S rRNA gene sequences placed all isolates within the family **Desulfurellaceae** of the class **Deltaproteobacteria**, with the closest cultured relative being **Hippea maritima** MH\(^2\) \(^T\) (~95–98 % gene sequence similarity). Phylogenetic analysis also identified several isolates with at least one intervening sequence within the 16S rRNA gene. The genomic DNA G+C contents of strains EP5-r\(^T\), KM1, Mar08-272r\(^T\) and Mar08-368r were 37.1, 42.0, 35.6 and 37.9 mol%, respectively. The new isolates differed most significantly from **H. maritima** MH\(^2\) \(^T\) in their phylogenetic placement and in that they were obligate thermoacidophiles. Based on these phylogenetic and phenotypic properties, the following two novel species are proposed: **Hippea jasoniae** sp. nov. (type strain Mar08-272r\(^T\)=DSM 24585\(^T\)=OCM 985\(^T\)) and **Hippea alviniae** sp. nov. (type strain EP5-r\(^T\)=DSM 24586\(^T\)=OCM 986\(^T\)).

Members of the class **Deltaproteobacteria** are physiologically diverse with eight described orders and thermophiles isolated from all of them (Kuever *et al.*, 2005). The order **Desulfurellales**, however, is the only order whose members are exclusively thermophiles. There is one family within this order, the family **Desulfurellaceae**, represented by two genera, **Desulfurella** and **Hippea**. Four species of the genus **Desulfurella** have been formally described from terrestrial thermal environments. All are neutrophiles that grow optimally between 52 °C and 60 °C, and conserve energy by sulfur or thiosulfate respiration or by pyruvate fermentation (Miroshnichenko *et al.*, 1998). The genus **Hippea**, on the other hand, is currently represented by one recognized species, **Hippea maritima** MH\(^2\) \(^T\), which was isolated from a shallow marine vent off the coast of Papua New Guinea (Miroshnichenko *et al.*, 1999). **H. maritima** MH\(^2\) \(^T\) is neutrophilic to moderately acidophilic (pH 5.7–6.5) and requires salt, yeast extract and sulfur for growth.

**Abbreviations:** ELSC, Eastern Lau Spreading Center; EPR, East Pacific Rise; GB, Guaymas Basin; IVS, intervening sequence(s); MAR, Mid-Atlantic Ridge.

The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene sequences reported in this paper are FR754496–FR754508.

Supplementary figures and tables are available with the online version of this paper.
Thermoacidophilic Deltaproteobacteria from deep-sea vents

Electron donors used for growth include molecular hydrogen and acetate. On average, H. maritima MH$_2$T shares 87% 16S rRNA gene sequence similarity with members of the genus Desulfdarella. Here, we describe the first deep-sea vent relatives of the genus Hippea, that are phylogenetically distinct and obligately thermoacidophilic.

Enrichment cultures targeting thermoacidophilic microorganisms were initiated from several deep-sea hydrothermal sulfide deposits that were collected with the HOV Alvin or ROV Jason 2 during research cruises to the East Pacific Rise (EPR) in 2004 and 2007, the Eastern Lau Spreading Center (ELSC) in 2005 and 2009, the Mid-Atlantic Ridge (MAR) in 2008 and the Guaymas Basin (GB) in 2009 (see Table S1 in IJSEM Online). Once shipboard, individual samples were processed and stored anaerobically as described previously (Göttz et al., 2002; Moussard et al., 2004; Reysenbach et al., 2006).

The medium used for enrichments was identical to that used by Reysenbach et al. (2006) and was prepared anaerobically under an atmosphere of N$_2$:CO$_2$ (80:20, v/v, 100 kPa). The pH was adjusted to 4.5 with sulfuric acid prior to autoclaving at 105 °C for 1 h to avoid melting the sulfur. Cysteine or sulfide (0.5 g l$^{-1}$) was added as a reducing agent from sterile stock solutions after autoclaving. For initial enrichments, 5 ml medium was inoculated with 0.5 ml sulfide-deposit slurry and incubated at 70 °C. Growth was detected after 2–3 days of incubation. Two different cell morphologies, small cocci (~1 μm in diameter) and short, motile rods (~1–3 μm in length) were observed in most enrichments when viewed using phase-contrast microscopy (BX60; Olympus). The cocci were subsequently isolated and identified as members of the genus ‘Aciduliprofundum’ (Reysenbach et al., 2006) through 16S rRNA gene sequencing (data not shown). Because species of the genus ‘Aciduliprofundum’ utilize peptides (Reysenbach & Flores, 2008), they were selected against by transferring the enrichments into medium lacking yeast extract and peptone but supplemented with acetate (as NaC$_2$H$_3$O$_2$; 15 mM) and 10 ml l$^{-1}$ vitamins (DSMZ medium 141). Pure cultures of the rods were obtained by at least two rounds of dilution to extinction or with the roll-tube method using Gelrite. Isolated colonies were small (~1–2 mm in diameter), translucent and spreading. The purity of all isolates was verified by routine observations using phase-contrast microscopy and sequencing of the 16S rRNA gene. Thirteen pure cultures were obtained with six from the MAR, four from ELSC, two from EPR and one from GB (Table S1). Upon isolation, cultures were maintained on the acetate/vitamin medium supplemented with 0.01% (w/v) yeast extract and grown at pH 4.5–5.0 and 65 °C.

The 16S rRNA gene of each isolate was amplified, purified and sequenced as described previously (Reysenbach et al., 2000). As primer 906F failed to amplify the 16S rRNA gene of the novel isolates, primers 1100R and 1114F were used for sequencing instead (Lane, 1991). Nearly complete 16S rRNA gene sequences were assembled using SeqMan software and were compared with the NCBI non-redundant database using BLAST (Altschul et al., 1990). The results of the BLAST search indicated that all 13 isolates were related to the marine thermophile Hippea maritima MH$_2$T (Miroshnichenko et al., 1999). The assembled sequences were deposited in GenBank under accession numbers FR754496–FR754508.

While the 16S rRNA gene sequence similarity between H. maritima MH$_2$T and the new isolates appeared to be relatively high, several of the isolates had large insertions that were not considered in BLAST and similarity analyses. Importing the sequences into ARB (Ludwig et al., 2004) and aligning them according to secondary structure constraints revealed that many of the isolates had at least one intervening sequence (IVS) in two distinct regions of their 16S rRNA genes (see Fig. S1). Isolates Mar08-272rT, Mar08-307r, Mar08-361r and Mar08-641r had two different IVS, one beginning at Escherichia coli nucleotide position 1025 (H1025) and another at 1448 (H1448) (Table S2). Isolate KM1 had one IVS at position H1025, while isolates Mar08-361r and Mar08-368r had one at position H1448. No IVS were detected in isolates LR3-DR, Lau09-781r, Lau09-1128r, EP5-rT, Epr07-159r or Guay09-253r. H. maritima MH$_2$T had an IVS in a different region of its 16S rRNA gene (H184) that was not reported in the initial characterization of this organism (Miroshnichenko et al., 1999). An environmental clone sequence (GenBank accession no. AM712337), previously detected at Brothers Seamount off the coast of New Zealand, also had two IVS, one at position H184 and another at H1448. Secondary structure predictions of the IVS were made using mfold (http://mfold.rna.albany.edu/?q=mfold/) and drawn using XRNA (http://rna.ucsc.edu/macenter/xrna/). Structure models of the IVS revealed probable stem–loop conformations with predicted free energy values between −49.80 and −58.90 kcal mol$^{-1}$ for the various IVS of the different organisms (Fig. S1). The G+C contents of the IVS were found to be significantly lower than for the remainder of the 16S rRNA genes (Table S2). Intervening sequences have most commonly been observed in 23S rRNA genes, although they have been observed in the 16S rRNA genes of several members of the Archaea and Bacteria (Baker et al., 2003; Bautista-Zapanta et al., 2009; Dewhirst et al., 2005; Tazumi et al., 2009, 2010; Teyssier et al., 2003; Villemur et al., 2007). In some members of the Archaea, IVS function as small nucleolar RNAs (snoRNAs) and guide rRNA processing (Dieci et al., 2009; Omer et al., 2000). Others have suggested that IVS in the H184 region may be involved in interactions with ribosomal proteins (Teyssier, et al., 2003). However, the exact role of IVS in members of the genus Hippea is unknown and beyond the scope of this study.

Neighbour-joining (Olsen correction, 500 bootstrap replicates) and maximum-likelihood (default parameters, 100 bootstrap replicates) analyses were conducted in ARB (Ludwig et al., 2004) and MEGA5 (Tamura et al., 2007),
respectively. Results from these analyses placed the isolates within the genus *Hippea* (Fig. 1). The 16S rRNA gene sequence similarity of all of the isolates to *H. maritima MH2* was ~95–98% when the IVS were masked from the analysis and when only homologous nucleotide positions were compared (1364 bp) (the alignment used for similarity matrix and tree construction is shown in Fig. S2). When the IVS were included, sequence similarity was significantly lower and ranged from ~81 to 93% (Fig. S3). Isolates from the same oceanic region (Atlantic, Eastern Pacific and Western Pacific) clustered together to form distinct biogeographical clades (Fig. 1). Nested within each clade were subclades that formed based on the specific vent field of isolation. *H. maritima MH2* fell within the Western Pacific Ocean clade and shared 97.9% sequence similarity with its closest relative, strain Lau09-1128r (93.3% with the H184 insert of *H. maritima MH2*) (Fig. 1). Similar biogeographic patterns have been seen for other thermophilic micro-organisms from terrestrial environments using a variety of phylogenetic markers (Papke et al., 2003; Whitaker et al., 2003).

Four of the isolates representing three different geographical and geological settings [MAR (Mar08-272rT and Mar08-368r), EPR (EP5-rT) and ELSC (KM1)] were selected for physiological and metabolic characterization (Table S1). All four isolates, except Mar08-272rT and Mar08-368r, shared less than 97% 16S rRNA gene sequence similarity with one another when the IVS were masked from the analysis (Fig. 1). The DNA G+C contents of strains EP5-rT, KM1, Mar08-272rT and Mar08-368r were 37.1, 42.0, 35.6 and 37.9 mol%, respectively, as determined by thermal denaturation (Table 1) (Marmur & Doty, 1962). Analysis of the polar lipid fatty acid composition of three isolates (EP5-rT, KM1 and Mar08-272rT) showed that they produced similar phospholipid fatty acids. This mainly comprised straight chain fatty acids.

![Figure 1](image-url)
Table 1. Comparison of physiological traits of four of the new isolates with *Hippea maritima MH2*<sup>T</sup>

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>Origin of isolation</td>
<td>9° N, EPR</td>
<td>Kilo Moana vent field, ELSC</td>
<td>Lucky Strike vent field, MAR</td>
<td>Lucky Strike vent field, MAR</td>
<td>Matupi Harbour, Papua New Guinea</td>
</tr>
<tr>
<td>Cell size (μm, length × width)</td>
<td>0.7–1.5 × 0.3–0.6</td>
<td>2.0–3.0 × 0.5–1.0</td>
<td>2.0–3.5 × 0.5–0.7</td>
<td>2.0–3.0 × 0.5–1.05</td>
<td>1.0–3.0 × 0.4–0.8</td>
</tr>
<tr>
<td>DNA G + C content (mol%)</td>
<td>37.1</td>
<td>41.1</td>
<td>35.6</td>
<td>37.3</td>
<td>-</td>
</tr>
<tr>
<td>16S rRNA gene sequence similarity to <em>H. maritima MH2</em>&lt;sup&gt;T&lt;/sup&gt; (%)</td>
<td>95.4</td>
<td>97.6</td>
<td>95.6</td>
<td>95.6</td>
<td>-</td>
</tr>
<tr>
<td>Temperature range (°C, optimum)</td>
<td>45–75 (60)</td>
<td>40–72 (60–65)</td>
<td>40–72 (60–65)</td>
<td>40–72 (60)</td>
<td>40–65 (52–54)</td>
</tr>
<tr>
<td>Doubling time at optimal temperature and pH (min)</td>
<td>166</td>
<td>167</td>
<td>96</td>
<td>66</td>
<td>ND</td>
</tr>
<tr>
<td>Average cell density at temperature and pH optima (cells ml&lt;sup&gt;-1&lt;/sup&gt;)</td>
<td>8.88 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>7.03 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>7.90 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>8.06 × 10&lt;sup&gt;7&lt;/sup&gt;</td>
<td>ND</td>
</tr>
<tr>
<td>pH range (optimum)</td>
<td>3.5–5.5 (4.5–5.0)</td>
<td>3.5–5.5 (4.5–5.0)</td>
<td>3.5–5.0 (4.5–5.0)</td>
<td>3.5–5.5 (4.5–5.0)</td>
<td>5.7–6.5 (5.8–6.2)</td>
</tr>
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<td>NaCl range (% w/v)</td>
<td>1–5</td>
<td>1–5</td>
<td>1–6</td>
<td>1–6</td>
<td>2–3&lt;sup&gt;*&lt;/sup&gt;</td>
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<tr>
<td>Substrates utilized:</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Casamino acids</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
<tr>
<td>Ethanol</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>Fumarate</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>(+)</td>
</tr>
<tr>
<td>Propionate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
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<tr>
<td>Succinate</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>(+)&lt;sup&gt;†&lt;/sup&gt;</td>
</tr>
</tbody>
</table>

*Range tested not reported.
†Tested as part of this study.

C<sub>16</sub> and C<sub>18</sub> fatty acids and high amounts of the iso and anteiso C<sub>17</sub> fatty acids and the C<sub>19:1</sub>ω9 fatty acid (Table S3). Direct polar lipid analysis by HPLC-ESI-MS (Pitcher et al., 2011) showed that the polar lipids mainly comprised diacylglycerols with phosphateylethanolamine and phosphatidylglycerol headgroups. Unless stated otherwise, all characterization experiments were conducted in triplicate at pH 4.5–5.0 and 65 °C in sealed 25 ml Balch tubes containing 5 ml media inoculated from fresh overnight cultures at 2.5–5% (v/v). Growth was determined by direct cell counts using a Petroff-Hauser counting chamber.

Isolates were tested for their ability to reduce a variety of inorganic electron acceptors with acetate (15 mM) acting as the electron donor. Electron acceptors tested included elemental sulfur (1%, w/v), O<sub>2</sub> (1–5%, v/v), sulfate (as Na<sub>2</sub>SO<sub>4</sub>; 0.1% w/v), thiosulfate (0.1%, w/v), nitrate (as Na<sub>NO</sub>3; 0.1%, w/v), nitrate (as Na<sub>NO</sub>O<sub>3</sub>; 0.1%, w/v), Fe<sup>3+</sup> (as ferric citrate; 5 mM) and arsenate (as Na<sub>2</sub>AsO<sub>4</sub>; 7H<sub>2</sub>O; 5 mM). All characterized isolates were strictly anaerobic and only able to use elemental sulfur as an electron acceptor (Table 1).

Isolates were tested for their ability to utilize a variety of organic carbon sources with and without CO<sub>2</sub> (N<sub>2</sub>, 100%) in the headspace and with sulfur acting as the sole electron acceptor. Vitamins were provided in all carbon substrate tests. Substrates were added at 0.1 and 0.02% (w/v; v/v for liquids) and included yeast extract, Bacto peptone, Casamino acids, glucose, starch, acetate, butyrate, formate, fumarate, lactate, succinate, propionate, pyruvate, ethanol, methanol and benzoate. All strains could use acetate, succinate, yeast extract and Casamino acids. Strains KM1, EP5-r<sup>T</sup> and Mar08–272<sup>T</sup> could use propionate while strains EP5-r<sup>T</sup> and the two MAR strains (Mar08–272<sup>T</sup> and Mar08–368r) could also use fumarate (Table 1). No difference in growth was observed at the different concentrations of carbon substrates (0.1 and 0.02%). Isolates were not able to grow on yeast extract and Casamino acids.
without elemental sulfur, indicating that they are not fermentative. Yeast extract was not required for growth if media was supplemented with vitamins. All isolates were also capable of autotrophic growth with H₂, CO₂, sulfur and vitamins.

For morphological analysis by thin-section transmission electron microscopy, cells were prepared as previously described (Hunter & Beveridge, 2005). For negatively stained whole mounts, 20 µl drops of culture were allowed to adsorb onto the copper grids for 2 min and were subsequently post-stained in 2% uranyl acetate. Isolates Mar08-272rᵀ (Fig. 2a), Mar08-368r (Fig. 2b) and KM1 (Fig. 2c) were rod-shaped, while isolate EP5-rᵀ was more ovoid in shape (Fig. 2d). The cell dimensions of each isolate varied (Table 1). Strains were all Gram-negative and the peptidoglycan sacculi were often difficult to visualize (white arrow, Fig. 2d). No intracellular membrane structures were observed, however strains Mar08-272rᵀ, Mar08-368r and KM1 contained electron-dense intracellular granules adjacent to the cytoplasmic membrane (black arrows, Fig. 2). These inclusions closely resembled the intracellular mixed-valence iron granules formed by *Shewanella putrefaciens* CN32 under anaerobic conditions (Glasauer et al., 2007). Consistent with this observation, when the new strains were centrifuged for DNA extraction, small black precipitates were observed in cell pellets. Negative stains of each isolate revealed polar flagella extending from the surfaces of most cells (Figs 2e, f).

The physiological and phylogenetic characteristics of the newly described isolates were distinct from *H. maritima*
MH$_2^+$ and each other. Unlike *H. maritima* which was isolated from shallow (<40 m) marine thermal environments, the novel strains from this study were all isolated from deep-sea vents (>1500 m). All of the new strains had very different pH and temperature ranges for growth when compared with *H. maritima* MH$_2^+$, which grows at pH 5.7–6.5 and at temperatures of 40–65 °C (Miroshnichenko *et al.*, 1999). In contrast, the newly described isolates were obligately acidophilic, growing at pH 3.5–5.5 (strain Mar08-272T only up to pH 5.0) and temperatures between 40 and 72 °C (strain EP5-rT grows between 45 and 75 °C). The new isolates also differed from *H. maritima* MH$_2^+$ and each other in their ability to utilize succinate and propionate (strains EP5-rT, KM1, Mar08-272T), and in their inability to utilize ethanol (strains EP5-rT, KM1, Mar08-272T and Mar08-368r) and fumarate (strain KM1). The genomic G+C content also varied between all of the new isolates and *H. maritima* MH$_2^+$ but all values were within ±5%. Additionally, based on their 16S rRNA gene phylogenies, two of the isolates represented distinct species (~95% 16S rRNA gene sequence similarity) (Table 1).

Based on the phenotypic (primarily acidophily, carbon substrate utilization and temperature range for growth) and phylogenetic differences between the new isolates and *H. maritima* MH$_2^+$, it is suggested that the description of the genus *Hippea* should be emended and two novel species are proposed, namely, *Hippea jasoniae* sp. nov. (type strain Mar08-272T) and *Hippea alviniae* sp. nov. (type strain EP5-rT).

**Emended description of the genus *Hippea***

Cells are Gram-negative rods or ovoid in shape. Moderate thermophiles. Neutrophiles to obligate acidophiles. Obligate anaerobes. Metabolize by reduction of elemental sulfur. Substrates utilized include H$_2$, volatile fatty acids, fatty acids and alcohols. Growth products are CO$_2$ and H$_2$S. The G+C content of the DNA of the type strain is 40 mol%. Inhabits shallow to deep submarine hydrothermal vents. The type species is *Hippea maritima*.

**Description of *Hippea jasoniae* sp. nov.**

*Hippea jasoniae* (ja.so.ni’a.e. N.L. fem. gen. n. jasoniae of Jason, named in honour of the ROV Jason 2 which collected the samples harbouring this strain and has been essential in the exploration of deep-sea hydrothermal environments).

Cells are motile, Gram-negative rods, 2.0–3.5 μm long and 0.5–0.7 μm wide. Moderate thermophiles (40–72 °C, optimal 60–65 °C). Obligately acidophilic (pH 3.5–5.0, optimal pH 4.5–5.0). Obligately anaerobic. Requires NaCl (1–5% w/v) for growth. Metabolizes by reduction of elemental sulfur. Growth substrates are H$_2$/CO$_2$, acetate, fumarate, succinate, propionate (not all strains), Casamino acids and yeast extract. Yeast extract is not required for growth if vitamins are provided.

The type strain, Mar08-272T (=DSM 24585T=OCM 985T), was isolated from the Lucky Strike (37° 17.5240’ N, 32° 16.5085’ W, 1624 m depth) hydrothermal vent field along the Mid-Atlantic Ridge in the Atlantic Ocean. The G+C content of the genomic DNA of the type strain is 35.6 mol%.

**Description of *Hippea alviniae* sp. nov.**

*Hippea alviniae* (al.vi.ni’a.e. N.L. fem. gen. n. alviniae of Alvin, named in honour of the ROV Alvin which collected the samples harbouring this strain).

Cells are motile, Gram-negative ovoid rods, 0.7–1.5 μm long and 0.3–0.6 μm wide. Moderate thermophiles (45–75 °C, optimal 60 °C). Obligately acidophilic (pH 3.5–5.5, optimal pH 4.5–5.0). Obligately anaerobic. Requires NaCl (1–5% w/v) for growth. Metabolizes by reduction of elemental sulfur. Growth substrates are H$_2$/CO$_2$, acetate, fumarate, succinate, propionate, Casamino acids and yeast extract. Yeast extract is not required for growth if vitamins are provided.

The type strain, EP5-rT (=DSM 24586T=OCM 986T), was isolated from ‘A’ vent (9° 46.5003’ N, 104° 16.8100’ W, 2520 m depth) along the East Pacific Rise in the eastern Pacific Ocean. The G+C content of the genomic DNA of the type strain is 37.1 mol%.

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**References**


