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Aurantimonas coralicida gen. nov., sp. nov., the causative agent of white plague type II on Caribbean scleractinian corals

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A bacterium previously isolated from a diseased colony of the scleractinian coral *Dichocoenia stokesi* (common name elliptical star coral), was subjected to a detailed polyphasic taxonomic characterization. The isolate designated as WP1^T (= CIP 107386^T, DSM 14790^T) was halophilic, strictly aerobic, and formed golden orange-pigmented colonies after prolonged incubation. Cells of WP1^T were Gram-negative, rod-shaped and showed a characteristic branching rod morphology. Chemotaxonomically, WP1^T was characterised by having Q-10 as the major respiratory lipoquinone, and *sym*-homospermidine as the main component in the cellular polyamine content. The predominant constituent in the cellular fatty acid profile was $C_{18:1}$ *ω7c* along with $C_{19:0}$ cyclo *ω8c*, and $C_{16:0}$. Other fatty acids in lower amounts were $C_{17:0}$, $C_{18:0}$, $C_{16:1}$ *ω7c*, $C_{20:1}$ *ω7c*, and $C_{18:1}$ 2-OH. The major polar lipids were phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine. Minor amounts of diphosphatidylglycerol, phosphatidylmonomethylethanolamine and phosphatidyldimethylethanolamine were present as well. The mol % G+C content of the genomic DNA was 66·3. Phylogenetic analysis of 16S rRNA gene sequence showed that WP1^T represents a separate subline of descent within the *Rhizobiales* of the *Alphaproteobacteria*.The new line of descent falls within the group of families that includes the *Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae* and *Phyllobacteriaceae* with no particular relative within this group. The 16S rRNA gene sequence similarity to all established taxa within this group was not higher than 92·0 % (with *Mesorhizobium mediterraneum*). To accommodate this emerging coral pathogen the creation of a new genus and species is proposed, *Aurantimonas coralicida*.

Keywords: coral bacterial pathogen, Caribbean scleractinian corals, *Dichocoenia stokesi*, white plague type II, *Alphaproteobacteria*, *Rhizobiales*, *Aurantimonas coralicida*

Abbreviations: DPG, diphosphatidylglycerol; PC, phosphatidylcholine; PDE, phosphatidyldimethylethanolamine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; Q-9, ubiquinone-9; Q-10, ubiquinone-10.

The GenBank accession number for the 16S rRNA gene sequence of *Aurantimonas coralicida* strain WP1^T is AJ065627.

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Introduction

In 1995 Richardson and co-workers documented a dramatic coral epizootic that occurred on reefs of the northern Florida Keys, which rapidly spread to infect 17 of the 43 species of scleractinian corals present. Mortality rates of up to 38 % of the most susceptible coral species *Dichocoenia stokesi* (the elliptical star coral) occurred within periods as short as 10 weeks (Richardson *et al*., 1998a). The disease was designated as white plague type II because of its similarity to an earlier epizootic characterised as plague, or white plague, that occurred on the same reefs in the 1970s (Dustan, 1977). Both epizootics emerged as a sudden occurrence of diseased coral colonies on Florida's reefs. The diseased corals exhibited active coral tissue death in which a sharp line between freshly exposed coral skeleton and apparently healthy coral tissue was present, and which migrated across coral colonies, eventually resulting in colony death. No pathogen was isolated in the original plague (now designated type I) outbreak. Microbiological studies conducted as part of the documentation of the 1995 white plague type II outbreak revealed that the disease was caused by a single Gram-negative bacterium, isolated as strain WP1 (Richardson *et al*., 1998a). At this time it was demonstrated that pure cultures of WP1 readily initiated disease activity in healthy corals in the laboratory, thus satisfying the procedures of Koch's postulates (Richardson *et al*., 1998b). Based on a BLAST search comparing a 300 bp sequence of the 16S rRNA gene sequence (accession no. AF143861) and a limited number of phenotypic tests the coral pathogen WP1^T was identified as a possibly new *Sphingomonas* species (Richardson *et al*., 1998a). Beyond it, a replication sequence comparison between the redetermined 16S rRNA gene sequence in the study presented here (accession no. AJ065627) and the originally deposited sequence AF143861 revealed that there was no significant degree of similarity (~82 %) between both sequences. In order to exclude any strain confusion we have followed the history and distribution of strain WP1 among our different laboratories carefully but we were not able to identify any problem. Also pathogenic, physiological and biochemical traits of the early WP1 and the strain we working with now agree perfectly. Similarities between the 16S rRNA gene sequences of WP1 indepently

determined in Vienna and in South Carolina, is 99·7 % confirming that both laboratories are working with the same strain. Therefore we conclude that we are working with the same strain originally isolated and described by Richardson *et al*. (1998a) and that the problem with the low sequence similarity is related to the sequence originally deposited in GenBank. The polyphasic study presented here, which included molecular, chemosystematic and standard bacteriological analyses, report on the actual taxonomic position of this emerging coral pathogen.

Methods

Source and isolation. Strain WP1^T (T = type strain) was isolated in August 1995 from a sample collected from a diseased colony of *D. stokesi* on a reef of the northern Florida Keys. Sampling was conducted underwater, while using SCUBA. A sterile 10 ml syringe equipped with a sterile 21 gauge needle was drawn along the line between apparently healthy coral tissue and exposed skeleton, with steady gentle suction applied. The syringe was immediately capped, and upon return to the surface placed in a cooler filled with freshly collected seawater. Upon return to shore, the contents of the syringe were placed in a sterile test tube, vortexed, and used to inoculate a dilution series (10^{-1} to 10^{-6}) of sterile seawater, from which 100 µl subsamples were plated onto Bacto marine agar 2216 (Difco). All incubations were conducted at room temperature.

Standard bacteriological characterisation. Strain WP1^T was routinely cultivated aerobically on Bacto marine agar 2216 at 28 °C, or otherwise as indicated in the text. To verify growth on different bacteriological media WP1^T was streaked onto Luria-Bertani agar (Atlas, 1993), Tryptone soy agar, MacConkey agar and R2A agar (Oxoid). The media were used in their original formulations and as marine versions containing 3·2 % (w/v) sea salts (Sigma). The ability to grow anaerobically was tested by means of a commercial atmosphere generation system (Anaero*Gen*™; Oxoid). Cell morphology was examined by phase-contrast microscopy (Leitz, Diaplan) from shake-flask cultures (150 r.p.m) grown overnight in Bacto marine broth 2216 (Difco). Scanning

electron microscopy (SEM) was performed on a Hitachi S4700 field emission scanning electron microscope at 5·0 kV. SEM samples were prepared by fixing intact colonies of the isolate in a 3·5 % glutaraldehyde solution (in 0·1 M sodium cacodylate buffer) for 18 hours. The samples were dehydrated using a series of 30-minute immersions in six different ethanol solutions (50, 70, 85, 95, and 100 %). Dehydrated samples were critical point dried (CO_2), mounted using carbon tape, and sputter coated with gold. For transmission electron microscopy (TEM) 2 µl bacterial suspension was placed onto a carbon-coated 400-mesh Ni grid (ATHENE SIRA, ∅ 3·05 mm, Smethurst High-light, UK). After 20 min absorption time the grid was fixed in a 2·5 % (w/v) glutaraldehyde-cacodylate buffer (pH 7·4). Subsequently the grid was rinsed 3 times in distilled water, stained with 1 % (w/v) uranylacetate (pH 4·2). Excess of stain was removed by touching the rim of the grid with a filter paper and air dried at room temperature. TEM samples were examined on a Philips EM 902 transmission electron microscope.

Growth at different temperatures was tested on Bacto marine agar 2216 plates incubated between 4–45 °C for as long as 2 weeks. The sensitivity against various antimicrobial agents (Table 1) was tested by the disk diffusion method using commercial antibiotic-impregnated disks (Oxoid) Briefly, 100 µl of a cell suspension (McFarland standard 0·5) in sterile 3·2 % seawater was plated onto Bacto marine agar 2216; after 48 to 72 h incubation at 28 °C any sign of growth inhibition was scored as sensitivity. Resistance was indicated if no inhibition zone was observed. Assay for cytolytic properties was performed on seawater supplemented (3·2 %, w/v) TSA plates containing 5 % (v/v) defibrinated sheep blood. Biochemical characterisation was carried out by following the standard methods of Smibert & Krieg (1994) supplemented by API 20E, API 20NE and API Zym galleries (bioMeriéux). API test systems were used according to the manufacturer's instructions except that (i) bacterial suspensions were prepared in autoclaved artificial sea water (40 g l^{-1} sea salts [Sigma] in demineralised water) and (ii) the reading was done after 5 h (API Zym) and up to

7 days (API 20E, API 20NE), respectively. The presence of cytochrome *c* oxidase was tested with Bactident-oxidase test strips (Merck). Metabolic fingerprinting was carried out using the BIOLOG system (Biolog; Hayward, Calif., USA). Briefly, five subcultures of WP1^T were grown for three days on GASW agar (Smith & Hayasaka, 1982). Subsequently, cell suspensions were prepared in sterile (3·2 %, w/v) artificial seawater to an optical density of 0·146 at 600 nm (OD_{600}). 96-well microtiter plates (Biolog GN1 MicroPlate™) containing 95 different carbon sources were inoculated with 150 µl of the adjusted cell suspension in each well and incubated at 30 °C for three days. A positive colorimetric reaction (the result of utilization of the sole carbon source and concomitant reduction of terazolium dye) was measured at 490 nm on an automated microplate reader (Molecular Devices, EMAX model). For this assay, any absorbance greater than 40 % of the control (sterile seawater) well was considered as positive.

Chemotaxonomic analyses. Respiratory lipoquinones were extracted from lyophilised cell material (~100 mg) with methanol:hexane (2:1, v/v) and were analysed by HPLC as described by Tindall (1990). Polyamines were extracted as described by Busse & Auling (1988), and were analysed according to Busse *et al*. (1997). Fatty acid methyl esters (FAME) were extracted and prepared by the standard protocol of the MIDI/Hewlett Packard Microbial Identification System (Sasser, 1990). The FAME profile was analysed by GLC using a GC-14A gas chromatograph (Shimadzu) as described by Groth *et al.* (1996). Polar lipids were extracted from ~100 mg lyophilised cell material by the modified Folch procedure devised by Bligh & Dyer (1959) and resolved in 250 µl chloroform/methanol (2:1, v/v). Two-dimensional TLC was carried out as described by Denner *et al*. (2001). For pigment analysis ~100 mg of cell material grown on Bacto marine agar 2216 were scraped from the agar surface and placed into a small (5 ml) Teflon-sealed glass vial. Subsequently, methanol (2 ml) was added to extract methanol-soluble pigments; after centrifugation (10,000 × *g*, 4 °C, 5 min) the supernatant was scanned (300-800 nm) on a Hitachi

S-2000 absorbance spectrophotometer. For DNA mol % G+C analysis genomic DNA was isolated from lyophilised cell material and was purified on hydroxyapatite according to the procedure of Cashion *et al*. (1977). The DNA was hydrolysed with P1 nuclease and the nucleotides dephosphorylised with bovine alkaline phosphatase (Mesbah *et al*., 1989). The resultant deoxyribonucleosides were then analysed by HPLC (Tamaoka and Komagata, 1984). Mol % G+C content of DNA was calculated from the ratio of deoxyguanosine and thymidine according to Mesbah *et al*. (1989).

Determination and analysis of the 16S ribosomal RNA gene sequence. Preparation of genomic DNA, enzymatic amplification of 16S rDNA, PCR, and sequencing were performed as described by Denner *et al*. (2001). Initial database searching was done by FASTA analysis (Pearson & Lipman, 1988; Pearson, 1990). Subsequently, relevant nucleotide sequences were retrieved from EMBL and GenBank databases, aligned manually using the program PILEUP (Devereux *et al.*, 1984) and edited to remove nucleotide positions of ambiguous alignment and gaps. A continuous stretch of 1301 nucleotides in the alignment was used in the pairwise evolutionary distance estimation (Jukes & Cantor, 1969). Phylogenetic dendrograms were constructed using the neighbour-joining method (Saitou & Nei, 1987) and confidence in the tree topology was determined using 1000 bootstrapped trees. The phylogenetic analyses were performed using the programs included in the PHYLIP software package (Felsenstein, 1995). Taxonomic nomenclature was used according to the most recent version of Bergey's Manual of Systematic Bacteriology (Bonne *et al.*, 2000).

Results and Discussion

Phylogeny of the coral pathogen

Sequence searches of GenBank and EMBL databases with the redetermined 16S rRNA gene sequence of WP1^T in this study showed that the bacterium is most closely related to strains and

species of the order *Rhizobiales* of the class *Alphaproteobacteria* (Garrity and Holt, 2000). The highest 16S rRNA gene sequence similarity (92·0%) was found to *Mesorhizobium mediterraneum* UPM-Ca36^T. Dendrograms of phylogenetic relationships inferred from neighbour-joining (Fig. 1) and both maximum-likelihood and maximum-parsimony analysis (data not shown) including a subset of *Alphaproteobacteria* species, showed that WP1^T represents a separate subline of descent within the *Rhizobiales*. Bootstrap analysis gave a 100 % confidence for this position. The new line of descent falls within the group of families that includes the *Rhizobiaceae*, *Bartonellaceae*, *Brucellaceae* and *Phyllobacteriaceae* with no particular relative within this group (Fig. 1), WP1^T may represent a novel family within the *Rhizobiales*.

Chemical biomarkers and chemotaxonomy of the coral pathogen

The DNA G+C content of WP1^T was found to be 66·3 mol %. Ubiquinones were the sole respiratory lipoquinones detected, with Q-10 predominating (99 %), Q-9 present in minor amounts (1 %). This quinone profile is characteristic for the majority of species within the class *Alphaproteobacteria* (Collins & Jones, 1981; Yokota *et al*., 1992; Busse *et al*., 1999). Fingerprinting of the cellular lipids by two-dimensional TLC revealed a complex composition consisting of diphosphatidylglycerol (DPG), phosphatidylethanolamine (PE), phosphatidylmonomethylethanolamine (PME), phosphatidyldimethylethanolamine (PDE), phosphatidylglycerol (PG), phosphatidylcholine (PC) and several unidentified lipids (Fig. 2). DPG, PE and PG are widely distributed amongst bacteria and thus of little value for chemotaxonomic purposes (Wilkinson, 1988). The *N*-methylated PE derivatives PME, PDE and PC are of considerable taxonomic interest. Most of the bacteria described to contain *N*-methylated derivatives of PE are actinomycetes or are Gram-negatives, particularly bacteria belonging to the class *Alphaproteobacteria* (Wilkinson, 1988; Busse *et al*., 1999). The polar lipid compositions of members of the *Rhizobiales* lineage are not entirely clear. All species investigated so far contained PE and PC in comparable amounts, DPG was also consistently present (Bunn & Elkan, 1970;

211 Kaneshiro & Marr, 1962; Thiele *et al*., 1968, 1973; Thompson *et al*., 1983; Kämpfer *et al*., 1999;
212 Choma & Komaniecka, 2002). Either or both of PME and PDE may also be formed: this applies
213 to *Agrobacterium tumefaciens* (Goldfine & Ellis, 1964), *Rhizobium leguminosarum* (Faizova *et*
214 *al*., 1971), *Brucella* spp. (Thiele *et al*., 1968, 1973; Kulikov & Dranovskaia, 1988), *Sinorhizobium*
215 *meliloti* (Thompson *et al*., 1983), *Aminobacter aminovorans*, *Pseudoaminobacter* spp. (Kämpfer
216 *et al*., 1999) and *Mesorhizobium* spp. (Choma & Komaniecka, 2002).
217
218 The cellular fatty acid profile of WP1^T was characterised by $C_{18:1}$ *ω7c* (76·9 %) along with $C_{19:0}$
219 cyclo *ω8c* (10·5 %), and $C_{16:0}$ (6·7 %). Additional fatty acids detected in lower amounts included
220 $C_{18:0}$ (1·5 %), $C_{16:1}$ *ω7c* (1·3 %), $C_{17:0}$ (0·6 %) and $C_{20:1}$ *ω7c* (0·5 %). The sole hydroxylated fatty
221 acid was $C_{18:1}$ 2-OH (2·0 %). The predominance of octadecenoic acids together with significant
222 amounts of a cyclic $C_{19:0}$ fatty acid is a typical feature for members of the *Rhizobiales* lineage
223 (Wilkinson, 1988; Moreno *et al*., 1990; Lechner *et al*., 1995; Jarvis *et al*., 1996; Kämpfer *et al*.,
224 1999; Dunfield *et al*., 1999; Tighe *et al*., 2000). The closest match in the MIDI fatty acid database
225 was *Ochrobactrum anthropi*, however, with a low (0·722) similarity index. The hydroxylated fatty
226 acid $C_{18:1}$ 2-OH detected in the fatty acid profile of WP1^T is not common amongst bacteria, but
227 interestingly this compound has been found in low quantities in *Agrobacterium* biovar 1 and
228 biovar 2, *Mesorhizobium huakuii*, *Mesorhizobium loti*, *Rhizobium leguminosarum*, *Rhizobium*
229 *hainanense*, *Rhizobium tropici* (Jarvis *et al*., 1996; Dunfield *et al*., 1999; Tighe *et al*., 2000),
230 *Ochrobactrum anthropi* (Lechner *et al*., 1995) and *Phyllobacterium myrsinacearum* (Mergaert *et*
231 *al*., 2002).
232
233 The main component (15·3 µmol g^{-1}, dry/wt) in the cellular polyamine pattern of WP1^T was *sym*-
234 homospermidine. Lesser amounts of spermidine (7·0 µmol g^{-1}, dry/wt) and putrescine (4·8 µmol
235 g^{-1}, dry/wt) were present as well. Generally, the polyamine patterns of all species of the

236 *Rhizobiales* which have been examined so far are dominated by *sym*-homospermidine (Busse &
237 Auling, 1988; Auling *et al*., 1991; Hamana & Matsuzaki, 1992; Hamana, K. & Takeuchi, 1998;
238 Kämpfer *et al*., 1999). This polyamine pattern is not consistent, however. For example both
239 *Ochrobactrum anthropi* and *Defluvibacter lusatiae* have either spermidine as the dominant
240 compound, or a combination of both putrescine and spermidine (Lechner *et al*., 1995; Fritsche *et*
241 *al*., 1999).
242
243 **Cultural, physiological and biochemical characteristics**
244 Colonies of the coral pathogen WP1^T on Bacto marine agar 2216 appeared opaque, circular,
245 entire, convex, smooth and golden orange in colour. Prior to pigment development (typically after
246 two days of growth) colonies were translucent. Pigment extraction (methanol) yielded peaks at λ
247 $_{max}$ 447 and 470-471 nm, and showed a slight inflexion at 424-427 nm. This spectral characteristic
248 is indicative of carotenoids (Schmidt *et al*., 1994). Cells were Gram-negative, rod-shaped (~1 µm
249 $\times$ 1·5-2·5 µm) and polar polytrichous flagellated (Fig. 3). Interestingly, cells of WP1^T exhibited a
250 branching rod morphology (Fig. 4) that is usually found among nonspore forming high G+C
251 Gram-positive bacteria such as *Arthrobacter* and *Corynebacterium* (Holt *et al*., 1994). This
252 morphotype is relatively rare among Gram-negative bacteria, but has been found in some species
253 of aerobic anoxygenic phototrophic bacteria (Yurkov & Beatty, 1998). Olson *et al*. (2002) recently
254 isolated from marine sponges several Gram-negative bacteria which also displayed a branching
255 rod morphology.
256
257 Cultivation experiments revealed that WP1^T is strictly aerobic and that cultures grew well on both
258 complex peptone based media (TSA, marine agar 2216, blood agar) and on nutrient-reduced
259 media such as R2A agar supplemented with 3·2 % (w/v) sea salts. WP1^T did not grow on media
260 without the addition of salts (NaCl). Growth occurred at 4 °C (3 to 4 weeks), 28 °C, and 37 °C,
261 but not at 45 °C or higher. There was no growth on MacConkey agar. The results of the standard

bacteriological characterisation are summarized in Table 1. WP1^T exhibited on blood agar a sharp clear zone of beta-haemolysis. This is of potential importance in the aetiology of white plague type II because this assay is indicative for cytolytic toxins (Rowe & Welch, 1994). Biochemical testing revealed further that WP1^T is strongly ureolytic, since the urease test was positive after a few hours of incubation. The formation of ammonia by the coral pathogen may potentially contribute to the pathogenesis (specifically by causing coral bleaching). Inhibition of photosynthesis by ammonia is well established (Abeliovich & Azov, 1976; Cohen *et al*., 1975; Warren, 1961). Ammonia acts as an uncoupler of photosynthesis by passing across membranes, thereby destroying the pH gradient across the thylakoid membrane (Smith & Raven, 1979; Velthuys, 1980). Very recently, Banin *et al*. (2001) demonstrated that the toxin P from the coral pathogen *Vibrio shiloi* (Kushmaro *et al*., 2001) rapidly inhibits photosynthesis of zooxanthellae of *Oculina patagonica* in the presence of NH_3.

Conclusions

Based upon the polyphasic taxonomic data obtained in this study, we conclude that coral bacterial pathogen WP1^T is a representative of an hitherto unknown marine taxon of the order *Rhizobiales* of the class *Alphaproteobacteria*. We propose the name *Aurantimonas coralicida* gen. nov., sp. nov., a formal description of this novel taxon is given below.

The *Rhizobiales* lineages include several bacteria such as *Bradyrhizhobium, Mesorhizobium, Rhizobium*, *Phyllobacterium*, *Brucella* and *Bartonella* spp. that are known to form symbiotic or pathogenic associations with plants and animals (Holt *et al*., 1994). In this respect the capability of *Aurantimonas coralicida* to initiate disease in corals is a further reference that specific procaryotic-eucaryotic association may have been important property shared by this group. To our knowledge this is the first description of a species of the *Rhizobiales* which is pathogenic for marine invertebrates. There is some evidence from our sequence database search by FASTA that

288 at least three unidentified bacterial strains closely related to *Aurantimonas coralicida* have been
289 isolated. These are strain SI85-9A1, a marine manganese-oxidising bacterium (Caspi *et al.*, 1996);
290 strain Eplume 4.J1, an isolate from a Pacific hydrothermal plume (Kaye & Baross, 2000); and
291 strain R7951 isolated from the polar sea (Mergeart *et al*., 2001). The 16S rRNA gene sequence
292 similarity of SI85-9A1 and R7951 to *Aurantimonas coralicida* WP1^T was 98·9 (in 1293 ungapped
293 nt positions) and 98·8 % (in 1292 ungapped nt positions), respectively. The phylogenetic
294 relationship between strains SI85-9A1, R7951 and *Aurantimonas coralicida* WP1^T is shown in
295 Figure 1. Strain Eplume 4.J1, of which only a partial (348 nt) 16S rRNA gene sequence is
296 available (accession no. AF251774), exhibited a sequence similarity of 99·7 % to WP1^T. These
297 three isolates may represent another species of *Aurantimonas*, or at least are novel strains of *A.*
298 *coralicida*.
299

Description of *Aurantimonas* gen. nov.

300 *Aurantimonas (*Au.ran.ti.mo'nas. M.L. *Aurantium* name and taxonomic name of the orange plant,
301 G. fem. n. *monas* monad, unicellular organism, N.L. fem. n. *Aurantimonas* orange colored
302 unicellular organism).
303 Gram-negative; endospores are not formed. Strictly aerobic. Catalase- and oxidase-positive.
304 Intracellular pigments (carotenoids) are produced; the visible absorption spectra of the pigment
305 (methanol extract) shows 2 peaks at λ_{max} 447 and 470-471 nm, and a slight inflexion at 424-427
306 nm. Sole respiratory lipoquinones present are ubiquinones, with Q-10 predominating; Q-9 may
307 account for about 1 % of the total.. The main cellular polyamine is *sym*-homospermidine; minor
308 amounts of putrescine and spermidine are present as well. Major polar lipids are
309 phosphatidylethanolamine, phosphatidylglycerol and phosphatidylcholine.
310 Diphosphatidylglycerol, phosphatidylmonomethylethanolamine and
311 phosphatidyldimethylethanolamine are present as secondary components. The predominant fatty

acid is $C_{18:1}$ *ω7c*, along with $C_{19:0}$ cyclo *ω8c* and $C_{16:0}$. Other fatty acids are $C_{17:0}$, $C_{18:0}$, $C_{16:1}$ *ω7c*, and $C_{20:1}$ *ω7c*. The sole hydroxylated fatty acid $C_{18:1}$ 2-OH. The G+C content of the DNA is 66·3 mol % (by HPLC).

Aurantimonas coralicida sp. nov.

Aurantimonas coralicida (co.ra.li´ci.da. L.n. coralium [red] coral, L. masc./fem. suffix -cida murderer, killer, N.L. masc./fem. n. *coralicida* coral killer).

Cells are Gram-negative, rod-shaped (on average: 1·5-2·5 μm × 1 μm) with a bulbous branching rod morphology. Motile by means of polar polytrichous flagellation. Colonies on marine agar 2216 are opaque, golden orange coloured, circular, entire, convex, and smooth. Prior to pigment development colonies are translucent. Urease is present. Details on nutritional, physiological and biochemical features of *Aurantimonas coralicida* are specified in Table 1. Chemotaxonomic characteristics are the same as those given in the genus description. **Source:** isolated from a diseased colony of the scleractinian coral *Dichocoenia stokesi* (elliptical star coral). Strain WP1 has been deposited as the type strain (= CIP 107386^T, DSM 14790^T).

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488 ***Table 1.*** Nutritional, physiological and biochemical characteristics of the coral pathogen WP1^T

Characteristics	**Reaction**
Oxidase	+
Catalase	+
Nitrate reduction	–
Indole formation	–
H_2S formation	–
Voges-Proskauer reaction	–
Acid from carbohydrates (API 20E)	
Glucose, rhamnose, and melibiose	+
Mannose, inositol, sorbitol, saccharose, amygdaline, and arabinose	–
Utilisation of single carbon sources (API 20NE)	
Glucose, arabinose, mannose, gluconate, and malate	+
Mannitol, *N*-acetylglucosamine, maltose, caproate, adipate, citrate, and phenylacetate	–
Hydrolysis of (API 20E, API 20NE)	
Gelatine and esculine	–
Enzymatic activities (API 20E, API Zym)	
Arginine dihydrolase, urease, alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, and naphtol-AS-BI-phosphohydrolase	+
Lysin decarboxylase, ornithin decarboxylase, lipase (C14), cystine arylamidase, valine arylamidase, trypsin, chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, α-glucosidase, β-glucosidase, *N*-acetyl-β-glucosaminidase, α-mannosidase, and α-fucosidase	–
Carbon source oxidation profile (Biolog GN1 MicroPlate)	
α-Cyclodextrin, dextrin, glycogen, Tween 40, Tween 80, L-arabinose, cellobiose, D-fructose, D-galactose, gentiobiose, α-D-glucose, α-D-lactose, lactulose, D-mannose D-melibiose, β-methyl-D-glucoside, D-raffinose, L-rhamnose, sucrose, D-trehalose, turanose, mono-methyl succinate, acetic acid, D-galacturonic acid, D-gluconic acid, alaninamide, L-alanine, L-alanyl glycine, L-glutamic acid, glycyl-L-aspartic acid, glycyl-L-glutamic acid, L-histidine, L-serine, uridine, and glycerol	+

N-Acetyl-D-galactosamine, *N*-acetyl-D-glucosamine, adonitol, L-fucose, maltose, D-psicose, methyl pyruvate, D,L-lactic acid, proprionic acid, L-asparagine, L-proline, L-threonine, and urocanic acid	(+)
D-Arabitol, L-erythritol, *m*-inositol, D-mannitol, D-sorbitol, xylitol, *cis*-aconitic acid, citric acid, formic acid, D-galactonic acid, lactone, D-glucosaminic acid, D-glucuronic acid, α-hydoxybutyric acid, β-hydroxybutyric acid, 7-hydroxybutyric acid, *p*-hydroxy phenylacetic acid, itaconic acid, α-keto butyric acid, α-keto glutaric acid, α-ketovaleric acid, malonic acid, quinic acid, D-saccharic acid, sebacic acid, succinic acid, bromo succinic acid, succinamic acid, glucoronamide, D-alanine, L-aspartic acid, hydroxy-L-proline, L-leucine, L-ornithine, L-phenylalanine, L-pyroglutamic, D-serine, D,L-carnitine, γ-amino butyric acid, inosine, thymidine, phenyl ethylamine, putrescine, 2-amino ethanol, 2,3-butenediol, D,L-α-glycerol phosphate, glucose-1-phosphate, and glucose-6-phosphate	–
Antimicrobial susceptibility pattern	
Sensitive to: amoxycillin/clavulanic acid (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (10 µg), fusidic acid (10 µg), gentamicin (10 µg), kanamycin (30 µg), nitrofurantoin (100 µg), streptomycin (10 µg), spectinomycin (10 µg), and tetracycline (10 µg).	
Resistant to: ampicillin (10 µg), bacitracin (10 µg), colistin sulphate (10 µg), furazolidone (50 µg), methicillin (5 µg), penicillin G (10 iu), polymyxin B sulphate (300 iu), vancomycin (10 µg), and lincomycin (15 µg).	

Legend to the Figures

Figure 1. Dendrogram based on 16S rRNA gene sequence data, indicating the phylogenetic position of the coral pathogen WP1^T within the class *Alphaproteobacteria*. The dendrogram was generated by neighbour-joining of Jukes-Cantor distances (Felsenstein, 1995) based on 1039 unambiguously aligned nucleotide sites. Scale bar, 10 nt substitutions per 100 nt positions. The root was defined by using *Bacillus subtilis* and *Escherichia coli* as the outgroup; tree construction was performed with TREEVIEW version 1.5.2 (Page, 1996).

Figure 2. Polar lipid fingerprints of the coral pathogen WP1^T after separation by two-dimensional TLC. DPG, diphosphatidylglycerol; PME, phosphatidylmonomethylethanolamine; PE, phosphatidylethanolamine; PDE, phosphatidyldimethylethanolamine; PG, phosphatidylglycerol; PC, phosphatidylcholine; L1-L3, unidentified lipids.

Figure 3. TEM micrograph of the coral pathogen WP1^T showing the type of flagellation.

Figure 4. SEM micrograph illustrating the bulbous branching rod morphology of the coral pathogen WP1^T.

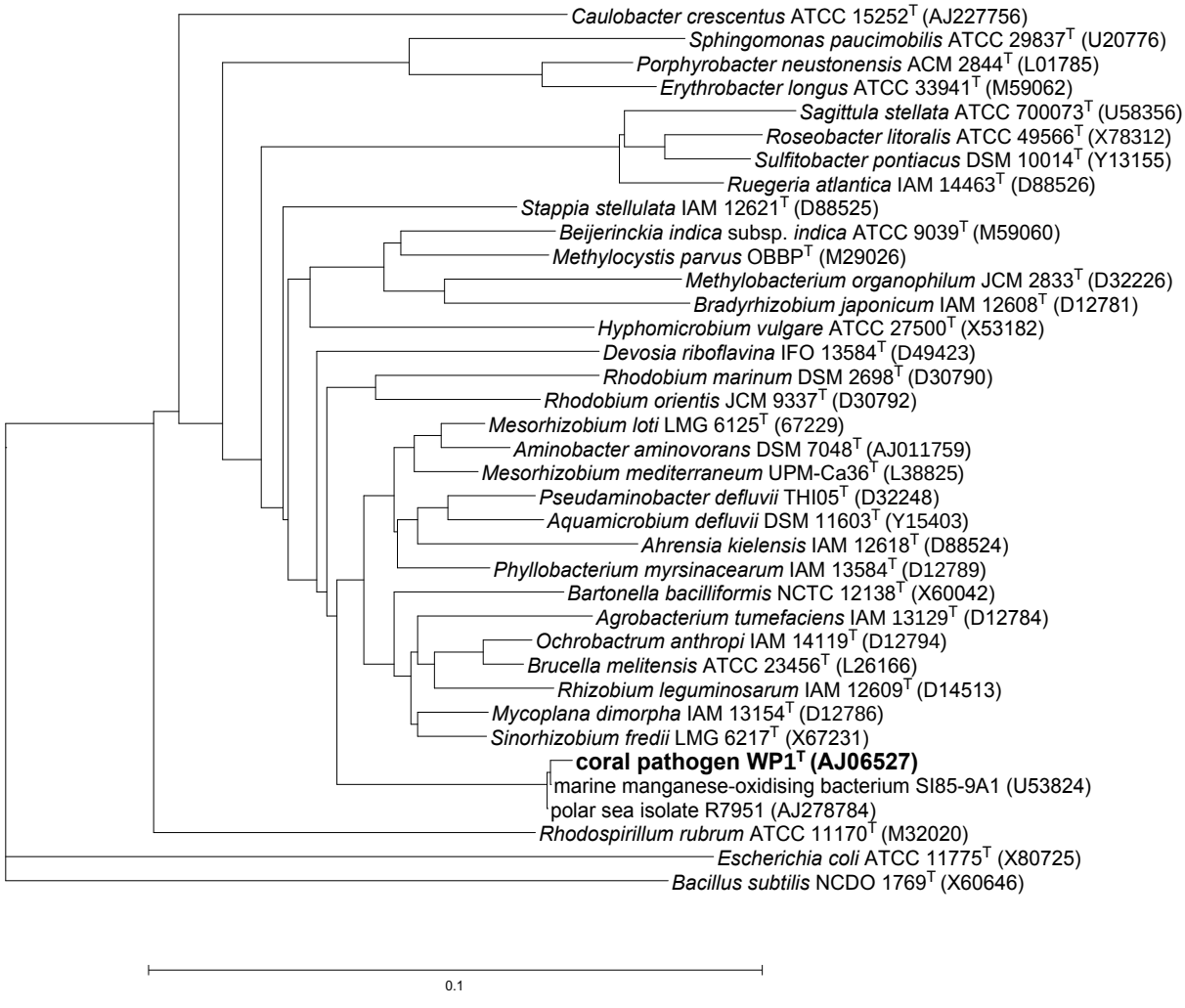

Caulobacter crescentus ATCC 15252^T (AJ227756)
Sphingomonas paucimobilis ATCC 29837^T (U20776)
Porphyrobacter neustonensis ACM 2844^T (L01785)
Erythrobacter longus ATCC 33941^T (M59062)
Sagittula stellata ATCC 700073^T (U58356)
Roseobacter litoralis ATCC 49566^T (X78312)
Sulfitobacter pontiacus DSM 10014^T (Y13155)
Ruegeria atlantica IAM 14463^T (D88526)
Stappia stellulata IAM 12621^T (D88525)
Beijerinckia indica subsp. *indica* ATCC 9039^T (M59060)
Methylocystis parvus OBBP^T (M29026)
Methylobacterium organophilum JCM 2833^T (D32226)
Bradyrhizobium japonicum IAM 12608^T (D12781)
Hyphomicrobium vulgare ATCC 27500^T (X53182)
Devosia riboflavina IFO 13584^T (D49423)
Rhodobium marinum DSM 2698^T (D30790)
Rhodobium orientis JCM 9337^T (D30792)
Mesorhizobium loti LMG 6125^T (67229)
Aminobacter aminovorans DSM 7048^T (AJ011759)
Mesorhizobium mediterraneum UPM-Ca36^T (L38825)
Pseudaminobacter defluvii THI05^T (D32248)
Aquamicrobium defluvii DSM 11603^T (Y15403)
Ahrensia kielensis IAM 12618^T (D88524)
Phyllobacterium myrsinacearum IAM 13584^T (D12789)
Bartonella bacilliformis NCTC 12138^T (X60042)
Agrobacterium tumefaciens IAM 13129^T (D12784)
Ochrobactrum anthropi IAM 14119^T (D12794)
Brucella melitensis ATCC 23456^T (L26166)
Rhizobium leguminosarum IAM 12609^T (D14513)
Mycoplana dimorpha IAM 13154^T (D12786)
Sinorhizobium fredii LMG 6217^T (X67231)
coral pathogen WP1^T (AJ06527)
marine manganese-oxidising bacterium SI85-9A1 (U53824)
polar sea isolate R7951 (AJ278784)
Rhodospirillum rubrum ATCC 11170^T (M32020)
Escherichia coli ATCC 11775^T (X80725)
Bacillus subtilis NCDO 1769^T (X60646)
0.1

Figure 2

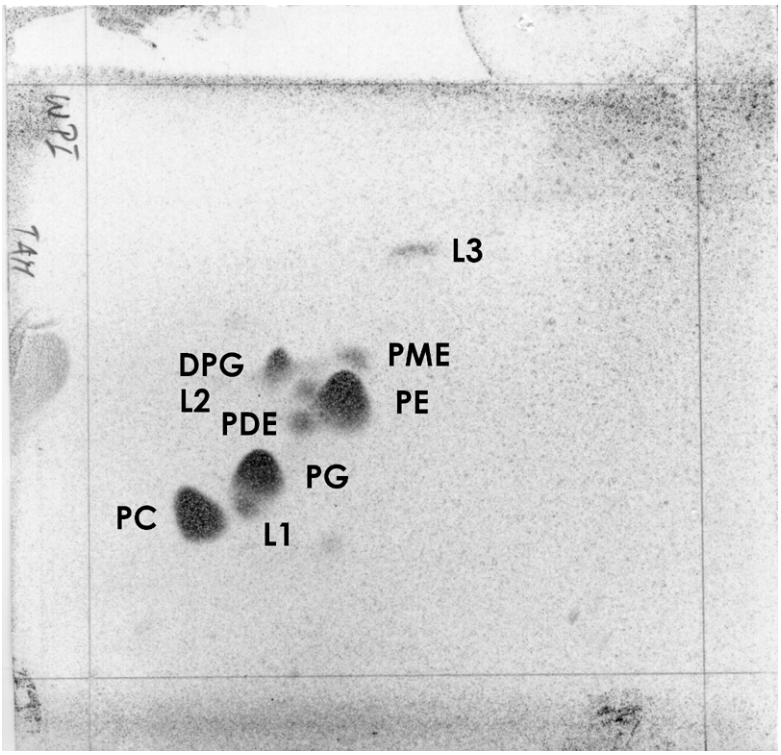
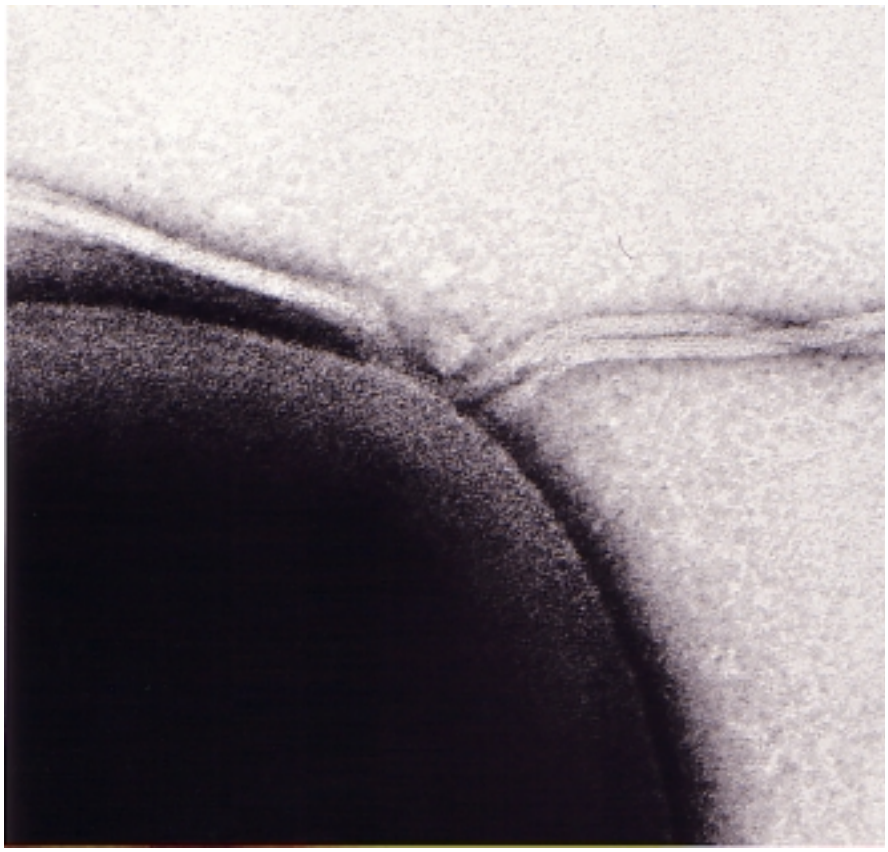


Figure 3



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563 ***Figure 4***

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