**Smaragdicoccus niigatensis** gen. nov., sp. nov., a novel member of the suborder *Corynebacterineae*

Kyoko Adachi,1 Atsuko Katsuta,1 Satoru Matsuda,1 Norihiko Misawa,1 Xue Peng,1 Yoshikazu Shizuri,1 Reiner M. Kroppenstedt,2 Akira Yokota3 and Hiroaki Kasai1

1Marine Biotechnology Institute, 3-75-1 Heita, Kamaishi, Iwate 026-0001, Japan
2DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Inhoffenstrasse 7b, D-38124 Braunschweig, Germany
3Institute of Molecular and Cellular Biosciences, University of Tokyo, 1-1-1 Yayoi, Bunkyo-ku, Tokyo 113-0032, Japan

A polyphasic taxonomic approach was applied to determine the taxonomic position of a hydrocarbon-degrading actinomycete, strain Hou_blue^T, which was isolated from soil samples collected from an oil spring in Niigata, Japan. The results of 16S rRNA and *gyrB* gene sequence comparisons indicated that strain Hou_blue^T represented a novel lineage in the suborder *Corynebacterineae*. Colonies were malachite green-like in colour on 1/10 trypticase soy agar and the cell morphology was coccoid in all growth phases. The cell-wall diamino acid and sugar indicated chemotype IV and variation A1\_c. The sugars of the peptidoglycan were glycolated. The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylinositol mannoside and some unspecified glycolipids. The organism contained two novel cyclic forms of menaquinone, smaragdiquinone A-8(H4, \(\omega_5\)-cycl) and smaragdiquinone B-8(H4, dicycl). The major fatty acids were cis-9-18:1 (34.46 %) and 16:0 (25.1 %). Small amounts of 10-methyl-branched fatty acids were also present (10-methyl-17:0, 0.17 %), but not tuberculostearic acid (10-methyl-18:0), which has been shown to be present in all nocardiae. Gas-chromatographic analysis of the mycolic acid revealed a carbon-chain length of C_{43}–C_{49}. The DNA G + C content was 63.7 mol%. On the basis of phenotypic and phylogenetic distinctness, the organism is proposed to represent a novel genus and species, *Smaragdicoccus niigatensis* gen. nov., sp. nov., with the type strain Hou_blue^T (=MBIC 06267^T =DSM 44881^T).

Strain Hou_blue^T was isolated from an enrichment culture containing petroleum-contaminated soil, obtained from the ground around a spurt of petroleum at Nishiyama-cho in Niigata, Japan, by using hexadecane as the sole carbon source. On W-medium (Peng et al., 2003) malachite green-like, round colonies of 1–3 mm diameter were obtained after incubation for 7 days in a hexadecane-saturated atmosphere at 25°C. Subcultivation was done on 1/10 trypticase soy agar (TSA; Difco) at 30°C for 7 days. On this medium, strain Hou_blue^T was able to grow at 4–37°C, but not at 45°C. Growth at 30°C was also observed on International Streptomyces Project (ISP) medium 2 (Daigo), medium 6 and TSA.

Gram-staining was performed as described by Gerhardt *et al.* (1994). Cell morphology was observed under a Nikon phase-contrast microscope at \(\times 1000\), with cells grown for 14 days at 30°C on 1/10 TSA. The size of the cells was determined by using a scanning electron microscope with specimens that were fixed with 2.5 % glutaraldehyde in 0.1 M cacodylate buffer containing several drops of 4 % osmium tetroxide for 1 h at room temperature. Suspensions were transferred to the surface of a polylysine-coated glass plate, and dehydrated in ethanol and t-butyl alcohol at room temperature. Preparations were sputter-coated with Pt/Pd on aluminium mounts and observed with a Hitachi S2500 scanning electron microscope (see Supplementary Fig. S1 in IJSEM Online). The 16S rRNA and *gyrB* genes were analysed as described by Katsuta *et al.* (2005). Sequences were aligned using CLUSTAL_X (Thompson *et al.*, 1997) based on aligned sequences supplied by RDP-II release 9 (Cole *et al.*,...
2005). A neighbour-joining phylogenetic tree based on genetic distances calculated using the Kimura two-parameter model was constructed with MEGA version 3.1 (Kumar et al., 2004). PHYML (version 2.4.4; Guindon & Gascuel, 2003) was used to construct a maximum-likelihood tree. The phylogenetic relationship based on the GTR substitution model was analysed, and a tree was built up from the neighbour-joining tree as the starter tree. The robustness of the topology was evaluated by using the maximum-likelihood method with bootstrap analysis based on 100 replications (Fig. 1). The 16S rRNA gene sequence of strain Hou_blueT was a continuous stretch of 1478 bp. Sequence similarity calculations after a BLAST search against GenBank indicated that the closest relatives of strain Hou_blueT were Nocardia africana (95.0%), Nocardia araoensis (94.5%), Nocardia arthritidis (94.8%), Nocardia beijingensis (94.7%), Nocardia elegans (94.9%), Nocardia takedensis (94.5%) and Nocardia paucivorans (94.5%). Twelve of 16 signature nucleotides of the 16S rRNA gene in Nocardiaceae (Stackebrandt et al., 1997) were identified in the 16S rRNA gene sequence of strain Hou_blueT. The gyrB gene-based phylogenetic tree is available as Supplementary Fig. S2 in IJSEM Online.

For analysis of cell-wall amino acids and sugars, cell walls were prepared from approximately 100 mg (dry weight) bacterial cells, as described by Schleifer & Kandler (1972). The amino acids in an acid hydrolysate of the cell walls were identified by using two-dimensional descending chromatography on cellulose TLC plates (Tokyo Kasei), following the

Fig. 1. Maximum-likelihood tree based on 16S rRNA gene sequences. The sequence of Corynebacterium glutamicum was used to root the tree. Numbers at branch points are bootstrap percentages based on 100 replications. Bar, 0.02 substitutions per nucleotide position.
method of Harper & Davis (1979), and by HPLC as their phenylthiocarbamoyl derivatives, with LC-10AD HPLC apparatus (Shimadzu) equipped with a Wakopak WS-PTC column (Wako Pure Chemical Industries, 1989). Whole-cell sugars were analysed using the method of Becker et al. (1964). The hydrolysate of whole cells of strain Hou_blue^1 contained the sugars arabinose, galactose, glucose and fucose, whereas the hydrolysate of the cell wall contained meso-diaminopimelic acid, alanine, glutamic acid and glycine in a molar ratio of approximately 0.5 : 3 : 1 : 2. This combination of cell-wall diamino acid and sugar indicated chemotype IV sensu Lechevalier & Lechevalier (1970) and variation A1 of Schleifer & Kandler (1972). The murein acyl type was determined by using a modification of the colorimetric method of Uchida & Aida (1977). In contrast to the original procedure, the whole-cell hydrolysate was neutralized by being passed through an ion-exchange column (Analytichem Bond Elut SCX; Varian). As expected for a member of the family Nocardiaceae, the sugars of the peptidoglycan were glycolated. Polar lipids were extracted, examined by two-dimensional TLC and identified using published procedures (Minnikin et al., 1977). The polar lipids were composed of diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, phosphatidylglycerol phosphatidylglycerol and some unspecified glycolipids. This pattern matched quite well with those reported by Minnikin et al. (1977) for members of the family Nocardiaceae. To determine the DNA base composition, DNA was extracted and purified by using a Genomic-tip and extraction, using the method of Miller (1982) with minor modifications (Kuykendall et al., 1988), from 40 mg cells that had been scraped from Petri dishes. The fatty acid methyl ester mixture was separated by using the Sherlock Microbial Identification System (MIS; Microbial ID) consisting of a 5980 gas chromatograph fitted with a 5 % phenylmethyl silicone capillary column (0.2 mm × 25 m), a flame-ionization detector, a 7673A automatic sampler and a Kayak XA computer (Hewlett Packard). The peaks were integrated automatically and the fatty acids present and their content (%) were calculated using the MIS software. The gas chromatographic parameters were as follows: carrier gas, ultra-high-purity hydrogen; column head pressure, 60 kPa; injection volume, 2 μl; column split ratio, 100 : 1; septum purge, 5 ml min^-1; column temperature, 170–270 °C at 5 °C min^-1; injection port temperature, 250 °C; and detector temperature, 300 °C. The fatty acid pattern of strain Hou_blue^7 did not match any of the patterns found previously for members of the genus Nocardia. Interestingly, only very small amounts of 10-methyl-branched fatty acids were present in strain Hou_blue^7 (10-methyl-17 : 0, 0.17 %) and, unlike all other nocardiae, tuberculostearic acid (10-methyl-18 : 0) was not present. In addition, a significant amount of iso-16 : 0 (8.12 %) was synthesized by strain Hou_blue^7; in contrast, this fatty acid, if present at all, has only been found previously in trace amounts among nocardiae and all other members of the suborder Corynebacterineae. Half of the fatty acid methyl ester extract (0.3 ml) was mixed with 0.1 ml N-methyl-N-(trimethylsilyl)-heptafluorobutyramide (MSHFBA) and trimethylchlorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel], yielding trimethylsilylated derivatives (TMS-MAME) of the -OH group of mycolic acid methyl ester (MAME) and trimethylcholorosilane (TMCS) [10 : 1 (v/v); Macherey and Nagel].
thickness using H₂ as the carrier gas and a split ratio of 50:1. The oven temperature was increased from 210 to 400 °C, at 10 °C min⁻¹, the final temperature being held for 10 min. The MIS apparatus was calibrated with a series of saturated TMS-MAMEs derived from Corynebacterium bovis DSM 20582T, Rhodococcus erythropolis DSM 43066T, Rhodococcus rhodnii DSM 43336T and Gordonia sp HU 44019. The chain length of the mycolic acids of strain Hou_blueT was shorter (C₄₃–C₄₉) than that found in other nocardiae (C₅₀–C₆₂), the pattern being very complex, i.e. highly unsaturated mycolic acids. It was therefore difficult to identify the numbers of the double bond. A detailed comparison of the chemotaxonomic data of strain Hou_blueT with those of other mycolic acid-containing genera is given in Table 1. Results of phenotypic characterization by using Biolog and API ZYM assays are given in the species description.

The results of 16S rRNA and gyrB gene sequence analyses, the cell wall and peptidoglycan characterization and the polar lipid analysis indicated that strain Hou_blueT belonged to the family Nocardiaceae. The profiles of quinones, fatty acids and mycolic acids indicated that strain Hou_blueT should be classified as representing a novel genus and species of the family Nocardiaceae, for which the name Smaragdicoccus niigatensis gen. nov., sp. nov. is proposed.

**Table 1.** Chemotaxonomic markers of Smaragdicoccus gen. nov. and other genera of the suborder Corynebacterineae

| Taxa: 1, strain Hou_blueT; 2, Nocardia; 3, Skermania; 4, Rhodococcus; 5, Gordonia; 6, Williamsia; 7, Mycobacterium; 8, Tsukamurella; 9, Segniliparus; 10, Turicella; 11, Dietzia; 12, Corynebacterium; 13, Corynebacterium ammnoniagenes. Chemotaxonomic characteristics summarized by Kämpfer et al. (1999) were updated. The whole-cell lysate of all organisms contained meso-diaminopimelic acid. +, Present; –, absent; ND, not determined. |
|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|-----------------|
| Characteristic  | 1               | 2               | 3               | 4               | 5               | 6               | 7               | 8               | 9*              | 10              | 11              | 12              | 13              |
| Acyl type†      | G               | G               | G               | G               | G               | G               | G               | ND              | ND              | A               | A               | ND              | ND              |
| Major          | SQA-8(H₄, o-cycl), SQB-8(H₄, o-cycl) | MK-8(H₄, o-cycl), MK-9(H₄, o-cycl) | MK-8(H₂, o-cycl), MK-9(H₂, o-cycl) | MK-8(H₂, o-cycl), MK-9(H₂, o-cycl) | MK-8(H₂, o-cycl), MK-9(H₂, o-cycl) | MK-10(H₂, o-cycl), MK-11(H₂, o-cycl) | MK-8(H₂, o-cycl), MK-9(H₂, o-cycl) | MK-8(H₂, o-cycl), MK-9(H₂, o-cycl) |
| Menaquinone    | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             | PE‡             |
| (no. of carbons)# | 63.7            | 64–72           | 68              | 63–73           | 63–69           | 64–65           | 70–72           | 67–68           | 68–72           | 65–72           | 73              | 51–67           | ND              |

*Data from Butler et al. (2005).
†A, Acetylated muramic acid; G, glycolated muramic acid.
‡Phosphatidylethanolamine.
§Present in C. bovis and Corynebacterium urealyticum (Kämpfer et al., 1999).
‖S, Saturated fatty acid; T, tuberculostearic acid; U, unsaturated fatty acid.
¶Tuberculostearic acid present in Corynebacterium ammoniagenes, C. bovis, Corynebacterium minutissimum, C. urealyticum and Corynebacterium variabile (Kämpfer et al., 1999).
#Number of carbon atoms in the mycolic acid molecule, range of homologous series of mycolic acids.
Cells are coccoid without branching (0.86 × 0.86 μm). Utilizes the following carbon sources after incubation for 14 days at 30°C: D-fructose, D-glucose, sodium n-butyrate and hexadecane. After prolonged incubation (1 month) growth occurs with sucrose. Better growth is observed on W-medium containing 0.1 than 1% of these carbon sources. The following carbon sources are not used for growth: L-arabinose, myo-inositol, D-mannitol, L-rhamnose, raffinose and D-xylene. Positive for activities of esterase (C4), esterase lipase (C8), lipase (C4), leucine arylamidase and naphthol-AS-BI-phosphohydrolase (API ZYM).

The DNA G+C content of the type strain is 63.7 mol%. The type strain is Hou_blueT (= MBIC 06267T = DSM 44881T), which was isolated from soil samples from an oil spring in Niigata, Japan.

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References


