***Roseiflexus castenholzii* gen. nov., sp. nov., a thermophilic, filamentous, photosynthetic bacterium that lacks chlorosomes**

Satoshi Hanada,¹ Shinichi Takaichi,² Katsumi Matsuura³ and Kazunori Nakamura¹

¹ Research Institute of Biological Resources, National Institute of Advanced Industrial Science and Technology (AIST), Tsukuba Central 6, 1-1-1 Higashi, Tsukuba 305-8566, Japan

² Biological Laboratory, Nippon Medical School, Kawasaki 211-0063, Japan

³ Department of Biology, Tokyo Metropolitan University, Hachioji 192-0307, Japan

Author for correspondence: Satoshi Hanada. Tel: +81 298 61 6590. Fax: +81 298 61 6587. e-mail: s-hanada@aist.go.jp

A novel thermophilic, photosynthetic bacterium, designated strain HLO8^T, was isolated from a bacterial mat in a Japanese hot spring. Morphologically, the isolate was an unbranched multicellular filament with a cell diameter of 0.8–1.0 µm. The bacterium was red to reddish-brown in colour and formed a distinct red bacterial mat in the natural environment. It was able to grow photoheterotrophically under anaerobic light conditions and also chemoheterotrophically under aerobic dark conditions. Optimal growth occurred at 50 °C and pH 7.5–8.0. The cells contained bacteriochlorophyll (Bchl) *a* and γ -carotene derivatives as photosynthetic pigments, but lacked Bchl *c* and chlorosomes. Cellular fatty acids in the isolate were mainly C16:0, C14:0 and C15:0. The major quinone was menaquinone-11. The DNA G+C content was 62.0 mol% (by HPLC). Phylogenetic analysis based on 16S rDNA sequencing suggested that the isolate belonged to the anoxygenic filamentous phototrophic bacteria represented by *Chloroflexus aurantiacus*, but was clearly distant from all members in this group (the sequence similarities between the isolate and its relatives were less than 83.8%). Based on genotypic and phenotypic data, the name *Roseiflexus castenholzii* gen. nov., sp. nov. is proposed for this isolate; the type strain is HLO8^T (= DSM 13941^T = JCM 11240^T).

Keywords: *Roseiflexus castenholzii*, gen. nov., sp. nov., anoxygenic photosynthesis, thermophiles, bacterial mats, gliding motility

INTRODUCTION

The multicellular filamentous phototrophic bacterium *Chloroflexus aurantiacus* was first described by Pierson & Castenholz (1974). Since this bacterium differed from the classical photosynthetic bacteria in many respects at the time, a new family of phototrophic bacteria was created for it (the family *Chloroflexaceae*; Trüper, 1976). Phylogenetic analysis based on 16S rRNA gene sequence data revealed that the bacterium is not closely related to any other photosynthetic bacterium and forms the deepest branch among phototrophic bacteria (Oyaizu *et al.*, 1987). The genus *Chloroflexus*, therefore, has received much attention as

a key organism in considering the origin of photosynthesis and the evolutionary process of photosynthetic systems. Another species, *Chloroflexus aggregans*, has been described in this genus (Hanada *et al.*, 1995).

The group represented by *Chloroflexus aurantiacus* was named the anoxygenic filamentous phototrophs (Pierson & Castenholz, 1995). This photosynthetic group includes some other genera: *Oscillochloris* (Keppen *et al.*, 1994, 2000), *Chloronema* (Dubinina & Gorlenko, 1975) and *Heliothrix* (Pierson *et al.*, 1985). In addition, several *Chloroflexus*-like bacteria have been observed in marine environments (Pierson *et al.*, 1994). Filamentous morphology and gliding motility are typical of these phototrophic bacteria, most of which possess light-harvesting apparatus called chlorosomes, which are intracellular vesicles containing bacteriochlorophyll (Bchl) *c* or *d*. This notable photosynthetic apparatus is regarded as a typical

Abbreviations: Bchl, bacteriochlorophyll; MK, menaquinone; RCR, Rabbit Creek Red.

The GenBank/EMBL/DBJ accession number for the 16S rRNA gene sequence of strain HLO8^T is AB041226.

feature in the anoxygenic filamentous phototrophs. An exception is *Heliothrix oregonensis* which has no chlorosomes. *H. oregonensis* is an interesting filamentous phototroph in that it contains Bchl *a* as the sole chlorophyll species, but has not been cultured axenically. This bacterium was described as a co-culture with the non-phototrophic bacterium, *Iso-sphaera pallida* (Pierson *et al.*, 1985).

For several years, distinct red-coloured bacterial mats consisting of filamentous phototrophic bacteria have been found in several neutral to alkaline hot springs in Japan. These filaments contained Bchl *a* and some carotenoids as photopigments, but no Bchl *c* has been detected. Morphological and spectroscopic features resembled those of *H. oregonensis*. Similar bacterial mats have also been observed in hot springs in Yellowstone National Park (Boomer *et al.*, 2000; Pierson & Castenholz, 1995). However, filamentous bacteria like *H. oregonensis* have not been successfully isolated, despite of the endeavours of many researchers.

Recently, a distinct red-coloured bacterial mat developed in Nakabusa hot spring in Japan [M. Sugiura, M. Takano and K. Toda (all Nagoya University), S. Kawakami (Gifu University) and S. Hanada]. It was predominantly composed of a filamentous phototrophic bacterium containing Bchl *a*. The filamentous bacterium was isolated from the bacterial mat. The isolate, designated strain HLO8^T, was a chlorosomeless, gliding, filamentous, phototrophic bacterium resembling *H. oregonensis*. In this paper, the distinct properties of strain HLO8^T are described and a new taxon is proposed for the isolate.

METHODS

Habitat and isolation. Strain HLO8^T was isolated from a bacterial mat in Nakabusa hot spring (Nagano Pref., Japan). The bacterial mat was well developed on a concrete wall in the outflow from the spring. The temperature and pH ranges of hot water in the site were 40–60 °C and pH 7.8–8.2, respectively. The bacterial mat mainly consisted of three zonal layers predominantly formed by phototrophic bacteria. Each of the layers had a distinctive coloration (dark green, brown and red in colour). The surface of the mat was mainly occupied by dark green and brown coloured layers. The dark green layer was composed mainly of thermophilic cyanobacteria and the brown layer was due to *Chloroflexus* sp. In addition to these, there was a distinct red layer underneath the dark green and brown layers. The red layer was on the concrete wall with a thickness of less than 10 mm. The red layer was mainly composed of a filamentous phototrophic bacterium with a diameter of 1 µm. The red layer contained Bchl *a* and γ -carotene derivatives as photo-synthetic pigments.

PE medium (Hanada *et al.*, 1995) was used for isolation of strain HLO8^T from the red layer. The red layer collected from the hot spring was gently homogenized and inoculated onto a 1.5% agar-containing PE plate (pH 8.0). The plate was incubated at 50 °C under anaerobic conditions in incandescent light (30 W m⁻²). Red to reddish-brown coloured colonies of strain HLO8^T were formed on the plate

after a few weeks. Enrichment of pure cultures was established using the same medium (pH 7.5) in screw-cap bottles at 50 °C under incandescent light (30 W m⁻²) or in flasks shaken vigorously (200 r.p.m.) in the dark at the same temperature. O2YE medium (pH 7.5) was also used to culture the strain. This medium contained (l⁻¹): 0.2% yeast extract (Difco), 0.38 g KH₂PO₄, 0.39 g K₂HPO₄, 0.5 g (NH₄)₂SO₄, 1 ml vitamin mixture and 5 ml basal salt solution. The vitamin mixture and basal salt solution were the same as those used in PE medium (Hanada *et al.*, 1995).

Morphology. The size, shape and ultrastructure of the cells were examined by phase-contrast microscopy and transmission electron microscopy. For transmission electron microscopy, a centrifuged cell pellet was fixed with 5% (v/v) glutaraldehyde and 1% (v/v) osmium tetroxide. Ultrathin sections of the sample embedded in epoxy resin (Kushida, 1980) were prepared with a Reichert ultra-microtome. Samples were stained with uranyl acetate and lead citrate, and examined using a Hitachi H-7000 transmission electron microscope. Gliding motility was determined by microscopically observing 1-week-old colonies on an agar plate (PE medium, pH 7.5), ensuring that the tips of filaments glided out.

Nutritional and physiological tests. For nutritional tests, 5 ml medium containing one of various compounds as the sole carbon and energy source to a final concentration of 0.25% (w/v) was used in a multi-well plate (12 wells). The medium (pH 7.5) basically contained (l⁻¹): 0.38 g KH₂PO₄, 0.39 g K₂HPO₄ as a buffer, 0.5 g (NH₄)₂SO₄ as a nitrogen source, 1 ml vitamin mixture and 5 ml basal salt solution (see above). The following 18 compounds were used for the test: aspartate, glutamate, glycine, acetate, butyrate, citrate, malate, pyruvate, succinate, glucose, mannose, ethanol, glycerol, lactate, methanol, mannitol, Casamino acids and yeast extract. All organic carbon sources were neutralized and sterilized before addition. Each plate was incubated at 50 °C under the following three conditions: anaerobically in the light, anaerobically in the dark and aerobically in the dark. Anaerobic conditions were achieved with an Aneropack system (Mitsubishi Gas Chemical). Photoautotrophic growth was tested with NaHCO₃ (50 mM) as a carbon source and Na₂S · 9H₂O (0.2 or 0.4 mM) or Na₂S₂O₃ (3 mM) as electron donor. The results (OD at 660 nm) were recorded after 1 week incubation. For estimation of the optimal temperature range for growth, the isolate was aerobically cultured in PE medium (pH 7.5) at the following temperatures: 35, 40, 45, 50, 55, 60 and 65 °C. Growth at various pH values was also determined at 50 °C in PE medium that was buffered by 10 mM potassium phosphate buffer (pH 6, 7 and 8) or 10 mM sodium glycine buffer (pH 9 and 10).

Spectroscopy and pigment analyses. Cells were collected from 1-week-old cultures in O2YE medium and were washed and disrupted by sonication (140 W, 3 min) in potassium phosphate buffer (100 mM KCl, 3 mM KH₂PO₄ and 2 mM K₂HPO₄, pH 7.0). Absorption spectra of ultrasonically disrupted cells and chloroform/methanol (2:1, v/v) extract were recorded with a Beckman DU 640 spectrophotometer. Pigments were extracted with acetone/methanol (7:2, v/v) and analysed by HPLC equipped with a μ Bondapak C18 column (8 × 100 mm, RCM type; Waters) with methanol as a mobile phase (Takaichi & Shimada, 1992).

Quinone and cellular fatty acid analyses. Quinones were extracted from freeze-dried cells with chloroform/methanol (2:1, v/v) and *n*-hexane. The extract was purified with a Sep-Pak Plus cartridge (Waters) and analysed by HPLC

equipped with a Zorbax ODS column (4.6 × 250 mm; Shimadzu) for identification (Tamaoka *et al.*, 1983).

Cellular fatty acids were converted to methyl esters by treatment with anhydrous methanolic HCl. The methyl esters were analysed with a Hitachi M7200A GC/3DQMS system equipped with a DB-5ms capillary column (30 m × 0.25 mm; J&W Scientific) coated with (5%-phenyl)-methylpolysiloxane (250 nm thickness). Helium was used as carrier gas at a flow rate of 1.5 ml min⁻¹. The column temperature profile was initially 100 °C for 1 min, then increased to 280 °C at a rate of 18 °C min⁻¹ and was finally held at 280 °C for 12 min.

DNA base composition and 16S rDNA analyses. The total DNA of strain HLO8^T was extracted according to a previously described method (Kamagata & Mikami, 1991) and digested with P1 nuclease using a GC kit (Yamasa Shoyu). The G + C content was measured with a Shimadzu model LC-6A HPLC system equipped with a CLC-ODS column (6 × 150 mm; Shimadzu).

The 16S rRNA gene (16S rDNA) fragment was amplified by PCR (Hiraishi, 1992; Hiraishi *et al.*, 1994) and sequenced directly with a dRhodamine dye terminator cycle sequencing kit (Applied Biosystems) and a model ABI 310 DNA sequencer. The obtained sequence and reference sequences were aligned and compared using the program CLUSTAL W, version 1.6 (Thompson *et al.*, 1994). The phylogenetic tree was constructed from the evolutionary distance matrix calculated by the neighbour-joining method (Saitou & Nei, 1987) with Kimura's two-parameter (Kimura, 1980). Neighbour-joining analysis was performed with the MEGA program (Kumar *et al.*, 1994). All gaps in the alignment were excluded to draw the tree.

RESULTS AND DISCUSSION

Morphology and photosynthetic pigments

The strain, designated strain HLO8^T, was isolated from the red-coloured bacterial mat in Nakabusa hot spring (40–60 °C). Colonies of the isolate formed on

Fig. 2. Absorption spectra of ultrasonically disrupted strain HLO8^T cells grown under aerobic and dark (solid line) and anaerobic and light (broken line) conditions. The dotted line shows the spectrum of chloroform/methanol (2:1, v/v) extract of the phototrophically grown cells. Spectra were recorded after 1 week incubation in O₂YE medium.

1.5% agar plates (PE medium) were red to reddish-brown in colour and showed gliding motility. The isolate was an unbranched multicellular filamentous bacterium of indefinite length with a diameter of 0.8–1.0 μm (Fig. 1, left). An electron micrograph of a thin section of the isolate revealed no evidence of intracellular vesicles or extensive internal membranes (Fig. 1, right).

Strain HLO8^T produced Bchls and the photosynthetic apparatus both under aerobic and anaerobic conditions. *In vivo* absorption spectra of ultrasonically disrupted cells (Fig. 2) grown aerobically (solid line) and anaerobically (broken line) showed two peaks (at 801 and 878 nm) in the IR region that may be due to Bchl *a*. In addition, absorption maxima at 479 and

Fig. 1. Phase-contrast photomicrograph (left; bar, 10 μm) and electron micrograph of ultrathin section (right; bar, 0.5 μm) of strain HLO8^T cells. Both cells were anaerobically grown in PE medium under incandescent light (30 W m⁻²) for 1 week.

Table 1. Phenotypic characteristics of strain HLO8^T and related anoxygenic filamentous phototrophic bacteria

Symbols: +, positive; –, negative; ND, not determined. Data were taken from the following references: *Chloroflexus* spp., Pierson & Castenholz (1974) and Hanada *et al.* (1995); *O. trichoides*, Keppen *et al.* (2000); *H. oregonensis*, Pierson *et al.* (1984, 1985).

Character	Strain HLO8 ^T	<i>Chloroflexus</i> spp.	<i>O. trichoides</i>	<i>H. oregonensis</i>
Morphology	Multicellular filaments	Multicellular filaments	Multicellular filaments	Multicellular filaments
Cell diameter (µm)	0.8–1.0	0.5–1.5	1.0–1.5	1.5
Gliding motility	+	+	+	+
Metabolism	Photoheterotroph, O ₂ respiration	Photoheterotroph, photoautotroph, O ₂ respiration	Photoautotroph, photoheterotroph	Photoheterotroph
Optimal growth temp. (°C)	50	55	28–30	40–55
Bchl	<i>a</i>	<i>a</i> , <i>c</i>	<i>a</i> , <i>c</i>	<i>a</i>
Chlorosomes	–	+	+	–
Peaks (nm) of <i>in vivo</i> spectrum (near IR)	878, 801	868, 808, 740	852, 748	865, 795
Major cellular fatty acids	C16:0, C14:0, C15:0	C18:0, C16:0, C18:1	C18:1, C16:0, C16:1	ND
Major quinone	MK-11	MK-10	MK-10	ND
DNA G + C content (mol %)	62.0	56.7–57.1	59.2	ND

590 nm were observed, but both spectra lacked a peak around 740 nm (Bchl *c*) that is typical of *Chloroflexus* species. The spectrum of the chloroform/methanol extract (dotted line; Fig. 2) indicated a peak at 770 nm of Bchl *a*. No peak at 670 nm (due to Bchl *c*) was found in the spectrum. Analysis of photopigments by HPLC clearly revealed the presence of Bchl *a* and the absence of Bchl *c* (data not shown). These findings suggest that the isolate has no chlorosomes (the light-harvesting apparatus containing Bchl *c*).

In morphological and spectroscopic respects, the newly isolated phototrophic bacterium resembled other anoxygenic filamentous phototrophs, including *Chloroflexus* spp. and its relatives. However, the isolate lacked the light-harvesting apparatus (chlorosome), whereas almost all phototrophic members in the group, i.e. *Chloroflexus* spp. (Pierson & Castenholz, 1974; Hanada *et al.*, 1995), *Oscillochloris trichoides* (Keppen *et al.*, 2000) and *Chloronema giganteum* (Dubinina & Gorlenko, 1975; Gorlenko, 1988), possess it as a notable feature. *H. oregonensis* is the sole chlorosome-lacking phototroph in the group (Pierson *et al.*, 1985). The new isolate, therefore, resembles *H. oregonensis* in lacking the distinct light-harvesting system. There is, however, an obvious difference in the absorption spectra of the near-IR region between the present isolate and *H. oregonensis*. The *in vivo* absorption spectrum of the isolate had distinct peaks at 878 and 801 nm due to Bchl *a* probably bound to membranous proteins. Those of *H. oregonensis* were slightly blue-shifted (peaks were at 865 and 795 nm). Strain HLO8^T also contained novel γ -carotene derivatives (including their glycoside esters) as the caro-

tenoids (identification of these carotenoids is under way).

Physiological properties

Strain HLO8^T was able to grow photoheterotrophically under anaerobic conditions in the light and chemoheterotrophically under aerobic conditions in the dark. Yeast extract was a good substrate for growth under both aerobic and anaerobic conditions. Citrate, lactate, glucose and Casamino acids were utilized as a sole carbon source only under anaerobic light conditions; however, cell yields were clearly less than that obtained with yeast extract. The other simple organic compounds, i.e. acetate, butyrate, malate, pyruvate, succinate, aspartate, glutamate, glycine, mannose, ethanol, glycerol and mannitol, as sole energy sources did not support growth of the isolate under aerobic or anaerobic conditions. For determination of photoautotrophic growth with bicarbonate as sole carbon source, Na₂S · 9H₂O (0.2 or 0.4 mM) or Na₂S₂O₃ (3 mM) were used as electron donors; the isolate did not show photoautotrophic ability under these conditions. No fermentative growth with glucose, yeast extract, Casamino acids or several other simple organic carbon sources was observed. Optimal growth occurred at 50 °C and at pH 7.5–8.0 under photoheterotrophic or chemoheterotrophic conditions. Good growth occurred at 45–55 °C. Growth did not occur above 60 °C and no practical growth was observed below 40 °C. The isolate grew between pH 6.0 and 9.0 and did not grow below pH 5.5 or above pH 9.5.

Fig. 3. Phylogenetic tree showing the relationship between strain HLO8^T and related species based on their 16S rDNA sequence. Species given in bold represent members of the anoxygenic filamentous phototrophic bacteria. Bootstrap confidence values from 100 bootstrap replicates are indicated at the branching points. Bar, 4 nt substitutions in 100 nt in 16S rDNA sequences. The accession number of each reference species is shown in parentheses. OS Type C (indicated by an asterisk) is a clone sequence retrieved from bacterial mats in Yellowstone by Weller *et al.* (1992).

Other chemotaxonomic characteristics

The major quinones, composition of cellular fatty acids and genomic G + C content of the isolate were analysed. These results are summarized in Table 1, along with phenotypic characteristics of *Chloroflexus* species and its relatives. The predominant quinone of the isolate was menaquinone (MK)-11. Cellular fatty acids in the isolate were composed of C16:0 (69.5 % of total fatty acids), C14:0 (19.9 %), C15:0 (5.8 %), C17:0 (1.8 %), iso-C17:0 (1.6 %), iso-C18:0 (0.8 %) and iso-C16:0 (0.6 %). Unsaturated fatty acids, e.g. C18:1 or C16:1, were not detected. The G + C content of strain HLO8^T determined by HPLC was 62.0 mol %.

Results of chemotaxonomic analyses also suggested differentiation of strain HLO8^T from related species (Table 1). The isolate contained MK-11 as the major quinone, whereas MK-10 was the predominant quinone in *Chloroflexus* spp. and *O. trichoides*. Cellular fatty acids of the isolate were entirely saturated, whereas the other members contained unsaturated fatty acids such as C18:1. The isolate also contained small amounts of iso-branched fatty acids that have not been detected in its relatives. The genomic G + C content of the isolate was 62.0 mol %, which was slightly higher than the G + C contents of its relatives (less than 60 mol % in *Chloroflexus* spp. and *O. trichoides*).

Phylogenetic position

Phylogenetic analysis based on 16S rDNA sequence showed that strain HLO8^T was related to the anoxygenic filamentous phototrophs (Fig. 3). The most

closely related sequence to that of strain HLO8^T was that of 'OS type C', which was not a sequence of an isolated strain but a 16S rDNA clone retrieved from Octopus Spring in Yellowstone (Weller *et al.*, 1992). The sequence similarity was significantly high (95.7 %; the compared length was 1087 bp).

The position of the isolate was, however, certainly distant from other members of this group. The sequence similarities between the isolate and its related species were follows: *Chloroflexus aurantiacus*, 83.2 % (the length compared in pairs was 1343 bp); *Chloroflexus aggregans*, 82.1 % (1356 bp); *O. trichoides*, 83.8 % (1375 bp); and *H. oregonensis*, 78.6 % (868 bp). The sequence similarities to these authentic species were less than 83.2 % and values were low enough for creation of a new genus for in this group (Stackebrandt & Goebel, 1994).

Several phenotypic properties of strain HLO8^T also supported the creation of a new taxon. There were obvious differences in *in vivo* spectra of the near-IR region between the present isolate and its relatives. The *in vivo* absorption spectrum of the isolate had distinct peaks at 878 and 801 nm due to Bchl *a*, probably bound to membranous proteins. Peaks in the absorption spectrum of *H. oregonensis* were slightly blue-shifted (peaks at 865 and 795 nm). In *Chloroflexus* spp., peaks were at 868 and 808 nm, with a prominent peak at 740 nm that was due to Bchl *c* (chlorosome). *O. trichoides* had peaks at 852 and 748 nm caused by Bchl *a* and *c*, respectively. Chlorosomes in the genus *Chloronema* contained Bchl *d* instead of Bchl *c* and showed an absorption peak at 720 nm (data for the genus *Chloronema* not shown in Table 1; Dubinina & Gorlenko, 1975; Gorlenko, 1988).

The following phenotypic findings also suggested differentiating strain HLO8^T from related species: (1) strain HLO8^T contained no chlorosome, a typical feature of most *Chloroflexus* spp. and relatives; (2) the isolate contained MK-11 as a major quinone; (3) cellular fatty acids of the isolate were entirely saturated, whereas related species contained significant amounts of unsaturated fatty acids; (4) the genomic G+C content of the isolate was 62.0 mol%, which was slightly higher than values for related species (less than 60 mol%). Based on these genotypic and phenotypic characteristics, the name *Roseiflexus castenholzii* gen. nov., sp. nov. is proposed for the isolate.

Distribution of chlorosome-less anoxygenic filamentous phototrophic bacteria

Strain HLO8^T formed a distinct red-coloured dense bacterial mat in the natural environment (Nakabusa hot spring in Japan). Similar red-coloured mats have been observed in other Japanese hot springs such as Meotobuchi (Tochigi Pref.; 57 °C, pH 6.8) and Atagawa (Shidzuoka Pref.; 44 °C, pH 8.0) hot springs. Both mats mainly consisted of filamentous bacteria like HLO8^T and had the same spectroscopic properties, showing peaks at 878 and 801 nm in the IR region of their *in vivo* spectra and the absence of a peak around 740 nm (Bchl *c*). Morphological and spectroscopic properties suggest that HLO8^T or a bacterium closely related to it also inhabit these two hot springs. Isolation of these strains is being attempted, but has not yet been achieved.

Boomer *et al.* (2000) recently reported on red-coloured mats in the alkaline hot springs of Yellowstone National Park. Filaments found in the mat were designated Rabbit Creek Red (RCR). The RCR filament seems to be similar to HLO8^T, but the following differences were noticed between these two bacteria: the diameter of the RCR filament was 1.0–1.5 µm, wider than that of HLO8^T; the cells of the filament were packed with stacked internal membranes; and the peaks in the IR region were clearly red-shifted (peaks at 913 and 807 nm). These differences suggest that the RCR filament is not the same species as HLO8^T, but a novel species related to it. The chlorosome-less, anoxygenic, filamentous, phototrophic bacteria, like HLO8^T, appear to be widespread in many hot springs in the world.

Description of *Roseiflexus* gen. nov.

Roseiflexus (ro.se.i.flex'us. L. adj. *roseus* rose-coloured; L. masc. n. *flexus* a bending, turning; N.L. masc. n. *Roseiflexus* rose-coloured bending).

Cells are unbranched, multicellular filaments showing gliding motility. Gram-negative. Bchl *a* and γ -carotene derivatives are present. Chlorosomes and Bchl *c* are absent. Cultures are red to reddish-brown in colour. Thermophilic, facultative phototroph that grows at 45–55 °C and pH 7–9. Aerobic/dark growth occurs at

full atmospheric oxygen tension. Neither photoautotrophic nor fermentative growth is observed. Major quinone is MK-11. Cellular fatty acids are entirely saturated, e.g. C14:0, C15:0 and C16:0. DNA G+C content is 62.0 mol% (by HPLC). Phylogenetic position is in the green, non-sulfur bacteria represented by *Chloroflexus aurantiacus*. Type species is *Roseiflexus castenholzii*.

Description of *Roseiflexus castenholzii* sp. nov.

Roseiflexus castenholzii (cas.ten.holz'i.i. N.L. gen. n. *castenholzii* of Castenholz, named after Richard W. Castenholz, an American microbiologist who notably contributed to our knowledge of thermophilic filamentous phototrophs).

Basic phenotypic characteristics are the same as those described for the genus. Filamentous bacterium of indefinite length with a diameter of 0.8–1.0 µm. Neither chlorosomes nor intracytoplasmic membranes are present. Optimum photoheterotrophic and chemoheterotrophic growth occurs at 50 °C and pH 7.5–8.0. Yeast extract is a good carbon source for growth. Citrate and glucose are utilized as sole energy source only under phototrophic conditions. Photopigments are Bchl *a* and γ -carotene derivatives; cell suspensions show absorption maxima at 479, 590, 801 and 878 nm. Pigmentation occurs under both aerobic and anaerobic conditions. Cellular fatty acids consist of C16:0 (69.5% total fatty acids), C14:0 (19.9%) and C15:0 (5.8%). Unsaturated fatty acids are not detected. Small amounts of iso-branched fatty acids are detected. Major quinone is MK-11. DNA G+C content is 62.0 mol% (by HPLC). Type strain is HLO8^T (= DSM 13941^T = JCM 11240^T).

Note added in proof

The identification of the carotenoids of this organism will be reported elsewhere (Takaichi *et al.*, 2002).

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