The genus *Saccharothrix* was first proposed by Labeda *et al.* (1984). Members of the genus are characterized chemo-taxonomically by a type III cell wall containing meso-diaminopimelic acid without glycine, galactose, rhamnose or mannose as diagnostic sugars in the whole-cell hydrolysates, the spore arrangement, the diamino acid of the peptidoglycan, the whole-cell hydrolysates, the predominant menaquinone and the phospholipid profile. Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain NEAU-yn17T should also be classified in the genus *Saccharothrix*, with *Saccharothrix saharensis* DSM 45456T (99.52% sequence similarity) and *Saccharothrix xinjiangensis* JCM 12329T (99.04%) as the nearest phylogenetic relatives. A combination of DNA–DNA hybridization results and some phenotypic characteristics indicated that strain NEAU-yn17T can be distinguished from its closest relatives. Therefore, strain NEAU-yn17T represents a novel species of the genus *Saccharothrix*, for which the name *Saccharothrix carnea* sp. nov. is proposed. The type strain is NEAU-yn17T (=CGMCC 4.7097T=DSM 45878T).

A novel actinobacterium, designated strain NEAU-yn17T, was isolated from a soil sample collected at the wastewater discharge site of a pesticide factory in Harbin, northern China, and characterized using a polyphasic approach. Morphological and chemotaxonomic properties of strain NEAU-yn17T were consistent with the description of the genus *Saccharothrix*, such as the predominant menaquinone and the phospholipid profile. Phylogenetic analysis based on 16S rRNA gene sequences demonstrated that strain NEAU-yn17T should also be classified in the genus *Saccharothrix*, with *Saccharothrix saharensis* DSM 45456T (99.52% sequence similarity) and *Saccharothrix xinjiangensis* JCM 12329T (99.04%) as the nearest phylogenetic relatives. A combination of DNA–DNA hybridization results and some phenotypic characteristics indicated that strain NEAU-yn17T can be distinguished from its closest relatives. Therefore, strain NEAU-yn17T represents a novel species of the genus *Saccharothrix*, for which the name *Saccharothrix carnea* sp. nov. is proposed. The type strain is NEAU-yn17T (=CGMCC 4.7097T=DSM 45878T).

### Saccharothrix carnea sp. nov., an actinobacterium isolated from soil

Chongxi Liu,† Xuejiao Guan,† Shurui Wang,† Junwei Zhao,† Haiyan Wang,† Hairong He,† Wensheng Xiang†,‡ and Xiangjing Wang†

†Key Laboratory of Agriculture Biological Functional Gene of Heilongjiang Provincial Education Committee, Northeast Agricultural University, No. 59 Mucai Street, Xiangfang District, Harbin 150030, PR China

‡State Key Laboratory for Biology of Plant Diseases and Insect Pests, Institute of Plant Protection, Chinese Academy of Agricultural Sciences, Beijing, PR China

The strain was isolated using the standard dilution plate method and grown on humic acid-vitamin agar (HV) (Hayakawa & Nonomura, 1987) supplemented with cycloheximide (50 mg l⁻¹) and nalidixic acid (20 mg l⁻¹). After 21 days of aerobic incubation at 28 °C, colonies were transferred and purified on International Streptomycetes Project (ISP) medium 3 (Shirling & Gottlieb, 1966) and Gause’s synthetic agar no. 1 (Atlas, 1993), and maintained as glycerol suspensions (20 %, v/v) at −80 °C. The type strains of *Saccharothrix saharensis* and *Saccharothrix xinjiangensis* were purchased from the German Collection of Microorganisms and Cell Cultures (DSMZ) and the Japan Collection of Microorganisms (JCM), respectively, and cultured under the same conditions for comparative analysis.

Morphological characteristics were observed by light microscopy (Nikon ECLIPSE E200) and scanning electron microscopy (Hitachi S-3400N) using cultures grown on ISP3 medium at 28 °C for 21 days. Cultural characteristics were determined on ISP media 2–7 (Shirling & Gottlieb, 1966) after 14 days at 28 °C. Colour determination was done with colour chips from the ISCC-NBS colour charts standard samples no. 2106 (Kelly, 1964). Growth at different temperatures (0, 4, 10, 20, 28, 30, 32, 35, 37, 40 and 45 °C) was determined on ISP3 medium after...
incubation for 14 days. Growth tests for pH range (pH 3–12) and NaCl tolerance (0–7 %, w/v, NaCl) were performed in modified YEME medium (Wang et al., 2013) at 28 °C for 5 days on a rotary shaker. Hydrolysis of Tween 80 and production of catalase and urease were tested as described by Smibert & Krieg (1994). The utilization of sole carbon and nitrogen sources, decomposition of cellulose, hydrolysis of starch and aesculin, reduction of nitrate, peptonization of milk, liquefaction of gelatin and production of H₂S were examined as described by Gordon et al. (1974) and Yokota et al. (1993).

Biomass for chemical studies was prepared by growing strain NEAU-yn17ᵀ in modified YEME medium in shake flasks at 28 °C for 5 days. Cells were harvested by centrifugation, washed with distilled water and freeze-dried. The isomer of dianimonopelidic acid in the cell-wall hydrolysates was derivatized and analysed by an HPLC method (Mckerrow et al., 2000) using an Agilent TC-C₁₈ Column (250 × 4.6 mm, i.d. 5 μm) with a mobile phase consisting of acetonitrile/0.05 mol l⁻¹ phosphate buffer pH 7.2 (15:85, v/v) at a flow rate of 0.5 ml min⁻¹. An Agilent G1321A fluorescence detector with 365 nm excitation and 455 nm longpass emission filters was used for peak detection. The whole-cell sugars were analysed according to the procedures developed by Lechevalier & Lechevalier (1980). The phospholipids were examined by two-dimensional TLC and identified using the method of Minnikin et al. (1984). Menaquinones were extracted from freeze-dried biomass and purified according to the method of Collins (1985). Extracts were analysed by an HPLC-UV method using an Agilent TC-C₁₈ Column (250 × 4.6 mm, i.d. 5 μm), typically at 270 nm. The mobile phase was acetonitrile/2-propanol (60:40, v/v), the flow rate was set to 1.0 ml min⁻¹ and the run time was 60 min. The injection volume was 20 μl, and the chromatographic column was controlled at 40 °C (Wu et al., 1989). The presence of mycolic acids was checked by the acid methanolysis method as described by Minnikin et al. (1980). To determine cellular fatty acid composition, strain NEAU-yn17ᵀ was cultivated in ISP2 medium in shake flasks at 28 °C for 5 days. Fatty acid methyl esters were extracted from the biomass as described by Gao et al. (2014) and were analysed by GC-MS using the method of Xiang et al. (2011).

Extraction of chromosomal DNA and PCR amplification of the 16S rRNA gene were carried out using a standard procedure (Kim et al., 2000). The PCR product was purified and cloned into the vector pMD19-T (Takara) and sequenced using an Applied Biosystems DNA sequencer (model 3730XL) and software provided by the manufacturer. The almost full-length 16S rRNA gene sequence of strain NEAU-yn17ᵀ (1511 bp) was obtained and aligned with multiple sequences obtained from the GenBank/EMBL/DBJ databases using CLUSTAL X 1.83 software. Phylogenetic trees were reconstructed using neighbour-joining (Saitou & Nei, 1987) and maximum-likelihood (Felsenstein, 1981) algorithms using MEGA software version 5.05 (Tamura et al., 2011). The stability of the topology of the phylogenetic trees was assessed using the bootstrap method with 1000 repetitions (Felsenstein, 1985). A distance matrix was generated using Kimura’s two-parameter model (Kimura, 1980). All positions containing gaps and missing data were eliminated from the dataset (complete deletion option). The 16S rRNA gene sequence similarities between strains were calculated on the basis of pairwise alignment using the EzTaxon-e server (Kim et al., 2012).

The G+C contents of the genomic DNA were determined using the thermal denaturation (T_m) method (Mandel & Marmur, 1968) with Escherichia coli JM109 DNA used as the control. DNA–DNA relatedness tests between strain NEAU-yn17ᵀ and S. saharensis DSM 45456ᵀ and S. xinjiangensis JCM 12329ᵀ were carried out as described by De Ley et al. (1970) under consideration of the modifications described by Huss et al. (1983), using a Cary 100 Bio UV/VIS-spectrophotometer equipped with a Peltier-thermostatted 6 × 6 multicell changer and a temperature controller with in-situ temperature probe (Varian). The concentration and purity of DNA samples were determined by measuring the OD at 260, 280 and 230 nm. The DNA samples used for hybridization were diluted to OD₂₆₀ around 1.0 using 0.1 × SSC (saline sodium citrate buffer) and then disrupted using a JY92-II ultrasonic cell disruptor (ultrasonic time 3 s, interval time 4 s, 90 times). The DNA renaturation rates were determined in triplicate in 2 × SSC at 70 °C.

Morphological observation of a 14 day culture of strain NEAU-yn17ᵀ grown on ISP3 medium revealed that it had the typical characteristics of the genus Saccharothrix. The substrate mycelium was well developed without fragmentation. The aerial mycelium was fragmented into rod-shaped spores (0.6–0.8 × 1.1–1.7 μm). The spores were non-motile and smooth (Fig. 1). Strain NEAU-yn17ᵀ exhibited good growth on ISP2, ISP3, ISP4, ISP6 and ISP7 media, but no growth on ISP5 medium. The colour of aerial mycelium was pale yellow on ISP6 and ISP7 media, light yellow on ISP3 medium, strong yellow ISP2 medium, and dark reddish orange on ISP4 medium. A yellow soluble pigment was produced on ISP3 medium (Fig. S1, available in the online Supplementary Material). Melanoid pigments were

Fig. 1. Scanning electron micrograph of cells of strain NEAU-yn17ᵀ grown on ISP3 medium for 21 days at 28 °C. Bar, 1 μm.
not produced on ISP6 or ISP7 media. Strain NEAU-yn17\textsuperscript{T} grew well between pH 6.0 and 11.0, with optimal growth at pH 7.0. The range of temperature for growth was determined to be 4–37 °C, with the optimum growth temperature at 28 °C. Strain NEAU-yn17\textsuperscript{T} grew in the presence of 0–3 % (w/v) NaCl. Detailed physiological characteristics are presented in the species description.

Strain NEAU-yn17\textsuperscript{T} contained meso-diaminopimelic acid in the cell wall. The whole-cell hydrolysate contained galactose, mannose, rhamnose and ribose. The phospholipid profile consisted of diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidylinositol and phosphatidylinositol mannoside (Fig. S2). The menaquinones detected were MK-9(H\textsubscript{4}) (84.2 %), MK-10(H\textsubscript{2}) (8.5 %), MK-9(H\textsubscript{2}) (3.9 %) and MK-9(H\textsubscript{0}) (3.3 %). The major cellular fatty acids were determined to be iso-C\textsubscript{15:0} (16.1 %), iso-C\textsubscript{16:0} (15.8 %), iso-C\textsubscript{17:0} (10.4 %), C\textsubscript{16:0} (9.4 %), anteiso-C\textsubscript{17:0} (9.0 %), C\textsubscript{18:0} (9.0 %), C\textsubscript{17:1\textit{c07c}} (9.0 %) and C\textsubscript{16:1\textit{c07c}} (5.6 %) (Table S1). Mycolic acids were not detected. The DNA G+C content was 74.5 \pm 0.3 mol\%. All the morphological characteristics and chemotaxonomic data showed that strain NEAU-yn17\textsuperscript{T} should be assigned to the genus \textit{Saccharothrix}. Identification using the EzTaxon-e server revealed that strain NEAU-yn17\textsuperscript{T} differed in morphological and chemotaxonomic characteristics as summarized in Table 1 and Fig. S1. The out-group used was \textit{Saccharothrix carnea} DSM 40225\textsuperscript{T} (X76965), for which the name \textit{Saccharothrix carneae} sp. nov. is proposed.

**Description of \textit{Saccharothrix carneae} sp. nov.**

\textit{Saccharothrix carneae} (car’ne.a. L. fem. adj. carneae of flesh, flesh-coloured).
Table 1. Differential characteristics of strain NEAU-yn17T and the most closely related type strains of species of the genus Saccharothrix

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>1</th>
<th>2</th>
<th>3</th>
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<tbody>
<tr>
<td>Reduction of nitrate</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Liquefaction of gelatin</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Peptonization of milk</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Production of cellulase</td>
<td>+</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Utilization of:</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Lactose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Ribose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>D-Sorbitol</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Sucrose</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Creatine</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>L-Tyrosine</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Growth at:</td>
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<tr>
<td>4 °C</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>45 °C</td>
<td>–</td>
<td>+</td>
<td>+</td>
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</table>

An aerobic and Gram-staining-positive actinobacterium. Aerial mycelium is fragmented into rod-shaped spores with a smooth surface on ISP3 medium. Good growth is observed on most of the media tested, but no growth is observed on ISP5 medium. The aerial mass colour is in the yellow colour-series. A yellow soluble pigment is observed on ISP5 medium. The diagnostic amino acid of the cell wall is meso-DAP. Whole-cell hydrolysates contain galactose, mannose, rhamnose and ribose. The predominant menaquinone is MK-9(H4). The polar lipid profile contains diphosphatidylglycerol, phosphatidylmonomethylethanolamine, phosphatidylethanolamine, phosphatidyl-D-1-lysine and phosphatidylglycerol. Mycolic acids are absent. Major fatty acids are iso-C15:0 and C16:0 iso-C17:0 anteiso-C16:0, C18:0 and C17:0 7c and C16:0 7c.

The type strain is NEAU-yn17T (=CGMCC 4,7097T=DSM 45878T), isolated from a soil sample collected at the wastewater discharge site of a pesticide factory in Harbin, northern China. The DNA G+C content of the type strain is 74.5±0.3 mol%.

Acknowledgements

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References


