The Journal of Antibiotics (2016) 69, 159–163
© 2016 Japan Antibiotics Research Association All rights reserved 0021-8820/16
www.nature.com/ja

ORIGINAL ARTICLE

Streptomyces hyaluromycini sp. nov., isolated from a tunicate (*Molgula manhattensis*)

Enjuro Harunari[1], Moriyuki Hamada[2], Chiyo Shibata[2], Tomohiko Tamura[2], Hisayuki Komaki[2], Chiaki Imada[3] and Yasuhiro Igarashi[1]

A novel Gram-stain-positive actinomycete, designated MB-PO13^T, was isolated from a tunicate (*Molgula manhattensis*) collected in Tokyo Bay, Japan, and its taxonomic position was studied by a polyphasic approach. Phylogenetic analysis based on 16S rRNA gene sequence comparisons revealed that strain MB-PO13^T was closely related to *Streptomyces graminisoli* JR-12^T (99.72% 16S rRNA gene sequence similarity) and *Streptomyces shenzhenensis* 172115^T (99.23%). The strain contained LL-diaminopimelic acid in the whole-cell hydrolysate. The predominant menaquinones were MK-9(H_8) and MK-9(H_6) and the major fatty acids were anteiso-$C_{15:0}$, iso-$C_{16:0}$, iso-$C_{14:0}$ and $C_{16:0}$. These data supported the affiliation of the novel strain to the genus *Streptomyces*. Meanwhile, results of DNA–DNA hybridization and physiological and biochemical tests indicated that strain MB-PO13^T was distinguished from known *Streptomyces* type strains. Therefore, strain MB-PO13^T represents a novel species of the genus *Streptomyces* for which the name *Streptomyces hyaluromycini* sp. nov. is proposed; the type strain is MB-PO13^T (=NBRC 110483^T =DSM 100105^T).
The Journal of Antibiotics (2016) **69,** 159–163; doi:10.1038/ja.2015.110; published online 4 November 2015

INTRODUCTION

The genus *Streptomyces* was originally proposed by Waksman and Henrici[1] and later unified with the genus *Streptoverticillium*.[2] The genus *Kitasatospora* was also unified to the genus *Streptomyces*[3] but was separated anew.[4] The genus *Streptomyces* is known as the most diverse genus of actinomycetes with more than 600 recognized species.[5] The genus is characterized by aerobic, Gram-stain-positive, formation of highly branched mycelia and spores, high DNA G + C content, presence of LL-diaminopimelic acid and absence of characteristic sugars in their cell wall. Numerous novel bioactive compounds have been isolated from marine-derived actinomycetes, especially, members of the genus *Streptomyces*, which have been recognized as an important source for drug discovery.[6,7,4] Most of marine-derived actinomycetes do not require seawater or salt supplementation for growth, and thus, it is assumed that they are transported from land into sea. However, the existence of some marine-adapted actinomycetes was indicated by phylogenetic analyses of marine actinomycete isolates and their secondary metabolism is suggested to be different from those of terrestrial counterparts.[8] Hyaluronidase is a target enzyme of anti-inflammatory and anti-allergic drug deveropment.[9] For example anti-allergic and anti-inflammatory agents such as disodium cromoglycate and glycyrrhizin show hyaluronidase inhibitory activity.[10,11] During the course of screening for hyaluronidase inhibitors from microbial metabolites, a novel actinomycete strain was isolated from a marine invertebrate. The objective of the present study is to clarify the taxonomic position of the isolate using a polyphasic approach.

MATERIALS AND METHODS

Bacterial strains and isolation

A novel actinomycete strain, designated MB-PO13^T, was isolated from a tunicate (*Molgula manhattensis*) collected in Tokyo Bay, Japan, as a producer of hyaluronidase inhibitor.[12] After the collection, the tunicate was immediately washed three times with sterilized natural seawater, cut into small pieces, and then crushed using a homogenizer (model POLYTRON PT2100; Kinematica, Luzern, Switzerland). Five hundred microliter of suspension of the tunicate was spread onto inorganic salts–starch agar (ISP 4) supplemented with nalidixic acid (20 mg l^{-1}) and cycloheximide (50 mg l^{-1}). After incubation at 27 °C for 14 days, a single colony was repeatedly transferred onto the same agar medium to obtain the pure isolate of strain MB-PO13^T. The strain was maintained on a yeast extract–malt extract medium (ISP 2). *Streptomyces graminisoli* NBRC 108883^T, *Streptomyces shenzhenensis* DSM 42034^T, *Streptomyces gramineus* NBRC 107863^T and *Streptomyces rhizophilus* NBRC 108885^T were used as reference strains.

Morphological, physiological and biochemical tests

Colony appearance was examined after incubation at 28 °C for 14 days. Cell morphology was observed under a light microscope (model BX-51; Olympus, Tokyo, Japan) and a scanning electron microscope (model JSM-6060; JEOL, Tokyo, Japan). Growth under anaerobic conditions was examined by incubating the strain in an anaerobic chamber with an O_2-absorbing and CO_2-generating agent (Anaero-Pack; Mitubishi Gas Chemical, Tokyo, Japan). Gram staining was performed as described by Hucker and Conn.[13] Catalase

[1]Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, Kurokawa, Imizu, Toyama, Japan; [2]Biological Resource Center, National Institute of Technology and Evaluation (NBRC), Kisarazu, Chiba, Japan and [3]Graduate School of Marine Science and Technology, Tokyo University of Marine Science and Technology, Minato-ku, Tokyo, Japan
Correspondence: Dr Y Igarashi, Biotechnology Research Center and Department of Biotechnology, Toyama Prefectural University, 5180 Kurokawa, Imizu, Toyama 939-0398, Japan. E-mail: yas@pu-toyama.ac.jp
Received 22 June 2015; revised 26 August 2015; accepted 29 September 2015; published online 4 November 2015

activity was determined by production of oxygen bubbles after the addition of a drop of 3% (v/v) aqueous hydrogen peroxide solution. The cultural properties of strain MB-PO13^T were observed according to the ISP guideline[14] on ISP 2, oatmeal agar (ISP 3), ISP 4, glycerol asparagine agar (ISP 5), peptone-yeast extract-iron agar (ISP 6), tyrosine agar (ISP 7), glucose – asparagine agar, nutrient agar, sucrose–nitrate agar, skim milk agar and yeast extract-starch agar after 14 days of incubation at 28 °C. Colors of aerial and substrate mycelia were determined using The Japanese Industrial Standard Common Color Names (JIS Z 8102: 2001). The temperature range and optimum temperature for growth were determined by incubating the strain at 5, 10, 15, 20, 28, 37, 42 and 50 °C on ISP 2 agar plates for 14 days. The pH range and the optimum pH for initial growth were determined by incubating at 28 °C in ISP 2 broth, of which pH was adjusted to 3 – 12 by 1 M HCl or 1 M Na_2CO_3. Tolerance to NaCl was tested by incubation on ISP 2 agar plates containing 2, 3, 5, 7, 9 and 12% (w/v) NaCl at 28 °C. Enzyme activity test was performed using API ZYM kit (bioMérieux, Tokyo, Japan) according to the supplier's instruction. Carbohydrate utilization was determined on Pridham – Gottlieb carbon utilization agar (ISP 9) supplemented with sterilized carbon sources.[14]

16S rRNA gene sequence determination and phylogenetic analysis

Genomic DNA of strain MB-PO13^T was extracted by modified bead-beating phenol – chloroform method.[15] PCR amplification was carried out with a Dice mini TP 100 (Takara Bio, Tokyo, Japan). Primers 27f (5′-AGA GTTTGATCMTGGCTCAG-3′) and 1492r (5′-TACGGYTACCTTGTTAC GACTT-3′) were used for PCR amplification of 16S rRNA gene.[16] The PCR product was purified with a Wizard SV Gel and PCR Clean-Up System (Promega, Madison, WI, USA), and sequenced with a BigDye cycle sequencing ready reaction kit (Applied Biosystems, Foster City, CA, USA) on an ABI PRISM 310 Genetic analyzer (Applied Biosystems). Phylogenetic neighbors were identified and pairwise 16S rRNA gene sequence similarities were calculated using EzTaxon-e server.[17] The CLUSTAL-W program[18] was used to align an almost complete 16S rRNA gene sequence (1429 nucleotides) of strain MB-PO13^T with the corresponding sequences from related *Streptomyces* species. Phylogenetic trees were reconstructed by the neighbor-joining,[19] maximum-likelihood[20] and maximum-parsimony[21] algorithms by using the MEGA 6.0 program.[22] The resultant tree topologies were evaluated by bootstrap analysis[23] based on 1000 replicates.

G + C content of DNA and DNA–DNA hybridization

Genomic DNAs for G + C content analysis and DNA – DNA hybridization were obtained using the method of Saito and Miura.[24] The G + C content was determined according to the method described by Hamada *et al.*[25] The microplate hybridization method developed by Ezaki *et al.*[26] was used to analyze the DNA – DNA relatedness. DNA – DNA hybridizations were carried out five times; after the highest and lowest values for each sample were excluded, the means of the remaining three values were obtained.

Chemotaxonomic tests

Biomass for chemotaxonomic studies was prepared by incubating the strains in flasks on a shaker (100 r.p.m.) at 28 °C for 72 h. Diaminopimelic acid (DAP) in whole-cell hydrolysate was analyzed by modified method of Hasegawa *et al.*[27] Whole-cell sugar and menaquinone compositions were analyzed according to the methods described previously.[25] The polar lipids were analyzed by TLC as described by Hamada *et al.*,[28] using chloroform – methanol-water (65:25:4, v/v/v) in the first direction and chloroform – acetic acid – methanol-water (80:18:12:5, v/v/v/v) in the second. Preparation and analysis of cellular fatty acid methyl esters were performed using the protocol of MIDI Sherlock Microbial Identification System[29] and gas chromatography (model 6890N; Agilent Technologies, Santa Clara, CA, USA) with the Sherlock MIDI software (version 6.2) and the ACTINO database (version 6.2; MIDI Inc., Newark, DE, USA).

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain MB-PO13^T is AB184533.

RESULTS AND DISCUSSION

Strain MB-PO13^T formed circular and cream color colonies and exhibited well developed, branched substrate and aerial mycelia. Fragmentation of mycelia was not observed. The aerial mycelia were branched monopodially and 0.4–0.6 μm in diameter. Spore chain was spiral type and contained about 6–10 helixes and 30–50 spores per chain. The spores were cylindrical, 0.5–0.8 × 1.0–1.5 μm in size and had a smooth surface (Figure 1). Sporulation was not observed on nutrient-rich media examined in this study. A number of spore chains were observed on three-fold diluted ISP 4 and ten-fold diluted ISP 2 agar plates. Sporangia were not observed. The substrate mycelia appeared in cream color after a few days, which later turned reddish when grown on all ISP media except for ISP 6, glucose – asparagine and yeast extract-starch agars (Table 1). In contrast to the reference strains, strain MB-PO13^T produced soluble reddish pigments on an ISP 2 agar plate (Supplementary Figure S1). The major component of the pigments was identified as an aromatic polyketide, hyaluromycin[9] (Supplementary Figure S2). Yellowish-white aerial mycelia were formed on ISP 4 medium, which became light gray at sporulation. Sporulation was not observed on other media. The strain grew at 10–37 °C (optimum temperature 28 °C) and at pH 4.0–9.0 (optimum pH 7.0). The strain exhibited growth with 0–2% (w/v) NaCl (optimum 0% NaCl). The strain was Gram-stain-positive, catalase-positive and did not grow under anaerobic conditions. The results of other physiological and biochemical analyses have been summarized in the species description.

Phylogenetic analysis, based on 16S rRNA gene sequences, showed that strain MB-PO13^T represented a member of the genus *Streptomyces*. In the neighbor-joining tree, strain MB-PO13^T and *S. graminisoli* formed a monophyletic cluster with a bootstrap resampling value of 89% (Figure 2). This cluster was supported in the trees generated by the maximum-likelihood and maximum-parsimony algorithms (Supplementary Figures S3 and S4). The highest similarity value was observed with *S. graminisoli* JR-12^T (99.72%), followed by *S. shenzhenensis* 172115^T (99.23%), *S. jiujiangensis* JXJ 0074^T (98.87%), *S. rhizophilus* JR-41^T (98.58%) and *S. gramineus* JR-43^T (98.53%). The DNA – DNA relatedness values between strain

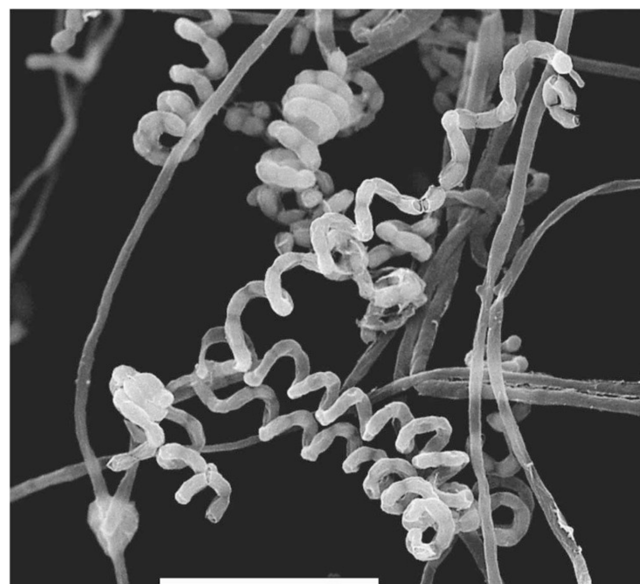

Figure 1 Scanning electron micrograph of strain MB-PO13^T grown on ten-fold diluted ISP 2 medium for 14 days at 28 °C. Bar, 10 μm.

MB-PO13T and the type strains of *S. graminisoli* and *S. shenzhenensis* were 40.6% (reciprocal reaction: 56.9%) and 22.4% (37.9%), respectively. These values are sufficiently below the 70% cutoff point of DNA – DNA relatedness that is a standard criterion for the assignment of bacterial strains to the same genomic species.[30] The G + C content of the genomic DNA of strain MB-PO13T was 73.6 mol%.

Table 1 Growth and cultural characteristics of strain MB-PO13T on various media

Medium	Growth	Soluble pigment	Color of mycelium: Aerial	Color of mycelium: Substrate
ISP 2	+	Red	None	Vivid red
ISP 3	++	Purple	None	Dark grayish red
ISP 4	+++	Purple	Light gray	Deep red
ISP 5	+	Red	Yellowish white	Very pale red
ISP 6	+	None	None	Black
ISP 7	+	None	None	Reddish black
Glucose – asparagine	+	Red	Yellowish white	Very pale yellowish red
Nutrient	+	Red	None	Grayish yellow
Sucrose – nitrate	−	−	−	−
Skim milk	+	None	None	Light grayish yellow
Yeast – starch	++	Red	None	Vivid red

+++, Good; ++, moderate; +, poor; −, no growth. The aerial mycelia are yellowish white in color, which become light gray at sporulation.

The whole-cell hydrolysate of strain MB-PO13T contained LL-DAP, glucose and mannose. The menaquinones were identified as MK-9 (H_8), MK-9(H_6), MK-9(H_4) and MK-9(H_{10}) (57:37:5:1). The principal polar lipids were diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol (Supplementary Figure S5). Six minor unidentified polar lipids were also detected. The major cellular fatty acids (>10%) were anteiso-$C_{15:0}$ (24.9%), iso-$C_{16:0}$ (23.4%), iso-$C_{14:0}$ (15.0%) and $C_{16:0}$ (10.7%; Supplementary Table S1). These chemotaxonomic features were in good accordance with those of the genus *Streptomyces*.

The results of the phylogenetic analyses based on 16S rRNA gene sequences suggested that strain MB-PO13T belonged to the genus *Streptomyces*, and its chemotaxonomic characteristics were also in good agreement with those of the genus *Streptomyces*. Meanwhile, DNA – DNA relatedness between strain MB-PO13T and the type strains of the closest species was low, and physiological and biochemical tests also distinguished strain MB-PO13T from these species (Table 2). On the basis of these findings, strain MB-PO13T was proposed to be classified as a representative of a novel species of the genus *Streptomyces*, with the name *Streptomyces hyaluromycini* sp. nov.

Description of *Streptomyces hyaluromycini* sp. nov.

Streptomyces hyaluromycini (hya.lu.ro.my.ci'ni. N. L. neut. n. *hyaluromycinum* hyaluromycin; N. L. gen. neut. n. *hyaluromycini* of the hyaluronidase inhibitor hyaluromycin).

Cells are aerobic and Gram-stain-positive. The aerial mycelia are branched and yellowish white in color, which became light gray at sporulation and the substrate mycelia are yellow to red. Smooth surface spores (0.5–0.8 × 1.0–1.5 μm) in spiral chains are formed when

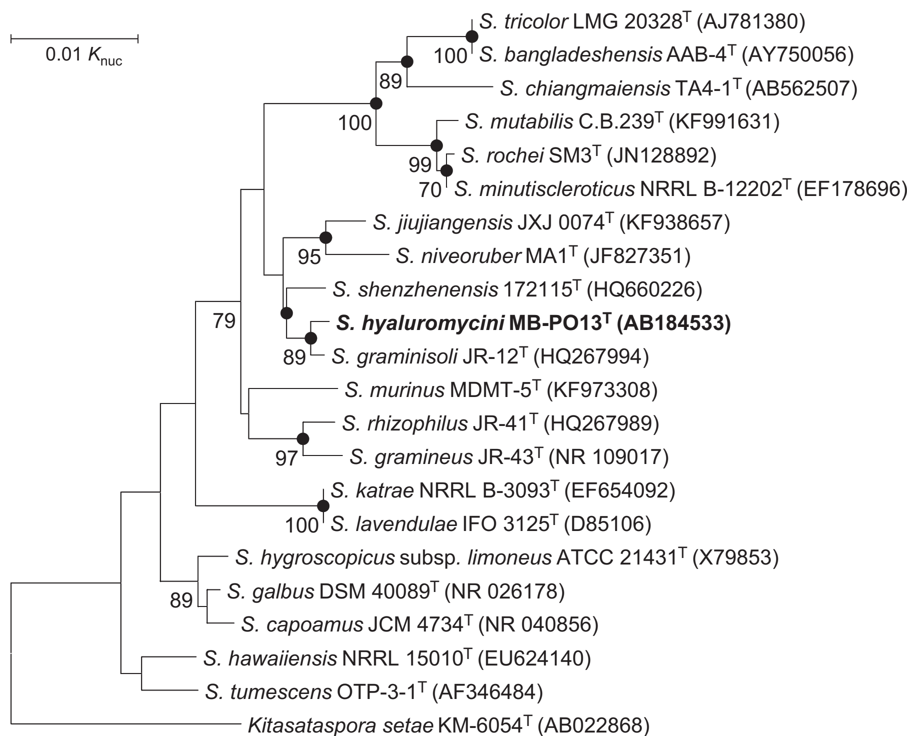

Figure 2 Neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain MB-PO13T and its taxonomic neighbors. *Kitasatospora setae* KM-6054T (AB022868) was used as the outgroup. Bootstrap values (>70%) based on 1000 replicates are shown at branch nodes. Filled circles indicate that the corresponding nodes are also recovered in the trees generated with the maximum-likelihood and maximum-parsimony algorithms (Supplementary Figures S3). Bar, 0.01 substitutions per nucleotide position.

Table 2 Differential characteristics of strain MB-PO13^{T} and its closest phylogenetic neighbors

Characteristic	*1*	*2*	*3*	*4*	*5*	*6*[a]
Spore surface	Smooth	Smooth[b]	Spiny[c]	Spiny[b]	Spiny[d]	Smooth
Characteristic on ISP 2						
Soluble pigment production	+	−	−	−	+	+
Aerial mycelium	None	Gray	White	None	Gray	White
Substrate mycelium	Vivid red	Pale yellow	Pale yellow	Pale yellow	Yellow	Gray yellow
Melanin production (ISP 6)	+	+[b]	−[c]	+[b]	+[d]	−
NaCl tolerance (%, w/v)	2	5[b]	3[c]	3[b]	3[d]	3
pH range	4–9	4–11[b]	5–7[c]	4–8[b]	4–8[d]	6–8
Carbon utilization						
Fructose	+	+	+	w	w	+
Glucose	+	+	+	−	+	−
Inositol	+	+	+	+	−	−
Mannitol	+	+	+	+	+	−
Raffinose	−	+	+	−	+	−
Rhamnose	+	+	+	−	+	−
Sucrose	−	w	+	−	−	−
Xylose	+	+	+	w	+	−
API ZYM						
α-Galactosidase	+	w	−	w	w	ND
N-Acetyl-β-glucosaminidase	w	+	−	+	+	ND
α-Mannosidase	−	+	−	−	−	ND
Major fatty acids (>10%)	C$_{14:0}$, ai-C$_{15:0}$, i-C$_{16:0}$, C$_{16:0}$	ai-C$_{15:0}$, i-C$_{16:0}$	i-C$_{15:0}$, ai-C$_{15:0}$, i-C$_{16:0}$	i-C$_{14:0}$, ai-C$_{15:0}$, i-C$_{16:0}$	i-C$_{14:0}$, i-C$_{15:0}$, ai-C$_{15:0}$, i-C$_{16:0}$	ai-C$_{15:0}$, i-C$_{16:0}$, ai-C$_{17:0}$
Polar lipids	DPG, PE, PI	PG, PC, PS, PI[b]	DPG, PE, PI[c]	DPG, PME, PG, PC, PS, PI[b]	PE, OH-PE, PC, PI, PIMs[d]	DPG, PE, APL, PI, PIMs, PIDM
DNA G + C content (mol%)	73.6	73.1	73.0	73.5[b]	70.5[d]	70.4

Abbreviations: APL, an unidentified aminophospholipid; DPG, diphosphatidylglycerol; OH-PE, hydroxyphosphatidylethanolamine; ND, no data; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; PI, phosphatidylinositol; PIDM, phosphatidylinositol dimannosides; PIMs, phosphatidylinositol mannosides; PME, phosphatidyl-*N*-methylethylethanolamine; PS, phosphatidylserine; w, weakly positive.
Strains: **1**, strain MB-PO13^{T}; **2**, *S. graminisoli* NBRC 108883^{T}; **3**, *S. shenzhenensis* DSM 42034^{T}; **4**, *S. rhizophilus* NBRC 108885^{T}; **5**, *S. gramineus* NBRC 107863^{T}; and **6**, *S. jiujiangensis* JXJ 0074^{T}.
Data are from this study unless otherwise stated. +, Positive; −, negative
[a]Zhang *et al.*[31]
[b]Lee & Whang.[32]
[c]Hu *et al.*[33]
[d]Lee *et al.*[34]

cultured on nutritionally poor media. Soluble red pigments are produced on ISP 2, ISP 3, ISP 4, ISP 7, glucose−asparagine, nutrient and yeast−starch media. Melanin pigments are produced on ISP 6 medium. Growth occurs at 10–37 °C (optimum 28 °C), at pH 4.0–9.0 (optimum pH 7.0) and in the presence of <2% NaCl (w/v). The API ZYM assays are positive for esterase (C4), leucine arylamidase, acid phosphatase, α-galactosidase, β-galactosidase and α-glucosidase but negative for valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, β-glucosidase, α-mannosidase and α-fucosidase. Weakly positive for alkaline phosphatase, esterase lipase (C8), naphtol-AS-BI-phosphohydrolase and *N*-acetyl-β-glucosaminidase activities. Utilizes L-arabinose, D-fructose, D-glucose, inositol, D-mannitol, rhamnose and D-xylose as sole carbon source for energy and growth, but not raffinose and sucrose (all at 1%, w/v). Hydrolyzes casein and starch, but not gelatin and cellulose. Peptidoglycan contains LL-DAP, glucose and mannose. The predominant menaquinones are MK-9(H_8) and MK-9(H_6). The major cellular fatty acids (>10% of total) are anteiso-C$_{15:0}$, iso-C$_{16:0}$, iso-C$_{14:0}$ and C$_{16:0}$. The principal polar lipids are diphosphatidylglycerol, phosphatidylethanolamine and phosphatidylinositol.

The type strain, MB-PO13^{T} (=NBRC 110483^{T} =DSM 100105^{T}), was isolated from a tunicate (*Molgula manhattensis*) collected at Tokyo Bay, Minato-ku, Tokyo, Japan. The DNA G + C content of the type strain is 73.6 mol%.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors are grateful to Dr Ken-ichiro Suzuki (NBRC) and NBRC staff for their assistance for chemotaxonomic study. This work was supported by the Japan Society for the Promotion of Science (JSPS) for Young Scientists (15K18692) and Institute for Fermentation, Osaka (IFO) for Young Scientists.

1 Waksman, S. A. & Henrici, A. T. The nomenclature and classification of the actinomycetes. *J. Bacteriol.* **46**, 337–341 (1943).
2 Witt, D. & Stackebrandt, E. Unification of the genera *Streptoverticillium* and *Streptomyces*, and amendation of *Streptomyces* Waksman and Henrici 1943, 339[AL]. *Syst. Appl. Microbiol.* **13**, 361–371 (1990).
3 Wellington, E. M. H., Stackebrandt, E., Sanders, D., Wolstrup, J. & Jorgensen, N. O. G. Taxonomic status of *Kitasatosporia*, and proposal unification with *Streptomyces* on the basis of phenotypic and 16S rRNA analysis and emendation of *Streptomyces* Waksman and Henrici 1943, 339[AL]. *Int. J. Syst. Bacteriol.* **42**, 156–160 (1992).
4 Zhang, Z., Wang, Y. & Ruan, J. A proposal to revive the genus *Kitastospora* (Ōmura, Takahashi, Iwai, and Tanaka 1982). *Int. J. Syst. Bacteriol.* **47**, 1048–1054 (1997).

5 Parte, A. C. LPSN–List of prokaryotic names with standing in nomenclature. http://www.bacterio.net/index.html (2015).
6 Zotchev, S. B. Marine actinomycetes as an emerging resource for the drug development pipelines. *J. Biotechnol.* **158**, 168–175 (2012).
7 Dharmaraj, S. Marine *Streptomyces* as a novel source of bioactive substances. *World J. Microbiol. Biotechnol.* **26**, 2123–2139 (2010).
8 Fenical, W. & Jensen, P. R. Developing a new resource for drug discovery: marine actinomycete bacteria. *Nat. Chem. Biol.* **2**, 666–673 (2006).
9 Petrey, A. C. & de la Motte, C. a. Hyaluronan, a crucial regulator of inflammation. *Front. Immunol.* **5**, 1–13 (2014).
10 Sakamoto, K., Nagai, H. & Koda, A. Role of hyaluronidase in immediate hypersensitivity reaction. *Immunopharmacology* **2**, 139–146 (1980).
11 Furuya, T. *et al.* Biochemical characterization of glycyrrhizin as an effective inhibitor for hyaluronidases from bovine testis. *Biol. Pharm. Bull.* **20**, 973–977 (1997).
12 Harunari, E. *et al.* Hyaluromycin, a new hyaluronidase inhibitor of polyketide origin from marine *Streptomyces* sp. *Mar. Drugs* **12**, 491–507 (2014).
13 Hucker, G. J. & Conn, H. J. Methods of gram staining. *Tech. Bull. NYSAES* **23**, 1–37 (1923).
14 Shirling, E. B. & Gottlieb, D. Methods for characterization of *Streptomyces* species. *Int. J. Syst. Evol. Microbiol.* **16**, 313–340 (1966).
15 Yu, Z. & Morrison, M. Improved extraction of PCR-quality community DNA from digesta and fecal samples. *Biotechniques* **36**, 808–812 (2004).
16 Heuer, H., Krsek, M., Baker, P., Smalla, K. & Wellington, E. M. H. Analysis of actinomycete communities by specific amplification of genes encoding 16S rRNA and gel-electrophoretic separation in denaturing gradients. *Appl. Environ. Microbiol.* **63**, 3233–3241 (1997).
17 Kim, O. S. *et al.* Introducing EzTaxon-e: A prokaryotic 16s rRNA gene sequence database with phylotypes that represent uncultured species. *Int. J. Syst. Evol. Microbiol.* **62**, 716–721 (2012).
18 Thompson, J. D., Higgins, D. G. & Gibson, T. J. CLUSTAL W: improving the sensitivity of progressive multiple sequence alignment through sequence weighting, position-specific gap penalties and weight matrix choice. *Nucleic Acids Res.* **22**, 4673–4680 (1994).
19 Saitou, N. & Nei, M. The neighbour-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol .Evol.* **4**, 406–425 (1987).
20 Felsenstein, J. Evolutionary trees from DNA sequences: a maximum likelihood approach. *J. Mol. Evol.* **17**, 368–376 (1981).
21 Fitch, W. M. Toward defining the course of evolution: minimum change for a specific tree topology. *Syst. Biol.* **20**, 406–416 (1971).
22 Tamura, K., Stecher, G., Peterson, D., Filipski, A. & Kumar, S. MEGA6: molecular evolutionary genetics analysis version 6.0. *Mol. Biol. Evol.* **30**, 2725–2729 (2013).
23 Felsenstein, J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* **39**, 783–791 (1985).
24 Saito, H. & Miura, K. I. Preparation of transforming deoxyribonucleic acid by phenol treatment. *Biochim. Biophys. Acta* **72**, 619–629 (1963).
25 Hamada, M. *et al. Luteimicrobium album* sp. nov., a novel actinobacterium isolated from a lichen collected in Japan, and emended description of the genus *Luteimicrobium. J. Antibiot.* **65**, 427–431 (2012).
26 Ezaki, T., Hashimoto, Y. & Yabuuchi, E. Fluorometric deoxyribonucleic acid-deoxyribonucleic acid hybridization in microdilution wells as an alternative to membrane filter hybridization in which radioisotopes are used to determine genetic relatedness among bacterial strains. *Int. J. Syst. Bacteriol.* **39**, 224–229 (1989).
27 Hasegawa, T., Takizawa, M. & Tanida, S. A rapid analysis for chemical grouping of aerobic actinomycetes. *J. Gen. Appl. Microbiol.* **29**, 319–322 (1983).
28 Hamada, M., Iino, T., Iwami, T. & Harayama, S. *Mobilicoccus pelagius* gen. nov., sp. nov. and *Piscicoccus intestinalis* gen. nov., sp. nov., two new members of the family Dermatophilaceae, and reclassification of *Dermatophilus chelonae* (Masters *et al.* 1995) as *Austwickia chelonae* gen. nov., comb. nov. *J. Gen. Appl. Microbiol.* **436**, 427–436 (2010).
29 Sasser, M. *Identification of Bacteria by Gas Chromatography of Cellular Fatty Acids, MIDI Technical Note 101* (MIDI Inc., Newark, DE, USA, 1990).
30 Wayne, L. G. *et al.* Report of the ad hoc committee on reconciliation of approaches to bacterial systematics. *Int. J. Syst. Bacteriol.* **37**, 463–464 (1987).
31 Zhang, B.-H. *et al. Streptomyces jiujiangensis* sp. nov., isolated from soil in South China. *Antonie Van Leeuwenhoek* **105**, 763–770 (2014).
32 Lee, H. J. & Whang, K. S. *Streptomyces graminisoli* sp. nov. and *Streptomyces rhizophilus* sp. nov., isolated from bamboo (*Sasa borealis*) rhizosphere soil. *Int. J. Syst. Evol. Microbiol.* **64**, 1546–1551 (2014).
33 Hu, H. *et al. Streptomyces shenzhenensis* sp. nov., a novel actinomycete isolated from mangrove sediment. *Antonie Van Leeuwenhoek* **100**, 631–637 (2011).
34 Lee, H. J., Han, S. I. & Whang, K. S. *Streptomyces gramineus* sp. nov., an antibiotic-producing actinobacterium isolated from bamboo (*Sasa borealis*) rhizosphere soil. *Int. J. Syst. Evol. Microbiol.* **62**, 856–859 (2012).

Supplementary Information accompanies the paper on The Journal of Antibiotics website (http://www.nature.com/ja)