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ORIGINAL ARTICLE

Streptomyces euryhalinus sp. nov., a new actinomycete isolated from a mangrove forest

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A Gram-positive, aerobic, non-motile actinomycete (strain MS 3/20^T) was isolated from the sediment of the Sundarbans mangrove forest in India. On International *Streptomyces* Project (ISP) medium 2, the isolate produced yellowish brown to red aerial hyphae that carried spiny-surfaced spores in a retinaculum-apertum arrangement. Whole-cell hydrolysate of the strain contained LL-diaminopimelic acid and galactose. Predominant menaquinones were MK-9(H_8) and MK-9(H_6). Diagnostic polar lipids were glycolipid, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, unidentified phospholipid and unidentified amino lipid. The major fatty acids were anteiso-$C_{15:0}$ (17.53%), iso-$C_{16:0}$ (23.89%) and anteiso-$C_{17:0}$ (10.29%). The strain showed 100% 16S ribosomal RNA (rRNA) gene sequence similarity with *Streptomyces variabilis* NBRC 12825^T, *Streptomyces erythrogriseus* LMG 19406^T, *Streptomyces griseoincarnatus* LMG 19316^T and *Streptomyces labedae* NBRC 15864^T. However, strain MS 3/20^T could be distinguished from these and seven other closely related species based on low levels of DNA–DNA relatedness (27.2–53.8%), supported by the unique banding pattern obtained from random amplified polymorphic DNA-PCR amplification and the distinctive matrix-assisted laser desorption/ionization–time-of-flight/mass spectrometry (MALDI-TOF/MS) profile of whole-cell proteins acquired for strain MS 3/20^T in comparison with its phylogenetic relatives. Disparate morphological, physiological and chemotaxonomic features, principally growth in NaCl, further corroborated the distinction of strain MS 3/20^T from other phylogenetic relatives. Strain MS 3/20^T is therefore suggested to be a novel species of the genus *Streptomyces*, for which the name *Streptomyces euryhalinus* sp. nov. is proposed. The type strain is MS 3/20^T (= CICC 11032^T = DSM 103378^T).
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INTRODUCTION

Waksman and Henrici proposed the formation of the genus *Streptomyces* belonging to the family *Streptomycetaceae* with the purpose of accommodating aerobic, Gram-positive and spore-forming actinomycetes.[1] At present, the genus comprises 611 species with validly published names, without considering homotypic and heterotypic synonyms (http://www.dsmz.de/bacterial-diversity/prokaryotic-nomenclature-up-to-date, last accessed 30 August 2016), qualifying it as the largest genus in the domain *Bacteria*. It is becoming progressively obvious that the taxonomic and metabolic diversity exhibited by streptomycetes is extraordinary, as novel and putatively new *Streptomyces* species are being continuously isolated from underexplored habitats, demonstrating that this genus is a valuable resource for new bioactive compounds.[2,3]

Mangroves occur along tropical and subtropical estuaries where fresh water and sea water mix together. Mangrove forests are vital sources for the discovery of novel actinomycetes, given that the major environmental determinant of the microbial community structure is salinity.[3,4] The continual changes in tidal gradient and salinity in the mangrove environment are considered to be drivers for microbial metabolic pathway diversity and can bring about the synthesis of atypical metabolites. This has encouraged the exploration of mangroves to identify novel microorganisms.[5] Several researchers have isolated novel streptomycetes from mangroves, such as *Streptomyces pluripotens*,[5] *Streptomyces qinglanensis*,[6] *Streptomyces sanyensis*,[7], *Streptomyces xiamenensis*[3] and *Streptomyces avicenniae*.[8] Earlier, we described *Streptomyces sundarbansensis* as the first validly described actinobacterium from the Sundarbans,[9] the largest mangrove forest in the world. Very recently, another research group explored this region for novel actinomycetes.[10]

Previously, we reported strain MS 3/20^T that expresses antimicrobial activity against Gram-positive and Gram-negative bacteria, molds, yeast and various multiple-drug resistant bacteria, including methicillin-resistant *Staphylococcus aureus*. The highly stable, active principle was purified, and a single compound was shown to possess broad-spectrum activity. Molecular characterization identified the active compound as a lipid.[11] We now present evidence that strain MS 3/20^T should be recognized as a new species that we propose be named *Streptomyces euryhalinus* MS 3/20^T.

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MATERIALS AND METHODS

Isolation and cultivation

Strain MS 3/20^T was isolated from sediment of the Lothian Island of the Sundarbans mangrove forest, India (Lat. 20°50′N, Long. 88°19′E), by serial dilution and plating followed by incubation at 28 °C for 4 days on an enrichment medium reported earlier.[11] The strain was preserved in − 80 °C as well as − 20 °C freezers as glycerol stocks (10–15%) after its isolation. The strain was also preserved by lyophilization. Working cultures of strain MS 3/20^T for morphological, physiological and biochemical studies were maintained at 0–4 °C. Biomass for molecular and chemotaxonomic analysis was obtained by culturing the strain on a modified medium described by Saha *et al.*[11] consisting of 2.0 g starch, 2.0 g glucose, 2.0 g soybean meal, 0.5 g yeast extract, 0.25 g NaCl, 0.32 g $CaCO_3$, 0.005 g $CuSO_4$, 0.005 g $MnCl_2$, 0.005 g $ZnSO_4$, 500 ml distilled water and 500 ml artificial sea water (pH 7.2) and incubating at 28 °C for 4 days. Strain MS 3/20^T was deposited in the Chinese Centre for Industrial Culture Collection (CICC 11032^T) and Leibniz Institute DSMZ–German Collection of Microorganisms and Cell Cultures (DSM 103378^T).

The reference strains *Streptomyces variabilis* NRRL B-3984^T, *Streptomyces erythrogriseus* NRRL B-3808^T, *Streptomyces griseoincarnatus* NRRL B-5313^T, *Streptomyces labedae* NRRL B-5616^T, *Streptomyces griseorubens* NRRL B-3982^T, *Streptomyces althioticus* NRRL B-3981^T, *Streptomyces griseoflavus* NRRL B-5312^T, *Streptomyces matensis* NRRL B-2576^T, *Streptomyces viridochromogenes* NRRL B-1511^T, *Streptomyces albogriseolus* NRRL B-1305^T and *Streptomyces paradoxus* NRRL B-3457^T were obtained as gifts from the ARS Culture Collection (NRRL), United States Department of Agriculture (Peoria, IL, USA) for comparative studies and were cultivated on International *Streptomyces* Project (ISP) 2 medium at 28 °C.

Phenotypic characterization

Spore chain morphology and spore surface ornamentation were studied by light (Leica DM750, Leica Microsystems, Buffalo Grove, IL, USA) and scanning electron (Jeol JSM-6700F, Jeol, Tokyo, Japan) microscopy following growth on ISP 2 medium at 28 °C for 14 days.[12] Colors of the aerial and substrate mycelia were determined on different ISP media (ISP 2, ISP 3, ISP 4, ISP 5 and ISP 6) by incubating at 28 °C for 2 to 3 weeks.[12] Tests for carbon source utilization were performed by supplementing a 1% carbon source in ISP 9 medium, and melanin production was assessed in ISP 6 medium following the protocols of Shirling and Gottlieb.[12] Utilization of amino acids as nitrogen sources, degradation of starch and gelatin, nitrate reduction and H_2S production were tested according to Williams *et al.*[13] Susceptibility/resistance to various antibiotics was measured by the disk diffusion plate technique. The effects of temperatures ranging from 15 to 45 °C (15–19 °C at intervals of 2 °C, 20–40 °C at intervals of 1 °C and 41–45 °C at intervals of 2 °C) on growth were observed visually for 14 days on ISP 2 medium. Growth over a pH range (1–12 at intervals of 0.5 units) and at different NaCl concentrations (0–25% w/v, at an increment of 1%) was recorded spectrophotometrically (OD_{600} nm) for 14 days on ISP 2 medium. Catalase activity was tested on modified Bennett's agar medium.[13] To test oxidase activity, MS 3/20^T was grown in modified Bennett's agar medium, and the color change was observed on oxidase strips (Sigma-Aldrich, St Louis, MO, USA). Esculin, L-tyrosine degradation and urea decomposition were observed as per Gordon *et al.*[14] Physiological characteristics were determined thrice in duplicate sets.

Chemotaxonomic characterization

Analyses of whole-cell sugars and isomers of diaminopimelic acid in MS 3/20^T peptidoglycan were performed using dried cell mass and identified by TLC following the protocol of Staneck and Roberts.[15] Respiratory quinones were identified by the China Center of Industrial Culture Collection (Beijing, China), applying FMIC-QO01-008 Analytical Method for Microbial Quinone Compounds.[16,17] Polar lipid analysis was performed by Microbial Culture Collection (MCC), Pune, India. Polar lipids were extracted with methanol, chloroform and saline solution (2:1:0.8, v/v).[18,19] The lipids were separated by two-dimensional TLC[20] and identified using the spraying agents ninhydrin, α-naphthol, Dragendorff, molybdenum blue and molybdophosphoric acid. Analysis of cellular fatty acids[21,22] was carried out by Royal Life Sciences (affiliated to MIDI Sherlock), Secundrabad, India. Cell mass at 40 mg was saponified, methylated and extracted to acquire fatty acid methyl esters. Separation and identification were carried out via the application of GC and the Sherlock Microbial Identification System (MIDI, Microbial ID, Newark, DE, USA). The DNA G+C content (mol%) was estimated by a thermal denaturation method following Marmur and Doty.[23]

16S rRNA gene sequencing and phylogenetic analysis

PCR amplification of the 16S ribosomal RNA (rRNA) gene of strain MS 3/20^T and its sequencing was carried out by the DSMZ Identification Service (Braunschweig, Germany).[24] Pairwise sequence similarity of the 16S rRNA gene sequence was determined on the EzTaxon-e-server (http://eztaxon-e.ezbiocloud.net/; last accessed 30 August 2016) to identify the close relatives of strain MS 3/20^T.[25] Reference strains for phylogenetic analysis were selected from the top hits of this determination. The 1503-bp 16S rRNA gene sequence of strain MS 3/20^T was aligned with the sequences of closely related *Streptomyces* type strains using the CLUSTAL W program[26] in Molecular Evolutionary Genetics Analysis (MEGA) 6 software.[27] The neighbor-joining[28] algorithm was used to construct the phylogenetic tree using MEGA 6.[27] Kimura's two-parameter model[29] was used to estimate the evolutionary distance matrix. Bootstrap analysis was performed with 1000 resamplings.[30]

DNA–DNA hybridization

DNA–DNA reassociation was performed thrice for species delineation by the dot blot method[31] with digoxigenin-labeled DNA using a DIG High Prime DNA detection kit (Roche Applied Science, Benzberg, Germany) following the manufacturer's instructions. Eleven strains that demonstrated the highest 16S rRNA similarities were selected for the DNA–DNA relatedness study. Dot intensities were measured using ImageJ software (http://rsb.info.nih.gov/ij/index.html); self-hybridization value was considered 100%.

RAPD-PCR amplification

Random amplified polymorphic DNA (RAPD-PCR) amplification and selection of primers were performed following Lee *et al.*[32] Three primers, AM50, AM62 and 70-34, were selected for the RAPD-PCR study. PCR amplification was carried out twice. The amplified PCR products were analyzed on an agarose gel after staining with ethidium bromide solution, and images were captured on an E-Gel imager (Thermo Fisher Scientific, Waltham, MA, USA).

MALDI-TOF/MS

Strains were grown for 3–4 days in ISP 2 medium to obtain the matrix-assisted laser desorption/ionization–time-of-flight/mass spectrometry (MALDI-TOF/MS) profiles of whole-cell proteins from strain MS 3/20^T and other type strains. Whole-cell proteins from actively growing cultures were extracted using ethanol, formic acid and acetonitrile. The extract was analyzed on an Autoflex TOF/TOF mass spectrometer (Bruker Daltonik GmbH, Bremen, Germany), and MALDI Biotyper software 3.0 (Bruker Daltonik GmbH) was used to visualize the mass spectra. This experiment was outsourced to MCC, Pune, India.

Nucleotide sequence accession number

The GenBank/EMBL/DDBJ accession number of the 16S rRNA gene sequence of strain MS 3/20^T is KR827683.

RESULTS AND DISCUSSION

The morphological characteristics of strain MS 3/20^T were typical of the genus *Streptomyces*. Scanning electron microscopy image at high (Figure 1) and low magnification (Supplementary Figures S4a–d) as well as light microscopy images under oil immersion (Supplementary Figures S4e and f) of strain MS 3/20^T grown on ISP 2 medium clearly showed long-chain spiny-surfaced spores in a retinaculum-apertum arrangement. The colors of the aerial and substrate mycelia of the strain on different ISP media are shown in Supplementary Table S1. The strain showed positive activity for catalase, oxidase and urea decomposition. It showed negative results for nitrate reduction, gelatin

liquefaction and melanin production. The strain was observed to be positive for esculin and starch degradation but negative for L-tyrosine degradation and hydrogen sulfide production. It demonstrated resistance to penicillin and susceptibility to ampicillin, polymixin B and neomycin. Arabinose, sucrose, lactose and sodium citrate were utilized by the strain as sole carbon sources, whereas sorbitol and sodium acetate were not utilized. The strain utilized tyrosine, leucine, isoleucine, valine, tryptophan and glutamine as sole amino acid sources. Growth was recorded between pH 5.0 to 9.0 and at temperatures between 24 and 35 °C. A whole-cell hydrolysate of the strain demonstrated the presence of LL-diaminopimelic acid and galactose. The polar lipids: glycolipid, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine (Dragendorff positive), unidentified phospholipid and unidentified amino lipid were observed in strain MS 3/20^T (Supplementary Figure S1). The menaquinones identified were MK-9(H_8): 52.3%, MK-9(H_6): 35.6%, MK-9(H_4): 8.5% and MK-9(H_2): 3.6%. The major cellular fatty acids present were anteiso-$C_{15:0}$ (17.53%), iso-$C_{16:0}$ (23.89%) and anteiso-$C_{17:0}$ (10.29%) (Supplementary Table S2). The DNA G+C content of strain MS 3/20^T was estimated at 77.3 mol%. These chemotaxonomic data revealed strain MS 3/20^T to be a member of the genus *Streptomyces*.

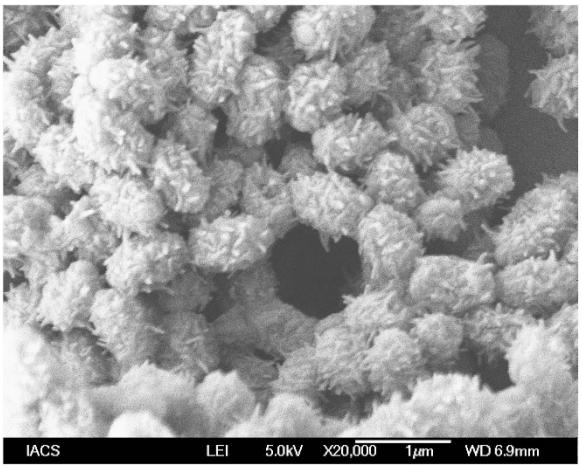

Figure 1 Scanning electron micrograph of strain MS 3/20^T grown on International *Streptomyces* Project 2 medium for 14 days at 28 °C.

The 16S rRNA gene sequence (KR827683) affiliated strain MS 3/20^T with the genus *Streptomyces*. The gene sequence showed 100% similarity with *S. variabilis* NBRC 12825^T (AB184884), *S. erythrogriseus* LMG 19406^T (AJ781328), *S. griseoincarnatus* LMG 19316^T (AJ781321) and *S. labedae* NBRC 15864^T (AB184704), thus creating ambiguity in the initial identity of MS 3/20^T. The limitations of the 16S rRNA gene sequence data alone for species delineation within the genus *Streptomyces* due to intraspecific variation, intragenomic heterogeneity, insufficient resolution capacity and inconsistency with DNA–DNA relatedness have been highlighted by several authors.[33–37] According to Labeda *et al.*,[38] it is impossible to recommend a definite16S rRNA gene sequence similarity value at which an unidentified actinobacterium may or may not be a novel species of the genus *Streptomyces*.

Other close relatives sharing more than 99% 16S rRNA gene sequence similarity were *S. griseorubens* NBRC 12780^T (AB184139) (99.73%), *S. althioticus* NRRL B-3981^T (AY999791) (99.52%), *S. griseoflavus* LMG 19344^T (AJ781322) (99.45%), *S. matensis* NBRC 12889^T (AB184221) (99.45%), *S. viridochromogenes* NBRC 3113^T (AB184728) (99.11%), *S. albogriseolus* NRRL B-1305^T (AJ494865) (99.05%), *S. heliomycini* NBRC 15899^T (AB184712) (99.04%), *S. paradoxus* NBRC 14887^T (AB184628) (99.04%) and *S. viridodiastaticus* NBRC 13106^T (AB184317) (99.04%). The neighbor-joining -phylogenetic tree (Figure 2) further confirmed that strain MS 3/20^T belongs to the genus *Streptomyces*; MS 3/20^T clustered with *S. griseoincarnatus* LMG 19316^T (AJ781321), *S. erythrogriseus* LMG 19406^T (AJ781328), *S. variabilis* NBRC 12825^T (AB184884) and *S. labedae* NBRC 15864^T (AB184704), thus reinforcing the close relationship among these five strains. Considering the insufficient

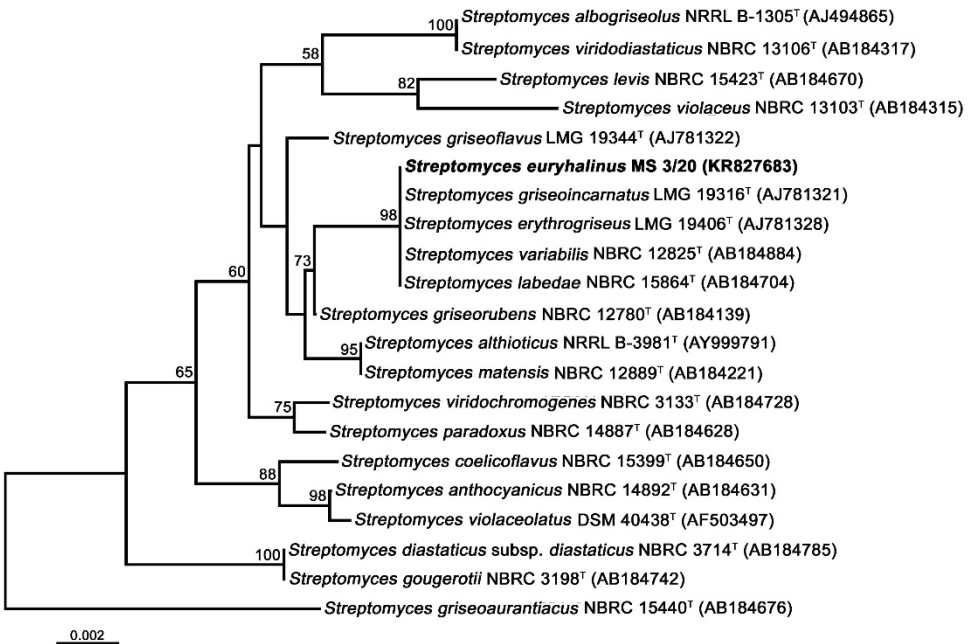

Figure 2 Neighbor-joining phylogenetic tree based on 16S ribosomal RNA (rRNA) gene sequences showing the position of *Streptomyces euryhalinus* MS 3/20^T within the genus *Streptomyces*. Numbers at nodes indicate levels of bootstrap support (percentages of 1000 replicates); only values of >50% are shown. GenBank accession numbers are given in parentheses. Bar, 0.002 nucleotide substitutions per site.

Table 1 Values of DNA–DNA hybridization between strain MS 3/20^T (accession no. KR827683) and its phylogenetically close relatives

			DNA–DNA hybridization (%)			
Relative strains	*NRRL accession numbers*	*Pairwise similarity of 16S rRNA (%)*	*I*	*II*	*III*	*Average value (± s.d.)*
S. variabilis	NRRL B-3984^T	100	28.4	36.8	29.6	31.6 (±4.6)
S. erythrogriseus	NRRL B-3808^T	100	24.2	39.3	27.0	30.2 (±8.0)
S. griseoincarnatus	NRRL B-5313^T	100	23.4	37.3	26.4	29.0 (±7.3)
S. labedae	NRRL B-5616^T	100	28.6	36.3	28.1	31.0 (±4.6)
S. griseorubens	NRRL B-3982^T	99.73	25.5	36.8	27.7	30.0 (±6.0)
S. althioticus	NRRL B-3981^T	99.52	35.1	38.9	32.4	35.5 (±3.3)
S. griseoflavus	NRRL B-5312^T	99.45	57.2	50.3	53.9	53.8 (±3.5)
S. matensis	NRRL B-2576^T	99.45	23.2	31.0	27.5	27.2 (±3.9)
S. viridochromogenes	NRRL B-1511^T	99.11	25.6	33.7	27.1	28.8 (±4.3)
S. albogriseolus	NRRL B-1305^T	99.05	25.0	31.3	26.9	27.7 (±3.2)
S. paradoxus	NRRL B-3457^T	99.04	27.8	35.0	28.2	30.3 (±4.0)

Abbreviation: rRNA, ribosomal RNA.

resolution capacity of the 16S rRNA molecule, Kämpfer *et al.*[36] cautioned that within the *Streptomyces* genus, the conclusions derived from simple treeing methods (such as the neighbor-joining algorithm) should be considered with prudence, as a 'tree is only a visual aid to place a novel species in its approximate neighborhood.'

Despite high 16S rRNA gene sequence similarity with other *Streptomyces* species, strain MS 3/20^T showed maximum DNA–DNA relatedness (53.8 ± 3.5%) with *S. griseoflavus* NRRL B-5312^T, significantly lower than the general 70% cutoff value for species differentiation[39] and the 80% value recommended for recognition of a novel species in the *Streptomyces* genus.[36] The DNA–DNA reassociation values of strain MS 3/20^T with the other phylogenetically close type strains varied between 27.2 and 35.5% (Table 1). This result clearly indicated that strain MS 3/20^T is different from the reference type strains and is a new species.

Fingerprinting techniques like RAPD can also be applied for the differentiation of species within a genus.[40] Consequentially, RAPD was performed to establish the differences between strain MS 3/20^T and other phylogenetically close neighbors that showed dissimilarities in DNA–DNA hybridization. The banding pattern of the randomly amplified DNA of strain MS 3/20^T exhibited recognizable differences compared with the patterns of its relatives (Supplementary Figure S2).

MALDI-TOF/MS is a recent technique applied for bacterial identification and is an effective tool for the differentiation of closely related species.[41] The MALDI-TOF/MS spectrum of whole-cell proteins of strain from MS 3/20^T was distinct from the corresponding spectra of other reference strains. Whole-cell proteins of strain MS 3/20^T displayed peaks at regions (*m/z* values) 2700, 3900, 4600, 5000, 5300, 7200, 8200, 8800 and 9400 (Supplementary Figure S3). A few of these *m/z* values were also present in other type strains, but they never occurred simultaneously. Peaks at regions (*m/z* values) 3300, 3600, 4100, 4800, 4900, 5400, 5800, 6600, 7300, 7400, 7700, 11 000, 12 000, 13 000, 15 000 and 18 000 were absent in strain MS 3/20^T but were present in other phylogenetically related strains (Supplementary Figure S3). In the genus *Streptomyces*, MALDI-TOF/MS data played an important role during the reclassification of *Streptomyces spheroides* and *Streptomyces laceyi*.[42] Thus, the RAPD-PCR banding pattern (Supplementary Figure S2) and MALDI-TOF/MS peak profile (Supplementary Figure S3) of strain MS 3/20^T demonstrated substantial disparities when compared with the corresponding data obtained from the closely related type strains, confirming the DNA–DNA hybridization results and further justifying strain MS 3/20^T as a novel species in the genus *Streptomyces*.

The morphological, physiological and biochemical characteristics of strain MS 3/20^T demonstrated 29 differentiating characteristics in total when compared with 11 phylogenetically close neighbors (Table 2). Out of the 29 characteristics, MS 3/20^T exhibited a minimum of 11 differentiating characteristics when compared with *S. griseoflavus* NRRL B-5312^T and a maximum of 18 distinguishing characteristics when compared with *S. erythrogriseus* NRRL B-3808^T and *S. griseoincarnatus* NRRL B-5313^T. Strain MS 3/20^T exhibited growth in media containing NaCl (range 0–20%), and this was the most vital discriminating property not observed for any other reference strain. Strain MS 3/20^T was different in many aspects from the four closely related species showing 100% similarity in the 16S rRNA gene sequence. The spore chain arrangement of strain MS 3/20^T was retinaculum-apertum, whereas the other four strains showed flexuous to straight arrangement (Table 2). The aerial mass color of strain MS 3/20^T cultivated on ISP 2 medium was yellowish brown to red, whereas that of *S. variabilis* NRRL B-3984^T, *S. erythrogriseus* NRRL B-3808^T and *S. labedae* NRRL B-5616^T was gray to yellow, and that of *S. griseoincarnatus* NRRL B-5313^T was red. On ISP 3 medium strain MS 3/20^T produced gray- to brown-colored aerial mycelia that was different from gray-colored aerial mass of *S. variabilis* NRRL B-3984^T and *S. labedae* NRRL B-5616^T, gray- to yellow-colored aerial mass of *S. erythrogriseus* NRRL B-3808^T and brown- to red-colored aerial mycelia of *S. griseoincarnatus* NRRL B-5313^T. Yellow- to brown-colored aerial mycelia was observed when strain MS 3/20^T was grown on ISP 4 medium, whereas *S. variabilis* NRRL B-3984^T and *S. labedae* NRRL B-5616^T displayed yellow- to gray-colored aerial mycelia, *S. erythrogriseus* NRRL B-3808^T presented yellow-colored aerial mass and *S. griseoincarnatus* NRRL B-5313^T yielded brown-colored aerial mass. The aerial mass color of strain MS 3/20^T grown on ISP 5 medium was yellow to brown that was dissimilar from *S. erythrogriseus* NRRL B-3808^T and *S. labedae* NRRL B-5616^T (gray to brown), *S. variabilis* NRRL B-3984^T (gray to yellow) and *S. griseoincarnatus* NRRL B-5313^T (brown to red). When cultivated on ISP 6 medium, strain MS 3/20^T and *S. griseoincarnatus* NRRL B-5313^T produced yellow- to brown-colored aerial mycelium, whereas *S. variabilis* NRRL B-3984^T, *S. erythrogriseus* NRRL B-3808^T and *S. labedae* NRRL B-5616^T produced gray-, brown-, brown- to gray-colored aerial

Table 2 Differential phenotypic characteristics of strain MS 3/20^T and its phylogenetically close relatives of the genus *Streptomyces*

Characteristic	***1***	***2***	***3***	***4***	***5***	***6***	***7***	***8***	***9***	***10***	***11***	***12***
Spore chain	RA	F–S[a]	S[a]	F–S[a]	S[a]	S[a]	S[a]	S[a]	RA–S[a]	S–RA[a]	F[a]	F–RA[a]
Spore surface	Spiny	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Spiny[a]	Warty[a]	Smooth[a]
Aerial mass color on ISP 2 medium	Yellowish brown to red	Gray to yellow	Gray to yellow	Red	Gray to yellow	Gray to yellow	Yellow	Yellowish brown to gray	Gray	Gray to yellow	Yellow to brown	Brown to red
Substrate mycelium color on ISP 2 medium	Yellow to brown	Brown	Yellow to brown	Brown to red	Brown to blue	Gray	Yellow to brown	Yellow to blue	Brown	Brown to blue	Brown	Brown
pH range for growth	5–9	5–10	5–10	5–10	5–9	5–10	6–10	5–10	5–10	5–10	6–10	5–10
Growth temperature range (°C)	24–35	24–37	25–40	22–37	25–37	25–40	25–37	24–37	24–37	25–37	24–40	24–37
Growth in NaCl concentration range (%)	0–20	0–12	0–9	0–9	0–11	0–10	0–9	0–10	0–10	0–10	0–12	0–8
Growth on sole carbon source												
Arabinose	+	–	–	–	–	–	+	+	–	–	–	+
Sucrose	+	+	–	+	+	–	+	+	+	+	+	–
Sodium acetate	–	+	+	–	+	–	+	–	+	+	–	–
Sodium citrate	+	+	–	–	–	+	+	+	–	–	+	–
Lactose	+	+	+	–	+	–	–	–	–	+	+	–
Sorbitol	–	+	–	+	+	–	–	–	–	–	–	+
Growth on sole amino acid as nitrogen source												
Tyrosine	+	+	–	–	+	+	–	+	+	+	–	+
Isoleucine	+	+	–	–	+	+	+	+	+	+	–	+
Glutamine	+	+	–	–	+	+	–	+	+	+	–	+
Tryptophan	+	+	+	+	–	+	–	+	+	+	–	+
Valine	+	+	–	–	+	+	–	+	+	+	+	+
Leucine	+	–	+	+	+	+	–	+	+	+	–	–
Antibiotic resistance/susceptibility												
Penicillin (10 units)	R	R	R	S	R	R	R	R	R	R	R	R
Ampicillin (10 μg)	S	R	R	R	R	R	R	R	R	R	R	R
Polymixin B (300 units)	S	S	S	S	R	S	S	R	R	S	S	R
Neomycin (30 μg)	S	R	S	S	S	S	S	S	R	S	S	S
Catalase activity	+	–	–	+	+	–	+	+	+	+	+	+
Oxidase activity	+	–	–	–	–	–	–	–	–	+	–	–
Nitrate reduction	–	–	+	–	+	+	+	+	+	+	+	–
Urea decomposition	+	–	–	+	–	–	+	+	–	–	–	–
Gelatin liquefaction	–	–	–	+	–	–	–	–	–	–	–	–
Melanin production	–	–	–	–	–	–	–	–	+	–	–	–

Abbreviations: F, flexuous; ISP, International *Streptomyces* Project; R, rectus; RA, retinaculum-apertum; S, spiral. In cases involving antibiotics: R, resistant; S, susceptible.
Strains: **1**, MS 3/20^T; **2**, *Streptomyces variabilis* NRRL B-3984^T; **3**, *Streptomyces erythrogriseus* NRRL B-3808^T; **4**, *Streptomyces griseoincarnatus* NRRL B-5313^T; **5**, *Streptomyces labedae* NRRL B-5616^T; **6**, *Streptomyces griseorubens* NRRL B-3982^T; **7**, *Streptomyces althioticus* NRRL B-3981^T; **8**, *Streptomyces griseoflavus* NRRL B-5312^T; **9**, *Streptomyces matensis* NRRL B-2576^T; **10**, *Streptomyces viridochromogenes* NRRL B-1511^T;**11**, *Streptomyces albogriseolus* NRRL B-1305^T; **12**, *Streptomyces paradoxus* NRRL B-3457^T.
Strain MS 3/20^T and four closely related strains that displayed 100% similarity of the 16S ribosomal RNA (rRNA) gene sequence with that of strain MS 3/20^T are shaded.
[a]Data from Kämpfer *et al.*[43]

masses, respectively. These dissimilarities are indicated in (Supplementary Table S1).

The color of the substrate mycelium produced by strain MS 3/20^T on ISP 2 medium was yellow to brown that was similar to *S. erythrogriseus* NRRL B-3808^T but different from *S. variabilis* NRRL B-3984^T (brown), *S. griseoincarnatus* NRRL B-5313^T (brown to red) and *S. labedae* NRRL B-5616^T (brown to blue). Strain MS 3/20^T produced yellow- to brown-colored substrate mycelium on ISP 3 medium that was similar to *S. erythrogriseus* NRRL B-3808^T but different from *S. variabilis* NRRL B-3984^T that displayed yellow- to gray-colored, *S. griseoincarnatus* NRRL B-5313^T that presented gray- to red-colored and *S. labedae* NRRL B-5616^T that showed brown- to red-colored substrate mycelia. Strain MS 3/20^T produced gray- to brown-colored substrate mycelium on ISP4 medium, whereas *S. variabilis* NRRL B-3984^T, *S. erythrogriseus* NRRL B-3808^T, *S. griseoincarnatus* NRRL B-5313^T and *S. labedae* NRRL B-5616^T produced brown, brown to pink, gray to yellow and yellow to brown-colored substrate mycelia, respectively. Yellowish brown to blue-colored substrate mycelium of strain MS 3/20^T was observed on ISP 5 medium, whereas *S. variabilis* NRRL B-3984^T produced brown-colored, *S. erythrogriseus* NRRL B-3808^T exhibited gray- to brown-colored, *S. griseoincarnatus* NRRL B-5313^T displayed red-colored and *S. labedae* NRRL B-5616^T presented gray- to brown-colored substrate mycelia. Strain MS 3/20^T produced brown- to red-colored substrate mycelium on ISP 6 media that was unlike *S. variabilis* NRRL B-3984^T (gray to blue), *S. erythrogriseus* NRRL B-3808^T (gray to brown), *S. griseoincarnatus* NRRL B-5313^T (red) and *S. labedae* NRRL B-5616^T (brown to blue). These dissimilarities are highlighted in Supplementary Table S1. The distinctive fatty acid, antesio $C_{17:1}$ was present in strain MS 3/20^T (3.28%) but absent in the other four closely related

strains (Supplementary Table S2). Strain MS 3/20^T grew in presence of sodium chloride 0–20% concentration, whereas the other four closely related strains failed to do so. Similarly, arabinose was utilized by strain MS 3/20^T as sole carbon source, whereas the other four strains could not. Strain MS 3/20^T was susceptible to ampicillin, whereas the four closely related strains were resistant to it. Furthermore, strain MS 3/20^T was positive for oxidase activity, whereas the other four strains were negative.

In conclusion, all data obtained from the experiments (DNA–DNA hybridization, RAPD banding pattern and MALDI-TOF/MS profile as well as morphological, physiological and biochemical characteristics) affiliated strain MS 3/20^T with the genus *Streptomyces* as a novel species for which the name *Streptomyces euryhalinus* MS 3/20^T sp. nov. is proposed.

Description of *Streptomyces euryhalinus* sp. nov.

Streptomyces euryhalinus (eu.ry.ha'linus Gr. adj. *eurus*, broad; Gr. n. *halos*, salt; L. suff.-*inus* suffix implying sense of belonging to; N.L. masc. adj. *euryhalinus*, living in a broad range of salinity).

Cells are Gram-positive, aerobic, non-motile. Spores are spiny, forming a retinaculum-apertum long chain. Grows luxuriantly on ISP 2, ISP 3, ISP 4, ISP 5 and ISP 6 media. Aerial mycelium is yellowish brown to red on ISP 2 medium, gray to brown on IPS 3 medium and yellow to brown on ISP 4, ISP 5 and ISP 6 media. Substrate mycelium color is yellow to brown on ISP 2 and ISP 3 media, gray to brown on ISP 4 medium, yellow-brown to blue on ISP 5 medium, brown to red on ISP 6 medium. Diffusible melanin pigment not produced in any ISP medium. Cell wall contains LL-DAP, and whole-cell hydrolysate shows the presence of galactose. Polar lipids present are glycolipid, phosphatidylglycerol, phosphatidylethanolamine, phosphatidylcholine, unidentified phospholipid and unidentified amino lipid. Major fatty acids are anteiso-$C_{15:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The predominant menaquinones are MK-9(H_6) and MK-9(H_8), whereas MK-9(H_4) and MK-9(H_2) are minor components. Positive for catalase, oxidase and urea decomposition. Negative for nitrate reduction, gelatin liquefaction and hydrogen sulfide production. Positive for esculin and starch degradation and negative for L-tyrosine degradation. Resistant to penicillin (10 units) and susceptible to ampicillin (10 μg), polymixin B (300 units), gentamicin (30 μg), tetracycline (30 μg), streptomycin (10 μg), kanamycin (30 μg), carbenicillin (100 μg), oleandomycin (15 μg), lincomycin (15 μg), vancomycin (30 μg), chloramphenicol (30 μg) and neomycin (30 μg). Utilizes arabinose, sucrose, lactose, sodium citrate, starch, mannose, xylose, inositol, cellobiose, trehalose, galactose, maltose and mannitol as sole carbon sources, but does not utilize sorbitol, sodium acetate and sodium succinate. Utilizes alanine, arginine, asparagine, glutamine, lysine, methionine, proline, threonine, histidine, tyrosine, leucine, isoleucine, valine, tryptophan and glutamine as sole amino acid sources, but does not utilize cystine. Grows in the presence of 0 to 20% NaCl (optimum 5%) at a temperature range between 24 and 35 °C (optimum 28 °C) and at a pH range between 5.0 and 9.0 (optimum 7.2–7.5).

The type strain MS 3/20^T (=CICC 11032^T =DSM 103378^T) was isolated from sediment of the Sundarbans mangrove forest in India. The genomic DNA G+C content of the type strain is 77.3 mol%.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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