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ARTICLE

Reclassification of *Nocardia* species based on whole genome sequence and associated phenotypic data

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Abstract

Type strains of 72 validated *Nocardia* species were phylogenetically analyzed based on the multilocus sequence analysis (MLSA) concatenated *atpD–groL1–groL2–recA–rpoA–secY–sodA–ychF*. Furthermore, their similarity based on digital DNA–DNA hybridization (dDDH) was calculated. *Nocardia soli*, *Nocardia cummidelens* and *Nocardia salmonicida*, *Nocardia nova* and *Nocardia elegans*, *Nocardia exalbida* and *Nocardia gamkensis*, and *Nocardia coubleae* and *Nocardia ignorata* formed coherent clades, respectively. Moreover, each set showed over 70% relatedness by dDDH and shared common phenotypic characteristics. Therefore, we propose a reclassification of *Nocardia soli and Nocardia cummidelens* as a later heterotypic synonym of *Nocardia salmonicida*, *Nocardia elegans* as a later heterotypic synonym of *Nocardia nova*, *Nocardia gamkensis* as a later heterotypic synonym of *Nocardia exalbida*, and *Nocardia coubleae* as a later heterotypic synonym of *Nocardia ignorata*.

Introduction

The genus *Nocardia*, first proposed by Trevisan [1], is a member of the family *Nocardiaceae*, suborder *Corynebacterineae* [2]. At the time of writing, the genus contains over 110 species with validly published names (http://www.bacterio.net/nocardia.html). The generic, medical, and industrial properties of the genus *Nocardia* has been reviewed by Goodfellow and Maldonado [3] in detail. Members of the genus are aerobic, Gram-stain positive, weakly acid-fast, non-motile, and mycolic acid-containing actinomycetes that form extensively branched mycelia and substrate hyphae that fragment into rod-shaped, non-motile elements. The genus is characterized chemotaxonomically by the presence of *meso*-diaminopimelic acid in the cell wall peptidoglycan, arabinose, and galactose as the characteristic sugars in the whole-cell hydrolysates (type IV), diphosphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, and phosphatidylinositol mannosides as diagnostic phospholipids (type PII), MK-8($H_{4,\omega\text{-cycl}}$) as the predominant menaquinone, straight-chain and unsaturated fatty acids and tuberculostearic acid as the major cellular fatty acids, and mycolic acid. Most species of the genus were isolated from soil sample, and some of them have been shown to be agents of human and animal diseases. Some *Nocardia* strains are known as producers of secondary metabolites with diverse biological activities and complex structures such as siderophores, polyketides, and terpenoids. Clarification of the taxonomic relationships among the

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Table 1 Genome feature of *Nocardia* strains used in this study

Species	Strain (NBRC number)	Genome size (Mb)	G+C content (mol%)	Accession number	Number of scaffold/ contig	N50
Nocardia abscessus	100374^T	8.41	68.2	BAFP00000000.1	274	94687
Nocardia acidivorans	108247^T	7.57	66.9	BDAW00000000.1	161	93005
Nocardia Africana	100379^T	7.81	67.9	BDAV00000000.1	93	379443
Nocardia alba	108234^T	7.28	67.7	BDAX00000000.1	62	231954
Nocardia altamirensis	108246^T	9.83	66.8	BDAY00000000.1	167	98520
Nocardia amamiensis	102102^T	8.24	67.4	BDBA00000000.1	487	34265
Nocardia amikacinitolerans	108937^T	7.65	68.5	BDAU00000000.1	108	197139
Nocardia anaemiae	100462^T	8.62	65.5	BDAZ00000000.1	134	130995
Nocardia aobensis	100429^T	7.56	68.0	BAFQ00000000.1	298	49166
Nocardia araoensis	100135^T	7.72	68.4	BAFR00000000.1	352	53602
Nocardia arizonensis	108935^T	7.20	67.9	BDCT00000000.1	99	159793
Nocardia arthritidis	100137^T	7.12	68.5	BDBB00000000.1	113	150833
Nocardia asiatica	100129^T	8.46	68.4	BAFS00000000.1	475	32247
Nocardia asteroides	15531^T	6.95	69.9	BAFO00000000.2	39	472953
Nocardia beijingensis	16342^T	7.48	68.9	BDBC00000000.1	113	134838
Nocardia brasiliensis	14402^T	8.90	68.2	BAFT00000000.2	115	125769
Nocardia brevicatena	12119^T	7.01	67.0	BAFU00000000.1	248	102039
Nocardia caishijiensis	108228^T	6.29	68.2	BDBE00000000.1	60	216896
Nocardia carnea	14403^T	7.50	67.1	BAFV00000000.1	126	147922
Nocardia cerradoensis	101014^T	7.60	68.2	BAFW00000000.1	388	37646
Nocardia concave	100430^T	8.93	67.7	BAFX00000000.1	206	92903
Nocardia coubleae	108252^T	6.62	67.9	BDBD00000000.1	40	284104
Nocardia crassostreae	100342^T	8.29	67.7	BDCH00000000.1	57	287529
Nocardia cummidelens	100378^T	7.50	67.1	BDBG00000000.1	60	250109
Nocardia cyriacigeorgica	100375^T	6.25	68.2	BAFY00000000.1	328	34812
Nocardia elegans	108235^T	7.54	67.9	BDBF00000000.1	117	129516
Nocardia exalbida	100660^T	7.37	68.6	BAFZ00000000.1	165	113130
Nocardia farcinica	15532^T	6.31	70.7	BDBJ00000000.1	194	84583
Nocardia flavorosea	108225^T	7.44	67.1	BDCG00000000.1	70	354858
Nocardia gamkensis	108242^T	7.71	68.4	BDBM00000000.1	120	142945
Nocardia grenadensis	108939^T	6.52	68.2	BDCJ00000000.1	71	251267
Nocardia harenae	108248^T	6.14	72.0	BDBH00000000.1	25	650297
Nocardia higoensis	100133^T	6.98	69.3	BAGA00000000.1	185	74558
Nocardia ignorata	108230^T	7.01	67.7	BDBI00000000.1	69	230637
Nocardia inohanensis	100128^T	8.12	67.8	BDBK00000000.1	26	562521
Nocardia jejuensis	103114^T	8.65	67.6	BDBU00000000.1	89	175270
Nocardia jiangxiensis	101359^T	10.45	66.8	BAGB00000000.1	174	193769
Nocardia jinanensis	108249^T	7.98	67.4	BDBO00000000.1	165	120704
Nocardia kruczakiae	101016^T	7.32	68.0	BDBL00000000.1	103	146771

Table 1 (continued)

Species	Strain (NBRC number)	Genome size (Mb)	G+C content (mol%)	Accession number	Number of scaffold/ contig	N50
Nocardia lijiangensis	108240^T	8.16	68.5	BDBP00000000.1	230	72042
Nocardia Mexicana	108244^T	8.96	68.6	BDBV00000000.1	180	99278
Nocardia mikamii	108933^T	7.56	68.0	BDCM00000000.1	42	420664
Nocardia miyunensis	108239^T	10.52	67.0	BDBQ00000000.1	208	93589
Nocardia niigatensis	100131^T	8.22	68.2	BAGC00000000.1	114	201374
Nocardia niwae	108934^T	7.31	68.8	BDCK00000000.1	105	206629
Nocardia nova	15556^T	7.85	67.9	BDBN00000000.1	198	72157
Nocardia otitidiscaviarum	14405^T	7.48	69.0	BAGD00000000.1	281	86449
Nocardia paucivorans	100373^T	6.00	66.6	BAGE00000000.1	128	224548
Nocardia pneumonia	100136^T	7.58	68.1	BAGF00000000.1	152	159154
Nocardia pseudobrasiliensis	108224^T	8.40	67.3	BDBS00000000.1	104	174575
Nocardia pseudovaccinii	100343^T	9.94	65.7	BDBY00000000.1	492	35813
Nocardia puris	108233^T	7.68	69.8	BDBW00000000.1	185	81313
Nocardia rhamnosiphila	108938^T	7.75	68.4	BDCL00000000.1	120	184444
Nocardia salmonicida	13393^T	8.25	67.0	BDBR00000000.1	99	169848
Nocardia shimofusensis	100134^T	6.33	69.1	BDBT00000000.1	75	234256
Nocardia sienata	100364^T	6.84	68.2	BDBX00000000.1	135	126075
Nocardia soli	100376^T	7.55	67.1	BDCB00000000.1	111	166395
Nocardia speluncae	108251^T	7.40	66.9	BDBZ00000000.1	61	214980
Nocardia takedensis	100417^T	8.03	69.5	BAGG00000000.1	266	96425
Nocardia tenerifensis	101015^T	9.73	68.4	BAGH00000000.1	615	29807
Nocardia terpenica	100888^T	8.63	68.3	BAGI00000000.1	4460	3019
Nocardia testacea	100365^T	7.27	68.5	BAGJ00000000.1	274	61620
Nocardia thailandica	100428^T	6.82	71.6	BAGK00000000.1	312	47211
Nocardia transvalensis	15921^T	8.38	69.2	BAGL00000000.1	128	147835
Nocardia uniformis	13702^T	8.77	66.0	BDCE00000000.1	144	113856
Nocardia vaccinii	15922^T	9.22	66.7	BDCC00000000.1	141	140170
Nocardia vermiculata	100427^T	6.69	67.0	BDCA00000000.1	80	178230
Nocardia veteran	100344^T	6.79	68.2	BAGM00000000.1	210	73162
Nocardia vinacea	16497^T	10.16	65.5	BAGN00000000.1	429	54832
Nocardia vulneris	108936^T	9.38	68.1	BDCI00000000.1	74	310852
Nocardia xishanensis	101358^T	7.69	68.4	BDCF00000000.1	124	135892
Nocardia yamanashiensis	100130^T	9.10	68.1	BDCD00000000.1	80	259867

The GenBank/EMBL/DDBJ accession numbers for the genome sequences of strains used in this study are shown in this table

members of this genus is important for clinical analysis and industrial use. Some molecular approaches have been applied to the identification of *Nocardia* strains [4–7]. The analysis of the 16S ribosomal RNA (rRNA) gene sequence still represents the backbone of taxonomic studies, but in some taxa, such as the class *Actinobacteria*, this gene is too conserved to distinguish two closely related species in many cases [8, 9]. The multilocus sequence analysis (MLSA) was

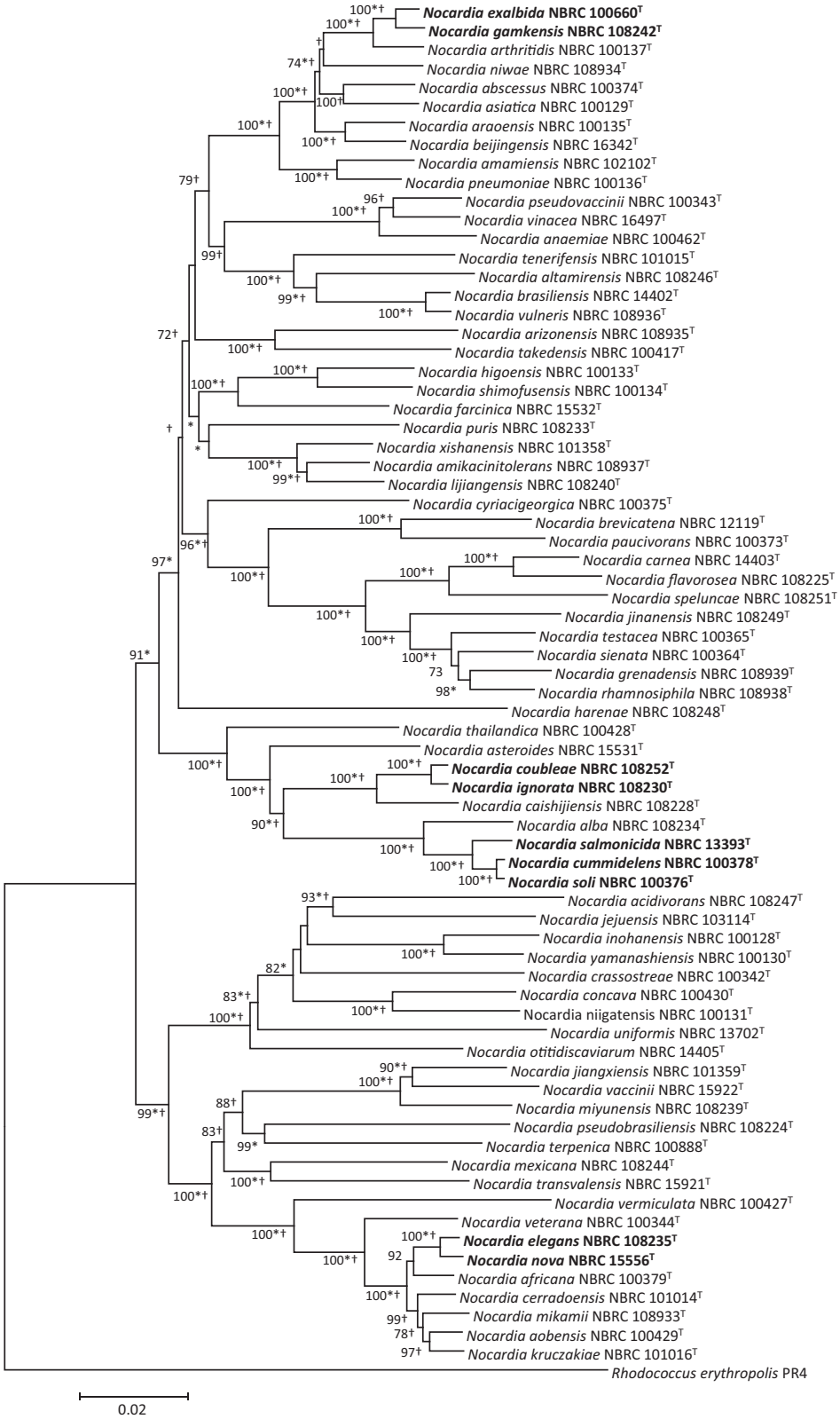

Fig. 1 Neighbor-joining phylogenetic tree of the genus *Nocardia* based on MLSA using concatenated *atpD–groL1–groL2–recA–rpoA–secY–sodA–ychF* gene sequences (9680 nucleotides (nt)). Numbers at nodes are bootstrap values based on 1000 resamplings (only values >70% are indicated). Asterisks indicate that the clades were recovered in the neighbor-joining tree using amino acid sequences, and daggers indicate that the clades were recovered in both the maximum-likelihood (nt) and the maximum-parsimony (nt) trees. Bar, 0.02% sequence divergence

recommended as a genetic method for species definition [10], and is generally used using various sets of genes for the definition of novel species and reclassification of the class *Actinobacteria* [11–16]. Recent studies demonstrated that genome-based methods are able to provide a conceptual framework for bacterial taxonomy of particular species.

These methods were also shown to be a digital DNA–DNA hybridization (dDDH) replacement for laboratory DNA–DNA hybridization (DDH), which is needed to describe new species [8, 17–24]. Approaches such as these have proven to be successful and are being increasingly adopted for the definition of novel species of the class *Actinobacteria* [25–28]. Previously, we investigated the taxonomic relationships of 26 *Nocardia* species based on MLSA and dDDH. We determined that the results obtained using these five methods correlated well with each other [29]. In this study, we show the phylogenetic relationships among the 72 *Nocardia* species analyzed by MLSA, and the reclassification of some *Nocardia* species based on whole genome sequence and associated phenotypic data.

Materials and methods

The strains of 72 validly proposed *Nocardia* species preserved at the Biological Resource Center, National Institute of Technology and Evaluation (NBRC), were used in this study (Table 1). DNA extraction was carried out as previously described [29]. Whole genome shotgun sequencing experiments were performed using the next-generation sequencing technique (Illumina MiSeq). The sequences were assembled using Newbler version 2.6 software and subsequently assessed using GenoFinisher software [30, 31]. The DNA sequences of genes encoding adenosine triphosphate (ATP) synthase subunit beta (*atpD*), chaperonin GroEL (two types of *groL*), DNA recombination and repair protein (*recA*), DNA-directed RNA polymerase subunit alpha (*rpoA*), preprotein translocase subunit SecY (*secY*), superoxide dismutase (*sodA*), and ribosome-binding ATPase (*ychF*) were extracted from each genome sequence. They were then concatenated as pseudo single sequences. Those concatenated sequences were used to perform a similarity search and phylogenetic analysis based on neighbor-joining (NJ), maximum-likelihood (ML), and maximum-parsimony (MP) algorithms using MEGA6 [32]. Genome-to-genome distance (GGD) [33] was computed on whole genome sequences to measure the genetic and evolutionary relatedness among strains, and to help consolidate the existing taxonomic ranks of bacterial strains. The GGD calculations were performed using the Genome-to-genome distance calculator, version 2 (available at http://ggdc.dsmz.de/), and expressed as a percent dDDH. Laboratorial DDH relatedness were determined by the microplate hybridization method developed by Ezaki et al. [34], using five replications. After the highest and lowest values for each sample were excluded, the mean was reported as the DDH relatedness. API Coryne, API ZYM, and API 50 CH strips were used as described by the manufacturer (bioMérieux). Conventional biochemical tests or API panels were incubated at 28 °C. Growth at 37 °C and 45 °C was assessed after incubation in ISP 2 medium [35] for 5 days. For analyses of chemotaxonomic characteristics, cells of *Nocardia* strains were grown in tryptic soy broth (TSB) for 3 days at 30 °C and harvested by centrifugation. Isoprenoid quinones were extracted by using the integrated procedure of Minnikin et al. [36] and analyzed using liquid chromatography mass spectrometry (LCMS; model LCMS-8030 and LC-20AD; Shimadzu) equipped with a Senshu-Pak Pegasil ODS-SP-100 column (100 × 2.0 mm i.d.; Senshu Scientific, Tokyo, Japan). Methanol–isopropanol was used as the mobile phase (34% isopropanol, 60 min) at the flow rate of 0.2 ml min^{-1} with ultraviolet detection at 275 nm. The preparation and analysis of cellular fatty acid methyl esters were performed using the protocol of the MIDI Sherlock Microbial Identification System [37] and a gas chromatograph (6890N; Agilent Technologies) with Sherlock MIDI software (version 6.2) and the TSBA6 database (version 6.2). Summed feature 3 detected in MIDI system was analyzed by GC/MS (6890N; Agilent Technologies).

Result and discussion

The genome sizes of *Nocardia* strains used in this study ranged from 6.00 (*N. paucivorans*) to 10.52 Mb (*N. miyunensis*), with an average of 7.86 Mb (Table 1). *N. vinacea* demonstrated the lowest DNA G+C content (65.5 mol%), whereas *N. harenae* demonstrated the highest (72.0 mol%).

The similarities of concatenated *atpD–groL1–groL2–recA–rpoA–secY–sodA–ychF* nucleotide (nt) sequences (total 9680 nt) among the tested strains ranged from 83.90% (between *N. flavorosea* and *N. inohanensis*) to 99.65% (between *N. cummidelens* and *N. soli*). The NJ phylogenetic tree derived from the nt sequences concatenated the eight housekeeping genes (Fig. 1). Of the 71 branches in the phylogenetic tree, 41 were supported by 100% bootstrap value and 46 were supported by the NJ phylogenetic tree based on the amino acid sequences (aa) that concatenated the eight housekeeping genes. There was excellent correlation between the phylogenetic relationships observed between species in the individual clades and those observed in a phylogenetic study of 190 clinical, 36 type, and 11 reference strains based on five-locus MLSA [6]. Phylogenetically, *N. cerradoensis*, *N. mikamii*, *N. kruczakiae*, *N. aobensis*, *N. nova*, *N. elegans*, and *N. africana* form a coherent clade. This clade included the species reported as *N. asteroides* Type III Drug Susceptibility Pattern [4, 38]. Their MLSA similarities ranged from 97.67% to 99.13%. Moreover, *N. gamkensis*, *N. exalbida*, and *N. arthritidis* (98.13 to 98.96% MLSA (nt) similarities); *N. cummidelens*, *N. soli*, and *N. salmonicida* (98.61 to 99.65% MLSA (nt) similarities); *N. coubleae and N. ignorata* (99.29% MLSA

Table 2 Phenotypic characteristics of *Nocardia elegans* NBRC 108235^T, *Nocardia nova* NBRC 15556^T, *Nocardia exalbida* NBRC 100660^T, *Nocardia gamkensis* NBRC 108242^T, *Nocardia cummidelens* NBRC 100378^T, *Nocardia soli* NBRC 100376^T, *Nocardia salmonicida* NBRC 13393^T, *Nocardia coubleae* NBRC 108252^T and *Nocardia ignorata* NBRC 108230^T

	N. elegans	*N. nova*	*N. exalbida*	*N. gamkensis*	*N. cummidelens*	*N. soli*	*N. salmonicida*	*N. coubleae*	*N. ignorata*
Growth at 37 °C	+	+	+	+	–	+	w	+	+
Nitrate reduction	+	+	+	+	w	+	w	+	+
Urea hydrolysis	w	w	w	w	+	+	+	+	+
Gelatin hydrolysis	+	w	+	w	+	+	w	+	+
Acid phosphatase	+	+	+	+	w	+	w	w	+
Cystine arylamidase	w	w	–	–	–	–	–	–	–
Esculin hydrolysis	+	+	–	–	w	w	w	–	–
Esterase (C-4)	–	–	+	+	+	+	+	+	+
Esterase lipase (C-8)	w	w	+	+	+	+	+	+	+
β-Galactosidase	w	w	–	–	–	–	–	–	–
α-Glucosidase	+	+	w	w	+	+	+	+	+
β-Glucosidase	+	+	–	–	+	+	+	–	–
Phosphohydrolase	+	+	w	+	w	w	w	w	w
Pyrazinamidase	+	w	+	w	w	w	+	–	–
Utilization of									
Glycerol	w	+	+	+	+	+	+	+	w
D-Fructose	w	w	+	+	+	+	+	–	+
N-Acetyl-glucosamine	–	–	+	+	+	+	+	+	+
Salicin	–	–	–	–	w	w	w	–	–
D-Trehalose	–	–	w	w	–	–	–	–	–
Potassium gluconate	–	–	–	–	–	–	–	w	+

+ positive, *w* weakly positive, − negative. All characteristics are determined in this study

All strains are positive for catalase and alkaline phosphatase, but negative for growth at 45 °C, *N*-acetyl-β-glucosaminidase, chymotrypsin, α-fucosidase, α-galactosidase, β-glucuronidase, lipase (C-14), α-mannosidase, pyrrolidonyl arylamidase or valine arylamidase

N. elegans NBRC 108235^T, *N. nova* NBRC 15556^T. *N. exalbida* NBRC 100660^T, *N. gamkensis* NBRC 108242^T, *N. cummidelens* NBRC 100378^T, *N. soli* NBRC 100376^T, and *N. salmonicida* NBRC 13393^T are positive for utilization of D-glucose, but negative for utilization of erythritol, D-oarabinose, L-arabinose, D-xylose, L-xylose, D-adonitol, methyl-β-D-xylopyranoside, D-mannose, L-sorbose, L-rhamnose, dulcitol, inositol, methyl-α-D-mannopyranoside, methyl-α-D-glucopyranoside, amygdalin, arbutin, D-lactose, D-melibiose, inulin, D-melezitose, D-raffinose, starch, glycogen, xylitol, gentiobiose, D-turanose, D-lyxose, D-tagatose, D-fucose, L-fucose, D-arabitol, L-arabitol, potassium 2-ketogluconate or potassium 5-ketogluconate

(nt) similarities); and *N. brasiliensis* and *N. vulneris* (99.01% MLSA (nt) similarities) also formed coherent clades. *N. coubleae* and *N. ignorata*, and *N. arthritidis*, *N. gamkensis* and *N. exalbida* were previously reported by McTaggart et al. [6] as two sets of type strains that form distinct clusters. Each clade was sustained in the ML and MP phylogenetic trees.

The dDDH relatedness among *N. cerradoensis*, *N. mikamii*, *N. kruczakiae*, *N. aobensis*, *N. nova*, *N. elegans*, and *N. africana* ranged from 45.6% to 75.1%. *N. nova* and *N. elegans* showed 75.1% relatedness (by dDDH), which is higher than the 70% cut-off point of DDH relatedness for the assignment of bacterial strains to the same genomic species [39]. This relatedness between *N. nova* and *N. elegans* was supported by laboratorial DDH (88 to 91%). Both G+C contents were 67.9 mol%. These strains assimilate glucose, but not arabinose, citrate, galactose, myo-inositol, mannitol, rhamnose, sorbitol, trehalose, or xylose. They however hydrolyze urea [40, 41]. The phenotypic characteristics determined in this study were similar for *N. nova* and *N. elegans* (Table 2). The similarity between the 16 S rRNA gene sequences of *N. nova* and *N. elegans* was only 98.2%, although the dDDH relatedness were 75.1%. Kim et al. [19] reported that 98.65% of 16S rRNA gene sequence similarity is the threshold for recognizing novel species using dDDH instead of laboratorial DDH, but the cut-off may not guarantee different genomic species status because there are exceptional cases owing to higher levels of intraspecies divergence of 16S rRNA gene sequences. Furthermore, the similarities between *N. nova* and *N. elegans* are within the threshold range (98.2% and 99.0% of 16S rRNA gene sequence similarity), which is on

the boundary for species delineation [22]. The major cellular fatty acids were $C_{16:0}$ (34.4–38.1%), $C_{18:1}$ ω9*c* (21.0–22.4%), $C_{18:0}$ 10-methyl (tuberculostearic acid (TBSA)) (17.0–18.8%), and $C_{18:0}$ (13.5–15.9%). Detailed fatty acid components are presented in Table S1. The predominant menaquinone of *N. nova* and *N. elegans* and *N. soli* was MK-8 (H 4ω-cycl).

The genomic relationships among *N. aobensis*, *N. cerradoensis*, and *N. kruczakiae* ranged from 59.8% to 65.3% relatedness (by dDDH). This finding correlated with that of Kageyama et al. [42] who reported that the DDH relatedness between *N. aobensis* and *N. cerradoensis* was 53% to 59%.

N. exalbida and *N. gamkensis* formed a coherent clade in MLSA phylogenetic tree, and showed 73.7% relatedness by dDDH. Although the 16S rRNA gene sequences similarity was high (99.4%), they had not been compared with each other in their original papers because they were proposed around the same time [43, 44]. The relatedness was also supported by laboratorial DDH (76 to 89%). Their GC content fell within the narrow range of 68.4 to 68.6 mol%. *N. gamkensis* was reported to weakly utilize galactose and mannose, and to grow at 45 °C [44]. However, their utilization and growth at 45 °C were negative for *N. gamkensis* similar to *N. exalbida* [43] (Table 2). The major cellular fatty acids were $C_{16:0}$ (30.0–34.6%), $C_{18:0}$ (16.9–23.9%), $C_{18:1}$ ω9*c* (16.7–20.4%), and $C_{18:0}$ 10-methyl (TBSA) (12.6–15.7%). Detailed fatty acid components are presented in Table S1. The predominant menaquinone of *N. exalbida* and *N. gamkensis* was MK-8 (H 4ω-cycl).

N. arthritidis isolated from the clinical samples of Japanese patients [45] was shown to be related to *N. exalbida*/*N. gamkensis* (62.2% to 62.4% relatedness by dDDH), but can utilize ribose unlike *N. exalbida* and *N. gamkensis*.

N. cummidelens also showed 92.9% relatedness by dDDH with *N. soli*. *N. salmonicida* showed similarities ranging from 78.8% to 79.3% relatedness (by dDDH) with *N. cummidelens* and *N. soli*. It was confirmed by laboratorial DDH relatedness (75 to 90%). They had almost same G+C contents (67.0 to 67.1 mol%). *N. cummidelens* and *N. soli* were reported as novel species forming a monophyletic clade in the 16S rRNA gene sequence tree together with *N. salmonicida* [46]. *N. cummidelens* and *N. soli* had 100% similarity of the 16S rRNA gene sequence, and they showed 99.5% similarities with *N. salmonicida*. *N. soli* reportedly utilizes rhamnose [46], and *N. salmonicida* utilizes mannitol and sorbitol [47]. In this study, *N. soli*, *N. cummidelens*, and *N. salmonicida* could not utilize rhamnose, mannitol, and sorbitol. Other phenotypic characteristics were also similar among *N. salmonicida*, *N. soli*, and *N. cummidelens* (Table 2). The major cellular fatty acids were $C_{16:0}$ (33.9–36.1%), $C_{18:0}$ 10-methyl (TBSA) (19.0–20.7%), $C_{18:1}$ ω9*c* (13.8–21.5%), and $C_{16:1}$ ω7*c* (9.7–15.4%). Detailed fatty acid components are presented in Table S1. The predominant menaquinone of *N. salmonicida, N. cummidelens,* and *N. soli* was MK-8 (H 4ω-cycl).

N. coubleae and *N. ignorata* showed 74.8% relatedness by dDDH. The laboratorial DDH between *N. coubleae* and *N. ignorata* tested in this study was also 79%, although this has previously been reported as 26 % [48]. The 16S rRNA gene sequence similarity was 99.4%. Their GC content fell within the narrow range of 67.7 to 67.9 mol%. *N. ignorata* was reported to grow at 45 °C [49]. However, it could not grow at this characteristic temperature in this study. A similar phenomenon was observed with *vcoubleae* (Table 2). Although *N. ignorata* was reported possessing MK-8(H_6) or MK-8(H_4cycl) as major menaquinone, it was not MK-8(H_6) but MK-8(H_4cycl) as with that of *N. coubleae* in this study. The major cellular fatty acids were $C_{16:0}$ (27.9–38.5%), $C_{18:0}$ 10-methyl (TBSA) (11.8–11.9%), $C_{18:0}$ (2.6–16.8%), $C_{16:1}$ ω7*c* (28.4–30.6%), and $C_{18:1}$ ω9*c* (10.5–14.2%). Detailed fatty acid components are presented in Table S1.

N. brasiliensis and *N. vulneris* showed 65.7% relatedness (by dDDH). This was consistent with the result of laboratorial DDH by Lasker et al. [50]. They reported that *N. vulneris* was readily distinguished phenotypically from *N. brasiliensis*, although it was in a transitional gray zone near the 70% threshold of DDH [50].

In conclusion, on the basis of genotypic and phenotypic data, it is evident that *N. soli* and *N. cummidelens* should be reclassified as later heterotypic synonyms of *N. salmonicida*, *N. gamkensis* as a later heterotypic synonym of *N. exalbida*, *N. coubleae* as a later heterotypic synonym of *N. ignorata*, and *N. elegans* as a later heterotypic synonym of *N. nova*.

Emended description of *Nocardia salmonicida* (ex Rucker 1949) Isik et al. 1999

The description is as that of Isik et al. [47] with the following amendments. Growth may occur at 37 °C. Positive for (in API ZYM and API coryne) catalase, urea hydrolysis, alkaline phosphatase, esterase (C-4), esterase lipase (C-8), α-glucosidase, and β-glucosidase. Utilizes D-glucose, glycerol, D-fructose and *N*-acetyl-glucosamine. MK-8(H_4cycl) is the predominant menaquinone. The major fatty acids are $C_{16:0}$, $C_{18:0}$ 10-methyl, $C_{18:1}$ ω9*c*, and $C_{16:1}$ ω7*c*.

The type strain, NBRC 13393^T (=ATCC 27463^T=CBS 694.72^T=CIP 104517^T=DSM 40472^T=JCM 4826^T=NRRL B-2778^T=NRRL B-12385^T), was a fish pathogen isolated from blueblack salmon (*Oncorhynchus nerka*) [47]. The names *Nocardia soli* (NBRC 100376^T) and *Nocardia cummidelens* (NBRC 100378^T) are later heterotypic synonyms.

Emended description of *Nocardia nova* Tsukamura 1983

The description is as that of Tsukamura [40] with the following amendments. Positive for (in API ZYM and API coryne) catalase, nitrate reduction, alkaline phosphatase, acid phosphatase, esculin hydrolysis,α-glucosidase, β-glucosidase, and phosphohydrolase. MK-8(H_4cycl) is the predominant menaquinone. The major fatty acids are $C_{16:0}$, $C_{18:1}$ ω9*c*, $C_{18:0}$ 10-methyl (TBSA), and $C_{18:0}$.

The type strain, NBRC 15556^T (=Tsukamura 23095^T=R.E. Gordon R443^T=ATCC 33726^T=CCUG 45939^T=CIP 104777^T=DSM 44481^T=JCM 6044^T=VKM Ac-1971^T), was a lung pathogenic bacterium [40]. The name *Nocardia elegans* (NBRC 108235^T) is a later heterotypic synonym.

Emended description of *Nocardia exalbida* Iida et al. 2006

The description is as that of Iida et al. [43]. with the following amendments. Growth may occur at 37 °C. Positive for (in API ZYM and API coryne) catalase, nitrate reduction, alkaline phosphatase, acid phosphatase, esterase (C-4), and esterase lipase (C-8). Utilizes D-glucose, glycerol, D-fructose, and *N*-acetyl-glucosamine. MK-8(H_4cycl) is the predominant menaquinone. The major fatty acids are $C_{16:0}$, $C_{18:0}$, $C_{18:1}$ ω9*c*, and $C_{18:0}$ 10-methyl (TBSA).

The type strain, NBRC 100660^T (=DSM 44883^T=IFM 0803^T=JCM 12667^T), was isolated from the bronchoalveolar lavage of an immunocompromised patient with lung abscess, in Chiba, Japan [43]. The name *Nocardia gamkensis* (NBRC 108242^T) is a later heterotypic synonym.

Emended description of *Nocardia ignorata* Yassin et al. 2001

The description is as that of Yassin et al. [49] with the following amendments. The predominant menaquinones is MK-8(H4cycl). Positive for (in API ZYM and API coryne) catalase, nitrate reduction, urea hydrolysis, gelatin hydrolysis, esterase (C-4), esterase lipase (C-8), and α-glucosidase. Utilizes D-glucose and *N*-acetyl-glucosamine. MK-8(H_4cycl) is the predominant menaquinone. The major fatty acids are $C_{16:0}$, $C_{18:0}$ 10-methyl (TBSA), $C_{18:0}$, $C_{16:1}$ ω7*c*, and $C_{18:1}$ ω9*c*.

The type strain, NBRC 108230^T (=CCUG 48296^T=DSM 44496^T=IMMIB R-1434^T=JCM 11764^T=NRRL B-24141^T), was originally identified as *Mycobacterium* sp. from a specimen sent to the clinical microbiological laboratory [49]. The name *Nocardia coubleae* (NBRC 108252^T) is a later heterotypic synonym.

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Compliance with ethical standards

Conflict of interest All authors declare that this research was conducted without any financial and commercial relationships with profit-making corporations.

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