Nakamurella intestinalis sp. nov., isolated from the faeces of Pseudorhynchus japonicus

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Abstract

One strain, designated 63MJ-1ᵀ, was isolated from fresh faeces of broad-winged katydids collected in Jinan-gun, Jeollabuk-do, the Republic of Korea. The organism grew in the range of 4–35°C (optimum, 28–30°C) and pH 6.0–9.0 (optimum, pH 7.0), and in the presence of 5% NaCl (w/v), but not in media containing 7% NaCl. According to the 16S rRNA gene sequence analysis, strain 63MJ-1ᵀ showed the highest sequence similarities with Nakamurella panacisegetis P4-7ᵀ (95.9%), Nakamurella endophytica 2Q3S-4-2ᵀ (95.8%) and Nakamurella multipartita DSM 44233ᵀ (95.7%). Phylogenetic trees also indicated that strain 63MJ-1ᵀ formed one robust cluster with members of the genus Nakamurella. The predominant quinone of strain 63MJ-1ᵀ was MK-8(H₄). Polar lipids were diphasphatidylglycerol, phosphatidylethanolamine, phosphatidylinositol, an unidentified aminophospholipid and two unidentified lipids. The major fatty acids were C₁₆:0, anteiso-C₁₅:0 and iso-C₁₅:0. The peptidoglycan type was A₁γ with meso-diaminopimelic acid as the diagnostic amino acid. The DNA G+C content was 64.6 mol %. Based on the phylogenetic, physiological and chemotaxonomic data, it was demonstrated that strain 63MJ-1ᵀ represents a novel species of the genus Nakamurella, for which the name Nakamurella intestinalis sp. nov. is proposed. The type strain is 63MJ-1ᵀ (=KACC 18662ᵀ=NBRC 111844ᵀ).

Originally, the genus Microsphaera was proposed for a strain isolated from activated sludge [1]. The new genus name Nakamurella was proposed as a substitute for Microsphaera Yoshimi et al. 1996, and then the new family name Nakamurellaceae substituting the former name Microsphaeraceae Rainey et al. 1997 was also proposed [2]. Members of the genus Nakamurella are characterized as aerobic, Gram-stain-positive, spherical, non-motile and non-spore-forming bacteria [1]. They contain meso-diaminopimelic acid in cell-wall peptidoglycan, MK-8(H₄) as the major quinone, and iso-C₁₆:0, iso-C₁₅:0 and C₁₈:1 Δ9 as the major fatty acids [1, 2]. At present, the genus Nakamurella includes six species, Nakamurella multipartita as type species of the genus, Nakamurella endophytica [3], Nakamurella flavida, Nakamurella lactea, Nakamurella panacisegetis [4] and Nakamurella silvestris [5]. Members of the genus Nakamurella have been isolated from soil, rock, sludge and plant bark [3–7].

Broad-winged katydids were collected in Jinan-gun, Jeollabuk-do, the Republic of Korea (35° 46′ 19″ N 127° 25′ 10″ E). Samples of fresh faeces from the insects were diluted serially in 0.85% (w/v) saline solution, spread on R2A agar (Difco) and incubated for 7 days at 28°C. Single colonies were selected and subcultured onto R2A agar plates to obtain pure cultures. One isolate, designated 63MJ-1ᵀ, was preserved in 20% (v/v) glycerol at −70°C.

The amplification of the 16S rRNA gene of strain 63MJ-1ᵀ was conducted using two universal primers, 9F and 1512R [8], and the PCR products were sequenced by Solgent (Daejeon, Republic of Korea). The nearly full length of the sequence (1457 bp) was obtained. The sequence was blasted using EzBioCloud [9], and the sequence similarity values were calculated. The sequence of strain 63MJ-1ᵀ represents the closely related 16S rRNA gene sequences were retrieved. The 16S rRNA gene sequences were aligned using the ARB software package [10]. Phylogenetic trees were reconstructed by the MEGA 6 program [11] with the neighbour joining [12], maximum parsimony [13] and maximum-likelihood [14] methods. The bootstrap values were calculated using MEGA 6 program [11] with the neighbour joining [12], maximum parsimony [13] and maximum-likelihood [14] methods.

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Cell morphology was examined by phase-contrast microscopy (AX10; Carl Zeiss) with cells grown at 28°C on R2A for 3 days. Gram staining was tested with the Difco Gram staining kit according to the manufacturer instructions. Temperature and pH range for growth were checked at various temperatures (4, 10, 15, 20, 28, 30, 37 and 42°C) and pH (pH 5.0–10.0 in increments of 1.0 pH units) on R2A medium. pH was adjusted using citrate/phosphate buffer (pH 4.0–7.0), Tris/hydrochloride buffer (pH 8.0–9.0) and carbonate/bicarbonate buffer (10.0). Growth with added salt was tested in R2A broth supplemented with NaCl (0, 1, 2, 3, 5, 7 and 10 %). Oxidase activity was tested by oxidation of 1% p-aminodimethylaniline oxalate, and catalase activity was determined by measurements of bubble production after the application of 3% (v/v) hydrogen peroxide solution. Hydrolysis of various substrates was tested using R2A agar medium supplemented with the following substrates: casein (1%, w/v), chitin (1%, w/v), carboxymethyl cellulose (1%, w/v), hypoxanthine (0.5%, w/v), starch (1%, w/v), Tween 80 (1%, w/v), tyrosine (0.1%, w/v) or xanthine (0.5%, w/v). A DNase test was conducted on DNase test agar (Difco). API 20NE, API ID 32GN and API ZYM kits (bioMérieux) were used to determine biochemical properties, assimilation of carbohydrates, acid production from carbohydrates and enzyme activities, according to the manufacturer’s instructions. Cells of strain 63MJ-1T were aerobic, Gram-stain-positive, non-flagellated, short rods (0.8–1.0 µm in width and 1.2–1.8 µm in length) (Fig. S1, available in the online Supplementary Material). Strain 63MJ-1T could be differentiated from the closely related species of the genus Nakamurella on the basis of habitat, growth conditions (temperature, pH and NaCl range for growth), substrate hydrolysis, assimilation of carbon sources and enzymic activities. Especially, strain 63MJ-1T assimilated a broad range of carbon sources (Table 1). The phenotypic comparison among strain 63MJ-1T and members of the genus Nakamurella is shown in Table 1.

![Neighbour-joining phylogenetic tree based on 16S rRNA gene sequences showing the phylogenetic position of strain 63MJ-1T and related species. Bootstrap values (expressed as percentages of 1000 replications) >70% are shown at branch points. Filled circles indicate branches that are also recovered in both maximum-likelihood and maximum-parsimony trees. Bar, 0.01 substitutions per nucleotide position.](https://www.microbiologyresearch.org/article-pdf/67/7/2970/2970-2974/2970-2974.pdf)
For the fatty acid analysis, strain 63MJ-1<sup>T</sup> and the closely related type strains N. endophytica KACC 18872<sup>T</sup>, N. flavida KACC 15065<sup>T</sup>, N. lactea KACC 14982<sup>T</sup>, N. multipartita KACC 20258<sup>T</sup> and N. panacisegetis KACC 18707<sup>T</sup> were grown on R2A agar medium at 28 °C for 2.5 or 3.5 days to the exponential phase depending on the strains [16]. Fatty acid methyl esters were prepared and analysed according to the standard protocol of MIDI (Sherlock Microbial Identification System, version 5.0) and identified by the TSBA 50 database of the Microbial Identification System. Menaquiones and polar lipids were extracted and purified according to the protocol of Minnikin et al. [17]. Individual polar lipids were separated by two-dimensional TLC and visualized by using the following reagents: 5% ethanolic
molybdophosphoric acid was used for detection of all lipids, ninhydrin reagent for lipids containing free amino groups, Zinzadze reagent for phosphorus-containing lipids and α-naphthol reagent for glycolipids. The DNA G+C content was determined by the fluorometric method [18] using SYBR Green 1 and a real-time PCR thermocycler (Bio-Rad). Biomass for peptidoglycan analysis was obtained by cultivation using trypticase soy broth (Difco) at 28 °C for 3 days. Amino acids and their isomers in cell-wall hydrolysates were analysed as described by Hamada et al. [19].

The predominant cellular fatty acids of strain 63MJ-1T were C16:0 (30.0%), anteiso-C15:0 (19.3%) and iso-C15:0 (16.0%) (Table 2). Compared with other closely related species, strain 63MJ-1T contained high amounts of C16:0, iso-C15:0 and summed feature 3, and a lower amount of anteiso-C17:0. Strain 63MJ-1T had MK-8(H4) as the predominant menaquinone, which was consistent with other species of the genus Nakamurella. Polar lipids consisted of diphosphatidylglycerol (DPG), phosphatidylinositolamine (PE), phosphatidylinositol (PI), an unidentified aminophospholipid (APL) and two unidentified lipids (Fig. S2). Members of the genus Nakamurella contain the polar lipids DPG, PE, APL and PI in common, and the presence and absence of polar lipids such as unidentified phospholipids, unidentified glycolipids, unidentified phosphoglycolipids or unidentified polar lipids is variable depending on species of the genus Nakamurella. The predominant cellular fatty acids of strain 63MJ-1T also had DPG, PE, APL and PI, with DPG and PE as the major polar lipids. And strain 63MJ-1T also contained two unidentified polar lipids, which were minor but specific for strain 63MJ-1T. The DNA G+C content of strain 63MJ-1T was 64.6 mol%, which was a little low compared with DNA G+C content values (67.5–74.3 mol%) of the genus Nakamurella. The peptidoglycan samples of strain 63MJ-1T contained alanine (Ala), glutamic acid (Glu) and diaminopimelic acid (A2pm) in a molar ratio of 1.4:1.0:0.4. Enantiomeric analysis of the peptidoglycan amino acids revealed the presence of D-Ala, L-Ala, D-Glu and meso-A2pm. These data suggested that the peptidoglycan type of strain 63MJ-1T was A1γ with meso-A2pm as a diagnostic amino acid. The peptidoglycan structure of all the members of the genus Nakamurella has been reported to contain meso-diaminopimelic acid as the diagnostic amino acid [1, 3–6], which was consistent with that shown for strain 63MJ-1T.

On the basis of phylogenetic analysis, phenotypic characteristics and chemotaxonomic properties, strain 63MJ-1T is considered to represent a novel species of the genus Nakamurella, for which the name Nakamurella intestinalis sp. nov. is proposed.

**DESCRIPTION OF NAKAMURELLA INTESTINALIS SP. NOV.**

Nakamurella intestinalis (in. tes. ti. na’lis. N.L. fem. adj. intestinals pertaining to the intestine).

Cells are aerobic, Gram-stain-positive, non-flagellated, short rods (0.8–1.0 µm in width and 1.2–1.8 µm in length). Colonies are white-coloured and round after incubation for 3 days on R2A agar at 28 °C. Catalase-positive and oxidase-negative. Hydrolyses aesculin, casein and gelatin, but does not hydrolyse chitin, carboxymethylcellulose, DNA, hypoxanthine, starch, Tween 80, tyrosine, urea or xanthine. Grows in the range of 4–35 °C (optimum, 28–30 °C) and pH 6.0–9.0 (optimum, pH 7.0). Growth occurs in media with NaCl up to 5% (w/v); no growth occurs with 7% NaCl. Negative for nitrate reduction, indole production, glucose fermentation and arginine dihydrolase. Assimilates D-glucose, L-arabinose, D-mannose, D-mannitol, maltose, potassium gluconate, malic acid, trisodium citrate, L-rhamnose, D-ribose, inositol, sucrose, lactic acid, L-alanine, potassium 5-ketogluconate, glycogen, L-serine, salicin, melibiose, L-fucose, D-sorbitol, L-histidine, potassium 2-ketogluconate, 3-hydroxybutyric acid and L-proline, but does not assimilate N-acetylg glucosamine, capric acid, adipic acid, phenylacetic acid, itaconic acid, suberic acid, sodium malonate, sodium acetate, 3-hydroxybenzoic acid, propionic acid, valeric acid or 4-hydroxybenzoic acid. Positive enzymic activity for alkaline phosphatase, esterase (C4), esterase lipase (C8), leucine arylamidase, acid phosphatase, naphthol-AS-BI-phosphohydrolase, α-glucosidase and β-glucosidase, but negative activity for lipase (C14), valine arylamidase, cystine arylamidase, trypsin, α-chymotrypsin, α-galactosidase, β-galactosidase, β-glucuronidase, N-acetyl-β-

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*Summed feature 3 contained iso-C15:0 2-OH and/or C16:1ω7c that cannot be separated by the MIDI system.
The predominant quinone is MK-8(H). Polar lipids are diphostadylglycerol, phosphatidylethanolamine, phosphatidylglycerol, and two unidentified phospholipids. The major fatty acids are C₁₆:0, anteiso-C₁₅:0 and iso-C₁₅:0. The peptidoglycan type is A1γ with meso-diaminopimelic acid as diagnostic amino acid.

The type strain, 63MJ-1T (=KACC 18662T=NBRC 111844T), was isolated from fresh faeces of broad-winged katydids collected in Jinan-gun, Jeollabuk-do, the Republic of Korea. The DNA G+C content of the type strain is 64.6 mol%.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References