Description of *Corynebacterium poyangense* sp. nov., isolated from the feces of the greater white-fronted geese (*Anser albifrons*)

Qian Liu¹, Guoying Fan², Kui Wu², Xiangning Bai¹, Xi Yang¹, Wentao Song², Shengen Chen², Yanwen Xiong¹*, and Haiying Chen²*

¹State Key Laboratory of Infectious Disease Prevention and Control, National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, Changping, Beijing 102206, P. R. China
²The Collaboration Unit for Field Epidemiology of State Key Laboratory of Infectious Disease Prevention and Control, Jiangxi Provincial Key Laboratory of Animal-origin and Vector-borne Diseases, Nanchang Center for Disease Control and Prevention, Nanchang 330038, P. R. China

Two novel Gram-positive, non-spore-forming, facultatively anaerobic, non-motile, and short rods to coccoid strains were isolated from the feces of the greater white-fronted geese (*Anser albifrons*) at Poyang Lake. The 16S rRNA gene sequences of strains 4H37-19² and 3HC-13 shared highest identity to that of *Corynebacterium uropygiale* Iso10¹ (97.8%). Phylogenetic and phylogenomic analyses indicated that strains 4H37-19² and 3HC-13 formed an independent clade within genus *Corynebacterium* and clustered with *Corynebacterium uropygiale* Iso10¹. The average nucleotide identity and digital DNA-DNA hybridization value between strains 4H37-19² and 3HC-13 and members within genus *Corynebacterium* were all below 95% and 70%, respectively. The genomic G + C content of strains 4H37-19² and 3HC-13 was 52.5%. Diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylglycerol (PI), phosphatidylcholine, and phosphatidylinositol mannosides (PIM) were the major polar lipids, with C₁₈:₀₁₉₀, C₁₆:₀, and C₁₄:₀ as the major fatty acids, and MK-8 (H₂), MK-8(H₃), and MK-9(H₃) as the predominant respiratory quinones. The major whole cell sugar was arabinose, and the cell wall peptidoglycan contained *meso*-diaminopimelic acid (meso-DAP). The polyphasic taxonomic data shows that these two strains represent a novel species of the genus *Corynebacterium*, for which the name *Corynebacterium poyangense* sp. nov. is proposed. The type strain of *Corynebacterium poyangense* is 4H37-19² (=GDMCC 1.1738¹ = KACC 21671¹).

**Keywords:** *Corynebacterium poyangense* sp. nov., feces, migratory bird, Poyang Lake, greater white-fronted geese

**Introduction**

*Corynebacterium* is the type genus of the family *Corynebacteriaceae*, order *Corynebacteriales*, class *Actinomycetia*, phylum *Actinomycetota* (Ludwig et al., 2015; Salam et al., 2020; Oren and Garrity, 2021). The genus *Corynebacterium* is composed of Gram-positive, non-motile, non-spore-forming, rod- or club-shaped, catalase-positive bacteria with a high G + C content (Bernard and Funke, 2015; Nouioui et al., 2018). As of 21 February 2022, the genus *Corynebacterium* included 136 species with validly published and correct names (https://lpsn.dsmz.de/genus/corynebacterium) (Parte et al., 2020). Species of *Corynebacterium* have been recovered from a variety of samples, such as humans, animals, soil, water, and food (Bernard and Funke, 2015). The type species, *Corynebacterium diphtheriae*, is a well-known human pathogen that causes diphtheria by multiplying and secreting diphtheria toxin (Sharma et al., 2019). However, *Corynebacterium glutamicum*, a non-pathogenic species, is commonly used as biochemical industrial producers of amino acids (Yu et al., 2021).

The greater white-fronted geese (*Anser albifrons*) belong to migratory birds which hold long-distance migration every year and might spread emerging and re-emerging pathogens across the world (Samuel et al., 2005; Boros et al., 2018; Xiang et al., 2019; Fukuda et al., 2021; Zhu et al., 2021). In the previous study, a novel bacterial genus (*Nanchangia*) and two novel species of genus *Corynebacterium*, i.e., *C. anserum*, and *C. heidelbergense*, were identified from feces of migratory birds (Braun et al., 2018; Liu et al., 2021a, 2021b). In this study, we isolated two strains 4H37-19² and 3HC-13, belonging to undescribed species within the genus *Corynebacterium*, from the feces of the greater white-fronted geese, and depicted the taxonomic characteristics of the two strains.

**Materials and Methods**

**Bacterial isolation and deposition**

Fecal samples of the greater white-fronted geese (*Anser albifrons*) were obtained from Poyang Lake in China. The specimens were homogenized and serially diluted (10⁻⁴–10⁻¹) in sterile phosphate-buffered saline. The diluted samples were spread on tryptone soya agar (TSA) and incubated at 37°C. Pure colonies were obtained by repeated subcultivations and stored at -80°C in 30% (v/v) glycerol stocks for further
identification. The representative isolates were deposited at Guangdong Microbial Culture Collection Center (GD MCC) of China and Korean Agricultural Culture Collection (KACC) under the accession numbers GDMCC 1.1738 and KACC 21671, respectively.

Phylogenetic analyses
For phylogenetic analyses, 16S rRNA gene sequences of strains 4H37-19T and 3HC-13 were amplified using primers 27F and 1492R, and then sequenced through the Sanger sequencing (Zhu et al., 2022). The obtained sequences were searched against the quality-controlled databases of 16S rRNA sequences using EzBioCloud service (Yoon et al., 2017). Phylogenetic trees were constructed using the MEGA-X program based on neighbor-joining (NJ), maximum-likelihood (ML), and minimum-evolution (ME) algorithms with 1,000 bootstrap replicates (Kumar et al., 2018). Mycobacterium tuberculosis H37RvT was used as the outgroup.

Whole-genome sequence analyses
Genomic DNA was extracted from pure culture using the Wizard Genomic DNA Purification kit (Promega). To obtain the complete genome of strain 4H37-19T, a combination of PacBio Sequel platform and Illumina NovaSeq platform was used. The draft genome of strain 3HC-13 and C. uropygieale Isol05 were sequenced on the Illumina NovaSeq platform. After filtering out the low-quality reads, the SPAdes optimizer Unicycler v0.4.8 (Wick et al., 2014) was used for de novo assembly. Multiple rounds of polishing were performed with Pilon 1.23 (Walker et al., 2014) in the Unicycler pipeline to correct small sequence errors. To further validate the taxonomic status of the two strains in the genus Corynebacterium, up-to-date bacterial core gene (UBCG, https://www.ezbiocloud.net/tools/ubcg) trees (Na et al., 2018; Kim et al., 2021) were constructed using the FastTree program with Mycobacterium tuberculosis H37RvT as the outgroup (Price et al., 2010). To evaluate the genomic relatedness, the digital DNA–DNA hybridization (dDDH) values and average nucleotide identity (ANI) values were calculated using the Genome-to-Genome Distance Calculator (GGDC) 2.1 (http://ggdc.dsmz.de/) (Meier-Kolthoff et al., 2022) and OrthoANI tool (Lee et al., 2016; Yoon et al., 2017), respectively. Gene annotation was performed using NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (Li et al., 2021) and Rapid Annotation using Subsystem Technology (RAST) server (https://rast.nmpdr.org/) (Brettin et al., 2015). The secondary metabolite biosynthesis gene clusters were predicted using anti-SMASH 6.0 (Blin et al., 2021). Carbohydrate-active enzyme features were analyzed in the dbCAN2 meta server, using DIAMOND, HMMER and eCAMI tools, respectively (https://bcb.unl.edu/dbCAN2/index.php) (Yin et al., 2012; Zhang et al., 2018).

Comparative analyses
Based on the 16S rRNA gene similarities, the four closely related type strains (C. uropygieale Isol05, C. choanae 200CH1T, C. jeikeium NCTC 11913T, and C. falsenii DSM 44353T) purchased from three culture collections (JCM, ATCC, and CCUG) were used as the reference strains for phenotypic, biochemical, and chemotaxonomic comparisons with strains 4H37-19T and 3HC-13. Comparative genome analyses and pairwise comparisons of ANI and dDDH values were also performed with genomic data of representative strains within the genus Corynebacterium publicly available from NCBI database. Whole-genome orthologous gene annotations and comparisons, including the genetic ontogeny of all predicted protein-coding genes, were conducted using OrthoVenn2 (Xu et al., 2019).

Growth conditions and morphological characterization
To determine the optimal growth conditions, strains 4H37-19T and 3HC-13 were cultured under various conditions. The growth temperatures were tested in tryptone soya broth (TSB) at 4, 10, 15, 20, 25, 30, 37, 45, 50, and 55°C, respectively. The salt tolerance was determined by culturing strains 4H37-19T and 3HC-13 in the presence of different NaCl concentrations (0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, and 12%, w/vol) in TSB. Growth was also evaluated at different pH values (4.0–11.0, at 1.0 pH unit intervals) using TSB. The pH values were re-adjusted after sterilization (121°C, 15 min) if necessary. The optimal growth conditions were determined by measuring the turbidity at 600 nm using Varian Cary 50 spectrophotometer (CARY-50, Agilent Technologies). The cells cultured under optimum conditions in TSB was used for following analyses, unless otherwise mentioned. Oxygen tolerance was evaluated in an anaerobic chamber in the presence of N2 (90%), H2 (5%), and CO2 (5%) for 1 week. Cell morphologies were observed under a transmission electron microscope (HT7700, Hitachi). Gram staining reactions and spore formation were observed under a light microscope (Eclipse 50i, Nikon) using a Gram staining kit (bioMerieux) and the malachite green staining method (Schaeffer and Fulton, 1933). Semi-solid medium containing 0.4% agar was used for motility testing. Catalase and oxidase activity were detected as previously described (Liu et al., 2021a). Antibiotic resistance was determined using K-B method (Bauer et al., 1966).

Biochemical and chemotaxonomic analyses
The biochemical characteristics of strains 4H37-19T and 3HC-13 were tested using API 50 CH (combined with API 50 CHB/E), the API ZYM system and API Coryne kits following the manufacturer’s instructions (bioMérieux). Cellular fatty acids of strains 4H37-19T and 3HC-13, and four reference strains were extracted, analyzed, and identified according to the previous studies (Sasser, 1990; Kim et al., 2021). The respiratory quinones of strain 4H37-19T was extracted and analyzed by HPLC as previously reported (Collins et al., 1977; Oh et al., 2020). The polar lipids of the isolate was analyzed by two-dimensional thin-layer chromatography (TLC) as previously described by Minnikin et al. (1984). Whole-cell sugars were obtained by hydrolyzing the cell harvests in 0.5 M sulfuric acid (100°C, 2 h), as described previously (Komagata and Suzuki, 1988). The cell wall peptidoglycan was analyzed as described previously (Schumann, 2011). The mycolic acids were extracted as previously described (Guerrant et al., 1981), then detected by gas chromatograph (HP 6890, Agilent) using an Ultra-2 chromatographic column (25 m by 0.2 mm
inside diameter and 0.33 μm liquid film thickness). The temperature of the injector and detector were 250°C and 300°C, respectively. The flow rate of the carrier gas (hydrogen) was 300 ml/min.

Accession numbers
The DDBJ/ENA/GenBank accession numbers for the 16S rRNA gene sequences of strains 4H37-19T and 3HC-13 are MN611115 and MN611764, respectively. The DDBJ/ENA/GenBank accession numbers for the whole-genome sequences of strain 4H37-19T, strain 3HC-13 and C. uropygiale Iso10T are CP046884, WWCB00000000, and JAKGSI0000000000, respectively.

Results and Discussion

Phylogenetic and phylogenomic analyses
Based on almost full-length 16S rRNA gene sequences comparisons against the EzBioCloud database, strains 4H37-19T and 3HC-13 were identified to be members of the genus Corynebacterium within family Corynebacteriaceae, and most

![Fig. 1. The neighbor-joining phylogenetic tree based on 16S rRNA gene sequences of strain 4H37-19T, strain 3HC-13, and closely related species. Bootstrap values (> 70%) based on 1,000 replicates are shown at branch nodes, with Mycobacterium tuberculosis H37Rv as an outgroup. Bar, 0.01 changes per nucleotide position. Strains from this study are highlighted in bold type and black circle.](image-url)
Table 1. The genomic features of strain 4H37-19T, strain 3HC-13, and phylogenetically related species

| Characteristics | Strain 1 | Strain 2 | Strain 3 | Strain 4 | Strain 5 | Strain 6 |
|-----------------|---------|---------|---------|---------|---------|---------|
| Size            | 2,617,997 | 2,559,826 | 2,460,278 | 2,986,773 | 2,526,027 | 2,719,616 |
| Contigs         | 1       | 9       | 10      | 1       | 2       | 2       |
| N50             | 2,617,997 | 828,747 | 515,070 | 2,986,773 | 2,516,825 | 2,677,607 |
| Number of genes | 2,465   | 2,386   | 2,293   | 2,331   | 2,235   | 2,075   |
| Number of CDSs  | 2,401   | 2,331   | 2,235   | 2,331   | 2,235   | 2,200   |
| G + C content (%) | 52.5    | 52.5    | 66.2    | 57.04   | 61.43   | 63.15   |
| rRNA genes (5S/16S/23S) | 12 (4, 4, 4) | 3 (1, 1, 1) | 3 (1, 1, 1) | 12 (4, 4, 4) | 9 (3, 3, 3) | 9 (3, 3, 3) |
| tRNA genes      | 49      | 49      | 52      | 51      | 50      | 50      |
| ncRNA genes     | 3       | 3       | 3       | 3       | 3       | 3       |
| Pseudo genes    | 43      | 37      | 23      | 28      | 50      | 35      |
| CRISPR count    | 1       | 1       | 0       | 2       | 1       | 1       |

Fig. 2. The UBCG tree of strain 4H37-19T, strain 3HC-13, and phylogenetically related strains. Gene Support Index (GSI) presented (> 70%) on nodes are the numbers of single gene trees supporting the branch. Mycobacterium tuberculosis H37RvT was used as an outgroup. Bar, 0.10 substitutions per nucleotide. Strains from this study are highlighted in bold type and black triangle.
Gene sequences showed that strains 4H37-19T and 3HC-13 could represent a novel species of the genus Corynebacterium. In addition, the phylogenetic tree based on 16S rRNA gene sequences showed that strains 4H37-19T and 3HC-13 formed a single clade and clustered with C. uropygiale Iso10T (Fig. 1; Supplementary data Figs. S1 and S2).

Whole-genome analyses showed that strains 4H37-19T and 3HC-13 contained 2,617,997 bp with a 52.5% DNA G + C content, and 2,559,826 bp with a 52.5% DNA G + C content, respectively. The genome of strain 4H37-19T contained 2,465 genes and 2,401 CDSs, which were different with the number of genes 2,386 and CDSs 2,331 of strain 3HC-13 genome. To compare strain 4H37-19T with strain 3HC-13 in detail, the conservation and variation were compared using MAUVE conserved domains, and orthologous genes were identified. A total of 13,460 SNPs were located within genomes, and some regions, including a total of 11 locally collinear blocks (LCBs) with minimum weight of 2,881 were generated. A total of 11913T (96.0%), C. falsenii DSM 44353T (96.0%), C. jeikeium NCTC 11913T (96.0%), C. falsenii DSM 44353T (96.0%) and C. urealyticum DSM 7109T (95.7%). These values were lower than 98.7%, the generally accepted threshold value for novel species (Rossi-Tamisier et al., 2015), suggesting that the two isolates could represent a novel species of the genus Corynebacterium. The dDDH and ANI values between stains 4H37-19T and 3HC-13 were 95.5% and 99.4%, indicating that the two isolates belonged to the same species.

### Table 2. Digital DNA-DNA hybridization (dDDH) and average nucleotide identity (ANI) values between genomes of the isolates and genomes of related strains

| Strains                          | Accession numbers | dDDH (%)  | ANI (%)  |
|---------------------------------|-------------------|-----------|----------|
|                                 |                   | 4H37-19T  | 3HC-13   | 4H37-19T  | 3HC-13   |
| C. poyangense 4H37-19T          | CP046884          | 100.0     | 95.5     | 100.0     | 99.4     |
| C. poyangense 3HC-13            | WWCB000000000     | 95.5      | 100.0    | 99.4      | 100.0    |
| C. choanae 200CH                | CP033896          | 27.3      | 25.9     | 68.5      | 68.7     |
| C. falsenii DSM 44353T          | CP007156          | 24.7      | 23.8     | 68.3      | 68.2     |
| C. jeikeium NCTC 11913T         | UFXO000000000     | 26.6      | 25.0     | 68.2      | 67.8     |
| C. urealyticum DSM 7109         | AM942444          | 23.3      | 22.0     | 68.8      | 67.7     |
| C. uropygiale JCM 32435T        | JAKGS000000000    | 18.5      | 18.1     | 70.6      | 70.8     |

Using the RAST server, the genome of strain 4H37-10T was annotated, 731 genes (29%) were further clustered into 24 subsystems. The most represented subsystem features were carbohydrates (201), amino acids and derivatives (193), protein metabolism (166), cofactors, vitamins, prosthetic groups, pigments (100), nucleosides and nucleotides (61), and DNA metabolism (46) (Fig. 3A). Screening the genes coding secondary metabolites showed that genome of strain 4H37-10T contained four (Regions 1–4) different genes clusters of secondary metabolites (Fig. 3B). Region 1 (649,498–670,421 nt, total 20,924 nt) and region 2 (2,183,106–2,193,576 nt, total 10,471 nt) displayed terpene and an unspecified ribosomally synthesised and post-translationally modified peptide product (RiPP) cluster types, respectively. Both region 1 and 2 were unable to identify the most similar known gene cluster. Region 3 (2,247,432–2,281,292 nt, total 33,861 nt) and region 4 (2,436,578–2,481,374 nt, total 44,797 nt) showed 5% and 8% similarity to pyrroloymycin A/pyrroloymycin B/pyrroloymycin C/pyrroloymycin D genes (BGCO000130) and stambomycin A / stambomycin B / stambomycin C / stambomycin D genes (BGCO000151), respectively.

The OrthoVenn2 analysis assigned 2,358 protein sequences from strain 4H37-19T to 2,255 orthologous clusters with 90 singletons, while 2,294 proteins from strain 3HC-13 were assigned to 2,234 clusters with 55 singletons. The Venn diagram (Supplementary data Fig. S4) showed 1,076 gene clusters shared by strains 4H37-19T and 3HC-13, and their closely related type strains. In addition, the six strains had 91 unique gene clusters, with strains 4H37-19T and 3HC-13 having two and zero unique gene cluster, respectively.

According to the results from dbCAN2 meta server, we identified a total of 88 genes encoding glycosyl transferases (GT), 75 genes encoding glycosyl hydrolases (GH), 22 genes encoding carbohydrate esterase (CE), 12 genes encoding carbohydrate-binding module (CBM), and 2 genes encoding auxiliary activities (AA) (Supplementary data Table S1).
Physiological, morphological, and biochemical features

Strains 4H37-19\textsuperscript{T} and 3HC-13 were Gram-stain-positive, facultatively anaerobic, non-motile and short rods to coccoid (0.2–0.4 × 0.6–0.9 μm; Supplementary data Fig. S5). Cells were catalase-positive and oxidase-negative. Colonies were creamy whitish, circular colonies with rough edges on TSA. Strains 4H37-19\textsuperscript{T} and 3HC-13 grew at 15–45°C, pH 6.0–9.0 and in the presence of 0–7.5% (w/vol) NaCl in TSB, with optimal growth at 37°C, pH 7.0 and in the presence of 0.5–1.5% (w/vol) NaCl. Antibiotic testing indicated that strains 4H37-19\textsuperscript{T} and 3HC-13 were susceptible to amikacin, ampicillin, cefazolin, chloramphenicol, ciprofloxacin, clindamycin, erythromycin,
gentamicin, kanamycin, penicillin G, streptomycin, sulfanilamide, tetracycline, and vancomycin.

The detailed biochemical characteristics of our isolates were described in the species descriptions, and the differential characteristics between strains 4H37-19T and 3HC-13 and their closely related Corynebacterium type strains were summarized in Table 3. The cell morphology of strains 4H37-19T and 3HC-13 were short rods to coccoid which were different with rod-club shape of C. uropygiale $\text{Iso10}^T$, C. jeikeium NCTC 11913T, and C. falsenii DSM 44353T, coccoid or irregular rod shape of C. choanae 200CHT. Strains 4H37-10T and 3HC-13, C. uropygiale $\text{Iso10}^T$, C. jeikeium NCTC 11913T, and C. falsenii DSM 44353T were non-spore-forming strains, except that C. choanae 200CHT was not determined (Table 3). Biochemical results indicated that strains 4H37-19T and 3HC-13 differed from their closely related neighbors by being positive for arbutin, L-arabinose, trypsin, $\alpha$-glucosidase, and $\beta$-glucuronidase (Table 3). Strains 4H37-19T and 3HC-13, C. jeikeium NCTC 11913T, and C. falsenii DSM 44353T were positive for alkaline phosphatase, while other related strains were negative. Corynebacterium uropygiale $\text{Iso10}^T$ and C. choanae 200CHT1 were positive for the reduction of nitrates, while the other related strains were negative, including our two isolates in this study. Furthermore, strains 4H37-19T and 3HC-13, and C. uropygiale $\text{Iso10}^T$ could utilize D-fructose, while the other related strains couldn’t.

### Chemotaxonomic characteristics

The detailed fatty acid profiles of our isolates and their closely related type strains were showed in Table 3. The major fatty acids (>10%) of strains 4H37-19T and 3HC-13 were C16:0 (both were 53.4%), C16:1ω7c (22.8% and 22.9%, respectively), and C18:2 (20.8% and 20.4%, respectively) (Table 4). Strain 4H37-19T contained MK-8 (H4) (38.3%), MK-8(H1)(36.4%), and MK-9(H2) (11.7%) as major respiratory quinones, which possessed the unique MK-8 (H4) that was absent in their closely related type strains, such as C. choanae 200CHT, and revealed different proportions of MK-8(H1) and MK-9(H2). (Bernard and Funke, 2015; Busse et al., 2019). The polar lipid profile of strain 4H37-19T was composed of diphasphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylinositol (PI), phosphatidylcholine (PC), phosphatidylglycerol mannosides (PIM), two unidentified phospholipids (PL), and two unidentified glycolipids (GL) (Supplementary data Fig. S6), which was similar to those of their closely related strains (Bernard and Funke, 2015). The whole-cell sugar of strain 4H37-19T consisted of arabinose (Supplementary data Fig. S7). The strain 4H37-19T included mycolic acids in cell wall.
Table 4. Composition of cellular fatty acids (%) of strain 4H37-19T, strain 3HC-13 and the closely related species

|                                | 1     | 2     | 3     | 4*    | 5     | 6     |
|--------------------------------|-------|-------|-------|-------|-------|-------|
| Saturated straight chain       |       |       |       | TR    |       | TR    |
| C10:0                          | -      | -     | -     | TR    | -     | TR    |
| C12:0                          | -      | -     | -     | TR    | -     | TR    |
| C14:0                          | -      | -     | -     | TR    | -     | TR    |
| C16:0                          | 22.8   | 22.9  | 23.2  | 41.5  | 34.3  | 30.9  |
| C17:0                          | -      | 2.5   | 3.8   | -     | -     | -     |
| C18:0                          | 20.8   | 20.4  | 19.8  | 6.7   | 10.5  | 11.8  |
| C20:0                          | 1.6    | 1.8   | -     | TR    | -     | -     |
| Saturated branched chain       |       |       |       |       |       |       |
| iso-C12:0                      | -      | 1.5   | -     | TR    | -     | TR    |
| iso-C13:0                      | -      | -     | -     | TR    | -     | TR    |
| iso-C14:0                      | -      | -     | -     | TR    | 0.5   | -     |
| iso-C16:0                      | -      | 1.4   | -     | -     | -     | -     |
| anteiso-C15:0                  | -      | -     | -     | TR    | -     | TR    |
| anteiso-C17:0                  | -      | 0.7   | -     | 0.5   | -     | -     |
| C18:0 10-methyl                | 0.5    | 0.5   | -     | -     | -     | -     |
| Unsaturated straight chain     |       |       |       |       |       |       |
| C16:ω9c                        | -      | -     | -     | -     | 1.6   | -     |
| C17:ω8c                        | -      | -     | 1.3   | -     | 1.4   | -     |
| C17:ω9c                        | -      | 1.2   | -     | -     | -     | -     |
| C18:ω9c                        | 53.4   | 53.4  | 35.2  | 51.8  | 21.8  | 51.0  |
| C20:ω9c                        | -      | -     | TR    | TR    | -     | -     |
| C20:ω6,ω9c                     | -      | -     | 0.8   | -     | TR    | 0.6   |
| Unsaturated branched chain     |       |       |       |       |       |       |
| C16:2OH                        | -      | -     | -     | TR    | -     | -     |
| C16:2OH                        | -      | -     | -     | TR    | -     | -     |
| C17:2OH                        | -      | -     | -     | TR    | -     | -     |
| C18:3OH                        | -      | -     | -     | 0.8   | -     | -     |
| C18:4OH                        | -      | -     | -     | TR    | -     | -     |
| C18:5OH                        | -      | -     | -     | -     | TR    | -     |
| iso-C15:1                      | -      | -     | -     | TR    | 0.6   | -     |
| Summed features*               | 3      | -     | 6.7   | -     | 1.2   | TR    |
| 4                              | -      | -     | 0.8   | -     | 0.7   | -     |
| 5                              | -      | -     | -     | -     | 12.9  | -     |
| 7                              | 0.7    | 0.7   | -     | -     | 0.9   | -     |
| 8                              | -      | -     | 4.1   | -     | 5.1   | -     |

*Data from Busse et al. (2019).
*Summed features were used when two or three fatty acids could not be separated using the Microbial Identification System. Summed feature 3 was comprised of C16:ω7c and C13:ω6c. Summed feature 8 was comprised of C18:ω7c and/or C13:ω6c.

and contained meso-diaminopimelic acid (meso-DAP) in the peptidoglycan.

Taxonomic conclusion

Taken together, the overall phylogenetic, genomic, physiological, biochemical, and chemotaxonomic findings distinguished strains 4H37-19T and 3HC-13 from their closely related species and suggested that they represent a novel species within the genus Corynebacterium. We propose the name Corynebacterium poyangense sp. nov. for strains 4H37-19T and 3HC-13.

Description of Corynebacterium poyangense sp. nov.

Corynebacterium poyangense (po.yang.en’s. N.L. neut. adj. poyangense, of or belonging to Poyang Lake from where the type strain was isolated)

Cells are Gram-stain-positive, non-spore-forming, facultatively anaerobic, non-motile and short rods to coccoid (0.2–0.4 × 0.6–0.9 μm). Colonies are creamy whiteish, circular colonies with rough edges on TSA at 37°C after 48 h. Cells grow at 15–45°C and pH 6.0–9.0 and in the presence of 0.5–1.5% (w/vol) NaCl. Optimal growth occurs at 37°C and pH 7.0 and in the presence of 0.5–1.5% (w/vol) NaCl. Cells are positive for acid phosphatase, alkaline phosphatase, esterase (C4), naphthol-AS-Bl-phosphohydrolase, trypsin, α-glucosidase, β-galactosidase, β-glucosidase, and β-glucuronidase, but negative for cystine arylamidase, hydrolysis, leucine arylamidase, lipase (C8), N-acetyl-β-glucosaminidase, reduction of nitrates, pyrrolnitrin, urease, valine arylamidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-glucosidase, and α-mannosidase. Cells can assimilate arbutin, esculin ferric citrate, D-fructose, D-glucose, D-maltose, D-mannose, D-ribose, D-trehalose, D-turanose, gentiobiose, gluconate, L-arabinose, and sorbose, and can assimilate amygdalin, erythritol, dulcitol, D-adenitol, D-arabinose, D-arabitol, D-cellobiose, D-fucose, D-lyxose, D-melezitose, D-melibiose, D-raffinose, D-tagatose, D-sorbitol, inositol, inulin, mannitol, methyl α-D-glucopyranoside, methyl α-D-mannopiranoside, methyl β-D-xylopyranoside, N-acetylglucosamine, galactose, glycerol, glycogen, lactose, L-arabitol, L-fucose, L-rhamnose, L-sorbose, salicin, starch, xylitol, or xylose. The major fatty acids are C18:1ω9c, C16:0, and C18:0, while MK-8 (H4), MK-8(H2), and MK-9(H2) are predominant respiratory quinones. The major polar lipids are DPG, PI, PC, and PIM. The major whole cell sugar was arabinose, and the cell wall included mycolic acids. The cell wall peptidoglycan contained meso-DAP. The genomic DNA G + C content is 52.5%.

The type strain is 4H37-19T (= GDMCC 1.1738T = KACC 21671T), isolated from the feces of the greater white-fronted geese (Anser albifrons) at Poyang Lake, China. The GenBank/EMBL/DDBJ accession numbers for the 16S rRNA gene and genome sequences of strains 4H37-19T strain 3HC-13 are MN611115 and MN611764 (16S rRNA gene), and CP-046884 and WWCB00000000 (genome), respectively.

Acknowledgements

This work was supported by grants from the National Science and Technology Major Project (2018ZX10301407-002), the Major Science and Project of Jiangxi Province (20201BBG-71010), and the Independent Research Project of State Key Laboratory of Infectious Disease Prevention and Control (2019SKLID311).

We express our sincere gratitude to the Jiangxi Province Department of Forestry for organizing the rescue of migratory birds and sampling. We are also grateful to the staff at Nanchang Center for Disease Prevention and Control who contributed to the sampling.
Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Statements

The migratory birds were live captured in Jiangxi province, China. All animals were subjected to non-invasive sampling (feces) and then released. We only collected feces for relevant microbiological studies. The animal welfare practices associated with this study were reviewed and approved by the Jiangxi Province Department of Forestry (No. 20181030).

References

Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493–496.

Bernard, K.A. and Funke, G. 2015. Corynebacterium. In Whitman, W.B., Rainey, F., Kämpfer, P., Trujillo, M., Chun, J., DeVos, P., Hedlund, B., and Dedysh, S. (eds.), Bergey’s Manual of Systematics of Archaea and Bacteria, pp. 1–70. John Wiley & Sons, Inc., New York, USA.

Blin, K., Shaw, S., Kloosterman, A.M., Charlop-Powers, Z., van Wezel, G.P., Medema, M.H., and Weber, T. 2021. antiSMASH 6.0: improving cluster detection and comparison capabilities. Nucleic Acids Res. 49, W29–W35.

Brettin, T., Davis, J.J., Disz, T., Edwards, R.A., Gerdes, S., Olsen, G.J., Busse, H.J., Kleinhagauer, T., Glaeser, S.P., Spergser, J., Kampfer, M.S., Braun, M.S., Wang, E., Zimmermann, S., and Wink, M.

Brecht, K.A. and Funk, G. 1977. Distribution of menaquinones in actinomycetes and corynebacteria. J. Gen. Microbiol. 100, 221–230.

Darling, A.E., Tritt, A., Eisen, J.A., and Facciotti, M.T. 2011. Mauve assembly metrics. Bioinformatics 27, 2756–2757.

Fukuda, A., Usui, M., Ushiyama, K., Shrestha, D., Hashimoto, N., Sakata, M.K., Minamoto, T., Yoshida, O., Murakami, K., Tamura, Y., et al. 2021. Prevalence of antimicrobial-resistant Escherichia coli in migratory greater white-fronted geese (Anser albifrons) and their habitat in Miyajimanuma, Japan. J. Wildl. Dis. 57, 954–958.

Guerrant, G.O., Lambert, M.A., and Moss, C.W. 1981. Gas-chromatographic analysis of mycotic acid cleavage products in mycobacteria. J. Clin. Microbiol. 13, 899–907.

Jackman, P. J. H., Pitcher, D. G., Pelczynska, S., and Borman, P. 1987. Classification of corynebacteria associated with endocarditis (group JK) as Corynebacterium jeikeium sp. nov. Syst. Appl. Microbiol. 9, 83–90.

Kim, K.R., Kim, K.H., Khan, S.A., Kim, H.M., Han, D.M., and Jeon, C.O. 2021. Lysobacter arenosi sp. nov. and Lysobacter solisilvae sp. nov. isolated from soil. J. Microbiol. 59, 709–717.

Komagata, K. and Suzuki, K.I. 1988. 4 Lipid and cell-wall analysis in bacterial systematics. In Colwell, R.R. and Grigorova, R. (eds.), Methods in Microbiology, pp. 161–207. Academic Press, Cambridge, Massachusetts, USA.

Liu, Q., Wu, K., Fan, G., Bai, L., Yang, X., Pan, Y., Cao, L., Song, W., Chen, S., Xiong, Y., et al. 2021a. Corynebacterium anserum sp. nov., isolated from the feces of greater white-fronted goose (Anser albifrons) at Poyang Lake, PR China. Int. J. Syst. Evol. Microbiol. 71. doi: 10.1099/ijsem.0.004637.

Liu, Q., Xue, L., Wu, K., Fan, G., Bai, L., Yang, X., Cao, L., Sun, H., Song, W., Pan, Y., et al. 2021b. Nanchangia anserum gen. nov., sp. nov., isolated from feces of greater white-fronted goose (Anser albifrons). Int. J. Syst. Evol. Microbiol. 71. doi: 10.1099/ijsem.0.004978.

Ludwig, W., Euzéby, J.P., and Whitman, W.B. 2015. Taxonomic outline of the phylum Actinobacteria. In Whitman, W.B. (ed.), Bergey’s Manual of Systematics of Archaea and Bacteria. pp. 1–4. John Wiley & Sons, Inc., Chichester, United Kingdom.

Luo, C., Rodriguez-R, L.M., and Konstantinidis, K.T. 2014. MyTaxa: an advanced taxonomic classifier for genomic and metagenomic sequence data. Nucleic Acids Res. 42, e73.

Meier-Kolthoff, J.P., Carbasse, J.S., Peinado-Olarte, R.L., and Göker, M. 2022. TYGS and LPNS: a database tandem for fast and reliable genome-based classification and nomenclature of prokaryotes. Nucleic Acids Res. 50, D801–D807.

Minnikin, D.E., O’Donnell, A.G., Goodfellow, M., Alderson, G., Athalye, M., Schaal, A., and Parlett, J.H. 1984. An integrated procedure for the extraction of bacterial isoprenoid quinones and polar lipids. J. Microbiol. Methods 2, 233–241.

Na, S.I., Kim, Y.O., Yoon, S.H., Ha, S., Baek, I., and Chun, J. 2018. UBCG: up-to-date bacterial core gene set and pipeline for phylogenetic tree reconstruction. J. Microbiol. 56, 280–285.

Naomi, I., Carro, L., García-López, M., Meier-Kolthoff, J.P., Woyke, T., Kyrpides, N. C., Pukall, R., Klenk, H.-P., Goodfellow, M., and Göker, M. 2018. Genome-based taxonomic classification of the phylum Actinobacteria. Front. Microbiol. 9, 2007.

Oh, Y.J., Kim, Y.J., Jo, H.E., Park, H.K., Lim, S.K., Kwon, M.S., and Choi, H.J. 2020. Lentibacillus cibarius sp. nov., isolated from kimchi, a Korean fermented food. J. Microbiol. 58, 387–394.

Oren, A. and Garrity, G.M. 2021. Valid publication of the names of forty-two phyla of prokaryotes. Int. J. Syst. Evol. Microbiol. 71, 3379–3393.

Parte, A.C., Sárdar Carbasse, J., Meier-Kolthoff, J.P., Reimer, L.C.,
and Göker, M. 2020. List of prokaryotic names with standing in nomenclature (LPSN) moves to the DSMZ. Int. J. Syst. Evol. Microbiol. 70, 5607–5612.

Price, M.N., Dehal, P.S., and Arkin, A.P. 2010. FastTree 2– approximately maximum-likelihood trees for large alignments. PLoS ONE 5, e9490.

Rossi-Tamisier, M., Benamar, S., Raoult, D., and Fournier, P.E. 2015. Cautionary tale of using 16S rRNA gene sequence similarity values in identification of human-associated bacterial species. Int. J. Syst. Evol. Microbiol. 65, 1929–1934.

Sasser, M. 1990. Identification of bacteria by gas chromatography of cellular fatty acids. MIDI Technical Note 101. MIDI Inc., Newark, Delaware, USA.

Schumann, P. 2011. 5- Peptidoglycan structure. In Rainey, F. and Oren, A. (eds.), Methods Microbiology, vol. 38, pp. 101–129. Academic Press, London, United Kingdom.

Sharma, N.C., Efstratiou, A., Mokrousov, I., Mutreja, A., Das, B., and Ramamurthy, T. 2019. Diphtheria. Nat. Rev. Dis. Primers. 5, 81.

Sjödén, B., Funke, G., Izquierdo, A., Akervall, E., and Collins, M.D. 1998. Description of some coryneform bacteria isolated from human clinical specimens as Corynebacterium falsenii sp. nov. Int. J. Syst. Bacteriol. 48, 69–74.

Walker, B.J., Abeel, T., Shea, T., Priest, M., Abouelil, A., Sakhikumar, S., Cuomo, C.A., Zeng, Q., Wortman, J., Young, S.K., et al. 2014. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. PLoS ONE 9, e112963.

Wick, R.R., Judd, L.M., Gorrie, C.L., and Holt, K.E. 2017. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput. Biol. 13, e1005595.

Xiang, X., Zhang, F., Fu, R., Yan, S., and Zhou, L. 2019. Significant differences in bacterial and potentially pathogenic communities between sympatric hooded crane and greater white-fronted goose. Front. Microbiol. 10, 163.

Xu, L., Dong, Z., Fang, L., Luo, Y., Wei, Z., Guo, H., Zhang, G., Gu, Y.Q., Coleman-Derr, D., Xia, Q., et al. 2019. OrthoVenn2: a web server for whole-genome comparison and annotation of orthologous clusters across multiple species. Nucleic Acids Res. 47, W52–W58.

Yin, Y., Mao, X., Yang, J., Chen, X., Mao, F., and Xu, Y. 2012. dbCAN: a web resource for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 40, W445–W451.

Yoon, S.H., Ha, S.M., Kwon, S., Lim, J., Kim, Y., Seo, H., and Chun, J. 2017. Introducing EzBioCloud: a taxonomically united database of 16S rRNA gene sequences and whole-genome assemblies. Int. J. Syst. Evol. Microbiol. 67, 1613–1617.

Yu, S., Zheng, B., Chen, Z., and Huo, Y.X. 2021. Metabolic engineering of Corynebacterium glutamicum for producing branched chain amino acids. Microb. Cell Fact. 20, 230.

Zhu, W., Zhou, J., Lu, S., Yang, Z., Busk, P.K., Xu, Y., and Yin, Y. 2018. dbCAN2: a meta server for automated carbohydrate-active enzyme annotation. Nucleic Acids Res. 46, W95–W101.