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Agromyces laixinhei sp. nov. isolated from bat feces in China[§]

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Three rod-shaped, Gram-stain-positive, and catalase-positive, phenotypically closely related isolates (HY052^T, HY050, and HY045) were obtained from fecal samples collected from bats in Guangxi province and Chongqing city of China. Circular, smooth, light-yellow colonies appeared on brain heart infusion plate after 24–48 h incubation at 28°C. The optimal pH for growth was between 6.0 and 7.5. Based on 16S rRNA, the three isolates were phylogenetically related to *Agromyces terreus* DS-10^T, *Agromyces aureus* AR33^T, *Agromyces salentinus* 20-5^T, *Agromyces allii* UMS-62^T, *Agromyces lapidis* CD55^T, and *Agromyces italicus* CD1^T. Moreover, based on 296 core genes, the phylogenomic tree indicated that the three isolates clustered together, closest to *Agromyces cerinus* VKM Ac-1340^T and *Agromyces fucosus* VKM Ac-1345^T but separated distantly from other *Agromyces* species. The average nucleotide identity values between strain HY052^T and other *Agromyces* species ranged from 79.3% to 87.9%, lower than the 95–96% threshold. Furthermore, the genome of strain HY052^T contains a circular chromosome of 3,437,203 bp with G + C content of 69.0 mol%. Main fatty acids were *anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$. The polar lipids comprised diphosphatidylglycerol, phosphatidylglycerol, and unidentified glycolipids. Rhamnose, ribose, and glucose were the primary cell wall sugars. The major peptidoglycan amino acids included alanine, glutamic acid, glycine, and 2,4-diaminobutyric acid. An additional remarkable difference from other *Agromyces* species is that MK-12 was the sole menaquinone in strain HY052^T. Based on results from the polyphasic characterizations performed in this study, our isolates are proposed to be members of a novel species in genus *Agromyces*, named *Agromyces laixinhei*. The type strain is HY052^T (= CGMCC 1.17175^T = JCM 33695^T).

***Keywords*:** *Agromyces laixinhei* sp. nov, bat feces, taxonomy

Introduction

The COVID-19 pandemic has put the possibility and danger of disease transmission from wildlife to humans in the spotlight again. Our laboratory has investigated the microbiome diversity of wild animals since 2014 to identify potentially pathogenic microbial species and dissect the process of disease transmission. So far, we have analyzed samples from Tibetan antelopes (Wang *et al.*, 2018), plateau pikas (Li *et al.*, 2020), snow finches (Ge *et al.*, 2020), and Tibetan wild asses (Huang *et al.*, 2019), and uncovered novel bacterial species facilitated by whole genome sequencing. Bats, the only flying mammal, are natural reservoirs of Ebola virus and SARS-like coronaviruses (Ge *et al.*, 2012, 2013). However, little is known about the bacterial species associated with bats (Pierlé *et al.*, 2015; Huang *et al.*, 2020), which prompted us to study the composition of their intestinal microbiota. To this end, three novel strains (HY052^T, HY050, and HY045) were isolated from two bat faecal samples and their taxonomic characterization were described.

The genus *Agromyces* was first proposed in 1969 (Gledhill and Casida, 1969), and amended later in 1992 and 2004, respectively (Zgurskaya *et al.*, 1992; Ortiz-Martinez *et al.*, 2004). Members of the genus *Agromyces* are aerobic, non-motile and Gram-stain-positive, with MK-11 and MK-12 as the predominant menaquinones, and *anteiso*-$C_{15:0}$, *anteiso*-$C_{17:0}$, and *iso*-$C_{16:0}$ as the primary fatty acids (Chen *et al.*, 2016; Corretto *et al.*, 2016; Huang *et al.*, 2016). As of the end of 2020, the genus is comprised of 36 species and two subspecies (http://www.bacterio.net/agromyces.html) with valid published names (Parte, 2018), mostly isolated from various environmental sources such as soil (Chen *et al.*, 2016; Huang *et al.*, 2016), the rhizosphere (Corretto *et al.*, 2016), caves and rocks, plant tissues, sea sediments, and catacombs. Studies directly addressing the importance of *Agromyces* in its associated ecosystems and its physiology are rare, however, *Agromyces* likely influences its environment in many ways, e.g., by interacting with other microbes (Casida, 1983). So far, most members of the genus are soil-associated, and some may be opportunistic pathogen (Sridhar *et al.*, 2015), only a few were isolated from other habitats, e.g., fermented seafood (Park *et al.*, 2010), gut of insect larvae and plateau pika (Heo *et al.*, 2020; Li *et al.*, 2020). However, there are a lot of unknowns about this genus that remain to be investigated. Interestingly, some of the products or metabolic capability of the genus *Agromyces* have huge industrial and ecological

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[§]Supplemental material for this article may be found at http://www.springerlink.com/content/120956.

potential, either by producing enzymes of higher quantity/quality (Yasuhira *et al.*, 2007; Mitsukawa *et al.*, 2018) or by providing the bioremediation of contaminated soil (Corretto *et al.*, 2016; Zhao *et al.*, 2016).

In this study, we report the phylogenetic position of our three novel strains (HY052^T, HY050, and HY045) and propose that they represent a new species of the genus *Agromyces* with a polyphasic taxonomic approach.

Materials and Methods

Isolation of bacterial strains and culture conditions

A novel bacterial species, *Apibacter raozihei* (Huang *et al.*, 2020), was recently isolated from *Hipposideros* and *Taphozous* spp. bat feces freshly collected between July and September in 2011 by Prof. Zhengli Shi's team (Wuhan Institute of Virology, Chinese Academy of Sciences). In this study, we described the isolation and characterization of another three strains (HY052^T, HY050, and HY045) recovered from the same batch of bat fecal samples. Each fecal sample (1.0 g) was suspended and diluted with 1,000 μl of sterile water, and then 150 μl of the diluents was spread onto brain heart infusion (BHI) agar medium and incubated at 10–30°C for 24–72 h. Different colonies that emerged on the plates were selected, purified, and stored. Bacterial strains were subjected to 16S rRNA gene sequence analysis, and three of them (HY052^T, HY050, and HY045) were selected for polyphasic characterization. Strain HY052^T (deposited in the China General Microbiological Culture Collection Center [CGMCC 1.17175] and Japan Collection of Microorganisms [JCM 33695]) was recovered from a *Taphozous* spp. fecal sample collected in Chongzuo City (22°20′54″N, 106°49′20″E) of Guangxi province while strains HY050 and HY045 were isolated from *Hipposideros* spp. feces in the Changshou District (30°02′15″N, 107°07′4″E) of Chongqing City, two locations a thousand miles apart.

Phenotypic and biochemical analysis

Phenotypic characteristics were assessed using a variety of tests on the three isolates, which were aerobically cultured in BHI medium at 28°C for 24–48 h. Gram-stain were performed with a kit according to the manufacturer's instructions. Morphological features of Gram-stained cells were observed using light and electron microscopy. Bacterial growth was monitored every 8 h for 144 h at different temperatures (4, 15, 25, 28, 30, 35, or 45°C), with a pH ranging from 3.0–12.0 (at increments of 1 pH unit adjusted with 1 M HCL or NaOH) and various concentrations of NaCl (0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 7.0, 8.0, 9.0, 10.0, or 11.0% [w/v]). The catalase reaction was determined by an ID Color Catalase kit and oxidase activity was tested with the oxidase reagent. BHI agar best supported growth, compared to plates of Reasoner's 2A Agar, Nutrient Agar, and Mueller-Hinton Broth medium. API 50CH, API 20E strips, and the API ZYM system (bioMérieux) were used following the manufacturer's instructions to test the carbon utilization, acid production and enzyme activity of substrates by strains that were grown on BHI for 2 days at 28°C; for carbohydrate fermentation tests, bacteria were suspended in API 50 CHB/E medium.

Phylogenetic analysis and whole-genome sequencing

To genetically identify the isolates, 16S rRNA genes of strains HY052^T, HY050, and HY045 were separately amplified by PCR using the universal primers 27F and 1429R (Delgado *et al.*, 2006; Jin *et al.*, 2013). The sequences of the amplified products were determined and compared with their corresponding sequences in the EzBioCloud database (Yoon *et al.*, 2017a) (http://www.ezbio-cloud.net) and GenBank (http://www.ncbi.nlm.nih.gov/blast) to locate its taxonomic position. After aligning the 16S rRNA gene sequences of the type strains in the genus *Agromyces* using the CLUSTAL_W program (Chen *et al.*, 2007), phylogenetic trees were constructed with three algorithms, Neighbor-Joining (NJ), Maximum-Likelihood (ML), and Maximum-Parsimony (MP), using MEGA X software (www.megasoftware.net) (Kumar *et al.*, 2016) with a bootstrap analysis of 1,000 replications (Kimura, 1980). Furthermore, the genomic DNA of the isolates (HY052^T, HY050, and HY045) and their closest relatives were extracted with the Wizard Genomic DNA Purification kit (Promega) according to the manufacturer's instructions.

The complete genome of strain HY052^T was sequenced with the PacBio single Molecule Real-Time technology (Berlin *et al.*, 2015), assembled *de novo* and analyzed using the HGAP v4 application (Pacific Biosciences, SMRT Link 6.0). Meanwhile, the rest of the strains (HY050, HY045, and all closely related strains) were sequenced on an Illumina sequencing platform. Genome sequence data were used for the following analyses. To verify their phylogenetic position within the genus *Agromyces*, a phylogenomic tree (Wee *et al.*, 2017) was constructed upon concatenation and alignment of the core genes in MAFFT, using the approximate ML algorithm in FastTree (Price *et al.*, 2009), then processed and edited it in Dendroscope 3 (Huson and Scornavacca, 2012). The 296 core genes were determined based on clustering 25 genomes (those of the three isolates, and 21 of the genus *Agromyces* plus *Arthrobacter globiformis* NBRC12137 as the outgroup) using CD-HIT with a 0.4 protein sequence identity threshold.

With the OrthoANIu algorithm (Yoon *et al.*, 2017b) and GGDC 2.1 (Meier-Kolthoff *et al.*, 2013), the ANI and dDDH values of these three isolates were calculated between them and the other related species in the genus *Agromyces*. After an alignment of the protein sequences by DIAMOND (Buchfink *et al.*, 2015), the phylogenetic classification of protein sequences was analyzed against the cluster of orthologous groups of proteins (COG) database (Galperin *et al.*, 2015). The threshold of protein identity was set at 0.4. Carbohydrate-active enzymes were predicted using the Carbohydrate-Active enZYmes database (CAZy) (Cantarel *et al.*, 2009). KEGG (Kyoto Encyclopedia of Genes and Genomes) pathway annotation was performed to analyze the function of target genes (Kanehisa *et al.*, 2016). After clustering by USEARCH 11 (Edgar, 2010), the Bacterial Pan Genome Analysis pipeline (BPGA) was used to analyze the pan-genome orthologous groups (POGs) of 24 available *Agromyces* genomes (those of the three isolates, and 21 of the genus *Agromyces*) with a 0.4 identity threshold for amino acid sequences. The Circos (http://circos.ca/) (Wyatt *et al.*, 2013) was used to build a circular genome map.

Chemotaxonomic analyses

Cellular fatty acids were extracted from saponified and methylated material with the Sherlock automatic bacterial identification system and analyzed with gas chromatography. Respiratory quinones were tested using reversed phase high

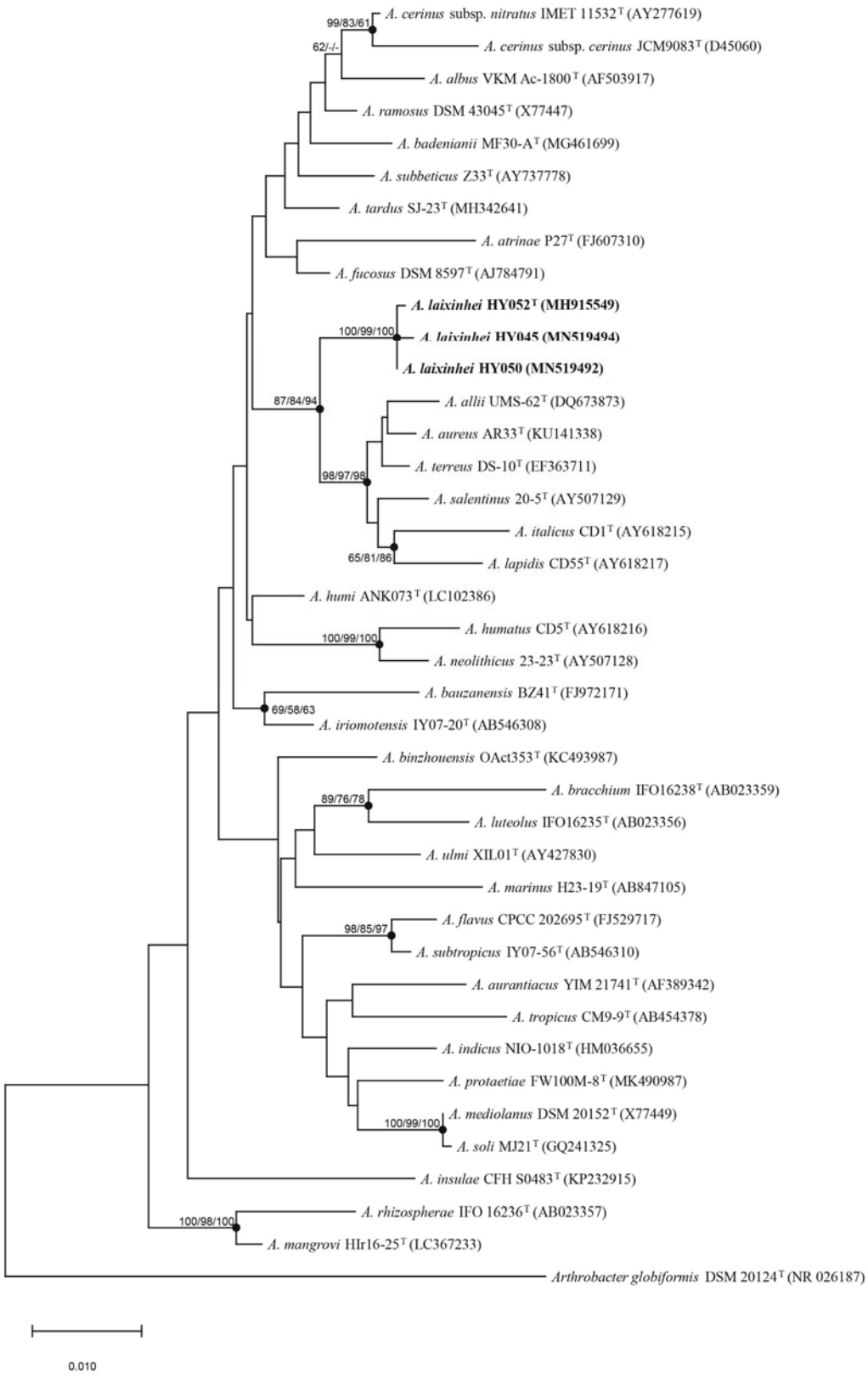

Fig. 1. Neighbor-joining tree based on the 16S rRNA gene sequences from *Agromyces* species. The tree shows the taxonomic position of strains HY052^T, HY050, and HY045 in the *Agromyces* genus. Filled circles mean that corresponding nodes are also recovered in trees generated using ML and MP methods. The numerals (values > 50% are noted) indicate percentage of bootstrap samplings as derived from 1,000 replications. Bar, 0.010 substitutions per nucleotide position. The sequence of *Arthrobacter globiformis* NBRC 12137^T serves as an outgroup.

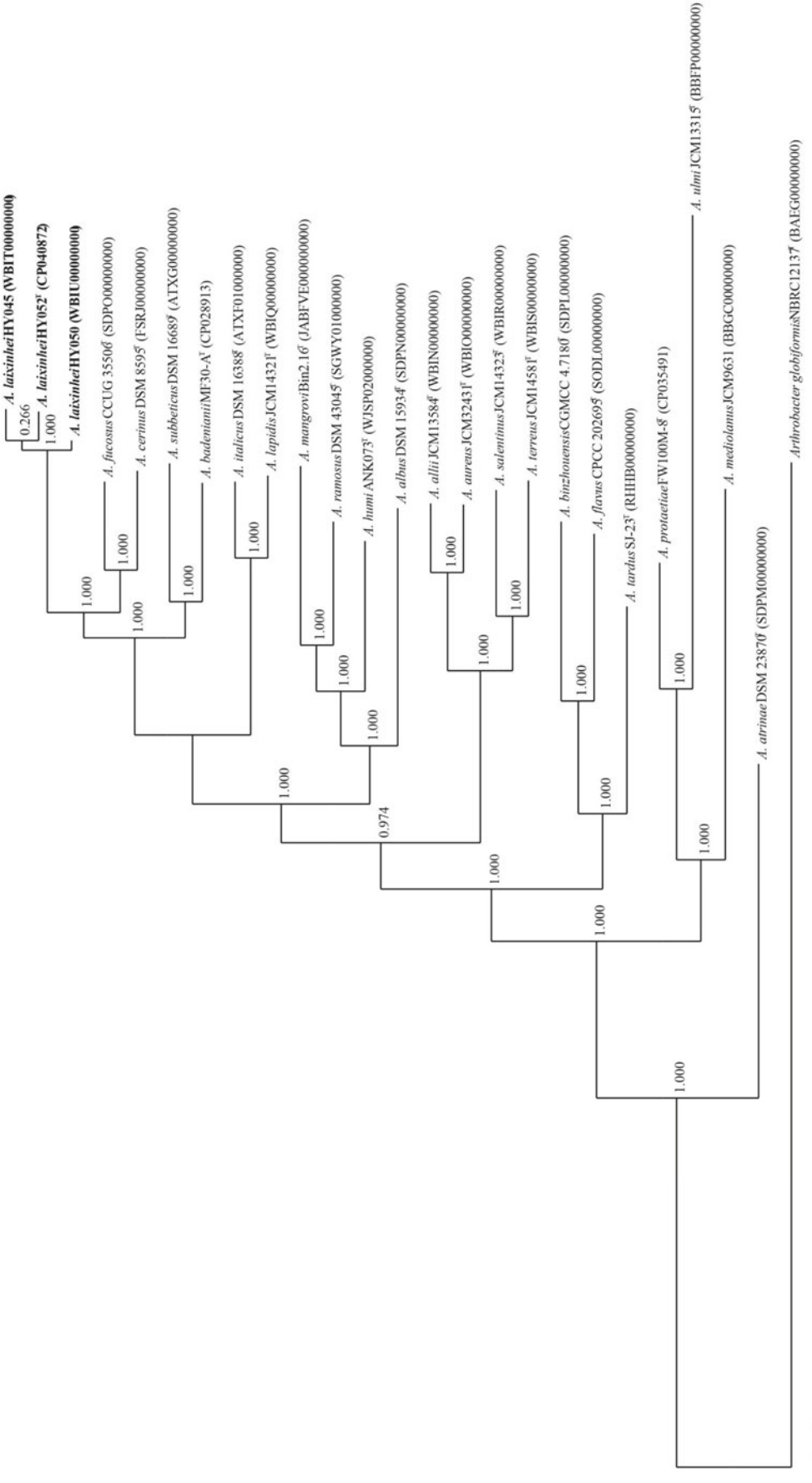

Fig. 2. Phylogenomic tree based on 296 core genes sequences of *Agromyces* species and closely related strains. The numbers in the tree indicate the reliability of each split with the Shimodaira-Hasegawa test calculated from 1,000 resamples. The sequence of *Arthrobacter globiformis* NBRC 12137^{T} serves as an outgroup.

performance liquid chromatography (HPLC). Polar lipids were analyzed with two-dimensional thin-layer chromatography (TLC). Peptidoglycan amino acids were measured with a Hitachi-8900 high speed amino acid analyzer. Cell wall sugars were examined with an established method (Hasegawa *et al.*, 1983).

Nucleotide sequence accession numbers

The GenBank accession numbers of strains HY052^T, HY050, and HY045 are MH915549, MN519492, and MN519494 (for the 16S rRNA gene) as well as CP040872, WBIU00000000, and WBIT00000000 (for the genome), respectively. Six Supplementary data Figures and four Supplementary data Tables are available with the online Supplementary Materials.

Results and Discussion

Phylogenetic analysis

BLAST analysis of the near full-length 16S rRNA gene sequences (1,491 bp) on the EzBioCloud database showed that the strain HY052^T were closely related to *A. terreus* DS-10^T (98.5%, similarity), *A. aureus* AR33^T (98.3%), *A. salentinus* 20-5^T (98.0%), and *A. allii* UMS-62^T (98.0%). By contrast, our isolates shared high similarity with each other (HY052^T *vs.* HY050, 99.9%; HY052^T *vs.* HY045, 99.8%; HY050 *vs.* HY045, 100%). The topologies of the three trees generated using NJ, ML, and MP methods were similar (Fig. 1, Supplementary data Figs. S1 and 2), with the three unknown isolates clustering together to form a distinct branch affiliated with members of the genus *Agromyces*. The closest cluster near that novel branch included six species of the genus *Agromyces* (*A. terreus* DS-10^T, *A. aureus* AR33^T, *A. salentinus* 20-5^T, *A. allii* UMS-62^T, *A. lapidis* CD55^T, and *A. italicus* CD1^T), are similar to the 16S rRNA gene BLAST results. The phylogenomic tree based on 296 core genes (Supplementary data Table S1) illustrated that our three isolates cluster together, closest to *A. cerinus* VKM Ac-1340^T and *A. fucosus* VKM Ac-1345^T but separated distantly from other *Agromyces* species. These results further support a novel species status for our isolates (Fig. 2). Thus, according to the above results, *A. allii* UMS-62^T (= JCM 13584^T), *A. aureus* AR33^T (= JCM 32431^T), *A. cerinus* VKM Ac-1340^T (= JCM 9083^T), *A. fucosus* VKM Ac-1345^T (= CCUG 35506^T), *A. italicus* CD1^T (= JCM 14320^T), *A. lapidis* CD55^T (= JCM 14321^T), *A. salentinus* 20-5^T (= JCM 14323^T), and *A. terreus* DS-10^T (= JCM 14581^T) were selected for further comparative tests and purchased from collection centers (Japan Collection of Microorganisms, Ibaraki, Japan, JCM; Culture Collection of University of Göteborg, Sweden, CCUG). ANI and dDDH values between strain HY052^T and the other two isolates were 99.1–99.2% and 92.3–93.4%, respectively, indicating that they belong to the same species. Moreover, the ANI and dDDH values between strains HY050 and HY045 were 98.9% and 90.8%, suggesting that the two isolates were clonally different. By contrast, the ANI values between strain HY052^T and other *Agromyces* species ranged from 79.3 to 87.9% (Table 1), lower than the 95–96% threshold for delineating species (Richter and Rossello-Mora, 2009; Chun *et al.*, 2018). Similarly, the dDDH values between strain HY052^T and other *Agromyces* species ranged from 22.5 to 31.0% (Table 1), also much lower than the threshold of 70% (Wayne, 1988). Both ANI and dDDH results (Table 1) demonstrated that our isolates represent a novel species in the genus *Agromyces*.

Whole genome characteristics and functional gene annotation

SMRT sequencing of strain HY052^T yielded a circular chromosome (no plasmid sequence was present) of 3,437,203 bp in size, containing 3,235 genes (including 45 tRNA and six rRNA genes) with a DNA G + C content of 69.0 mol%, which is similar to the genomic characteristics of strains HY050 and HY045 (Supplementary data Table S2). However, the G +

Table 1. The 16S rRNA gene similarity, dDDH, ANI results between HY052^T and related strains

Species	16S rRNA similarity (%)	Whole genome GenBank accession No.	G + C (mol%)	dDDH (%)	ANI (%)
HY052^T	-	CP040872	69.0	-	-
HY050	99.9	WBIU00000000	69.0	92.3	99.1
HY045	99.8	WBIT00000000	68.9	93.4	99.2
A. terreus DS-10^T	98.5	WBIS00000000	71.1	23.0	80.1
A. aureus AR33^T	98.3	WBIO00000000	70.4	23.0	85.1
A. salentinus 20-5^T	98.0	WBIR00000000	70.9	22.5	79.3
A. allii UMS-62^T	98.0	WBIN00000000	70.8	23.1	79.7
A. iriomotensis IY07-20^T	97.9	-	-	-	-
A. ramosus KCC A-0108^T	97.9	SGWY00000000	71.3	23.6	85.1
A. lapidis CD55^T	97.7	WBIQ00000000	70.9	23.9	81.1
A. marinus H23-8^T	97.7	-	-	-	-
A. neolithicus 23-23^T	97.6	-	-	-	-
A. tardus SJ-23^T	97.6	RHHB00000000	71.8	23.4	80.1
A. italicus CD1^T	97.5	WBIP00000000	70.2	23.8	85.3
A. humatus CD5^T	97.5	-	-	-	-
A. subbeticus Z33^T	97.2	ATXG00000000	69.1	27.0	86.3
A. cerinus VKM Ac-1340^T	97.2	FSRJ00000000	70.0	31.0	87.9
A. fucosus VKM Ac-1345^T	97.1	SDPO00000000	70.1	30.2	87.5

- indicates that a public genome does not exist in GenBank, so there is no G + C content, dDDH, and ANI value.

C content range (68.9–69.0 mol%) of our three strains was slightly lower than those of other related species (70.0–71.1 mol%). A pan-genome analysis showed that the strains HY-052^T, HY050, and HY045 have 73, 113, and 104 unique genes as well as 2,654, 2,665, and 2,725 accessory genes, respectively (Supplementary data Table S3). In particular, the 73 unique genes to strain HY052^T are notably fewer than those unique to other strains in the genus *Agromyces* (115–1880 genes). Of these 73 unique genes, 27 are assumed to encode for unique hypothetical proteins, including deadenylate cyclase, polyketide cyclase, bacterial regulatory proteins and others.

The distribution of genes in the KEGG functional categories showed that our strains and those of the eight closely related species possess many similar pathways in carbohydrate metabolism and amino acid metabolism (Supplementary data Table S4). Of note, the strain HY052^T has a complete shikimate pathway, almost the exclusive biochemical way to obtain aromatic compounds. This pathway enables it to utilize phosphoenolpyruvate and erythrose-4P as raw material to produce not only aromatic amino acids but also aromatic precursors for biosynthesis of secondary metabolites (Lai *et al.*, 2017). Thus, researchers are striving to leverage the shikimate pathways of microbial systems for bulk and fine chemical production (Noda and Kondo, 2017; Averesch and Kromer, 2018).

A CAZy analysis of 135 genes in the strain HY052^T genome that encode carbohydrate active enzymes revealed that 64 (47.4%) are predicted to be glycoside hydrolases (GH), 39 (28.9%) to be glycosyltransferases (GT), and 28 (20.7%) to be carbohydrate-binding modules (CBM), a profile within the range of its closest relatives (43.1–52.6% for GH, 23.7–33.9% for GT, and 15.9–20.9% for CBM). Among all carbohydrate active enzymes, GH are the most abundant for the purposes of degrading polysaccharides into smaller products (Berlemont and Martiny, 2016). Strain HY052^T has genes encoding up to 29 GH-like enzyme families, consistent with its biochemical profile (Table 2) of being able to utilize β-galactosidase (GH1), α-glucosidase (GH13), β-glucosidase (GH3), and N-acetyl-β-glucosaminidase (GH84).

A total of 2,759 (HY052^T), 2,782 (HY050), and 2,815 (HY-045) genes were assigned in the COG database. Among the 23 COG functional categories (Supplementary data Fig. S3) the genomes of strains HY052^T, HY050, and HY045 were comprised of roughly 10% of genes belonging to the four largest categories (Supplementary data Fig. S4), i.e., amino acid transport and metabolism (10.0/10.4/10.0%), carbohydrate transport and metabolism (9.1/9.0/9.2%), transcription (10.1/9.8/9.9%), and general functions (10.3/10.0/10.3%). These strains had profiles similar to their closest neighbors (9.9–10.9%, 9.4–11.2%, 8.8–10.8%, and 9.9–11.5%).

Phenotypic and biochemical characteristics

The strain HY052^T cells were aerobic, Gram-stain-positive, oxidase-negative, catalase-positive, non-spore-forming, non-flagellated rods 0.3–0.7 × 0.3–3.0 μm in size (Supplementary data Fig. S5) that formed circular, smooth, and light-yellow colonies. The growth of strains HY052^T, HY050, and HY045 occurred at 15 and 35°C (28°C was optimum) but not at 4 or 45°C. Growth also occurred at pH 5.0 and 9.5 (6.0–7.5 was optimum) but not at pH 4.0 or 10.0. Finally, growth oc-

Table 2. Phenotypic characteristics that differentiate *Agromyces laixinhei* sp. nov. from phylogenetically related species

Strains: 1, *A. allii* JCM 13584^T; 2, *A. italicus* JCM 14320^T; 3, *A. lapidis* JCM 14321^T; 4, *A. salentinus* JCM 14323^T; 5, *A. terreus* JCM 14581^T; 6, *A. aureus* JCM 32431^T; 7, *A. fucosus* CCUG 35506^T; 8, *A. cerinus* JCM 9083^T. All strains were grown in BHI medium, 28°C.
+, Positive; -, negative; w, weakly positive; NA, not available.

Characteristic	HY052^T	HY050	HY045	1	2	3	4	5	6	7	8
Optimal temperature (°C)	28	28	28	30[a]	28[b]	28[b]	20–28[c]	26[d]	28[e]	26–30[f]	28[f]
Halotolerance (NaCl; %, w/v)	4.5	4.5	4.5	3[a]	4[b]	4[b]	4[c]	6[d]	3[e]	4[f]	7[f]
Optimal pH range	6.0–7.5	6.0–7.5	6.0–7.5	6.5–7.5[a]	5–9.5[b]	5–9.5[b]	NA	4.5–10.5[d]	6.5–7.5[e]	NA	NA
Major menaquinone	12	12	12	11,12[a]	12,13[b]	12,13[b]	12,11[c]	10,11,12[d]	11,10,12[e]	12[f]	NA
Enzyme activity:											
Esterase (C4)	-	-	-	-	+	+	w	-	-	-	+
Valine arylamidase	-	-	-	-	-	w	-	-	w	w	-
Cystine arylamidase	-	-	-	-	-	w	-	-	w	w	-
Acid phosphatase	-	-	-	-	-	-	-	-	w	-	w
β-Galactosidase	-	-	-	w	w	-	+	-	+	w	+
N-Acetyl-β-glucosaminidase	w	w	+	-	-	-	-	-	-	-	-
Gelatinase	-	-	-	-	+	-	-	-	-	+	-
Citrate utilization	+	w	w	-	-	-	-	w	-	-	-
Acid production from:											
D-Galactose	-	-	-	-	w	+	+	+	+	w	+
D-Glucose	-	-	-	+	+	+	+	+	+	+	+
L-Rhamnose	-	-	-	+	-	-	+	+	+	w	+
D-Mannitol	+	+	+	-	-	-	-	-	w	-	-
D-Turanose	-	-	-	-	w	-	w	w	w	w	w
Potassium gluconate	+	w	w	-	w	-	-	-	-	-	-
Potassium 5-keto-gluconate	+	w	w	-	-	-	-	-	-	-	-

[a] data from (Jung *et al.*, 2007); [b] data from (Jurado *et al.*, 2005b); [c] data from (Jurado *et al.*, 2005a); [d] data from (Yoon *et al.*, 2008); [e] data from (Corretto *et al.*, 2016); [f] data from (Zgurskaya *et al.*, 1992).

curred in the presence of 0 to 4.5% (w/v) NaCl (Table 2). The differential biochemical characteristics of strain HY052^T from closely related species are summarized in Table 2. In addition, all species (HY052^T, HY050, HY045, and all eight related type strains) produced acid from polychrome esculine citrate, maltose, starch, and glycogen but not from amygdalin, arabinose, D-adonitol, D-arabitol, D-fucose, D-sorbitol, D-tagatose, dulcitol, D-xylose, erythritol, glucose, inositol, inositol, L-arabitol, L-sorbose, L-xylose, melezitose, melibiose, methyl α-D-glucopyranoside, methyl β-D-xylopyranoside, potassium 2-keto-gluconate, sorbitol rhamnose, sucrose, or xylitol. Moreover, all tested species were positive for naphthol-AS-BI-phosphohydrolase, β-galactosidase, and acetoin production but negative for alkaline phosphatase, lipase (C14), trypsin, α-chymotrypsin, β-glucuronidase, α-mannosidase, α-fucosidase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urea hydrolysis, deaminase, or indole production.

Chemotaxonomic characteristics

As detailed in Table 3, the major cellular fatty acids of strains HY052^T, HY050, and HY045 were *anteiso*-$C_{15:0}$ (36.9%, 37.2%, 38.0%) and *anteiso*-$C_{17:0}$ (31.8%, 35.4%, 35.7%), in contrast to the lower average proportion of *anteiso*-$C_{17:0}$ in the related type strains (25.9%). MK-12 is the sole menaquinone in strain HY052^T, distinctively different from most *Agromyces* species with more than one menaquinone, either predominant or minor (Table 2). The major polar lipids (Supplementary data Fig. S6) were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), and unidentified glycolipids (GL1-2). Rhamnose, ribose, and glucose were the primary cell wall sugars for strain HY052^T. The peptidoglycan amino acids in HY052^T included alanine, glutamic, glycine, and 2,4-diaminobutyric acid.

Taxonomic conclusion

The topologies based on core genes and 16S rRNA gene sequences of strains HY052^T, HY050, and HY045 using NJ, ML, and MP methods (Fig. 1, Supplementary data Figs. S1 and 2) indicated that they are members of the genus *Agromyces*, while these three unknown strains clustered together and were distinguishable from its closest phylogenetic neighbors (*A. terreus* DS-10^T, *A. aureus* AR33^T, *A. salentinus* 20-5^T, *A. allii* UMS-62^T, *A. lapidis* CD55^T, *A. italicus* CD1^T, *A. cerinus* VKM Ac-1340^T, and *A. fucosus* VKM Ac-1345^T). Moreover, strains HY052^T, HY050, and HY045 can were distinguished from the related type strains and each other by ANI and dDDH (Table 1), along with other differences among gene similarity, and the genome sequence characteristics of strain HY052^T as well as the related type species (Table 1 and Supplementary data Table S2). The growth temperature, pH, and NaCl range of strains HY052^T, HY050, and HY045 were like other related strains in the genus *Agromyces*. However, in sharp contrast to the results from their closest relatives, only these three strains were negative for β-galactosidase activity and acid production from D-glucose, in addition to being positive for N-acetyl-β-glucosaminidase activity and acid production from potassium 5-keto-gluconate (Table 2). Despite the homogeneous biochemical profile of our three novel isolates (Table 2), there are some small variations: strain HY052^T was positive for citrate utilization as well as acid production from both potassium gluconate and potassium 5-keto-gluconate whereas the other two strains were only weak positives for all these three test. Conversely, strain HY045 was positive for N-acetyl-β-glucosaminidase while the other two were weak positive.

Based on the phylogenetic, physiological, and chemotaxonomic characterizations, we suggest that strains HY052^T, HY050, and HY045 represent a novel species of the genus *Agromyces*, for which the name *Agromyces laixinhei* sp. nov. is proposed.

Description of *Agromyces laixinhei* sp. nov.

Agromyces laixinhei (lai.xin.he´i. N.L. gen. n. *laixinhei* of Dr. Xin-He Lai, for his persistent enthusiasm in microbiology, significant contribution to *Francisella* pathogenesis research and unwavering commitment to bacterial taxonomy).

Cells are aerobic, Gram-stain-positive, catalase-positive, nonmotile, rod-shaped, 0.3–0.7 × 0.3–3.0 μm in size, forming circular, smooth and light-yellow colonies. Growth occurs after 24–48 h at temperatures between 15 and 35°C (optimum, 28°C) on BHI plates with an optimal pH between 6.0 and 7.5. Growth is modest in the presence of 4.5% (w/v) NaCl (optimum, 0.5–1.0%). Oxidase negative. Main fatty acids are *anteiso*-$C_{15:0}$ and *anteiso*-$C_{17:0}$. MK-12 is the sole menaquinone. Polar lipids comprise DPG, PG, and GL1-2. Rhamnose, ribose and glucose are the primary cell wall sugars. The major peptidoglycan amino acids are alanine, glutamic acid, glycine, and 2,4-diaminobutyric acid. Acid is produced from D-mannitol, D-xylose, glycerol, glycogen, L-arabinose, maltose, polychrome esculine citrate, potassium 5-keto-gluconate (weakly by strains HY050 and HY045), potassium gluconate (weakly by strains HY050 and HY045), salicin, and starch. Weak acid production from arbutin, D-

Table 3. **Fatty acid compositions of *Agromyces laixinhei* sp. nov. and closely related strains**
Strains: 1, *A. allii* JCM13584^T; 2, *A. italicus* JCM 14320^T; 3, *A. lapidis* JCM 14321^T; 4, *A. salentinus* JCM 14323^T; 5, *A. terreus* JCM14581^T; 6, *A. aureus* JCM 32431^T; 7, *A. fucosus* CCUG 35506^T; 8, *A. cerinus* JCM 9083^T; ND, not detected.

Fatty acid	HY052^T	HY050	HY045	1	2	3	4	5	6	7	8
iso-$C_{15:0}$	11.0	10.9	9.3	6.2	5.7	6.9	11.1	16.4	6.6	11.7	8.7
anteiso-$C_{15:0}$	**36.9**	**37.2**	**38.0**	**33.9**	**39.5**	**35.0**	**31.7**	**28.7**	**42.3**	**42.6**	**42.9**
anteiso-$C_{15:1}$ A	3.4	ND	1.2	6.3	6.3	6.7	6.1	3.6	3.7	0.4	5.5
iso-$C_{16:0}$	9.8	9.9	9.2	20.1	20.4	22.4	13.9	16.0	19.9	11.5	9.8
iso-$C_{17:0}$	5.1	5.5	4.3	2.9	1.2	1.9	4.9	8.1	2.2	3.7	3.1
anteiso-$C_{17:0}$	**31.8**	**35.4**	**35.7**	**27.1**	**24.3**	**24.5**	**28.4**	**23.2**	**22.7**	**27.6**	**27.6**

Bolded numbers indicate the composition of the predominant fatty acid.

arabinose, D-fructose, D-mannose, and trehalose. However, acid is not produced from amygdalin, cellobiose, D-adonitol, D-arabitol, D-fucose, D-galactose, D-glucose, D-ribose, D-sorbitol, D-tagatose, D-turanose, dulcitol, D-xylose, erythritol, gentiobiose, inositol, inulin, lactose, L-arabitol, L-fucose, L-rhamnose, L-sorbose, L-xylose, melezitose, melibiose, methyl β-D-xylopyranoside, methyl α-D-glucopyranoside, methyl α-D-mannopyranoside, N-acetylglucosamine, potassium 2-keto-gluconate, raffinose, sucrose, or xylitol. Positive for leucine arylamidase, naphthol-AS-BI-phosphohydrolase, α-glucosidase, and N-acetyl-β-glucosaminidase (weakly in strains HY052^T and HY050). Weakly positive for β-glucosidase. Negative for acid phosphatase, alkaline phosphatase, cystine arylamidase, esterase (C4), esterase lipase (C8), lipase (C14), trypsin, valine arylamidase, α-chymotrypsin, α-fucosidase, α-galactosidase, α-mannosidase, β-galactosidase, or β-glucuronidase. Positive for citrate utilization (weakly by strains HY050 and HY045) and acetoin production but negative for tryptophan deaminase, arginine dihydrolase, lysine decarboxylase, ornithine decarboxylase, H_2S production, urea hydrolysis, deaminase, indole production or gelatinase. Also negative for utilization of glucose, mannitol, inositol, sorbitol, rhamnose, sucrose, melibiose, amygdalin, or arabinose.

The type strain HY052^T (= CGMCC 1.17175^T = JCM 33695^T), isolated from bat fecal sample of the *Taphozous* spp. in the Guangxi province of China, has a DNA G + C content of 69.0 mol%. The GenBank accession numbers of strains HY-052^T, HY050, and HY045 are MH915549, MN519492, and MN519494 (for the 16S rRNA gene sequence), as well as CP040872, WBIU00000000, and WBIT00000000 (for the-whole genome), respectively.

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Conflict of Interest

The authors declare that there are no conflicts of interest.

Ethical Statement

The ethical practice was approved by Ethical Committee of the National Institute fo Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention (# ICDC-2016004).

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