Preliminary Communication

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Whole-genome analysis of *Pandoraea* species strains from cystic fibrosis patients

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Aim: Genetic characterization of *Pandoraea* strains recovered from cystic fibrosis patients. **Materials & methods:** The whole-genome sequence of 12 *Pandoraea* strains was determined using Illumina technology. The position of the strains within the genus *Pandoraea* was analyzed using selected partial gene sequences, core genome multi-locus sequence typing and average nucleotide identity analysis. Furthermore, the sequences were annotated. **Results:** The results show that some strains previously identified as *Pandoraea pnomenusa*, *Pandoraea sputorum*, *Pandoraea oxalativorans* and *Pandoraea pulmonicola* belong to novel species. The strains did not harbor acquired antibiotic resistance genes but encoded an OXA-type ß-lactamase. **Conclusion:** The taxonomy of the genus *Pandoraea* needs to be revised.

First draft submitted: 7 February 2019; Accepted for publication: 14 October 2019; Published online: 25 November 2019

Keywords: cystic fibrosis • *Pandoraea* • taxonomy • WGS

Currently, up to ten species of *Pandoraea* have been described: *Pandoraea apista*, *Pandoraea faecigallinarum*, *Pandoraea norimbergensis*, *Pandoraea oxalativorans, Pandoraea pnomenusa*, *Pandoraea pulmonicola*, *Pandoraea sputorum*, *Pandoraea terrae*, *Pandoraea thiooxydans* and *Pandoraea vervacti*. *Pandoraea* spp. strains are usually found in soil and water but at least four species (*P. apista*, *P. pnomenusa*, *P. pulmonicola* and *P. sputorum*) have also been isolated from respiratory samples from cystic fibrosis (CF) patients [1–10]. Moreover, in at least one case, mortality was attributed to a lung infection of a CF patient with *P. pnomenusa* [11]. Despite their role in CF, their potential biotechnology applications and the availability of 17 whole-genome sequences (WGS), relatively little is known about *Pandoraea* spp.

The aim of the study was to classify 12 *Pandoraea* strains obtained from 11 CF patients and a non-CF patient and their relationship to other species in this genus as taxonomically correct identification is important to study the role of different bacterial species in cystic fibrosis and other diseases.

Materials & methods

Strains

A total of 12 *Pandoraea* spp. strains were sequenced; 11 strains were obtained from CF patients in Northern Ireland (n = 2), Spain (n = 6) and The Netherlands (n = 3), and one (16–535164) from a Dutch patient with chronic obstructive pulmonary disease (Supplementary Table 1).

Data on stage of disease and *Pseudomonas aeruginosa* co-infection is not available for the anonymized Spanish and UK strains. These strains were collected from CF patients in multicenter studies, but further patient data were anonymized. The three CF patients from The Netherlands were all adults with late-stage disease and were co-infected with *P. aeruginosa*. The Dutch chronic obstructive pulmonary disease patient also had late-stage disease and had previously been colonized with *P. aeruginosa,* but not at the time *Pandoraea* was cultured. All four Dutch isolates were recovered from sputum samples.

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Samples and patient data were collected in compliance with the Declaration of Helsinki ICH-GCP, the Declaration of Tapei regarding Health Databases and Biobanks and with local and European regulations for collection and handling of patient data. Since the study concerned retrospectively collected anonymized patient data and bacterial strains, informed consent at the individual patient level was not required for this study. In addition, the Spanish and UK strains were collected in accordance with their local ethics guidelines and described in prior studies. Results from these studies have been previously published [12–14]. Furthermore, in The Netherlands, use and analysis of bacterial strains with anonymized patient data do not require approval from institutional review boards/ethics committees.

Whole genome sequencing

Bacterial DNA was purified using the Qiacube with the DNeasy Blood & Tissue kit with the enzymatic lysis protocol (Qiagen, CA, USA) and used to prepare a library for sequencing with the MiSeq or Nextseq (Illumina, CA, USA) platforms, using the NexteraXT library prep kit (Illumina). Contigs were assembled with SPAdes genome assembler v.3.6.2. The assembled contigs were analyzed for the presence of resistance genes by ResFinder (last accessed 28 June 2018) from the Center for Genomic Epidemiology (DTU, Copenhagen, Denmark) [15].

Core genome multi-locus sequence typing

In total, 17 public genomes were used to create a core genome multi-locus sequence typing (cgMLST) scheme for the *Pandoraea* genus (Supplementary Table 2). The WGS of *P. apista* TF81F4 (NZ_CP010518.3) was used as the reference genome. Other public genomes were aligned to find homologous genes using Basic Local Alignment Search Tool (BLAST), where a query gene had a homolog if it completely overlaps with the reference gene and had an identity of at least 90% (BLAST version 2.2.12 [16]).

Average nucleotide identity

Average nucleotide identity (ANI) among the genomes was calculated using ANIb algorithm of pyani tool [17], which uses nucleotide BLAST alignment for whole genome alignment. Genomes are fragmented into genomic fragments of 1020 bases long and after pairwise alignments of all fragments of each genome, ANI was calculated as the percentage of nucleotide identity for matching regions of all genomes. In biclustering analysis of ANI scores, complete linkage was used as a hierarchical clustering method with the Euclidean distance metric. Heatmap of all genomes was generated using biclustering, where a color scale bar shows the pairwise ANI for values below 75% with gray color and values above 75% are shown in a color range starting from blue (ANI 75%) through white to red (ANI 100%). In the heatmap, each *Pandoraea* species is shown in a different color next to a leaf node of a tree.

Phylogenetic trees of partial sequences

Neighbor-joining trees of the bla_{OXA} genes and partial *gyrB* and 16S rDNA gene sequences were generated with Clustal Omega (https://www.ebi.ac.uk/Tools/msa/clustalo/) and MEGA X [18–21]. KODON (Applied Maths, Belgium) was used for whole genome alignments. The accession numbers of the sequences used are listed in Supplementary Table 2.

Genome annotation

Annotation of the WGS of the 12 strains was performed with Rapid Annotation using Subsystem Technology (RAST) [22].

Data availability

This whole-genome shotgun project has been deposited at European Nucleotide Archive (ENA) under the project number PRJEB30961. The version described in this paper is the first version.

Results & discussion

Species identification

Three strains, 16–535641, 16–535642 and 16–543519 were not identified to species level before whole genome sequencing using MALDI-TOF, because of insufficient scores. BLAST alignment of the WGS against the GenBank database identified the first two strains as *P. apista* and the third as *P. pulmonicola*. Identification of the other strains are included in Supplementary Table 1.

Genome characteristics

The genomes were sequenced with an average coverage of 36–78-times and the number of contigs ranged from 40–161 (Supplementary Table 1). The sequencing results show that the total length of the genomes sequenced correlated with the species: *P. apista* has a genome size of approximately 5.4–5.5 Mb, *P. sputorum* 5.6–5.9 Mb, *P. pnomenusa* approximately 5.4 MB and *P. pulmonicola* approximately 4.8 Mb with the exception of 16–535646, which had a total assembled sequence length of 5.8 Mb (Supplementary Table 1). The GC content also correlated with species, except for *P. pulmonicola* 16–535646, which had a GC content of 64.32% compared with 66.06–66.13% for the other strains of this species (Supplementary Table 1). These discrepancies for 16–535646 suggest that it may belong to a different species than *P. pulmonicola*.

cgMLST

A cgMLST scheme for the *Pandoraea* genus with 342 core genes was generated. Analysis of the 12 WGS against this scheme showed that strains belonging to different species clustered together (Supplementary Figure 1). However, deep branches are present between *P. pulmonicola* 16–535646 and the other three strains and also between *P. sputorum* 16–535640 and 16–540164 compared with the two other strains. ANI analysis of the sequences confirmed that *P. pulmonicola* 16–535646 was different from the other *P. pulmonicola* strains (Supplementary Figure 2). It also confirmed that the four *P. sputorum* strains fall into two related groups.

Although WGS are not available for all ten species, a cgMLST of available sequences was generated (Figure 1). The length of the branches again suggests that 16–535646 does not belong to *P. pulmonicola* but also that *P. pulmonicola* DSM 16583 is related to this strain. WGS alignment shows that there is no difference in gene content between these two strains. However, strain 16–535646 came from Spain whereas the other one was isolated in Canada. The *P. sputorum* strains belong to two different groups, possibly subspecies or may be even different species. However, strains 16–535647 and DSM 21091 are closely related. WGS alignment shows no difference in gene content between the strains, but DSM 21091 was isolated in the USA and the other strain in Spain. *P. oxalativorans* also clusters closely with *P. sputorum*.

The cgMLST data show that *P. pnomenusa* strains 6399 and 7641 cluster differently from the other strains of this species in this analysis. Either these two strains or the three other strains do not belong to *P. pnomenusa*. However, *P. pnomenusa* strains 16–535644 and DSM 16536 are closely related. WGS alignment shows no additional genes. Furthermore, strain *P. apista* 16–535642 from Spain and DSM 16535 from Denmark are closely related. Genome sequence alignment shows that 16–535642 contains approximately 4.3 kb additional sequence. Strain E26, which had only been identified at the genus level, appears to belong to *P. pnomenusa*.

bla$_{OXA}$ gene analysis

Besides the whole genome, sequences of a number of *bla*$_{OXA}$ (encoding OXA-family β-lactamases), *gyrB* (encoding the B-subunit of DNA gyrase) and 16S rRNA gene sequences from additional *Pandoraea* strains are available, although not all sequences were present for each strain. To further understand the phylogenetic relationships between *Pandoraea* strains, we also analyzed these sequences.

All strains in our study contained a *bla*$_{OXA}$ gene. In addition to the sequences obtained in this study, sequences of *bla*$_{OXA}$ genes from other *Pandoraea* species present in GenBank were compared. The genes cluster according to the species, but the genes are not identical within species (Figure 2). However, it should be noted that *P. pulmonicola* 16–535646 and DSM 16583 cluster separately from the other strains of this species. Also, the *P. sputorum* strains are split into two groups with a clustering identical to that obtained by cgMLST. Additionally, all *P. pnomenusa* and *Pandoraea* spp. E26 strains created a distinct cluster, which is in agreement with the cgMLST clustering.

gyrB gene analysis

A phylogenetic analysis of *gyrB* gene sequences shows that *P. pulmonicola* 16–535646, DSM 16583 and LMG18106 cluster together, but separately from the other *P. pulmonicola* strains (Figure 3). The *P. sputorum* strains again are split into two groups with a clustering identical to that obtained by cgMLST. Strains from *P. oxalativorans* clustered together with *P. sputorum* strains, which is comparable with the cgMLST clustering. Genomospecies 2 also falls into this cluster. The two *P. pnomenusa* strains 6399 and 7641 clustered completely differently from the other *P. pnomenusa* strains. This part of our analysis is confirmed by the recent description of these isolates as a novel species, *Pandoraea fibrosis*, during the preparation of our manuscript [23]. The sequences for *P. faecigallinarum*, *P. vervacti* and

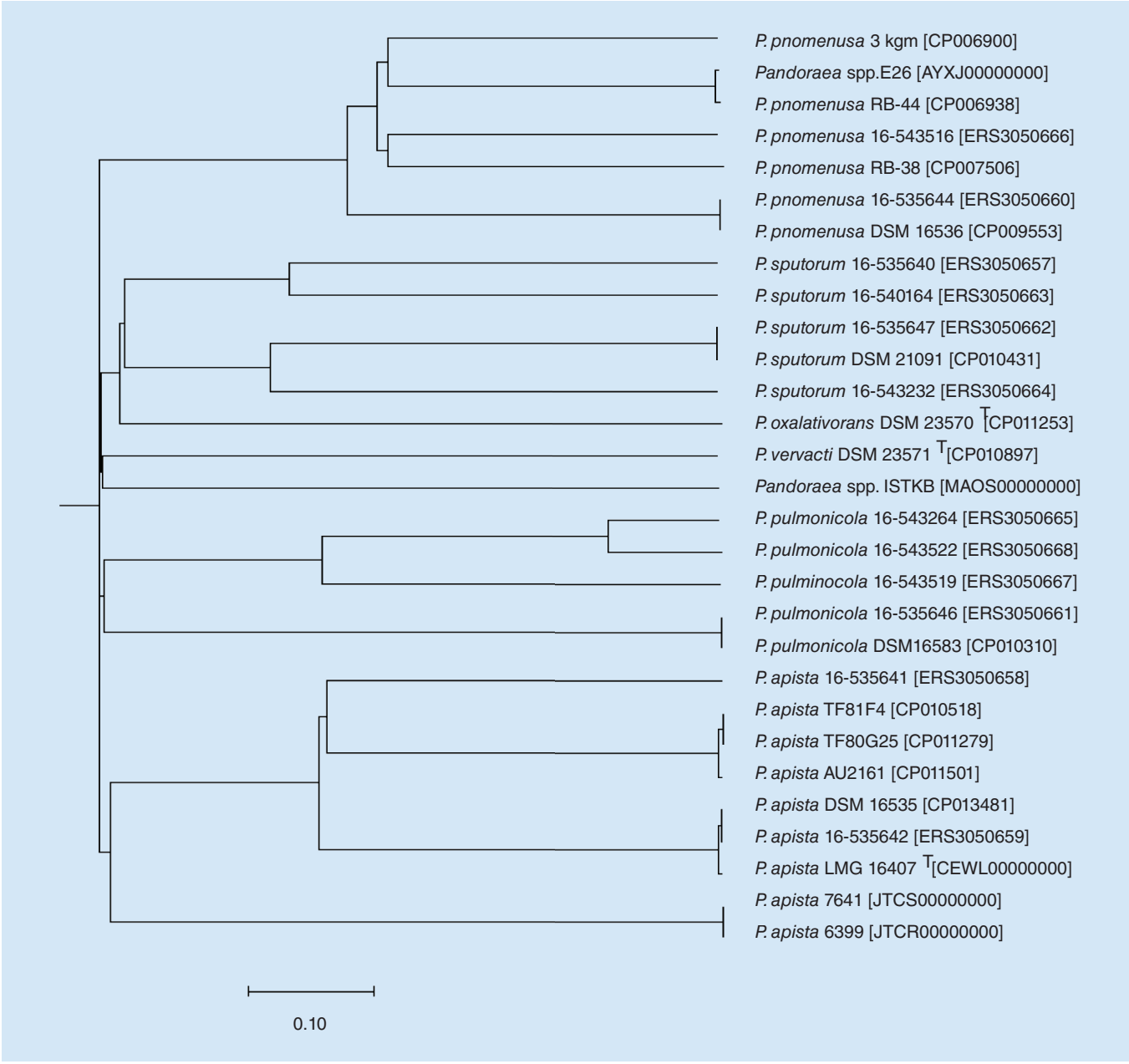

Figure 1. Phylogenetic tree of 12 isolates from this study and 17 isolates with publicly available genomes. The tree was created using presence/absence profile of the 342 core genes (core genome multi-locus sequence typing) of the publicly available 17 *Pandoraea* genomes (see Materials & methods section for more details).

genomospecies three strains clustered closely together. Also, *P. thiooxydans*, *P. terrae* and *P. norimbergensis* strains have closely related *gyrB* sequences.

16S rRNA gene analysis

The analysis of 16S rDNA sequences was divided into two different regions, because in some cases only limited sequence data were available. A first fragment of 960 sbp lacked the sequence from 16–535646 and 16–535644 (Figure 4A) and a second fragment of 896 bp lacked the sequences of 16–543516 and 16–543522 (Figure 4B). The different clustering of *P. pulmonicola* 16–535646 in the cgMLST and with bla_{OXA} and *gyrB* is not observed with 16S rRNA sequences. Unfortunately, only the second region of 16–535646 was available (Figure 4B). The *P. sputorum* strains again clustered into two different groups comparable with those described for bla_{OXA} and *gyrB*.

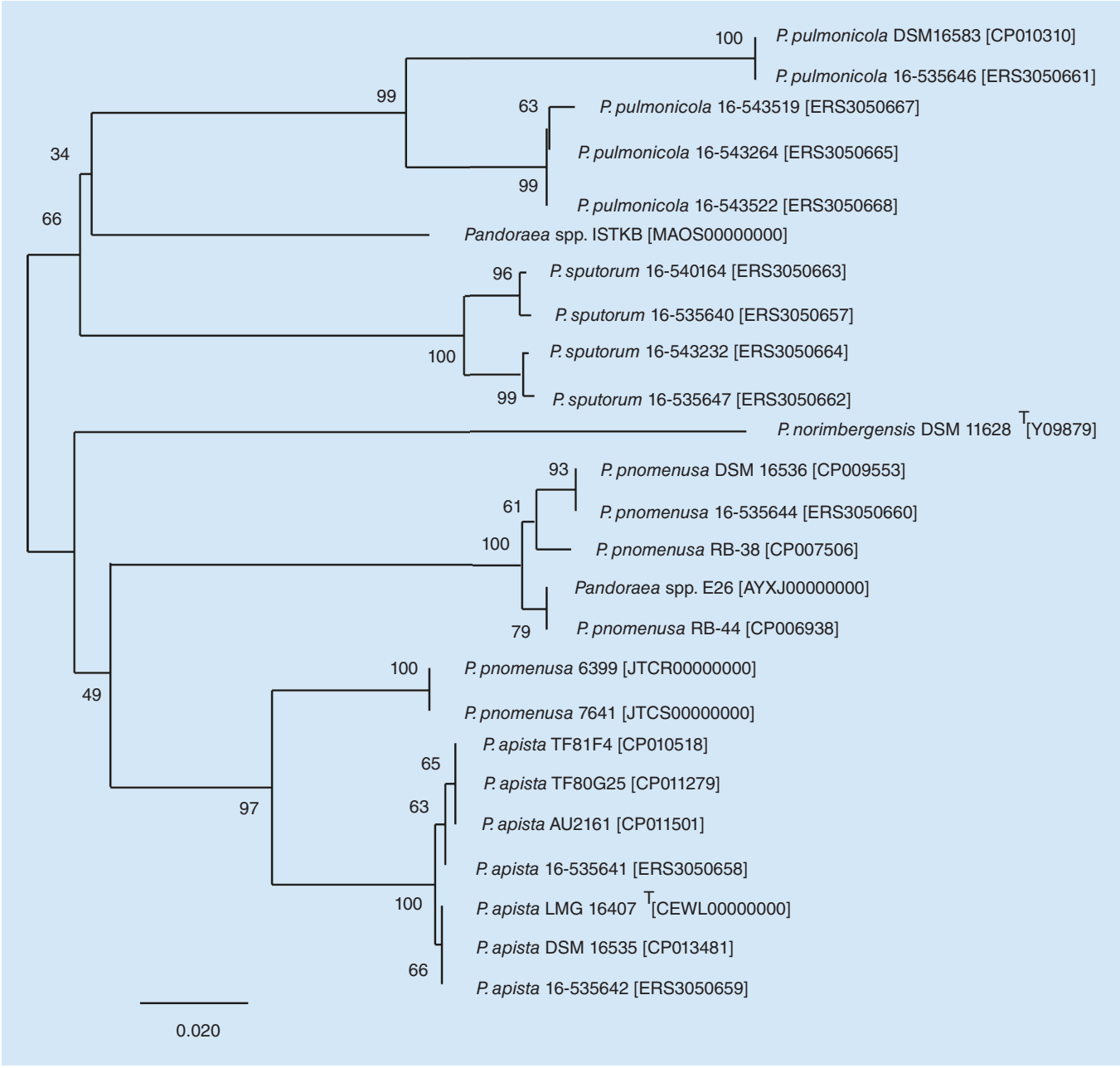

Figure 2. Neighbor-joining tree of *bla*$_{OXA}$ sequences. The optimal tree with the sum of branch length = 0.66186054 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [19]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [20] and are in the units of the number of base substitutions per site. The analysis involved 25 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 292 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [21]. Data taken from [18].

However, the *P. oxalativorans* strain also clustered with two *P. sputorum* strains based on the sequence of each region, similar to the *gyrB* sequences. Also, genomospecies 2 strains clustered with the *P. sputorum* strains as it did using the *gyrB* sequences. *Pandoraea* spp. ISTKB also clusters in this group as did genomospecies 3, although somewhat separately. Genomospecies 4 clusters with *P. norimbergensis* and genomospecies 1 clusters differently with sequences of both fragments as did *P. terrae*, *P. vervacti*, *P. thiooxydans* and *P. faecigallinarum*. The *P. pnomenusa* strains 6399 and 7641 again clustered differently from the other strains of this species. However, they cluster differently in the

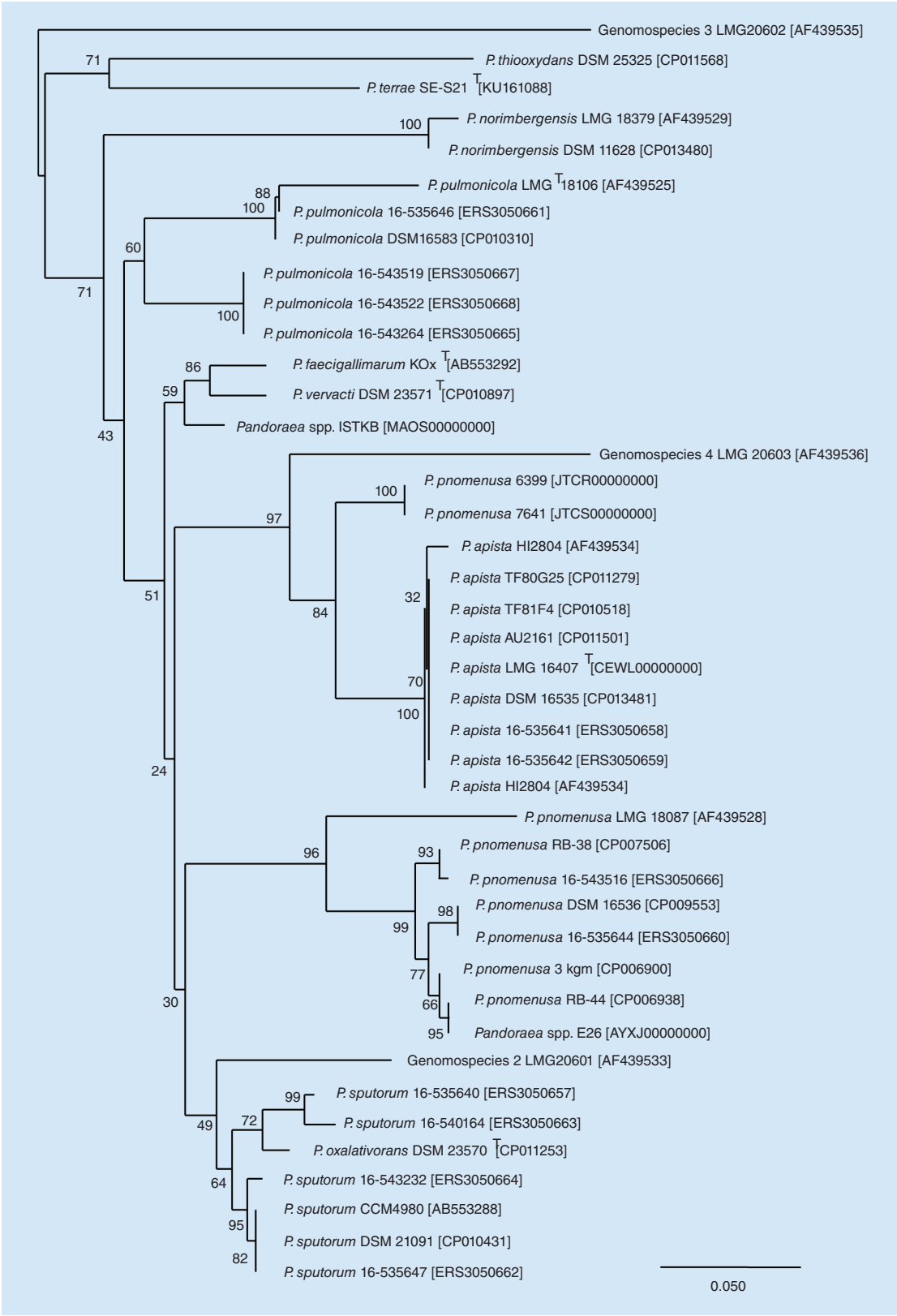

Figure 3. Neighbor-joining tree of partial *gyrB* sequences [18]. The optimal tree with the sum of branch length = 1.40428879 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [19]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [20] and are in the units of the number of base substitutions per site. The analysis involved 42 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There was a total of 398 positions in the final dataset. Evolutionary analyses were conducted in MEGA X [21].
Data taken from [18].

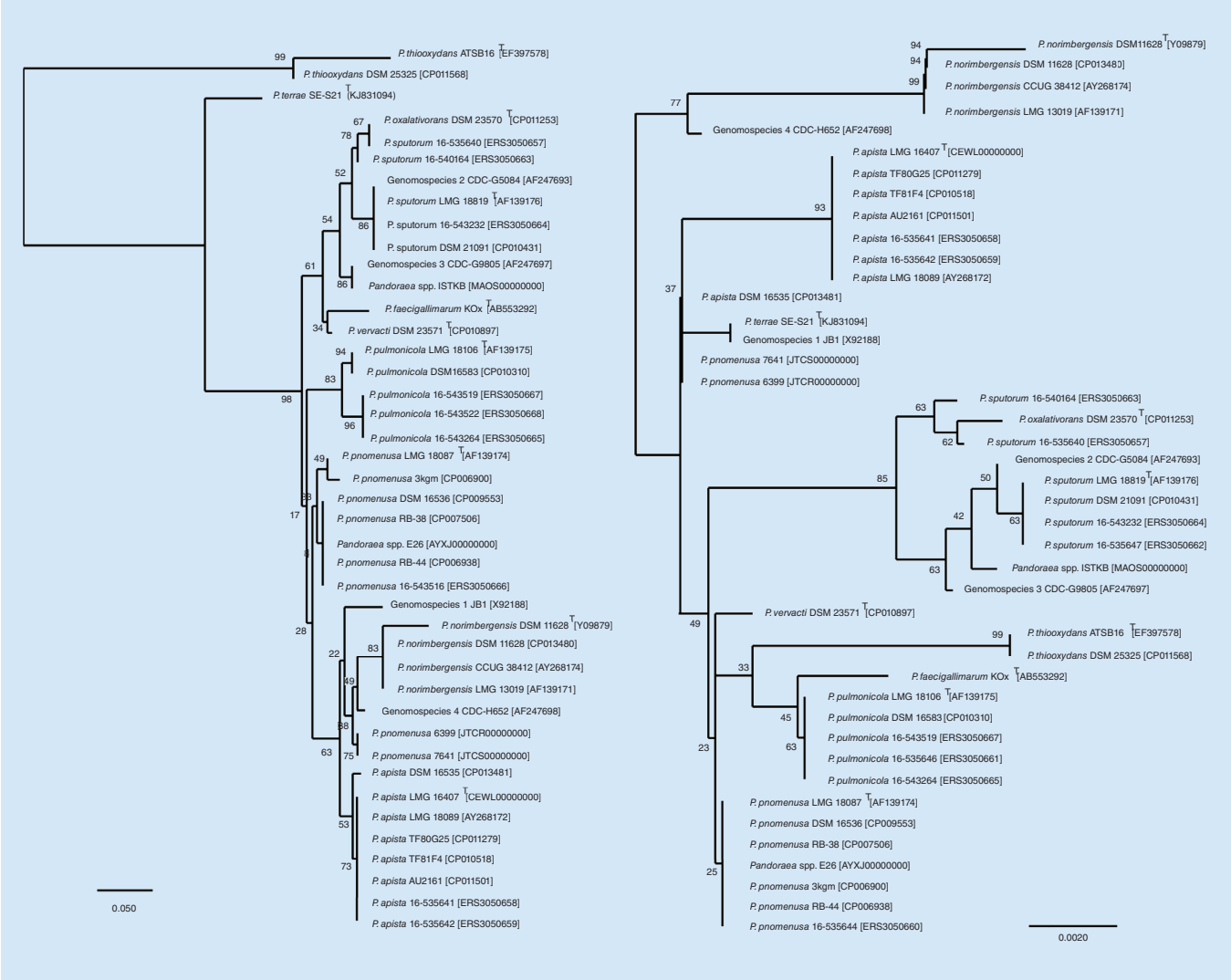

Figure 4. Neighbor-joining trees of the 16S ribosomal DNA gene sequence. (A) Neighbor-joining tree of the first part of the 16S ribosomal DNA gene sequence. **(B)** Neighbor-joining tree of the second part of the 16S ribosomal DNA gene sequence. The evolutionary history was inferred using the neighbor-joining method [18]. The optimal tree with the sum of branch length = 0.10332359 and = 0.03749079 (Figure 4A & B, respectively) is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [19]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the maximum composite likelihood method [20] and are in the units of the number of base substitutions per site. The analyses involved 42 and 43 nucleotide sequences, respectively. All ambiguous positions were removed for each sequence pair. There was a total of 962 and 896 positions for Figure 4A & B, respectively. Evolutionary analyses were conducted in MEGA X [21].

phylogenetic tree for each 16S sequence fragment. This part of our analysis is confirmed by the recent description of these isolates as a novel species, *P. fibrosis*, during the preparation of our manuscript [24].

ANIb analysis

An ANIb can be used to delineate species and a score below 95–96% can be considered a different species [25]. When an ANIb analysis was performed using the 12 whole genomes, sequenced as a part of this study, and the 17 publicly available WGS, the genus *Pandoraea* appeared to be composed of 8–10 species (Figure 5). As observed with the phylogenetic analyses, the *P. apista* strains cluster together. However, strains identified as *P. pnomenusa* fall into two groups. Strains 6399 and 7641 do not belong to the species, whereas the other strains do because they cluster with the type strain of that species. *Pandoraea* spp. E26 also belongs to *P. pnomenusa*. *P. sputorum* and *P. oxalativorans*, form a complex that either consists of 3 species or subspecies consistent with the other analyses. Based

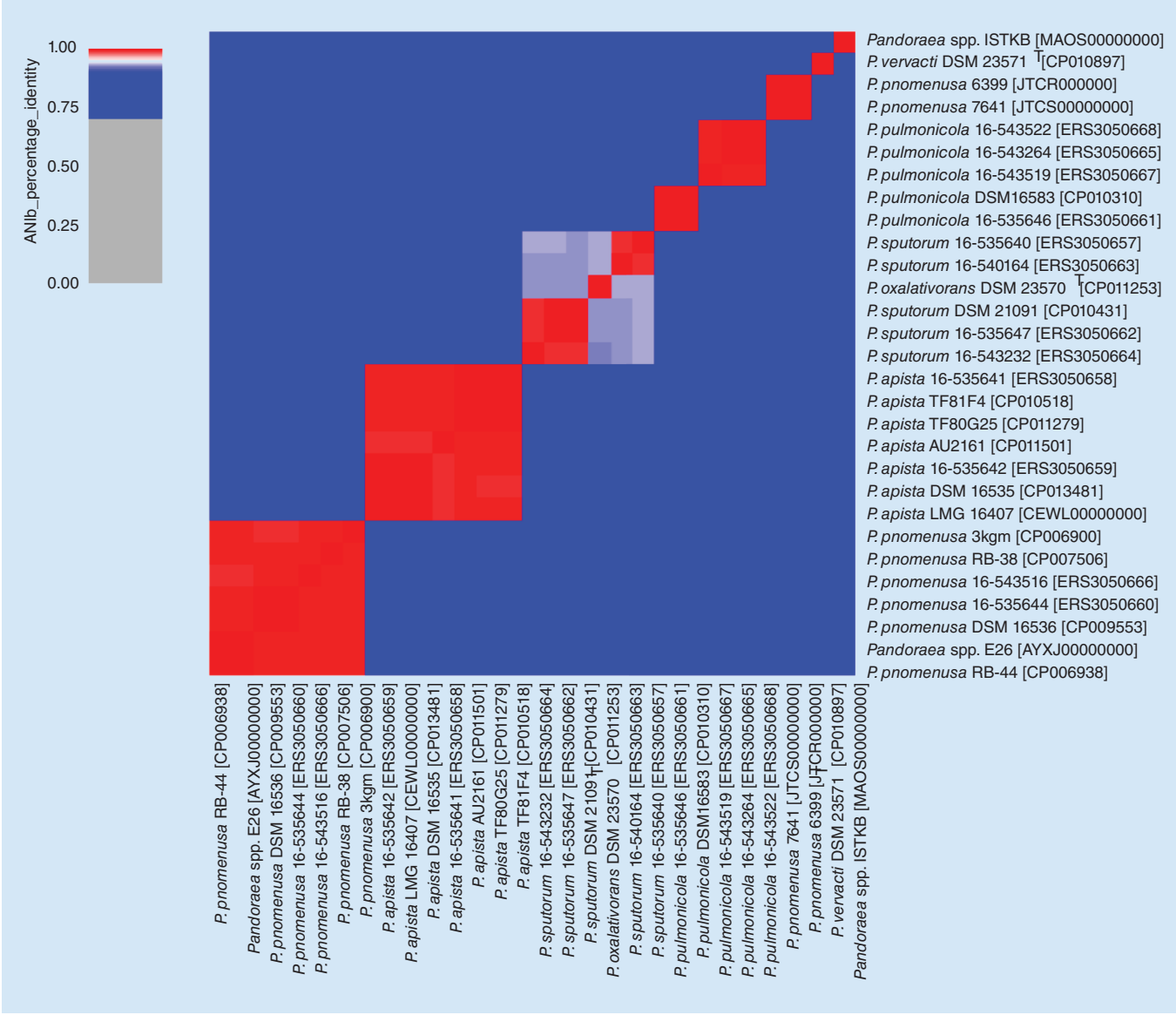

Figure 5. Heatmap based on the percentage of average nucleotide identity for all 29 *Pandoraea* isolates that were analyzed in this study. The color bar shows the percentage of average nucleotide identity between any two isolates starting from blue (75%) through white to red (100%).

on the analyses of the 16S rRNA sequences, strains 16–535647 and 16–543232 appear to belong to *P. sputorum* since they cluster with the type strain, whereas the other strains belong to a different species or the same species but a different subspecies. *P. pulmonicola* appears to consist of two species. *P. vervacti* is a separate species and *Pandoraea* spp. ISTKB belongs to a species for which no WGS was available or a novel species; the latter option appears more likely, since the strain did not cluster with described species in the other analyses.

Annotation

The annotation with RAST showed no major differences in most metabolism and cell function categories within species with the exception of *Pandoraea* spp. 16–535646 where, in the category 'Motility and Chemotaxis', more proteins were identified compared with the other strains (Supplementary Table 1), again suggesting that this isolate belongs to a new species. Depending on the isolate, 44–71 virulence genes were identified (Supplementary Table 1). Phage proteins were identified in all strains except those of *P. pulmonicola* (Supplementary Table 1). No virulence-related genes were identified. No proteins involved in cell division and cell cycle or plasmid-related functions were

identified. Whether the absence of proteins reflects true absence of these functions or is covered by different proteins remains to be seen.

All our strains harbored a bla_{OXA} gene. *P. apista* encoded bla_{OXA153}, *P. pnomenusa* bla_{OXA151} or bla_{OXA152}, *P. pulmonicola* bla_{OXA156}, bla_{OXA158} or bla_{OXA159}, and *P. sputorum* bla_{OXA154} or bla_{OXA155} (Supplementary Figure 1). These species/bla_{OXA}-type combinations have been described before [24]. However, we also identified bla_{OXA158} and bla_{OXA159} in *P. pulmonicola*. Besides the intrinsic bla_{OXA} genes, no other resistance genes were detected using ResFinder or RAST, with the exception of five strains belonging to four species that encode a putative class C β-lactamase. The amino acid sequences are different for each isolate (data not shown). The minimal inhibitory concentrations of the strains for aztreonam, ceftazidime, ciprofloxacin, colistin, cotrimoxazole, imipenem, meropenem and tobramycin were tested earlier [26], but no relationship with species or the presence of a putative AmpC β-lactamase for the β-lactam antibiotics was present (data not shown); however, differences in the promotor region can be an explanation. Possibly, efflux pumps in combination with porin mutations play a role in antibiotic resistance similar to that reported for *P. aeruginosa* [27]. Unfortunately, except for the OXA-family β-lactamases, no data on antibiotic resistance mechanisms are available and insufficient genome sequences are available to identify mutations involved in antibiotic resistance.

Plasmid analysis

Although no plasmid-related functions were identified with RAST, our sequences, when compared with eight published *Pandoraea* plasmid sequences (GenBank accession numbers pPV15C: P010898; pPO70-1: CP011518; pPO70-2: CP011519, pPO70-3: CP011520; pPO70-4: CP011521; pPF72-1: CP011808; pPF72-2: CP011809; pPA35: CP013482), showed that only *P. apista* 16–535642 shared approximately 67 kb of DNA with the 77 kb pPA35 plasmid. The shared sequences encoded for conjugal transfer related proteins and hypothetical proteins (data not shown). pPA35 was described as plasmid of *P. apista* DSM 16535 [28].

Conclusion

In summary, the sequence data show the need to revisit the taxonomy within the genus *Pandoraea*. Speciation within the genus *Pandoraea* is not reliable when using only (partial) individual genes (e.g., 16S rRNA genes or *gyrB*) and further characterization using WGS is needed to confirm speciation. Based on the clustering of the *P. pulmonicola* type strain LMG18106 with 16–535646 and DSM 16583 for *gyrB* compared with the other strains described, we propose that strains 16–543264, 16–543519 and 543522 belong to a new species. *P. sputorum* 16–543232, 16–535647 and DSM12091 cluster with the type strain in agreement with clustering using the other data. This implies that the two other strains do not belong to *P. sputorum* and belong either to a new species or subspecies; *P. oxalativorans* belongs to this complex and may be a subspecies. Our analyses of *P. pnomenusa* strains 6399 and 7641 are in agreement with the recent description of these strains as the novel species *P. fibrosis*. *Pandoraea* spp. E26 belongs to *P. pnomenusa*. The status of species for which only gene sequences were available can only be ascertained when (sufficient) whole-genome sequences are available.

Summary points

- Pandoraea species are environmental bacteria but important pathogens in cystic fibrosis patients.
- These species are underinvestigated.
- The genomic sequences of 12 isolates were investigated.
- Several undescribed species were identified.
- The isolates do not harbor acquired antibiotic resistance genes.
- A revision of the taxonomy is required.

Supplementary data

To view the supplementary data that accompany this paper please visit the journal website at: www.futuremedicine.com/doi/suppl/10.2217/fmb-2019-0038

Acknowledgments

We thank Antonio Oliver from Servicio de Microbiología, Hospital Universitario Son Espases, for providing four of the six *Pandoraea* strains from Spain.

Financial & competing interests disclosure

This study was funded by Innovative Medicines Initiative Joint Undertaking – European Union's Seventh Framework Programme (FP7/2007–2013) and EFPIA companies in kind contribution (no. # 115721-1). The authors have no other relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript apart from those disclosed.

No writing assistance was utilized in the production of this manuscript.

Ethical conduct of research

Samples and patient data were collected in compliance with the Declaration of Helsinki ICH-GCP, the Declaration of Tapei regarding Health Databases and Biobanks and with local and European regulations for collection and handling of patient data. Since the study concerned retrospectively collected anonymized patient data and bacterial strains, informed consent at the individual patient level was not required for this study. In The Netherlands, use and analysis of bacterial strains with anonymized patient data does not require approval from institutional review boards/ethics committees. The Spanish and UK strains were collected in prior studies, in accordance with their local ethics guidelines; results from these studies have been previously published [12,13].

References

Papers of special note have been highlighted as: • of interest; •• of considerable interest

1. Chan KG, Yin WF, Goh SY. Complete genome sequence of *Pandoraea pnomenusa* 3kgm, a quorum-sensing strain isolated from a former landfill site. *Genome Announc.* 2, e00427–14 (2014).
2. Chan KG, Yong D, Ee R *et al.* Complete genome sequence of *Pandoraea oxalativorans* DSM 23570(T), an oxalate metabolizing soil bacterium. *J. Biotechnol.* 219, 124–1255 (2016).
3. Coenye T, Falsen E, Hoste B *et al.* Description of *Pandoraea* gen. nov. with *Pandoraea apista* sp. nov., *Pandoraea pulmonicola* sp. nov., *Pandoraea pnomenusa* sp. nov., *Pandoraea sputorum* sp. nov. and *Pandoraea norimbergensis* comb. nov. *Int. J. Syst. Evol. Microbiol.* 50, 887–899 (2000).
4. Dupont C, Aujoulat F, Chiron R, Condom P, Jumas-Bilak E, Marchandin H. Highly diversified *Pandoraea pulmonicola* population during chronic colonization in cystic fibrosis. *Front. Microbiol.* 8, 1892 (2017).
5. Ee R, Ambrose M, Lazenby J, Williams P, Chan KG, Roddam L. Genome sequences of wwo *Pandoraea pnomenusa* isolates recovered 11 months apart from a cystic fibrosis patient. *Genome Announc.* 3, e01389–14 (2015).
6. Fernández-Olmos A, Morosini MI, Lamas A *et al.* Clinical and microbiological features of a cystic fibrosis patient chronically colonized with *Pandoraea sputorum* identified by combining 16S rRNA sequencing and matrix-assisted laser desorption ionization-time of flight mass spectrometry. *J. Clin. Microbiol.* 50, 1096–1098 (2012).
7. Jeong SE, Lee HJ, Jia B, Jeon CO. *Pandoraea terrae* sp. nov., isolated from forest soil, and emended description of the genus *Pandoraea* Coenye *et al.* 2000. *Int. J. Syst. Evol. Microbiol.* 66, 3524–3530 (2016).
8. Martina PF, Martínez M, Frada G *et al.* First time identification of *Pandoraea sputorum* from a patient with cystic fibrosis in Argentina: a case report. *BMC Pulm. Med.* 17, 33 (2017).
9. Millard AD, Westblade LF, LiPuma JJ *et al.* Draft genome sequence of the *Pandoraea apista* LMG 16407 type strain. *Genome Announc.* 3, e01300–e01315 (2015).
10. Yong D, Ee R, Lim YL *et al.* Complete genome sequence of *Pandoraea thiooxydans* DSM 25325^{T}, a thiosulfate-oxidizing bacterium. *J. Biotechnol.* 217, 51–52 (2016).
11. Stryjewski ME, LiPuma JJ, Messier RH Jr, Reller LB, Alexander BD. Sepsis, multiple organ failure, and death due to *Pandoraea pnomenusa* infection after lung transplantation. *J. Clin. Microbiol.* 41, 2255–2257 (2003).
12. de Dios Caballero J, Del Campo R, Royuela A *et al.* Bronchopulmonary infection-colonization patterns in Spanish cystic fibrosis patients: results from a national multicenter study. *J. Cyst. Fibros.* 15, 357–365 (2016).
13. Muhlebach MS, Hatch JE, Einarsson GG *et al.* Anaerobic bacteria cultured from cystic fibrosis airways correlate to milder disease: a multisite study. *Eur. Respir. J.* 52, 1800242 (2018).
14. McCaughey G, McKevitt M, Elborn JS *et al.* Antimicrobial activity of fosfomycin and tobramycin in combination against cystic fibrosis pathogens under aerobic and anaerobic conditions. *J. Cyst. Fibros.* 11, 163–172 (2012).
15. Zankari E, Hasman H, Cosentino S *et al.* Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 67, 2640–2644 (2012).
16. Altschul SF, Gish W, Miller W, Myers EW, Lipman DJ. Basic local alignment search tool. *J. Mol. Biol.* 215, 403–410 (1990).
17. Pritchard L, Glover RH, Humphris S, Elphinstone JG, Toth IK. Genomics and taxonomy in diagnostics for food security: soft-rotting enterobacterial plant pathogens. *Anal. Methods* 8, 12–24 (2016).
18. Saitou N, Nei M. The neighbor-joining method: a new method for reconstructing phylogenetic trees. *Mol. Biol. Evol.* 4, 406–425 (1987).

19. Felsenstein J. Confidence limits on phylogenies: an approach using the bootstrap. *Evolution* 39, 783–791 (1985).

20. Tamura K, Nei M, Kumar S. Prospects for inferring very large phylogenies by using the neighbor-joining method. *Proc. Natl Acad. Sci. USA* 101, 11030–11035 (2004).

21. Kumar S, Stecher G, Li M, Knyaz C, Tamura K. MEGA X: molecular evolutionary genetics analysis across computing platforms. *Mol. Biol. Evol.* 35, 1547–1549 (2018).

22. Overbeek R, Olson R, Pusch GD *et al.* The SEED and the rapid annotation of microbial genomes using subsystems technology (RAST). *Nucleic Acids Res.* 42, D206–D214 (2014).

23. See-Too WS, Ambrose M, Malley R *et al. Pandoraea fibrosis* sp. nov., a novel *Pandoraea* species isolated from clinical respiratory samples. *Int. J. Syst. Evol. Microbiol.* 69, 645–651 (2019).

24. Richter M, Rosselló-Móro R. Shifting the genomic gold standard for the prokaryotic species definition. *Proc. Natl Acad. Sci. USA* 106, 19126–19131 (2009).

•• **Describes the relationship between whole-genome sequences and the species concept for bacteria.**

25. Schneider I, Bauernfeind A. Intrinsic carbapenem-hydrolyzing oxacillinases from members of the genus *Pandoraea*. *Antimicrob. Agents Chemother.* 59, 7136–7141 (2015).

• **Describes the variety of OXA β-lactamases present in *Pandoraea* species.**

26. Díez-Aguilar M, Ekkelenkamp M, Morosini MI *et al.* Antimicrobial susceptibility of non-fermenting Gram-negative pathogens isolated from cystic fibrosis patients. *Int. J. Antimicrob. Agents* 53, 84–88 (2019).

27. Puzari M, Chetia P. RND efflux pump mediated antibiotic resistance in gram-negative bacteria *Escherichia coli* and *Pseudomonas aeruginosa*: a major issue worldwide. *World J. Microbiol. Biotechnol.* 33, 24 (2017).

28. Yong D, Tee KK, Yin WF, Chan KG. Characterization and comparative overview of complete sequences of the first plasmids of *Pandoraea* across clinical and non-clinical strains. *Front. Microbiol.* 7, 1606 (2016).

• **Plasmids play an important role in the evolution and adaption of bacteria and may play an important role in *Pandoraea* species.**