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ORIGINAL PAPER

Genome-based reclassification of *Anoxybacillus salavatliensis* Cihan et al. 2011 as a later heterotypic synonym of *Anoxybacillus gonensis* Belduz et al. 2003

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Abstract In the present study, we aim to clarify the taxonomic positions of *Anoxybacillus salavatliensis* DSM 22626^T and *Anoxybacillus gonensis* G2^T by using whole genome phylogenetic analysis, biochemical and chemotaxonomic characteristics. The genome sequences of *A. salavatliensis* DSM 22626^T was not available in any database, so it was sequenced in this study. In phylogenetic trees drawn using whole genome sequences and 16S rRNA gene sequences, *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T clade together and showed high sequence similarity (99.3%) based on 16S rRNA gene. The average amino acid identity, average nucleotide identity and digital DNA–DNA hybridization values between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were found to be greater than the threshold values for species demarcation. Further, the phylogenomic analysis based on the core genome of the strains under study confirmed that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T formed a monophyletic clade. Most phenotypic and chemotaxonomic features between both strains were almost identical except for a few exceptions. The present results show that *A. salavatliensis* DSM 22626^T is a later heterotypic synonym of *A. gonensis* G2^T.

Keywords *Anoxybacillus gonensis* · *Anoxybacillus salavatliensis* · Genome-based reclassification

Introduction

The genus *Anoxybacillus,* belonging to the phylum Firmicutes*,* was proposed by Pikuta et al. (2000) with *Anoxybacillus pushchinoensis* as the type species and its description was considerably emended by Pikuta et al. (2003). At the time of writing, this genus comprised 24 species with validly published names and three species with not validly published names (http://www.bacterio.net). *Anoxybacillus* species are widely distributed and isolated from geothermally heated environments. The taxonomy of *Anoxybacillus* members was predominantly based on 16S rRNA gene sequence analysis and DNA–DNA hybridization (DDH). However, it is widely known that the resolving power of 16S rRNA gene analysis often shows limited variation for discrimination of closely related species, such as the *Anoxybacillus* species. DDH is time-consuming and labor-intensive method

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and it is impossible to establish a central database. Phylogeny using whole-genome sequences-based metrics such as average nucleotide identity (ANI), digital DDH, and average amino acid identity (AAI) have become important tools for the delineation of prokaryotic taxa (Orata et al. 2018), and is being used for the reclassifcation of several bacterial taxa (Liu et al. 2019; Rao et al. 2022).

The type strain G2^T of *Anoxybacillus gonensis* was isolated from hot spring in Turkey by Belduz et al. in 2003 and described as validly named species based on a polyphasic taxonomic approach. *Anoxybacillus salavatliensis* DSM 22626^T was isolated from a high temperature well-pipeline sediment sample in Turkey by Cihan et al. (2011) and was validated in IJSEM (Validation List No. 138; Euzéby 2011). In the original article, Cihan et al. (2011) proposed *A. salavatliensis* DSM 22626^T as a new species in the genus *Anoxybacillus* based mainly on DNA–DNA hybridization values between *A. salavatliensis* DSM 22626^T and *A. kamchatkensis* DSM 14988^T, *A. amylolyticus* DSM 15939^T. Phylogenetic tree based on 16S rRNA gene sequences in the original article showed that *A. salavatliensis* DSM 22626^T, *A. gonensis* G2^T, *A. kamchatkensis* DSM 14988^T, *A. ayderensis* AB04^T and *A. thermarum* DSM 17141^T clustered together. In the orginal article, Cihan et al. (2011) stated that *A. salavatliensis* DSM 22626^T could be clearly differentiated from all of the closely related *Anoxybacillus* species (*A. kamchatkensis* DSM 14988^T, *A. thermarum* DSM 17141^T, *A. ayderensis* AB04^T, *A. gonensis* G2^T, *A. flavithermus* DSM 2641^T and *A. amylolyticus* DSM 15939^T) based on the results of Rep-PCR and ITS fingerprinting. Also, in the original article Cihan et al. (2011) determined DDH values between *A. salavatliensis* DSM 22626^T and *A. kamchatkensis* DSM 14988^T, *A. amylolyticus* DSM 15939^T, they did not determine DDH values between *A. salavatliensis* DSM 22626^T and the other closely related *Anoxybacillus* species (A. *thermarum* DSM 17141^T, *A. ayderensis* AB04^T, *A. gonensis* G2^T, *A. flavithermus* DSM 2641^T). During our genome-based analysis, we observed that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T shared similar features; as a result, we attempted to clarify the relationship between that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T through genomics-based methods, biochemical and chemotaxonomic characteristics. The data presented in this study provides evidence that *A. salavatliensis* DSM 22626^T is later heterotypic synonym of *A. gonensis* G2^T.

Materials and methods

A. salavatliensis DSM 22626^T was purchased from the German Collection of Microorganisms and Cell Cultures GmbH (DSMZ). *A. gonensis* G2^T was isolated by us and validly published, so *A. gonensis* G2^T was obtained from our own laboratory collection. Two type strains were grown on trypticase soy agar (TSA) incubated at 50 °C for 24 h.

The genome sequencing of *A. salavatliensis* DSM 22626^T was performed in this study, while genome sequences of *A. gonensis* G2^T (JRZG00000000) was downloaded from NCBI Database (https://www.ncbi.nlm.nih.gov/genome/). For whole genome sequencing, genomic DNA was isolated from culture of *A. salavatliensis* DSM 22626^T by using the QIAamp DNA Mini Kit according to the manufacturer's instructions (Qiagen, Hilden-Germany). Whole-genome sequencing of *A. salavatliensis* DSM 22626^T was performed on an Illumina HiSeq 2500 next-generation platform with a 250-bp paired-end sequencing protocol by MicrobesNG (http://www.microbesng.uk, Birmingham, United Kingdom). Assemblies of raw sequence data were achieved using the full SPAdes assembly strategy on the PATRIC web server (https://patricbrc.org/) (Wattam et al. 2017). The draft genome sequences were annotated by using the Rapid Annotations Using Subsystems Technology (RAST) server (Aziz et al. 2008). The obtained draft genome sequences were deposited in the National Centre for Biotechnology Information (NCBI) database under accession number JANGZY000000000.

The 16S rRNA gene sequence identity between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was compared using the pairwise alignment feature implemented on the EZBioCloud server (https://www.ezbiocloud.net/tools/pairAlign). The 16S rRNA gene sequences of closely related type strains were dowloaded from EzBioCloud server at https://www.ezbiocloud.net/ (Yoon et al. 2017a) and edited by using the BioEdit software (Hall 1999). Multiple sequence alignment of 16S rRNA gene sequences was performed using the ClustalW (Thompson et al. 1994). Evolutionary distances were calculated with the Kimura's two-parameter model (Kimura 1980). The

phylogenetic trees were generated in Mega-X using neighbor-joining (Saitou and Nei 1987), maximum parsimony (Kluge and Farris 1969) and maximum-likelihood method (Felsenstein 1981), with bootstrap values based on 1000 replications.

The phylogenetic analysis of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was carried out using the type strain genomes server pipeline (TYGS, https://tygs.dsmz.de/) (Meier-Kolthoff and Göker 2019). The digital DNA-DNA hybridization (dDDH) value between the draft genome sequences of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was calculated with the Formula 2 of the online Genome-to-Genome Distance Calculator at http://ggdc.dsmz.de/distcalc2.php (Meier-Kolthoff et al. 2013). Average nucleotide identity (ANI) values were calculated for evaluating the genetic relationship between between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T by using the orthoANIu algorithm and an online ANI calculator (www.ezbiocloud.net/tools/ani) (Lee et al. 2016; Yoon et al. 2017b). A phylogenetic tree based on whole-genome sequences was constructed using the TYGS web server (https://tygs.dsmz.de/) (Meier-Kolthof and Göker 2019). The amino acid identity (AAI) value was calculated with CompareM (https://github.com/dparks1134/CompareM). For the phylogenetic and pangenome analyses, the genomes of *A. salavatliensis* DSM 22626^T, *A. gonensis* G2^T and all other *Anoxybacillus* species registered in RefSeq were re-annotated using Prokka 1.14.5 with default settings to avoid bias resulted from diferent annotations (Seemann 2014). The phylogenetic trees were then constructed using the 'insert genome into species tree app' (version 2.2.0), utilizing the FastTree 2 algorithm (Price et al. 2010). The pangenome was constructed using the genome as mentioned earlier set by 'build pangenome with OrthoMCL app' (v2.0) available at KBase platform (https://www.kbase.us/) (Arkin et al. 2018). The pangenome-based phylogenomic analysis was performed by the 'phylogenetic pangenome accumulation (v1.4.0) app' (Li et al. 2003; Arkin et al. 2018).

The API 20E, API 50CH strips and Vitek2 Bacilli Identification Card (BCL) microtest systems (bioMérieux) were used to evaluate the biochemical properties of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T according to the manufacturer's instructions. The polar lipids of strain *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were extracted from 100 mg freeze-dried cells by using two-dimensional thin-layer chromatography (TLC) according to the method of Tindall (1990a, 1990b). Polar lipids were separated by two-dimensional TLC on silica gel. The first direction was developed with chloroform:methanol:water (65:25:4, v/v) and the second direction with chloroform:methanol:acetic acid:water (80:12:15:4, v/v). Detection was performed using 5% ethanolic molybdophosphoric acid for the total lipids, molybdenum blue for phospholipids, ninhydrin for aminolipids, and α-naphthol for glycolipids (Tindall et al. 2007). Standard lipids (dihosphatidylglycerol [DPG], phosphatidic acid [PA], phosphatidylglycerol [PG], phosphatidylethanolamine [PE], phosphatidylcholine [PC], phosphatidylserine [PS], and phosphatidylinositol [PI]) were used as reference. Isoprenoid quinones were extracted and purified from freeze-dried cells by following the procedure of Collins (1985) and analysed by high performance liquid chromatography (HPLC).

Results and discussion

The phylogenetic analysis based on whole genome sequences has clarified the taxonomic inconsistence of prokaryotic taxa; as a result, several bacterial species have been reclassified (Orata et al. 2018). In the present study, the taxonomic relationship of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was re-evaluated by using whole-genome phylogenetic analysis, biochemical and chemotaxonomic features. *A. salavatliensis* DSM 22626^T was isolated from a high temperature well-pipeline sediment sample in Turkey; *A. gonensis* G2^T was isolated from hot spring in Turkey.

In the original article, Cihan et al. (2011) stated that the Rep-PCR and ITS fingerprinting profiles differentiated *A. salavatliensis* DSM 22626^T from all of the closely related *Anoxybacillus* species: *A. kamchatkensis* DSM 14988^T, *A. thermarum* DSM 17141^T, *A. ayderensis* AB04^T, *A. gonensis* G2^T, *A. flavithermus* DSM 2641^T and *A. amylolyticus* DSM 15939^T. In the original article, *A. salavatliensis* DSM 22626 ^{T} formed a cluster with *A. gonensis* G2^T, *A. kamchatkensis* DSM 14988^T, *A. ayderensis* AB04^T and *A. thermarum* DSM 17141^T in the phylogenetic tree based on 16S rRNA gene sequences. However,

in the original article Cihan et al. (2011) determined DDH values between *A. salavatliensis* DSM 22626^T and *A. kamchatkensis* DSM 14988^T, *A. amylolyticus* DSM 15939^T, they did not determine DDH values between *A. salavatliensis* DSM 22626^T and the other closely related *Anoxybacillus* species.

In the present study, we determined the pairwise nucleotide sequence alignment (16S rRNA gene sequence) between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was 99.3% with a mismatch of ten nucleotides. Also, in the present study, we reconstructed the phylogenetic trees based on 16S rRNA gene sequences and determined that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T clustered together in the neighbour-joining phylogenetic tree with high bootstrap resampling values of 97% (Fig. 1). Topologies of phylogenetic trees built according to the maximum-likelihood and maximum-parsimony algorithms also supported the results of the neighbour-joining algorithm (Fig. S1, S2). Further, in the phylogenomic tree (Fig. 2) *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T formed a

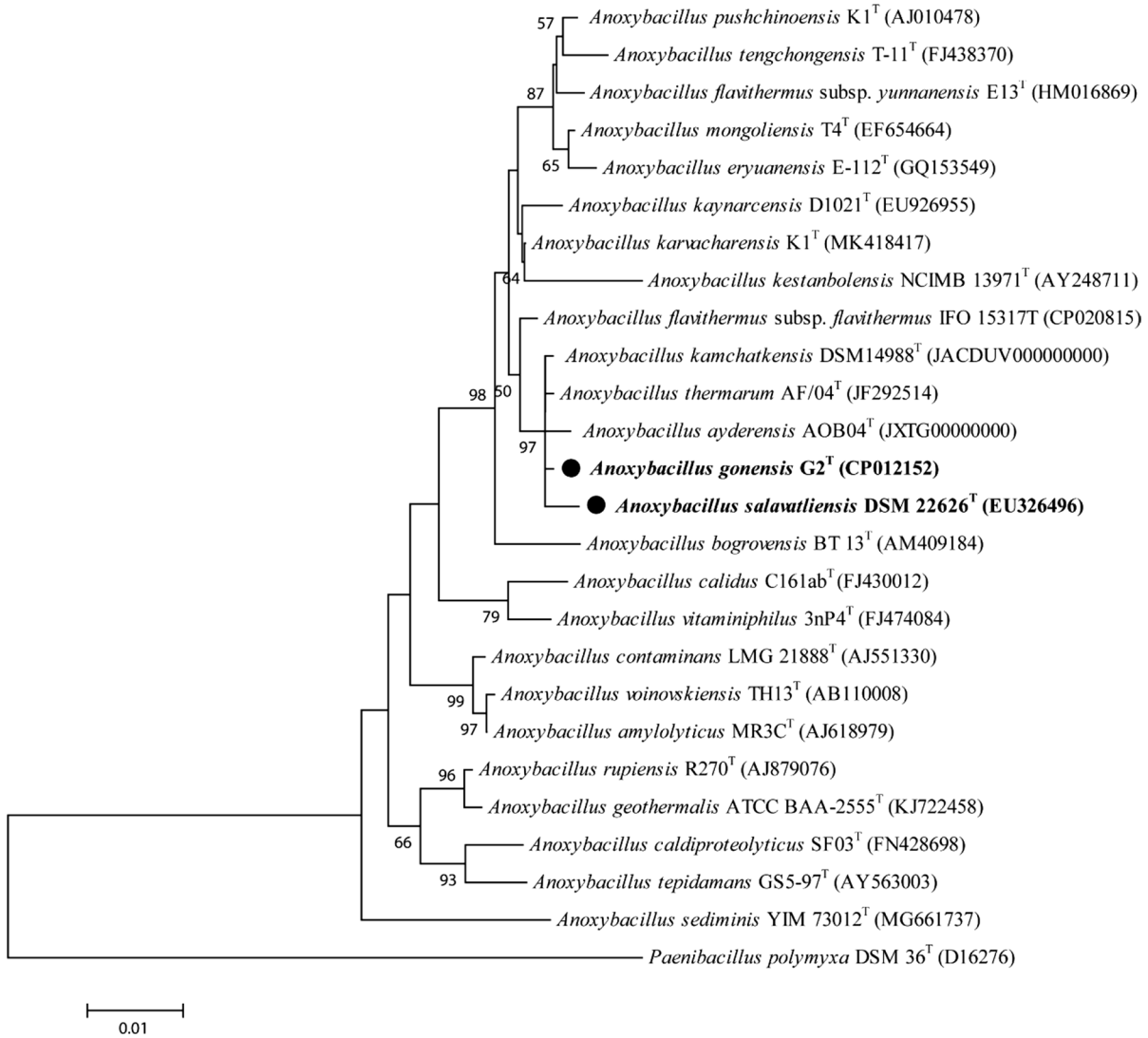

Fig. 1 Neighbour-joining (NJ) tree constructed based on 16S rRNA gene sequences available from the GenBank database. Bootstrap values (expressed as percentages of 1000 replications) greater than 50% are shown at branch points. Bar, 0.01 represents substitutions per nucleotide position. *Paenibacillus polymyxa* DSM 36^T was used as the outgroup

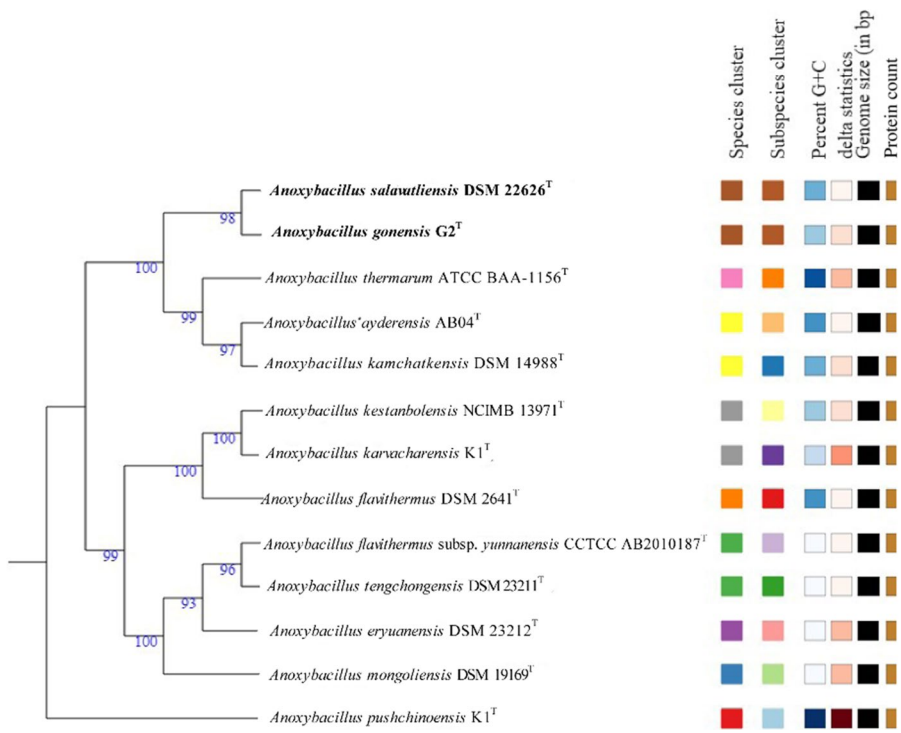

Fig. 2 Phylogenetic tree based on whole-genome sequences of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T and related reference strains. The tree was inferred with FastME 2.1.6.1 (Lefort et al. 2015) from genome blast distance phylogeny (GBDP) distances calculated from genome sequences using the TYGS server (https://tygs.dsmz.de) (Meier-Kolthoff and Göker 2019) The branch lengths are scaled in terms of GBDP distance formula d5. The numbers at branches are GBDP pseudo-bootstrap support values ≥ 64% from 100 replications with an average branch support of 97.7%. The tree was rooted at the midpoint (Farris 1972)

robust branch different from other type strains of this genus with high bootstrap resampling values of 98%. The ANI value between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was 97.98% which was greater than the threshold value (95–96%) for species demarcation (Richter and Rosselló-Móra 2009), confirming that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were highly phylogenetically closely related. The calculated AAI value between the *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was 98.1% and this value is also clearly above the suggested cut-offs for species delineation (AAI > 95%) (Luo et al. 2014), confirming that they belong to the same species. Also, digital DNA–DNA hybridization (DDH) analyses indicated that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T exhibited 81.0% dDDH value which is higher than the cut-off (70%) used to classify bacterial strains to the same species (Wayne et al. 1987), further confirming that *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T should belong to the same genomic species. Pangenomic analysis of the *Anoxybacillus* species, including *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T, revealed 6,138 orthologous clusters that constituted the pangenome. The numbers of core genes, strain-specifc genes (singleton) and accessory genes (partial) were 1867, 2623, and 1648, respectively. According to the pangenome-based phylogenomic analysis, *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T formed a monophyletic clade and shared 2381 core genes (Fig. 3).

In addition, this conclusion has been also confirmed by a comparation of phenotypic and chemotaxonomic features between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T. In API 20E, API 50CH and Vitek2 BCL system, *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T shared similar biochemical features with few exceptions (Table 1). For example, acid production from D-galactose, D-mannose, esculin ferric citrate and, ellman, palatinose, β-glucosidase were negative for *A. gonensis* G2^T, while positive for *A. salavatliensis* DSM 22626^T. β-galactosidase, α-galactosidase and acid production from methyl-alpha-D-mannopyranoside and maltotriose were positive for *A. gonensis* G2^T, while negative for *A. salavatliensis* DSM 22626^T. Both species were shown positive for ONPG hydrolysis, tyrosine arylamidase, nitrate reduction, Leucine-arylamidase, gelatinase, phenylalanine arylamidase, Ala-Phe-Pro-Arylamidase, *α*-glucosidase, acid production from D-Xylose, D-Mannitol, D-Glucose, D-Fructose, methyl-a-D-glucopyranoside, salicin,

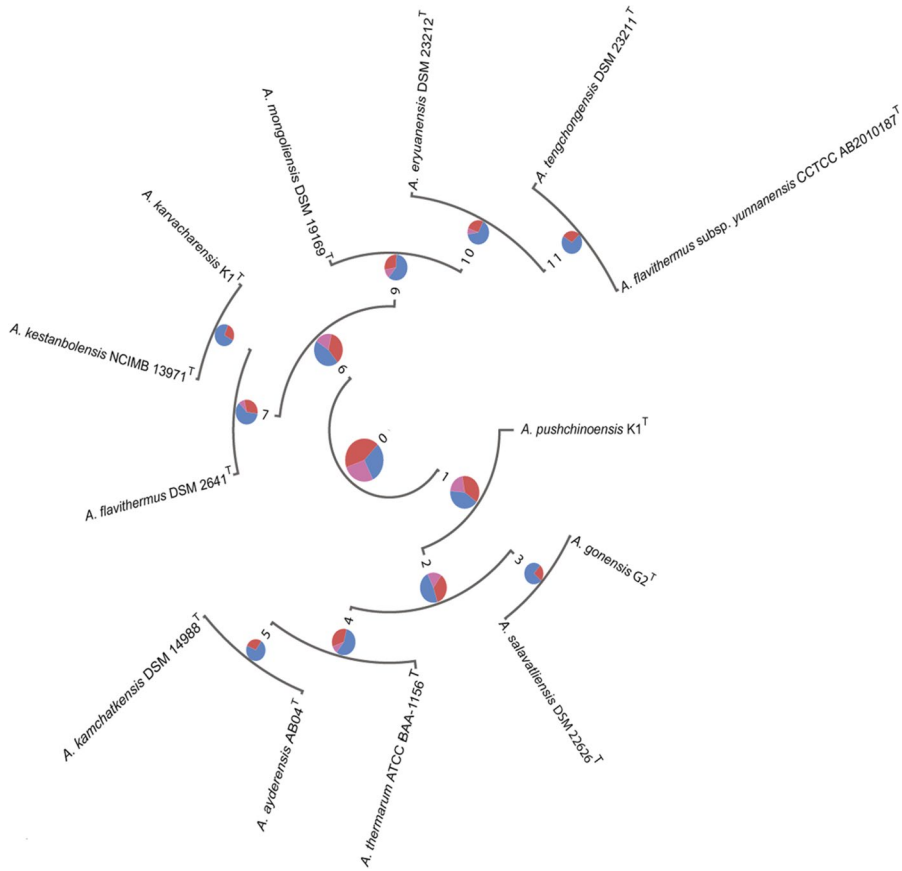

Fig. 3 Pangenome-based phylogenomic analysis of *Anoxybacillus* species. Orthologous gene sets within a pangenome are partitioned into three categories: core (blue), singleton (red), and partial pangenome (pink). Pangenome-based phylogenomic analysis was created by the OrthoMCL and phylogenetic pangenome accumulation (v1.4.0) app. (Color figure online)

D-Cellobiose, D-Maltose, D-Melibiose, D-Sucrose, D-Trehalose, inulin, D-Melezitose, D-Raffinose, starch, glycogen, D-Turanose. *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were shown negative for arginine dihydrolase, tryptophan deaminase, citrate utilization, urease, lysine decarboxylase, Voges–Proskauer, L-Lysine arylamidase, ornithine decarboxylase, alanine arylamidase, indole production (tryptophanase), pyruvate, hydrogen sulfide production, β-xylosidase, L-Aspartate arylamidase, L-Proline arylamidase, L-Pyrrolydonyl arylamidase, phosphoryl choline, β-*N*-acetyl-glucosaminidase, cyclodextrine, Methyl-D-Xyloside, α-mannosidase, glycine arylamidase, *N*-acetyl-glucosamine, kanamycin resistance, β-mannosidase, growth in 6.5% NaCI, oleandomycin resistance, polymyxin B and acid production from inositol, L-Rhamnose, D-Tagatose, D-Ribose, putrescine, glycerol, erythritol, D-Arabinose, L-Arabinose, L-Xylose, D-Xylose, methyl-D-Xylopyranoside, L-Sorbose, dulcitol, D-Sorbitol, amygdalin, arbutin, D-Lactose, xylitol, gentiobiose, D-Lyxose, D-Fucose, L-Fucose, D-Arabitol, L-Arabitol, potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate. A total of 91 of phenotypic test performed using the API 50CH, API 20E and Vitek2 BCL system. It was determined that there was a difference between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T in only 10 tests and the difference value was 11%.

In the original articles, the polar lipids of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were not determined (Belduz et al. 2003; Cihan et al. 2011). In the present study, the polar lipids found in *A. salavatliensis* DSM 22626^T were diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE), unidentified phospholipid-1 (PL1), and unidentified phospholipid-2 (PL2), whereas *A. gonensis* G2^T consisted of DPG, PG, PC, PE and PL1. Polar lipid composition showed very similar profile between two species (Fig. 4). The respiratory quinone of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T was menaquinone MK-7. Most of the chemotaxonomic and phenotypic

Table 1 The biochemical characteristics of *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T

	A. salavatliensis DSM 22626^T	*A. gonensis* G2^T
β-glucosidase	+	–
β-galactosidase	–	+
α-galactosidase	–	+
Gelatinase	+	+
Tyrosine arylamidase	+	+
α-glucosidase	+	+
Leucine-arylamidase	+	+
β-xylosidase	–	–
α-mannosidase	–	–
B-mannosidase	–	–
L-Aspartate arylamidase	–	–
L-Proline arylamidase	–	–
β-*N*-acetyl-glucosaminidase	–	–
Alanine arylamidase	–	–
Tryptophanase	–	–
Lysine decarboxylase	–	–
Tryptophan deaminase	–	–
Acid production from:		
D-Galactose	+	–
D-Mannose	+	–
Maltotriose	–	+
D-Xylose	+	+
D-Fructose	+	+
D-Mannitol	+	+
D-Glucose	+	+
D-Maltose	+	+
D-Sucrose	+	+
D-Melibiose	+	+
D-Tagatose	–	–
L-Arabinose	–	–
D-Sorbitol	–	–
D-Ribose	–	–
L-Xylose	–	–
D-Ribose	–	–

+ Positive; –, negative

features between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T were almost identical except for a few exceptions as is shown in Table 1 and Fig. 4. The disagreement for phenotypic and chemotaxonomic was probably due to their different ecological niches.

The present results demonstrate the synonym between *A. salavatliensis* DSM 22626^T and *A. gonensis* G2^T. Based on genomic, phylogenetic and chemotaxonomic comparison, we propose that *A. salavatliensis* DSM 22626^T Cihan et al. 2011 should be reclassified as a later heterotypic synonym of *A. gonensis* G2^T Belduz et al. 2003. The type strain is G2^T (=NCIMB 13933 T=NCCB 100040 T) and A343 (DSM 22,626=NCIMB 14,579) is an additional strain of *A. gonensis.*

Emended description of ***A. gonensis*** Belduz et al. (2003)

The description is the same as given by Belduz et al. (2003) with the following modification.

The respiratory quinone is menaquinone MK-7. Major polar lipids include diphosphatidylglycerol (DPG), phosphatidylglycerol (PG), phosphatidylcholine (PC), phosphatidylethanolamine (PE) and unidentified phospholipid-1 (PL1). In API 50CH, API 20E and Vitek2 BCL system, the following activities were positive for tyrosine arylamidase, ONPG hydrolysis, Leucine-arylamidase, nitrate reduction, Ala-Phe-Pro-Arylamidase, gelatinase, phenylalanine arylamidase, *β*-galactosidase, *α*-galactosidase, *α*-glucosidase, acid production from D-Xylose, D-Mannitol, D-glucose, D-Fructose, Methyl-*α*-D-Glucopyranoside, salicin, D-Cellobiose, D-Maltose, D-Melibiose, D-Sucrose, D-Trehalose, inulin, D-Melezitose, D-Raffinose, starch, glycogen, D-Turanose, methyl-alpha-D-mannopyranoside and maltotriose. Negative for urease, arginine dihydrolase, tryptophan deaminase, citrate utilization, Voges–Proskauer, ornithine decarboxylase, indole production (tryptophanase), *β*-xylosidase, L-Lysine arylamidase, lysine decarboxylase, L-Aspartate arylamidase, phosphoryl choline, L-Proline arylamidase, ellman, L-Pyrrolydonyl arylamidase, pyruvate, alanine arylamidase, hydrogen sulfide production, *β*-*N*-acetyl-glucosaminidase, cyclodextrine, kanamycin resistance, palatinose, *β*-glucosidase, Methyl-D-Xyloside, *α*-mannosidase, *N*-acetyl-glucosamine, *β*-mannosidase, glycine arylamidase, growth in 6.5% NaCI, oleandomycin resistance, polymyxin B and acid production from inositol, D-Galactose, D-Mannose, esculin ferric citrate, L-Rhamnose, D-Tagatose, D-Ribose, putrescine, glycerol, erythritol, D-Arabinose, L-Arabinose, L-Xylose, D-Xylose, Methyl-D-Xylopyranoside, L-Sorbose, dulcitol, D-Sorbitol, amygdalin, arbutin, D-Lactose, xylitol, gentiobiose, D-Lyxose, D-L-Fucose, D-and L-Arabitol,

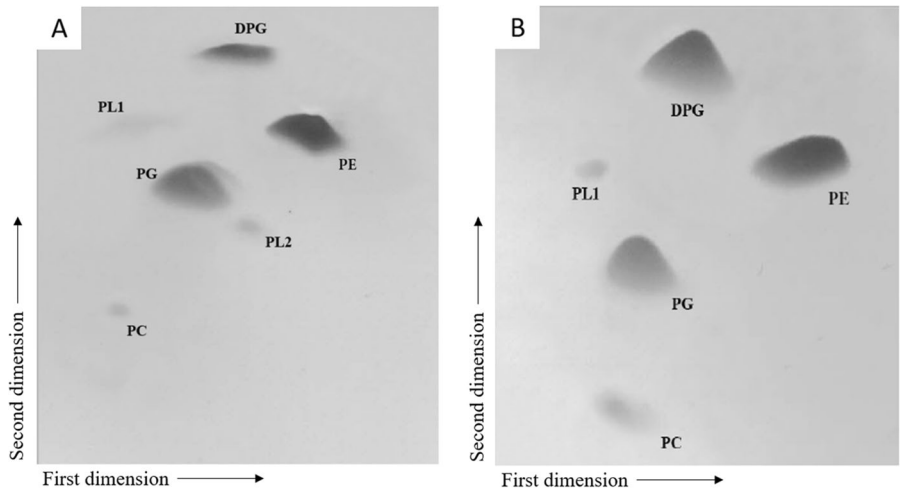

Fig. 4 Two-dimensinal thin-layer chromatogram of polar lipids of **A** *A. salavatliensis* DSM 22626^T and **B** *A. gonensis* G2^T. Abbreviations: DPG, diphosphatidylglycerol; PG, phosphatidyeglycerol; PE, phosphatidylethanolamine; PC, phosphatidylcholine; APL, unidentified aminophospolipid; PL, unidentified phospholipid

potassium gluconate, potassium 2-ketogluconate, potassium 5-ketogluconate. The DNA G+C content of the type strain G2^T (=NCIMB 13933 T=NCCB 100040 T) is 41.46 mol%.

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Author contributions KIB designed the study. KIB, HIG and SC performed genome analysis and analysed the data. KIB, HIG and AOB performed the phenotypic and chemotaxonomic analysis. KIB wrote the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by any of the authors.

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