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RESEARCH ARTICLE

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Alkalicoccobacillus porphyridii sp. nov., isolated from a marine red alga, reclassification of *Shouchella plakortidis* and *Shouchella gibsonii* as *Alkalicoccobacillus plakortidis* comb. nov. and *Alkalicoccobacillus gibsonii* comb. nov., and emended description of the genus *Alkalicoccobacillus* Joshi *et al*. 2022

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Abstract

A Gram-stain-positive alkali-tolerant and strictly aerobic bacterium, designated strain P16^T, was isolated from a marine red alga, *Porphyridium cruentum*, in the Yellow Sea, Republic of Korea. Cells were motile rods with peritrichous flagella and exhibited catalase and oxidase activities. The optimal growth of strain P16^T was observed to occur at 30 °C and pH 8.0 and in the presence of 2.0% (w/v) NaCl. Menaquinone-7 was identified as the sole respiratory quinone. Strain P16^T contained anteiso-$C_{15:0}$, iso-$C_{15:0}$, iso-$C_{14:0}$ and iso-$C_{16:0}$, and diphosphatidylglycerol, phosphatidylglycerol and phosphatidylethanolamine as major cellular fatty acids and polar lipids, respectively. The G+C content of strain P16^T was 40.8mol%. Strain P16^T was most closely related to *Shouchella plakortidis* P203^T, *Shouchella gibsonii* DSM 8722^T and *Alkalicoccobacillus murimartini* LMG 21005^T with 98.1, 98.1 and 98.0% 16S rRNA gene sequence similarities, respectively. Phylogenetic analyses based on 16S rRNA gene and genome sequences revealed that strain P16^T, *S, plakortidis*, *S. gibsonii* and *A. murimartini* formed a single phylogenetic lineage cluster, and genomic relatedness analyses showed that they are different species. Based on phylogenetic, phenotypic, chemotaxonomic and molecular features, strain P16^T represents a novel species of the genus *Alkalicoccobacillus*, for which the name *Alkalicoccobacillus porphyridii* sp. nov. is proposed. The type strain is P16^T (=KACC 19520^T=JCM 32931^T). In addition, *S. plakortidis* and *S. gibsonii* are reclassified as *Alkalicoccobacillus plakortidis* comb. nov. (type strain P203^T=DSM 19153^T=NCIMB 14288^T) and *Alkalicoccobacillus gibsonii* comb. nov. (type strain PN-109^T=ATCC 700164^T=DSM 8722^T=KCCM 41407^T), respectively.

INTRODUCTION

The genus *Bacillus* of the family *Bacillaceae* was divided into six novel genera including the genus *Alkalihalobacillus* by Patel and Gupta [1]. The genus *Alkalihalobacillus* was further reclassified into eight genera, namely, *Alkalihalobacillus, Alkalihalobacterium*, *Halalkalibacter*, *Alkalihalophilus*, *Halalkalibacterium*, *Pseudalkalibacillus*, *Alkalicoccobacillus* and *Shouchella*, by Joshi *et al.* [2] based on overall genome sequence relatedness. At the time of writing, the genus *Alkalicoccobacillus* includes only a single species *Alkalicoccobacillus murimartini*, which was isolated from a mural painting in a church [3]. Cells of the genus *Alkalicoccobacillus* are catalase- and oxidase-positive, Gram-stain-positive and motile rods with flagellar and menaquinone-7 (MK-7) is their major respiratory quinone. In this study, we isolated a presumably novel species belonging to the genus *Alkalicoccobacillus*, designated strain P16^T, from a micro red marine alga, *Porphyridium cruentum*. In addition, we found that *Shouchella plakortidis* [3] and *Shouchella gibsonii* [4] are closely related to *A. murimartini* based on their 16S rRNA gene sequences, suggesting that their

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Keywords: *Alkalicoccobacillus porphyridii*; new taxa; red alga; *Alkalicoccobacillus plakortidis*; *Alkalicoccobacillus gibsonii*; reclassification.
Abbreviations: AAI, average amino acid identity; AF, aligment fraction; ANI, average nucleotide identity; DDH, DNA–DNA hybridization; DPG, diphosphatidylglycerol; LB, Luria–Bertani; MA, marine agar; MB, marine broth; MK, menaquinone; ML, maximum-likelihood; MP, maximum-parsimony; NJ, neighbour-joining; PE, phosphatidylethanolamine; PG, phosphatidylglycerol; POCP, percentage of conserved proteins; R2A, Reasoner's 2A.
The GenBank accession number for the 16S rRNA gene of strain P16^T is MN080901, and those for the genome sequences of strains P16^T, KCCM 41407^T and DSM 19153^T are VLXZ00000000, JAEUZA000000000 and JAMQJY000000000, respectively.
Five supplementary figures and one supplementary table are available with the online version of this article.

reclassification into the members of the genus *Alkalicoccobacillus* may be necessary. Therefore, we taxonomically characterized strain P16^T as representing a novel species of the genus *Alkalicoccobacillus* by using a polyphasic approach and proposed the reclassification of *S. plakortidis* and *S. gibsonii* as *Alkalicoccobacillus plakortidis* comb. nov. and *Alkalicoccobacillus gibsonii* comb. nov., respectively.

ISOLATION AND ECOLOGY

Strain P16^T was isolated from a culture of a micro red marine alga, *Porphyridium cruentum*, which was isolated from the Yellow Sea, Repulic of Korea (36° 54′ 15.9″ N 126° 11′ 52.8″ E), as described previously [5]. In brief, the algal culture was mechanically homogenized and serially diluted in artificial seawater (20 g NaCl, 2.9 g $MgSO_4$, 4.53 g $MgCl_2{\cdot}6H_2O$, 0.64 g KCl and 1.75 g $CaCl_2{\cdot}2H_2O$ per litre). Aliquots of each serial dilution were spread on 1/5 strength marine agar (MA; BD) and incubated aerobically at 30 °C for 3 days. The 16S rRNA genes of the colonies grown on agar media were amplified via PCR using the 16S rRNA gene universal primers F1 (5′-AGAGTTTGATCMTGGCTCAG-3′) and R13 (5′-TACGGYTACCTTGTTACGACTT-3′) [5]. The amplified PCR products were double-digested using two restriction enzymes (*Hae*III and *Hha*I) at 37 °C for 4 h and representative PCR amplicons showing different fragment patterns were selected and partially sequenced using the 16S rRNA gene universal primer 340F (5′-CCTACGGGAGGCAGCAG-3′) [5] at Macrogen (Seoul, Republic of Korea). The resulting 16S rRNA gene sequences were compared with those of validated type strains using the nucleotide similarity search program in the EzBioCloud server (www.ezbiocloud.net/identify/) [6]. From this analysis, the putative novel strain of the genus *Alkalicoccobacillus*, designated strain P16^T, was selected for further phenotypic and phylogenetic analyses and preserved at −80 °C in marine broth (MB; BD) containing 15% (v/v) glycerol. *Shouchella plakortidis* DSM 19153^T, *Shouchella gibsonii* KCCM 41407^T and *Alkalicoccobacillus murimartini* DSM 19154^T, purchased from their culture collection centres, were used as reference strains for the comparison of phenotypic properties and fatty acid compositions.

16S rRNA GENE SEQUENCE PHYLOGENY

The 16S rRNA gene amplicon of strain P16^T amplified using the F1 and R13 primers was further sequenced using the universal primers 518R (5′-ATTACCGCGGCTGCTGG-3′) and 805F (5′-GATTAGATACCCTGGTAGTC-3′). Three partial 16S rRNA gene sequences obtained by sequencing using the 340F, 518R and 805F primers were assembled, and an almost-complete 16S rRNA gene sequence (1475 nucleotides) of strain P16^T was obtained. The 16S rRNA gene sequence similarity values between strain P16^T and the closely related type strains were calculated using the EzBioCloud server. The 16S rRNA gene sequences of strain P16^T and closely related type strains were then aligned using Infernal software (version 1.1.4) with the covariance model of Rfam family RF00177 [7]. Next, phylogenetic trees with bootstrap values (1000 replications) were reconstructed based on the maximum-likelihood (ML), neighbour-joining (NJ), and maximum-parsimony (MP) algorithms using the MEGA11 software [8].

Comparative analysis of the 16S rRNA gene sequences revealed that strain P16^T was most closely related to *S. plakortidis* P203^T, *S. gibsonii* DSM 8722^T and *A. murimartini* LMG 21005^T with 98.1, 98.1, and 98.0% sequence similarities, respectively. Phylogenetic analysis based on the ML algorithm showed that strain P16^T formed a cluster with *S. plakortidis* P203^T, *S. gibsonii* DSM 8722^T and *A. murimartini* LMG 21005^T with a high bootstrap value (98%) (Fig. 1). Phylogenetic analyses based on the NJ and MP algorithms also suggest that strain P16^T, *S. plakortidis* P203^T, *S. gibsonii* DSM 8722^T and *A. murimartini* LMG 21005^T may form a single phylogenetic lineage (Fig. S1, available in the online version of this article). In a previous study [2], *S. plakortidis* was classified as a heterotypic synonym of *Shouchella oshimensis* [9] based on genome sequence relatedness analysis. However, phylogenetic analyses based on the 16S rRNA gene sequences clearly showed that *S. plakortidis* and *S. oshimensis* were phylogenetically distinct, suggesting that they may be different genus or species members. The 16S rRNA gene sequence similarities between strain P16^T and the closely related type strains were lower than the threshold value (98.65%) for bacterial species delineation [10], suggesting that strain P16^T may represent a novel species of the genus *Alkalicoccobacillus*. In addition, the tight clustering of *S. plakortidis* and *S. gibsonii* with *A. murimartini*, the type species of the genus *Alkalicoccobacillus*, suggests that *S. plakortidis* and *S. gibsonii* may need to be reclassified as members of the genus *Alkalicoccobacillus*.

GENOME FEATURE AND PHYLOGENOMIC ANALYSIS

Genomic DNA was extracted from strain P16^T, *S. gibsonii* KCCM 41407^T and *S. plakortidis* DSM 19153^T using the phenol-chloroform extraction and ethanol precipitation method [11]. Genomic DNA extracted from strain P16^T and *S. gibsonii* KCCM 41407^T was sequenced by an Illumina Hiseq X 10 instrument to generate 151 bp paired-end sequencing reads at Macrogen, while genomic DNA extracted from *S. plakortidis* DSM 19153^T was sequenced using an Oxford Nanopore Minion platform in the laboratory. The sequencing reads obtained via Illumina HiSeq and Minion sequencing were *de novo*-assembled using standalone SPAdes (version 3.13.0) [12] and Unicycler (version 0.4.9b) [13], respectively. The quality of the assembled draft genomes was evaluated based on their completeness and contamination rates using CheckM software (version 1.0.4) [14]. The obtained whole genome sequences of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were deposited in GenBank and annotated using the NCBI Prokaryotic Genome Annotation Pipeline [15]. To infer the phylogenetic positions of strain P16^T, *S.*

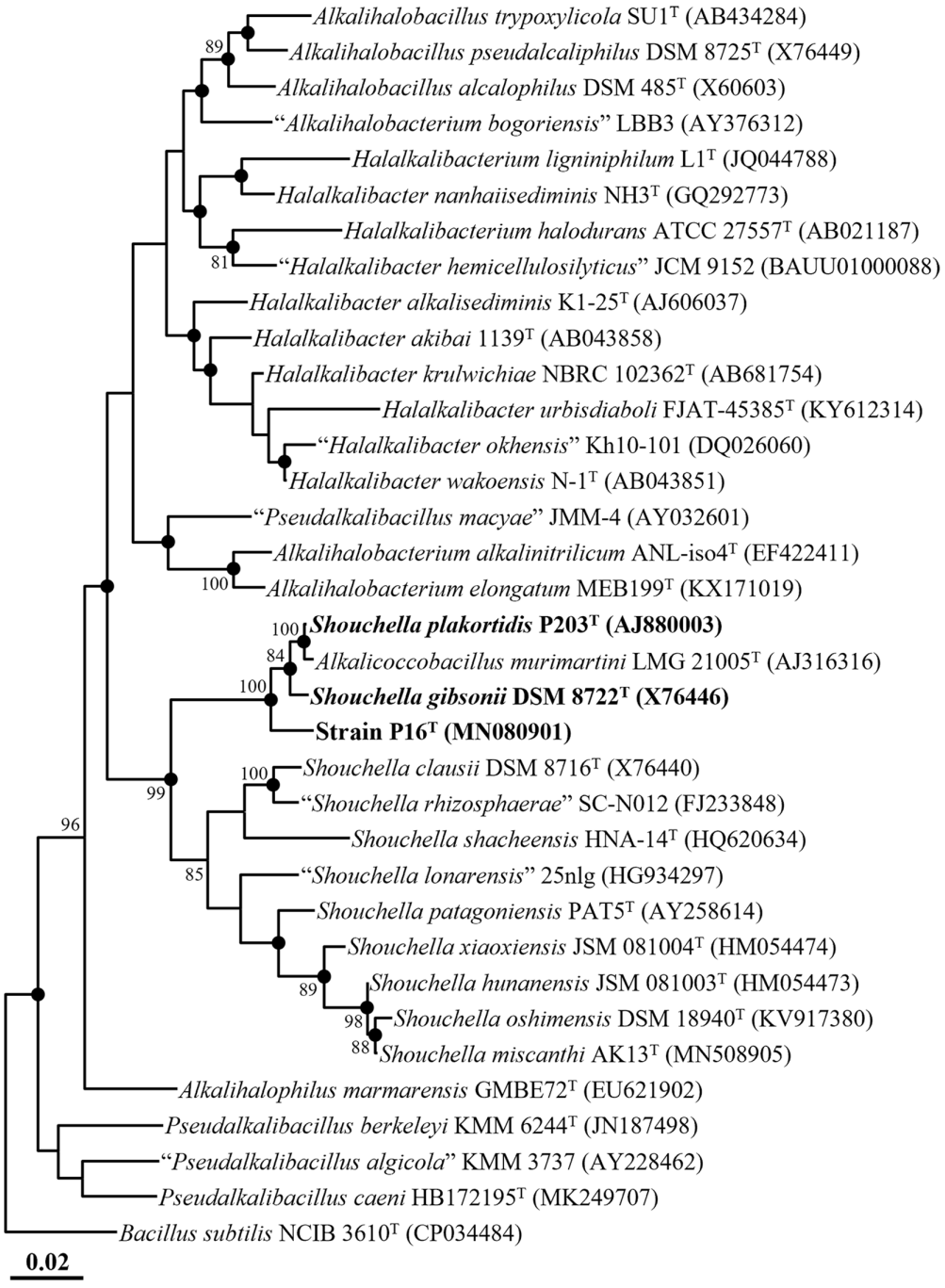

Fig. 1. Maximum-likelihood tree showing the phylogenetic positions of strain P16^T, *Shouchella plakortidis* P203^T and *Shouchella gibsonii* DSM 8722^T, based on their 16S rRNA gene sequences. Bootstrap values over 70% are shown on the nodes as percentages of 1000 replicates. Filled circles (●) indicate that the corresponding nodes recovered by the neighbour-joining and maximum-parsimony algorithms. *Bacillus subtilis* NCIB 3610^T (CP034484) was used as an outgroup. The scale bar equals 0.02 changes per nucleotide.

plakortidis DSM 19153^T and *S. gibsonii* KCCM 41407^T, a phylogenomic tree with bootstrap values (1000 replications) based on the concatenated nucleotide sequences of 81 housekeeping core genes was reconstructed using the Up-to-date Bacterial Core Genes 2 (UBCG2) pipeline (http://leb.snu.ac.kr/ubcg2) [16] and visualized using MEGA11 software. The average nucleotide identity (ANI) and digital DNA–DNA hybridization (DDH) values among strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T were calculated using the Orthologous ANI Tool online (www.ezbiocloud.net/tools/orthoani) [17] and the Genome-to-Genome Distance Calculator version 2.1 (https://ggdc.dsmz.de/ggdc.php) with formula 2 [18],

Table 1. General genomic features and genome relatedness analysis of strain P16^T and the closely related type strains

Strains: 1, P16^T (VLXZ00000000); 2, *Shouchella plakortidis* DSM 19153^T (JAMQJY000000000); 3, *Shouchella gibsonii* KCCM 41407^T (JAEUZA000000000); 4, *Alkalicoccobacillus murimartini* DSM 19154^T (IMG-taxon 2926524226). The genomes of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were sequenced in this study, whereas the genome sequence information of strain DSM 19154^T was obtained from the IMG database.

Feature		**1**	**2**	**3**	**4**
Genome size (kb)		4383	4366	3938	4011
Genome status		Draft	Draft	Draft	Draft
No. of contigs		499	9	33	14
N50 value (kb)		312.2	274.1	971.7	898.5
G+C content (mol%)		40.8	39.5	40.4	39.7
No. of genes		4543	4672	4105	4259
No. of protein coding genes (CDS)		4328	3999	3904	4118
No. of tRNA genes		68	73	68	73
			Digital DDH value (%)*		
ANI value (%)†	**Strain**	**1**	**2**	**3**	**4**
	1	–	**19.5**	**18.3**	**19.1**
	2	75.0	–	**19.9**	**23.1**
	3	73.2	75.8	–	**18.8**
	4	74.7	79.9	74.8	–

*Digital DDH value, DNA–DNA hybridization value (highlighted in bold); ANI value, average nucleotide identity value (normal).

respectively. Average amino acid identity (AAI), the percentage of conserved proteins (POCP) and alignment fraction (AF) among strain P16^T, *S. plakortidis* DSM 19153^T, *S. gibsonii* KCCM 41407^T and closely related type strains were analysed using the EzAAI tool (version 1.2.1) with default options [19], the method described by Qin *et al.* [20] and a server-based ANIcalculator (https://ani.jgi.doe.gov/html/calc.php) [21], respectively. Functional analysis of strain P16^T was performed using the KEGG annotation server [22] and the bacterial version of antiSMASH 6.0 [23].

Since *S. plakortidis* and *S. oshimensis*, which have been classified as heterotypic synonyms, were phylogenetically distant according to the 16S rRNA gene sequence-based analyses, the genome of *S. plakortidis* DSM 19153^T was sequenced again in this study. Although the genome sequence information deposited as *S. plakortidis* DSM 19153^T is present in GenBank (LJJD00000000), this genome sequence information was considered to be the genome information of another bacterial strain (probably closely related to *S. oshimensis*). In addition, because the genome information of *S. gibsonii* is not available, the genome of *S. gibsonii* KCCM 41407^T was sequenced in this study. The *de novo* assemblies of the genome sequencing reads of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T resulted in 449, 9 and 33 contigs, respectively, with N50 values of 312.2, 274.1 and 971.7 kb, respectively. The average genome coverages of the sequencing data of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were 763×, 103× and 617×, respectively. The genome sizes of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were 4383, 4366 and 3938 kb, respectively, and their G+C contents were 40.8, 39.5 and 40.4 mol%, respectively. The completeness and contamination rates of the draft genomes of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were 98.2, 98.0 and 98.7% and 1.9, 0.8, nd 0.2%, respectively, which satisfied the criteria (≥90 and ≤10%, respectively) to be considered high-quality genomes [14]. The sequencing summaries and general genomic features of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T are presented in Table 1. The general genomic features of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T were similar.

Phylogenomic analysis based on the concatenated amino acid sequences of 92 bacterial housekeeping core genes also showed that strain P16^T, *S. plakortidis* DSM 19153^T, *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T formed a single phylogenetic lineage (Fig. 2); this was also consistent with the findings based on the 16S rRNA gene sequence-based analyses. In addition, the phylogenomic tree clearly showed that *S. plakortidis* and *S. oshimensis* were phylogenetically distant, as shown by results of 16S rRNA gene sequence-based analyses, indicating that they were not heterotypic synonyms. Since the genome information deposited as the genome of *A. murimartini* (LGUH00000000) in GenBank was considered not to be the correct genome of *A. murimartini*, IMG-taxon genome information (accession no. 2926524226) was used instead of the GenBank genome information in the phylogenomic and genome relatedness analyses. The ANI and digital DDH values among strain P16^T, *S. plakortidis*

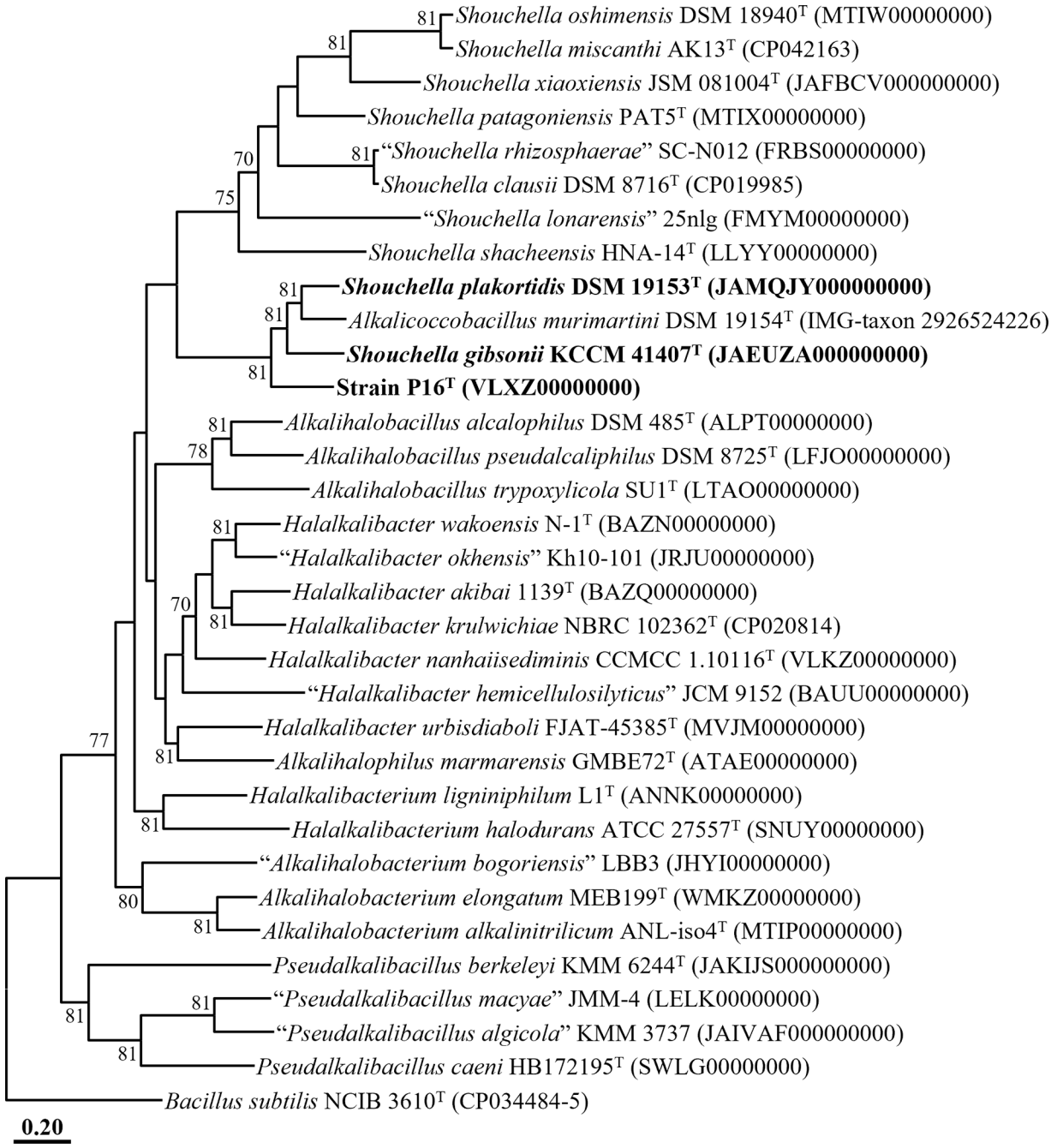

Fig. 2. A maximum-likelihood tree showing the phylogenetic positions of strain P16^T, *Shouchella plakortidis* DSM 19153^T and *Shouchella gibsonii* KCCM 41407^T, based on the concatenated amino acid sequences of 92 housekeeping core genes. Bootstrap values (> 70 %) based on 1000 replicates are shown on the branch nodes. *Bacillus subtilis* NCIB 3610^T (CP034484-5) was used as an outgroup. The scale bar equals 0.20 changes per amino acid.

DSM 19153^T, *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T were less than 79.9 and 23.1%, respectively, which were lower than the prokaryotic species delineation thresholds (ANI, ~95%; digital DDH, 70%) [24, 25], suggesting that strain P16^T, *S. plakortidis* DSM 19153^T, *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T are different species.

The AAI and POCP values between strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T and *A. murimartini* DSM 19154^T were 71.4–75.8% and 77.5–78.7%, respectively, whereas the AAI and POCP values between strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T and *S. clausii* DSM 8716^T were 48.0–54.5% and 54.0–60.2%, respectively (Table S1). These results suggested that the genomes of strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T are more closely related to the genome of *A. murimartini* DSM 19154^T (the type species of the genus *Alkalicoccobacillus*) compared to the

genome of *S. clausii* DSM 8716^T (the type species of the genus *Shouchella*) and they should be classified into members of the genus *Alkalicoccobacillus* and not of the genus *Shouchella*. In addition, it was reported that a combination of AF representing a complementary measure of genetic relatedness of a pair of genomes based on gene contents and ANI values can be also used for delineating a prokaryotic genus [21]. The AF and ANI plots clearly showed that strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T were closely related to *A. murimartini* (Fig. S2A), and they were well separated from *S. clausii* (Fig. S2B), which also supported that strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T should be classified into the genus *Alkalicoccobacillus* and not the genus *Shouchella*.

ALGAL SYMBIOSIS-ASSOCIATED GENES

Genomic analysis of strain P16^T showed that it harbours genes encoding enzymes capable of synthesizing riboflavin from GTP and ribulose 5-phosphate (*ribABDEH* and *ribBEH*, respectively), pyridoxal 5′-phosphate from D-ribose 5-phosphate (*pdxST*), pantothenate from pyruvate (*ilvCDM* and *panBCE*) and folate from GTP (*folABCEKP* and *phoD*). Since vitamins play essential roles in central metabolism in marine algae [26], the ability of strain P16^T to produce vitamins may have beneficial effects on the growth of its algal hosts. Strain P16^T harbours four putative secondary metabolite biosynthetic gene clusters, including a putative T3PKS cluster (locus_tag: FN960_RS15855) encoding putative chalcone and stilbene synthase, bacteriocin family protein (FN960_RS 16425) and class I lanthipeptide synthetic gene cluster (FN960_RS12755–60) responsible for producing antimicrobial products, which may contribute to protecting algal hosts from pathogens. For coping with salt stress, halophilic and halotolerant bacteria accumulate compatible solutes, such as glycine betaine, glutamate, proline, ectoine and hydroxyectoine, in their cytoplasm [27]. Strain P16^T contained genes that synthesize glycine betaine from choline (*betAB*), glutamate from 2-oxo-glutarate and glutamine (*gltBD*), proline from glutamate (*proABC*), ectoine from L-aspartate-β-semialdehyde (*ectABC*) and hydroxyectoine from ectoine (*ectD*). Therefore, the ability of strain P16^T to produce compatible solutes may confer tolerance to salt stress in its algal hosts.

MORPHOLOGY AND PHYSIOLOGY

Growth of strain P16^T was assessed on several bacteriological agar media: MA, Reasoner's 2A (R2A) agar (BD), Luria–Bertani (LB; BD) agar, nutrient agar (BD) and tryptic soy agar (BD) at 30 °C for 3 days. R2A agar, LB agar, nutrient agar and tryptic soy agar were supplemented with NaCl at a final concentration of 2% (w/v). The growth of strain P16^T was tested on MA at different temperatures (4, 10, 15, 20, 25, 30, 37, 40 and 45 °C) for 3 days and in MB with different pH levels (4.5–11.5, at 0.5 pH unit intervals) at 30 °C for 3 days. MB media with different pH values, below pH 5.0, 6.0–8.0 and above pH 9.0, were prepared using sodium citrate, Na_2HPO_4/NaH_2PO_4 and Tris-HCl buffers, respectively [28], and their pH was re-adjusted, after sterilization (121 °C for 15 min) if necessary. Growth at different NaCl concentrations (0–10% at 1.0% intervals) was assessed for 3 days in MB, which was prepared in the laboratory according to the MB medium composition. Gram staining was performed using the bioMérieux Gram stain kit according to the manufacturer's instructions and the endospores were examined according to the Schaeffer–Fulton staining method [29]. Bacterial motility was observed using the hanging drop method. Motility, cell morphology and the presence of flagella were investigated via phase-contrast microscopy (Carl Zeiss) and transmission electron microscopy (JEM-1010, JEOL) using cells grown on MA at 30 °C for 3 days. The endospore-forming ability of strain P16^T was investigated by culturing the strain in MB supplemented with $MnCl_2$ (5 mg l^{-1}) for 7 days and observing under an optical microscope (Carl Zeiss) after malachite green staining [30]. The catalase and oxidase activities of strain P16^T were tested based on its capability to produce oxygen bubbles in 3% (v/v) aqueous hydrogen peroxide solution (Junsei) and oxidize 1% (w/v) tetramethyl-*p*-phenylenediamine (Merck), respectively [30]. Anaerobic growth was assessed on MA at 30 °C for 21 days under anaerobic conditions using the GasPak Plus system (BBL). The capabilities of strain P16^T and the three reference strains to hydrolyse Tween 20, Tween 80, casein, tyrosine and aesculin were tested on MA, as described previously [30]. Acid production from carbohydrates by strain P16^T and the three reference strains was investigated using an API 50CHB strip (bioMérieux), according to the manufacturer's instructions.

Strain P16^T grew well on MA and grew slowly on LB agar and nutrient agar containing 2% NaCl but did not grow on R2A agar and tryptic soy agar containing 2% NaCl. Growth of strain P16^T was observed at 10–40 °C (optimum, 30 °C) and pH 5.0–11.0 (optimum, pH 8.0) and in the presence of 0–6% (w/v) NaCl (optimum, 2 %). Cells of strain P16^T were observed to be Gram-stain-positive mobile rods with peritrichous flagella of approximately 0.5–0.8 µm width and 2.0–2.3 µm length (Fig. S3). Spore formation of strain P16^T was not observed. Anaerobic growth was not observed after 21 days of incubation. Some phenotypic properties of strain P16^T, including Gram-stain-positive, oxidase and catalase activities, and hydrolysis of casein, aesculin, Tween 80 and tyrosine, were similar to those of the reference strains. In contrast, other properties such as growth temperature and pH, salt tolerance, endospore formation and acid production from glycerol, L-rhamnose, D-sorbitol, xylitol, turanose and D-arabitol differentiated strain P16^T from the closely related type strains (Table 2). Although sporulation by strain P16^T was not observed, genes related to sporulation, such as *spo0A*, *ssp* and *dspA/B*, were identified in the genome of strain P16^T, which suggests that strain P16^T may have sporulation potential.

Table 2. Phenotypic comparisons between strain P16^T and the closely related type strains

Strains: 1, P16^T (this study); 2, *Shouchella plakortidis* DSM 19153^T [3]; 3, *Shouchella gibsonii* KCCM 41407^T [4]; 4, *Alkalicoccobacillus murimartini* DSM 19154^T [3]. All strains are positive for the following characteristics: motility, Gram-staining, activity* of catalase and oxidase, hydrolysis* of casein and aesculin, and acid production* from L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, D-mannitol, amygdalin, arbutin, aesculin, salicin, sucrose, gluconate, 2-keto-gluconate and 5-keto-gluconate. All strains are negative for the following characteristics: hydrolysis* of Tween 20, Tween 80 and tyrosine, and acid production* from D-adonitol, L-sorbose, dulcitol, tagatose, D-fucose, L-fucose and L-arabitol. +, Positive; –, negative.

Characteristic	1	2	3	4
Isolation source	Red algae	Sponge	Soil	Wall mural
Flagellum type	Peritrichous	Peritrichous	Polar	Polar
Spore formation	–	+	+	+
Range for growth:				
Temperature (°C)	10–40	4–30	10–37	10–30
pH	5–11	6.5–10	7–8.5	7–10
NaCl (%)	0–6	0–12	0–9	0–4
Acid production from:*				
Inositol	+	+	+	–
Methyl *α*-D-glucoside, cellobiose, maltose, raffinose, D-galactose	+	+	–	+
Trehalose	+	–	+	+
Starch, glycogen, gentiobiose	+	–	+	–
Glycerol	+	–	–	+
L-Rhamnose, D-sorbitol	+	–	–	–
Xylitol	–	+	+	+
Turanose, D-arabitol	–	+	+	–
Melibiose, methyl *α*-D-mannoside, lactose, *N*-acetylglucosamine, melezitose	–	+	–	+
Inulin, methyl *β*-D-xyloside	–	–	+	+
Erythritol, L-xylose, D-xylose	–	–	+	–
D-Arabinose	–	–	–	+

*Data from this study.

CHEMOTAXONOMY

The isoprenoid quinone of strain P16^T was extracted according to the method of Minnikin *et al.* [31] and analysed using an HPLC system (LC-20A, Shimadzu) equipped with a reverse-phase column (250×4.6 mm, Kromasil, Akzo Nobel) and a diode array detector (SPD-M20A, Shimadzu), as described previously [32]. For cellular fatty acid analysis, strain P16^T and the reference strains were cultivated in MB at 30 °C and the microbial cells were harvested at the same growth stage (exponential phase; optical density at 600 nm=0.8). The fatty acids of the microbial cells were saponified, methylated and extracted according to the standard MIDI protocol. Fatty acid methyl esters were analysed using a gas chromatograph (Hewlett Packard 6890; USA) and identified using the RTSBA6 database of the Microbial Identification System (Sherlock ver. 6.0B) [33]. The cell-wall peptidoglycan of strain P16^T and *A. murimaritini* DSM 19154^T was analysed according to the procedure described by Schleifer and Kandler [34], and the presence of *meso*-diaminopimelic acid was confirmed using a standard compound purchased from Sigma-Aldrich. The polar lipids of strain P16^T were analysed via two-dimensional thin layer chromatography using cells harvested during the exponential growth phase, as previously described by Minnikin *et al.* [31]. The following reagents were used to detect the different polar lipids: 10% ethanolic molybdophosphoric acid (for total polar lipids), ninhydrin (for aminolipids), Dittmer–Lester reagent (for phospholipids) and *α*-naphthol (for glycolipids). The presence or absence of diphosphatidylglycerol (DPG), phosphatidylglycerol (PG) and phosphatidylethanolamine (PE) were verified using standard polar lipid compounds purchased from Sigma-Aldrich.

The only respiratory quinone detected in strain P16^T was menaquinone-7 (MK-7), which is in line with that detected in the members of the family *Bacillaceae* [2]. The major cellular fatty acids (>5% of the total fatty acids) of strain P16^T were anteiso-$C_{15:0}$

Table 3. Cellular fatty acid composition (%) of strain P16^T and the closely related type strains

Strains: 1, P16^T; 2, *Shouchella plakortidis* DSM 19153^T; 3, *Shouchella gibsonii* KCCM 41407^T; 4, *Alkalicoccobacillus murimartini* DSM 19154^T. All data were obtained from this study. Data are expressed as percentages of total fatty acids and fatty acids with proportions less than 1.0% in all strains are not shown. The major components (>5.0 %) are highlighted in bold. tr, trace amount (<1.0 %); –, not detected.

Fatty acid	1	2	3	4
Saturated:				
$C_{14:0}$	1.7	1.2	1.6	TR
$C_{16:0}$	3.8	2.9	**5.7**	2.7
Unsaturated:				
$C_{16:1}\,\omega 11c$	–	TR	–	1.0
Branched:				
iso-$C_{14:0}$	**9.8**	**5.8**	**7.7**	3.7
iso-$C_{15:0}$	**13.5**	**17.5**	**10.7**	–
anteiso-$C_{15:0}$	**52.8**	**59.9**	**53.0**	**76.9**
iso-$C_{16:0}$	**8.4**	3.2	**10.0**	1.9
iso-$C_{17:0}$	1.1	1.3	1.4	1.9
anteiso-$C_{17:0}$	4.0	4.2	4.7	4.5

(52.8%), iso-$C_{15:0}$ (13.5%), iso-$C_{14:0}$ (9.8%) and iso-$C_{16:0}$ (8.4 %), which are generally consistent with those of *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T (Table 3). However, the presence of iso-$C_{15:0}$ as the major fatty acid (>10%) in strain P16^T, *S. plakortidis* DSM 19153^T and *S. gibsonii* KCCM 41407^T was clearly differentiated as it was absent from *A. murimartini* DSM 19154^T. *meso*-Diaminopimelic acid was identified in the cell-wall peptidoglycan of strain P16^T and *A. murimaritini* DSM 19154^T (Fig. S4), suggesting that *Alkalicoccobacillus* strains may have *meso*-diaminopimelic acid as the type diamino acid. DPG, PG and PE were detected as the major polar lipids in strain P16^T, and two other unidentified lipids were also detected as minor polar lipids (Fig. S5). The presence of DPG, PG and PE as the major polar lipids of strain P16^T was in agreement with those reported in the closely related species, *S. plakortidis* [3] and *S. gibsonii* [4].

TAXONOMIC CONCLUSION

Phylogenetic inference based on the 16S rRNA gene and whole-genome sequences demonstrated that strain P16^T is a member of the genus *Alkalicoccobacillus*. In addition, 16S rRNA gene sequence similarities, genome comparisons based on ANI and digital DDH values and some phenotypic and physiological characteristics support its assignment as representing a novel species of the genus *Alkalicoccobacillus*, for which the name *Alkalicoccobacillus porphyridii* sp. nov. is proposed. In addition, phylogenetic analysis based on 16S rRNA gene and whole-genome sequences, genome sequence relatedness (ANI and digital DDH values) and physiological and chemotaxonomic features (fatty acid and polar lipid profiles) also suggest that *Shouchella plakortidis* and *Shouchella gibsonii* should be reclassified as *Alkalicoccobacillus plakortidis* comb. nov. and *Alkalicoccobacillus gibsonii* comb. nov., respectively.

DESCRIPTION OF *ALKALICOCCOBACILLUS PORPHYRIDII* SP. NOV.

Alkalicoccobacillus porphyridii (por.phy.ri'di.i N.L. gen. n. *porphyridii* of *Porphyridium*, referring to the isolation of the type strain from the red alga, *Porphyridium cruentum*).

Cells are Gram-stain-positive, strictly aerobic, alkali-tolerant and motile rods with peritrichous flagella. Colonies on MA are ivory in colour, slightly convex and circular with a smooth surface. Growth occurs at 10–40 °C (optimum, 30 °C) and pH 5.0–11.0 (optimum, pH 8.0) and in the presence of 0–6% (w/v) NaCl (optimum, 2%). Oxidase and catalase are positive. Casein and aesculin are hydrolysed, but Tween 20, Tween 80 and tyrosine are not. Cells are positive for acid production from L-arabinose, D-ribose, D-xylose, D-glucose, D-fructose, D-mannose, D-mannitol, amygdalin, arbutin, aesculin, salicin, sucrose, gluconate, 2-keto-gluconate, 5-keto-gluconate, inositol, methyl α-D-glucoside, cellobiose, maltose, raffinose, D-galactose, trehalose, starch, glycogen, gentiobiose, glycerol, L-rhamnose and D-sorbitol, but negative for the utilization of D-adonitol, L-sorbose, dulcitol, tagatose, D-fucose, L-fucose, L-arabitol, xylitol, turanose, D-arabitol, melibiose, methyl α-D-mannoside, lactose, *N*-acetylglucosamine, melezitose, inulin, methyl β-D-xyloside, erythritol, L-xylose, D-xylose and D-arabinose. MK-7 is identified as the sole respiratory

isoprenoid quinone. Major cellular fatty acids are anteiso-$C_{15:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$. The cell-wall peptidoglycan contains *meso*-diaminopimelic acid. DPG, PG and PE are major polar lipids. The DNA G+C content and genomic size of the type strain are 40.8mol% and 4383 kb, respectively.

The type strain is P16^T (=KACC 19520^T=JCM 32931^T), isolated from the marine red alga *Porphyridium cruentum* in the Republic of Korea. The GenBank accession numbers of the 16S rRNA gene and genome sequences of this strain are MN080901 and VLXZ00000000, respectively.

DESCRIPTION OF *ALKALICOCCOBACILLUS PLAKORTIDIS* COMB. NOV.

Basonym: *Bacillus plakortidis* Borchert *et al*. 2007

The description is as given for *Bacillus plakortidis* by Borchert *et al.* [3] with the following amendments. The major fatty acids are iso-$C_{14:0}$, iso-$C_{15:0}$ and anteiso-$C_{15:0}$. The DNA G+C content and genomic size of the type strain are 39.5mol% and 4366 kb, respectively.

The type strain is P203^T (DSM 19153^T=NCIMB 14288^T). The GenBank accession numbers of the 16S rRNA gene and genome sequences of this strain are AJ880003 and JAMQJY000000000, respectively.

DESCRIPTION OF *ALKALICOCCOBACILLUS GIBSONII* COMB. NOV.

Basonym: *Bacillus gibsonii* Nielsen *et al*. 1995

The description is as given for *Bacillus gibsonii* by Nielsen *et al.* [4] with the following amendments. The major fatty acids are $C_{16:0}$, iso-$C_{14:0}$, iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$. The DNA G+C content and genomic size of the type strain are 40.4mol% and 3938 kb, respectively.

The type strain is PN-109^T (ATCC 700164^T=DSM 8722^T=KCCM 41407^T). The GenBank accession numbers of the 16S rRNA gene and genome sequences of this strain are X76446 and JAEUZA000000000, respectively.

EMENDED DESCRIPTION OF THE GENUS *ALKALICOCCOBACILLUS* JOSHI *ET AL*. 2022

This description is based on that given by Joshi *et al.* [2], with the following amendments. The optimum growth NaCl range is 2–7% (w/v). The major fatty acids are anteiso-$C_{15:0}$, iso-$C_{14:0}$, iso-$C_{16:0}$ and anteiso-$C_{17:0}$. The DNA G+C contents and genomic sizes are 39.5–40.8mol% and 3938–4383 kb, respectively.

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

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