**Pseudomonas nanhaiensis** sp. nov., a lipase-producing bacterium isolated from deep-sea sediment of the South China Sea

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**Abstract** A bacterial lipase producing bacterium, designated SCS 2–3, was isolated from deep-sea sediment of the South China Sea. Phylogenetic analysis based on the 16S rRNA sequence revealed that strain SCS2-3 belonged to the genus *Pseudomonas* and had 98.56% similarity to *P. xinjiangensis* NRRL B-51270 T as the closest relative strain. MLSA using four protein-coding genes (*dnaK*, *gyrA*, *recA*, and *rpoB*) showed strain SCS 2–3 to form a separate branch. ANI and in silico DDH values between strain SCS 2–3 and related type strains of *Pseudomonas* were less than 81.51% and 23.80%, respectively. Genome comparison showed that strain SCS 2–3 shared 1875 core gene families with other eight closely related type strains in *Pseudomonas*, and the number of strain-unique genes was 263. Through gene annotations, genes related to lipase were found in the genome. Furthermore, a combination of phenotypic, chemotaxonomic, phylogenetic and genotypic data clearly indicated that strain SCS 2–3 represents a novel species of the genus *Pseudomonas*, for which the name *Pseudomonas nanhaiensis* sp. nov. is proposed. The type strain is SCS 2–3 T (= GDMCC 1.2219 T = JCM 34440 T).

**Keywords** *Pseudomonas nanhaiensis* sp. nov. · Deep-sea · Sediment · Lipase

**Introduction**

*Pseudomonas* is one of the most studied species of bacteria and is also the genus with the largest number of species (George et al. 2005). The name *Pseudomonas* was first proposed by professor Migula of the Karlsruhe Institute in Germany at the end of 19th century. They are Gram-negative, rod-shaped and polar-flagellated bacteria. The genus *Pseudomonas* belongs to class *Gammaproteobacteria*, order *Pseudomonadales*, family *Pseudomonadaceae* and *Pseudomonas aeruginosa* is described as the type species. At the time of writing, genus *Pseudomonas* includes about 396 species with 21 subspecies (https://lpsn.dsmz.de/search?word=Pseudomonas). *Pseudomonas* species are ubiquitous environmental organisms that occupy several niches, including soils, marine environments, fresh water, plants and animals (Khan et al. 2010; Peix et al. 2009). *Pseudomonas* species are of great interest for biotechnological applications due to
their versatile metabolic machinery and their potential for adaptation to fluctuating environmental conditions (Novik et al. 2015). Some of the species have the ability to degrade aromatic compounds (Ma et al. 2012), and others are able to denitrify (He et al. 2016). Moreover, some species can promote plant-growth (Ke et al. 2019), whereas others act as pathogens to plants (Oueslati et al. 2019) and animals (Driscoll et al. 2007).

Additionally, *Pseudomonas* are an excellent source of various extracellular enzymes (Gilbert 1993). Especially, *Pseudomonas* lipases are commonly applied in various industries (Gupta et al. 2004). Lipases (EC 3.1.1.3), triacylglycerol acylhydrolases, are an important group of hydrolases, which catalyze the hydrolysis of long-chain triacylglycerols at the lipid-water interface to liberate fatty acids and glycerol (Jaeger et al. 1999). Lipases can also reverse this reaction in an aqueous and non-aqueous media. Lipases are applied amongst others in food technology, detergents, waste disposal, biosensor modulations, chemical industry and biomedical sciences (Pandey et al. 1999). A number of *Pseudomonas* lipases have been launched successfully in the market, such as Lumafast from *P. mendocina*, Lipomax from *P. alcaligenes*, Chiro CLEC-PC from *P. cepacia* and Lipase AH from *P. fluorescens* (Gupta et al. 2004; Nagarajan 2012; Liu et al. 2017; Phukon et al. 2020).

During our screening for lipase-producing bacteria, we were able to isolate a bacterial strain SCS 2–3 from deep sea sediment from the South China Sea. Based on phenotypic, chemotaxonomic, phylogenetic, genomic and metabolic characters, we classified strain SCS 2–3 as the genus *Pseudomonas*, and strain SCS 2–3 appears to represent a novel species. The putative ability of lipase production of stain SCS 2–3 was studied comparative genomics.

### Materials and methods

#### Sample collection, isolation of bacterial strain and culture conditions

The deep-sea sediment sample was collected from the South China Sea (depth of 1305 m, E 117° 56.6283', N 20° 59.2010'). Briefly, 1 g sediment sample was enriched in 50 ml 2216E liquid medium supplemented with olive oil emulsion for 72 h at 28 °C, 150 rpm. Then, 200 μl of the enrichment was transferred to fresh medium and was cultured at 28 °C, 150 rpm for 72 h. This was routinely repeated three times. The supernatant of last enrichment was serially diluted (10⁻⁵ to 10⁻⁷) with PBS buffer (KH₂PO₄ 0.2 g, Na₂HPO₄·12H₂O 2.9 g, NaCl 8 g, KCl 0.2 g, pH 7.0). 100 μl of each diluted sample was spread on 2216E agar plates and incubated at 28 °C for 48 h. The colonies were aseptically picked and were subcultured on Rhodamine B-olive oil agar plates (Kouker and Jaeger 1987) for selecting lipase producing strains.

#### Phenotypic and biochemical analyses

Colony and cellular morphology were examined by eye and by scanning electron microscope (SU8010, Hitachi, Japan) and transmission electron microscope (H-7650, Hitachi, Tokyo, Japan). A method for Gram-stain reaction determination was modified from Buck’s method (Buck 1982). Growth was monitored at various temperatures (4, 15, 20, 25, 28, 30, 33, 37, 40, 45, and 50 °C) on 2216E agar medium. Tolerance to different NaCl concentrations (0–10%, in increments of 1%, w/v, NaCl) and pH range (pH 4.0–11.0, at intervals of 1 unit) were performed at 28 °C, for 7 days (Guo et al. 2020). Anaerobic growth was tested in an MGCAnaeroPouch-Anaero (Mitsubishi, Tokyo, Japan) at 28 °C for 7 days on 2216E agar medium. Catalase and oxidase activities were investigated in 3% (v/v) H₂O₂ and using commercial strips (Huankai, Guangzhou, China) according to the manufacturer’s instruction, respectively. Additional enzyme activities and carbon source utilization assays were examined by using API 20NE, API ZYM (bioMerieux, Marcy-l’Etoile, French) and Biolog plates kits (Hayward, CA, USA), respectively, following the manufacturer’s instruction.

#### Chemotaxonomic analysis

For analysis of the chemotaxonomic features of strain SCS 2–3, a series of experiments were carried out to determine the content of the respiratory quinones, polar lipids, and fatty acids of closely related type strains (*P. xinjiangensis* NRRL B-51270ᵀ and *P. sabulinigri* JCM 14963ᵀ) and SCS 2–3. Respiratory quinones of the studied strain were extracted and analyzed via the HPLC system. Polar lipids of strain
SCS 2–3 were extracted and examined by two-dimensional TLC. The fatty acids were extracted, quantified, and analyzed using the microbial identification system with strain SCS 2–3 and related type strains.

**16S rRNA gene amplification and phylogenetic analysis**

Genomic DNA was extracted and purified by a commercial bacterial genomic DNA isolation kit (Magen, Guangzhou, China). PCR amplification of 16S rRNA gene was carried out using bacterial primers F27 and R1492 (Weisburg et al. 1991). The amplified fragments were cloned into a cloning-vector pJET1.2/Blunt Vector (Thermo Scientific, Waltham, MA, USA) and sequenced by Sangon Biotech (Shanghai, China). The 16S rRNA gene sequence of strain SCS 2–3 was compared with the EzBioCloud server (Yoon et al. 2017) and closely related type strains within the top 30 of 16S rRNA gene sequences were included in the phylogenetic tree analysis. The phylogenetic tree was constructed using the CLustalW algorithm (Hung and Weng 2016) from the MEGA version X software package (Kumar et al. 2018) using the neighbor-joining (NJ), maximum likelihood (ML), and maximum parsimonv (MP) methods followed by bootstrap analysis with 1000 bootstrap replications.

**MLSA based on housekeeping genes**

Multilocus sequence analysis (MLSA) was performed using the method of Maiden et al. (1998). The sequences of four protein-coding genes, dnaK (1923 bp), gyrA (2676 bp), recA (1041 bp), and rpoB (4068 bp), were obtained from the genome sequences (see below). A phylogenetic tree of concatenated sequences (9708 bp) was reconstructed using the neighbor-joining method. Calculation of distances and multiple alignments and construction of the neighbor-joining phylogenetic tree was done with the same methods as those of 16S rRNA gene sequences.

The housekeeping genes of closely related type strains with ≥ 97% 16S rRNA sequence similarity with strain SCS 2–3 are available in EzBioCloud database (Yoon et al. 2017), and include *P. xinjiangensis* NRRL B-51270T, *P. sabulinigri* JCM 14963T, *P. populi* KBL-4-9T, *P. gallaeciensis* V113T, *P. pelagia* CL-AP6T, *P. pachastrellae* JCM 12285T, *P. phragmitis* S-6-2T, *P. abyssi* MT5T and *P. salina* XCD-X85T.

**Genome sequencing, de novo assembly and annotation**

For genomic DNA extraction and sequencing, strain SCS 2–3 was inoculated from glycerol stocks in TBS liquid medium, and grown for 24 h at 28 °C, 200 rpm. Then, bacteria were washed in 1 × PBS and collected by centrifugation at 5000 rpm for 10 min at 4 °C. The genome of SCS 2–3 was extracted and sequence by Majorbio Bio-pharm Technology Co., Ltd (Shanghai, China) on PacBio and illumina HiSeq × 10 platform. A high-quality data set with a corresponding sequencing depth of 100-fold was generated.

The scan map of the bacterial genome is created using SOAPdenovo2 (Luo et al. 2012) and the complete map of the bacterial genome is assembled using canu and SPAdes (Bankevich et al. 2012). Glimmer (Delcher et al. 2007) and GeneMarkS (Besemer and Borodovsky 2005) were used to predict coding sequences (CDS) and plasmid genes, respectively. tRNA and rRNA were predicted using tRNAscan-SE v2.0 (Chan and Lowe 2019) and Barrnap, respectively. Function annotation of SCS 2–3 was obtained from Non-Redundant Protein (NR), SwissProt (Bairoch and Apweiler 2000), Pfam (Finn et al. 2014), Clusters of Orthologous Group (COG) (Jensen et al. 2007), Gene Ontology and Kyoto Encyclopedia of Genes (Kanehisa et al. 2016) databases using BLASTp and the same BLAST thresholds. Additionally, the CAZymes were identified, classified and annotated using CAZy database (Lombard et al. 2014).

**Comparative genomic analysis**

Bacterial Pan Genome Analysis (BPGA), a fast and efficient computational pipeline was used to draw a neighbor-joining phylogenetic tree (Chaudhari et al. 2016). For comparative genomics, the genome sequences of closely related *Pseudomonas* strains were obtained from the EzBioCloud genome database (Yoon et al. 2017) and NCBI genome database. And CDSs of these strains were clustered by USEARCH with identity cut-off at 50% (Edgar 2010). This analysis utilized all default parameters. The genome
average nucleotide identity (ANI) values and digital DNA-DNA hybridization (dDDH) values were calculated using OrthoANI (Lee et al. 2016) and the Genome-to-Genome Distance Calculator (GGDC 2.1) (Meier-Kolthoff et al. 2013), respectively.

Lipase sequence analysis

In our study, ClustalW2 was employed to analyze multiple sequence alignments. Phylogenetic analysis was accomplished using MEGA X with the neighbor-joining method. A bootstrap analysis with 1000 replicates was applied to estimate the reliability of the tree. And visualization was carried out using ESPript 3.1 (Robert and Gouet 2014), which divulge crucial dissimilitude in the lipase as compared to other species. Homology modeling was constructed using the SWISS-MODEL sever (Waterhouse et al. 2018).

Lipase activity

Lipase activity was measured using p-nitrophenyl palmitate (pNPP) as substrate according to the method described by Zhang et al. (2021). Briefly, reaction mixture contained 550 μL of substrate test solution (500 μm pNPP; 50 mM Tris–HCl, pH 8) and 50 μL of crude enzyme. The reaction was incubated for 5 min at 30 °C. Enzyme activity was determined by measuring hydrolysis production of p-nitrophenol (pNP) based on spectrophotometric analysis at 410 nm.

Results and discussion

Morphological and physiological characteristics

In the present study, strain SCS 2–3 was isolated from deep sea sediment sample from the South China Sea. After 3 days of incubation at 28 °C, colonies were yellow, circular and convex with entire edges using tryptic soy broth agar. Cell morphology and size were examined by electron microscopy. SCS 2–3 cells were rod-shaped, measuring approached 0.4 μm in width and more than 1.5 μm in length on the scanning electron microscope (SEM) and transmission electron microscope (TEM) (Figs. 1a and b). Negative-stain transmission electron microscopy showed that cells also contained one polar flagellum (Fig. 1c). Cells of strain SCS 2–3 were Gram-stain-negative and aerobic.

The strain SCS 2–3 was capable of growth at temperatures between 10 and 45 °C, and the strain grew well at pH values between 5.0 and 10.0. The strain SCS 2–3 was tolerant to 10% (w/v) NaCl. In addition, strain SCS 2–3 was resistant to Rifamycin SV and Vancomycin. By comparison with two closely related type strains, strain SCS 2–3 differed in a combination of negative oxidase activity and positive glucuronamide metabolism and nitrate reduction (Table 1).

Chemotaxonomic characteristics

The major isoprenoid quinone detected in aerobically grown strain SCS 2–3 was ubiquinone 9, which is consistent with other species in the genus Pseudomonas. The polar lipids compositions of strain SCS 2–3 were diphosphatidylglycerol, phosphatidylglycerol, phosphatidyethanolamine and two unknown phospholipids (Supplementary Fig. S1). The cellular fatty acid composition of strain SCS 2–3 and two closely related type strains were similar. All strains had the same predominant fatty acids (> 10%) albeit
in varying quantities (Supplementary Table S1). The major cellular fatty acids in strain SCS 2–3 were C18:1ω6c/ω7c, C16:1ω6c/ω7c, and C16:0. Strain SCS 2–3 also has unique fatty acid anteiso C17:1ω9c which was not detected in other 2 close relatives. The different fatty acid profile is also an indication to distinguish strain SCS 2–3 from other Pseudomonas species.

**Phylogenetic characteristics**

The complete 16S rRNA gene sequence (1532 bp) of strain SCS 2–3 was compared with the top-30 type strains using phylogenetic analysis. On the basis of 16S rRNA gene sequence homology, the closest relatives were determined to be *P. xinjiangensis* NRRL B-51270T (98.56%), *P. sabulinigri* JCM 14963T (97.94%), *P. populi* KBL-4-9T (97.67%), *P. gallaeciensis* V113T (97.29%), *P. pelagia* CL-AP6T (97.26%), *P. pachastrellae* JCM 12285T (97.19%), *P. phragmitis* S-6-2T (97.19%), *P. abyssi* MT5T (97.15%) and *P. salina* XCD-X85T (97.12%). The NJ phylogenetic tree revealed that strain SCS 2–3 clustered with members of the genus Pseudomonas and formed a monophyletic clade with *P. xinjiangensis* NRRL B-51270T (Fig. 2).

To further confirm the phylogenetic position of strain SCS 2–3, we performed MLSA based on four protein-coding fragments of the *dnaK*, *gyrA*, *recA*, and *rpoB* gene sequence (Supplementary Fig. S2). The sequence similarities between strain SCS 2–3 and related Pseudomonas type strain are summarized in Table 2. The most closely related Pseudomonas type strain was *P. xinjiangensis* NRRL B-51270T with 88.3% sequence similarity, and the strain SCS 2–3 formed a clear branch (Supplementary Fig. S2).

| Characteristic                  | 1   | 2   | 3   |
|---------------------------------|-----|-----|-----|
| Sodium butyrate                 |     | w   |     |
| All data are from this study    |     |     |     |
| Strains: 1, SCS 2–3; 2, *P. xinjiangensis* NRRL B-51270T; 3, *P. sabulinigri* JCM 14963T |
| + positive, w weakly positive, − negative |

*Table 1 continued*

| Characteristic                  | 1   | 2   | 3   |
|---------------------------------|-----|-----|-----|
| Sodium butyrate                 |     | w   |     |
| All data are from this study    |     |     |     |
| Strains: 1, SCS 2–3; 2, *P. xinjiangensis* NRRL B-51270T; 3, *P. sabulinigri* JCM 14963T |
| + positive, w weakly positive, − negative |
**Genomic characteristics**

The complete genome sequence of strain SCS 2–3 was assembled into a circular chromosome with 3,653,147 bp (Fig. 3). The DNA G + C content of strain SCS 2–3 was 61.22% well within the range (58–71%) for the genus *Pseudomonas* and close to the 60.7% of *P. xinjiangensis* NRRL B-51270T. SCS 2–3 contained 51 tRNAs and 9 rRNAs (Table 3). A length of 3,287,676 bp genes was found based on gene
Prediction, and 90% was covered by coding sequences. The GC contents of the gene and intergenic region were 61.70% and 56.89%, respectively. Furthermore, 3379 CDSs were contained in the genome, and the average length of gene was 972.97 bp. CDSs were further annotated in NR, Swiss-Port, Pfam, COG, kEGG, GO and CAZy.

Table 2  Sequence similarity, ANI and dDDH of strain SCS 2–3 and the related type strains in genus *Pseudomonas*

| Strains            | Strain SCS 2–3 (%) | 16S rRNA | dnaK | gyrA | recA | rpoB | ANI | dDDH |
|--------------------|--------------------|----------|------|------|------|------|-----|------|
| *P. xinjiangensis* NRRL B-51270 T | 98.56 | 87.7 | 87.3 | 86.1 | 89.9 | 88.3 | 81.51 | 23.80 |
| *P. sabulinigri* JCM 14963 T | 97.94 | 85.7 | 75.9 | 84.8 | 87.1 | 83.4 | 74.59 | 19.50 |
| *P. galleaeciensis* V113 T | 97.29 | 84.1 | 78.2 | 85.4 | 86.9 | 83.6 | 74.56 | 19.70 |
| *P. pelagia* CL-AP6 T | 97.26 | 83.6 | 76.3 | 86.3 | 85.4 | 82.5 | 74.23 | 19.50 |
| *P. pachastrellae* JCM 12285 T | 97.19 | 84.6 | 77.2 | 85.3 | 87.0 | 83.4 | 74.68 | 19.80 |
| *P. phragmitis* S-6-2 T | 97.19 | 85.4 | 75.4 | 85.1 | 86.6 | 83.0 | 75.43 | 19.60 |
| *P. abyssi* MTS T | 97.15 | 84.2 | 78.3 | 85.0 | 86.7 | 83.5 | 74.48 | 19.90 |
| *P. salina* XCD-X85 T | 97.12 | 84.6 | 75.9 | 83.9 | 85.8 | 82.5 | 73.79 | 18.90 |

**Fig. 3** The whole genome sequences of strain SCS 2–3. The genome map is composed of seven circles. From outside to inside, each circle displays information regarding the genome of (1) forward CDS, (2) reverse CDS, (3) forward COG function classification, (4) reverse COG function classification, (5) nomenclature and locations of predictive secondary metabolite clusters, (6) G + C content and (7) GC skew.
Genomic comparison with closely related bacterial strains

The genome of strain SCS 2–3 was compared to the available genome of eight closely related bacterial type strains. This comparison revealed that the genome size of strain SCS 2–3 (3.65 Mb) was larger than that of \textit{P. xinjiangensis} NRRL B-51270\textsuperscript{T} (3.54 Mb), but smaller than those of \textit{P. sabulinigri} JCM 14963\textsuperscript{T}, \textit{P. gallaeciensis} V113\textsuperscript{T}, \textit{P. pelagia} CL-AP6\textsuperscript{T}, \textit{P. pachastrellae} JCM 12285\textsuperscript{T}, \textit{P. phragmitis} S-6-2\textsuperscript{T}, \textit{P. abyssi} MT5\textsuperscript{T}, and \textit{P. salina} XCD-X85\textsuperscript{T} (see Table 3). The number of CDSs of strain SCS 2–3 (3379) was similar to that of \textit{P. xinjiangensis} NRRL B-51270\textsuperscript{T}, but smaller than those of the other strains.

The distribution of genes into COG categories was similar in all nine genomes (Supplementary Fig. S3). The 9 genomes were analyzed with BPGA, which indicated that \textit{Pseudomonas} species have open pangenome, where continuously new genes are being added. In the pangenome, the addition of new genomes, leads to an increase in accessory and unique genes, and a decrease in core genes number (Supplementary Fig. S4). To measure phylogenetic relationships, the CDSs of each strain were used for analysis, and the core genes of individual strain were calculated. The resulting phylogenetic analysis indicated that strain SCS 2–3 formed a monophyletic clade with \textit{P. xinjiangensis} NRRL B-51270\textsuperscript{T} (Supplementary Fig. S5). The core genome of the 9 strains contained 1857 (10.6\%) shared orthologous coding sequences, while 11,946 (67.9\%) genes were represented in the accessory genome, and 3798 (21.6\%) were identified.

### Table 3 General features of the analyzed genomes of \textit{Pseudomonas} strains

| Genome                  | GenBank assembly no | Isolation origin                          | No. of contigs | Total bases | No. of CDSs | No. of rRNAs | No. of tRNAs | GC content | Ref                                      |
|-------------------------|---------------------|--------------------------------------------|----------------|-------------|-------------|--------------|--------------|------------|-----------------------------------------|
| \textit{Pseudomonas} nanaensis SCS 2–3 | CP073751            | deep sea sediment                          | 1              | 3,653,147   | 3,379       | 9            | 51          | 61.2\%    |                                         |
| \textit{P. xinjiangensis} NRRL B-51270\textsuperscript{T} | LT629736            | desert sand                               | 1              | 3,537,092   | 3,280       | 9            | 50          | 60.7\%    | (Liu et al. 2009)                       |
| \textit{P. sabulinigri} JCM 14963\textsuperscript{T} | LT629763            | black sand                                | 1              | 4,030,203   | 3,637       | 9            | 51          | 59.9\%    | (Kim et al. 2009)                      |
| \textit{P. gallaeciensis} V113\textsuperscript{T} | FN995250            | crude-oil-contaminated intertidal sand sample | 18             | 4,246,542   | 3,919       | 3            | 46          | 61.5\%    | (Mulet et al. 2018)                    |
| \textit{P. pelagia} CL-AP6\textsuperscript{T} | AROI010000066       | culture of Pyramimonas gelidicola established from the Antarctic | 81             | 4,642,307   | 4,261       | 3            | 46          | 57.4\%    | (Hwang et al. 2009)                    |
| \textit{P. pachastrellae} JCM 12285\textsuperscript{T} | MUBC01000081        | marine sponge                              | 55             | 3,934,694   | 3,663       | 8            | 49          | 61.2\%    | (Romanenko et al. 2005)                |
| \textit{P. phragmitis} S-6-2\textsuperscript{T} | CP020100            | petroleum polluted river Host: sediment    | 1              | 4,035,153   | 3,703       | 9            | 51          | 60.1\%    | (Li et al. 2020)                       |
| \textit{P. abyssi} MT5\textsuperscript{T} | MF962536            | Deep Sea Water                             | 79             | 4,322,744   | 3,999       | 4            | 46          | 61.2\%    | (Wei et al. 2018)                      |
| \textit{P. salina} XCD-X85\textsuperscript{T} | KC762324            | Salt lake                                 | 45             | 4,256,268   | 3,858       | 10           | 49          | 57.5\%    | (Zhong et al. 2015)                    |
as strain-unique genes (Supplementary Fig. S5). Functional COG annotation revealed that the core genome had a higher proportion of genes classified in COG categories J (translation, ribosomal structure, and biogenesis), E (Amino acid transport and metabolism), H (Coenzyme transport and metabolism), and O (Posttranslational modification, protein turnover, chaperones), all associated with basic metabolic functions. The accessory genome and strain-specific genes were biased toward COG categories T (Signal transduction mechanisms), V (Defense mechanisms), K (Transcription) and P (Inorganic ion transport and metabolism) (Supplementary Fig. S6), which were probably related to the adaption of *Pseudomonas* strains to various kinds of environmental stresses.

DNA–DNA hybridization is required for discrimination of microbial species in cases where 16S rRNA gene sequence similarities are 97% or higher (Spencer et al. 1984). To determine the taxonomic position of strain SCS 2–3, digital DNA–DNA relatedness with other *Pseudomonas* type strains was calculated and showed that strain SCS 2–3 had less than 23.8% DNA–DNA relatedness (Table 2). The ANI between genome sequences of strain SCS 2–3 and closely related type strains (16S rRNA gene similarity > 97%) varied between 73.79 and 81.51% (Table 2). The ANI values are lower than the 95% threshold value (Thompson et al. 2013) and the dDDH values are significantly lower than the 70% threshold value (Wayne et al. 1987). Thus, based on the presented phenotypic and genomic data, we propose the creation of new *Pseudomonas* species with strain SCS 2–3 as its type strain (see below).

**KEGG annotations**

KEGG function-based classification and potential metabolism analysis was used to identify the major metabolic pathways of strain SCS 2–3. As shown in Supplementary Fig. S7, this analysis revealed 39 functional groups. Metabolism contained the most numbers of genes, followed by environmental information processing. In KEGG metabolism annotations of strain SCS 2–3, amino acid metabolism and carbohydrate metabolism, contained 191 and 162 genes, respectively (Supplementary Fig. S7). For these metabolisms, there were some dominant pathways, such as pyruvate metabolism (ko00620) and glyoxylate and dicarboxylate metabolism (ko00630).

Lipid metabolism contained 69 genes. Fatty acid degradation (ko00071, 24 genes), fatty acid biosynthesis (ko00061, 22 genes) and glycerophospholipid metabolism (ko00564, 18 genes) were the dominant pathways. Also, glycerolipid metabolism (ko00561) contained 10 genes including triacylglycerol lipase (EC:3.1.1.3).

Genomic analysis of strain SCS 2–3 supported its phenotypic characteristics. Only one gene encoding the CcoQ protein which is a part of the cbb3-type cytochrome c oxidase complex was present in strain 2–3. Thus, strain SCS 2–3 showed no ability to utilize oxygen as a sole electron acceptor. Some denitrification genes were found in its genome, including *nar*, *nor*, *nas*, *nir* and *nrt*, suggesting that the strains have the ability to utilize nitrate as a sole electron acceptor. Bacterial reduction of nitrate to nitrite was ensured by the presence of the operons encoding putative nitrate reductases (*NarX*, *NarG*, *NarH*, *NarJ*, *NarI*).

Lipase-producing capability and lipase sequence analysis for strain SCS 2–3

The lipase production of strain SCS 2–3 was tested on rhodamine B agar plates for 72 h at 28 °C (Supplementary Fig. S9). Strain SCS 2–3 formed an orange fluorescent zone around the colonies indicative of the lipase activity. The results of lipase activity assay revealed that strain SCS 2–3 was able to hydrolyze *p*-nitrophenyl palmitate.

In general, lipases from *Pseudomonas* have been classified into three categories, and they all belong to family I (Rosenau and Jaeger 2000). Family I.1 lipases have molecular masses in the range 30–32 kDa and display a higher sequence similarity to the *Pseudomonas aeruginosa* lipase. The crystal structure of the *Pseudomonas aeruginosa* lipase was resolved (Nardini et al. 2000) providing the first structure in the lipases of Family I.1. Lipases from family I.2 are characterized by a slightly larger size (33 kDa) due to an insertion in the amino acid sequence forming an anti-parallel double β-strand at the surface of the molecule (Noble et al. 1993; Kim et al. 1997). The expression in an active form of lipases belonging to family I.1 and I.2 depends on a molecular chaperone named lipase-specific foldase (Lif) and these lipases
provide classic signal peptide for secretion through cell membrane. Lipases from family I.3 have a higher molecular mass than lipases from family I.1 and I.2 and the absence of an N-terminal signal peptide and of Cys residues. The secretion of these lipases occurs through a three-component ATP-bind-cassette.

Table 4 Protologue description of *Pseudomonas nanhaiensis* sp. nov

| Description of the new taxon and diagnostic traits |  |
|--------------------------------------------------|--|
| Certain species are Gram-stain-negative, aerobic, motile, rods, catalase positive, oxidase negative, and have a diameter varying from 0.4 to 0.6 μm. The colonies are yellow, circular and convex with entire edges on tryptic soy broth agar. Growth occurs at temperatures in the range 10–45 °C. Cells can grow in a range of pH from 5.0–12.0. Cells can grow without NaCl but also be tolerant to 10% (w/v) NaCl. Cells can reduce nitrate to nitrite. Positive for hydrolysis of Tween 80, and negative for amylopectin, casein, gelatin and urea. This strain has a positive reaction was observed for alkaline phosphatase, esterase (C4), lipase esterase (C8), lipid enzyme (C14) and leucine arylamidase. It is able to utilize D-fructose-6-PO4, D-galacturonic acid, D-glucuronic acid and glucuronamide as carbon sources for growth. The major fatty acids of strain SCS 2–3 are C18:1 ω6c/ω7c, C16:1 ω6c/16:1 ω7c, and C16:0. The predominant isoprenoid quinone detected in aerobically grown cells of strain SCS 2–3 is ubiquinone 9. The polar lipids compositions of strain SCS 2–3 were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylethanolamine and two unknown phospholipids.

Country of origin China
Region of origin The South China Sea
Source of isolation Deep-sea sediment
Sampling date 08/11/2018
Latitude 20° 59.2010’ N
Longitude 117° 56.6283’ E
16S rRNA gene accession nr MZ027638
Genome accession number GenBank = CP073751
Genome status Complete
Genome size 3653 kbp
GC mol% 61.22
Number of strains in study 1
Information related to the Nagoya Protocol Not applicable
Designation of the type strain SCS 2–3^T
Strain collection numbers GDMCC 1.2219^T = JCM 34440^T
transporter system (Rosenau and Jaeger 2000; Zhao et al. 2007). There are 9 genes of Pseudomonas lipases in UniprotKB database (Consortium, 2019) (Supplementary Table S2).

Analysis of the genome of strain SCS 2–3 identified one lipase gene (Gene ID: gene0160). The lipase gene encoded a 311 amino acid sequence with a theoretical molecular mass of 32.86 kDa. This lipase gene sequence was compared with the reference sequences, and NJ phylogenetic tree was constructed (Fig. 4a) and showed that the lipase of strain SCS 2–3 belongs to family I.1. Using in silico techniques the structure and potential function of the lipase encoded in gene0160 was analysed. The structural protein sequence of the lipase is shown in Fig. 4b. The FASTA sequence of the lipase was entered into the SWISS-MODEL server. Based on the sequence, the structure of the molecule was predicted (Fig. 4c). The most appropriate template for homology modeling is the crystal structure of the lipase (Accession No. 1ex9.1.A) from P. aeruginosa. The identity of amino acid sequence was 82.69%. The 3D structure showed a variant of the α/β hydrolase fold, and the Ser108, Asp255 and His277 as the catalytic active center (Figs. 4b and c).

Conclusion

The phylogenetic and phenotypic analysis of the new strain SCS 2–3 exhibited several distinct traits compared to other strains of the genus Pseudomonas. Hence strain SCS 2–3 is a novel species of the genus Pseudomonas for which the name Pseudomonas nanhaiensis sp. nov. is suggested, its protologue description is listed in Table 4. Furthermore, strain SCS 2–3 showed lipolytic activity using rhodamine B agar plates and the lipase from strain SCS 2–3 was identified in its genome.

Author contributions Z.Z. and W.Z. designed research and project outline. Y.P., Y.Z., MR.C., and M.C. performed isolation, deposition and polyphasic taxonomy. Y.P. and Y.Y. performed genome analysis. W.L. and W.Z. contributed to the polar lipid analysis. Y.P. and Z.Z. drafted the manuscript. M.L. revised the manuscript. All authors read and approved the final manuscript.

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Declarations

Conflict of interest The authors declare that there are no conflicts 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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