Investigation of the thermophilic mechanism in the genus *Porphyrobacter* by comparative genomic analysis

Lin Xu¹,², Yue-Hong Wu¹, Peng Zhou¹, Hong Cheng¹, Qian Liu¹ and Xue-Wei Xu¹,³*

**Abstract**

**Background:** Type strains of the genus *Porphyrobacter* belonging to the family *Erythrobacteraceae* and the class *Alphaproteobacteria* have been isolated from various environments, such as swimming pools, lake water and hot springs. *P. cryptus* DSM 12079¹ and *P. tepidarius* DSM 10594¹ out of all *Erythrobacteraceae* type strains, are two type strains that have been isolated from geothermal environments. Next-generation sequencing (NGS) technology offers a convenient approach for detecting situational types based on protein sequence differences between thermophiles and mesophiles; amino acid substitutions can lead to protein structural changes, improving the thermal stabilities of proteins. Comparative genomic studies have revealed that different thermal types exist in different taxa, and few studies have been focused on the class *Alphaproteobacteria*, especially the family *Erythrobacteraceae*. In this study, eight genomes of *Porphyrobacter* strains were compared to elucidate how *Porphyrobacter* thermophiles developed mechanisms to adapt to thermal environments.

**Results:** *P. cryptus* DSM 12079¹ grew optimally at 50 °C, which was higher than the optimal growth temperature of other *Porphyrobacter* type strains. Phylogenomic analysis of the genus *Porphyrobacter* revealed that *P. cryptus* DSM 12079¹ formed a distinct and independent clade. Comparative genomic studies uncovered that 1405 single-copy genes were shared by *Porphyrobacter* type strains. Alignments of single-copy proteins showed that various types of amino acid substitutions existed between *P. cryptus* DSM 12079¹ and the other *Porphyrobacter* strains. The primary substitution types were changes from glycine/serine to alanine.

**Conclusions:** *P. cryptus* DSM 12079¹ was the sole thermophile within the genus *Porphyrobacter*. Phylogenetic analysis and amino acid frequencies indicated that amino acid substitutions might play an important role in the thermophily of *P. cryptus* DSM 12079¹. Bioinformatic analysis revealed that major amino acid substitutional types, such as changes from glycine/serine to alanine, increase the frequency of α-helices in proteins, promoting protein thermostability in *P. cryptus* DSM 12079¹. Hence, comparative genomic analysis broadens our understanding of thermophilic mechanisms in the genus *Porphyrobacter* and may provide a useful insight in the design of thermophilic enzymes for agricultural, industrial and medical applications.

**Keywords:** *Porphyrobacter*, Comparative genomics, Thermophiles, Adaptational mechanism, Amino acid substitution

* Correspondence: xuxw@sio.org.cn
¹ Key Laboratory of Marine Ecosystem and Biogeochemistry, Second Institute of Oceanography, State Oceanic Administration, 310012 Hangzhou, People’s Republic of China
³ Ocean College, Zhejiang University, 316021 Zhoushan, People’s Republic of China
Full list of author information is available at the end of the article

© The Author(s). 2018 Open Access This article is distributed under the terms of the Creative Commons Attribution 4.0 International License (http://creativecommons.org/licenses/by/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided you give appropriate credit to the original author(s) and the source, provide a link to the Creative Commons license, and indicate if changes were made. The Creative Commons Public Domain Dedication waiver (http://creativecommons.org/publicdomain/zero/1.0/) applies to the data made available in this article, unless otherwise stated.
Background
There are nine species in the genus Porphyrobacter at the time of writing [1–9], eight species of which have been validated (except for Candidatus Porphyrobacter meromictius) according to the List of Prokaryotic Names with Standing in Nomenclature [10]. Porphyrobacter species live in diverse environments, including hot springs [4, 6], fresh water [2, 7], stadium seats [1], seawater [5, 8, 9], and swimming pools [3]. Among all type strains within the family Erythrobacteraceae, P. cryptus and P. tepidarius are the only two species isolated from geothermal environments. Moreover, the genus Porphyrobacter has potential applications, such as hydrocarbon degradation [11], algalytic activity [12] and bioleaching [13]. Type strains isolated from hot springs may provide an insight to how these bacteria have adapted to thermal environments. This information may help us design thermophilic enzymes for many important applications.

Thermophiles are microorganisms whose optimum temperature for growth exceeds 45 °C [14]. Thermophiles are widely distributed [14–16] and have been isolated from various geothermal environments, such as artificial hot environments [17–19], marine hydrothermal vents [20–22] and hot springs [23–25]. The thermal stabilities of proteins play an important role in the survival of thermophiles [14, 26]. Amino acid substitutions in protein sequences can improve the thermal stabilities of proteins, which are beneficial changes that help microorganisms adapt to the environment [27–29]. Compared with the proteins of mesophiles, the proteins of thermophiles have a larger fraction of residues in α-helices, enhancing the stability of proteins [30]. Moreover, the proteins of thermophiles often have hydrophobic residues in the protein interior, promoting conformational stability in the inner part of proteins [15, 31]. Therefore, it is worth studying amino acid substitutions in the proteins of thermophiles that exhibit a close phylogenetic relationship to elucidate their adaptational mechanisms to geothermal environments and guide site-directed substitutions in the engineering of industrial enzymes to improve their thermostability.

Next-generation sequencing (NGS) technology generates massive data sets, which offer a convenient approach to studying amino acid substitutions in the protein sequences of microbes. Nishio et al. [32] studied the amino acid substitutions of Corynebacterium efficiens with 45 °C as an upper temperature limit for growth (ULTG) in comparing the genomes of C. efficiens (ULTG: 40 °C) and C. glutamicum and pointed out that three substitutions, changing the amino acid from lysine to arginine, serine to alanine or serine to threonine, enhanced the thermostability of proteins. Takami et al. [33] first sequenced the complete genome of a thermophilic Bacillus-related species, Geobacillus kaustophilus HTA426, with 60 °C as an optimal temperature for growth (OTG) and compared its genome with those of five mesophilic (OTG: 20–45 °C) Bacillus-related species, respectively. However, the analyses have been performed [32–34], few studies have been focused on the class Alphaproteobacteria, especially the family Erythrobacteraceae. To interpret the thermophilic mechanisms in this taxon, here, we sequenced the genomes of five Porphyrobacter type strains using a NGS platform and performed comparative genomic and statistical analysis among eight genomes of the genus Porphyrobacter type strains.

Results and discussion
Range and optimum growth temperature of type strains belonging to the genus Porphyrobacter.

Except for P. cryptus DSM 12079T and P. tepidarius DSM 10594T, other Porphyrobacter strains, whose optimal temperature for growth was in the range of 30–35 °C, could not grow at 45 °C (Fig. 1). The optimal growth temperatures for P. cryptus DSM 12079T and P. tepidarius DSM 10594T were 50 and 45 °C, respectively (Fig. 1). Furthermore, the ranges for the growth of P. cryptus DSM 12079T and P. tepidarius DSM 10594T were 40–60 and 35–50 °C, respectively (Fig. 1); the upper limits of these ranges were higher than those of other Porphyrobacter species.

Although the optimal temperatures for the growth of P. cryptus DSM 12079T and P. tepidarius DSM 10594T were higher than those of other Porphyrobacter strains, P. cryptus DSM 12079T was the only thermophile within the genus Porphyrobacter based on the definition of a thermophile,
which has an optimal temperature that exceeds 45 °C [14]. Notably, P. cryptus DSM 12079T could grow above 50 °C, whereas P. tepidarius DSM 10594T could not.

**Comparative genomic and phylogenomic analyses**

The genome size, number of coding sequences (CDSs), and G + C content of the genus *Porphyrobacter* were 2.9–4.3 Mbp, 2839–4042 and 63.6–67.9 mol%, respectively (Table 1). Compared with other *Porphyrobacter* strains, *P. cryptus* DSM 12079T had the smallest genome size and the second lowest number of CDSs (Table 1). However, the p values of significant tests on genome size and the number of CDSs between *P. cryptus* DSM 12079T and other *Porphyrobacter* strains were 0.056 and 0.100, respectively, which showed that *P. cryptus* was not significantly different from other *Porphyrobacter* species in terms of genome size and the number of CDSs. Therefore, the small genomic size and reduced number of CDSs were not related to the thermophily of *P. cryptus* DSM 12079T.

The number of unique OCs harbored by each *Porphyrobacter* strain varied. *P. mercurialis* CoronadoT had the most unique OCs, and *P. cryptus* DSM 12079T, being a thermophile, harbored the fewest unique OCs (Fig. 2). It was found that over 80% of the unique OCs held by *P. cryptus* DSM 12079T were shared by the closest relatives belonging to the orders *Rhizobiales, Rhodobacterales, Rhodospirillales* and *Sphingomonadales*, which do not include typical thermophiles.

Analysis of the genus *Porphyrobacter* showed that their pan-genome was open (Fig. 2), which suggests that the genus *Porphyrobacter* colonizes multiple environments [35], matching the isolation sources of the genus *Porphyrobacter* (Table 1). It was found that 1434 OCs were shared by all eight *Porphyrobacter* strains (Fig. 2). When screening the single-copy shared OCs of eight *Porphyrobacter* strains and *Altererythrobacter epoxidivorans* CGMCC 1.7731T, the number of shared OCs decreased to 1322. A maximum-likelihood phylogenetic tree reconstructed based on 1322 shared protein sequences revealed that *P. cryptus* DSM 12079T formed a distinct and independent clade from other *Porphyrobacter* strains (Fig. 3). The phylogenetic distance from other members of the same genus may be the result of amino acid substitutions that have been selected for to allow the organism to adapt to thermal environments.

**Analysis of amino acid substitutions**

There were 274 amino acid substitutions in *P. cryptus* DSM 12079T, and the total number that varied amongst all strains was 5698 (Fig. 4, Additional files 1 and 2). Among the whole amino acid substitutions, the major variations (> 3.0%) were changes from valine to isoleucine, asparatic acid to glutamic acid, lysine to arginine, serine to

| Table 1 | Habitats and genomic information of the type strains belonging to the genus *Porphyrobacter* |
|---------|---------------------------------|
| Type Strain | Habitat          | Contigs Number | Total (bp) | N50 Value | G + C Content (mol%) | CDSs Number |
| P. colymbi JCM 18338T | Swimming Pool | 53 | 4,309,164 | 443,561 | 66.5 | 4045 |
| P. cryptus DSM 12079T | Hot Spring   | 34 | 2,954,426 | 268,332 | 67.8 | 2852 |
| P. dokdonensis DSM 17193T | Seawater   | 13 | 2,996,446 | 2,986,864 | 64.8 | 2838 |
| P. donghaensis DSM 16220T | Seawater   | 11 | 3,372,281 | 911,943 | 66.2 | 3152 |
| P. mercurialis CoronadoT | Stadium Seat | 10 | 3,482,341 | 2,089,745 | 67.3 | 3154 |
| P. neustonensis DSM 9434T | Lake         | 1 | 3,090,363 | 3,090,363 | 65.3 | 2902 |
| P. sanguineus JCM 20691T | Seawater   | 39 | 3,018,448 | 462,701 | 63.6 | 2884 |
| P. tepidarius DSM 10594T | Hot Spring | 32 | 3,241,088 | 372,825 | 65.9 | 3104 |
Fig. 2 Pan-genomic curve (a) showing the relationship between OC number and genome number. Schematic view (b) of the core and unique OCs of Porphyrobacter species. Central number indicates the shared OCs of the genus Porphyrobacter, whereas the ambient number represents the unique OCs harboured by each Porphyrobacter species.

Fig. 3 Maximum-likelihood tree based on the concatenation of 1322 protein sequences showing the phylogenetic relationship of type strains belonging to the genus Porphyrobacter. Bootstrap values are based on 100 replicates. Bar, 0.1 substitutions per amino acid position. Red branch shows the clade within P. cryptus DSM 12079T. Altererythrobacter epoxidivorans CGMCC 1.7731T was used as an outgroup (not shown).

Fig. 4 Hot spot map of amino acid variations of Porphyrobacter cryptus. Amino acids located on the vertical axis represent substituting amino acids, whereas amino acids lying on the horizontal axis are substituted amino acids. A, alanine; C, cysteine; D, aspartic acid; E, glutamic acid; F, phenylalanine; G, glycine; H, histidine; I, isoleucine; K, lysine; L, lysine; M, methionine; N, asparagine; P, proline; Q, glutamine; R, arginine; S, serine; T, threonine; V, valine; W, tryptophan; Y, tyrosine.
alanine, and glycine to alanine. When compared with proteins from other thermophiles, where the common amino substitutions reported were changes from lysine to arginine and serine to alanine, the number of variations from valine to isoleucine, aspartic acid to glutamic acid, and glycine to alanine were higher here than in other studies [32–34]. Due to bidirectional substitutions, the substituted/substituting ratio analysis indicated that amino acids were most frequently changed to alanine, which resulted in a net substitutional number that was higher for alanine (a ratio above one) than for other amino acids (Fig. 5, Additional file 3).

In view of amino acid substitutions causing variations in amino acid frequencies, principal component analysis (PCA) of the amino acid frequency revealed that *P. cryptus* DSM 12079^T^ was distinct from other *Porphyrobacter* strains (Fig. 6, Additional file 4), which indicated that the amino acid frequencies of *P. cryptus* DSM 12079^T^ were also different from other strains with PC1 and PC2 accounting for 43 and 40% of the total variances, respectively. It was observed that the frequencies of asparagine, aspartic acid, glutamine, methionine, serine, threonine, tyrosine and valine were the lowest in *P. cryptus* DSM 12079^T^ proteins, whereas the frequencies of alanine, arginine, leucine and proline were the highest in this strain (Fig. 7). The *t*-tests performed on the frequency of these amino acids showed that the *p* values were all lower than 1.0 × 10^{-4}, indicating that these differences were significant.

The results showed that the major amino acids with net gains were alanine, arginine, glutamic acid, isoleucine and serine based on the substituted/substituting residues and amino acid frequency analyses (Figs. 5 and 7). Substitutions of these five amino acids occurred in 1039 proteins out of all 1405 shared and orthologous proteins in the genus *Porphyrobacter*.

Except for 12 overly long proteins that failed to be analyzed in secondary structure predictions, the percentages of α-helices, β-sheets, random coils and turns were identical in the 291 original and substituted protein pairs. However, the percentage of α-helices were clearly lower when the substitutional proteins were compared with their corresponding original proteins in *P. cryptus* DSM 12079^T^ (Additional file 5). Furthermore, 37 conserved proteins were selected through a comparative genomic study of selected Alphaproteobacteria genomic data. Except for five proteins not showing amino acid substitutions, the percentage of α-helices decreased in 18 proteins (Additional file 6). Previous studies showed that net increases in α-helix content could enhance the thermal stability of proteins [36, 37]. It is speculated that a net increase in α-helix content plays a key role in raising protein thermostability in the genus *Porphyrobacter*.

Further analysis of substitutional proteins with decreased ratios of α-helix regions indicated that 68.3% (348/509), 41.1% (209/509) and 39.8% (203/509) of these proteins had increased percentages of β-sheets, random coils and turns, respectively (Fig. 8). It was found that alanine was the most commonly substituted amino acid (43.9%, 739/1682) among the five major substituted amino acids in substitutional proteins, resulting in fewer amino acids forming α-helix, and the most common
replacement amino acids were glycine (20.8%, 154/739) and serine (21.4%, 158/739). In addition, \textit{P. cryptus} DSM 12079T had a distinctly higher frequency ($p = 4.75 \times 10^{-6}$) of alanine than the other \textit{Porphyrobacter} species (Fig. 7).

Comparisons of secondary structures of \textit{P. cryptus} DSM 12079T proteins in which alanine was replaced by glycine/serine indicated that variations of secondary structures occurred in 57.1% (88/154) and 42.4% (67/158) of alanine to glycine/serine sites, respectively. It was investigated whether \( \alpha \)-helices significantly changed into other secondary structures, and secondary structure changes occurred in 89.8% (79/88) of alanine to glycine sites and 94.0% (63/67) of alanine to serine sites. Previous studies showed that alanine was the best helix-forming amino acid [27] and that it was found at a higher frequency in thermophiles than in other organisms [38]. The side chain of alanine is methyl (-CH$_3$), which promotes the interactions of neighboring residues in proteins and contributes to packing in protein structures [39]. In addition, hydrophobic residues located in the protein interior enhance conformational...
stability in the inner part of proteins [31]. Glycine and serine are both hydrophilic amino acids, and they can decrease the core hydrophobicity of proteins. Moreover, glycine tends to increase molecular flexibility by weakening protein stability at higher temperatures [40]; and serine can be released from proteins at higher temperatures, which results in unstable protein structures [41, 42]. Hence, alanine substituting for glycine or serine might promote the formation of α-helices in proteins, which could increase protein thermostability within P. cryptus DSM 12079T.

The analysis of neighboring amino acids of the substituted alanine indicated that hydrophobic amino acids occurred mostly in the N-terminus (41.1%, 58/141) and C-terminus (42.6%, 60/141), and this occurrence was higher than for other amino acid types. Furthermore, alanine, isoleucine and leucine were frequently located in the N-terminus, whereas alanine, leucine and proline were always found in the C-terminus among the neighboring hydrophobic amino acids of the substituted alanine. In addition, it was also found that arginine and glycine were located in neighboring N- and C-terminal ends.

Conclusions
Comparative genomics analysis of the thermophile Porphyrobacter cryptus DSM 12079T with seven non-thermophilic Porphyrobacter strains broadened our knowledge of bacterial thermophilic mechanisms, especially in the class Alphaproteobacteria. Phylogenomic analysis of the genus Porphyrobacter and alignments of proteins encoded by shared single-copy genes revealed that amino acid substitutions play an important role in the thermophily of P. cryptus DSM 12079T. As a whole, substitutions from glycine/serine to alanine in P. cryptus DSM 12079T proteins increased the frequency of α-helices in these proteins, which could promote protein thermostability and ensure the growth of P. cryptus DSM 12079T at high temperatures. It is hoped that the findings of this study will help in the design of thermostable proteins using techniques such as site-directed mutagenesis.

Methods
Range and optimum of growth temperature
Seven type strains of the Porphyrobacter species were purchased from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (P. cryptus DSM 12079T, P. dokdonensis DSM 17193T, P. neustonensis DSM 9434T and P. tepidarius DSM 10594T) and Japan Collection of Microorganisms (P. colympi JCM 18338T and P. sanguineus JCM 20691T). All type strains were cultured in appropriate media [2–6, 8, 9] and maintained in glycerol suspensions (30%, v/v) at –80 °C. The temperature range for growth (4, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55 and 60 °C) was inspected in the appropriate medium of each strain. The absorbance at a wavelength of 600 nm for Porphyrobacter cultures was measured using an ultraviolet and visible spectrophotometer (TU-1810, Persee).

Whole-genomic sequencing, assembly and annotation
Except for the genomic sequences of P. cryptus DSM 12079T, P. mercurialis CoronadoT and P. neustonensis DSM 9434T were acquired under NCBI GenBank accession numbers AUHC00000000, JTDN0000000 [43] and CP016033 [44], respectively. Genomic DNA of the five Porphyrobacter type strains was extracted according to the protocol of AxyPrep™ Bacterial Genomic DNA Miniprep Kit (Axygen®, Corning) and detected through 0.6% (w/v) agarose gel electrophoresis with λ-HindIII digest DNA (Takara, Dalian). Whole-genomic sequencing was carried out using Solexa paired-end sequencing technology (HiSeq2000 system, Illumina, USA) based on a whole-genome shotgun strategy in Anoroad Gene Technology Co. Ltd. (Beijing). Raw reads containing ≥9 bp of N were removed and trimmed by removing adaptor sequences. Clean reads were assembled using ABYSS 1.9.0 [45]. The ORFs were predicted by Glimmer v.3.0 [46] based on assembled genomes and then annotated by using the online Rapid Annotation using Subsystems Technology (RAST) server [47]. Clusters of Orthologous Groups (COGs) protein annotations were carried out by the online WebMGA server [48, 49].

Comparative-genomic and phylogenomic analyses
The predicted proteins of eight Porphyrobacter species as well as Altererythrobacter epoxidivorans CGMCC 1.7731T (GenBank accession number: CP012669) were compared through OrthoMCL version 2.0 based on OrthoMCL algorithm [50, 51] to detect orthologous clusters (OCs) among the genomes of nine species. Pan- and core-genomic analyses were performed using in-house shell and R scripts (Additional file 7). The BLASTp searches [52] of unique OCs harbored by each Porphyrobacter species against a nr database were used to detect the closest relatives with thresholds of the identity above 70% and coverage not less than the half-length of query annotated proteins. Moreover, conserved proteins were selected by comparative genomic analysis with other Alphaproteobacteria genomes (Additional file 8) using OrthoMCL version 2.0 [50, 51].

Single-copy shared OCs of eight Porphyrobacter species and Altererythrobacter epoxidivorans were screened by in-house shell scripts (Additional file 7). Each single-copy shared OC was aligned by using MAFFT version 7 [53]. Aligned sequences refined by trimAL version 1.4.1 [54] were concatenated manually. Then, a phylogenetic tree was constructed by maximum-likelihood [55] algorithms with IQ-TREE 1.6.1 software [56] based on concatenated aligned single-copy OCs with bootstraps analysis set to
Amino acid substitutional analyses
Single-copy shared OCs of eight *Porphyrobacter* strains were screened and aligned according to the methods described above. Each site of the aligned protein sequences was detected through MEGA7 software [57] to determine a mutational site, which was defined as cases where *P. cryptus* DSM 12079T held a different amino acid and other *Porphyrobacter* species had another identical amino acid. The amino acid content of each *Porphyrobacter* species was summarized, and principal components analysis (PCA) was carried out by using the `psych` package within R programming language (https://cran.r-project.org/web/packages/psych/index.html). Proteins in *P. cryptus* DSM 12079T involved in major types of amino acid substitutions with net positive variation were screened using R scripts. Substitutional proteins were created manually by replacing the substituting amino acids with the substituted ones. Original and substitutional proteins were submitted to Chou & Fasman algorithm (http://www.biogem.org/tool/chou-fasman/) to predict their secondary structures based on the Chou & Fasman algorithm [58, 59]. Variations about the percentages of α-helices, β-sheets, random coils and turns in the original and substitutional proteins were summarized to check intrinsic mechanisms leading to the thermophily of *P. cryptus* DSM 12079T.

Additional files

| Additional file 1 | Alignment results of protein amino acid sequences encoded by single-copy genes found in eight Porphyrobacter strains. (ZIP 15.10 kb) |
|-------------------|-------------------------------------------------------------------------------------------------|
| Additional file 2 | Summary of site-directed amino acid variations of *Porphyrobacter cryptus* DSM 12079T. (XLSX 16 kb) |
| Additional file 3 | Summary of the substituted/substituting amino acid in *Porphyrobacter cryptus* DSM 12079T. (XLSX 8 kb) |
| Additional file 4 | Frequency of each amino acid in Porphyrobacter type strains. (XLSX 10 kb) |
| Additional file 5 | Summary of protein secondary structure variations in site-directed substitutions. (XLSX 99 kb) |
| Additional file 6 | Summary of protein secondary structure variations in conserved proteins among Alphaproteobacteria bacteria. (XLSX 12 kb) |
| Additional file 7 | In-house shell and R scripts for pair- and core-genomic analyses and screening single-copy shared OCs. (DOCX 13 kb) |
| Additional file 8 | Summary of sources of Alphaproteobacteria strains applied in investigations of conserved proteins. (XLSX 10 kb) |

Abbreviations
CDSs: Coding sequences; NGS: Next-generation sequencing; OTG: Optimal temperature for growth; UTLG: Upper temperature limit for growth

Funding
This work was supported by grants from the National Natural Science Foundation of China (No. 31770204 and No. 41406174), the Scientific Research Fund of the Second Institute of Oceanography, State Oceanic Administration (U1527), the Natural Science Foundation of Zhejiang Province (LR17D060001) and the National Program for Support of Top-Notch Young Professionals. The funding bodies had no role in the analysis, data interpretation and manuscript writing.

Availability of data and materials
The GenBank accession numbers for *Porphyrobacter colymbi* JCM 18338T, *Porphyrobacter doldanensis* DSM 17193T, *Porphyrobacter donghaensis* DSM 16220T, *Porphyrobacter sanguineus* JCM 20691T and *Porphyrobacter tepidarius* DSM 10594T are MUYK00000000, MUYI00000000, MUYG00000000, MUHY00000000 and MUYJ00000000, respectively.

Authors’ contributions
X-WX conceived and designed the study. LX and Y-HW carried out the laboratory work. LX, PZ, QL and HC performed bioinformatic analysis. LX wrote the manuscript. All authors reviewed and approved the final manuscript.

Ethics approval and consent to participate
Not applicable.

Competing interests
The authors declare that they have no competing interests.

Publisher’s Note
Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Author details
1Key Laboratory of Marine Ecosystem and Biogeochemistry, Second Institute of Oceanography, State Oceanic Administration, 310012 Hangzhou, People’s Republic of China. 2College of Life Sciences, Zhejiang Sci-Tech University, 310018 Hangzhou, People’s Republic of China. 3Ocean College, Zhejiang University, 316021 Zhoushan, People’s Republic of China.

Received: 23 August 2017 Accepted: 14 May 2018
Published online: 23 May 2018

References
1. Coil DA, Flanagan JC, Stump A, Alexiev A, Lang JM, Eisen JA. Porphyrobacter merunculis sp. nov., isolated from a stadium seat and emended description of the genus Porphyrobacter. PeerJ. 2015;3:e1400.
2. Fuerst JA, Hawkins JA, Holmes A, Sly LI, Moore CJ, Stackebrandt E. Porphyrobacter neustonensis gen. Nov. sp. nov., an aerobic bacteriochlorophyll-synthesizing budding bacterium from fresh water. Int J Syst Bacteriol. 1993;43:125–34.
3. Furuhata K, Edagawa A, Miyamoto H, Kawakami Y, Fukuyama M. Porphyrobacter colymbi sp. nov. isolated from swimming pool water in Tokyo, Japan. J Gen Appl Microbiol. 2013;59:245–50.
4. Hanada S, Kawase Y, Hiraishi A, Takachi S, Matsuura K, Shimada K, Nagashima KV. Porphyrobacter tepidarius sp. nov., a moderately thermophilic aerobic photosynthetic bacterium isolated from a hot spring. Int J Syst Bacteriol. 1997;47:408–13.
5. Hiraishi A, Yonemitsu Y, Matsuura M, Shin YK, Kuraishi H, Kawahara K. Characterization of *Porphyrobacter sanguineus* sp. nov., an aerobic bacteriochlorophyll-containing bacterium capable of degrading biphenyl and dibenzofuran. Arch Microbiol. 2002;178:456–66.
6. Rainey FA, Silva J, Nobre NF, Silva MT, da Costa MS. *Porphyrobacter cryptus* sp. nov., a novel slightly thermophilic, aerobic bacteriochlorophyll a-containing species. Int J Syst Evol Microbiol. 2003;53:35–41.
7. Rathgeber C, Yurkova N, Stackebrandt E, Schumann P, Humphrey E, Beatty JT, et al. *Porphyrobacter merunculis* sp. nov., an appended bacterium, that produces bacteriochlorophyll a. Curr Microbiol. 2007;55:356–61.
