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Application of qPCR assays based on haloacids transporter gene *dehp2* for discrimination of *Burkholderia* and *Paraburkholderia*

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Abstract

Background: A major facilitator superfamily transporter Dehp2 was recently shown to be playing an important role in transport and biodegradation of haloacids in *Paraburkholderia caribensis* MBA4, and Dehp2 is phylogenetically conserved in *Burkholderia* sensu lato.

Results: We designed both *Burkholderia* sensu stricto-specific and *Paraburkholderia*-specific qPCR assays based on *dehp2* and 16S rRNA, and validated the qPCR assays in 12 bacterial strains. The qPCR assays could detect single species of *Burkholderia* sensu stricto or *Paraburkholderia* with high sensitivity and discriminate them in mixtures with high specificity over a wide dynamic range of relative concentrations. At relatively lower cost compared with sequencing-based approach, the qPCR assays will facilitate discrimination of *Burkholderia* sensu stricto and *Paraburkholderia* in a large number of samples.

Conclusions: For the first time, we report the utilization of a haloacids transporter gene for discriminative purpose in *Burkholderia* sensu lato. This enables not only quick decision on proper handling of putative pathogenic samples in *Burkholderia* sensu stricto group but also future exploitation of relevant species in *Paraburkholderia* group for haloacids biodegradation purposes.

Keywords: *Burkholderia*, *Paraburkholderia*, Haloacids transporter, Dehp2, qPCR, Pathogenicity

Background

Burkholderia sensu lato includes versatile members that have dramatic different living-styles and occupy diverse ecological niches [1–6]. The most infamous species are *Burkholderia pseudomallei* and *Burkholderia mallei* which cause melioidosis and glanders in animals and humans [7–10]. Besides that, *Burkholderia cepacia* complex (Bcc) includes many closely-related opportunistic pathogens such as *Burkholderia cenocepacia* and *Burkholderia multivorans* [11–14]. There are also many species isolated from the environment with biotechnological application potentials, such as plant growth promotion, antibiotics production, and biodegradation of pollutants [4, 5, 15–17], which are generally defined as "plant-beneficial-environmental (PBE) cluster" [18–20]. Phylogenetic analysis based on single gene such as 16S rRNA, *recA*, *fur*, *acdS*, *hisA* and *rpsU* have revealed the complexity of their evolutionary relationships [21–26]. Based on assessment of conserved sequence indels, a new *Paraburkholderia* genus was created to include the diverse environmental isolates while *Burkholderia* sensu stricto includes *B. mallei*, *B. pseudomallei*, Bcc members and other pathogenic members [27]. The complicated taxonomy of *Burkholderia* sensu lato has attracted great attention, and large scale phylogenomic study has been suggested [28]. In accordance with this suggestion, a recent study systematically analyzed the conserved sequences in 92 *Burkholderia* sensu lato species and demonstrated the existence of 5 lineages: *Burkholderia*

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sensu stricto, *Paraburkholderia*, *Caballeronia*, the newly described genus *Robbsia* [29], and the lineage represented by *Paraburkholderia rhizoxinica* [30]. Assessment of the phylogenetic position of new isolates or samples containing *Burkholderia* or *Paraburkholderia* could be useful for further analysis, and there have been such reports based on molecular techniques such as PCR [22, 31–34], multi-locus sequence typing (MLST) [35–38] and qPCR assays [39–41]. As qPCR could sensitively quantitate the target and is accessible to more places compared with sequencing-based approaches, it has great application potentials in the phylogenetic studies of *Burkholderia* sensu lato.

Paraburkholderia caribensis (formerly *Burkholderia caribensis*) MBA4 is a bacterium with the ability to degrade environmental pollutant haloacids [42]. Besides the hydrolytic enzyme dehalogenase, membrane transporters that mediate active uptake of haloacids are also important for effective biodegradation [43–45]. Although structurally similar, haloacetate is transported with a different system compared with acetate [46]. We have recently revealed that *P. caribensis* MBA4 harbors two haloacids transporters, Deh4p and Dehp2, which show overlapping but not identical substrate specificities [45, 47]. The expression of *dehp2* is strictly regulated in response to the presence of haloacids in its growing environment, and the ~ 100 bp upstream non-coding region of *dehp2* is highly conserved in *Burkholderia* sensu lato [48]. Three strains from other environmental *Paraburkholderia* species, namely *P. caribensis* LMG 18531, *Paraburkholderia phymatum* (formerly *Burkholderia phymatum*) STM815, and *Paraburkholderia xenovorans* (formerly *Burkholderia xenovorans*) LB400, gained the ability to degrade haloacids with the introduction of dehalogenase Deh4a, and haloacids-inducible haloacids transport activities were observed in accordance with haloacids-inducible expressions of *dehp2* orthologs, strongly suggesting their roles as haloacids transporters [16]. Dehp2 thus represents a group of conserved transporters in *Burkholderia* sensu lato, and the phylogenetic tree based on Dehp2 clearly show two clades corresponding well to *Burkholderia* sensu stricto and *Paraburkholderia* [43]. This provides the ground of exploiting Dehp2 for discrimination of *Burkholderia* sensu stricto and *Paraburkholderia*.

In this study, we tested the utilization of *dehp2* as a phylogenetic marker for quick discrimination of putative pathogenic/opportunistic pathogenic *Burkholderia* sensu stricto and mainly environmental-derived *Paraburkholderia*. We designed qPCR assays that target the regions of *dehp2* and 16S rRNA conserved in *Burkholderia* sensu stricto or *Paraburkholderia* and validated their performance in 12 strains of bacteria from *Burkholderia* sensu lato. The results showed that *dehp2* could be used
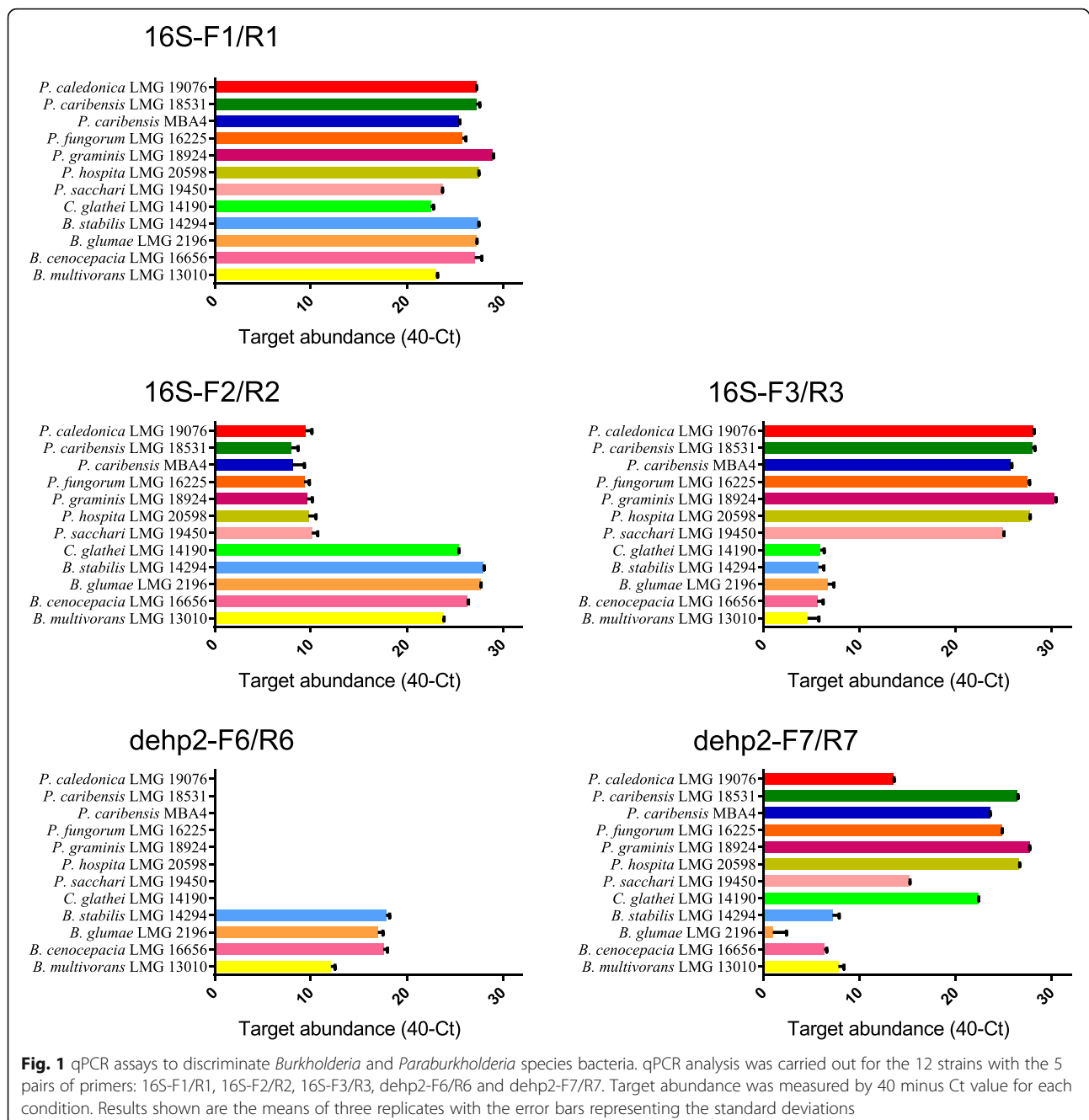
as a discriminative marker similarly as 16S rRNA, and assays based on both markers produced more reliable results with high specificity and sensitivity. This is the first report on the utilization of a haloacids transporter as a discriminative marker in *Burkholderia* sensu lato, which will be useful for further clinical or biotechnological studies.

Results

Quick detection of *Burkholderia* sensu stricto and *Paraburkholderia* by qPCR assays

As described previously, both 16S rRNA and *dehp2* phylogenetic trees display two major groups which could discriminate *Burkholderia* sensu stricto and *Paraburkholderia* species [5, 43]. We first designed three pairs of qPCR primers based on 16S rRNA: 16S-F1/R1 to target the region conserved in *Burkholderia* sensu lato, 16S-F2/R2 to target the region conserved in *Burkholderia* sensu stricto, and 16S-F3/R3 to target the region conserved in *Paraburkholderia*. Similarly, we designed dehp2-F6/R6 and dehp2-F7/R7 to target *Burkholderia* sensu stricto-specific and *Paraburkholderia*-specific regions of *dehp2*. To ensure that the primers cover all the sequence variations, we incorporated degenerate bases during primer design, which have been a common practice in microbial studies [49], such as microbial population taxonomy [50], diversity of functional genes related to antibiotic or arsenite resistance, etc. [51, 52].

We first analyzed the performances of the 5 pairs of primers against the 12 strains using a single strain as the template for each qPCR reaction (Fig. 1). For the *Burkholderia* sensu lato-conserved primer pair 16S-F1/R1, all 12 strains showed similar amplification efficiency. The *Burkholderia* sensu stricto-specific primer pair 16S-F2/R2 had statistically higher amplification efficiency with the 4 *Burkholderia* strains and *C. glathei* LMG 14190, while the *Paraburkholderia*-specific 16S-F3/R3 primer pair showed statistically higher amplification efficiency with the 7 *Paraburkholderia* strains. For *dehp2*, the *Burkholderia* sensu stricto-specific primer pair dehp2-F6/R6 had statistically higher amplification efficiency with the 4 *Burkholderia* strains, while the *Paraburkholderia*-specific dehp2-F7/R7 primer pair showed statistically higher amplification efficiency with the 7 *Paraburkholderia* strains and *C. glathei* LMG 14190. It should be pointed out that the amplification efficiency of qPCR assays will be affected by degenerate primers, but the amplification efficiencies of our discriminative qPCR assays show such dramatic differences between *Burkholderia* sensu stricto and *Paraburkholderia* group that they still enable effective discrimination of the two group even with the use of degenerate primers. The performances of primers targeting *dehp2* were comparable to 16S rRNA-based primers, suggesting *dehp2* could be used

Fig. 1 qPCR assays to discriminate *Burkholderia* and *Paraburkholderia* species bacteria. qPCR analysis was carried out for the 12 strains with the 5 pairs of primers: 16S-F1/R1, 16S-F2/R2, 16S-F3/R3, dehp2-F6/R6 and dehp2-F7/R7. Target abundance was measured by 40 minus Ct value for each condition. Results shown are the means of three replicates with the error bars representing the standard deviations

as a marker for discrimination of *Burkholderia* sensu stricto and *Paraburkholderia*.

The above results clearly showed that the 4 pairs of discriminative primers work well in regard to their ability to discriminate *Burkholderia* sensu stricto and *Paraburkholderia*. The interesting species is *C. glathei*, as qPCR patterns based on 16S rRNA assays were similar to *Burkholderia*, while qPCR patterns based on *dehp2* assays were similar to *Paraburkholderia*. This seemingly contradictory results showed the uniqueness of

this species, which is supported by its recent transfer to a new genus *Caballeronia* [53].

Specific discrimination of *Burkholderia* and *Paraburkholderia* from mixtures by qPCR assays

The above results showed that the qPCR assays are able to discriminate whether the samples contain *Burkholderia* or *Paraburkholderia* species when we used a single bacterial species as the qPCR template. For environmental or clinical samples collected without further cultivation and

isolation, it is common that they contain different bacterial species. To test whether our qPCR assays are capable of specific discrimination of *Burkholderia* or *Paraburkholderia* species, we mimicked such conditions by preparing mixtures of gDNA from known species. We first tested three conditions: equal concentration mixture of 2 *Burkholderia* species, mixture of 2 *Paraburkholderia* species, and mixture of 1 *Burkholderia* species and 1 *Paraburkholderia* species. Our qPCR assays could clearly tell whether there are only *Burkholderia*, only *Paraburkholderia*, or both genera in the samples, which are also consistent with values calculated from qPCR results of single species (Fig. 2a).

In addition to the equal concentration mixtures, we further assessed mixtures of *Burkholderia* and *Paraburkholderia* species at constant total concentration but different ratios. qPCR showed that for relative ratios of 1:125, 1:25, 1:5, 1:1, 5:1, 25:1 and 125:1 of *B. cenocepacia* LMG 16656 to *P. caribensis* LMG 18531, all the 4 pairs of discriminative primers exhibited specific detection (Fig. 2b). We then used the Delta-Ct values between the *Burkholderia* sensu stricto-specific and *Paraburkholderia*-specific primers (16S-F3/R3 vs. 16S-F2/R2, dehp2-F7/R7 vs. dehp2-F6/R6) to monitor their ability to discriminate the two genera. This approach could also avoid possible interference by other closely related bacteria. For the dynamic range tested, high correlation coefficients were observed for Delta-Ct values against different ratios of the two genera for both 16S rRNA and *dehp2* (Fig. 2c). The results showed that our qPCR assays can specifically discriminate *Burkholderia* and *Paraburkholderia* species with a wide dynamic range of relative ratios.

Illustrative visualization of the qPCR data by hierarchical clustering (HC) and principal component analysis (PCA)

We then used HC and PCA to analyze the qPCR results for more straightforward visualization. For the qPCR data collected against single species or mixtures as described above, we first used HC to construct a heat-map. Both the
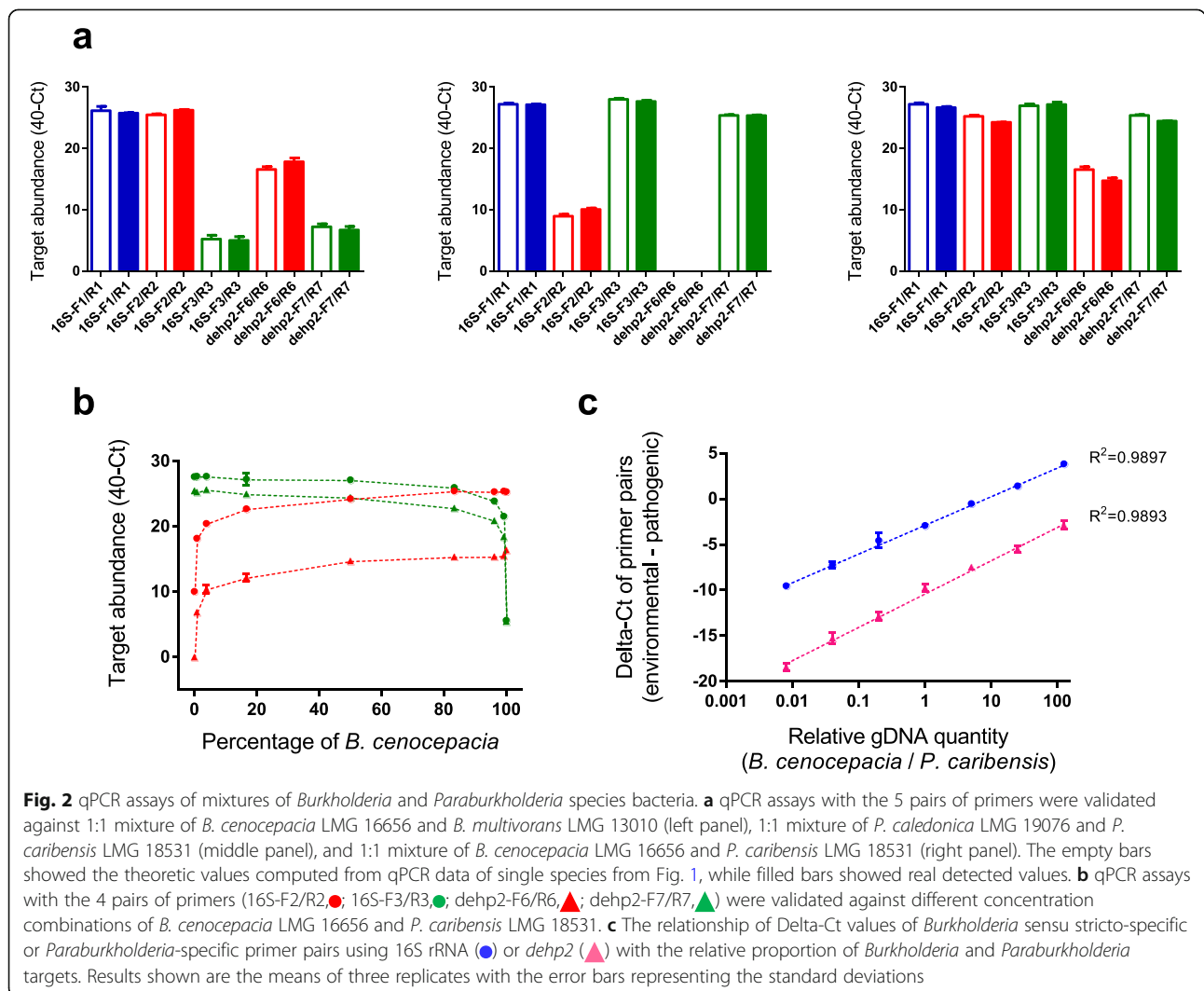

Fig. 2 qPCR assays of mixtures of *Burkholderia* and *Paraburkholderia* species bacteria. **a** qPCR assays with the 5 pairs of primers were validated against 1:1 mixture of *B. cenocepacia* LMG 16656 and *B. multivorans* LMG 13010 (left panel), 1:1 mixture of *P. caledonica* LMG 19076 and *P. caribensis* LMG 18531 (middle panel), and 1:1 mixture of *B. cenocepacia* LMG 16656 and *P. caribensis* LMG 18531 (right panel). The empty bars showed the theoretic values computed from qPCR data of single species from Fig. 1, while filled bars showed real detected values. **b** qPCR assays with the 4 pairs of primers (16S-F2/R2, ●; 16S-F3/R3, ●; dehp2-F6/R6, ▲; dehp2-F7/R7, ▲) were validated against different concentration combinations of *B. cenocepacia* LMG 16656 and *P. caribensis* LMG 18531. **c** The relationship of Delta-Ct values of *Burkholderia* sensu stricto-specific or *Paraburkholderia*-specific primer pairs using 16S rRNA (●) or *dehp2* (▲) with the relative proportion of *Burkholderia* and *Paraburkholderia* targets. Results shown are the means of three replicates with the error bars representing the standard deviations

primer pairs and samples were clustered relevant to the genus they belong, facilitating easy interpretation of the results (Fig. 3a). The uniqueness of *C. glathei* LMG 14190 is also shown in the heat-map. For PCA score plot, the positions of the mixtures are straightforward display of their relative proportion of *Burkholderia* or *Paraburkholderia* (Fig. 3b). For PCA loading plot, the positions of the primer pairs also indicate whether they are targeting *Burkholderia* sensu lato-conserved, *Burkholderia* sensu stricto-specific or *Paraburkholderia*-specific region (Fig. 3c). In general, HC and PCA displays of the qPCR results facilitate easy detection of the presence and relative quantity of *Burkholderia* and *Paraburkholderia* species.

High sensitivity of the qPCR assays for detection of *Burkholderia* and *Paraburkholderia*

Our qPCR assays are able to specifically discriminate *Burkholderia* and *Paraburkholderia* species in complicated samples, and the amplification specificities of the 4 discriminative PCR assays were further confirmed by using gDNA of *E. coli* DH5α as negative control (Additional file 1: Figure S1). We then checked their sensitivity at detecting the target species. We prepared serial diluted bacterial gDNA of *Burkholderia* and *Paraburkholderia*, and for a dynamic range of six orders of magnitude, all 4 pairs of primers worked consistently (Fig. 4). The targets were approaching several-copies to even single-copy per qPCR assay for the lowest concentration tested (calculated to be < 10 copies μl^{-1}), and all primer pairs were performing well except for *Burkholderia* sensu stricto-specific dehp2-F7/R7, which is relatively less efficient compared with the others. This is reasonable, as haloacids transporter gene is a good marker for environmental rather than pathogenic species. The above results showed that our qPCR assays are highly sensitive for detection of low-quantity of *Burkholderia* and *Paraburkholderia* target in the samples.
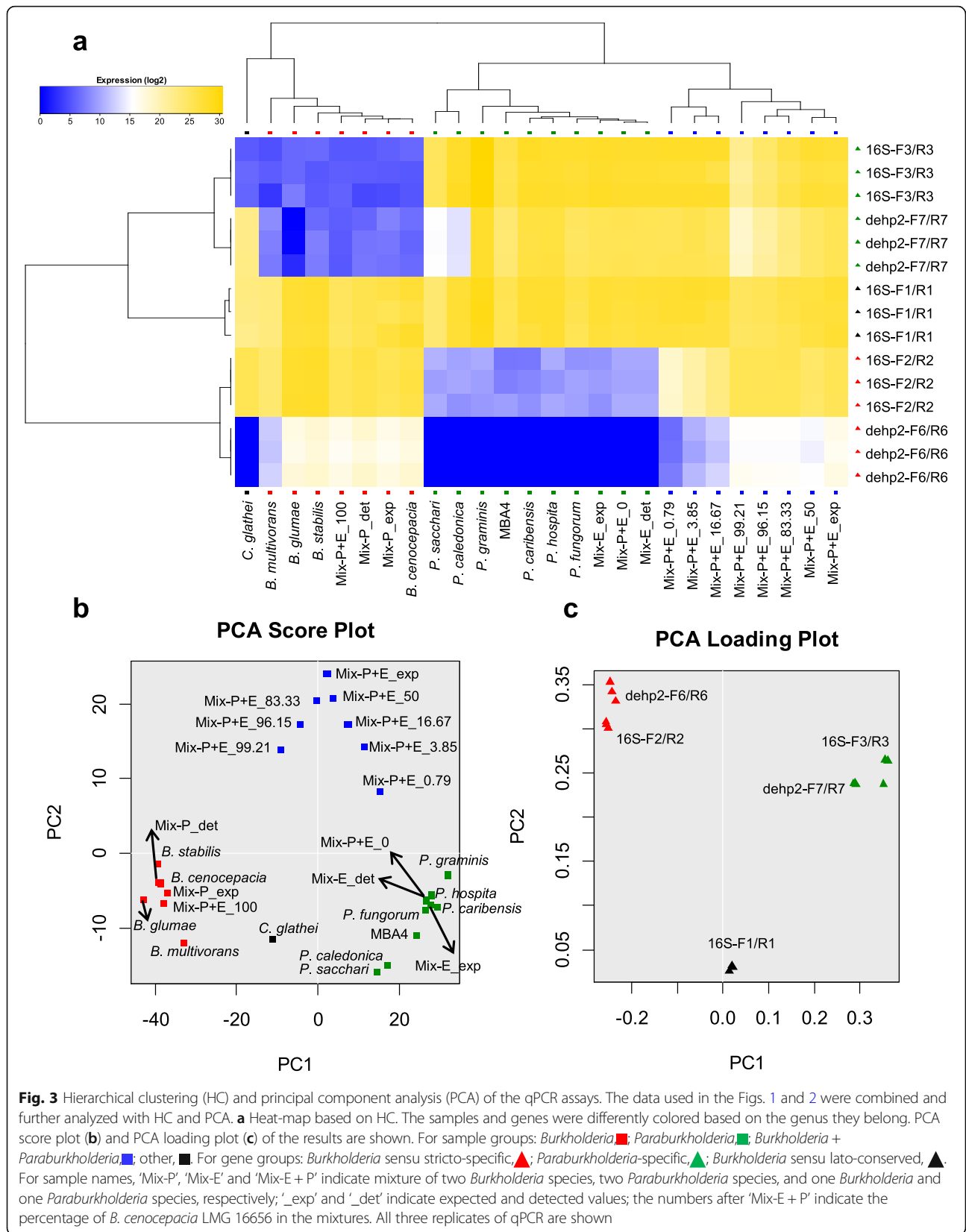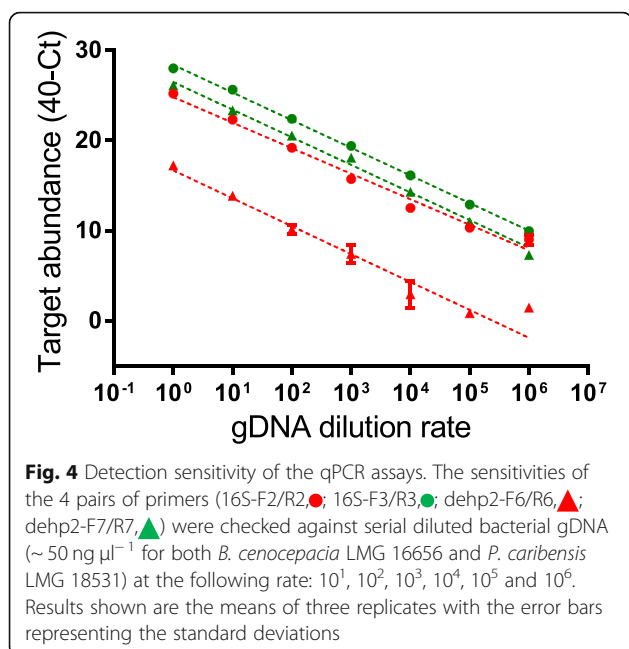
Discussion

Considering the pathogenic potentials of some species in *Burkholderia* sensu lato, it is useful to first have a quick assessment before further analysis such as sequencing, especially when there is a large number of specimens. As some bacteria may not be cultured successfully, qPCR assay should be more sensitive than culture-dependent detection methods. On the other hand, although high throughput sequencing is more powerful to give a global view of genomes, qPCR can actually show very consistent results towards specific targets at much lower cost [54]. Moreover, qPCR is also easier to handle and accessible to more places compared with sequencing-based identification approaches. With these advantages, there have been many qPCR based methods for detection or discrimination purposes in *Burkholderia* sensu lato. For

example, qPCR assay based on Type III Secretion System enabled quick and accurate identification of *B. pseudomallei* [39], and qPCR assay Bu550 that targets a 7 kb locus was able to discriminate *B. ubonensis* from its close neighbor *B. pseudomallei* [55]. Multi-target qPCR assays were able to detect the presence of Bcc members at the resolution of species from sputum specimens [40]. Our study represents the first application of a haloacids transporter gene to discriminate *Burkholderia* and *Paraburkholderia*, which will be useful for not only detecting pathogenic species but also screening environmental species that can be exploited for bioremediation of haloacids.

It has been suggested that phylogenetic relationship could not be reliably established based on single gene [56], and the inclusion of more independent targets increased the sensitivity compared with single target method for identification of *B. pseudomallei* [41, 57]. In this study, we also considered this issue and utilized both the haloacids transporter gene *dehp2* and 16S rRNA. Indeed, there were in-consistence between results obtained from assays based on *dehp2* and 16S rRNA in regard to *C. glathei*, which was transferred to a novel genus recently [53], further supporting the reliability of the assays. Moreover, our qPCR assays were designed to target regions conserved in both pathogenic *Burkholderia* sensu stricto species and environmental *Paraburkholderia* species, and the Delta-Ct values between *Burkholderia* sensu stricto-specific and *Paraburkholderia*-specific assays could be utilized to calculate the relative ratio of the two genera in mixtures. The combination of qPCR assays that target both *Burkholderia*-specific and *Paraburkholderia*-specific regions in *dehp2* and 16S rRNA could rule out possible inference by other related environmental bacteria. High sensitivity and specificity of such assays were observed for both *dehp2* and 16S rRNA over a broad dynamic range of mixtures from both genera.

Our previous work has established Dehp2 as a haloacids transporter [43, 47], and the successful application of this gene to discriminate *Burkholderia* sensu stricto and *Paraburkholderia* further proved its importance for *Burkholderia* sensu lato. As haloacids are not the natural nutrients for pathogenic *Burkholderia* species and even some of the environmental *Paraburkholderia* species, the presence of this transporter showed gene expression rewiring and adaption potentials of bacteria to their living environment. Further analysis of this transporter gene among the two genera, such as evolutionary analysis of key amino acid residues and comparative assessment of the promoter regions between pathogenic and environmental species should provide precious clues for understanding of the transport mechanisms and technological exploitation of relevant species to efficiently degrade haloacids without causing pathogenic risks.

Fig. 3 Hierarchical clustering (HC) and principal component analysis (PCA) of the qPCR assays. The data used in the Figs. 1 and 2 were combined and further analyzed with HC and PCA. **a** Heat-map based on HC. The samples and genes were differently colored based on the genus they belong. PCA score plot (**b**) and PCA loading plot (**c**) of the results are shown. For sample groups: *Burkholderia*, ■; *Paraburkholderia*, ■; *Burkholderia* + *Paraburkholderia*, ■; other, ■. For gene groups: *Burkholderia* sensu stricto-specific, ▲; *Paraburkholderia*-specific, ▲; *Burkholderia* sensu lato-conserved, ▲. For sample names, 'Mix-P', 'Mix-E' and 'Mix-E + P' indicate mixture of two *Burkholderia* species, two *Paraburkholderia* species, and one *Burkholderia* and one *Paraburkholderia* species, respectively; '_exp' and '_det' indicate expected and detected values; the numbers after 'Mix-E + P' indicate the percentage of *B. cenocepacia* LMG 16656 in the mixtures. All three replicates of qPCR are shown

Fig. 4 Detection sensitivity of the qPCR assays. The sensitivities of the 4 pairs of primers (16S-F2/R2,●; 16S-F3/R3,●; dehp2-F6/R6,▲; dehp2-F7/R7,▲) were checked against serial diluted bacterial gDNA (~ 50 ng μl^{-1} for both *B. cenocepacia* LMG 16656 and *P. caribensis* LMG 18531) at the following rate: 10^1, 10^2, 10^3, 10^4, 10^5 and 10^6. Results shown are the means of three replicates with the error bars representing the standard deviations

It should be pointed out that, however, qPCR assay is just a first step for full understanding of the bacteria or samples. As has been cautioned, phylogenetic positions not necessarily confirm whether a *Burkholderia* or *Paraburkholderia* species is pathogenic or not [5, 6, 58]. The results based on this qPCR assay should raise our attention to putative pathogenic species that must be handled carefully, and the samples suggested to be in the environmental group should also be systematically assessed before wide technological applications. Another fact that should be emphasized is that we only tested the qPCR assays in 12 bacterial strains, which represent a relatively small sampling of the > 100 of strains from *Burkholderia* sensu lato. Further assessment of the assays in a larger sample pool of *Burkholderia* sensu lato members will be beneficial for the research field and broad applications of the assays.

Conclusions
In this study we designed qPCR assays based on haloacids transporter Dehp2 as well as 16S rRNA, which enable quick discrimination of *Burkholderia* species and *Paraburkholderia* species with high sensitivity and specificity. Results obtained with the qPCR assays will facilitate more specific handling in regard to the putative pathogenicity of the samples and also exploitation of relevant species for haloacids bioremediation.

Methods
Bacterial strains and extraction of genomic DNA
We used 12 strains from 11 species from *Burkholderia* sensu lato in this study, namely *Paraburkholderia*

caledonica LMG 19076, *P. caribensis* LMG 18531, *P. caribensis* MBA4, *Paraburkholderia fungorum* LMG 16225, *Paraburkholderia graminis* LMG 18924, *Paraburkholderia hospita* LMG 20598, *Paraburkholderia sacchari* LMG 19450, *Caballeronia glathei* LMG 14190, *Burkholderia stabilis* LMG 14294, *Burkholderia glumae* LMG 2196, *B. cenocepacia* LMG 16656 and *B. multivorans* LMG 13010, which were gifts from Molecular Microbiology Laboratory of The University of Hong Kong (Table 1). Genomic DNAs (gDNAs) from the 12 strains were extracted with a G-spin™ Genomic DNA Extraction Kit (iNtRON). The concentrations of the gDNA were adjusted to 50 ~ 100 ng μl^{-1}.

Primer design of 16S rRNA and *dehp2*
The 16S rRNA and *dehp2* sequences from the above described strains were retrieved from NCBI. For the haloacids transporter gene *dehp2*, the sequences are available in 6 species (*P. caribensis*, *P. fungorum*, *B. stabilis*, *B. glumae*, *B. cenocepacia*, and *B. multivorans*), and the sequences are currently unknown in the other 5 species (*P. caledonica*, *P. graminis*, *P. hospita*, *P. sacchari* and *C. glathei*). The sequences of 16S rRNA or *dehp2* were aligned using

Table 1 Bacterial strains used in this study

Bacterial strains	Description	References
E. coli DH5α	Negative control for primer validation	Takara
P. caledonica LMG 19076	Bacterium isolated from the rhizosphere	[61]
P. caribensis LMG 18531	Exopolysaccharide-producing bacterium isolated from vertisol	[62, 63]
P. caribensis MBA4	Haloacids-degrading bacterium isolated from soil	[42, 64, 65]
P. fungorum LMG 16225	Bacterium isolated from the white-rot fungus	[61]
P. graminis LMG 18924	Bacterium isolated from rhizosphere of grasses	[66]
P. hospita LMG 20598	Bacterium isolated from B-horizon soil	[67]
P. sacchari LMG 19450	Polyhydroxyalkanoate-accumulating bacterium isolated from soil	[68]
C. glathei LMG 14190	Bacterium isolated from lateritic soil	[53, 66, 69]
B. stabilis LMG 14294	Pathogenic bacterium isolated from sputum of a cystic fibrosis patient, Bcc member	[70]
B. glumae LMG 2196	Plant pathogen	[71]
B. cenocepacia LMG 16656	Pathogenic bacterium isolated from a cystic fibrosis patient, Bcc member	[72]
B. multivorans LMG 13010	Pathogenic bacterium isolated from sputum of a cystic fibrosis patient, Bcc member	[73]

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ClustalW [59]. Three pairs of qPCR primers were then designed based on 16S rRNA: 16S-F1/R1 to target the region conserved in *Burkholderia* sensu lato, 16S-F2/R2 to target the region conserved in *Burkholderia* sensu stricto, and 16S-F3/R3 to target the region conserved in *Paraburkholderia*. We designed dehp2-F6/R6 and dehp2-F7/R7 to target *Burkholderia* sensu stricto-specific and *Paraburkholderia*-specific regions of *dehp2*. The sequences of the 5 pairs of primers are shown in Table 2.

qPCR

qPCR was carried out using SYBR® Premix Ex Taq™ (Clontech) on the StepOnePlus system (Applied Biosystems) with a two-step method: initial denaturation of 95 °C for 30 s; 40 cycles of 95 °C for 5 s and 66 °C for 30 s. A melting curve program was also included to verify the specificity of the amplified products. Three replicates were set up for each condition, and negative controls were also included to monitor possible contaminations. To measure the amplification efficiency, 40 minus Ct values were used, which represent logarithmic transformed target abundance as previous described [54, 60]. To check the ability of combination of primer pairs to discriminate *Burkholderia* sensu stricto and *Paraburkholderia*, the difference between Ct values derived from 16S rRNA primers ($Ct_{16S-F3/R3}$ - $Ct_{16S-F2/R2}$), or between those derived from *dehp2* primers ($Ct_{dehp2-F7/R7}$ - $Ct_{dehp2-F6/R6}$) were calculated. For qPCR reactions, besides the single strains analyzed, we also tested mixtures of different strains. To check the sensitivity of qPCR assays, serial diluted gDNA of *B. cenocepacia* LMG 16656 was used as template for 16S-F2/R2 and dehp2-F6/R6, while serial diluted gDNA of *P. caribensis* LMG 18531 was used as template for 16S-F3/R3 and dehp2-F7/R7.

Table 2 Primers used in this study

Primers	Sequence (5′ to 3′)[a]
16S-F1	GGTAATACGTAGGGTGC**R**AGCGTT
16S-R1	CAC**M**AATGCAGTTCCCAGGTT**R**AG
16S-F2	GGAGGAATACCGATGGCGAAGG
16S-R2	TTACTAAGGAAATGAATCCCCAACAAC
16S-F3	ACAAGCGGTGGATGATGTGGAT
16S-R3	TGTGTTA**Y**GGCTCCCTTTCGG
dehp2-F6	**R**CA**Y**TCGCCGATGACG**RS**
dehp2-R6	GGA**R**AAGAAGCTCTTGCTGAT**R**T
dehp2-F7	**R**C**M**TGGGGCTGGCGCATT
dehp2-R7	GTCCGG**R**TT**S**GCGATCACGAC

[a]Degenerated bases are shown in bold

Visualization of data by HC and PCA

HC and PCA analysis of the qPCR data were performed using the SINGuLAR™ Analysis Toolset R package (Fluidigm).

Additional file

Additional file 1: Figure S1 Validation of the amplification specificity of the discriminative PCR primers. (PDF 226 kb)

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References
1. Coenye T, Vandamme P. Diversity and significance of *Burkholderia* species occupying diverse ecological niches. Environ Microbiol. 2003;5(9):719–29.

2. Compant S, Nowak J, Coenye T, Clement C, Ait Barka E. Diversity and occurrence of *Burkholderia* spp. in the natural environment. FEMS Microbiol Rev. 2008;32(4):607–26.

3. Vial L, Chapalain A, Groleau MC, Deziel E. The various lifestyles of the *Burkholderia cepacia* complex species: a tribute to adaptation. Environ Microbiol. 2011;13(1):1–12.

4. da Silva K, Cassetari Ade S, Lima AS, De Brandt E, Pinnock E, Vandamme P, Moreira FM. Diazotrophic *Burkholderia* species isolated from the Amazon region exhibit phenotypical, functional and genetic diversity. Syst Appl Microbiol. 2012;35(4):253–62.

5. Depoorter E, Bull MJ, Peeters C, Coenye T, Vandamme P, Mahenthiralingam E. *Burkholderia*: an update on taxonomy and biotechnological potential as antibiotic producers. Appl Microbiol Biotechnol. 2016;100(12):5215–29.

6. Eberl L, Vandamme P. Members of the genus *Burkholderia*: good and bad guys. F1000Res. 2016;5:1007.

7. Ussery DW, Kiil K, Lagesen K, Sicheritz-Ponten T, Bohlin J, Wassenaar TM. The genus *Burkholderia*: analysis of 56 genomic sequences. Genome Dyn. 2009;6: 140–57.

8. Galyov EE, Brett PJ, DeShazer D. Molecular insights into *Burkholderia pseudomallei* and *Burkholderia mallei* pathogenesis. Annu Rev Microbiol. 2010;64:495–517.

9. Chewapreecha C, Holden MT, Vehkala M, Valimaki N, Yang Z, Harris SR, Mather AE, Tuanyok A, De Smet B. Le hello S *et al*: global and regional dissemination and evolution of *Burkholderia pseudomallei*. Nat Microbiol. 2017;2:16263.

10. Limmathurotsakul D, Golding N, Dance DA, Messina JP, Pigott DM, Moyes CL, Rolim DB, Bertherat E, Day NP, Peacock SJ, et al. Predicted global distribution of *Burkholderia pseudomallei* and burden of melioidosis. Nat Microbiol. 2016;1:15008.

11. Tsang JS. Molecular biology of the *Burkholderia cepacia* complex. Adv Appl Microbiol. 2004;54:71–91.

12. Chiarini L, Bevivino A, Dalmastri C, Tabacchioni S, Visca P. *Burkholderia cepacia* complex species: health hazards and biotechnological potential. Trends Microbiol. 2006;14(6):277–86.

13. Mahenthiralingam E, Baldwin A, Dowson CG. *Burkholderia cepacia* complex bacteria: opportunistic pathogens with important natural biology. J Appl Microbiol. 2008;104(6):1539–51.

14. Vandamme P, Dawyndt P. Classification and identification of the *Burkholderia cepacia* complex: past, present and future. Syst Appl Microbiol. 2011;34(2):87–95.

15. O'Sullivan LA, Mahenthiralingam E. Biotechnological potential within the genus *Burkholderia*. Lett Appl Microbiol. 2005;41(1):8–11.

16. Su X, Deng L, Kong KF, Tsang JS. Enhanced degradation of haloacid by heterologous expression in related *Burkholderia* species. Biotechnol Bioeng. 2013;110(10):2687–96.

17. Paungfoo-Lonhienne C, Lonhienne TG, Yeoh YK, Donose BC, Webb RI, Parsons J, Liao W, Sagulenko E, Lakshmanan P, Hugenholtz P, et al. Crosstalk between sugarcane and a plant-growth promoting *Burkholderia* species. Sci Rep. 2016;6:37389.

18. Estrada-de los Santos P, Rojas-Rojas FU, Tapia-García EY, Vásquez-Murrieta MS, Hirsch AM. To split or not to split: an opinion on dividing the genus *Burkholderia*. Ann Microbiol. 2016;66(3):1303–14.

19. Suarez-Moreno ZR, Caballero-Mellado J, Coutinho BG, Mendonca-Previato L, James EK, Venturi V. Common features of environmental and potentially beneficial plant-associated *Burkholderia*. Microb Ecol. 2012;63(2):249–66.

20. Gyaneshwar P, Hirsch AM, Moulin L, Chen WM, Elliott GN, Bontemps C. Estrada-de Los Santos P, gross E, dos reis FB, Sprent JI *et al*: legume-nodulating betaproteobacteria: diversity, host range, and future prospects. Mol Plant-Microbe Interact. 2011;24(11):1276–88.

21. Mahenthiralingam E, Bischof J, Byrne SK, Radomski C, Davies JE, Av-Gay Y, Vandamme P. DNA-based diagnostic approaches for identification of *Burkholderia cepacia* complex, *Burkholderia vietnamiensis*, *Burkholderia multivorans*, *Burkholderia stabilis*, and *Burkholderia cepacia* genomovars I and III. J Clin Microbiol. 2000;38(9):3165–73.

22. Payne GW, Vandamme P, Morgan SH, LiPuma JJ, Coenye T, Weightman AJ, Jones TH, Mahenthiralingam E. Development of a *recA* gene-based identification approach for the entire *Burkholderia* genus. Appl Environ Microbiol. 2005;71(7):3917–27.

23. Lynch KH, Dennis JJ. Development of a species-specific *fur* gene-based method for identification of the *Burkholderia cepacia* complex. J Clin Microbiol. 2008;46(2):447–55.

24. Onofre-Lemus J, Hernandez-Lucas I, Girard L, Caballero-Mellado J. ACC (1-aminocyclopropane-1-carboxylate) deaminase activity, a widespread trait in *Burkholderia* species, and its growth-promoting effect on tomato plants. Appl Environ Microbiol. 2009;75(20):6581–90.

25. Papaleo MC, Perrin E, Maida I, Fondi M, Fani R, Vandamme P. Identification of species of the *Burkholderia cepacia* complex by sequence analysis of the *hisA* gene. J Med Microbiol. 2010;59(Pt 10):1163–70.

26. Frickmann H, Neubauer H, Loderstaedt U, Derschum H, Hagen RM. *rpsU*-based discrimination within the genus *Burkholderia*. Eur J Microbiol Immunol. 2014;4(2):106–16.

27. Sawana A, Adeolu M, Gupta RS. Molecular signatures and phylogenomic analysis of the genus *Burkholderia*: proposal for division of this genus into the emended genus *Burkholderia* containing pathogenic organisms and a new genus *Paraburkholderia* gen. nov. harboring environmental species. Front Genet. 2014;5:429.

28. de Lajudie PM, Young JPW. International committee on systematics of prokaryotes Subcommittee for the Taxonomy of *Rhizobium* and *Agrobacterium* minutes of the meeting, Budapest, 25 august 2016. Int J Syst Evol Microbiol. 2017;67(7):2485–94.

29. Lopes-Santos L, Castro DBA, Ferreira-Tonin M, Correa DBA, Weir BS, Park D, Ottoboni LMM, Neto JR, Destefano SAL. Reassessment of the taxonomic position of *Burkholderia andropogonis* and description of *Robbsia andropogonis* gen. Nov., comb. nov. Antonie Van Leeuwenhoek. 2017;110(6):727–36.

30. Beukes CW, Palmer M, Manyaka P, Chan WY, Avontuur JR, van Zyl E, Huntemann M, Clum A, Pillay M, Palaniappan K, et al. Genome data provides high support for generic boundaries in *Burkholderia* Sensu Lato. Front Microbiol. 2017;8:1154.

31. Wong-Villarreal A, Caballero-Mellado J. Rapid identification of nitrogen-fixing and legume-nodulating *Burkholderia* species based on PCR 16S rRNA species-specific oligonucleotides. Syst Appl Microbiol. 2010;33(1):35–43.

32. Peeters C, Daenekindt S, Vandamme P. PCR detection of *Burkholderia multivorans* in water and soil samples. BMC Microbiol. 2016;16(1):184.

33. Koh SF, Tay ST, Sermswan R, Wongratanacheewin S, Chua KH, Puthucheary SD. Development of a multiplex PCR assay for rapid identification of *Burkholderia pseudomallei*, *Burkholderia thailandensis*, *Burkholderia mallei* and *Burkholderia cepacia* complex. J Microbiol Meth. 2012;90(3):305–8.

34. Zakharova I, Teteryatnikova N, Toporkov A, Viktorov D. Development of a multiplex PCR assay for the detection and differentiation of *Burkholderia pseudomallei*, *Burkholderia mallei*, *Burkholderia thailandensis*, and *Burkholderia cepacia* complex. Acta Trop. 2017;174:1–8.

35. Baldwin A, Mahenthiralingam E, Thickett KM, Honeybourne D, Maiden MC, Govan JR, Speert DP, Lipuma JJ, Vandamme P, Dowson CG. Multilocus sequence typing scheme that provides both species and strain differentiation for the *Burkholderia cepacia* complex. J Clin Microbiol. 2005; 43(9):4665–73.

36. Spilker T, Baldwin A, Bumford A, Dowson CG, Mahenthiralingam E, LiPuma JJ. Expanded multilocus sequence typing for *Burkholderia* species. J Clin Microbiol. 2009;47(8):2607–10.

37. Estrada-de los Santos P, Vinuesa P, Martinez-Aguilar L, Hirsch AM, Caballero-Mellado J. Phylogenetic analysis of *Burkholderia* species by multilocus sequence analysis. Curr Microbiol. 2013;67(1):51–60.

38. Price EP, MacHunter B, Spratt BG, Wagner DM, Currie BJ, Sarovich DS. Improved multilocus sequence typing of *Burkholderia pseudomallei* and closely related species. J Med Microbiol. 2016;65(9):992–7.

39. Novak RT, Glass MB, Gee JE, Gal D, Mayo MJ, Currie BJ, Wilkins PP. Development and evaluation of a real-time PCR assay targeting the type III secretion system of *Burkholderia pseudomallei*. J Clin Microbiol. 2006;44(1):85–90.

40. Martinucci M, Roscetto E, Iula VD, Votsi A, Catania MR, De Gregorio E. Accurate identification of members of the *Burkholderia cepacia* complex in cystic fibrosis sputum. Lett Appl Microbiol. 2016;62(3):221–9.

41. Gohler A, Trung TT, Hopf V, Kohler C, Hartleib J, Wuthiekanun V, Peacock SJ, Limmathurotsakul D, Tuanyok A, Steinmetz I. Multitarget quantitative PCR improves detection and predicts cultivability of the pathogen *Burkholderia pseudomallei*. Appl Environ Microbiol. 2017;83(8):e03212–6.

42. Tsang JSH, Sallis PJ, Bull AT, Hardman DJ. A monobromoacetate dehalogenase from *Pseudomonas cepacia* MBA4. Arch Microbiol. 1988; 150(5):441–6.

43. Su X, Li R, Kong KF, Tsang JS. Transport of haloacids across biological membranes. Biochim Biophys Acta. 2016;1858(12):3061–70.

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44. Jing NH, Wahab RA, Hamdan S, Huyop F. Cloning and DNA sequence analysis of the haloalkanoic permease uptake gene from *Rhizobium* sp. RC1. Biotechnology. 2010;9(3):319–25.

45. Yu M, Faan YW, Chung WY, Tsang JS. Isolation and characterization of a novel haloacid permease from *Burkholderia cepacia* MBA4. Appl Environ Microbiol. 2007;73(15):4874–80.

46. Su X, Kong KF, Tsang JS. Transports of acetate and haloacetate in *Burkholderia* species MBA4 are operated by distinct systems. BMC Microbiol. 2012;12:267.

47. Su X, Tsang JS. Existence of a robust haloacid transport system in a *Burkholderia* species bacterium. Biochim Biophys Acta. 2013;1828(2):187–92.

48. Su X, Li R, Tsang JS. The 228 bp upstream non-coding region of haloacids transporter gene *dehp2* has regulated promoter activity. Gene. 2016;593(2):322–9.

49. Telenius H, Carter NP, Bebb CE, Nordenskjold M, Ponder BA, Tunnacliffe A. Degenerate oligonucleotide-primed PCR: general amplification of target DNA by a single degenerate primer. Genomics. 1992;13(3):718–25.

50. Hugerth LW, Wefer HA, Lundin S, Jakobsson HE, Lindberg M, Rodin S, Engstrand L, Andersson AF. DegePrime, a program for degenerate primer design for broad-taxonomic-range PCR in microbial ecology studies. Appl Environ Microbiol. 2014;80(16):5116–23.

51. Cai L, Liu G, Rensing C, Wang G. Genes involved in arsenic transformation and resistance associated with different levels of arsenic-contaminated soils. BMC Microbiol. 2009;9:4.

52. Fouhy F, Ross RP, Fitzgerald GF, Stanton C, Cotter PD. A degenerate PCR-based strategy as a means of identifying homologues of aminoglycoside and beta-lactam resistance genes in the gut microbiota. BMC Microbiol. 2014;14:25.

53. Dobritsa AP, Samadpour M. Transfer of eleven species of the genus *Burkholderia* to the genus *Paraburkholderia* and proposal of *Caballeronia* gen. Nov. to accommodate twelve species of the genera *Burkholderia* and *Paraburkholderia*. Int J Syst Evol Microbiol. 2016;66(8):2836–46.

54. Su X, Shi Y, Zou X, Lu Z-N, Xie G, Yang JYH, Wu C-C, Cui X-F, He K-Y, Luo Q, et al. Single-cell RNA-Seq analysis reveals dynamic trajectories during mouse liver development. BMC Genomics. 2017;18:946.

55. Price EP, Sarovich DS, Webb JR, Ginther JL, Mayo M, Cook JM, Seymour ML, Kaestli M, Theobald V, Hall CM, et al. Accurate and rapid identification of the *Burkholderia pseudomallei* near-neighbour, *Burkholderia ubonensis*, using real-time PCR. PLoS One. 2013;8(8):e71647.

56. Tayeb LA, Lefevre M, Passet V, Diancourt L, Brisse S, Grimont PA. Comparative phylogenies of *Burkholderia*, *Ralstonia*, *Comamonas*, *Brevundimonas* and related organisms derived from *rpoB*, *gyrB* and *rrs* gene sequences. Res Microbiol. 2008;159(3):169–77.

57. Trung TT, Hetzer A, Gohler A, Topfstedt E, Wuthiekanun V, Limmathurotsakul D, Peacock SJ, Steinmetz I. Highly sensitive direct detection and quantification of *Burkholderia pseudomallei* bacteria in environmental soil samples by using real-time PCR. Appl Environ Microbiol. 2011;77(18):6486–94.

58. Peeters C, Meier-Kolthoff JP, Verheyde B, De Brandt E, Cooper VS, Vandamme P. Phylogenomic study of *Burkholderia glathei*-like organisms, proposal of 13 novel *Burkholderia* species and emended descriptions of *Burkholderia sordidicola*, *Burkholderia zhejiangensis*, and *Burkholderia grimmiae*. Front Microbiol. 2016;7:877.

59. Larkin MA, Blackshields G, Brown NP, Chenna R, McGettigan PA, McWilliam H, Valentin F, Wallace IM, Wilm A, Lopez R, et al. Clustal W and Clustal X version 2.0. Bioinformatics. 2007;23(21):2947–8.

60. Livak KJ, Wills QF, Tipping AJ, Datta K, Mittal R, Goldson AJ, Sexton DW, Holmes CC. Methods for qPCR gene expression profiling applied to 1440 lymphoblastoid single cells. Methods. 2013;59(1):71–9.

61. Coenye T, Laevens S, Willems A, Ohlen M, Hannant W, Govan JR, Gillis M, Falsen E, Vandamme P. *Burkholderia fungorum* sp. nov. and *Burkholderia caledonica* sp. nov., two new species isolated from the environment, animals and human clinical samples. Int J Syst Evol Microbiol. 2001;51(Pt 3):1099–107.

62. Achouak W, Christen R, Barakat M, Martel MH, Heulin T. *Burkholderia caribensis* sp. nov., an exopolysaccharide-producing bacterium isolated from vertisol microaggregates in Martinique. Int J Syst Bacteriol. 1999;49:787–94.

63. Pan Y, Kong KF, Tsang JS. Complete genome sequence of the exopolysaccharide-producing *Burkholderia caribensis* type strain MWAP64. Genome Announc. 2016;4(1):e01636–15.

64. Pan Y, Kong KF, Tsang JS. Draft genome sequence of the haloacid-degrading *Burkholderia caribensis* strain MBA4. Genome Announc. 2014;2(1):e00047–14.

65. Pan Y, Kong KF, Tsang JS. Complete genome sequence and characterization of the haloacid-degrading *Burkholderia caribensis* MBA4. Stand Genomic Sci. 2015;10:114.

66. Viallard V, Poirier I, Cournoyer B, Haurat J, Wiebkin S, Ophel-Keller K, Balandreau J. *Burkholderia graminis* sp. nov., a rhizospheric *Burkholderia* species, and reassessment of [*Pseudomonas*] *phenazinium*, [*Pseudomonas*] *pyrrocinia* and [*Pseudomonas*] *glathei* as *Burkholderia*. Int J Syst Bacteriol. 1998;48(Pt 2):549–63.

67. Goris J, Dejonghe W, Falsen E, De Clerck E, Geeraerts B, Willems A, Top EM, Vandamme P, De Vos P. Diversity of transconjugants that acquired plasmid pJP4 or pEMT1 after inoculation of a donor strain in the A- and B-horizon of an agricultural soil and description of *Burkholderia hospita* sp. nov. and *Burkholderia terricola* sp. nov. Syst Appl Microbiol. 2002;25(3):340–52.

68. Brämer CO, Vandamme P, Da SL, Gomez JG, Steinbüchel A. *Burkholderia sacchari* sp. nov., a polyhydroxyalkanoate-accumulating bacterium isolated from soil of a sugar-cane plantation in Brazil. Int J Syst Evol Microbiol. 2001; 51(Pt 5):1709–13.

69. Zolg W, Ottow JC. *Pseudomonas glathei* sp. nov., a new nitrogen-scavening rod isolated from acid lateritic relics in Germany. J Comp Neurol. 1975; 164(1):287–99.

70. Vandamme P, Mahenthiralingam E, Holmes B, Coenye T, Hoste B, De Vos P, Henry D, Speert DP. Identification and population structure of *Burkholderia stabilis* sp. nov. (formerly *Burkholderia cepacia* genomovar IV). J Clin Microbiol. 2000;38(3):1042–7.

71. Urakami T, Itoyoshida C, Araki H, Kijima T, Suzuki KI, Komagata K. Transfer of *Pseudomonas plantarii* and *Pseudomonas glumae* to *Burkholderia* as *Burkholderia* spp. and description of *Burkholderia vandii* sp. nov. Int J Syst Bacteriol. 1994;44(2):235–45.

72. Holden MT, Seth-Smith HM, Crossman LC, Sebaihia M, Bentley SD, Cerdeno-Tarraga AM, Thomson NR, Bason N, Quail MA, Sharp S, et al. The genome of *Burkholderia cenocepacia* J2315, an epidemic pathogen of cystic fibrosis patients. J Bacteriol. 2009;191(1):261–77.

73. Vandamme P, Holmes B, Vancanneyt M, Coenye T, Hoste B, Coopman R, Revets H, Lauwers S, Gillis M, Kersters K, et al. Occurrence of multiple genomovars of *Burkholderia cepacia* in cystic fibrosis patients and proposal of *Burkholderia multivorans* sp. nov. Int J Syst Bacteriol. 1997;47(4):1188–200.