

Supplementary Material

Supplementary Table

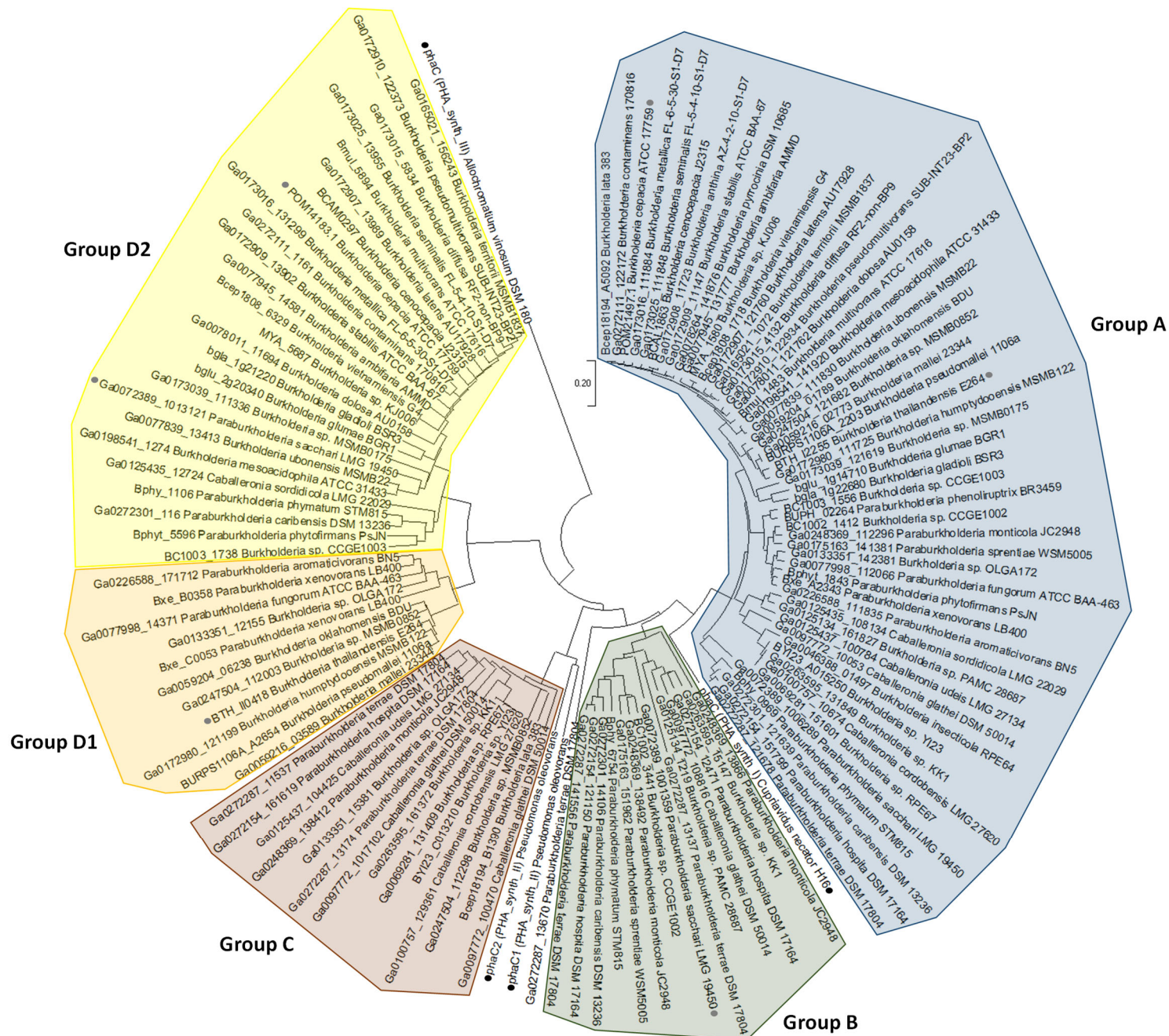
Table S1. Assimilation of substrates commonly used for PHA production in 37 *Burkholderia* sensu lato type and representative strains.

Strain	Substrate assimilation								Reference
	Scr	Man	Glu	Fru	Xyl	Ara	Gln	Gly	
<i>Burkholderia cepacia</i> ATCC 25416 ^T	+	+	+	+	+	+	+	+	[1]
<i>Burkholderia contaminans</i> MS14	nr	nr	nr	nr	nr	nr	nr	nr	-
<i>Burkholderia cenocepacia</i> J2315	+	+	+	nr	+	nr	nr	nr	[2]
<i>Burkholderia stabilis</i> ATCC BAA-67 ^T	-	+	+	nr	nr	+	nr	nr	[3]
<i>Burkholderia pyrrocinia</i> DSM 10685 ^T	+	nr	nr	+	nr	+	nr	nr	[4]
<i>Burkholderia vietnamiensis</i> LMG 10929 ^T	+	+	+	+	+	+	nr	+	[5]
<i>Burkholderia ambifaria</i> AMMD ^T	nr	+	+	nr	+	+	+	nr	[6]
<i>Burkholderia stagnalis</i> LMG 28156 ^T	+	+	nr	nr	+	+	nr	nr	[7]
<i>Burkholderia multivorans</i> ATCC 17616	-	+	+	+	+	+	-	+	[2,8]
<i>Burkholderia thailandensis</i> E264 ^T	nr	nr	nr	nr	nr	+	nr	+	[9,10]
<i>Burkholderia mallei</i> ATCC 23344 ^T	+	+	+	-	-	-	+	+	[5,9,11]
<i>Burkholderia glumae</i> LMG 2196 ^T	+	+	+	+	+	+	+	-	[1]
<i>Burkholderia plantarii</i> ATCC 43733 ^T	-	+	+	+	+	+	nr	+	[1]
<i>Paraburkholderia graminis</i> PHS1	+	nr	nr	+	nr	+	nr	nr	[4]
<i>Paraburkholderia caledonica</i> PHRS4	-	nr	+	+	+	+	nr	nr	[6]
<i>Paraburkholderia aromaticivorans</i> BN5 ^T	+	+	+	+	nr	+	+	+	[12]
<i>Paraburkholderia xenovorans</i> LB400 ^T	-	+	+	+	+	-	+	nr	[13]
<i>Paraburkholderia phytofirmans</i> PsJN ^T	+	nr	+	+	+	nr	nr	nr	[14]
<i>Paraburkholderia fungorum</i> ATCC BAA-463 ^T	-	nr	+	+	+	+	nr	nr	[6]

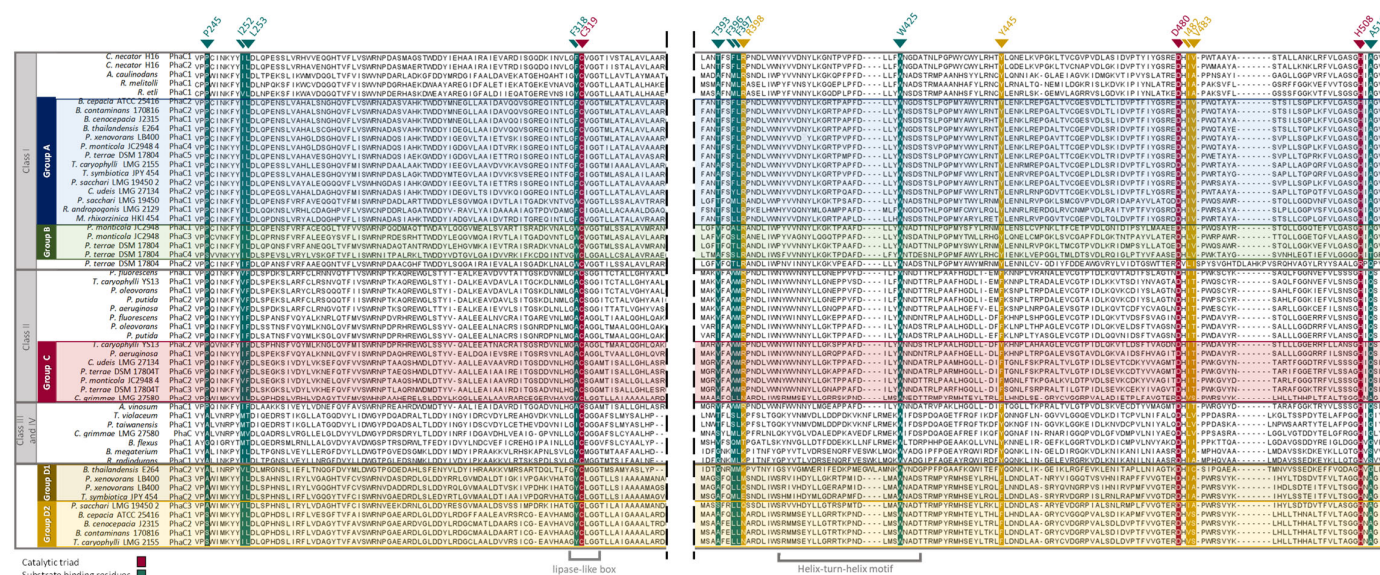
<i>Paraburkholderia caffeinilytica</i> CF1 ^T	-	-	-	+	+	+	-	+	[15]
<i>Paraburkholderia sprengelii</i> WSM5005 ^T	nr	+	+	nr	nr	+	+	nr	[13]
<i>Paraburkholderia megapolitana</i> LMG 23650 ^T	+	+	+	nr	nr	+	+	nr	[8,13]
<i>Paraburkholderia terrae</i> DSM 17804 ^T	-	+	+	nr	nr	+	+	nr	[16]
<i>Paraburkholderia hospita</i> DSM 17164 ^T	-	+	+	nr	-	+	+	nr	[13]
<i>Paraburkholderia phymatum</i> STM 815 ^T	nr	+	+	nr	nr	+	+	nr	[13]
<i>Paraburkholderia sacchari</i> LMG 19450 ^T	+	+	+	+	+	+	+	+	[4]
<i>Paraburkholderia tropica</i> LMG 22274 ^T	-	+	+	+	+	+	+	+	[13]
<i>Caballeronia sordidicola</i> LMG 22029	-	+	+	-	nr	+	+	-	[17]
<i>Caballeronia udeis</i> LMG 27134 ^T	-	+	+	+	nr	+	+	-	[17]
<i>Caballeronia glathei</i> LMG:14190 ^T	-	+	+	+	nr	+	+	-	[17]
<i>Caballeronia insecticola</i> RPE64 ^T	nr	+	+	nr	nr	+	+	nr	[18]
<i>Caballeronia cordobensis</i> LMG 27620 ^T	-	+	nr	nr	nr	nr	+	nr	[19]
<i>Caballeronia grimmiae</i> LMG 27580 ^T	nr	+	+	nr	nr	+	+	nr	[13]
<i>Trinickia caryophylli</i> LMG 2155 ^T	+	-	+	+	+	+	nr	+	[1]
<i>Trinickia symbiotica</i> JPY-345 ^T	nr	+	+	+	nr	+	nr	nr	[20]
<i>Mycetohabitans rhizoxinica</i> HKI 454 ^T	nr	nr	-	nr	nr	nr	nr	+	[20]
<i>Robbsia andropogonis</i> LMG 2129 ^T	-	+	nr	+	nr	+	nr	nr	[21]

Substrates claimed to be assimilated (+) or not (-) and those not found in literature (not reported: nr). Scr: sucrose; Man: mannitol; Glu: *D*-glucose; Fru: *D*-fructose; Xyl: *D*-Xylose; Ara: *L*-Arabinose; Gln: Gluconate; Gly: glycerol.

Supplementary Figures



Supplementary Figure 1. Phylogenetic tree for PhaC of representative *Burkholderia sensu lato* species. The phylogenetic tree was obtained by the neighbor joining method using MEGA-X software based on sequence alignments calculated by CLUSTALW. PhaC sequences were retrieved from IMG/M database considering similarity by the chance expectation values and scores of BLAST probing. Confidence in phylogenetic inference was assessed using non-parametric bootstrap resampling.



Supplementary Figure 2. Multiple sequence alignment of PHA synthases of four phylogenetic groups in *Burkholderia sensu lato* genomes. The conserved amino acidic residues and the lipase box-like identified in PHA synthases sequences are highlighted. PHA synthases of class I, II, III and IV are included as reference. The alignment was made using MAFFT.

>CP039287.1:c1498517-1498218 *Cupriavidus necator* H16

GGGCGCGGGCAATCCGCGATGGTAGACCCCTGCATGGCCCTCGCCGAGCGCCCCGGAGTGGC
 GTCACAGCCGCTCCCGTGTATCGCCAGCAACGTTGTTTGTGCA**TTGCACAAAATCCA**CTTGACA
TTGGATCTGCGCCCC**TAAATAGGAATGTTTTCGGCG**CACCAATAAGAAATGCGCCTTGACC
 CACCCAACGCCTGGGCTGGCCGAATCGGGCACAACACCGTACAGGCCCTGACATCTAGGCGGCT
 TAATTTGCTAGACCTTGAAGTTCACCACTGGAGACCAGCA**ATG**

>CP000270.1:3203820-3204119 *Paraburkholderia xenovorans* LB400

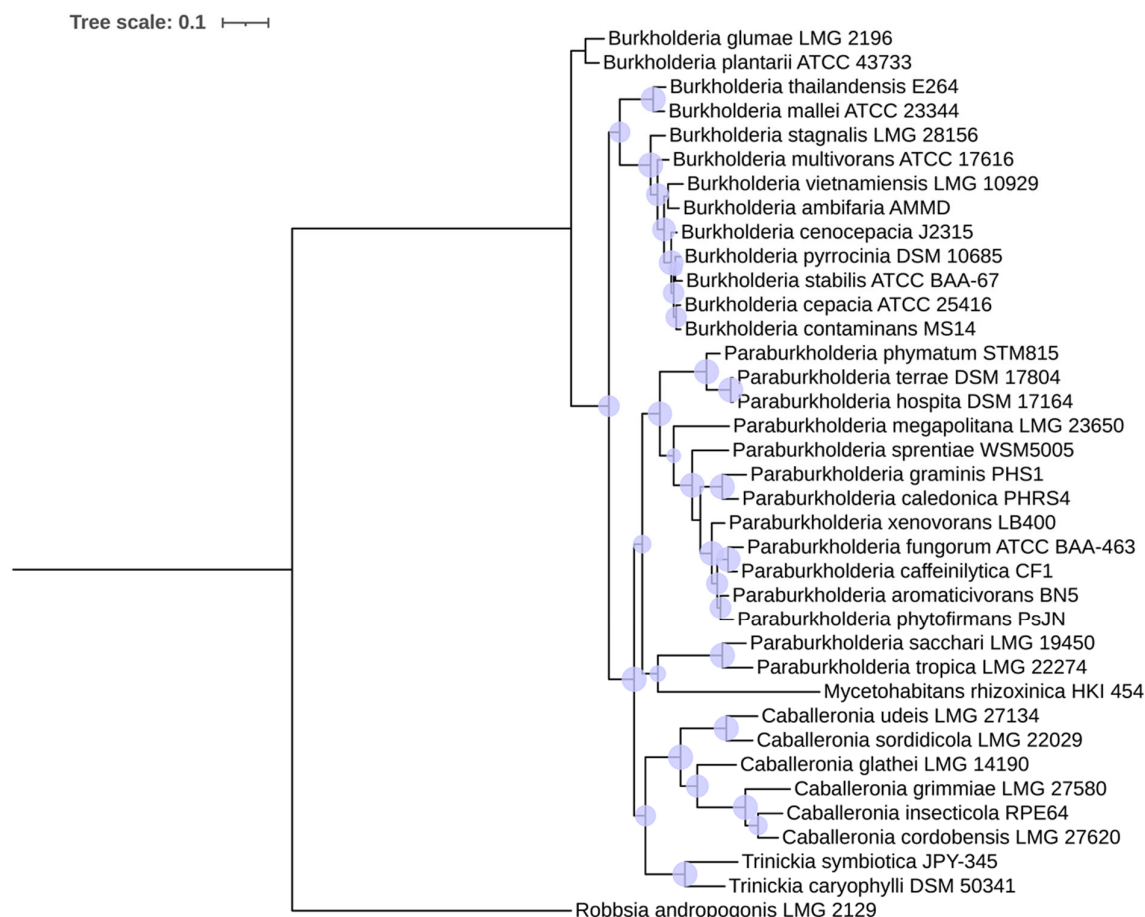
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CTGCGTCACATGAACGTCATGCCAACGCATAACGTCTTGTTTTATCAGTAGTTTCTTAATATG 29
GTGCGACGCACAAAAAGGACTTGACATTGGTGACGCCATCCTAAAAATGGGAACCATGCTGC 30
GATGCACAAAACATCGCGGTCCGGCGGGGCGCATCACTATCAATGTGTCTCACCCTCACCCAA 31
TGCGGTCCGCACAGACAGACCGCGATCCAGGAGCGTAAACCATG 32
>JTDB02000008.1:c192093-191794 *Paraburkholderia sacchari* LMG 33
19450 34
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AAAAATAGGCTCCATTGCTGCGTTGACAAAGCACGTCGCCGCAAGATTCCCCAGCGCAGTGCCC 44
TTTACCCCGCATCGACGCGATCGCTTCCAGGAGCTTGACATG 45
>CP009435.1:3550343-3550642 *Burkholderia glumae* LMG 2196 46
AAACCCGCGATGGGCGCGCTTTGCCAAGGGCGTCGCGAGTCAGGCGCATCGAGCCGTCTCATGC 47
AATGCCGCGGACCCGAACCGATTTTCATCCGGAACGATACAAGCCATTGTTTTTCCGGAATAAT 48
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ATCGCCGCGAAGCCGGCGATCGTCAATCAGGAGCCAAAATCATG 51
52
53
>JFHE01000079.1:6667-6966 *Caballeronia grimmiae* LMG 27580 54
CAACATGCGCCAACGCAGCCGCGCGCAGCATGGGACTCGCCGCTTTTCGCTAGTCCACGTCGGT 55
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>FCOK02000074.1:c21237-20938 *Caballeronia udeis* LMG 27134 60
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GACCCGCGCTGATGATGGGTGCAATCTTTCAGGAGCGTGACATG 65
>VJZF01000001.1:c156642-156343 *Trinickia caryophylli* DSM 50341 66
AGGAAGGCCGCCATCGAAGGGTCCGTCGCCCTTTTACGCGCGACGCAGCAAAATCCATTCCAAGC 67
GCCCTTCGCCGCTGCCCTACCATTCCCTCGTAGCACCTTGTTTTTATGCAGAATCCTTTGAATTA 68
GACAGAATTGTGCATCGCACCAAACAGCGCTTGACTTTTGTGCACGACGGCATAGAATAGGTCT 69

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TGCATCTGCGCCAGACAGGTCGATTTTCAGGAGCGAAAACATG
>PTIR01000018.1:c93163-92864 Trinickia symbiotica JPY-345
GGCGGGAGCGTGGGCGCGATCGTATCACGAGACTGGTGCACGAGGGCGAAGCACTGCCGCATTT
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CCCTAACATTGTGCAATGCACAAAAATCGCGTTGACATTTGGTCAAGTGAGCCTAAAAATAGGAT
CCGTTGTTGCGACGCACAAAACGCGTCGGCTAGACGACGCGATCAAAGTTTTGCCATTCCACCC
CCCGGTCTGCGCCAGACAGGCCGACTATCAGGAGCGAAAACATG

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Supplementary Figure 3. Conserved motif in the putative promoter sequence of the *phaP* gene in 8 selected *Burkholderia sensu lato* strains and the model PHA-producer *C. necator* H16. The 300 bp *phaP* upstream regions, containing putative regulatory sequences, were recovered from 8 genomes of *Burkholderia* s.l. strains, including two species per main genera (*Burkholderia*, *Paraburkholderia*, *Caballeronia* and *Trinickia*), and from *C. necator* H16 genome, in which the repression of *phaP* expression by the PhaR regulator has been reported [22], and altogether used for a consensus motif search. The web-based MEME suite of motif-based sequence analysis tools version 5.3.3 [23] was used for motif discovery utilizing standard settings. The logo and consensus sequence of a 57 bp-motif identified in the nine putative regulatory regions included is shown in the top. The 300 bp *phaP* upstream region of each strain is also shown including every motif (in color) and the ATG initiation codon of each gene (in bold inside the box). The PhaR binding sites determined by DNase I footprinting experiments in *C. necator* H16 [22] are also shown in gray boxes. The accession numbers and coordinates of each sequence are included in the header of the fasta format



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Supplementary Figure 4. Evolutionary relationships among concatenated PhaA, PhaB and PhaC protein homologues encoded by the conserved canonical *phaCAB* gene cluster of the 37 *Burkholderia sensu lato* genomes. Maximum likelihood (ML) topology provided by IQ-TREE [24] employing ModelFinder as model-selection method [25] and Ultrafast Bootstrap Approximation as bootstrap approach [26] with the -m TEST, -bb 1000 and -alrt 1000 options. Sequence alignments for phylogenetic reconstruction were calculated with MAFFT online service using Auto (FFT-NS-1, FFT-NS-2, FFT-NS-i or L-INS-i; depends on data size) strategy [27]. ML topology is shown with SH-like approximate likelihood ratio support values (n = 1000) given at each node (values >50% are shown). Visualization and edition of phylogenetic trees were performed by the Interactive Tree Of Life (iTOL) online tool [28]

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