

Figure S1. Circular plots of the plasmid pBHB. Rings from the outside to the inside: 1 and 2: ORFs on the leading and complementary strands, respectively. The putative genes are represented by 24 colors based on COG assignments. 3: G+C content with violet areas (below average) and red areas (above average). 4: GC-skew curve in cyan (negative value) and pink (positive value).

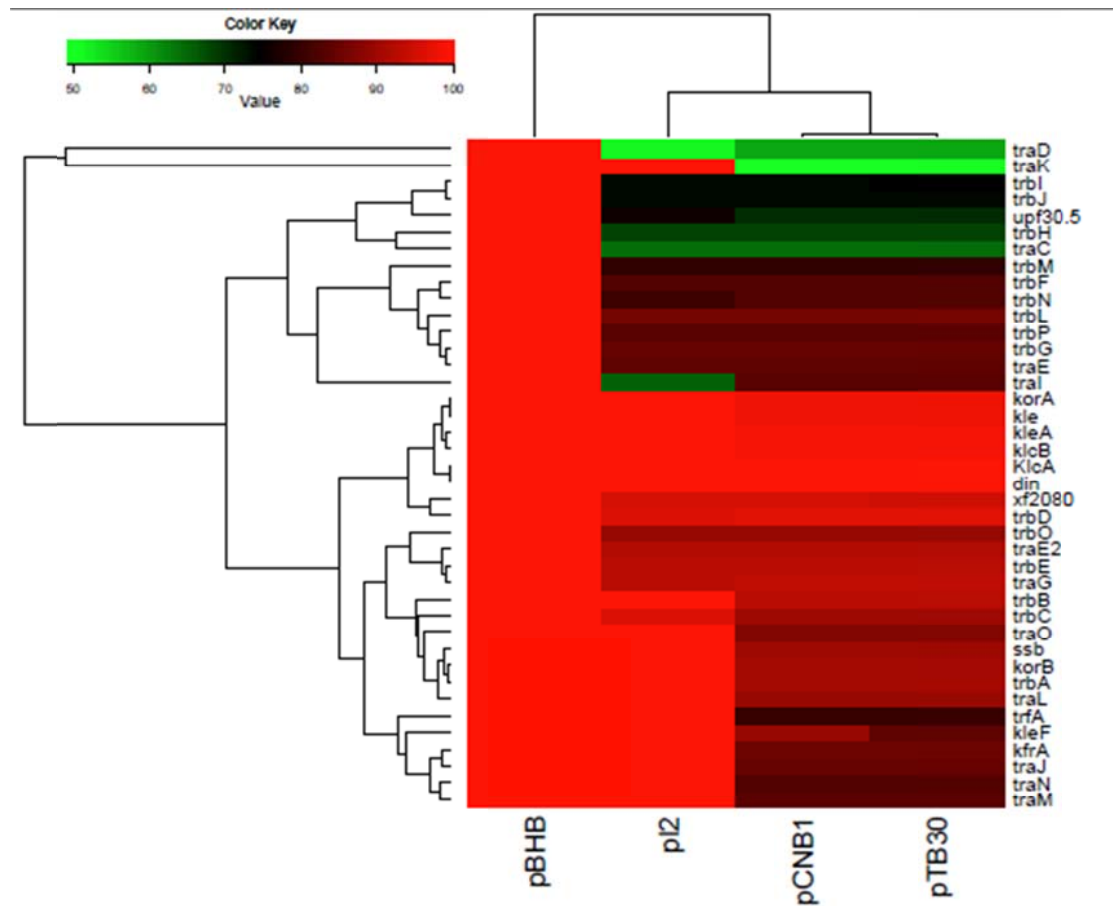


Figure S2. Neighbor joining dendrograms built using the Jaccard distance matrix values between phylogenetic profiles of the 40 proteins in the dataset obtained with an identity threshold of 50% from the four *Comamonas* plasmids. Red, increase in identity; green, decrease in identity.

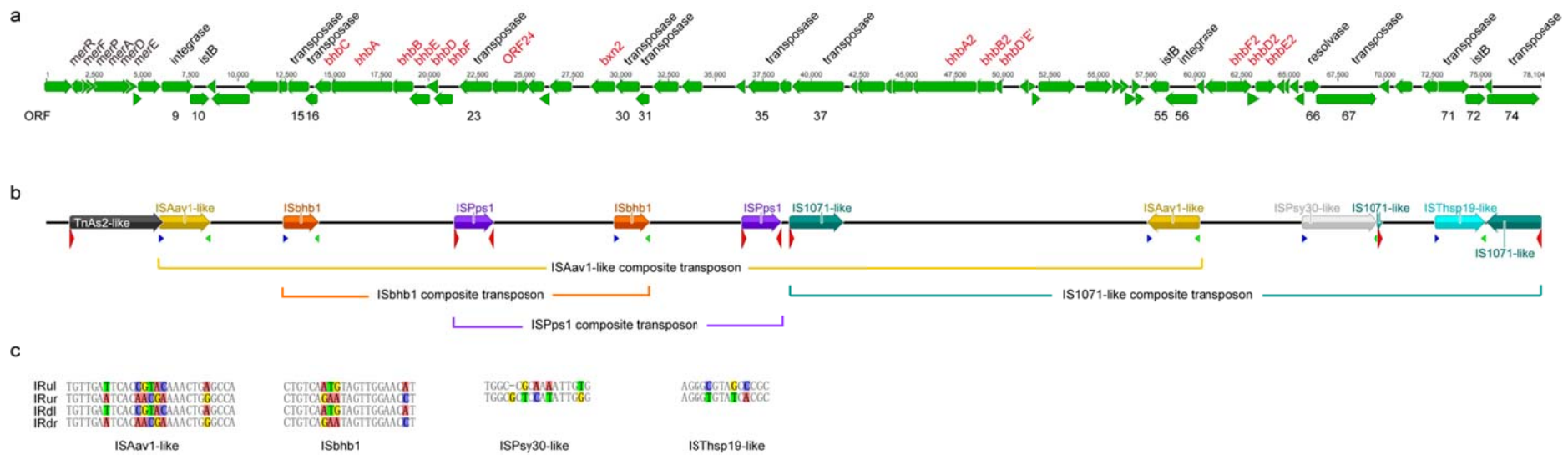


Figure S3. The distribution of transposons and ISs in the plasmid pBHB. (a) Genes involved in the bromoxynil catabolism and ORFs in the ISs are marked in red above the cutline and in black below the cutline. (b) Structure and annotation of the ISs and composite transposon. The large red triangles refer to the left/right ends of the ISs that are identical to previously reported ISs. The small blue and green triangles identify the left and right inverted repeats, respectively. For transposon TnAs2 and the IS1071-like element, only the right end was detected, whereas both left and right ends were found in ISPPs1. (c) Alignment of the inverted repeat (IR) sequences flanking ISAav1-like element, ISbhb1, ISPsy30-like and ISThsp19-like. IRul/IRur, left/right IR of the upstream elements of the two IS copies; IRdl/IRdr, left/right IR of the downstream elements of the two IS copies.

Table S1. Primers used in this study

Primer	Sequence (5'-3') [†]	Sequence amplified
bhbA-f	<u>CAAGGATCCCCTTCAATGGCCGCCA</u> (BamHI)	<i>bhbA</i>
bhbA-r	<u>GCATCTAGATTACATGTCCAGCATCAC</u> (XbaI)	
bhbB-f	<u>CAAGGATCCATTTTACAACAAGGAGAC</u> (BamHI)	<i>bhbB</i>
bhbB-r	<u>CATCTAGATTTAGATCTTTACTCTTTGATGCCGCGT</u> (XbaI, BglII)	
bhbA2-f	<u>GCAGGATCCAGGAAAGTCAGAGGCTTC</u> (BamHI)	<i>bhbA2</i>
bhbA2-r	<u>GCATCTAGATTAGAGGTTGAGAACGATCT</u> (XbaI)	
bhbB2-f	<u>GCAGGATCCACACGAACACTGGAGA</u> (BamHI)	<i>bhbB2</i>
bhbB2-r	<u>CATCTAGATTTAGATCTCTATTCCCTAATCCCACG</u> (XbaI, BglII)	
bhbB-f	<u>CAAGGATCCATTTTACAACAAGGAGAC</u> (BamHI)	<i>bhbAB</i>
bhbA-r	<u>GCATCTAGATTACATGTCCAGCATCAC</u> (XbaI)	
bhbB2-f	<u>GCAGGATCCACACGAACACTGGAGA</u> (BamHI)	<i>bhbA2B2</i>
bhbA2-r	<u>GCATCTAGATTAGAGGTTGAGAACGATCT</u> (XbaI)	
A-RT-f	CACGCTCGCGCTGAAGGTG	143-bp fragment of <i>bhbA</i> for real-time qPCR
A-RT-r	GGTAGTTTCGACCGGTGTAG	
B-RT-f	ATGGCTACACCTTCCTCGTC	187-bp fragment of <i>bhbB</i> for real-time qPCR
B-RT-r	TTGGCGTAGGTCAGCAGATC	
A2-RT-f	GTAGCCACCAGTCGATCCAC	157-bp fragment of <i>bhbA2</i> for real-time qPCR
A2-RT-r	TCCGACGGAATTTACCTCG	
B2-RT-f	TGATTGCCGCTGAAGCTGTC	184-bp fragment of <i>bhbB2</i> for real-time qPCR
B2-RT-r	TGACGTGCATCACTCACCAG	
trfA-RT-f	TGAACAAGATGGCCGAGCAG	175-bp fragment of <i>trfA</i> for real-time qPCR
trfA-RT-r	AACAGCGAGAGCTGCATGTC	

[†]Specified restriction sites are underlined.

Table S2. Similarity of the complete sequences between the four *Comamonas* metabolic plasmids

	pBHB	pI2	pTB30	CNB1
pBHB		62% [†] (99% [‡])	57% (84%)	55% (84%)
pI2	40% (99%)		74% (96%)	60% (97%)
pTB30	36% (84%)	72% (96%)		55% (99%)
CNB1	40% (84%)	70% (97%)	65% (99%)	

[†]The percentage of the complete sequence from the column plasmid that is conserved.

[‡]The similarity between the conserved sequences.