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Title: Degradation of recalcitrant polyurethane and xenobiotic additives by a selected landfill microbial community and its biodegradative potential revealed by proximity ligation-based metagenomic analysis

Supporting information:

Number of pages 9

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Table S1. Effects of BP8 biodegradative activity on the PE-PU-A copolymer analyzed by Differential Scanning Calorimetry.

Culture time (days)	Tg (°C)	Tm-I (°C)	Tm-II (°C)	Tm-III (°C)	Tc (°C)
Non-inoculated	50.2	70.0	210.6	398.1	459.6
5	39.5	68.8	211.0	408.7	478.2
10	46.0	68.0	210.1	407.9	480.2
15	38.1	70.5	211.1	393.2	479.9
20	46.2	78.8	213.9	392.5	476.2

Tg = glass transition temperature; Tm = melting temperature; Tc = crystallization temperature.

Table S2. Molecular weight and polydispersity index of the PE-PU-A copolymer during cultivation with the BP8 community.

Culture time (days)	Tg (°C)	Tm-I (°C)	Tm-II (°C)	Tm-III (°C)	Tc (°C)
Non-inoculated	50.2	70.0	210.6	398.1	459.6
5	39.5	68.8	211.0	408.7	478.2
10	46.0	68.0	210.1	407.9	480.2
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20	46.2	78.8	213.9	392.5	476.2

Tg = glass transition temperature; Tm = melting temperature; Tc = crystallization temperature.

Table S3. General features of the deconvoluted genomes from the BP8 metagenome.

Cluster ID	Genome Size (bp)	Num Contigs	Contig N50	[a]Comple-teness (%)	[b]Relative Abun-dance (%)	Novelty Score (%)	GC (%)	Identification	Genes assigned	Proteins assigned
1	4 275 656	282	51 004	89.4	57.7	1.6	67.8	*Paracoccus* sp. BP8	4 225	4 073
2	2 157 639	388	7 081	95.6	3.7	98.7	47.3	*Chryseobacterium* sp. BP8.2	2 253	2 185
3	5 478 545	1098	6 493	95.5	12.5	99.2	48.1	*Parapedobacter* sp. BP8.3	5 310	5 173
4	2 790 120	158	39 967	97.7	3.6	94.0	71.3	[c]Microbacteriaceae bacterium BP8.4	2 850	2 705
5	2 916 513	1146	2 823	71.0	22.5	2.5	58.4	*Ochrobactrum intermedium* BP8.5	3 472	3 162

[a]Completeness was calculated based on 40 single copy gene markers [36]. All the genomes' drafts have at least 18 tRNAs and, except for cluster 5, at least 1 rDNA gene copy.
[b]Relative abundance was normalized according to the reads distribution along the deconvoluted taxon.
[c]For Microbacteriaceae bacterium BP8.4 no further classification was possible even that nine single-copy markers were analyzed.

Table S4. [a]Phylogenetic relatedness of the bacterial species from the BP8 community identified by Hi-C metagenome deconvolution

Clusters/ Clasification/ GeneBank Acc. Num.	Organism	Assembly	Genome size (Mb)	GC content (%)	[a]ANI value (%)	Proteins encoded in the genomes
Cluster 1/ *Paracoccus* sp. BP8 GCA_003852815.1	*Paracoccus* sp. J39	GCA_000518925.1	4.42837	68.08	98.73	4131-4993
	Paracoccus denitrificans	GCA_000203895.1	5.23619	66.78	90.24	
	Paracoccus aminovorans	GCA_001546115.1	4.58940	67.50	85.59	
	Paracoccus halophilus	GCA_900111785.1	4.00871	65.20	82.17	
	Paracoccus yeei	GCA_002073635.2	4.82967	67.08	81.95	
Cluster 2/ *Chryseobacterium* sp. BP8.2 GCA_003852805.1	*Chryseobacterium koreense*	GCA_001045435.1	3.15420	40.10	69.38	3000-4810
	Chryseobacterium camelliae	GCA_002770595.1	4.37635	41.80	68.86	
	Chryseobacterium luteum	GCA_000737785.1	4.71855	37.30	68.70	
	Chryseobacterium sp. 52	GCA_002754245.1	5.29882	37.00	68.49	
	Chryseobacterium antarcticum	GCA_000729985.1	3.12366	36.10	68.10	
Cluster 3/ *Parapedobacter* sp. BP8.3 GCA_003852785.1	*Parapedobacter indicus*	GCA_900113765.1	6.15523	48.00	80.23	3796-4949
	Parapedobacter koreensis	GCA_900109365.1	5.54776	48.20	74.24	
	Parapedobacter luteus	GCA_900168055.1	4.82992	49.30	73.66	
	Parapedobacter composti	GCA_900112315.1	4.62202	50.00	73.32	
Cluster 4/ Microbacteriaceae bacterium BP8.4 GCA_003852775.1	*Micrococcales bacterium* 72-143	GCA_001898835.1	3.33048	71.60	85.07	2535-3047
	[b]*Leifsonia* sp. Leaf336	GCA_001423695.1	4.15779	69.60	74.40	
	[b]*Microcella alkaliphila*	GCA_002355395.1	2.70284	68.40	74.10	
	[b]*Plantibacter* sp. H53	GCA_001650455.1	4.01278	69.40	73.98	
	[b]*Herbiconiux* sp. YR403	GCA_000799285.1	3.60404	62.00	71.66	
	[c]*Arthrobacter cupressi*	GCA_900099975.1	4.0588	67.00	70.82	
Cluster 5/ *Ochrobactrum intermedium* BP8.5 GCA_003852825.1	*Ochrobactrum intermedium* LMG 3301	GCA_000182645.1	4.72539	57.70	98.48	3645-4502
	Ochrobactrum anthropi	GCA_000017405.1	5.20578	56.15	87.48	
	Ochrobactrum oryzae OA447	GCA_002943495.1	4.46700	56.20	87.19	
	Brucella ceti	GCA_000590795.1	3.27803	57.27	82.03	
	Ochrobactrum sp. P6BS-III	GCA_002016635.1	5.25313	56.00	81.19	

[a] Phylogenetic relatedness was calculated based on ANI value with OrthoANIu algorithm**.** ANI values higher than 95% indicate that the compared genomes are the same species [39].
[b] Members of Microbacteriaceae family, order Micrococcales.
[c] Member of Micrococcaceae family, order Micrococcales.

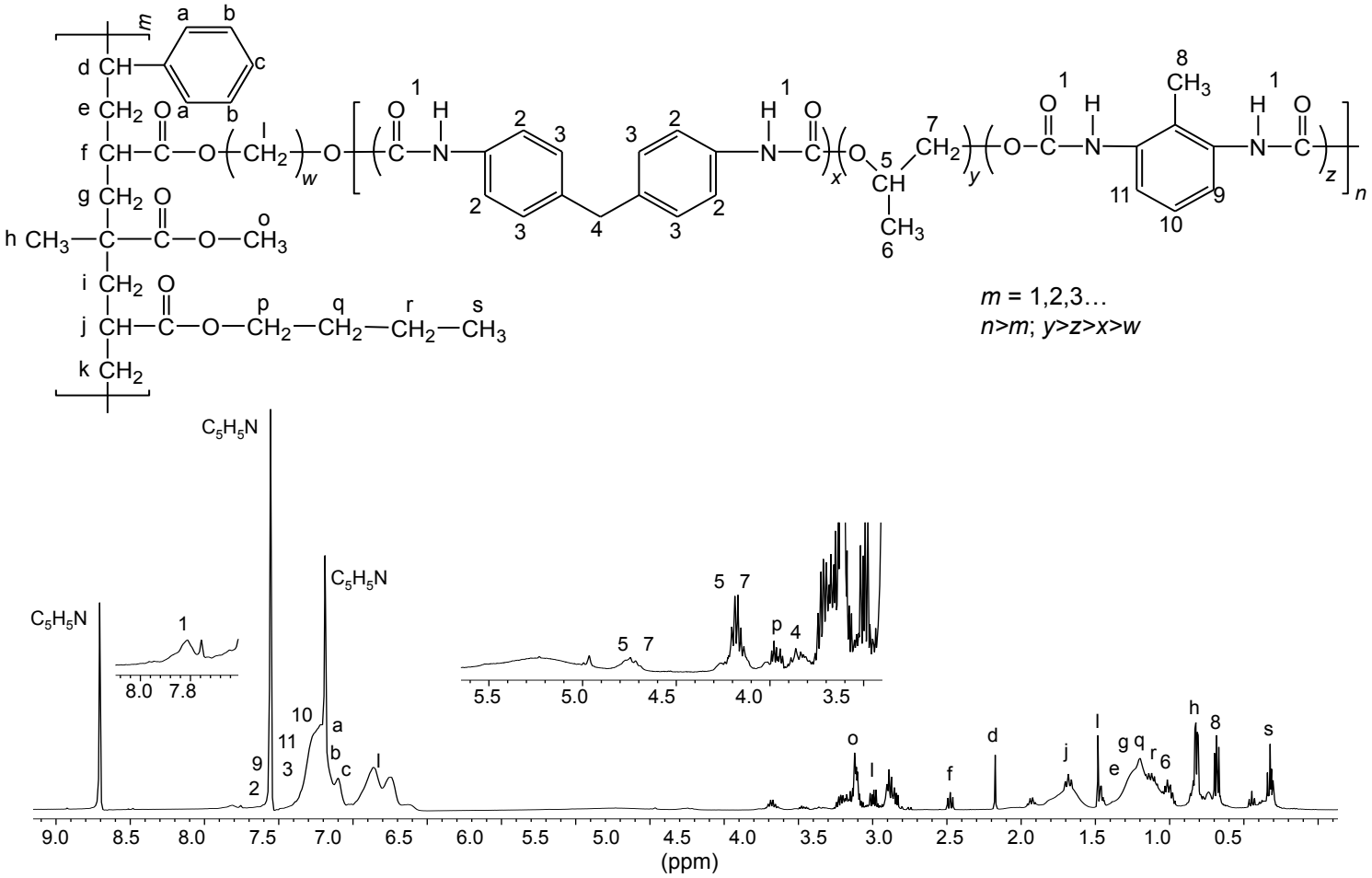

Figure S1. Proposed chemical structure for the PE-PU-A copolymer present in PolyLack®. This structure was proposed based on the ^{1}H-NMR analysis of dried PolyLack®, the information included in the manufacturer technical manual [31], the GC-MS analysis (Figure 2), and the most frequent acrylates used in the synthesis of these types of copolymers [45, 46]. Synthesis of PE-PU-A copolymers starts by the polycondensation of polyols (polypropylene glycol) (y moiety) and diisocyanates (TDI and MDI) (x and z moieties) followed by end capping with acrylates' mixture (m moiety). From the most frequently used acrylates we selected methyl methacrylate, butyl acrylate, hydroxy acrylate and styrene as representatives in this structure. In the ^{1}H-NMR spectrum, chemical shifts are provided in parts per million from $SiMe_4$ as internal reference. Signal 1 is assigned to carbamate groups (NH-COO); signals a, b, c, 2, 3, 9-11 are assigned to the aromatic protons; signals 4 and 8 correspond to the protons of methylene (CH_2) and methyl (CH_3) groups in MDI and TDI, respectively; signals 5-7 correspond to PPG; signals l correspond to the hydroxyl proton (CH_2-O) and methylene groups (CH_2) in the chain of hydroxy-acrylate; signals f, j, o and p correspond to the acrylic groups (CH-COO, CH_2-COO or CH_3-COO), signals d (CH), e, g, i, k, q, r (CH_2), h and s (CH_3) are assigned to methylene and methyl groups in the acrylate mixtures.

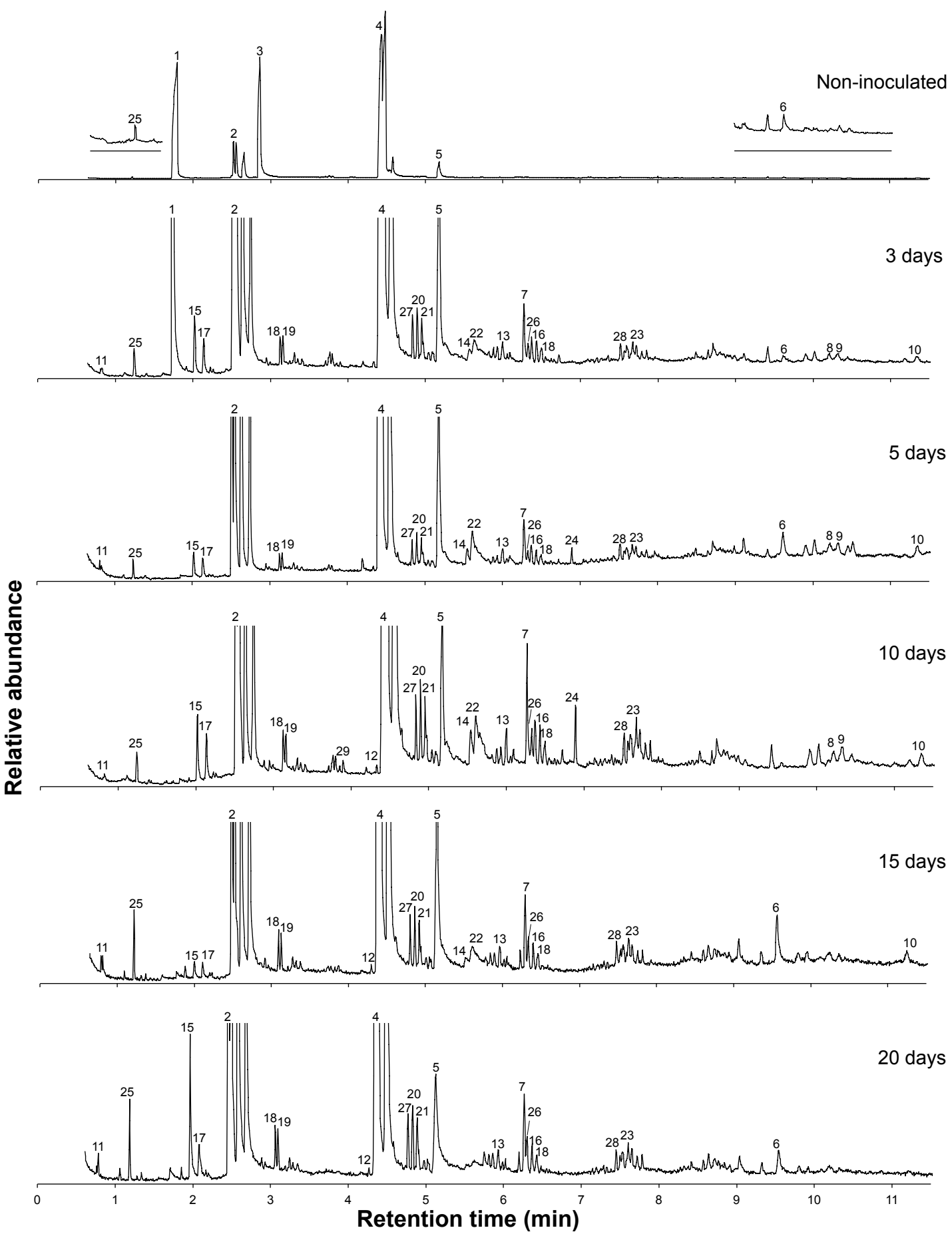

Figure S2. Chromatograms of cell-free supernatants from BP8 cultures grown in MM-PolyLack. Numbered signals designate compounds identified by mass spectrometry listed in Figure 2. Chromatogram of non-inoculated sample is at original scale, whereas the other chromatograms are at larger scale.

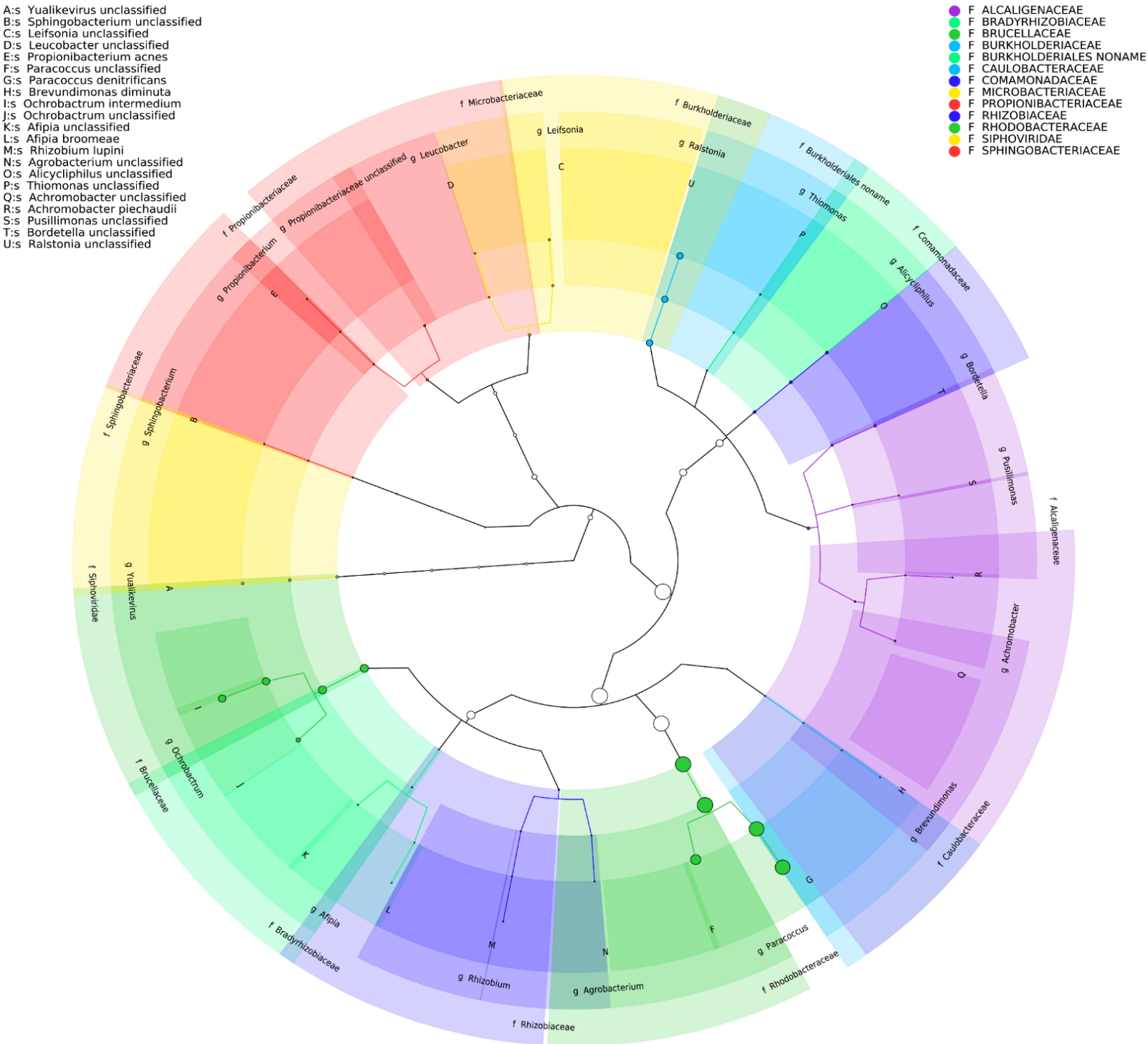

Figure S3. Taxonomic cladogram of BP8 community microbial diversity profiled with MetaPhlAn. Circles size is proportional to the taxon relative abundance. The most abundant taxa were *Paracoccus* genus (83.3%) and *Ochrobactrum* genus (8.7%). Families are color-labeled and predicted species diversity is indicated by capital letters [Asnicar F, Weingart G, Tickle TL, Huttenhower C, Segata N. Compact graphical representation of phylogenetic data and metadata with GraPhlAn. PeerJ. 2015;3:e1029].

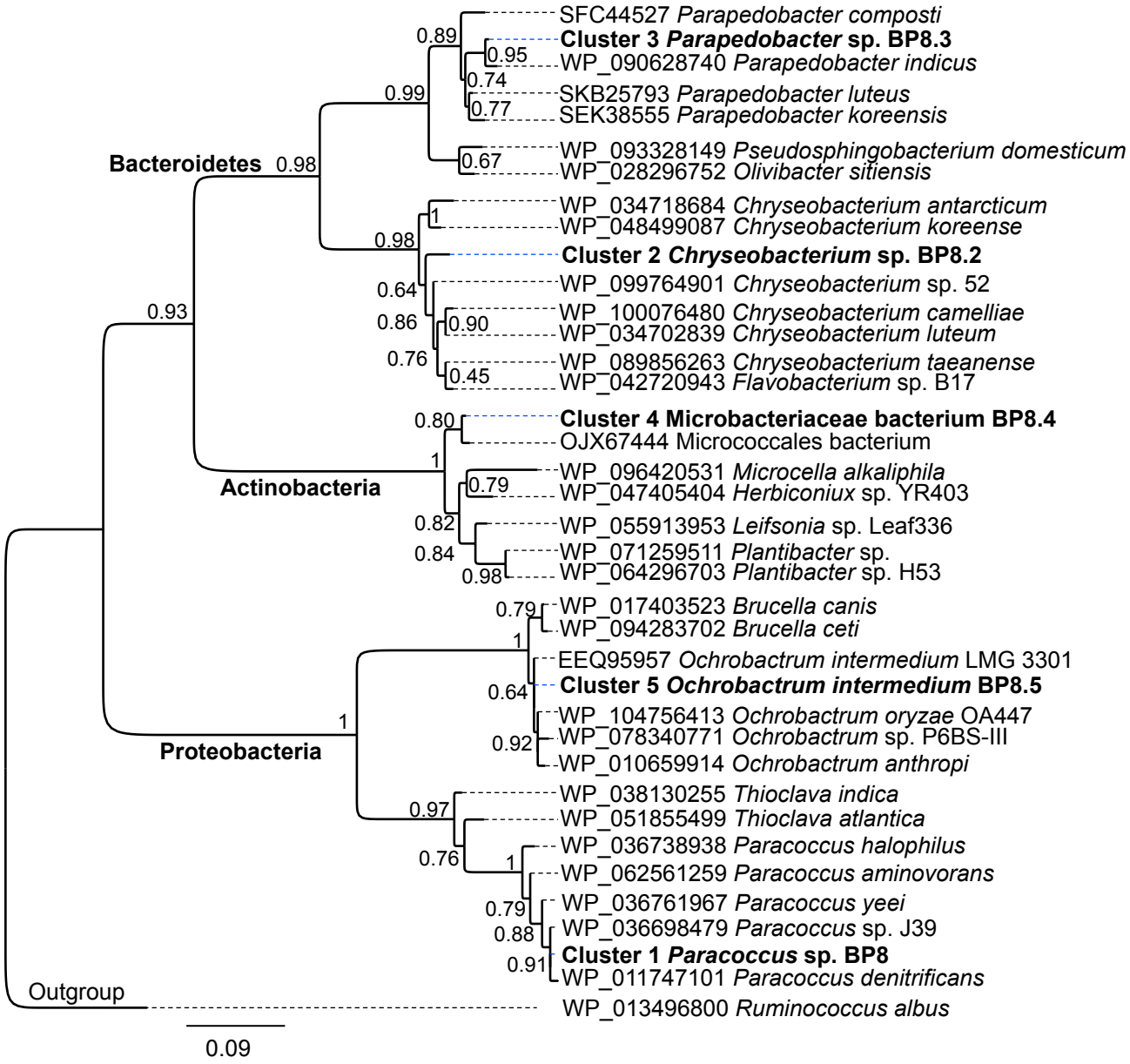

Figure S4. Maximum likelihood phylogeny for taxonomic delimitation of the deconvoluted genomes from the BP8 metagenome. This analysis was performed with three phylogenetic markers, ribosomal protein L3, ribosomal protein L5 and DNA gyrase A subunit, which generated similar results. Here we present the analysis for ribosomal protein L3. Branch support values are indicated in the corresponding nodes. Bar indicates the number of expected substitutions per site under the WAG + G model. A sequence of *Ruminococcus albus* (Firmicutes) was used as outgroup. Key genome clusters are highlighted in bold and different Phyla are indicated at the left. Sequences for L3 ribosomal proteins of the deconvoluted genomes are accessible in the NCBI GenBank under accession numbers RQP07704.1, RQP15098.1, RQP16503.1, RQP08603.1 and RQP16393.1 for clusters 1 to 5, respectively.