Supporting Information

Biopolymers from urban organic waste: influence of the solid retention time to cycle length ratio in the enrichment of a mixed microbial culture (MMC)

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Number of pages: 5

Numbers of figures: 1

Numbers of tables: 2

Results and Discussion

Microbiome composition

The majority of the bacterial taxa obtained from the samples taken under steady-state operating conditions are indicated in Figure S1. The analyses revealed distinct bacterial profiles over different runs. The relative abundance of putative PHA-storing bacteria selected under different runs varied and decreased with the increase of the SRT/CL ratio (Figure 1; Table S1), with lowest evenness and biodiversity values in Ae3 (Table S2).

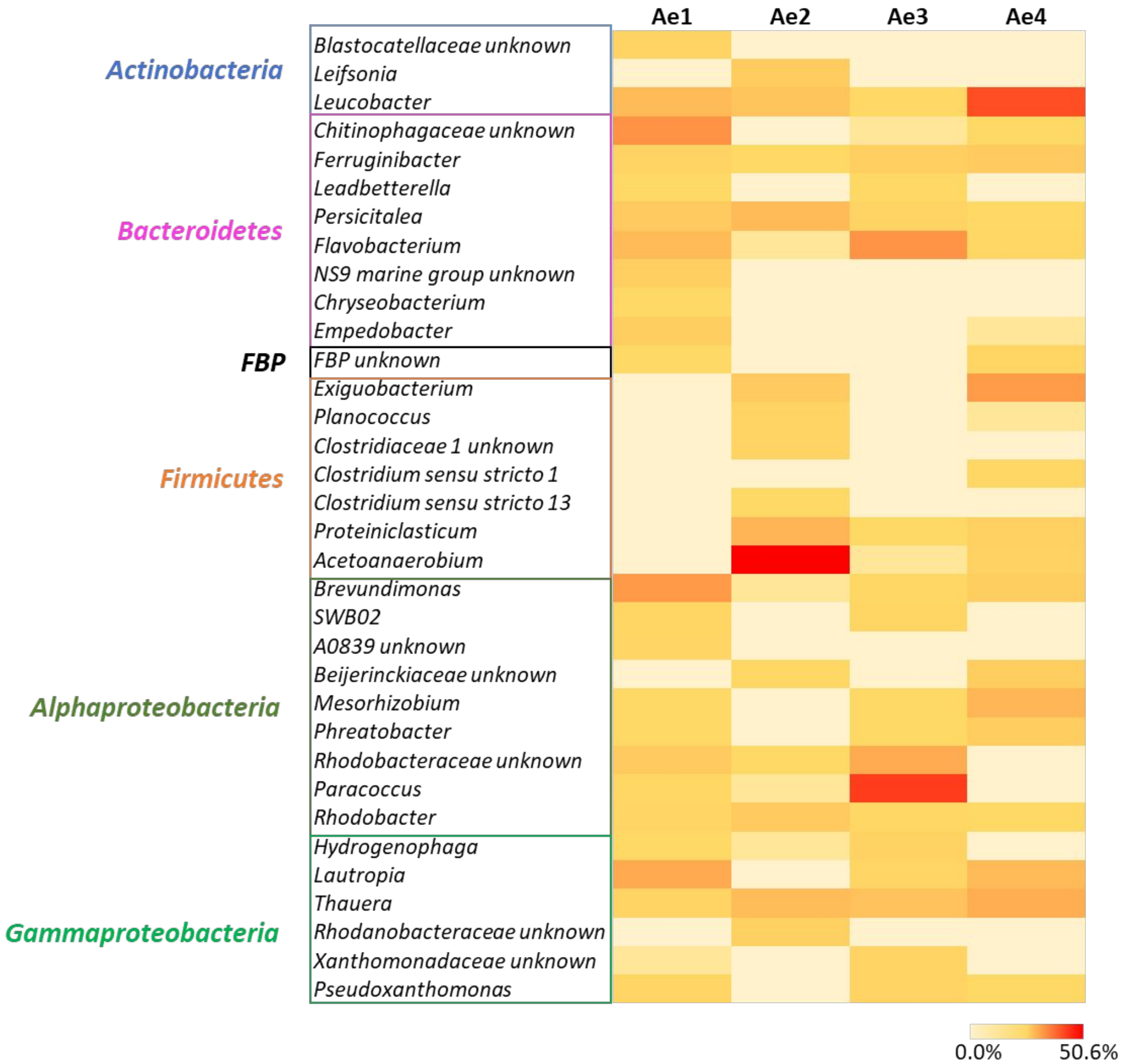

Figure S1. Frequency heat-map of bacterial communities at genus level: the colour intensity in each cell shows the relative abundance (relative abundance of total reads ≥1% in at least one sample).

Table S1. Relative abundance (% of total reads) of bacterial taxa known for their capability to store PHA. For each taxonomic group, a reference reporting the ability to store PHA is also provided.

phylum	class	order	family	genus	Ae1	Ae2	Ae3	Ae4	Reference
Actinobacteria	Actinobacteria	Micrococcales	Microbacteriaceae	Leifsonia	0.0	3.4	0.0	0.0	[1]
Bacteroidetes	Bacteroidia	Cytophagales	Spirosomaceae	Leadbetterella	0.3	0.0	0.6	0.0	[2]
Bacteroidetes	Bacteroidia	Flavobacteriales	Flavobacteriaceae	Flavobacterium	7.4	0.1	16.3	0.8	[3]
Firmicutes	Bacilli	Bacillales	Family XII	Exiguobacterium	0.0	3.5	0.0	14.5	[4]
Proteobacteria	Alphaproteobacteria	Caulobacterales	Caulobacteraceae	Brevundimonas	15.0	0.1	0.7	2.8	[5]
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Paracoccus	0.8	0.1	36.8	0.0	[6]
Proteobacteria	Alphaproteobacteria	Rhodobacterales	Rhodobacteraceae	Rhodobacter	1.0	3.7	0.7	0.3	[7]
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Burkholderiaceae	Hydrogenophaga	0.2	0.1	1.7	0.0	[8]
Proteobacteria	Gammaproteobacteria	Betaproteobacteriales	Rhodocyclaceae	Thauera	1.4	6.9	5.6	10.4	[9]
Proteobacteria	Gammaproteobacteria	Xanthomonadales	Xanthomonadaceae	Pseudoxanthomonas	0.9	0.0	1.6	0.3	[10]

Table S2. Biodiversity indexes.

	Ae1	Ae2	Ae3	Ae4
Dominance_D	0.1	0.1	0.2	0.0
Simpson_1-D	0.9	0.9	0.8	1.0
Shannon_H	3.8	3.3	3.0	3.5
Evenness_e^H/S	0.3	0.3	0.2	0.5

References

[1] Kumar, V.; Thakur, V.; Kumar, S.; Singh, D. Bioplastic reservoir of diverse bacterial communities revealed along altitude gradient of Pangi-Chamba trans-Himalayan region. *FEMS Microbiol. Lett*. **2018**, *365*. https://doi.org/10.1093/femsle/fny144.

[2] Coats, E.R.; Watson, B.S.; Brinkman, C.K. Polyhydroxyalkanoate synthesis by mixed microbial consortia cultured on fermented dairy manure: Effect of aeration on process rates/yields and the associated microbial ecology. *Water Res*. **2016**, *106*, 26-40. https://doi.org/10.1016/j.watres.2016.09.039.

[3] Dionisi, D.; Beccari, M.; Di Gregorio, S.; Majone, M.; Petrangeli Papini, M.; Vallini, G. Storage of biodegradable polymers by an enriched microbial community in a sequencing batch reactor operated at high organic load rate. *J. Chem. Technol. Biotechnol.* **2005**, *80*, 1306-1318. https://doi.org/10.1002/jctb.1331.

[4] Kung, S.S.; Chuang, Y.C.; Chen, C.H.; Chien, C.C. Isolation of polyhydroxyalkanoates-producing bacteria using a combination of phenotypic and genotypic approach. *Lett. Appl. Microbiol*. **2007**, *34*, 364-371. https://doi.org/10.1111/j.1472-765X.2006.02090.x.

[5] Bhuwal, A.K.; Singh, G.; Aggarwal, N.K.; Goyal, V.; Yadav, A. Isolation and Screening of Polyhydroxyalkanoates Producing Bacteria from Pulp, Paper, and Cardboard Industry Wastes. *Int. J. Biomater.* **2013**, Article ID 752821. https://doi.org/10.1155/2013/752821.

[6] Sawant, S.S.; Salunke, B.K.; Kim, B.S. Degradation of corn stover by fungal cellulase cocktail for production of polyhydroxyalkanoates by moderate halophile *Paracoccus* sp. LL1. *Bioresour. Technol.* **2015**, *194*, 247-255. https://doi.org/10.1016/j.biortech.2015.07.019.

[7] Jiang, Y.; Chen, Y.; Zheng, X. Efficient polyhydroxyalkanoates production from a waste-activated sludge alkaline fermentation liquid by activated sludge submitted to the aerobic feeding and discharge process. *Environ. Sci. Technol.* **2009**, *43*, 7734-7741. https://doi.org/10.1021/es9014458.

[8] Valentino, F.; Morgan-Sagastume, F.; Fraraccio, S.; Corsi, G.; Zanaroli, G.; Werker, A.; Majone, M. Sludge minimization in municipal wastewater treatment by polyhydroxyalkanoates (PHA) production. *Env. Sci. Pollut. Res. Int.* **2015**, *22*, 7281-7294. DOI: 10.1007/s11356-014-3268-y.

[9] Serafim, L.S.; Lemos, P.C.; Rossetti, S.; Levantesi, C.; Tandoi, V.; Reis, M.A.M. Microbial community analysis with a high PHA storage capacity. *Water Sci. Technol.* **2006**, *54*, 183-188. https://doi.org/10.2166/wst.2006.386.

[10] Mwamburi, S.M.; Mbatia, B.N.; Remmy, K.; Kirwa, E.M.; Noah, N.M. Production of polyhydroxyalkanoates by hydrocarbonaclastic bacteria. *Afr. J. Biotechnol.* **2019**, *18*, 352-364. https://doi.org/10.5897/AJB2019.16763.