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Eronen-Rasimus, E.[a,b]#, Hultman, J.[a], Hai, T.[c], Pessi, I.S.[a], Collins, E.[d], Wright, S.[c], Laine, P., Viitamäki, S.[a], Lyra, C.[a], Thomas, D.N.[e,f], Golyshin, P.N.[c], Luhtanen, A.-M.[1,g], Kuosa, H.[b] and Kaartokallio, H[b].

[a] *University of Helsinki, Faculty of Agriculture and Forestry, Department of Microbiology, Helsinki, Finland, PO BOX 56 (Viikinkaari 9), FI-00014 University of Helsinki, Finland*

[b] *Finnish Environment Institute (SYKE), Marine Research Centre, Helsinki, Finland*

[c] *Bangor University, School of Natural Sciences, Bangor, UK*

[d] *University of Manitoba, Centre for Earth Observation Science, Winnipeg, Canada*

[e] *University of Helsinki, Faculty of Biological and Environmental Sciences, Ecosystems and Environment Research Programme, Helsinki, Finland*

[f] *Bangor University, School of Ocean Sciences, Bangor, UK*

[g] *University of Helsinki, Faculty of Biological and Environmental Sciences, Molecular and Integrative Biosciences Research Programme, Helsinki, Finland*

#Address correspondence to eeva.eronen-rasimus@helsinki.fi

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Running head: Sea-ice bacteria produce poly-3-hydroxyalkaonoic acids

ABSTRACT

Poly-3-hydroxyalkanoic acids (PHAs) are bacterial storage polymers commonly used in bioplastic production. Halophilic bacteria are industrially interesting organisms as their salinity tolerance and psychrophilic nature lowers sterility requirements and subsequent production costs. We investigated the PHA synthesis in two bacterial strains, *Halomonas sp.* 363 and *Paracoccus sp.* 392, isolated from Southern Ocean sea ice and elucidated the related PHA biopolymer accumulation and composition with various approaches, such as transcriptomics, microscopy and chromatography. We show that both bacterial strains produce PHAs at 4 °C when the availability of nitrogen and/or oxygen limited growth. The genome of *Halomonas* sp. 363 encoded three *phaC* synthase genes and transcribed genes along three PHA pathways (I–III), whereas *Paracoccus sp.* 392 carry only one *phaC* gene and transcribed genes along one pathway (I). Thus *Halomonas* sp. 363 has versatile repertoire of *phaC* genes and pathways enabling native production of both short- and medium chain length PHA products.

IMPORTANCE

Plastic pollution is one of the most topical threats to the health of the World Ocean. One recognized way to alleviate the problem is to use degradable bioplastic materials in high-risk applications. PHA is a promising bioplastic material as it is non-toxic and fully produced and degraded by bacteria. Sea ice is an interesting environment for prospecting novel PHA-producing organisms, since traits advantageous to lower production costs, such as tolerance for high salinities and low temperatures are common. We show that two sea-ice bacteria, *Halomonas* sp. 363 and *Paracoccus* sp. 392, are able to produce various types of PHA from inexpensive carbon sources. *Halomonas* sp. 363 is an especially interesting PHA-producing organism, since it has three different synthesis pathways to produce both short- and medium-chain length PHAs.

INTRODUCTION

Poly-3-hydroxyalkanoic acids (PHAs), the most common bacterial storage polymers, can be utilized as renewable and biodegradable plastics (1). Industrially, the challenge is to produce PHAs from inexpensive, non-related carbon (C) skeletons structurally different from those of PHA C sources such as glucose, for which marine bacteria, including *Halomonas* spp., have shown considerable potential (2–7). Moreover, the recent focus on marine plastic pollution has given rise to an urgent need to develop sustainable alternatives for petrochemical plastics at competitive prices (8). PHA is one of the most promising alternative materials, because it is biocompatible; i.e., non-toxic for living organisms, and bacteria are able to synthesize and degrade it completely with hydrolases and depolymerases (9–11). In particular, medium-chain-length (MCL)-PHAs and co-polymers are more flexible and easier to process, thus making them the polymers preferred for industrial applications (12).

Halophilic and psychrophilic bacteria display advantages as potential bioplatforms for PHA production, because both high salinity tolerance and growth at low temperatures reduce the risk of contamination during cultivation and associated production costs (6,13–15). Recent studies have shown that sea-ice bacteria possess PHA granules and synthase genes (16,17), suggesting that PHA production is ecologically relevant to microbial populations inhabiting sea ice. Thus, sea ice, known for the rapidly fluctuating environmental conditions, including combined high salinities (up to 216 ‰ at -21 °C, Eicken et al. 2000) and low temperatures (18), is a promising biome in which to prospect for new PHA-producing bacteria.

PHAs are linear polyesters that accumulate in hydrophobic cytoplasmic inclusion bodies that many bacteria use for C and energy storage (19–21). PHAs are ideal storage polymers; they are highly

reduced and by their low solubility have negligible effect on osmotic pressure regulation in the cell (19). PHAs also enhance survival during environmental stress such as oxygen (O_2) deficiency, ultraviolet (UV) radiation, salinity and cold (21–26), all of which are encountered in sea ice(17). Environmental stressors cause oxidative stress in the bacteria, increasing the concentrations of reactive-oxygen species (ROS) in cells (27). These can be further detoxified enzymatically with antioxidants such as superoxidase dismutase and catalase, some of which use nicotinamide adenine dinucleotide phosphate NAD(P)Hs as cofactors (27). During O_2 deficiency, PHA can act as a sink for reducing power, because the NAD(P)H produced in glucose catabolism cannot be oxidized, which leads to high NAD(P)H/NAD(P) ratios and subsequent channelling of NAD(P)H to NAD(P)H-dependent *phaB* and PHA production (19,28–30). Therefore, PHAs are used by bacteria to maintain cellular redox balance by either synthesizing or depolymerizing PHA; i.e., storing or producing reduced equivalents (19,21,26,28,29,31). Most commonly, PHAs are produced when nutrient availability is not balanced; e.g., when nitrogen (N) or phosphorus limits the growth but there is excess C available, leading to channelling of the surplus acetyl-coenzyme A (CoA) and NAD(P)H to PHA production (20,32). Again, nutrient limitation is a well-recorded feature in sea ice habitats (33).

We investigated the conditions and cellular basis for the PHA production in two bacterial strains newly isolated from Southern Ocean sea ice, *Halomonas* sp. 363 (Gammaproteobacteria) and *Paracoccus* sp. 392 (Alphaproteobacteria). We verified the PHA production, using transcriptomes, microscopy and gas chromatography-mass spectrometry (GC-MS). We show that these two sea-ice bacteria can produce various types of PHAs from inexpensive C sources under N-limitation and co-limitation of N and O_2 at low temperature.

RESULTS

Aim of the study was to investigate the conditions and cellular basis for the PHA production in two bacterial strains isolated from Southern Ocean sea ice, *Halomonas* sp. 363 (Gammaproteobacteria) and *Paracoccus* sp. 392 (Alphaproteobacteria). Shaker flask batch-culture experiments were conducted with *Halomonas* sp. 363 and *Paracoccus* sp. 392 under both N-limited and N-replete conditions (Fig. S1).

PHA genes

The closed circular genome of *Halomonas* sp. 363 was comprised of 5.6 Mb and that of *Paracoccus* sp. 392 3.03 Mb along with 18 plasmids (range of plasmid length 0.003–0.33 Mb, complete genome 4.5 Mb). Both strains harboured all the genes (*phaA, phaB* and *phaC)* essential for PHA production (Fig. 1, Fig. S2 and S3). In addition, both strains included the phasin (*phaP*) and depolymerase (*phaZ*) genes, while *Paracoccus* sp. 392 also carried the regulator protein gene *phaR* (Fig. 2). One of the *Paracoccus* sp. 392 *phaZ* genes was carried by a plasmid (Fig. 1, Table S2). In *Halomonas* sp. 363, the PHA genes were scattered around the genome, as also observed in other *Halomonas* strains (34–36) whereas in *Paracoccus* sp. 392, two gene clusters (*phaRPCZ* and *phaAB*) were identified (Fig. 1) in accordance with a previous study (37).

Based on annotations with RAST (38) and PROKKA (39), *Halomonas* sp. 363 harbours three *phaC* genes (*phaC*, *phaC1* and *phaC2*); however, *phaC* was annotated only with RAST (Table S1). The predicted CDS showed non-specific matching with the Class III PHA synthase (TIGR01836, 201–415 bp) based on the National Centre for Biotechnology Information (NCBI) Conserved Domain Database (CDD). In addition, there was a stretch in the CDS (from AA 183–244/bp 549–732), which resulted in a 100% protein Basic Local Alignment Search Tool (BLASTp) hit against nr database to the *phaC* gene in *Halomonas* (EHA17034.1). Moreover, the *phaC* gene was much larger (2544 bp) than the synthase genes in general (1622–1973 bp) (40). Exceptionally large *phaC*

genes have also been detected in other *Halomonas* strains (7,34), as well as strains with three *phaC* genes (41). Since the *phaC* gene appears to be conserved in *Halomonas* spp. (Fig. S4), the results suggest it is a true gene.

In addition, *Halomonas* sp. 363 carried two copies of *phaB* genes as the halophilic archaeon, *Haloferax mediterranei* (42). This may have resulted from *Halomonas* having both NADPH-dependent *phaB* genes for anabolic PHA production and another NADH-dependent *phaB* genes for PHA production under fermenting, O_2-limited conditions, as suggested previously (30).

Transcriptomes and PHA granule formation

In total, ~834.2 million reads (~173 Gb) were obtained with Nextseq. *Halomonas* sp. 363 transcribed genes for all three main PHA production pathways (I–III), of which the transcription of Pathway I was highest (Fig. 2 and 4B, Fig. S5, Tab. S3). *Paracoccus* sp. 392 transcribed genes only for a pathway I (Fig. 2 and 4D, Tab. S4). In both strains, the PHA genes were transcribed in the N-limited 1 wk treatments (Fig. 3 B and D). By day 5, all *phaC* gene transcription levels increased significantly in *Halomonas* sp. 363 (one-way analysis of variance (ANOVA) *phaC* $p = 0.00142$, $F = 61.64$; *phaC1* $p = 0.0157$, $F = 16.26$; *phaC2* $p = 0.018$, $F = 14.96$, Fig. S6A), however, the increase in *phaC1* and *phaC2* transcription was much lower than in *phaC*. No such increase was observed in *Paracoccus* sp. 392 (Fig. 3D and Fig. S6C). In addition, in *Halomonas* sp. 363, *phaC* and *PhaC2* gene transcription levels were significantly greater at the end of the N-replete 3 wks treatment than on Day 5 in N-replete 1 wk treatment (one-way ANOVA, *phaC* $p = 0.00424$, $F = 34.31$; *phaC2* $p = 0.00108$, $F = 71.22$, Fig. 3B and Fig. S6B) with the highest *phaC* activity observed throughout the experiment (Fig. 3B and 4D). However, it should be noted that the transcription of *PhaC* gene in *Halomonas* sp. 363 was ~10 times higher than *PhaC1* and *PhaC2* (Figure 3B).

In the N-limited 1 wk treatment in *Halomonas* sp. 363 from Day 2 onwards and from Day 12 onwards in the N-replete 3 wks treatment the glutamine synthetase gene (*glnA*, NLJJMJOO_00241)

151 was up-regulated as an indicator of N deficiency, (Fig. 3A). The N-limitation likely induced up-
152 regulation of N uptake genes *nasD* (NLJJMJOO_01038 and NLJJMJOO_01039), *nrgA*
153 (NLJJMJOO_04706) and *yhdW* (NLJJMJOO_04689, Fig 3A and Fig. S2) and *narK*
154 (NLJJMJOO_01066). The *NarK* gene encodes a transporter responsible for nitrite/nitrate uptake
155 across the cytoplasmic membrane, *nasD* a subunit of assimilatory nitrite reductase, *nrgA* an
156 ammonium transporter and *yhdW* encodes an amino-acid transporter. The nutrient limitation
157 appeared to be more severe in the N-limited 1 wk treatment than in the N-replete 3 wks treatment in
158 *Halomonas* sp. 363, since the cells were larger in the latter indicating that cells were not suffering
159 for severe N-limitation (Fig. S7 C and D).
160 In *Paracoccus* sp. 392, the expression levels of *glnA* did not increase until at Day 4 in the N-limited
161 1 wk treatment (Fig. 3D), indicating that *Paracoccus* sp. 392 likely used stored cellular N after
162 transfer to the N-limited medium.
163 In addition to N limitation, the increases in the expression of superoxide dismutase (*sodM*),
164 catalase-peroxidase 1 (*katG1*), activator for hydrogen peroxide-inducible genes (*oxyR*) and hypoxic
165 response protein 1 (*hrp1*) genes (Fig. 3C) indicated O_2 deficiency in the N-replete 3 wks treatments
166 in *Halomonas* sp. 363. Facultative anaerobes use superoxidase dismutase with catalase, or
167 peroxidase, to protect anaerobic metabolism in the presence of O_2 (43). Rapid increase in *phaC*
168 expression coinciding with the up-regulation of antioxidant and N limitation genes suggest that co-
169 limitation of N and O_2 induced an increase in PHA production in the N-replete 3 wk treatment.
170 High cell densities combined with low rotation speed (120 rpm) lead to microaerobic conditions
171 and enhanced PHA accumulation in cultures (30,44).
172
173 **PHA composition**
174
175 *Halomonas* sp. 363 produced mainly poly-3-hydroxybutyrate (PHB) (up to 47 % w/w, Tab. 1) from
176 glucose and gluconate. Under N-limited conditions, trace amounts of beta-hydroxyvaleric (3-HV)

and beta-hydroxydodecanoic (HDD) acid moieties were observed, although not quantified. Interestingly, the *Halomonas* sp. 363 N-replete 1 wk treatment also accumulated PHB (~17% w/w, Table 1). *Paracoccus* sp. 392 produced poly(3-hydroxybutyrate-co-3-hydroxyvalerate) (PHBV) co-polymer with a range of 8.7 % w/w 3-HB and 4.5 % (w/w) 3-HV, while the N-replete treatment produced similar molarities of 3-HB and 3-HV (Table 1) from glucose and gluconate.

Table 1. Extracted PHA biopolyesters in *Halomonas* sp. 363 and *Paracoccus* sp. 392 cultured in glucose and gluconate under N-limited 1 wk and N-replete 1 wk conditions. PHA = poly-3-hydroxyalkanoic acid, 3-HB = 3-hydroxybutyrate, 3-HV = 3-hydroxyvalerate, 3-HDD = 3-hydroxydodecanoate, ND = not detected

		% of dry matter		
Strains	**Treatment**	**% 3-HB**	**% 3-HV**	**%3-HDD**
Halomonas sp. 363	N-limiting 1 wk	45.00	traces	traces
	N-replete 1 wk	17.19	ND	ND
Paracoccus sp. 392	N-limiting 1 wk	8.71	4.51	ND
	N-replete 1 wk	8.52	4.17	ND

DISCUSSION

PHAs are one of the most promising bioplastic materials, because they are fully synthetised and degraded by bacteria (11). We investigated PHA production and *pha*-gene transcription in two bacterial strains, *Halomonas* sp. 363 and *Paracoccus* sp. 392, isolated from Southern Ocean sea ice, using shaker flask batch-culture experiments under N-limiting and N-replete growth conditions with glucose and gluconate as a carbon source. *Halomonas* sp. 363 produced mainly PHB, but trace amounts of PHBV and 3HDD were also detected, whereas *Paracoccus* sp. 392 produced PHBV. Since *Halomonas* sp. 363 tolerates high salinities and low temperatures, can exploit inexpensive carbon sources as well as has three actively transcribed pathways (I–III) to produce PHAs with

197 indications of MCL-PHA and co-polymer production, *Halomonas* sp. 363 is an especially
198 promising candidate for industrial PHA production.
199
200 **PHA genes and growth conditions**
201 PHA granules have a hydrophobic core, with amorphous PHA enclosed by a phospholipid layer that
202 contains PHA synthase, depolymerase, phasin and regulatory proteins embedded and attached
203 (20,40,45). The key enzyme in PHA production is synthase (*phaC*) (40), which is divided into four
204 classes (I–IV), based on the substrate specificity, subunit composition and sequence homology
205 (10,40). Class I, III and IV synthases use short-chain-length (SCL)-HA-CoAs (C_3–C_5) whereas
206 Class II synthases use medium-chain-length MCL-HA-CoAs (C_6–C_{14}) as a substrates for
207 polymerizing PHAs (40). *Halomonas* sp. 363 encoded three *phaC* genes and produced SCL-PHA
208 (PHB) in both N-limited and N-replete 1 wk treatments, as well as in the N-replete 3 wks treatment
209 with combined N and O_2 limitation. Based on microscopy and transcriptomes, the highest PHA
210 yield was obtained under the combined N and O_2 limitation, which occurred due to the low rotation
211 speed of shake flasks in N-replete 3 wks treatment. However, since the N-replete 3 wks treatment
212 was not analysed with GC-MS, the result is based only for higher transcription level of *phaC* gene
213 and visual inspection of micrographs. In addition, trace amounts of MCL-PHA (3HDD) and co-
214 polymer PHBV were observed. MCL-PHA and co-polymers are more flexible and have more
215 desirable properties for industrial purposes; e.g., thermoplastic moulding compared with SCL-PHAs
216 (12). Based on the MCL-PHAs detected, *Halomonas* sp. 363 apparently has synthase genes from
217 different classes. Previously, *Halomonas* spp. *phaC* genes are regarded as Class I, since they encode
218 only enzymes producing SCL-PHAs and co-polymers (6,7,36,48-50), whereas MCL-PHAs are
219 almost exclusively produced by *Pseudomonas* species or mutant strains (32). Interestingly,
220 *Pseudomonas stutzerii phaC2* has very low substrate specificity and is capable of producing both
221 SCL-PHAs and MCL-PHAs (3,46,47). In all, *Halomonas* sp. 363 appears to be the first wild-type

222 strain, which has been experimentally shown to possess the native capability for producing both
223 SCL- and MCL-PHAs. However, further investigations are needed to directly link the genes to the
224 PHA production observed and to determine the synthase class.
225
226 *Paracoccus* sp. 392 was comprised of the Class I *phaC* gene and produced low amounts of PHBV,
227 both in the N-limited 1 wk and N-replete 1 wk treatments. However, based on *glnA* expression, N
228 limitation was initiated only on Day 5, likely explaining the small difference in PHA yield between
229 the N-limited and N-replete treatments. Although bacteria more commonly produce PHA under
230 nutrient-limiting conditions, these mechanisms vary, and evidence is available that bacteria can also
231 produce PHA when nutrients are not exhausted (20,32,51). Another reason for the low PHBV
232 concentration may be that the strains were cultured on glucose and gluconate and for the valerate
233 production, bacteria also need to use cell-derived substrates, such as amino acids, to produce
234 propionyl-CoA precursor (52). PHBV production in *Paracoccus* spp. has also been observed in
235 previous studies (52,53), although they are better known as a PHB producers (6,37).
236
237
238 **PHA pathways**
239 PHAs are diverse and produced along several different pathways (I–VIII) from various C sources,
240 including carbohydrates, amino acids, fatty acids and CO_2 (54,64,65). There are two main pathways
241 from sugars; Pathway I and III, which begin as acetyl-CoA as a precursor (65). In this study,
242 glucose and gluconate were used as a C sources for the bacteria, which are processed along
243 Pathway I, producing SCL-PHAs and co-polymers, and along the Fatty-Acid Biosynthesis (FAB)
244 Pathway III, producing MCL-PHAs and co-polymers (32,51,65–70). In *Halomonas* sp. 363, both
245 Pathway I and III genes were actively expressed, whereas in *Paracoccus* sp. 392 only Pathway I
246 was expressed. However, the transcription of the Pathway I genes was several times higher than for

247 Pathway III. Accordingly, *Halomonas* sp. 363 accumulated mostly SCL-PHA (PHB), however also
248 showing indications of possible MCL-PHA (3HDD) and co-polymer production (PHBV), whereas
249 *Paracoccus* sp. 392 accumulated only PHBV co-polymer (Table 1). The Class II PHA synthases
250 (Pathway III) are capable of using exclusively CoA-linked 3-hydroxy acids (HAs), and thus a
251 transacylating enzyme is needed to link FAB and PHA synthesis (66–69,71). *PhaG* catalyses the
252 conversion of (R)-3-hydroxyacyl-ACP to (R)-3-hydroxyacyl-CoA, which is further used as a
253 substrate for *phaC* (66–68,71). However, evidence is available that in bacteria lacking the *phaG*
254 gene, *rhlA* (72) and *fabD* as well as *fabH* (73) may substitute to produce substrates for PHA
255 synthase. In *Halomonas* sp. 363, all necessary genes for Pathway III, except *phaG*, were annotated
256 and expressed; however, it also encoded *rhlA*, f*abD* and *fabH* genes.
257
258 In addition to these two pathways, MCL-PHAs are produced from fatty acids along the Fatty-Acid
259 Degradation (FAD) Pathway II (65). Interestingly, *Halomonas* sp. 363 also encoded all the genes
260 necessary for Pathway II. Thus, *Halomonas* sp. 363 uses two fully annotated pathways to produce
261 MCL-PHAs from both sugars and fatty acids. FAD genes have also been annotated from
262 *Halomonas* SF 2003 (36). Since only trace amounts of 3HDD were detected in *Halomonas* sp. 363,
263 it may be a product of Pathway II derived from bacterial debris. Ecologically, the conversion of
264 fatty acids to PHA likely occurs in sea ice, because sea-ice algae provide abundant fatty acids as
265 bacterial C sources (18,74).
266
267 **Conclusions**
268
269 PHA production was observed in two Southern Ocean sea-ice bacteria *Halomonas* sp. 363 and
270 *Paracoccus* sp. 392. Both strains produced PHAs from glucose and gluconate under N-limited and
271 N-replete conditions at 4 °C. Moreover, *Halomonas* sp. 363 also produced PHAs under combined

N- and O_2-limitation. *Halomonas* sp. 363 is a particularly versatile organism with regards to PHA production, harbouring genes for each of the three main pathways, as well as the native capability for producing both SCL and MCL-PHAs. In addition, it has several qualities that are considered industrially valuable by offsetting production costs, including the production of PHAs from inexpensive C sources under low aeration without compromising the cell size, as well as very flexible salinity and temperature tolerances.

Material and Methods

Bacterial strains

Experiments were conducted with two Antarctic sea-ice bacteria, *Paracoccus* sp. 392 (Alphaproteobacteria) and *Halomonas* sp. 363 (Gammaproteobacteria) isolated from Southern Ocean sea ice (isolation described in 75). First, the strains were inoculated from a glycerol stock on modified ZoBell agar (5 g peptone, 1 g yeast extract, 15 g agar, 33 g Instant Ocean® sea salt, 1000 ml milli-Q (MQ) water, autoclaved at 121 °C for 20 min) (76). Single colonies were then inoculated into 50 ml liquid ZoBell medium (5 g peptone, 1 g yeast extract, Instant Ocean ® sea salt, 1000 ml MQ water, autoclaved at 121 °C for 20 min (76) for pre-growth at +4 °C to turbidity optical density (OD) 0.7-1.2 (*Halomonas* sp. 363: 3 days, *Paracoccus* sp. 392: 6 days) in three replicates. The OD could not be measured reliably from *Paracoccus* sp. 392, because the bacterial cultures were too heterogenous and organized in tight aggregates. From each culture, 1 ml of *Halomonas* sp. 363 and 8 ml of *Paracoccus* sp. 392 were inoculated into the N-replete experimental units (two from each; i.e., control and N-limitation treatment) for the Phase I biomass accumulation (Fig. S1).

Experimental set up

PHA-production was examined in 200-ml shaker flask batch cultures in the dark at 4 °C on an orbital shaker set at 120 rpm, with three replicates for each treatment. The bacteria were cultured in two phases (Fig. S1): in Phase I, six replicates from both strains, were inoculated from the pre-growth media to the 200-ml N-replete Mineral Media (MM, modified from (77), DocS1). In Phase I, the bacteria were cultured, achieving an OD of 0.7–1.2 on N-replete MM to accumulate biomass. In Phase II, the cells were pelleted (13 000 g, 3 min, 4 °C) and three were inoculated to N-limited MM (modified from (77), DocS1) to induce PHA production (N-limited 1 wk treatment) and three to N-replete MM as a negative control (N-replete 1 wk treatment). After the cells were collected and transferred to new media (Day 1), their growth was followed for 4 days and sampled daily for 5 days for transcriptomes (2 ml) and Nile-blue microscopy (1 ml in 1.25% of glutaraldehyde). Surprisingly, *Halomonas* sp. 363 produced PHA under N-replete conditions, so an additional experiment (N-replete 3 wks) was conducted to observe the effects of natural nutrient depletion on PHA production. Bacterial strains were prepared and cultured the same way as for the N-replete 1 wk, but the cells were not pelleted or resuspended, and the incubation time was extended to 19 days. Samples were collected once per week for 3 weeks (Days 1, 5, 12, 19).

Microscopy

PHA production was verified microscopically. Samples for Nile-blue staining were stored in electron microscopy-grade glutaraldehyde (final concentration of 1.25%) at 4 °C. The Nile-blue preparates were prepared according to (78). In short, 10 µl from the stock, were pipetted onto microscopic slides, spread out and dried for 15 min in a laminar-flow hood. The slides were flamed and immersed into pre-heated, 0.2-µm-filtered Nile-blue for 10 min (water bath, 55 °C). The slides were rinsed with MQ water and incubated in 8% acetic acid, at room temperature (RT) for 1 min. The samples were analysed with epifluorescence microscopy under green-light excitation (Leica Aristoplan; Leica Biosystems GmbH, Wetzlar, Germany).

Gas chromatography

The PHA content and composition in the PHA biopolymers (PHB, polyhydroxyvalerate (PHV) and polyhydroxyoctanoate (PHO) as standards as well as from the biomasses of *Paracoccus* sp. 392 and *Halomonas* sp. 363 were determined with gas chromatography-mass spectrometry (GC-MS) as described below. The cells were collected (13 000 g, 3 min, 4 °C) from the N-limited 1 wk and N-replete 1 wk treatments, and washed with N-limiting growth medium and freeze-dried for 20 h (1 mbar >, +0.035 mbar final dry for 2 h). In all, 10 mg of lyophilized cells (or 1 mg of isolated PHAs, respectively) were subjected to methanolysis, which was done in a mixture of 2 ml high-performance liquid chromatography (HPLC)-grade chloroform and 2 ml methanol containing 15% (vol/vol) sulphuric acid, as suggested previously(79,80). The samples were diluted 50 times with n-hexane of HPLC grade. The initial structural assignments of the methylesters obtained were based on their retention times compared with those of authentic standards of practical (PA) grade, including methyl (S)-(R)-3-hydroxybutyrate 98% from Alfa Aesar (Thermo Fisher Scientific, Haverhill, MA, USA),(-)-methyl-(R)-3-hydroxyvalerate , 98% from Sigma Aldrich (now Millipore Sigma, Burlington, MA, USA), methyl-3-hydroxyhexanoate of from Sigma Aldrich and methyl-(S)-3-hydroxyoctanoate from Key Organics Ltd, Camelford, Cornwall, UK (ordered through Sigma Aldrich).

For each analysis we applied a hexane blank for monitoring the thermocycle and purities of the column. The authentic structures of the monomers were determined by GC-MS, using a model Agilent Technologies LDA UK Ltd (Stockport, Cheshire SK8 3GR, UK) with a capillary column of type Agilent HP-5MS UI 30 m, 0.25 mm, and the carrier gas: 99.9999% purity helium at a constant flow of 1.2 ml/min. The temperature program was modified with an initial temperature of 40 °C with a hold of 2 minutes, followed by a ramp of 20 °C/min to 140 °C and a second ramp of 40 °C/min to 300 °C, then a hold at 300 °C for 3 minutes, giving a total run time of 14 minutes. For the

detector settings, a transfer line temperature of 250 °C and mass-to-charge ratio (m/z) scanning range of 50–300 were applied.

DNA extraction, library preparation and sequencing

DNA was extracted from 1ml of ZoBell growth media with a DNeasy UltraClean Microbial Kit (QIAGEN, Hilden, Germany) and stored at -80 °C. Whole-genome large-insert (16 kbp (*Paracoccus* sp. 392), 14 kbp (*Halomonas* sp. 363)) PacBio libraries for the RSII instrument were prepared, using a DNA Template Prep Kit 2.0 and DNA/Polymerase Binding Kit P6 according to the manufacturer's protocol. Both samples were sequenced individually in a Single-Molecule, Real-Time (SMRT) cell. Dual-indexed paired-end genomic DNA (gDNA) libraries were prepared according to the Illumina Nextera® DNA Library Prep Guide (Illumina Inc., San Diego, CA, USA), except that half of the tagment DNA Enzyme 1 (TDE1) was used per reaction. An Illumina NextSeq500 instrument was used to sequence the DNA fragments in a paired-end manner (170 + 132bp).

RNA extraction, cDNA translation and library preparation and sequencing

RNA was extracted, using the cetyltrimethylammonium bromide-polyethylene glycol (CTAB-Peg) DNA/RNA extraction protocol (81), after which the RNA was purified with a AllPrep DNA/RNA Kit (QIAGEN, Hilden, Germany). The libraries were prepared according to the manufacturer's instructions with a NEBNext® Ultra™ II RNA Library Prep Kit for Illumina (#E7770, NEW ENGLAND Biolabs Inc.), using NEBNext® Multiplex Oligos for Illumina® 96 Index Primers (#E6609S, NEW ENGLAND Biolabs Inc.) and NEBNext Sample Purification Beads (#E7767S, NEW ENGLAND Biolabs Inc.). Paired-end (75 + 75) sequencing was performed on an Illumina Nextseq500 instrument.

Bioinformatics pipeline

Genomes

The PacBio reads were assembled, using the Hierarchical Genome Assembly Process 3 (HGAP3) implemented in smartportal 2.3.0 (Pacific Biosciences, Menlo Park, CA, USA), using default parameters. The sequences obtained were manually inspected and circularized, using the GAP4 Staden package (82). Chromosomal DNA sequencing was set to start from the *dnaA* gene. The Illumina short reads were first quality checked with FastQC (83) and then filtered, using cutadapt (v. 1.14 (84) using the following three criteria: 1) adapter sequence removal, 2) low-quality base from the 3' end of the read (-q 25) removal and 3) minimum read length (-m 50) set to 50 bp. The filtered Illumina short reads were mapped against the circularized sequences with bwa mem (v. 0.7.17) (85), sorted and indexed with SAMtools (v. 1.7) (86). Reads that did not map to the reference sequences given were selected and assembled separately with spades (v. 3.11.1)(87), using the -careful option. Sequences from the spades assembly were circularized in GAP4. Finally, all sequences were polished using pilon (v. 1.16) (88).

The sequences were annotated against the Kyoto Encyclopedia of Genes and Genomes (KEGG) (86, April 2018) (89) with KEGG-tools2.0 (90), PROKKA (1.13) (39) and RAST with default parameters (2.0) (38).

Transcriptomes

The quality of the raw reads was analysed with FastQC (83). The primers were removed with Cutadapt (v. 1.10 with Python 2.7.3)(84,91), using a quality score of 20 and minimum length 30. The complementary DNA (cDNA) was annotated against PROKKA (1.13)(39), and the trimmed reads were mapped against the PROKKA-annotated genes (ffn-file) with Bowtie2 (v.1.2.2)(92), and sorted and indexed with SAMtools (v. 1.4)(86).

Statistics

Differences between the treatments for selected genes were tested with one-way analysis of variance (ANOVA, function 'aov', R-core package with R4.0.2 (93). Variance of homogeneity ($p > 0.05$) was tested with Levene's test (package 'car', in R4.0.2) and normality ($p > 0.05$) with Shapiro-Wilk normality test (93). Tests were done only for *Halomonas* sp. 363, since one of the *Paracoocus* sp. 392 replicates didn't grow thus making the statistical tests unreliable.

For further analyses, the rRNA-associated transcripts were removed and abundances were normalized against the single-copy gene *rpoB* and the relative abundances were calculated. Data cleaning was done, using the package tidyverse (1.3.0) (94). The graphics were done with R4.0.2 (93), using ggplot2 (3.3.2) (95) and pheatmap (1.0.12) (96).

Data and code availability

The data reported in this article is available in Supplementary tables 1-2. The raw RNA-seq fastq sequence data files are deposited in the European Nucleotide Archive (ENA) study PRJEB41946 under accessions: ERS5465044 (SAMEA7708542) - ERS5465113 (SAMEA7708611) and closed genomes of *Halomonas* sp. 363 and *Paracoccus* sp. 392 under ERS5472646 (SAMEA7725270) and ERS5472645 (SAMEA7725269), respectively.

All scripts for to processing RNA-seq data are available in Supplementary Material: PHA_experiment_bioinformatics.html and R-scripts at https://github.com/elxerone/PHA_experiments.

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References

1. Koller M, Maršálek L, de Sousa Dias MM, Braunegg G. 2017. Producing microbial polyhydroxyalkanoate (PHA) biopolyesters in a sustainable manner. N Biotechnol 37: 24–38.
2. Shrivastav A, Mishra SK, Shethia B, Pancha I, Jain D, Mishra S. 2010. Isolation of promising bacterial strains from soil and marine environment for polyhydroxyalkanoates (PHAs) production utilizing Jatropha biodiesel byproduct. Int J Biol Macromol 47: 283–287.

3. Chen GQ, Hajnal I, Wu H, Lv L, Ye J. 2015. Engineering biosynthesis mechanisms for diversifying polyhydroxyalkanoates. Trends Biotechnol 33: 565–574.
4. Takahashi RYU, Castilho NAS, Silva MACD., Miotto MC, Lima AODS. 2017. Prospecting for marine bacteria for polyhydroxyalkanoate production on low-cost substrates. Bioeng 4: 60.
5. Kucera D, Pernicová I, Kovalcik A, Koller M, Mullerova L, Sedlacek P, Mravec F, Nebesarova J, Kalina M, Marova I, Krzyzanek V, Obruca S. 2018. Characterization of the promising poly (3-hydroxybutyrate) producing halophilic bacterium *Halomonas halophila*. Bioresour Technol 256: 552–556.
6. Mitra R, Xu T, Xiang H, Han J. 2020. Current developments on polyhydroxyalkanoates synthesis by using halophiles as a promising cell factory. Microb Cell Fact 19: 1–30.
7. Thomas T, Sudesh K, Bazire A, Elain A, Tan HT, Lim H, Bruzaud S, PHA Production and PHA Synthases of the Halophilic Bacterium *Halomonas* sp. SF2003. Bioeng 7: 29.
8. Lebreton LC, Van Der Zwet J, Damsteeg JW, Slat B, Andrady A, Reisser J. 2017. River plastic emissions to the world's oceans. Nat Commun 8: 15611.
9. Jendrossek D, Handrick R. 2002. Microbial degradation of polyhydroxyalkanoates. Annu Rev Microbiol 56: 403–432.
10. Pötter M, Steinbüchel A. 2005. Poly (3-hydroxybutyrate) granule-associated proteins: impacts on poly (3-hydroxybutyrate) synthesis and degradation. Biomacromolecules 6: 552–560.
11. Verlinden RA, Hill DJ, Kenward MA, Williams CD, Radecka I. 2007. Bacterial synthesis of biodegradable polyhydroxyalkanoates. J Appl Microbiol 102: 1437–1449.

12. Poirier Y, Brumbley SM. 2010. Metabolic engineering of plants for the synthesis of polyhydroxyalkanaotes. In Plastics from bacteria (pp. 187–211). Springer, Berlin, Heidelberg.
13. Tan D, Xue YS, Aibaidula G, Chen GQ. 2011. Unsterile and continuous production of polyhydroxybutyrate by *Halomonas* TD01. Bioresour Technol 102: 8130–8136.
14. Chen GQ, Jiang XR. 2018. Next generation industrial biotechnology based on extremophilic bacteria. Curr Opin Biotechnol 50: 94-100.
15. Kumar V, Kumar S, Singh D. 2020. Microbial polyhydroxyalkanoates from extreme niches: Bioprospection status, opportunities and challenges. Int J Biol Macromol 147: 1255–1267.
16. Kaartokallio H, Søgaard DH, Norman L, Rysgaard S, Tison JL, Delille B, Thomas DN. 2013. Short-term variability in bacterial abundance, cell properties, and incorporation of leucine and thymidine in subarctic sea ice. Aquat Microb Ecol 71: 57–73.
17. Pärnänen K, Karkman A, Virta M, Eronen-Rasimus E, Kaartokallio H. 2015. Discovery of bacterial polyhydroxyalkanoate synthase (*PhaC*)-encoding genes from seasonal Baltic Sea ice and cold estuarine waters. Extremophiles 19197_206.
18. Thomas DN, Dieckmann GS. 2002. Antarctic sea ice–a habitat for extremophiles. Science 295: 641–644.
19. Dawes EA, Senior PJ, The Role and Regulation of Energy Reserve Polymers in Micro-organisms. Adv Microb Physiol 10: 135–266.
20. Sudesh K, Abe H, Doi Y. 2000. Synthesis, structure and properties of polyhydroxyalkanoates: biological polyesters. Prog Polym Sci 25: 1503–1555.
21. López NI, Pettinari MJ, Nikel PI, Méndez BS. 2015. Polyhydroxyalkanoates: much more than biodegradable plastics. Adv Appl Microbiol 93: 73–106.
22. Soto G, Setten L, Lisi C, Maurelis C, Mozzicafreddo M, Cuccioloni M, Angeletti M,

492 Ayub ND. 2012. Hydroxybutyrate prevents protein aggregation in the halotolerant
493 bacterium *Pseudomonas* sp. CT13 under abiotic stress. Extremophiles 16: 455–462.
494 23. Obruca S, Sedlacek P, Krzyzanek V, Mravec F, Hrubanova K, Samek O, Kucera D,
495 Benesova P, Marova I. 2016. Accumulation of poly (3-hydroxybutyrate) helps bacterial
496 cells to survive freezing. PloS one 11: e0157778.
497 24. Obruca S, Sedlacek P, Koller M, Kucera D, Pernicova I. 2018. Involvement of
498 polyhydroxyalkanoates in stress resistance of microbial cells: Biotechnological
499 consequences and applications. Biotechnol Adv 36: 856–870.
500 25. Slaninova E, Sedlacek P, Mravec F, Mullerova L, Samek O, Koller M, Hesko O. 2018.
501 D. Kucera, I. Marova, S. Obruca, Light scattering on PHA granules protects bacterial cells
502 against the harmful effects of UV radiation. Appl Microbiol Biotechnol 102: 1923–1931.
503 26. Tribelli P, López N 2018. Reporting Key Features in Cold-Adapted Bacteria. Life 8: 8–12.
504 27. Cabiscol Català E, Tamarit Sumalla J, Ros Salvador J. 2000. Oxidative stress in bacteria and
505 protein damage by reactive oxygen species. Int J Microbiol 3: 3–8.
506 28. Senior PJ, Dawes EA. 1971. Poly-p-hydroxybutyrate biosynthesis and the regulation of
507 glucose metabolism in *Azotobacter beijernickii*. Biochem J 1–12.
508 29. Anderson AJ, Dawes EA. 1990. Occurrence, metabolism, metabolic role, and industrial uses
509 of bacterial polyhydroxyalkanoates. Microbiol Mol Biol Rev 54: 450–472.
510 30. Ling C, Qiao GQ, Shuai BW, Olavarria K, Yin J, Xiang RJ, Song KN, Shen YH, Guo Y,
511 Chen GQ. 2018. Engineering NADH/NAD+ ratio in *Halomonas bluephagenesis* for
512 enhanced production of polyhydroxyalkanoates (PHA). Metab Eng 49: 275–286.
513 31. Ayub ND, Tribelli PM, López NI. 2009. Polyhydroxyalkanoates are essential for
514 maintenance of redox state in the Antarctic bacterium *Pseudomonas* sp. 14–3 during low
515 temperature adaptation. Extremophiles 13: 59–66.

516 32. Prieto A, Escapa IF, Martínez V, Dinjaski N, Herencias C, de la Peña F, Tarazona N,
517 Revelles O. 2016. A holistic view of polyhydroxyalkanoate metabolism in *Pseudomonas*
518 *putida*. Environ Microbiol 18: 341–357.
519 33. Meiners KM, Michel C. 2017. Dynamics of nutrients, dissolved organic matter and
520 exopolymers in sea ice. Sea Ice *3*: 415–432.
521 34. Cai L, Tan D, Aibaidula G, Dong XR, Chen JC, Tian WD, Chen GQ. 2011.
522 Comparative genomics study of polyhydroxyalkanoates (PHA) and ectoine relevant genes
523 from *Halomonas* sp. TD01 revealed extensive horizontal gene transfer events and co-
524 evolutionary relationships. Microb Cell Fact 10: 1–15.
525 35. Kutralam-Muniasamy G, Corona-Hernandez J, Narayanasamy RK, Marsch R, Pérez-
526 Guevara F. 2017. Phylogenetic diversification and developmental implications of poly-(R)-
527 3-hydroxyalkanoate gene cluster assembly in prokaryotes. FEMS Microbiol. Lett., 364,
528 fnx135.
529 36. Thomas T, Elain A, Bazire A, Bruzaud S. 2019. Complete genome sequence of the
530 halophilic PHA-producing bacterium *Halomonas* sp. SF2003: insights into its
531 biotechnological potential. World J Microb Biot 35: 1–14.
532 37. Olaya-Abril A, Luque-Almagro VM, Manso I, Gates AJ, Moreno-Vivián C, Richardson DJ,
533 Roldán MD. 2018. Poly (3-hydroxybutyrate) hyperproduction by a global nitrogen regulator
534 *NtrB* mutant strain of *Paracoccus denitrificans* PD1222. FEMS Microbiol. Lett 365:
535 fnx251.
536 38. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S,
537 Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil
538 LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O,
539 Vonstein V, Wilke A, Zagnitko O. 2008. The RAST Server: rapid annotations using
540 subsystems technology. BMC genomics: 9: 1–15.

541 39. Seemann T. 2014. Prokka: Rapid Prokaryotic Genome Annotation, Bioinformatics 15:
542 2068–9.
543 40. Rehm BH. 2003. Polyester synthases: natural catalysts for plastics. Biochem J 376: 15–33.
544 41. Williamson A, De Santi C, Altermark B, Karlsen C, Hjerde E. 2016. Complete genome
545 sequence of *Halomonas* sp. R5-57. Stand Genom Sci 11: 1–9.
546 42. Feng B, Cai S, Han J, Liu H, Zhou J, Xiang H. 2010. Identification of the *phaB* genes and
547 analysis of the PHBV precursor supplying pathway in *Haloferax mediterranei*. Wei sheng
548 wu xue bao= Acta Microbiol Sin 50: 1305–1312.
549 43. Slonczewski L, Foster W, Gillen M. 2009. Chapter 5: Environmental Influence and Control
550 of Microbial Growth. In Microbiology An Evolving Science (WW Norton Company. Inc.,
551 New York) pp. 149–178.
552 44. Tolosa L, Kostov Y, Harms P, Rao G. 2002. Noninvasive measurement of dissolved oxygen
553 in shake flasks. Biotechnol Bioeng 80: 594–597.
554 45. Rehm BH, Steinbüchel A. 1999. Biochemical and genetic analysis of PHA synthases and
555 other proteins required for PHA synthesis. Int J Biol Macromol 25: 3–19.
556 46. Chen JY, Liu T, Zheng Z, Chen JC, Chen GQ. 2004. Polyhydroxyalkanoate synthases
557 *PhaC1* and *PhaC2* from *Pseudomonas stutzeri* 1317 had different substrate specificities.
558 FEMS Microbiol Lett 234: 231–237.
559 47. Chen JY, Song G, Chen GQ. 2006. A lower specificity PhaC2 synthase from *Pseudomonas*
560 *stutzeri* catalyses the production of copolyesters consisting of short-chain-length and
561 medium-chain-length 3-hydroxyalkanoates. Antonie Van Leeuwenhoek, 89: 157–167.
562 48. Quillaguamán J, Doan-Van T, Guzmán H, Guzmán D, Martín J, Everest A, Hatti-Kaul R.
563 2008. Poly (3-hydroxybutyrate) production by *Halomonas boliviensis* in fed-batch culture.
564 Appl Microbiol Biotechnol 78: 227–232.

565 49. Chen Y, Chen XY, Du HT, Zhang X, Ma YM, Chen JC, Ye JW, Jiang XR, Chen GQ. 2019.
566 Chromosome engineering of the TCA cycle in *Halomonas bluephagenesis* for production of
567 copolymers of 3-hydroxybutyrate and 3-hydroxyvalerate (PHBV). Metab Eng 54: 69–82.
568 50. Ye J, Hu D, Yin J, Huang W, Xiang R, Zhang L, Wang X, Han J, Chen GQ. 2020.
569 Stimulus response-based fine-tuning of polyhydroxyalkanoate pathway in
570 *Halomonas*. Metab Eng 57: 85–95.
571 51. Kato M, Bao HJ, Kang CK, Fukui T, Doi Y. 1996. Production of a novel copolyester of 3-
572 hydroxybutyric acid and medium-chain-length 3-hydroxyalkanoic acids by Pseudomonas sp.
573 61-3 from sugars. Appl Microbiol Biotechnol 45: 363–370.
574 52. Madison LL, Huisman GW. 1999. Metabolic engineering of poly (3-hydroxyalkanoates):
575 from DNA to plastic. Microbiol Mol Biol 63: 21–53.
576 53. Yamane T, Chen XF, Ueda S. 1996. Polyhydroxyalkanoate synthesis from alcohols during
577 the growth of *Paracoccus denitrificans*. FEMS Microbiol Lett 135: 207–211.
578 54. Chanprateep S, Abe N, Shimizu H, Yamane T, Shioya S. 2001. Multivariable control of
579 alcohol concentrations in the production of polyhydroxyalkanoates (PHAs) by *Paracoccus*
580 *denitrificans*. Biotechnol. Bioeng., 74: 116–124.
581 55. Bowman JP. 2013. "Sea-Ice Microbial Communities" in *The Prokaryotes* E. Rosenberg, E.
582 F. DeLong, S. Lory, E. Stackebrandt, F. Thompson Eds. (Springer, Berlin, Heidelberg)
583 https://doi.org/10.1007/978-3-642-30123-0_46
584 56. Wessel AK, Arshad TA, Fitzpatrick M, Connell JL, Bonnecaze RT, Shear JB, Whiteley M.
585 2014. Oxygen limitation within a bacterial aggregate. *MBio*, **5**..
586 57. Kaartokallio H. 2001. Evidence for active microbial nitrogen transformations in sea ice
587 (Gulf of Bothnia, Baltic Sea) in midwinter. Polar Biol 24: 21–28.
588 58. Rysgaard S, Glud RN. 2004. Anaerobic N_2 production in Arctic sea ice. Limnol Oceanogr
589 49: 86–94.

590 59. Rysgaard S, Glud RN, Sejr MK, Blicher ME, Stahl HJ. 2008. Denitrification activity and
591 oxygen dynamics in Arctic sea ice. Polar Biol 31: 527–537.
592 60. Eronen-Rasimus E, Luhtanen AM, Rintala JM, Delille B, Dieckmann G, Karkman A, Tison
593 JL. 2017. An active bacterial community linked to high *chl-a* concentrations in Antarctic
594 winter-pack ice and evidence for the development of an anaerobic sea-ice bacterial
595 community. ISME J 11: 2345–2355.
596 61. Müller S, Vähätalo AV, Stedmon CA, Granskog MA, Norman L, Aslam SN, Underwood
597 GJC, Dieckmann GS, Thomas DN. 2013. Selective incorporation of dissolved organic
598 matter (DOM) during sea ice formation. Mar Chem 155: 148–157.
599 62. Meiners KM, Vancoppenolle M, Thanassekos S, Dieckmann GS, Thomas DN, Tison JL,
600 Arrigo KR, Garrison DL, McMinn A, Lannuzel D, van der Merwe P, Swadling KM,
601 Smith Jr. WO, Melnikov I, Raymond B. 2012. Chlorophyll *a* in Antarctic sea ice from
602 historical ice core data. Geophys Res Lett 39.
603 63. Leu E, Mundy CJ, Assmy P, Campbell K, Gabrielsen TM, Gosselin M, Juul-Pedersen T,
604 Gradinger R. 2015. Arctic spring awakening–Steering principles behind the phenology of
605 vernal ice algal blooms. Prog Oceanogr 139: 151–170.
606 64. Steinbüchel A, Lütke-Eversloh T. 2003. Metabolic engineering and pathway construction
607 for biotechnological production of relevant polyhydroxyalkanoates in microorganisms.
608 Biochem Eng J 16: 81–96.
609 65. Chen GQ. 2010. Plastics completely synthesized by bacteria: polyhydroxyalkanoates. In
610 Plastics from bacteria (pp. 17–37). Springer, Berlin, Heidelberg.
611 66. Rehm BHA, Krüger N, Steinbüchel A. 1998. A new metabolic link between fatty acid de
612 novo synthesis and polyhydroxyalkanoic acid synthesis. J Biol Chem 273: 24044–24051.
613 67. Rehm BH, Mitsky TA, Steinbüchel A. 2001. Role of fatty acid de novo biosynthesis in
614 polyhydroxyalkanoic acid (PHA) and rhamnolipid synthesis by pseudomonads:

615 establishment of the transacylase (*PhaG*)-mediated pathway for pha biosynthesis in
616 *Escherichia coli*. Appl Environ Microbiol 67: 3102–3109.
617 68. Fiedler S, Steinbüchel A, Rehm BH. 2000. *PhaG*-mediated synthesis of poly (3-
618 hydroxyalkanoates) consisting of medium-chain-length constituents from nonrelated carbon
619 sources in recombinant *Pseudomonas fragi*. Appl Environ Microbiol 66: 2117–2124.
620 69. Borrero-de Acuña JM, Bielecka A, Häussler S, Schobert M, Jahn M, Wittmann C, Jahn D,
621 Poblete-Castro I. 2014. Production of medium chain length polyhydroxyalkanoate in
622 metabolic flux optimized *Pseudomonas putida*. Microb Cell Fact 13: 88–15.
623 70. Mozejko-Ciesielska J, Pokoj T, Ciesielski S. 2018. Transcriptome remodeling of
624 *Pseudomonas putida* KT2440 during mcl-PHAs synthesis: effect of different carbon sources
625 and response to nitrogen stress. J Ind Microbiol Biotechnol 45: 433–446.
626 71. Hoffmann N, Steinbüchel A, Rehm BH. 2000. The *Pseudomonas aeruginosa phaG* gene
627 product is involved in the synthesis of polyhydroxyalkanoic acid consisting of medium-
628 chain-length constituents from non-related carbon sources. FEMS Microbiol Lett 184: 253–
629 259.
630 72. Gutiérrez-Gómez U, Servín-González L, Soberón-Chávez G. 2019. Role of β-oxidation and
631 de novo fatty acid synthesis in the production of rhamnolipids and polyhydroxyalkanoates
632 by *Pseudomonas aeruginosa*. Appl Microbiol Biotechnol 103: 3753–3760.
633 73. Taguchi K, Aoyagi Y, Matsusaki H, Fukui T, Doi Y. 1999. Over-expression of 3-ketoacyl-
634 ACP synthase III or malonyl-CoA-ACP transacylase gene induces monomer supply for
635 polyhydroxybutyrate production in *Escherichia coli* HB101. Biotechnol Lett 2: 579–584.
636 74. Leu E, Wiktor J, Søreide JE, Berge J, Falk-Petersen S. 2010. Increased irradiance reduces
637 food quality of sea ice algae. Mar Ecol Prog Ser 411: 49–60.

638 75. Luhtanen AM, , Eronen-Rasimus E, Oksanen HM, Tison JL, Delille B, Dieckmann GS,
639 Rintala JM, Bamford DH. 2018. The first known virus isolates from Antarctic sea ice have
640 complex infection patterns. FEMS Microbiol. Ecol., 94, fiy028.
641 76. ZoBell CE. 1946. Marine microbiology. A monograph on hydrobacteriology. *Chronica*
642 *Botanica Company*, Waltham.
643 77. Schlegel HG, Kaltwasser H, Gottschalk G. 1961. Ein Submersverfahren zur Kultur
644 wasserstoffoxydierender Bakterien: Wachstumsphysiologische Untersuchungen. Archiv fur
645 Mikrobiologie 38: 209–222.
646 78. Ostle A, Holt JG. 1982. Nile Blue A as a Fluorescent Stain for Poly-3-Hydroxybutyrate.
647 Appl Environ Microbiol 44: 238–241.
648 79. Steinbüchel A, Wiese S. 1992. A *Pseudomonas* strain accumulating polyesters of 3-
649 hydroxybutyric acid and medium-chain-length 3-hydroxyalkanoic acids. Appl Microbiol
650 Biotechnol 37: 691-697.
651 80. Hai T, Lange D, Rabus R, Steinbüchel A. 2004. Polyhydroxyalkanoate (PHA)
652 Accumulation in Sulfate-Reducing Bacteria and Identification of a Class III PHA Synthase
653 (*PhaEC*) in *Desulfococcus multivorans.* Appl Environ Microbiol 70: 4440–4448.
654 81. Viitamäki S. 2019. The activity and functions of soil microbial communities across a
655 climate gradient in Finnish subarctic. University of Helsinki, Microbiology, Pro-gradu
656 thesis.
657 82. Staden R, Judge DP, Bonfield JK.2003. Managing Sequencing Projects in the GAP4
658 Environment. Introduction to Bioinformatics. A Theoretical and Practical Approach. Eds.
659 Stephen A. Krawetz and David D. Womble. Human Press Inc., Totawa, NJ 07512.
660 83. Andrew S. 2010. FastQC: a quality control tool for high throughput sequence data.
661 https://github.com/s-andrews/FastQC.

84. Martin M. 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet J 17: 10–12.

85. Li H, Durbin R. 2009. Fast and accurate short read alignment with Burrows-Wheeler Transform. Bioinformatics 25: 1754–60.

86. Li H, Handsaker B, Wysoker A, Fennell T, Ruan J, Homer N, Marth G, Abecasis G, Durbin R. 2009. The sequence alignment/map format and SAMtools. Bioinformatics 25: 2078–2079.

87. Nurk S, Bankevich A, Antipov D, Gurevich A, Korobeynikov A, Lapidus A, Prjibelsky A, Pyshkin A, Sirotkin A, Sirotkin Y, Stepanauskas R, McLean J, Lasken R, Clingenpeel SR, Woyke T, Tesler G, Alekseyev MA, Pevzner PA. 2013. Assembling Genomes and Mini-metagenomes from Highly Chimeric Reads *In* Research in Computational Molecular Biology Deng M., Jiang R., Sun F., Zhang X. (eds) RECOMB 2013. (Springer, Berlin, Heidelberg 2013) vol 7821.

88. Walker BJ, Abeel T , Shea T, Priest M, Abouelliel A, Sakthikumar S, Cuomo CA, Zeng Q, Wortman J, Young SK, Earl AM. 2014. Pilon: An Integrated Tool for Comprehensive Microbial Variant Detection and Genome Assembly Improvement. PLoS ONE, 9: e112963.

89. Kanehisa M, Goto S. 2000 KEGG: Kyoto Encyclopedia of Genes and Genomes. Nucleic Acids Res 28: 27–30.

90. Pessi IS. 2019. KEGG-tools v2.0: A tool to parse the results of BLAST/DIAMOND similarity searches made against the KEGG GENES prokaryotes database. GitHub Repository.

91. Van Rossum G, Drake Jr FL. 1995. Python reference manual. Centrum voor Wiskunde en Informatica Amsterdam.

92. Langmead B, Salzberg S. 2012. Fast gapped-read alignment with Bowtie 2. Nat Methods 9: 357–359.

687 93. R Core Team. 2020. R: A language and environment for statistical computing. R Foundation
688 for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.
689 94. Wickham H, Averick M, Bryan J, Chang W, D'Agostino McGowan L, François R,
690 Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen TL, Miller E, Bache SM,
691 Müller K, Ooms J, Robinson D, Seidel DP, Spinu V, Takahashi K, Vaughan D, Wilke C,
692 Woo K, Yutani H. 2019. Welcome to the Tidyverse. Journal of Open Source Software 4:
693 1686.
694 95. Wickham H. 2016. ggplot2: Elegant Graphics for Data Analysis. Springer-Verlag New
695 York. ISBN 978-3-319-24277-4, https://ggplot2.tidyverse.org.
696 96. Kolde R, Kolde MR. 2015. Package 'pheatmap'. R Package, 1: 790.
697 97. Davis JJ, Wattam AR, Aziz RK, Brettin T, Butler R, Butler RM, Chlenski P, Conrad N,
698 Dickerman A, Dietrich EM, Gabbard JL, Gerdes S, Guard A, Kenyon RW, Machi D, Mao
699 C, Murphy-Olson D, Nguyen M, Nordberg EK, Olsen GJ, Olson RD, Overbeek JC,
700 Overbeek R, Parrello B, Pusch GD, Shukla M, Thomas C, VanOeffelen M, Vonstein V,
701 Warren AS, Xia F, Xie D, Yoo H, Stevens R. 2020. The PATRIC Bioinformatics Resource
702 Center: expanding data and analysis capabilities. Nucleic Acids Res. Jan 8: 48(D1):D606-
703 D612. PMID: 31667520. PMCID: PMC7145515.
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Table 1. Extracted PHA biopolyesters in *Halomonas* sp. 363 and *Paracoccus* sp. 392 cultured in glucose and gluconate under N-limited 1 wk and N-replete 1 wk conditions. PHA = poly-3-hydroxyalkanoic acid, 3-HB = 3-hydroxybutyrate, 3-HV = 3-hydroxyvalerate, 3-HDD = 3-hydroxydodecanoate, ND = not detected

		% of dry matter		
Strains	**Treatment**	**% 3-HB**	**% 3-HV**	**%3-HDD**
Halomonas sp. 363	N-limiting 1 wk	45.00	traces	traces
	N-replete 1 wk	17.19	ND	ND
Paracoccus sp. 392	N-limiting 1 wk	8.71	4.51	ND
	N-replete 1 wk	8.52	4.17	ND

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Fig. 1. Annotated poly-3-hydroxyalkanoic acid (PHA) metabolic genes in the sea-ice bacteria *Halomonas* sp. 363 and *Paracoccus* sp. 392. The genome annotations against KEGG (86, April 2018) (89), PROKKA (1.13) (39) and RAST (2.0) (38) are listed in Table S1-2.

Fig. 2. Actively transcribed genes putatively associated with poly-3-hydroxyalkanoic acid (PHA) synthesis in the sea-ice bacterial strains *Halomonas* sp. 363 and *Paracoccus* sp. 392. *Halomonas* sp. 363 has putatively three different pathways (Pathway I–III) to produce both short-chain-length (SCL) and medium-chain-length (MCL) PHAs, whereas *Paracoccus* sp. 392 only SCL-PHA via Pathway I. FAB= fatty-acid biosynthesis, FAD=fatty-acid degradation. The genes and their annotations are listed in Table S5. *PhaC1-3* genes are not specified for the Figure, since we have not proven the Pathways with knock-out mutant strains.

Fig. 3 A**ctively transcribed genes from sea-ice bacterial strains *Halomonas* sp. 363 and *Paracoccus* sp. 392**. **associated with** (A) Nitrogen cycle in *Halomonas* sp. 363, (B) Poly-3-hydroxyalkanoic acid (PHA) production in *Halomonas* sp. 363, (C) Oxygen limitation in *Halomonas* sp. 363 and (D) PHA production and nitrogen limitation in *Paracoccus* sp. 392. The sequences were normalized against *rpoB*, after which the rRNA-associated genes were removed and the relative percentage counted. Note different scales! The complete transcriptome annotations against KEGG (86, April 2018) (89), PROKKA (1.13) (39) and RAST (2.0) (38) are listed in Table S3-4.

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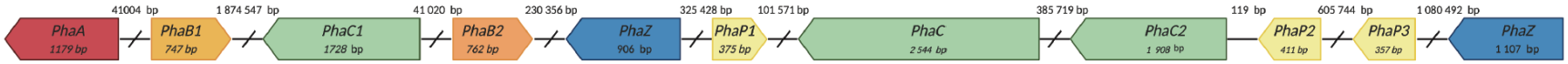
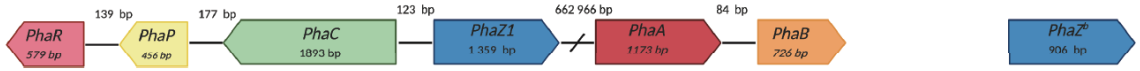
Halomonas sp. 363



Paracoccus sp. 392



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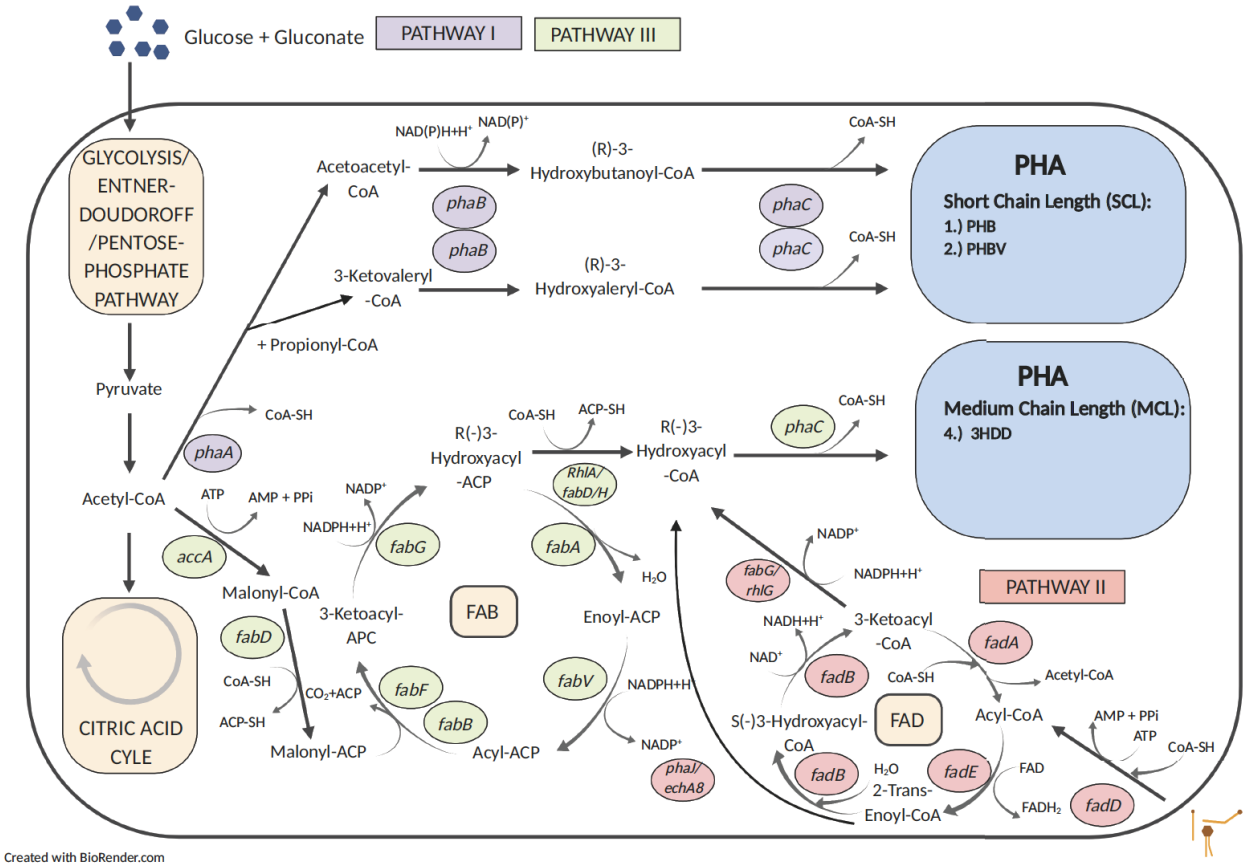
Glucose + Gluconate
PATHWAY I
PATHWAY III
GLYCOLYSIS/ ENTNER-DOUDOROFF /PENTOSE-PHOSPHATE PATHWAY
Pyruvate
Acetyl-CoA
CITRIC ACID CYLE
Acetoacetyl-CoA
3-Ketovaleryl-CoA
+ Propionyl-CoA
(R)-3-Hydroxybutanoyl-CoA
(R)-3-Hydroxyaleryl-CoA
PHA
Short Chain Length (SCL):
1.) PHB
2.) PHBV
PHA
Medium Chain Length (MCL):
4.) 3HDD
R(-)3-Hydroxyacyl-ACP
R(-)3-Hydroxyacyl-CoA
Malonyl-CoA
3-Ketoacyl-APC
Malonyl-ACP
Acyl-ACP
Enoyl-ACP
FAB
PATHWAY II
3-Ketoacyl-CoA
S(-)3-Hydroxyacyl-CoA
2-Trans-Enoyl-CoA
Acyl-CoA
Acetyl-CoA
FAD
phaA
phaB
phaB
phaC
phaC
phaC
accA
fabG
fabA
RhlA/ fabD/H
fabD
fabF
fabB
fabV
phaJ/ echA8
fabG/ rhlG
fadA
fadB
fadB
fadE
fadD
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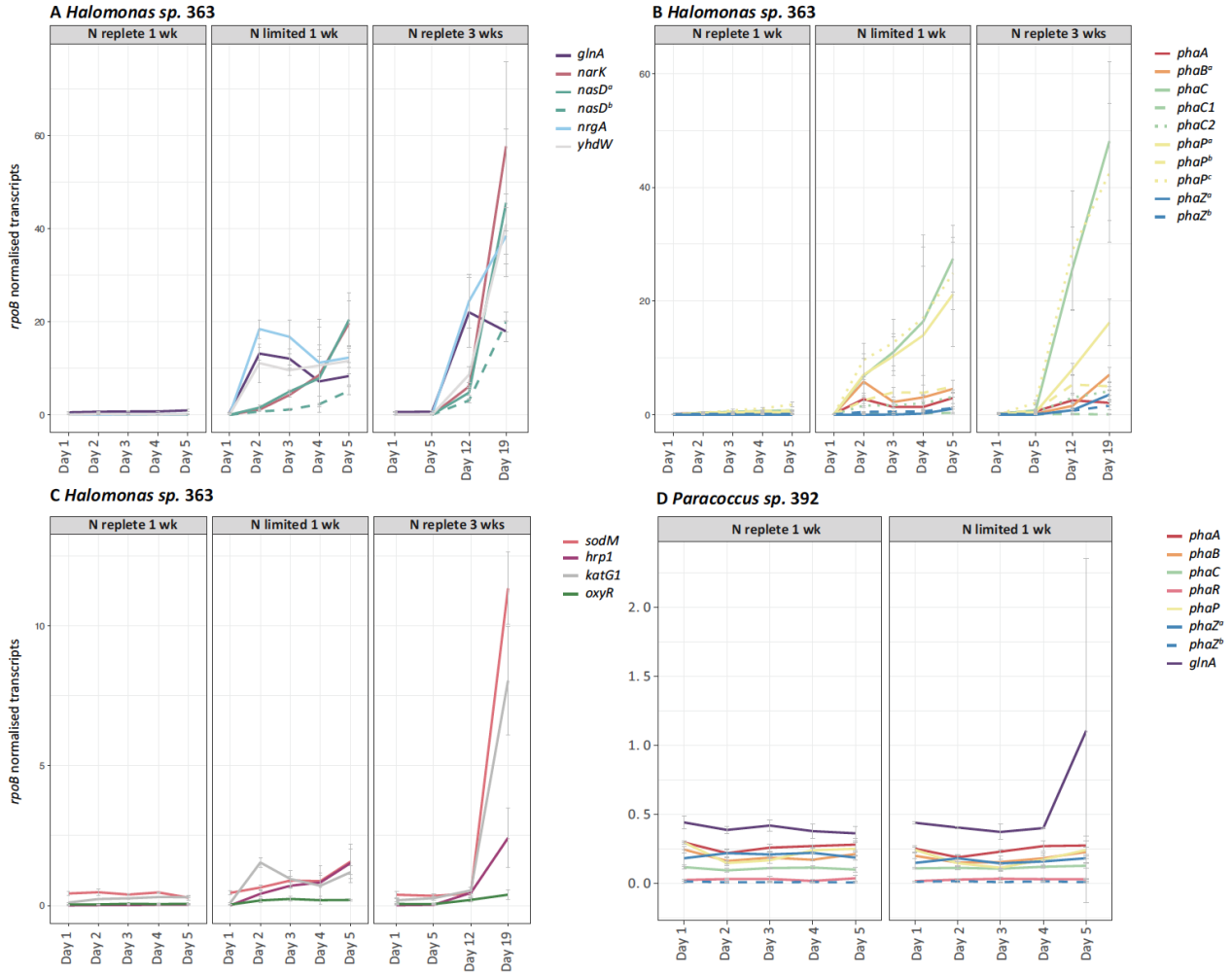
A Halomonas sp. 363
N replete 1 wk
N limited 1 wk
N replete 3 wks
rpoB normalised transcripts
glnA
narK
nasD^a
nasD^b
nrgA
yhdW
B Halomonas sp. 363
phaA
phaB^a
phaC
phaC1
phaC2
phaP^a
phaP^b
phaP^c
phaZ^a
phaZ^b
C Halomonas sp. 363
sodM
hrp1
katG1
oxyR
D Paracoccus sp. 392
phaA
phaB
phaC
phaR
phaP
phaZ^a
phaZ^b
glnA
Day 1
Day 2
Day 3
Day 4
Day 5
Day 12
Day 19

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