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## **Characterization of the SUF FeS cluster synthesis machinery in the amitochondriate eukaryote *Monocercomonoides exilis***

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HAL Authorization

1 **Comprehensive analysis of the microbial consortium in**  
2 **the culture of flagellate *Monocercomonoides exilis***

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17

## 18 **Abstract**

19 *Monocercomonoides exilis* is the only known amitochondriate eukaryote, making it an  
20 excellent model for studying the implications of mitochondrial reduction from a cellular and  
21 evolutionary point of view. Although *M. exilis* is an endobiotic heterotroph, it can grow *in*  
22 *vitro*, albeit with an uncharacterized and complex prokaryotic community. All attempts to  
23 grow *M. exilis* axenically have been unsuccessful. Here, we use metagenomic sequencing at  
24 different time points during culture growth to describe the composition and dynamics of this  
25 community. We assembled genomes of 24 from at least the 30 different bacterial species  
26 within. Based on DNA read abundances, *M. exilis* represents less than 1.5%, and the  
27 representation of dominant bacterial members changes over time. Genome-scale metabolic  
28 reconstruction, differential expression analysis and measurements of metabolites in the media  
29 showed that the community depends on organic carbon oxidation, fermentation, and  
30 hydrogen production without methanogenesis. This is consistent with the rapid decline of  
31 amino acids, nucleotides, glyceraldehyde, lactate, fatty acids, and alcohols in the media. The  
32 community depends on recycling the external supply of amino acids since it has a limited  
33 capacity to fix nitrogen gas and lacks ammonia oxidizers to close the nitrogen cycle. With the  
34 senescence of the culture, we observe changes in the expression of several metabolic  
35 pathways in *M. exilis*, particularly those adapting to starvation. We do not reveal any clear  
36 metabolic link to explain the dependence of *M. exilis* on prokaryotes.

## 37 **Introduction**

38           The gut microbiota refers to the community of microorganisms inhabiting the  
39 gastrointestinal system. Several studies have proved the influence of this community on  
40 human health (Fan and Pedersen, 2021; Jandhyala et al., 2015; Thursby and Juge, 2017), but  
41 these have mainly focused on the prokaryotic component since this represents the majority of  
42 cells and biomass. Yet, the animal gut microbiota also comprises viruses and eukaryotes,  
43 including unicellular protists. The effect of protists on the function and processes in the  
44 intestine, as well as their metabolic interactions with prokaryotes, are essentially unknown,  
45 except for some medically and veterinary important parasites, e.g., *Entamoeba histolytica*,  
46 *Giardia intestinalis*, *Cryptosporidium* spp., *Spironucleus salmonicida*, *Blastocystis* spp.  
47 (Partida-Rodriguez et al., 2021; von Huth et al., 2021). However, these represent only a  
48 fraction of the intestinal protist diversity and their presence often leads to pathological states  
49 that significantly depart from the conditions found in the healthy gut.

50           Many intestinal protists can be successfully maintained in xenic cultures using a  
51 medium such as TYSGM-9 (Diamond, 1982; Hamann et al., 2016) and for many, this is the  
52 only feasible method of *in vitro* maintenance, given that the process of axenisation, i.e.,  
53 removal of other unwanted organisms, has not been achieved. Xenic cultivation depends on  
54 establishing a stable community in culture, typically derived from the original sample, i.e.,  
55 gut content or stool, which can be maintained for years by regular transfers. Whereas the  
56 community may be derived from the sampled environment, its eventual composition will  
57 most probably reduce as it adapts to the culture conditions (nutrient source, oxygen  
58 concentration, etc.) and thus will only distantly resemble the situation in the host gut. Still,  
59 the culture represents a simple and tractable model system from which information about  
60 these complex communities may be obtained. To our knowledge, such attempts are rare  
61 (Hamann et al., 2017).

62           In the present study, we aim to characterize the culture community derived from the  
63 stool of *Chinchilla lanigera*. It contains a single eukaryote, the commensal bacterivorous  
64 flagellate *Monocercomonoides exilis*, for which a quality genome draft is available and  
65 functionally annotated (Karnkowska et al., 2019, 2016; Treitli et al., 2021). This species  
66 belongs to Oxymonadida (Preaxostyla, Metamonada), known as inhabitants of animal  
67 intestines (Hampl, 2017; Treitli et al., 2018). The whole group, including *M. exilis*, has lost  
68 mitochondria, which is unique among eukaryotes and raises interest in their biochemistry and  
69 physiology (Karnkowska et al., 2016; Novák et al., 2023). While other oxymonad species  
70 harbor symbionts (Hampl, 2017) and specific roles and interactions have been partially  
71 elucidated, e.g. *Streblomastix strix*, a protist that takes part in the complex community of the  
72 hindgut of termites and is involved in cellulose digestion (Treitli et al., 2019), no symbiotic  
73 interaction is known for *M. exilis*. Light and electron microscopy has demonstrated that  
74 species of *Monocercomonoides* actively feed on prokaryotes (Treitli et al., 2018), hence  
75 acting as predators within the intestinal microbiota. The effect and scale of this grazing on the  
76 prokaryotic community and the level of prey selectivity are unknown.

77           We used a multi-omic approach to obtain a first insight into the processes of this  
78 multipartite culture community over five days of growth. Namely, we determined the species  
79 composition and changes in species abundances through time, predicted the metabolism  
80 pathways, followed gene expression changes of the most abundant members as the culture  
81 aged, and finally, measured the composition and changes of selected metabolites in the  
82 media. With all these data in hand, we attempted to reconstruct the major community  
83 biochemical processes and searched for potential interactions between its members.

84

## 85 **Results**

### 86 **Growth of the culture and sampling of DNA, RNA and media**

87           The development of the culture was followed for seven days in an experiment  
88 performed in triplicates (Figure 1). An aliquot of the bacterized medium, i.e., medium in  
89 which *Citrobacter portucalensis* grew overnight, representing day 0, and aliquots of days 2,  
90 3, and 5 representing the exponential, stationary, and decline phases of *M. exilis* growth, were  
91 chosen to sample metagenomic, metatranscriptomic, and metabolomic data.

## 92 **Community composition**

93           Using metagenomic reads generated from triplicates of each sampling point, we  
94 assembled 24 metagenome-assembled genomes (MAGs) corresponding to the prokaryotic  
95 members of the culture community (Figure 2, Supplementary Table 1). In addition to the  
96 assembled MAGs, we identified six additional bacterial species based on the presence of 16S  
97 rRNA genes. However, their read abundances were too low to assemble them into clean  
98 MAGs (Supplementary Table 1). No viral or plasmid DNA was identified in the  
99 metagenomic data. This suggests that the community consists of one eukaryote and at least  
100 30 bacterial species, representing seven phyla, without any archaeal or viral component.

101           The quality of the 24 assembled MAGs varied, with the lowest completeness being  
102 around 83% and the vast majority having genome completeness greater than 95%  
103 (Supplementary Table 1). The estimated contamination levels are overall lower than 5%,  
104 except for *Deltaproteobacteria* sp. MAG, whose contamination was estimated to be 11.8%  
105 (Supplementary Table 1). This MAG was the least abundant during the whole experiment.  
106 We calculated the relative read abundance of each bacterium and *M. exilis* for days 2, 3, and  
107 5 of culture. While *M. exilis* represented only 0.25% to 1.23% of the reads, the seven most  
108 abundant bacterial species comprised 80 – 90% of the reads, and the remaining bacterial  
109 species never exceeded 1% of the reads per species (Figure 2 and Supplementary Table 1).  
110 We observed a switch in the dominant groups throughout the culture growth. Not considering  
111 *Citrobacter portucalensis* (Gammaproteobacteria), which was present in the medium before

112 inoculation of the community (“bacterization” of the medium), the most abundant bacterium  
113 on day two was *Fusobacterium varium* (Fusobacteriota). On days three and five, the  
114 abundance of this bacterium decreased, and representatives of the phylum Bacteroidota  
115 dominated. *M. exilis* reached its peak of 1.23% on day 3 (Figure 2).

#### 116 ***Monocercomonoides exilis* metabolic capacities**

117 Our analysis of *M. exilis* metabolism identified 933 enzymatic reactions, 31 transport  
118 and 148 enzymatic pathways (Supplementary Figure 1). As previously published  
119 (Karnkowska et al., 2019, 2016; Treitli et al., 2021), *M. exilis* is equipped with a limited  
120 repertoire for energy metabolism relying on glycolysis to produce pyruvate, which is  
121 converted into acetyl-coenzyme A and further to acetate and ethanol. Our analysis showed  
122 that *M. exilis* can obtain glucose from the degradation of glycogen, maltose or starch but can  
123 also digest chitin and (1,3)-  $\alpha$ -D-glucans present in fungal cell walls and peptidoglycan  
124 present in bacterial cell walls, which corroborates its bacterivorous feeding mode  
125 (Supplementary Table 2). The biosynthesis of amino acids and nucleotides is limited and  
126 dependent on conversion and salvage pathways from compounds acquired from the diet or  
127 via transporters. However, *M. exilis* can synthesize dNTPs from NTPs using ribonucleoside-  
128 triphosphate reductase (Karnkowska et al., 2019; Novák et al., 2023).

#### 129 **Bacterial community metabolic capacities**

130 To better understand the metabolic potential of the 24 assembled bacterial genomes,  
131 we reconstructed the metabolism of each MAG using EggNOG- mapper and Pathway-Tools.  
132 Our results showed a higher number of pathways and reactions compared to *M. exilis*, with  
133 considerable diversity in the number of enzymatic reactions, transporters and pathways (from  
134 443 in *Citrobacter portucalensis* to 218 in *Peptostreptococcus russellii*) (Supplementary  
135 Table 1). The metabolic maps of the seven most abundant MAGs are provided in  
136 Supplementary Figures 2 – 8. The number of reactions and pathways identified in closely

137 related MAGs was very similar, even if the assembly quality differed between MAGs. This  
138 suggests that our metabolic analyses were not heavily affected by the level of fragmentation  
139 of the MAG assemblies.

140 We analyzed the roles of community members within the carbon, nitrogen, sulfur, and  
141 iron cycles of the community. The results showed that the community likely depends on the  
142 oxidation of organic carbon compounds (mainly amino acids and complex carbon  
143 compounds), fermentation, and H<sub>2</sub> generation (Figure 3). The community does not perform  
144 methanogenesis, and the produced H<sub>2</sub> is probably oxidized to water. Our analyses identified  
145 *Phascolarctobacterium* sp. as the only community member able to fix N<sub>2</sub> to ammonium.  
146 However, the abundance of this species is low (Figure 2 and Supplementary Table 1) so the  
147 N<sub>2</sub> fixation most likely does not significantly contribute to the ammonium pool. Another  
148 potential source of ammonium could be the Dissimilatory Nitrate Reduction to Ammonium  
149 (DNRA) pathway (Kaviraj et al., 2024). Components of this pathway, periplasmic nitrate  
150 reductase (NapAB) or the nitrate reductase A (NarGHI) and cytochrome c552 nitrite  
151 reductase (NrfAH and NrfABCDEF), are present in multiple members of the community  
152 (Figure 3, Supplementary Table 3, Supplementary Figures 2, 4 – 7). However, we failed to  
153 identify the source of the nitrates and nitrites. These anions are not added to the cultivation  
154 medium, and we did not identify enzymes producing nitrate or nitrite either by oxidation of  
155 ammonia derived from the amino acid and nucleotide decay or by the biosynthesis of nitrite  
156 from aspartate (Sugai et al., 2016). In conformity with the above, the expression of the  
157 DNRA pathway in most bacteria, for which the transcriptomic data are available, is  
158 extremely low and in the two species, which express it (*B. thetaiotaomicron* and  
159 *Parabacteroides* sp.), it is down-regulated in later days (Supplementary Table 3,  
160 Supplementary Figures 2, 4 – 7). We conclude that the community does not run a complete  
161 nitrogen cycle but depends on the ammonium derived from amino acids and nucleotides

162 supplemented to the medium or originating from dead cells with a small addition through the  
163 fixation of nitrogen gas.

164 In contrast to the carbon and nitrogen cycles, the sulfur cycle is complete. It is  
165 maintained by *Desulphovibrio* sp, the only member able to reduce sulfate and oxidize sulfite.  
166 We identified eight species that encode the sulfide:quinone oxidoreductase, suggesting that  
167 they accumulate sulfur in globules within the cytoplasm (Arieli et al., 1994). The reduction of  
168 iron is another important activity in the community, with 13 species involved in the process  
169 (Figure 3). In contrast, iron oxidation activity was identified only for *Saezia sanguinis*.  
170 (Figure 3).

#### 171 **The consumption and production of compounds by the community**

172 Triplicates of the cell-free medium were sampled at each time point and their  
173 composition was analyzed by non-targeted metabolomics. LC-MS/MS and GCxGC-MS  
174 covered a broad range of structural classes of metabolites, detecting 270 distinctive  
175 compounds altogether (Supplementary Table 4). Of these, 171 take part in the biological  
176 reactions listed on the MetaCyc database (Caspi et al., 2014); hence, they were the only ones  
177 used for the follow-up analyses (Supplementary Table 4).

178 On the day of inoculation (day 0), the medium was already affected by an overnight  
179 growth of *C. portucalensis*. Yet it was relatively abundant in nucleotides and their derivatives,  
180 glyceraldehyde, lactate, fatty acids, alcohols and amino acids, except asparagine, aspartate,  
181 cysteine, glutamine and serine, which were below the detection limit at this time point  
182 (Supplementary Table 4). On day 2, a drastic evanesce of some compounds was registered,  
183 specifically nucleotides and their derivatives, most amino acids, glyceraldehyde, glyceric acid,  
184 malate, citric acid, and citrulline, suggesting that the community had consumed them. On the  
185 contrary, a spectrum of short-chain fatty acids and aromatic compounds putatively derived  
186 from the degradation of amino acids, such as tyrosine, phenylalanine and tryptophan, was

187 observed in the media on this day (Supplementary Table 4). The measurements displayed  
188 little changes between days 2, 3, and 5, suggesting that the composition of cell-free media  
189 stabilized.

190 We integrated the synthesis and consumption of compounds into an *in silico*  
191 metabolic capacity prediction, both for *M. exilis* and the bacterial community, using the  
192 Metage2Metabo (m2m) pipeline (Belcour et al., 2020). In these predictions, the culture days  
193 were compared pairwise, i.e., day 0 vs. day 2, day 2 vs. day 3 and day 3 vs. day 5. We then  
194 classified compounds either as seeds if detected on the first day in pairwise comparison or as  
195 targets if they were detected only or their concentration increased on the second sampling day  
196 of that pair. This was performed using measured differences in media composition,  
197 presence/absence and concentration changes of compounds between sampling days. These  
198 results are summarized in Figure 4. From the fact that *M. exilis* represents less than 1.5% of  
199 the community metagenomic reads, we infer that its metabolic power is low and the  
200 prokaryotic component converts most of the metabolites. Nevertheless, in subsequent  
201 analyses, we sought to stress out the metabolic capabilities of this protozoan, which was our  
202 main point of interest. Between day 0 and day 2, 137 compounds were classified as seeds and  
203 92 as targets, of which *M. exilis* can synthesize 63 by itself. However, the metabolic  
204 capacities of *M. exilis* are not limited to the target compounds, as it can synthesize other  
205 important compounds like pyruvate, amino acids, medium-chain fatty acids, and vitamins. An  
206 additional 29 target compounds can be synthesized via metabolic interactions of *M. exilis* and  
207 the bacterial community (Figure 4). Among these compounds, there are several amino acids.  
208 In this pairwise comparison, m2m did not identify any bacteria as an essential member of the  
209 community. However, two Bacteroidota bacteria were classified as key members (species  
210 occurring in at least one of the minimal community models) (Figure 4). Between day 2 and  
211 day 3, we identified 141 compounds as seeds and 72 as targets. According to the m2m

212 pipeline, *M. exilis* can synthesize 68 targets independently and 51 compounds through  
213 metabolic interaction with the community (Figure 4). In this comparison, no bacterial species  
214 were considered essential or key members of the community. The last comparison, day 3 vs.  
215 day 5, showed a similar result. *M. exilis* could synthesize 54 of the 55 compounds identified  
216 as targets using 140 compounds as seeds. The interaction between *M. exilis* and the bacterial  
217 community allows the synthesis of 49 more compounds and none of the bacteria were  
218 considered essential or key members (Figure 4).

### 219 ***Monocercomonoides exilis* differential expression analysis**

220 To investigate how *M. exilis* metabolism changes during the growth curve, we also  
221 compared the expression of transcripts between days 2, 3 and 5, again pairwise. Most  
222 significant changes in gene expression were observed between days 2 and 5, while  
223 comparisons between consecutive days revealed less significant changes (i.e., day 3 vs. day 2  
224 and day 5 vs. day 3). 2,236 genes were up-regulated and 2,431 down-regulated between days  
225 2 and 5 (Supplementary Table 1), of which the majority, 1703 and 1,381 genes, respectively,  
226 are annotated as hypothetical; hence their function could not be deciphered.

227 The changes in expression of all functionally annotated metabolic enzymes and  
228 pathways are shown in Supplementary Figure 1 and those discussed below are shown in  
229 Figures 5 and 6 in more detail. We observed an increase in the expression of importers of  
230 sugars such as  $\alpha$ -D-xylose and *sn*-glycerol-3-phosphate towards day 5. Connected to this,  
231 there was an increment in the expression of enzymes responsible for the degradation of  
232 starch, (1 $\rightarrow$ 4)- $\alpha$ -glucan, chitin and other polysaccharides (Figure 5 and Supplementary Table  
233 2). Furthermore, the interconversion between glucose-1-P and glucose-6-P was up-regulated,  
234 which may relate to the increased trehalose biosynthesis from glucose-6-P. Trehalose is often  
235 used as carbohydrate storage and as a stress protector (Iturriaga et al., 2009). All three  
236 pathways for trehalose synthesis were up-regulated on day 5, including the synthesis directly

237 from  $\alpha$ -maltose, performed by both  $\alpha$ - and  $\beta$ -amylases. The synthesis of glycogen, another  
238 storage polysaccharide, was also up-regulated. The expression of most genes involved in  
239 carbohydrate metabolism and glycolysis remained unchanged. However, some, such as  
240 phosphoenolpyruvate carboxykinase (PEPCK), exhibit down-regulation, suggesting that  
241 glycolysis is regulated to produce pyruvate via the up-regulated pyruvate kinase (PK). Later,  
242 this pyruvate is converted to acetyl-CoA by pyruvate-ferredoxin oxidoreductase (PFOR) and  
243 the up-regulated hydrogenases (HYD) re-oxidize ferredoxins, allowing the cycle to continue.  
244 The expression of enzymes of extended glycolysis and ethanol fermentation, i.e., acetate  
245 synthase (ACS) producing acetate and aldehyde and alcohol dehydrogenases (ALDH, Adh)  
246 producing ethanol, decreases greatly on day 5 for all but one paralogue of ALDH  
247 (Supplementary Table 5). ACS1 was consistently the most expressed of these enzymes in all  
248 three time points and replicates. Overall, these expression data suggest that acetate and  
249 hydrogen are the preferred metabolic end products at all three time points. In contrast to  
250 glycolysis, the pentose phosphate pathway may be down-regulated due to the repression of  
251 the ribulose-phosphate 3-epimerase (RPE) (Figure 5).

252 Genes responsible for the phosphorylation of (d)NDP, DNA and RNA  
253 polymerization, and protein ubiquitination were up-regulated on day 5 (Supplementary  
254 Figure 1). In contrast, nucleoside import, and the salvage pathway of adenosine were down-  
255 regulated. Similarly, the mevalonate pathway for the synthesis of isoprenoids from  
256 acetoacetyl-CoA was down-regulated (Figure 5 and Supplementary Figure 1). Most amino  
257 acid importers displayed lower expression or showed no significant change in expression  
258 (Figure 6). This relates to the fact that amino acid content in the media significantly  
259 decreased from day 2. Asparagine, serine, and cysteine biosynthesis were down-regulated, as  
260 was the ATP-producing arginine deaminase pathway (Figure 6).

261 Another system that showed significant changes during cultivation was the  
262 endomembrane system. Among the proteins involved in the regulation of endomembrane  
263 transport, some show significant changes in expression (Supplementary Table 6). The  
264 proteins involved in the early secretory pathway show little change, but several proteins  
265 involved in the late steps (Law et al., 2022; Shimizu and Uemura, 2022; Tanaka et al., 2017)  
266 were significantly up-regulated on day 5 (Supplementary Table 6). These include proteins  
267 involved in trans-Golgi-network (TGN) to plasma membrane trafficking (DSCR3A and  
268 exocyst complex components Exo84, Sec5 and Sec15, DSCR), multivesicular body  
269 formation and sorting (Vsp2, 4a-c, 23, 24a and 60b, charged multivesicular body protein 7),  
270 plasma membrane uptake (AP-2a, TSET complex protein TPLATE subunit B),  
271 endocytic/digestive pathway (Vps34bc, Vps41b) and endosomes to TGN transport (Vps51b,  
272 TRAPP component Bet3 paralogue A, B, syntaxin 6D and 16). On the contrary, four Rab7  
273 paralogues were consistently down-regulated, implying a decrease in lysosome biogenesis on  
274 day 5 (Bucci et al., 2000).

275 At the end of the growth curve, we observed a decline in the number of *M. exilis* cells.  
276 Our data showed that most of the enzymes involved in the apoptosis pathway (Teulière et al.,  
277 2020) do not show significant expression change (Supplementary Table 7). This suggests that  
278 the decline in cell density in later days is not due to programmed cell death but most likely  
279 due to uncontrolled decay combined with slow growth caused by nutrient deficiencies.

### 280 **Bacterial community differential expression analysis**

281 Our data only allowed to perform differential expression analysis for the seven most  
282 abundant bacteria: *B. fragilis*, *B. thetaiotaomicron*, *B. intestinalis*, *Parabacteroides* sp., *K.*  
283 *gyiorum*, *Fusobacterium varium* and *C. portucalensis*. These species represent 80-90% of the  
284 community DNA reads, a proxy measure of their abundance. These bacteria showed a  
285 variable number of up- or down-regulated genes (Supplementary Table 1); for example, only

286 9% of *B. intestinalis*. transcripts changed in expression between day 2 vs. day 5, while in  
287 *Parabacteroides* sp., it was 51%. Changes in the expression of metabolic enzymes and  
288 pathways of these bacteria are indicated in Supplementary Figures 2 – 8.

289 We focused specifically on pathways absent in *C. portucalensis* but present in other  
290 members of the community, as these may explain why we failed to grow *M. exilis* with only  
291 *C. portucalensis* as food. First, *F. varium* and *Tissierella* sp. can ferment lysine to acetate,  
292 butanoate and ammonia, while in *C. portucalensis*, this pathway is incomplete. Furthermore,  
293 *B. intestinalis*, *S. sanguinis*, *K. gyiorum*, *Phascolarctobacterium* sp. and *Tissierella* sp.  
294 encode genes for the synthesis of the essential polyamine spermidine and the phospholipid  
295 phosphatidylcholine. While phosphatidylcholine is one of the major components of cell  
296 membranes (Zhukov and Popov, 2023), spermidine is involved in an array of biological  
297 processes, such as maintenance of membrane potential, control of intracellular pH, lipid  
298 metabolism and regulation of cell growth, proliferation, and death (Michael, 2016). In both  
299 cases, our metabolic analysis fails to identify a pathway that can synthesize these compounds  
300 *de novo* in *M. exilis*, suggesting that the eukaryote may depend on the uptake of these  
301 compounds from these prokaryotes.

302 Iron uptake is another relevant function for which no pathway is known in *M. exilis*.  
303 Several bacteria encode for transporters of  $\text{Fe}^{2+}$ , the anaerobic Feo import system, the aerobic  
304 Efe system and the Sit system (Braun and Hantke, 2013) (Supplementary Table 8). Our data  
305 showed that in all bacteria with expression data, only the Sit system in *K. gyiorum* is up-  
306 regulated between day 2 and day 5. In the rest of the bacteria, all the  $\text{Fe}^{+2}$  transporters are  
307 down-regulated or not significantly differentially expressed between these days  
308 (Supplementary Figures 2 – 8). Of importance is also another type of molecules used for iron  
309 chelation in bacteria known as siderophores. These iron-chelating molecules are secreted into  
310 the environment and their iron-bound forms are subsequently selectively imported (Kramer et

311 al., 2020). Our data showed that some, but not all, community members produce  
312 siderophores, *C. portucalensis* and *S. sanguinis* synthesize enterobactin, while *F. varium*  
313 synthesizes staphylopin, and *K. gyiorum* a periplasmic iron-binding protein. Interestingly, all  
314 bacteria, but not *M. exilis*, encode transport systems for siderophores or transferrin. Of these,  
315 we identified components of the Ton uptake system (TonB, ExbB and ExbD) (Braun and  
316 Hantke, 2013) in most gram negative bacteria except for ExbB absent from *Morganella*  
317 *morganii* (Supplementary Table 8). Consistently, we identified several TonB-dependent  
318 receptors and transporters, namely the enterobactin transporters FepA and FbpABC and the  
319 transferrin receptor TbpA in many members of the community. The presence of these  
320 proteins and protein complexes makes the transport of siderophores or heme groups across  
321 the outer membrane possible (Braun and Hantke, 2013). Our data showed that the Ton uptake  
322 system and the receptor TbpA are up-regulated in *F. varium*, *K. gyiorum* and members of the  
323 Bacteroidota in later days of the culture (Supplementary Figures 3 – 5, 7,8). The described  
324 pattern of presence and absence suggests that the bacterial community contains only a few  
325 species able to synthesize siderophores and all the other members are “cheaters” that uptake  
326 siderophores produced by others (Butaite et al., 2017; Leventhal et al., 2019). From this point  
327 of view, the siderophores' producers are key players in the prokaryotic community but do not  
328 facilitate the uptake of iron into *M. exilis*. Once iron is imported into the cell, it cannot be  
329 kept free in the cytoplasm. One solution would be its coordination within different kinds of  
330 porphyrins (Bryant et al., 2020; Dailey et al., 2017; Layer, 2021). We observed two main  
331 porphyrins, siroheme and heme *b*. Heme *b* can be synthesized aerobically or in an oxygen-  
332 independent manner, while siroheme is synthesized only independently of oxygen (Dailey et  
333 al., 2017; Layer, 2021). Our data showed that *C. portucalensis* can synthesize both  
334 compounds. Still, under anaerobic conditions, the synthesis of heme *b* is down-regulated,  
335 while that of siroheme is up-regulated on day 5 (Supplementary Figure 2). The opposite

336 situation was observed in *Bacteroides fragilis*, where oxygen-independent synthesis of heme  
337 *b* was up-regulated, yet siroheme synthesis underwent down-regulation (Supplementary  
338 Figure 4). Some bacteria in the community can synthesize heme *b* from siroheme. However,  
339 we lacked transcriptome data for their assessment due to their low abundance.

340

## 341 **Discussion**

342 The only study of chinchilla gut microbiota composition (O' Donnell et al., 2017)  
343 reports Betaproteobacteria as the dominant phylum and *Parabacteroides* and *Barnesiella* as  
344 the dominant genera. Our study of an *M. exilis* culture derived from the gut of chinchilla  
345 identified 30 bacterial species, of which *Fusobacterium varium* was the most abundant at the  
346 beginning of the experiment, steadily replaced by the Bacteroidetes group, represented by  
347 *Bacteroides fragilis*, *Parabacteroides* sp. and *B. thetaiotaomicron* on day 5. *Parabacteroides*  
348 sp. was the second most abundant bacteria on day 5, yet we failed to detect any *Barnesiella*  
349 species. Only two Betaproteobacteria species were identified in our study, but they are not  
350 among the most abundant species. At the level of bacterial phyla, Bacteroidota and Bacillota  
351 are the most abundant in our culture, which, on a broad scale, resemble the composition of  
352 the gut microbiota of Guinea pigs (Crowley et al., 2017). These comparisons indicate similar  
353 compositions of the prokaryotic community between the gut of caviomorph rodents and our  
354 *M. exilis* culture, yet the culture community has changed due to long-term cultivation.

355 Given that *M. exilis* represents around 1% of the read percentage, the biochemical  
356 processes in the community are driven by prokaryotes. Of the three main cycles, only the  
357 sulfur cycle seems to be complete and contains reactions converting all basic inorganic sulfur  
358 compounds (Figure 3). Our analyses infer that the community dominantly oxidizes organic  
359 carbon substrates, performs fermentation and generates hydrogen, which is further oxidized  
360 to water (Figure 3). Since the ability to fix carbon from CO<sub>2</sub> is present only in *Eubacterium*

361 *maltosivorans*, the least abundant species, the community depends on the supply of organic  
362 carbon. This is consistent with our observation that organic carbon sources (glyceraldehyde,  
363 lactate, fatty acids, alcohols and most amino acids) present on day 0 are quickly consumed.

364         The community does not run a complete nitrogen cycle. While there is a small level of  
365 N<sub>2</sub> fixation due to the contribution of *Phascolarctobacterium* sp., this bacterium is among the  
366 less abundant members of the community, and most likely, its N<sub>2</sub> fixation does not cover the  
367 demands for ammonium. Another pathway for ammonium synthesis, the DNRA pathway  
368 using the periplasmic nitrate reductase or the nitrate reductase A, and the membrane-bound  
369 nitrate reductase nrf, is encoded by some bacterial species (Kaviraj et al., 2024). This  
370 pathway uses nitrate as electron acceptor and allows building membrane potential (Cabello et  
371 al., 2019). However, in all the bacteria with transcriptomic data, the DNRA is not expressed  
372 or downregulated in later days. The nitrogen sources for building a biomass thus are amino  
373 acids and nucleobases, which are components of the growth medium and are measurable on  
374 the day 0 with a small supplement of ammonia synthesized by *Phascolarctobacterium* sp.

375         The metabolomic analyses of the soluble media clearly showed that on day 2, when  
376 the population of *M. exilis* only began to grow, essentially all soluble carbon sources,  
377 nucleotides and most amino acids had been already consumed (Supplementary Table 4). The  
378 fact that *M. exilis* maintains active growth after day 2 strongly suggests that the protist uses  
379 bacterial prey as the major source of carbon, nitrogen, and energy to build its biomass. A  
380 putative exception could be arginine, which was detected in later days and may serve as a  
381 potential substrate for energy metabolism (Schofield et al., 1992; Yarlett et al., 1996).  
382 Grazing on bacteria has been previously documented by electron microscopy (Treitli et al.,  
383 2018) and the fact that the protist encodes lysozymes amongst a broad array of glycosyl  
384 hydrolases (Supplementary Table 2) also provides molecular tools for feeding on bacteria.  
385 There is no consistent pattern that would indicate up- or down-regulation of lysozymes as the

386 culture ages. The protist, however, displays a significant up-regulation of several genes  
387 involved in the late pathways of the endomembrane system on day 5. At the same time  
388 expression of many genes encoding for proteins regulating these processes remained  
389 unchanged and, notably, four paralogues of Rab7 small GTPase, known for its role in  
390 lysosome activation (Bucci et al., 2000), were significantly down-regulated on day 5  
391 (Supplementary Table 6). We interpret this pattern as an indication of up-regulation of  
392 exocytosis and secretion rather than bacterial feeding, which is also consistent with the  
393 decrease in *M. exilis* cell counts at this stage of the culture.

394 Most *M. exilis* genes that exhibit differential expression completely lack functional  
395 annotation, providing us with a rather large transcriptomic dark matter consisting of approx.  
396 three thousand genes that may convey some functional and adaptive value to the protist, but  
397 we know absolutely nothing about it. Among the known metabolic genes, we observed an up-  
398 regulation of the importers of sugars,  $\alpha$ -D-xylose and *sn*-glycerol-3-phosphate, which is  
399 likely the reaction of the cell to the absence of these substrates. Another functional category  
400 of genes up-regulated on day 5 is the enzymes responsible for starch degradation and  
401 synthesis of trehalose and glycogen, indicating the onset of starvation. The degradation of  
402 nucleosides and nucleotides and the biosynthesis of 5-phospho- $\alpha$ -D-ribose 1-diphosphate  
403 (PRPP) are up-regulated, suggesting an increment in the storage of nucleotides and phosphate  
404 donors. On the other hand, glycolysis, extended glycolysis, fermentation, pentose phosphate  
405 pathway, amino acids biosynthesis, arginine deaminase pathway and mevalonate pathway  
406 involved in isoprenoid biosynthesis are generally down-regulated on day 5, with few  
407 exceptions. The same holds for the synthesis of very long and ultra-long fatty acids and the  
408 aminoacyl-tRNA synthetases, indicating the decline of proteosynthesis.

409 Although we hoped to find some metabolic dependencies between *M. exilis* and  
410 prokaryotes, potentially explaining the inability to grow the flagellate axenically using

411 nutrients dissolved in the medium, we did not reveal any clear link. This obviously does not  
412 mean that some have remained unobserved or unnoticed, as the amount and complexity of the  
413 data are significant. The *in silico* metabolic capacity prediction by Metage2Metabo (Figure 4)  
414 has shown that the compounds detected in the media at the time points can be synthesized by  
415 the reactions and pathways found in the community. Importantly, none of the community  
416 members has been found to be essential at any time point, though some may provide key  
417 compounds for others, e.g., siderophores. This suggests that the community is based on broad  
418 redundant foundations, which certainly contributes to its long-term stability. However, we  
419 infer that carbon and nitrogen could be limited factors that can disturb this tendency.

420

## 421 **Material and methods**

### 422 **Cell culturing**

423 700 mL of TYSGM-9 medium in 2x 1 L glass bottles were inoculated with 500  $\mu$ L of  
424 *Citrobacter portucalensis* culture before the inoculation of the whole community. This strain  
425 of *C. portucalensis* was previously isolated from the original culture during the isolation of  
426 *M. exilis*. After inoculation, the flasks were closed and allowed to grow standing at 37°C. The  
427 next day, the two flasks were merged in a 2 L bottle and mixed thoroughly. 45 mL of culture  
428 were removed and used to isolate DNA, RNA and metabolomic analyses of day 0. The rest of  
429 the medium was inoculated with approx. 125 mL of *M. exilis* culture ( $2.5 \times 10^5$  cells.mL<sup>-1</sup>).  
430 After inoculation, the media was thoroughly mixed and divided into 50-mL tubes containing  
431 45 mL of media, closed, and incubated at 37°C. Each day, three tubes were removed, one for  
432 each replicate. From each tube, 20 mL of culture were used for DNA isolation, 20 mL for  
433 RNA isolation and 5 mL for metabolomics. Before each nucleic acid isolation, *M. exilis* cells  
434 were counted using a Neubauer cytometer.

### 435 **DNA isolation**

436 All DNA isolations were performed using the Dneasy Blood and Tissue Kit (Qiagen).  
437 Each culture was centrifuged at 1500 *xrcf* for 10 min at 4°C. The supernatant was separated  
438 into a fresh 50 mL tube and centrifuged again at 6000 *xrcf* for 10 min at 4°C. The pellet from  
439 the first centrifugation was resuspended in 200 µL of PBS and DNA was isolated using the  
440 cultured cells protocol of the Dneasy Blood and Tissue Kit (Qiagen). The second pellet,  
441 obtained after centrifugation at 6000 *xrcf*, was resuspended in 200 µL of PBS and DNA was  
442 isolated using the gram-positive protocol of the Dneasy Blood and Tissue Kit (Qiagen). For  
443 each isolation, the DNA was eluted in 100 µL of elution buffer. The DNA from the two  
444 pellets was merged into one DNA sample. The quality of the DNA was estimated using  
445 Nanodrop and the concentration was measured using the QuantiFluor ONE dsDNA System  
446 (Promega).

#### 447 **RNA isolation**

448 Each culture was centrifuged at 1500 *xrcf* for 10 min at 4°C. The supernatant was  
449 separated into a fresh 50 mL tube and centrifuged again at 6000 *xrcf* for 10 min at 4°C. Both  
450 pellets were resuspended together in 1 mL Tri-Reagent (Sigma-Aldrich) and the total RNA  
451 was isolated according to the manufacturer's procedure. The obtained RNA pellet was  
452 resuspended in 60 µL of RNA-grade H<sub>2</sub>O and heated at 37°C for 10 min to allow full  
453 resuspension.

454 The total RNA was further DNase-treated. For this purpose, we added 6 µL of  
455 rDNase buffer and 0.6 µL rDNase (Macherey-Nagel) per sample and incubated at 37°C for  
456 10 min. Samples were subsequently re-isolated with Tri-Reagent (Sigma-Aldrich) as before  
457 and finally resuspended in 30 µL of RNA-grade H<sub>2</sub>O. RNA concentration was measured  
458 using Nanodrop.

#### 459 **Identification of compounds within the growth medium**

460 For metabolomics, cells were pelleted as described for DNA and RNA isolation (see  
461 above). The supernatant obtained in the second centrifugation was filtered (Whatman  
462 polycarbonate filter, 3  $\mu\text{m}$  pore size)-sterilized and stored at  $-80^{\circ}\text{C}$ . Metabolites released to  
463 the media were determined using a non-targeted metabolomics approach. To capture both  
464 volatiles and non-volatiles, a combination of two techniques was employed – Liquid  
465 Chromatography analysis in connection with Orbitrap Fusion mass spectrometer (LC-  
466 MS/MS; Orbitrap Fusion, Q-OT-qIT, Thermo Scientific) and two-dimensional  
467 comprehensive gas chromatography in connection with mass spectrometer (GCxGCTOF-MS;  
468 Pegasus 4D, Leco Corp.).

469 For LC-MS/MS, media samples harvested in triplicates were precipitated with  
470 acetonitrile in a ratio of 4:1 (ACN:media) and centrifugated for 20 min at 16000 *xrcf* at  $5^{\circ}\text{C}$ .  
471 100  $\mu\text{L}$  of each supernatant was collected, fully evaporated and dissolved in 50  $\mu\text{L}$  10% (v/v)  
472 ACN. 15  $\mu\text{L}$  were injected into a ProntoSIL column, 150 x 3.0 mm, 3  $\mu\text{m}$  (Bischoff  
473 Chromatography). Compounds were eluted under the following conditions: 0 - 3 min 100%  
474 A, 3 - 18 min linear gradient to 100% B, 18 - 20 min 100% B using mobile phases. A: 2%  
475 (v/v) ACN, 10 mM  $\text{HCOONH}_4$ , 0.1% (v/v) FA; B: 99% (v/v) ACN, 0.1% (v/v) FA with a  
476 flow of  $0.4 \text{ mL}\cdot\text{min}^{-1}$ . The separated compounds were ionized in an electrospray ion source in  
477 positive polarity mode. Master scans of precursors from 80 to 1000 m/z were performed in  
478 Orbitrap at 120 K resolution with an intensity threshold filter  $2.0 \times 10^4$ . Tandem MS was  
479 performed by isolation in the quadrupole, HCD fragmentation with a stepped collision energy  
480 of 15, 30, and 45% and an isolation window of 1.6 m/z. The MS2 ions were analysed and  
481 detected in Orbitrap with a set resolution of 30k and a max injection time of 300 ms. The  
482 dynamic exclusion duration occurred every 20 s with 10 ppm tolerance around the selected  
483 precursor. The data processing, including chromatographic peaks alignment, was performed  
484 using Compound Discoverer 3.3 (Thermo Scientific). Quality control samples were employed

485 for the data correction. The mzCloud libraries were used as a tool for a fragmentation spectra  
486 comparison and the compound structure assignment.

487 Volatiles were analyzed using GCxGC-MS and a headspace solid phase  
488 microextraction (HS-SPME) on fiber (DVB/CAR/PDMS\_grey; Supelco, USA). A volume of  
489 1 mL of media samples was placed in a 20 mL glass vial and 10  $\mu$ L of an internal standard  
490 (2,2,2-trifluoro ethanol, Fluka, 0.11 mg.mL<sup>-1</sup>) was added. Samples were incubated for 10 min  
491 at 40°C before extraction. The extraction was carried out for 5 minutes. The volatiles were  
492 analyzed using a combination of polar and mid-polar separation columns for the separation  
493 (primary column: Stabilwax-DA (30 m x 0.25 mm, Restek, USA); secondary column BPX-  
494 50 (1.38 m x 0.1 mm, SGE, Australia)). Other parameters were set as follows: inlet  
495 temperature 220°C, split 5 modes, constant He flow 1 mL.min<sup>-1</sup>, modulation time 3 s (hot  
496 pulse 0.7 s), modulation temperature offset with respect to the secondary oven 20°C. The  
497 temperature program applied on the primary oven was 30°C (hold 1.5 min), then increased to  
498 110°C (8°C.min<sup>-1</sup>), followed by an increase to 250°C (25°C.min<sup>-1</sup>) to 250°C (hold 5 min). The  
499 temperature offset applied on the secondary column was +5°C. Transferline temperature was  
500 held at 280°C. The mass spectrometer was equipped with an Electron Ionization ion source  
501 (energy of 70eV was applied), and Time-of-Flight analyser enabling a united mass resolution.  
502 The scanned mass range was 29 – 400 *m/z*. The ion source chamber was held at 250°C.

503 To extend the range of compounds analyzable by gas chromatography to polar non-  
504 volatiles, the media samples underwent two consecutive derivatization procedures –  
505 oximation (25 mg.mL<sup>-1</sup> of methoxyamine hydrochloride in anhydrous pyridine  
506 hydrochloride, Sigma-Aldrich) and silylation. Two different agents were used for silylation:  
507 N,O-Bis(trimethylsilyl)trifluoroacetamide with trimethylchlorosilane (BSTFA + 1%  
508 (v/v)TMSC) and N-tert-Butyldimethylsilyl-N-methyl trifluoroacetamide (MTBSTFA), both  
509 from Sigma-Aldrich.

510 For silylation using BSTFA + 1% (v/v) TMSC, 50  $\mu\text{L}$  were transferred to a 2 mL  
511 glass vial and 2  $\mu\text{L}$  of internal standard were added (Adonitol, 424  $\mu\text{L}\cdot\text{mL}^{-1}$ ). The liquid was  
512 dried in a vacuum concentrator and reconstituted back by adding 30  $\mu\text{L}$  of anhydrous  
513 pyridine and 30  $\mu\text{L}$  of an oximation agent. The samples were then incubated in a  
514 Thermomixer (Eppendorf) at 40°C for 2 hours at constant shaking (1500 rpm), after which 40  
515  $\mu\text{L}$  of neat solution of BSTFA + 1% (v/v) TMSC mixture were added to the samples and  
516 were further incubated at 70°C for 30 min at constant shaking (1500 rpm). Before analysis,  
517 400  $\mu\text{L}$  of hexane was added to the sample. For silylation using MTBSTFA, the  
518 derivatization process is essentially the same as the derivatization using BSTFA + TMSC  
519 except that 30  $\mu\text{L}$  of media sample are used, plus 5  $\mu\text{L}$  of nor-Valine (550  $\mu\text{L}\cdot\text{mL}^{-1}$ ) as an  
520 internal standard and 20  $\mu\text{L}$  of oximation agent.

521 Metabolites were analyzed as oximated trimethylsilylated or tert-butyl silylated  
522 derivatives. A combination of nonpolar and mid-polar separation columns was used for the  
523 separation; a primary Rxi-5SIL MS column (30 m x 0.25 mm, Restek, USA) and a secondary  
524 BPX-50 column (1.39 m x 0.1 mm, SGE, Australia). Other parameters were set as follows:  
525 inlet temperature 290°C, splitless mode, constant He flow 1  $\text{mL}\cdot\text{min}^{-1}$ , modulation time 3s  
526 (hot pulse 0.7s), modulation temperature offset with respect to the secondary oven 15°C. The  
527 temperature program applied to the primary oven was 50°C (hold 1 min), then increased  
528 ( $8^\circ\text{C}\cdot\text{min}^{-1}$ ) to 320°C (hold 2 min). The temperature offset applied on the secondary column  
529 was +5°C. Transferline temperature was held on 280°C. The scanned mass range was 85 –  
530 800  $m/z$ . The ion source chamber was held at 280°C. Data were processed in ChromaTOF  
531 v4.5 software. Detected analytes were quantified relatively after normalization to the internal  
532 standards. Metabolites detected by all the methods used were identified by comparison of  
533 their mass spectra with those available in the main NIST mass library, Fiehn's mass library of  
534 silylated compounds, and in-house-built libraries. The retention index was determined using

535 linear hydrocarbons. In the cases where the same compounds were detected in both  
536 derivatizations, the highest signal was taken to the final table.

### 537 **Library preparation and sequencing**

538 For the DNA samples, sequencing libraries were prepared from 1 µg of gDNA using  
539 the Illumina TruSeq DNA PCR free (Illumina) library preparation kit according to the  
540 manufacturer's protocol. The prepared libraries were sequenced on an Illumina HiSeq 4000  
541 with 2x150 bp reads.

542 For RNA, 1 µg of total RNA per sample was used for library preparation. Ribosomal  
543 RNA depletion was performed using the NEBNext rRNA Depletion Kit for Bacteria (New  
544 England Biolabs), spiked with a custom oligo pool. The oligo pool was designed based on  
545 bacterial and eukaryotic ribosomal sequences that were identified from previous sequencing  
546 projects of *M. exilis* (Karnkowska et al., 2016; Treitli et al., 2021). After rRNA depletion,  
547 libraries were prepared using the NEBNext® Ultra™ Directional RNA Library Prep Kit  
548 (New England Biolabs) according to the manufacturer's protocol. The prepared libraries were  
549 sequenced using Illumina NextSeq 500 with 2x75bp reads.

### 550 **Metagenome assembly and binning**

551 The metagenomics raw reads were quality-checked with FASTQC v0.11.5 and  
552 trimmed using Trimmomatic v0.39 (Bolger et al., 2014) (ILLUMINACLIP:TruSeq3-PE-  
553 2.fa:2:30:10, LEADING:20, TRAILING:20, SLIDINGWINDOW:5:20 MINLEN:50). To  
554 reduce complexity during assembly, the eukaryotic reads were removed by mapping the reads  
555 against the *M. exilis* genome (Treitli et al., 2021) using BMap (minidentity=0.98,  
556 idfilter=0.98) included in BBTools v38.90 (<https://sourceforge.net/projects/bbmap/>) and  
557 taking only unmapped reads.

558 The filtered reads were assembled independently for each sample using metaSPAdes  
559 v3.14.0 (Nurk et al., 2017) with k-mers 21,33,55,77,99,127. A reiterative process was used

560 for binning metagenome-assembled genomes (MAGs) within the samples. First, contigs  
561 above 2kbp were binned using MaxBin v2.2.7 (Wu et al., 2016) (minimum probability 0.9,  
562 marker set 107) and tetraESOM (Dick et al., 2009). Then, bins were manually checked based  
563 on the markers identified by MaxBin using Blastp against nr database (April 2021). For each  
564 potential MAG, rRNA genes were identified using Prokka v1.14.6 automatic annotation  
565 pipeline (Seemann, 2014) and used as controls together with the marker genes. Lastly, reads  
566 corresponding to trustful bins (markers and rRNA pointing to the same species) were  
567 removed from the data set by mapping the reads back to the identified bins using BBSplit  
568 (minidentity=0.98, idfilter=0.98) included in BBTools v38.90. This process was iterated and  
569 stopped when no new clean MAG was created. In total, 24 prokaryotic MAGs were  
570 assembled. The contribution of each prokaryotic MAG and *M. exilis* to the whole community  
571 per sample was calculated as the percentage of reads mapped to each genome assembly.

572 To identify possible viral particles and plasmids in the culture, the filtered reads were  
573 assembled independently for each sample using metaviralSPAdes (Antipov et al., 2020) and  
574 metaplasmidSPAdes (Antipov et al., 2019), respectively, with k-mers 21,33,55,77,99,127.  
575 Candidates contigs were verified using viralVerify tool ([github.com/ablab/viralVerify.git](https://github.com/ablab/viralVerify.git))  
576 and Prokka v1.14.6.

### 577 **Identification of prokaryotic MAGs**

578 The taxonomic classification of the 24 prokaryotic MAGs was computed using  
579 classify\_wf, included in GTDB-Tk (Chaumeil et al., 2022; Eddy, 2011; Harris et al., 2020;  
580 Hyatt et al., 2010; Matsen et al., 2010; Ondov et al., 2016; Parks et al., 2020; Price et al.,  
581 2010; Shaw and Yu, 2023; Sukumaran and Holder, 2010) with standard parameters. The  
582 completeness and contamination of every MAG were estimated based on the  
583 presence/absence and number of copies of the 107 marker genes used by MaxBin (see  
584 above).

## 585 **Annotation and metabolic capacities of prokaryotic MAGs**

586 The annotation of the 24 prokaryotic MAGs was performed using Prokka v1.14.6.  
587 The functional annotation of each MAG was made using EggNOG-mapper v1.0.3-35 on  
588 emapper DB 2.0 (Cantalapiedra et al., 2021; Huerta-Cepas et al., 2019) (standard parameter  
589 except minimum % of query coverage: 50 and minimum % of subject coverage: 50).  
590 EggNOG-mapper predictions and Prokka annotations were combined using emapper2gbk,  
591 included in the Metage2Metabo pipeline (Belcour et al., 2020), to create a compatible input  
592 for Pathway-Tools v24.5 (Karp et al., 2020).

593 A metabolic database was created for each MAG using Pathologic (Karp et al., 2011),  
594 included in Pathway-Tools (standard parameters except name matching that was turned off).  
595 All databases were manually curated using the Assign Probable Enzymes, Transport  
596 Inference Parser and Rescore Pathways tools included in Pathologic. Predicted pathways  
597 were manually checked to remove highly incomplete pathways.

## 598 **Identification of metabolic capacities of *Monocercomonoides exilis***

599 The genome of *M. exilis* was downloaded from NCBI. The metabolic capacities of  
600 this species were predicted using Pathologic, included in Pathway-Tools, with standard  
601 parameters. The resulting database was manually curated using the Assign Probable  
602 Enzymes, Transport Inference Parser and Rescore Pathways tools included in Pathologic.  
603 Information from previous curations (Karnkowska et al., 2019, 2016; Treitli et al., 2021) and  
604 EggNOG-mapper was included at this step.

605 Carbohydrate-metabolizing enzymes were identified by computing searches against  
606 CAZy databases using METABOLIC-G with default parameters (Zhou et al., 2022).

## 607 **Transcriptome processing and identification of differentially expressed genes**

608 Meta-transcriptomic raw reads were quality-checked with FASTQC v0.11.5 and  
609 trimmed using Trimmonatic v0.39 (ILLUMINACLIP:TruSeq3-PE-2.fa:2:30:15,

610 LEADING:15, TRAILING:15, SLIDINGWINDOW:4:15 MINLEN:50), resulting in 47  
611 million read pairs per sample on average (except for sample D5R3 where the number of read  
612 pairs was 77 million). Reads were mapped and the expression of transcripts of prokaryotes  
613 and *M. exilis* was quantified using Salmon (Patro et al., 2017) with default parameters.

614 Due to the low mapping rate and a low number of reads, the differential expression  
615 was assessed only for *M. exilis* and the seven most abundant prokaryotic genomes:  
616 *Bacteroides fragilis*, *Bacteroides thetaiotaomicron*, *Bacteroides intestinalis*, *Parabacteroides*  
617 sp., *Kerstersia gyiorum*, *Fusobacterium varium* and *Citrobacter portucalensis*. Raw reads  
618 were re-mapped onto the eight selected genomes using Bowtie2 (Langmead and Salzberg,  
619 2012). Reads mapping to protein-coding sequences were counted with the FeatureCounts  
620 program of the subread package v2.0.3 (Liao et al., 2014). Read counts were normalized  
621 using the DESeq2 package v1.32.0 (Love et al., 2014) in R v4.1.1 (R Core Team, 2021).  
622 Normalisation was based on taxon-specific scaling (Christel et al., 2018; Klingenberg and  
623 Meinicke, 2017).

#### 624 **Identification of the major biochemical cycles in the community**

625 The metabolic capacities of the prokaryotic community were also analyzed using  
626 METABOLIC-C (Zhou et al., 2022) with default parameters, except tax genus, to understand  
627 their roles within the community's major biochemical cycling. We manually verified the  
628 results concerning the carbon, nitrogen, sulfur, and iron cycle by combining the results from  
629 METABOLIC-C and EggNOG-mapper from previous analyses.

#### 630 **Identification of the metabolic relationships between *M. exilis* and the prokaryotic** 631 **community**

632 All identified reactions in *M. exilis* and the 24 prokaryotic MAGs metabolic database  
633 were exported as SBML format from Pathway-Tools. The Metacom tool implemented in the  
634 metage2metabo (m2m) pipeline was used to identify metabolic interactions between *M. exilis*

635 and the rest of the community. In this analysis, the sampling days were compared pairwise,  
636 with *M. exilis* defined as the host. In each pairwise comparison, compounds that could be  
637 identified in the first sampling point were defined as seeds. Meanwhile, those whose  
638 concentration increased in the second sampling point were defined as targets.

639

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647 resources provided by the e-INFRA CZ project (ID:90254), supported by the Ministry of  
648 Education, Youth and Sports of the Czech Republic.

649

## 650 **Competing Interest**

651 The authors declare that they have no conflict of interest.

652

## 653 **Data Availability Statement**

654 DNA reads, RNA reads and the complete 24 MAGs are available under a BioProject  
655 with accession number PRJEB70623. MAGs are available with accession numbers from  
656 ERS17699982 to ERS17699999.

657

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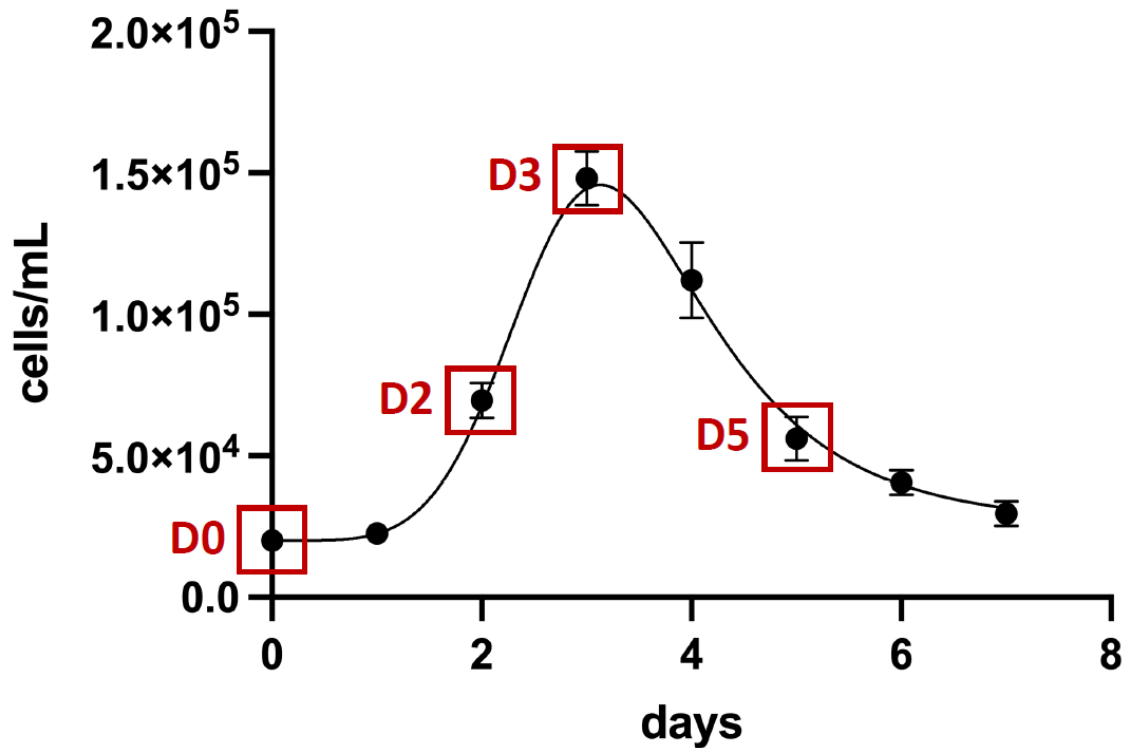
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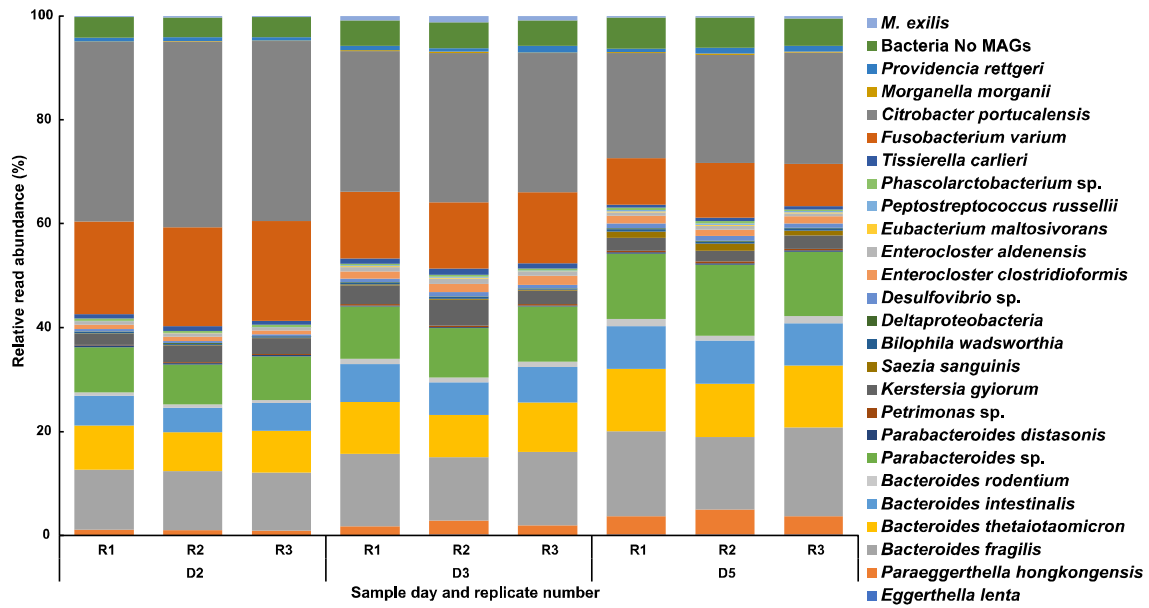


860 **Figure legends**



861

862 **Figure 1: Growth curve of *Monocercomonoides exilis* and sampling points.** Concentration  
863 of *M. exilis* (cells/mL<sup>-1</sup>) grown in TYSGM-9 medium for seven days. Samples for thorough  
864 analysis were taken on days 0, 2, 3 and 5, as highlighted by red squares. Error bars provide  
865 standard deviations of the values from three measurements.

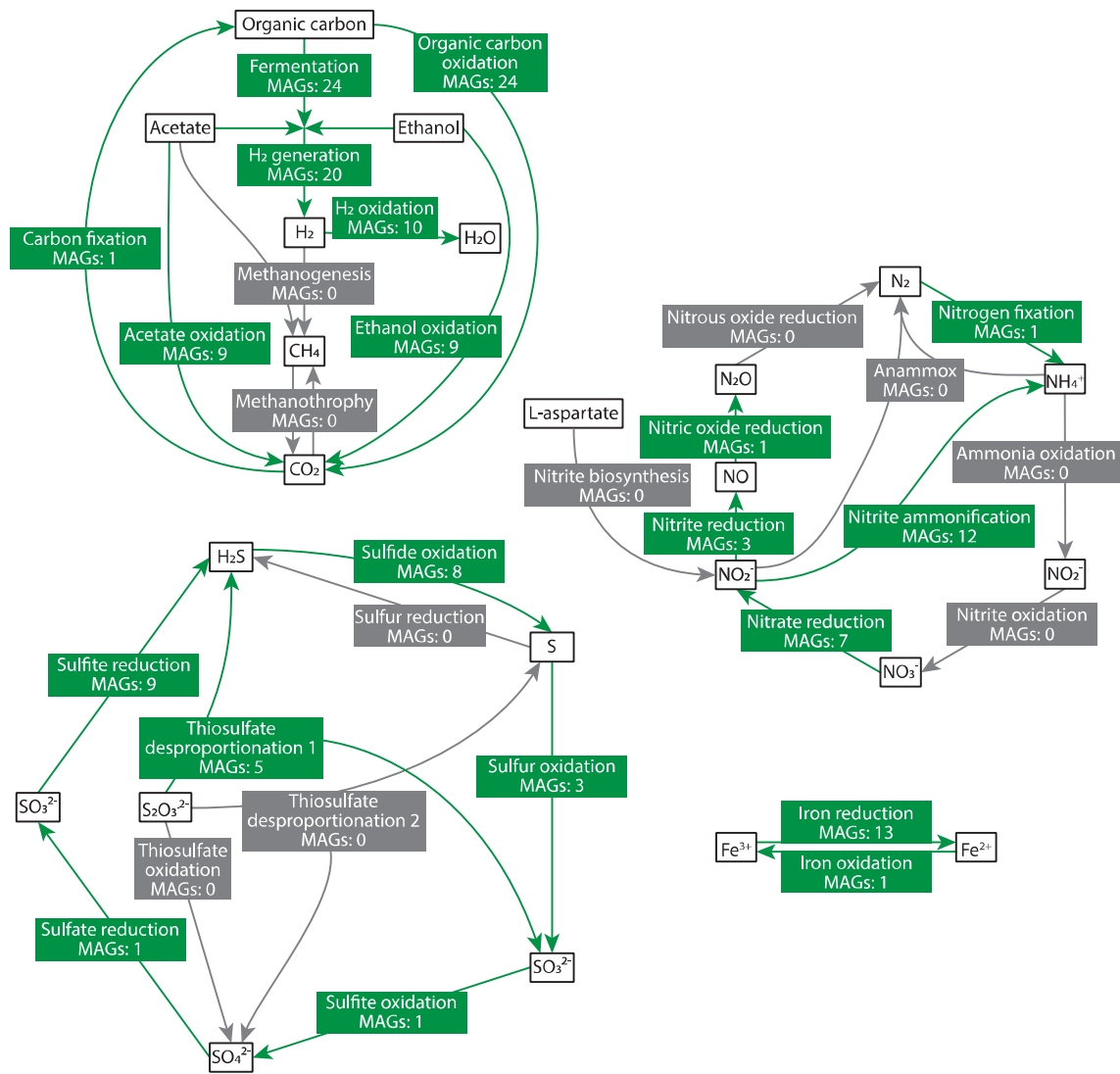


866

867 **Figure 2: Relative read abundance of the members of the community.** Each member of

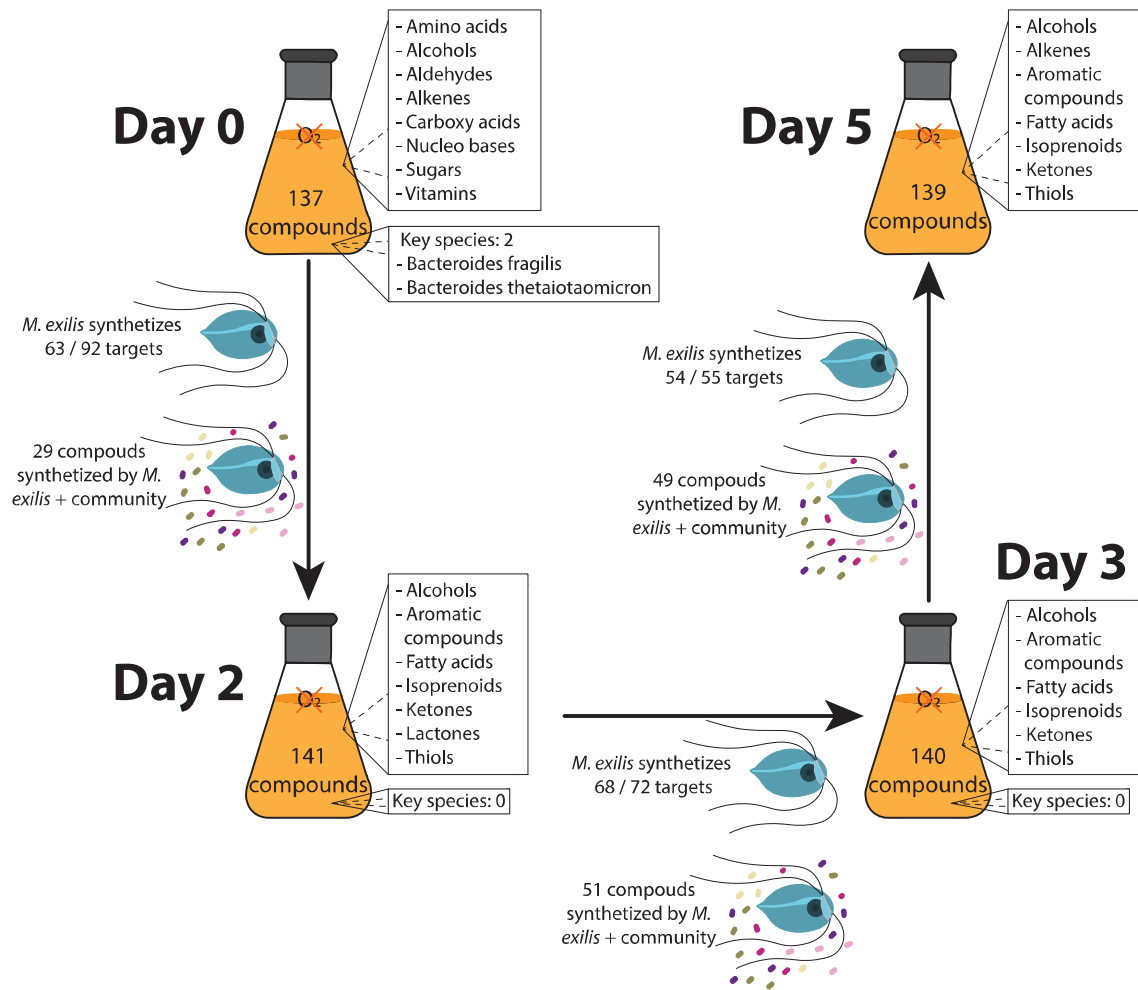
868 the community is represented as relative read abundance (%) corresponding to its genome on

869 each day and replicate.



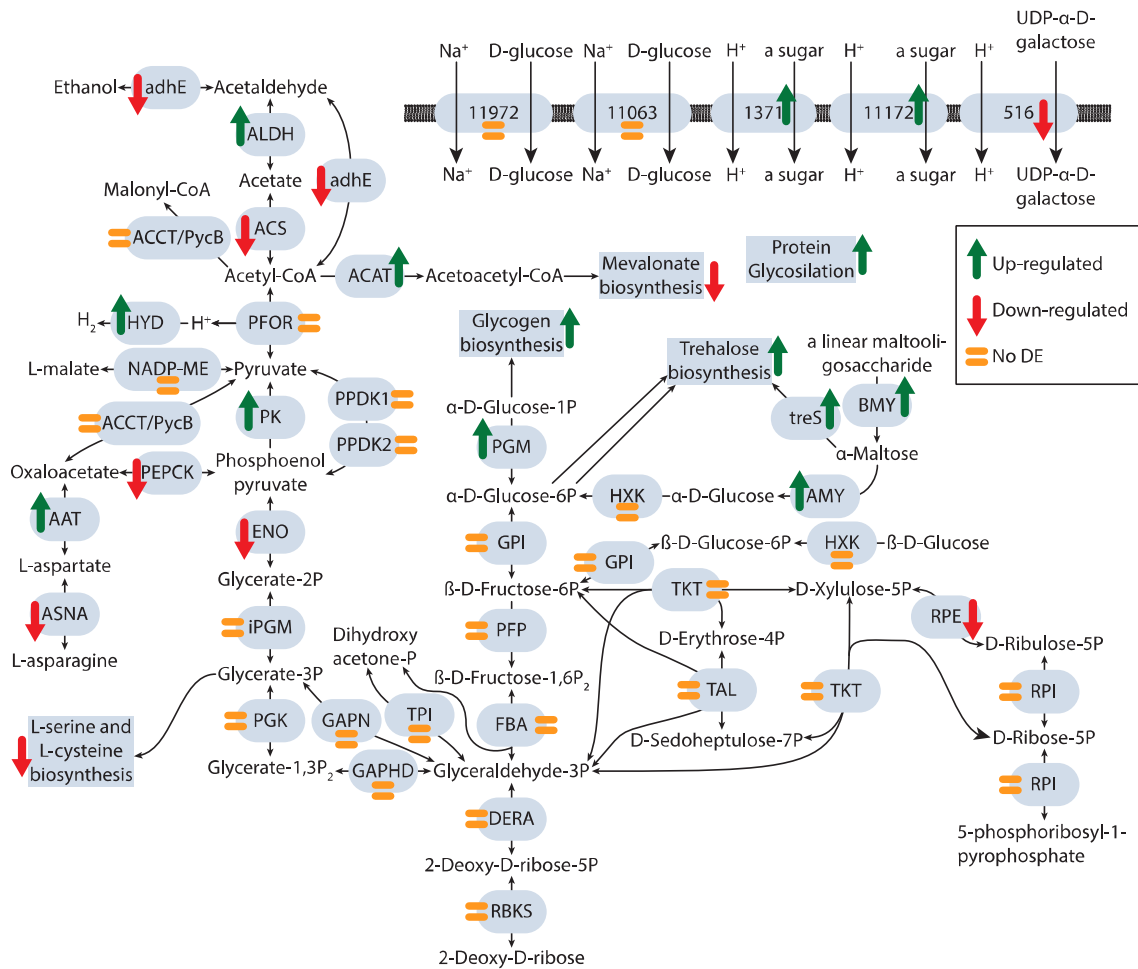
870

871 **Figure 3: Potential contribution of the bacterial community to the biochemical cycling**  
 872 **processes of carbon, nitrogen, iron and sulfur.** Labels represent the main steps. Present  
 873 steps are identified in green. Absent steps are identified in light grey. MAGs: number of  
 874 MAGs responsible for a step.

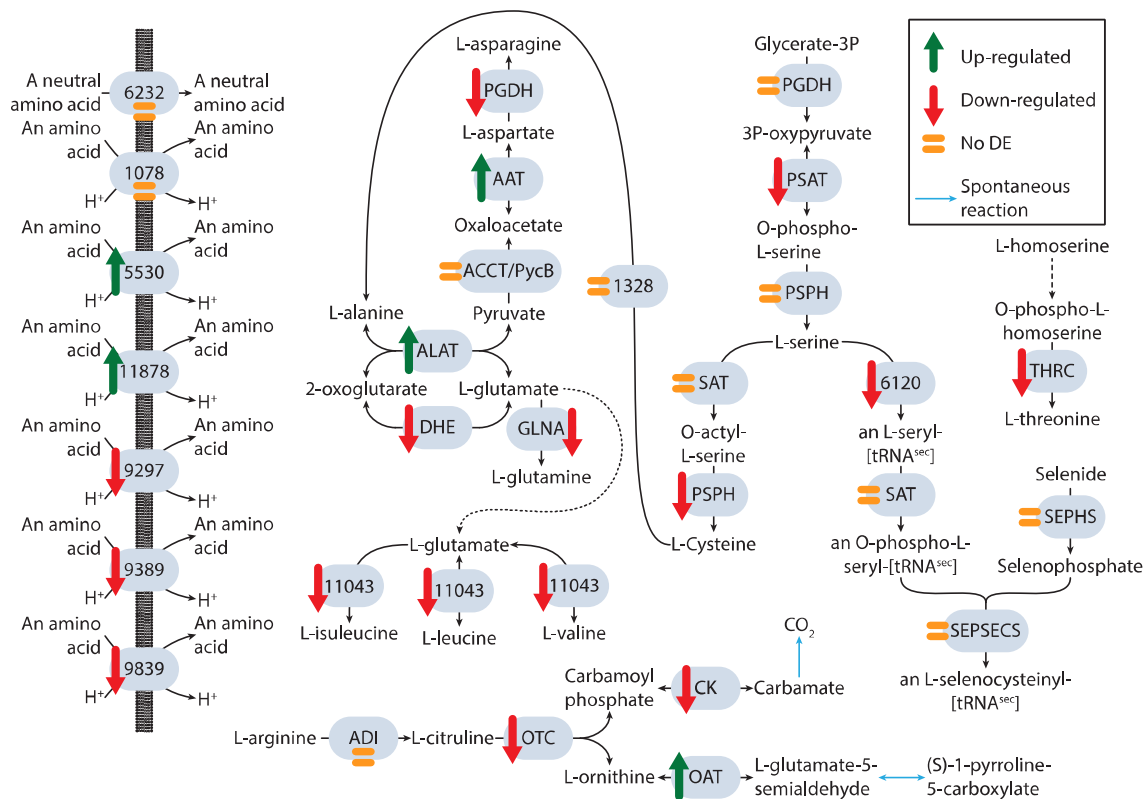


875

876 **Figure 4: Schematic summary of changes in the culture medium.** Chemical classification  
 877 of significant compounds of identified compounds per day (Supplementary Table 4), number  
 878 of compounds exhibiting an increase in concentration to the previous day (targets), number of  
 879 targets that *M. exilis* can synthesize independently, and number of compounds that can be  
 880 synthesized because of the interaction between *M. exilis* and the bacterial community are  
 881 shown. Key bacterial species identified by the m2m pipeline are shown for each pairwise  
 882 comparison.



895 Phosphoenolpyruvate carboxykinase; AAT: Aspartate aminotransferase; ASNA: Asparagine  
 896 synthetase; ENO: Enolase; iPGM: 2,3-bisphosphoglycerate independent phosphoglycerate  
 897 mutase; PGK: Phosphoglycerate kinase; GAPDH: Glyceraldehyde-3-phosphate  
 898 dehydrogenase; GAPN: NAD(P)-dependent glyceraldehyde-3-phosphate dehydrogenase;  
 899 TPI: Triose phosphate isomerase; DERA: Deoxyribose-phosphate aldolase; RBKS:  
 900 Ribokinase; FBA: Fructose-bisphosphate aldolase; PFP: Phosphofructokinase  
 901 (pyrophosphate-based); GPI: Glucose-6-phosphate isomerase; PGM: Phosphoglucomutase;  
 902 BMY:  $\beta$ -amylase; AMY:  $\alpha$ -amylase; treS: Maltose alpha-D-glucosyltransferase/ alpha-  
 903 amylase; HXK: Hexokinase; RPKD; ribose-phosphate diphosphokinase; RPI: Ribose-5-  
 904 phosphate isomerase; RPE: Ribulose-phosphate 3-epimerase; TKT: Transketolase; TAL:  
 905 Transaldolase.



909 importers, amino acid biosynthesis and arginine catabolism. Up-regulated genes and  
910 processes are marked with a green arrow pointing up. Down-regulated genes and processes  
911 are marked with a red arrow pointing down. Non-differentially expressed genes and  
912 processes are marked with an orange equal symbol. Spontaneous reactions are shown in blue.  
913 PGDH: Phosphoglycerate dehydrogenase; PSAT: Phosphoserine transaminase; PSPH:  
914 putative phosphoserine phosphatase; SAT: Serine O-acetyltransferase; SEPHS:  
915 Selenophosphate synthetase; SEPSECS: O-phospho-L-seryl-tRNA[Sec]:L-selenocysteinyl-  
916 tRNA synthase; THRC: Threonine synthase; ASNA: Asparagine synthetase; ALAT: Alanine  
917 aminotransferase; ACCT/PycB: putative malonyl-CoA:pyruvate transcarboxylase; AAT:  
918 Aspartate aminotransferase; ASNA: Asparagine synthetase; DHE: Glutamate dehydrogenase;  
919 GLNA: Glutamine synthetase; ADI: Arginine deiminase; OTC: Ornithine transcarbamylase:  
920 CK: Carbamate kinase; OAT: Lysine/Ornithine aminotransferase.