Abstract: The bacterial consumption of viruses not been reported on as of yet even though bacteria feed on almost anything. Viruses are widely distributed but have no acknowledged active biocontrol. Viral biomass undoubtedly reintegrates trophic cycles; however, the mechanisms of this phase still remain unknown. $^{13}$C-labelled T4 phages monitor the increase of the density of the bacterial DNA concomitant with the decrease of plaque forming units. We used $^{12}$C T4 phages as a control. T4 phage disappearance in wastewater sludge was found to occur mainly through predation by Aeromonadacea. Phage consumption also favours significant in situ bacterial growth. Furthermore, an isolated strain of Aeromonas was observed to grow on T4 phages as sole the source of carbon, nitrogen, and phosphorus. Bacterial species are capable of consuming bacteriophages in situ, which is likely a widespread and underestimated type of biocontrol. This assay is anticipated as a starting point for harnessing the bacterial potential in limiting the diffusion of harmful viruses within environments such as in the gut or in water.

Keywords: bacteriophage; Aeromonas; stable isotope probing

1. Introduction

For any type of bacteria, the presence of viruses may present a significant opportunity for feeding. Indeed, viruses represent 0.2 gigatons of carbon on Earth [1]. For example, the major capsid protein of the T4-like bacteriophage family is one of the most prevalent proteins in the biosphere [2]. Therefore, phages represent a major potential carbon source that bacteria may tap into. Furthermore, viruses are also a potential source of phosphorus [3].

No bacterium preying on viruses have been described even though bacterial extracellular proteases are able to degrade certain bacteriophages in anaerobic wastewater treatment plants, in pure cultures [4], and in soil [5]. In seawater, the only reported biotic pressure arises from marine ciliates that have been co-incubated with viruses and bacteria [6]. This observation is also supported by the recent discovery of viral DNA in free-living eukaryotic cells [7].

Here, we show that specific bacteria can indeed degrade T4 bacteriophages in situ, and we confirm this observation in pure culture.

2. Materials and Methods

2.1. Preparation of the $^{13}$C-Labeled T4 Bacteriophages

T4-phage particles labeled with $^{13}$C were produced on Escherichia coli B cells (DSM 613) grown in M9 minimal medium with $^{13}$C-glucose as the sole carbon source. The M9 medium was prepared using M9, Minimal Salts, 5X (Sigma-Aldrich, St. Louis, MO, USA), by
adding MgSO\(_4\) (Sigma-Aldrich) and CaCl\(_2\) (Sigma-Aldrich) at final concentrations of 1 mM and D-Glucose at a final concentration of 10 g/L. Moreover, additional salts were added to favor phage adsorption (a solution of CaCl\(_2\) 0.5 M and MgCl\(_2\) 1M diluted 1000 times in the culture medium). More precisely, starting from an \(E.\ coli\) stock of cells frozen in LB and glycerol, two successive overnight pre-cultures were grown in LB medium (LB broth, Fisher). Subsequently, 5 × 20 mL of M9 minimal medium containing D-Glucose-\(^{13}\)C\(_6\) as the sole carbon source (10 g/L) were each inoculated with 20 \(\mu\)L of the second \(E.\ coli\) pre-culture; approximately 1500 T4-phage particles (DSM 4505, in PFU) were added. Finally, 20 \(\mu\)L of a solution containing 0.5M CaCl\(_2\) and 1M MgCl\(_2\) was also added in each case to favor phage adsorption.

After 30 h of incubation at 37 °C under agitation, the T4 phage particles were collected: the cultures were centrifuged for 15 min at 5000 × g and at a temperature of 10 °C. The supernatants were collected and filtered with 0.22 \(\mu\)m pore-sized PES filters (Millipore, Burlington, MA, USA). They were subsequently incubated overnight in 8% w/v PEG 6000 and 0.5 M NaCl solution at 4 °C to precipitate viral particles. The supernatants were centrifuged at 20,000 × g for 30 min at 4 °C. The pellets were suspended in SM buffer (100 mM NaCl, 8 mM MgSO\(_4\), 50 mM Tris pH 7.5) and were centrifuged once more at 20,000 × g for 4h at 4 °C. The viral particles were finally suspended in 1.4 mL of SM buffer and were stored at 4 °C before use (Appendix C Figure A3 for details). To obtain unlabeled T4 phage particles, the same procedure was used, except unlabeled glucose was employed in the M9 minimal medium.

2.2. Incubation of \(^{13}\)C-Labeled T4 Bacteriophages with Aerobic Sludge

A 100 mL sample was taken from an aerobic wastewater treatment plant and was stabilized without the addition of substrate for one week at room temperature.

The T4 phages were incubated at 20 °C with 200 \(\mu\)L of the initial sample from the wastewater treatment plant under 50 rpm agitation. The initial concentration in the \(^{13}\)C bottle was 2.24 × 10\(^{10}\) T4 phages in 5 mL, i.e., 4.48 × 10\(^9\) T4 phages/mL. The PFU titers of the obtained; \(^{13}\)C- and unlabeled T4 stock solutions were determined on a bacterial lawn of \(E.\ coli\) cells (DSM 613) using the soft-agar overlay technique. More precisely, 5 \(\mu\)L of T4 phage stock solutions and 10-fold serial dilutions of those solutions were plated on a soft layer containing 7.5 g/L of Agar and \(E.\ coli\) cells (DSM 613) that had been pre-cultured in LB medium and in LB-Agar plates (15 g/L of Agar, Sigma-Aldrich). After a short drying period, the Petri dishes were incubated at 37 °C over 24 h in static conditions. The PFU titers were determined by counting the visible plaques and by calculating the concentration in the original stock solutions.

2.3. DNA Extraction and 16S rDNA Analysis

The three samples (the initial sample from the wastewater treatment plant, the \(^{12}\)C bottle after 24 h of incubation, and the \(^{13}\)C bottle after 24h of incubation) were used for ribosomal 16S DNA sequencing and analysis. After the addition of 3 × 10\(^5\) copies of internal standard [8], bead beating lysed the microbial cells, and the DNA was purified using the ZR-96 Soil Microbe DNA kit according to the manufacturer’s description (Zymo Research, Irvine, CA, USA). The V4-V5 region was amplified from purified genomic DNA with the primers 515F (5′-CTTTCCCTACACGACGCTCTTCCGATCTGTGYCAGCMGCCGCGGTA) and 928R (5′-GGAGTTCAGACGTGTGCTCTTCCGATCTCCCCGGYCAATTCTMTTTRAGT) using 30 amplification cycles with an annealing temperature of 65 °C (an amplicon of 510 bp, although length varies depending on the organisms). Because the Illumina MiSeq technology enables paired 300-bp reads, the ends of each read overlap and can be stitched together to generate extremely high-quality, full-length reads of the entire V4-V5 region in a single run. Single multiplexing was performed using a homemade 6 bp index, which were added during a second PCR with 12 cycles using a forward primer (AATGATACGGCGACCACGAGATCTACACTCTTTCCCTACACGAC) and a reverse primer (CAAGCAGAAGACCGCATACGAGAT-index-GTGACTGGAGTTCAAGACGTGT). The
resulting PCR products were purified and were loaded onto the Illumina MiSeq cartridge according to the manufacturer’s instructions. The quality of the run was checked internally using PhiX control as recommended by manufacturer, and then each pair-end sequence was assigned to its sample with the help of the previously integrated index. Each pair-end sequence was assembled using Flash software [9] using at least a 10bp-overlap between the forward and reverse sequences. The absence of contamination was checked with a negative control during the PCR (water as the template). The quality of the stitching procedure was controlled using four bacterial samples that are run routinely in the sequencing facility in parallel to the current samples.

The resulting sequences were analyzed using the DADA2 pipeline (maxN = 0, truncQ = 2, trimLeft = c(17,17), pool = “pseudo”) [10] with the Silva 138 database [11]. Chimeras were removed by means of the DADA2 using the consensus method. Normalisation was performed using the internal standard, and the total bacterial 16S rDNA was measured using qPCR (see below).

2.4. Detailed Calculations of the $^{13}$C Mass Balance

This paragraph explains the mass balance in detail. For a simpler explanation, here, we will focus on the calculations for the Amplicon Sequence Variant ASV1 (because it corresponds to the Aeromonas_isolate_007 that we used to confirm the consumption of T4 phages in pure culture).

**STEP1:** using qPCR, we determined the absolute abundance of total bacteria in the $^{13}$C bottle at the beginning of the experiment and at the end of the experiment. This could be conducted because we spiked $3 \times 10^5$ copies of a synthetic DNA standard to 200 µL of the initial sample [8], which we extracted immediately and quantified using 16S the universal primers 515F-928R with the Illumina adapters. We also quantified the spiked synthetic DNA standard by qPCR [8]. The ratio between the internal standard and the total bacterial 16S rDNA indicates the number of 16S rDNA copies in the extraction tube from the $^{13}$C bottle independently from the DNA recovery yield, which was estimated to be $2.35 \times 10^8$ copies of 16S rDNA. For an accurate mass balance, we also considered the fact that 240 µL were removed for the PFU measurements during the experiment, so we estimated that $2.47 \times 10^8$ copies of 16S rDNA would have been present at the end of the experiment in the $^{13}$C bottle if no sampling had been performed.

**STEP2:** Using the absolute abundance of total 16S rDNA from STEP1 and 16S bar-coding, we converted the proportion of each ASV into the absolute abundance of each ASV at the beginning and at the end of the experiment, thereby estimating the number of 16S rDNA copies produced during the experiment for each ASV. ASV1 was undetectable in the 3748 sequences of 16S rRNA genes obtained at the beginning of the experiment. At the end, 303 sequences out of 3578 (i.e., 8%) belonged ASV1, while 3578 sequences correspond to $2.47 \times 10^8$ copies of 16S rDNA, so we estimated that we had $2.47 \times 10^8 \times 303/3578 = 2.09 \times 10^7$ copies of *Aeromonas* 16S rDNA at the end of the experiment. For the two instances where the final 16SrDNA abundances were lower than the initial 16S rDNA abundances (possibly due to part of the population dying combined with another part showing small growth), we neglected the contribution of these ASVs to the $^{13}$C mass balance.

**STEP3:** We converted the increase in the 16S rDNA from STEP2 into the number of cells using a database that associates a 16S rDNA copy number with each bacterial genus [12]. This database associated ASV1 with 10 copies of 16S rDNA, so the $2.09 \times 10^7$ copies of 16S rDNA that were estimated in STEP2 actually correspond to $2.09 \times 10^6$ *Aeromonas* cells. It should be noted that the estimated generation time is within a realistic range: ASV 1 was undetectable at $t_0$, so if only 1 cell of ASV 1 (*Aeromonas* sp.) was present at the beginning of the experiment, 22 generations would have been needed in the course of the experiment to produce $2 \times 10^6$ cells, i.e., 62 min per generation. If ASV 1 was just below the detection limit ($1/3748 \times 9.78 \times 10^7)/10 = 2600$), the same rationale estimates an average growth rate of 125 min per generation.
STEP 4: We converted the number of cells to their carbon content. It should be noted that 30 fg of carbon was measured for the dried *Aeromonas* cells using a Leco CHN analyzer [12], but we assumed the same value for every ASV because the cellular carbon content varied between 20 and 40 fg, depending on the bacterial species. Assuming that each cell contained 30 fg of carbon, we converted the number of cells produced during the course of the experiment to a total carbon reservoir at the end of the experiment ($^{13}$C + $^{12}$C). *Aeromonas* had $2.09 \times 10^7$ cells at the end and a negligible amount at the beginning (undetectable). We then estimated the amount of total carbon that was captured by the growth of ASV1. For example, $2.09 \times 10^7$ *Aeromonas* cells translate into $6.28 \times 10^{-8}$ g of total carbon content.

STEP 5: Using the shifts in buoyant density between the $^{12}$C- and the $^{13}$C-bottles, we estimated the labeling level of each ASV so that we could estimate their contribution to the $^{13}$C mass balance. We compared the buoyant density of each ASV in the $^{13}$C bottle to the density of in the $^{12}$C bottle by fitting a normal curve to the absolute numbers of the 16S rRNA genes that were detected in each fraction. Since we measured the density of each fraction by refractometry, the mean of the normal curve is the best estimate of the actual buoyant density of the ASV. For example, the 16S DNA of ASV1 have a mean density of 1.72 in the $^{12}$C bottle and 1.75 in the $^{13}$C bottle with good fits ($R^2 = 0.98$ and 0.89). As mentioned in the text, ASV2 (identified as *Tolumonas* sp.) was not abundant enough in the $^{12}$C bottle to fit a normal curve on its distribution across the gradient, so we used its theoretical density based on its GC content (which shows a 15% error). The shift between the $^{12}$C- and $^{13}$C-densities was converted into a percentage of $^{13}$C by dividing by 0.036 [13]. The labeling level of ASV1 was $(1.75 - 1.72)/0.036 = 85\%$.

STEP 6: To complete the mass balance of the $^{13}$C-atoms of the labeled T4 phages, we multiplied the total carbon content of each species by its labeling level. For example, ASV1 (*Aeromonas* sp.) represents $6.28 \times 10^{-8} \times 0.85 = 5.33 \times 10^{-8}$ g of carbon.

STEP 7: To estimate the mass of the carbon needed to account for the carbon content observed for each species, we assumed that the carbon use efficiency for each ASV was 33\% (see pure culture experiment). It should be noted that we measured the yield for ASV1, and we then assumed the same yield for every ASV. Once corrected by the carbon use efficiency (i.e., the bacterial yield), the estimation of the $^{13}$C needed by each species was compared to the $3.2 \times 10^{-6}$ g of $^{13}$C incorporated in the $2.28 \times 10^{15}$C-labeled T4 phages (since each T4 viral particle contained $1.49 \times 10^{-16}$ g C, and we assumed that they were 100\% labeled with $^{13}$C because of their production method). For example, we estimated $2.09 \times 10^8$ newly synthesized ASV1 cells, which accounted for $6.28 \times 10^{-8}$ g $^{13}$C and therefore corresponded to 5\% of the $^{13}$C atoms that were initially present. Adding the contributions of the nine most-labeled ASVs accounted for 41\% of the initial mass of $^{13}$C.

2.5. Isolation of *Aeromonas_isolate_007* and Subsequent Experiments

Following the stable isotope probing experiment, we could isolate a strain of *Aeromonas* sp. (corresponding to the amplicon sequence variant ASV1) from the initial sample using the Aeromonas Isolation Agar medium (Sigma 17118) with ampicillin since Aeromonads are resistant to ampicillin. Therefore, we could confirm that *Aeromonas* sp. was indeed able to assimilate the carbon of the T4 phages. Furthermore, we could show that *Aeromonas* could use T4 phages as a carbon and nitrogen source, with a 33\% yield, and we also completed a scan the genome of *Aeromonas_isolate_007* for the putative mechanisms by which *Aeromonas* could capture and digest the T4 phage proteins and transfer the generated peptides into the intracellular space.

We incubated 50 *Aeromonas* cells with $10^{11}$ T4 phages in 1mL of SM buffer without gelatin (100 mM NaCl, 8 mM MgSO$_4$, 50 mM Tris HCl) at 20 °C to confirm the consumption of T4 phages by *Aeromonas* sp.
2.6. Sequencing of *Aeromonas_Isolate_007*

*Aeromonas* DNA was fragmented by sonication and sequencing adaptors were ligated. A total of eight cycles of PCR were applied to amplify the libraries. Library quality was assessed using an Advanced Analytical Fragment Analyzer, and the libraries were quantified by QPCR using the Kapa Library Quantification Kit. DNA-seq experiments were performed on an Illumina MiSeq using a paired-end read length of 2 × 300 pb with the Illumina MiSeq Reagent Kits v3. The sequences were quality trimmed with fastp v0.20.05, assembled by Spades v3.14.16 after removing the residual phiX by using bowtie2 v2.3.5.17 and filtering scaffolds smaller than the right insert size quantile 525 nt and coverage smaller than 50 ×.

3. Results
3.1. Stable Isotope Probing Experiment with T4 Bacteriophage
3.1.1. T4 Phages Support Bacterial Growth

To search for bacteriophage consumption activity, we chose wastewater because it contains a high bacterial diversity, high nutrient degradation/turnover rate, and a high microbial metabolic rate. In this work, the stable isotope probing method was applied by adding 2.2 × 10^{10} {^{13}}C-labelled T4 phages to 200 µL of sludge corresponding to 10^{8} bacteria cells. The enumerated T4 phages decreased by 99% in 24 h (Table 1), at which point the bacterial 16S rDNA genes were analyzed.

### Table 1. Decrease of the free {^{13}}C and {^{12}}C bacteriophages by PFU of the supernatant. We indicate the absolute numbers of phages and their percentages compared to t0.

| Time (min) | T4 Phages in {^{12}}C Bottle | T4 Phages in {^{13}}C Bottle | T4 Phages in {^{12}}C Bottle | T4 Phages in {^{13}}C Bottle |
|-----------|-------------------------------|-------------------------------|-------------------------------|-------------------------------|
| 0         | 2.8 × 10^{10}                 | 2.2 × 10^{10}                 | 100%                          | 100%                          |
| 24        | 5.2 × 10^{8}                  | 9.4 × 10^{5}                  | 1.9%                          | 4.2%                          |
| 122       | 2.6 × 10^{7}                  | 4.1 × 10^{6}                  | 0.9%                          | 1.8%                          |
| 445       | 3.2 × 10^{7}                  | 3.3 × 10^{5}                  | 1.2%                          | 1.5%                          |
| 1375      | 5 × 10^{2}                    | 5.7 × 10^{5}                  | 0%                            | 0%                            |

The decrease of T4 phages is concomitant with a 2.35-fold increase of the 16S rDNA genes (Table 2). Indeed, the comparison of the 16S and internal standard qPCR curves indicates that the initial sample contained 1.798^{23.462}/1.828^{13.215} = 326-fold more bacterial 16S rDNA copies than the internal standard, so the tube contained 3 × 10^{5} × 326 = 9.78 × 10^{7} copies of bacterial 16S at the beginning of the experiment. At the end of the experiment, a comparison of the 16S and internal standard qPCR curves indicates that bacterial 16S rDNA were 783-fold more abundant than the spiked synthetic standard, leading to an estimated 2.35 × 10^{8} copies of bacterial 16S rDNA genes in the {^{13}}C bottle. For an accurate mass balance, we also considered the fact that 240 µL were taken out for PFU measurements during the experiment, so we estimated that 2.47 × 10^{8} copies of 16S rDNA would have been present at the end of the experiment in the {^{13}}C bottle if no sampling had been performed. Since we used the LinReg software, we also accounted for the slight individual variations in qPCR efficiency, which were between 80 and 84% for the internal standard and between 80 and 82% for the bacterial 16S rRNA genes, respectively. In total, we estimated a global biomass increase of 2.35-fold. This global increase regroups the ASVs that were initially abundant and that have a tendency towards slight growth with the ASVs that are initially rare and grow massively.
Table 2. Quantification of the bacterial density with the internal standard at the beginning and at the end of the experiment.

| Sample Name    | Primers | Efficiency | Cycle Threshold | Spiked Internal Standard | 16S rDNA |
|----------------|---------|------------|-----------------|--------------------------|----------|
| Initial sample | Internal standard | 79.8%    | 23.462          | 3 × 10^5 copies           | 9.78 × 10^7 copies |
|                | V4V5     | 82.8%      | 13.215          |                          |          |
| Final 13C sample | Internal standard | 80.1%    | 14.133          | 3 × 10^5 copies           | 2.35 × 10^8 copies |
|                | V4V5     | 84.5%      | 24.461          |                          |          |

The bacterial growth concomitant to the decrease of T4 phages changes the composition of the microbial community: In particular ASV1 and ASV2 strongly increased (Figure 1 and Table 3), suggesting that these bacterial species are more adapted to the consumption of bacteriophages, assuming that any other substrates were consumed during the stabilization period before the experiment.

![Figure 1](image1.png)

Figure 1. Bacterial growth on T4 phages. (A): Identification of 13C-labeled bacteria: the 13C-labeled T4 (red) were incubated with a microbial community of a wastewater treatment plant in the same conditions as the 12C control (blue). (B): Bacteria present in each sample: the bar plots show the growth of each ASVs based on the 16S rDNA copies, detailing the nine bacteria assimilating T4 phages.

Table 3. Taxonomic affiliation of the ASVs that are significantly labeled with 13C. This table indicates the taxonomy of the labeled ASV performed by DADA2 with the Silva138 dataset. The taxonomy was also checked by means of blasting on the NCBI database.

| seq.ID.x | Class      | Order          | Family           | Genus        |
|----------|------------|----------------|------------------|--------------|
| ASV 2    | Gammaproteobacteria | Aeromonadales | Aeromonadaceae | Tolumonas Aeromonas |
| ASV 1    | Gammaproteobacteria | Aeromonadales | Aeromonadaceae | NA           |
| ASV 12   | Ignavibacteria    | Ignavibacteriales | PHOS-HE36 | NA           |
| ASV 21   | Ignavibacteria    | Ignavibacteriales | PHOS-HE36 | NA           |
| ASV 79   | Bacteroidia       | Chitinophagales | Sapropiraceae | Haliscomenobacter |
| ASV 20   | Bacteroidia       | Chitinophagales | Sapropiraceae | NA           |
| ASV 67   | Anaerolineae     | Ardenticatenales | NA     | NA           |
| ASV 7    | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | NA           |
| ASV 9    | Gammaproteobacteria | Burkholderiales | Rhodocyclaceae | Dechloromonas |

3.1.2. Increase of DNA Density of 9 Microbial Species after 24 h

The assimilation of phages by specific members of the bacterial community is confirmed by the increase in the DNA density of nine bacterial species as they assimilate the 13C labeled bacteriophages (Figure 2). About 41% of the 13C atoms initially present in the T4 phages were accounted for in the bacterial biomass. However, only 9 out of the 4046 microbial species—or more accurately, the Amplicon Sequence Variant (ASVs)—were labelled by the 13C initially contained in the T4 phages, thus suggesting that the incorporation of T4
phage is not a widespread ability. This incorporation is in agreement with the 2.35-fold growth and the disappearance of the T4 phages.

The two main degraders of the T4 phages were ASV1 (identified as *Aeromonas* sp.) and ASV2 (identified as *Tolumonas* sp.), which accounted for 5% and 29% of the $^{13}$C atoms found in the bacterial biomass, respectively. Both belong to the *Aeromonadaceae* family and exhibit strong growth rates. Indeed, both rose from undetectable levels to 51% of the biomass, while the density of their DNA increased because they incorporated $^{13}$C atoms from the isotopically labeled T4 phages. For example, the $2 \times 10^6$ *Aeromonas* cells present after 24 h contained 85% of the $^{13}$C atoms in their DNA, the density of which shifted from 1.72 g/mL to 1.75 g/mL in the bottle with the $^{13}$C-labeled T4 bacteriophages.

The mean buoyant density of ASV 2 (*Tolumonas* sp.) was estimated with the theoretical value in the $^{12}$C bottle, as ASV2 did not grow sufficiently in that bottle to fit a reliable Gaussian fit. The pie charts indicate the ratios of the nine $^{13}$C-labeled ASV in the $^{13}$C and $^{12}$C bottles after 24 h, and their proportion in the initial sample is reported in the X axis. For example, the pie of *Aeromonas* is roughly balanced because ASV 1 (*Aeromonas* sp.) represents 19% and 8% in the 12C and 13C bottles, respectively. In contrast, ASV 2 (*Tolumonas* sp.) only grew substantially in the $^{13}$C bottle (red).

**Figure 2.** The amount of DNA for the top nine ASVs (in ng) is plotted against the density of the Cesium gradient (in g/mL). The blue color is in the control bottle. The red color indicates the amount of DNA in the bottle supplemented with 3.2 µg of $^{13}$C in the $^{13}$C-labeled T4 bacteriophages. The dots indicate the actual measures performed after 24 h, i.e., the amount of DNA of each ASV (Qbit was combined with the 16S rDNA sequencing) in each fraction, the density of which was measured by means of refractometry. The lines indicate the Gaussian distributions to accurately estimate the mean buoyant density. For example, the pie of *Aeromonas* is roughly balanced because ASV 1 (*Aeromonas* sp.) represents 19% and 8% in the 12C and 13C bottles, respectively. In contrast, ASV 2 (*Tolumonas* sp.) only grew substantially in the $^{13}$C bottle (red).
from the isotopically labelled T4 phages. For example, the $2 \times 10^6$ Aeromonas cells present after 24 h contained 85% of the $^{13}$C atoms in their DNA, the density of which shifted from 1.72 g/mL to 1.75 g/mL in the bottle with the $^{13}$C-labeled T4 phages. The 16S rRNA sequences assigned to Aeromonas represented 19% and 8% of the total reads in the $^{12}$C and the $^{13}$C bottles, respectively, thus revealing a consistent growth from initially undetectable levels (Figure 1) in addition to the increase of their DNA density (Figure 2).

In addition to the Aeromonadaceae family, two species (ASV12 and 21) belonging to the Ignavibacteriales PHOS-HE36 family, although labelled with medium strength (49 and 71%) and negligible growth (0 and $5.87 \times 10^6$ synthetized cells, respectively), still gathered 5% of the $^{13}$C atoms (Table 4). The last five species with significant DNA density shifts (ASV7, 9, 20, 67, and 79) accounted for the remaining 1% of the $^{13}$C atoms, but their labelling level being below 25% may have resulted from indirect labelling.

**Table 4.** $^{13}$C mass balance of the isotopically labeled T4 bacteriophages. This table indicates the rationale for the $^{13}$C mass balance following the steps described above. For example, ASV 1 (Aeromonas sp.) represents 8% of the $2.47 \times 10^8$ 16S rDNA copies found at the end of the experiment, which represents 5% of the total amount of 13C present in the initial T4 phages because the 30fgC-cells labeled at 85% needed $5.3 \times 10^{-8}$ g of $^{13}$C if we consider a 33% yield.

| Description                                      | ASV 1     | ASV 2     | ASV 7     | ASV 9     | ASV 12    | ASV 20    | ASV 21    | ASV 67    | ASV 79    |
|--------------------------------------------------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|-----------|
| **STEP 1** Absolute number of 16S rDNA copies in | 9.78 $\times 10^7$ | 2.47 $\times 10^8$ |           |           |           |           |           |           |
| the initial sample                               |           |           |           |           |           |           |           |
| **STEP 2** Counts in the initial sample           | 0         | 0         | 148       | 132       | 197       | 123       | 0         | 46        | 53        |
| (out of 3748 sequences)                          |           |           |           |           |           |           |           |           |
| **STEP 3** Relative initial abundance of 16S     | 0%        | 0%        | 4%        | 4%        | 5%        | 3%        | 0%        | 1%        | 1%        |
| rDNA of each ASV                                 |           |           |           |           |           |           |           |           |
| **STEP 4** Absolute number of 16S rDNA copies in | 0         | 0         | $3.86 \times 10^6$ | $3.44 \times 10^6$ | $5.14 \times 10^6$ | $3.21 \times 10^6$ | 0         | $1.20 \times 10^6$ | $1.38 \times 10^6$ |
| the initial sample of each ASV                   |           |           |           |           |           |           |           |           |
| **STEP 5** Absolute number of 16S rDNA copies in | $2.09 \times 10^6$ | $1.07 \times 10^6$ | $2.55 \times 10^6$ | $4.28 \times 10^6$ | $3.59 \times 10^6$ | $3.80 \times 10^6$ | $3.80 \times 10^6$ | $2.83 \times 10^6$ | $3.80 \times 10^6$ |
| the final sample of each ASV                     |           |           |           |           |           |           |           |           |
| **STEP 6** Number of newly synthetized 16S copies | $2.09 \times 10^7$ | $1.07 \times 10^6$ | 0         | $8.36 \times 10^3$ | 0         | $5.87 \times 10^3$ | $3.80 \times 10^6$ | $1.63 \times 10^6$ | $2.41 \times 10^6$ |
| **STEP 7** Number of newly synthetized cells of | 2.09 $\times 10^6$ | 1.07 $\times 10^6$ | 0         | $2.09 \times 10^3$ | 0         | $2.94 \times 10^3$ | $3.80 \times 10^6$ | $8.15 \times 10^6$ | $1.21 \times 10^6$ |
| each ASV [14]                                    |           |           |           |           |           |           |           |           |
| **STEP 8** Carbon content (g/cell)                | 3.00 $\times 10^{-14}$ |           |           |           |           |           |           |           |
| **STEP 9** Total Carbon content in each ASV (g)  | $6.28 \times 10^{-8}$ | $3.20 \times 10^{-7}$ | 0         | $6.27 \times 10^{-9}$ | 0         | $8.81 \times 10^{-9}$ | $1.14 \times 10^{-7}$ | $2.45 \times 10^{-9}$ | $3.62 \times 10^{-9}$ |
Table 4. Cont.

| Description                                      | ASV 1 | ASV 2 | ASV 7 | ASV 9 | ASV 12 | ASV 20 | ASV 21 | ASV 67 | ASV 79 |
|--------------------------------------------------|-------|-------|-------|-------|--------|--------|--------|--------|--------|
| Mean 12C density (g/mL)                          | 1.72  | 1.7   | 1.72  | 1.72  | 1.68   | 1.71   | 1.68   | 1.72   | 1.72   |
| Goodness_fit_in_13C (R²)                         | 0.98  | 0.4   | 0.99  | 0.97  | 0.99   | 0.97   | 0.98   | 0.88   | 0.89   |
| Mean corrected 13C density                       | 1.72  | 1.71  | 1.72  | 1.72  | 1.68   | 1.71   | 1.68   | 1.72   | 1.72   |
| Mean 13C density                                 | 1.73  | 1.74  | 1.73  | 1.73  | 1.7    | 1.71   | 1.7    | 1.73   | 1.72   |
| Goodness_fit_in_13C (R²)                         | 0.89  | 0.99  | 0.9   | 0.89  | 0.98   | 0.93   | 0.99   | 0.94   | 0.94   |
| Labeling Level (%)                               | 85%   | 95%   | 10%   | 14%   | 71%    | 19%    | 49%    | 16%    | 23%    |
| 13C carbon content in each ASV (g)                | 5.3 × 10⁻⁸ | 3.0 × 10⁻⁷ | 0    | 8.8 × 10⁻¹⁰ | 0    | 1.7 × 10⁻⁹ | 5.6 × 10⁻⁸ | 3.9 × 10⁻⁹ | 8.3 × 10⁻⁹ |
| Carbon use efficiency (i.e., Bacterial yield)    |       |       |       |       |        |        |        |        | 0.33   |
| Contribution to the 13C mass balance (out of the 3.2 µg of 13C in the bacteriophages) | 5%<sup>1</sup> | 29%<sup>1</sup> | 0%<sup>1</sup> | 0%<sup>1</sup> | 0%     | 0%     | 5%     | 0%     | 1%     |
| 13C mass balance                                 |       |       |       |       |        |        |        |        | 41%    |

<sup>1</sup>, 83% of the predation of T4 phages is due to Gammaproteobacteria, which also include E. coli, the natural host of T4 phages.

3.2. Validation in a Pure Culture Experiment

3.2.1. Pure Culture with High Phages Concentrations

To confirm the quality of Aeromonas sp. as a predator of T4 phages, an Aeromonas-selective medium was used for retrieving an Aeromonas colony from the initial sludge. It is called Aeromonas_isolate_007 in the text below. The analysis of the whole genome confirmed that this isolate belongs to an intermediate clade between the Aeromonas media and Aeromonas rivipollensis species and that it has 10 copies of 16S rDNA based on the coverage ratio. Aeromonas_isolate_007 was incubated with T4 phages only as a substrate. Starting with 50 resting bacterial cells, the population reached 1.63 × 10⁸ cells after 24 h at 20 °C while consuming 10¹¹ T4 phages (Figure 3). No growth was observed when the T4 phages were absent from the SM buffer, and no degradation was observed when the bacterial cells were absent.

Figure 3. Aeromonas sp. growth on T4 phages: Aeromonas_isolate_007 isolated on the Aeromonas Isolation Agar medium grew on T4 phages as the sole carbon and nitrogen source. When a few Aeromonas cells were incubated with 10¹¹ T4 phages, the colony forming units (red) increased while the plaque-forming units (blue) decreased. The Aeromonas cell control in SM buffer without phages (grey) confirms that Aeromonas cannot use the Tris from the SM buffer as a carbon source.
3.2.2. Pure Culture with Low Phages Concentrations

Aeromonas sp. could also capture T4 phages when their concentrations were comparable with environmental conditions: $7 \times 10^4$ T4 phages/mL decreased to $2 \times 10^3$ T4 phages/mL when incubated with Aeromonas_isolate_007 cells. No decrease in the T7 phages have been observed in similar experiments where the T4 phages were replaced by T7 phages (data not shown).

3.2.3. Sequencing Aeromonas_isolate_007

Aeromonas_isolate_007 had a genome of 4,667,413 nt in 29 scaffolds with a N50 of 947,468 nt and an average size of 160,945 nt. This represents 4172 genes, and the number of 16S rRNA copies was estimated to be 10.89 with the coverage, which is in accordance with the Vetrovsky database. The whole genome sequencing of Aeromonas_isolate_007 by Illumina Miseq narrows down the phylogeny of the strain (Appendix A Figure A1) and offers suggestions with respect to degradative enzymes that may help bacteriophage digestion (Appendix A Figure A2).

4. Discussion

Aeromonas cells are widely distributed [15], including in wastewater treatment plants, where their abundance is around 0.1% [16]. Interestingly, Aeromonas cells have an S-layer [17] that is associated with lipopolysaccharides [18] and an outer membrane protein C (Appendix A Figure A2 and Appendix B Table A1), which are known to bind the T4 phages to the surface of E.coli cells [19]. Once captured at the surface, the phage is likely degraded by several extracellular enzymes, including DNase and protease [20]. For example, metallo- and serine-proteases found in Aeromonas are involved in the degradation of large molecules such as albumin, earning the nickname of “Jack-of-all-trades” due to this enzymatic versatility [21]. Finally, Aeromonas possesses transporters to uptake the resulting amino acids and peptides [21].

Bacterial predation on bacteriophages is rich in consequences because bacteriophages can control the abundance of specific bacterial species. Indeed bacteriophage decay is mainly considered abiogenic via adhesion to particulate material, chemical inactivation, or degradation by solar radiation or passive grazing by flagellates [6]. Here, we showed that there might be a non-negligible fraction of phage degradation that could be due to low-abundant bacterial species.

Furthermore, the diversity in bacteriophages could be partly related to the presence of phage-specific bacterial predation. Indeed, the bacterial predators of T4 phages do not appear to consume T7 bacteriophages. Therefore, a rapid increase of a specific phage in the environment could be specifically controlled by a phage-eating bacterium, forming a killing-the-killer loop. This is especially true as the two main degraders in our experiment were initially undetectable. Therefore, the control of the T4 phages at the end of our experiment is likely to be more pronounced than at the beginning of the experiment, thereby potentially changing the dynamics of E. coli and T4 if T4 was controlling E. coli in our environment.

In conclusion, specific bacteria that are capable of eliminating specific viruses changes our vision of food webs and represent a noteworthy avenue to explore to control harmful bacteriophages that disrupt dairy fermentation.

Author Contributions: Conceptualization, J.-J.G. and O.Z.; methodology, A.B., J.-J.G., and O.Z.; software, M.A.; validation, L.C., M.-A.P., J.-J.G., and O.Z.; formal analysis, J.-J.G. and O.Z.; investigation, A.B., J.-J.G., M.-A.P., H.N., and O.Z.; resources, A.B. and O.Z.; data curation, L.C.; writing—original draft preparation, J.-J.G. and O.Z.; writing—review and editing, all.; visualization, O.Z.; supervision, J.-J.G. and O.Z.; project administration, J.-J.G. and O.Z.; funding acquisition, O.Z. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.
Informed Consent Statement: Not applicable.

Data Availability Statement: High-throughput sequencing data have been deposited on NCBI (https://www.ncbi.nlm.nih.gov/bioproject) (accessed on 11 August 2021) under accession number PRJNA650397, and the genome of Aeromonas_isolate_007 is accessible with the BioSample accession number SAMN17689348.

Acknowledgments: We thank Caroline Achard for insights on bacteriophage capture and Martin Beaumont and Amira Bousleh for the qPCR of the internal standard. Electronic microscopy work benefited from the facilities and expertise of MIMA2 MET-GABI, INRA, Agroparistech, 78352 Jouy-en-Josas, France. Sequencing was performed in collaboration with the GeT core facility, Toulouse, France (http://get.genotoul.fr) (accessed on 11 August 2021), supported by the France Génomique National infrastructure, funded as part of “Investissement d’avenir” program managed by the Agence Nationale pour la Recherche (contract ANR-10-INBS-09). Figures were created by Biorender.

Conflicts of Interest: The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Appendix A

The analysis of the whole genome confirms that Aeromonas_Isolate_007_151020 belongs to an intermediate clade between the Aeromonas media and Aeromonas rivipollensis species.

Figure A1. Phylogenetic tree of Aeromonas_Isolate_007_151020.
Figure A2. Schematic representation of the capture, digestion, and absorption of the T4 phages by *Aeromonas* sp. Since T4 phages are large particles (not to scale in the figure), we assume that *Aeromonas* captures the T4 phages due to its similarity with the cell wall of *E. coli*. Once captured, the proteins of the capsid may be digested by the extracellular proteases present in *Aeromonas* sp. and the DNA could be degraded as well. The products of these degradations could be ingested by *Aeromonas* via common transporters.

Appendix B

The functional annotation was performed with RAST8, eggNOG-mapper (v2.0.0)9 and TMHMM10 to predict the extracellular location of the proteins. The results are reported in the table below.
Table A1. Annotation of the *Aeromonas* proteins putatively involved in the capture, digestion, and absorption of T4 phages, which are reported in the table below.

| Putative Function | RAST Protein Id | Contig Id | RAST Function | TMHMM Inside | TMHMM Transmembrane | TMHMM Outside |
|-------------------|-----------------|-----------|---------------|--------------|----------------------|---------------|
| CAPTURE           | fig|642.770.peg.2774 NODE_2_length_947468_cov_91.192050 | Outer_membrane_porin_OmpC | 1 | 1 | 1 |
|                   | fig|642.770.peg.1967 NODE_1_length_1964260_cov_90.024368 | 1 | 0 | 1 |
|                   | fig|642.770.peg.3181 NODE_3_length_439005_cov_94.027299 | 0 | 0 | 1 |
|                   | fig|642.770.peg.202 NODE_1_length_1964260_cov_90.024368 | putative_extracellular_serine_protease | 0 | 0 | 1 |
|                   | fig|642.770.peg.309 NODE_1_length_1964260_cov_90.024368 | Uncharacterized_protease_YhbU | 0 | 0 | 1 |
|                   | fig|642.770.peg.653 NODE_1_length_1964260_cov_90.024368 | Tail-specific_protease_precursor_(EC_3.4.21.102) | 0 | 0 | 1 |
|                   | fig|642.770.peg.664 NODE_1_length_1964260_cov_90.024368 | Lon_protease_homolog_YchZ | 0 | 0 | 1 |
|                   | fig|642.770.peg.903 NODE_1_length_1964260_cov_90.024368 | ATP-dependent_protease_La_(EC_3.4.21.53)_Type_I | 0 | 0 | 1 |
|                   | fig|642.770.peg.904 NODE_1_length_1964260_cov_90.024368 | ATP-dependent_Clp_protease_ATP-binding_subunit_ClpX | 0 | 0 | 1 |
|                   | fig|642.770.peg.905 NODE_1_length_1964260_cov_90.024368 | ATP-dependent_Clp_protease_proteolytic_subunit_ClpP_(EC_3.4.21.92) | 0 | 0 | 1 |
|                   | fig|642.770.peg.919 NODE_1_length_1964260_cov_90.024368 | Protease_III_precursor_(EC_3.4.24.55) | 0 | 0 | 1 |
|                   | fig|642.770.peg.976 NODE_1_length_1964260_cov_90.024368 | ATP-dependent_Clp_protease_ATP-binding_subunit_ClpA | 0 | 0 | 1 |
|                   | fig|642.770.peg.1312 NODE_1_length_1964260_cov_90.024368 | Protease_II_(EC_3.4.21.83) | 0 | 0 | 1 |
|                   | fig|642.770.peg.1793 NODE_1_length_1964260_cov_90.024368 | Uncharacterized_protease_YogQ | 0 | 0 | 1 |
| Extracellular     | fig|642.770.peg.1989 NODE_1_length_1964260_cov_90.024368 | Vibriolysin__extracellular_zinc_protease_(EC_3.4.24.25)_@_Pseudolysin__extracellular_zinc_protease_(EC_3.4.24.26) | 0 | 0 | 1 |
| DIGESTION of      | fig|642.770.peg.2147 NODE_2_length_947468_cov_91.192050 | Uncharacterized_protease_YdcP | 0 | 0 | 1 |
| large proteins    | fig|642.770.peg.3280 NODE_3_length_439005_cov_94.027299 | Protease_II_(EC_3.4.21.83) | 0 | 0 | 1 |
|                   | fig|642.770.peg.3795 NODE_5_length_206066_cov_93.597574 | ATP-dependent_hsl_protease_ATP-binding_subunit_HslU | 0 | 0 | 1 |
|                   | fig|642.770.peg.3796 NODE_5_length_206066_cov_93.597574 | ATP-dependent_protease_subunit_HslV_(EC_3.4.25.2) | 0 | 0 | 1 |
|                   | fig|642.770.peg.324 NODE_1_length_1964260_cov_90.024368 | Oligopeptidase_A_ | 0 | 0 | 1 |
|                   | fig|642.770.peg.3795 NODE_5_length_206066_cov_93.597574 | Oligopeptidase_A_(EC_3.4.24.70) | 0 | 0 | 1 |
|                   | fig|642.770.peg.667 NODE_10_length_72653_cov_95.226534 | Xaa-Pro_dipeptidase_PepQ_(EC_3.4.13.9) | 0 | 0 | 1 |
|                   | fig|642.770.peg.308 NODE_1_length_1964260_cov_90.024368 | Uncharacterized_peptidase_U32_family_member_YhbV | 0 | 0 | 1 |
|                   | fig|642.770.peg.319 NODE_1_length_1964260_cov_90.024368 | Peptidase_B_(EC_3.4.11.23) | 0 | 0 | 1 |
|                   | fig|642.770.peg.324 NODE_1_length_1964260_cov_90.024368 | Peptidase_B_(EC_3.4.11.23) | 0 | 0 | 1 |
|                   | fig|642.770.peg.496 NODE_1_length_1964260_cov_90.024368 | Aminopeptidase_PepA-related_protein | 0 | 0 | 1 |
|                   | fig|642.770.peg.654 NODE_1_length_1964260_cov_90.024368 | Membrane_alanine_aminopeptidase_N_(EC_3.4.11.2) | 0 | 0 | 1 |
|                   | fig|642.770.peg.1189 NODE_1_length_1964260_cov_90.024368 | Oligopeptidase_F-like_protein | 0 | 0 | 1 |
|                   | fig|642.770.peg.1360 NODE_1_length_1964260_cov_90.024368 | Tripeptide_aminopeptidase_(EC_3.4.11.4) | 0 | 0 | 1 |
|                   | fig|642.770.peg.1462 NODE_1_length_1964260_cov_90.024368 | Probable_endopeptidase_NlpC | 0 | 0 | 1 |
| Putative Function | RAST Protein Id | Contig Id | RAST Function | TMHMM Inside | TMHMM Transmembrane | TMHMM Outside |
|------------------|----------------|-----------|---------------|--------------|---------------------|---------------|
| **Extracellular DIGESTION of large proteins** | | | | | | |
| | fig|642.770.peg.1502 | NODE\_1\_length\_1964260\_cov\_90.024368 | FIG00905\_D\_D-carboxypeptidase\_family\_protein | 0 | 0 | 1 |
| | fig|642.770.peg.1638 | NODE\_1\_length\_1964260\_cov\_90.024368 | Peptidase\_M23/M37\_family | 0 | 0 | 1 |
| | fig|642.770.peg.1722 | NODE\_1\_length\_1964260\_cov\_90.024368 | Membrane\_proteins\_related\_to\_metalloendopeptidases | 0 | 0 | 1 |
| | fig|642.770.peg.1792 | NODE\_1\_length\_1964260\_cov\_90.024368 | L\_D-transpeptidase\_>YbiS | 0 | 0 | 1 |
| | fig|642.770.peg.1981 | NODE\_1\_length\_1964260\_cov\_90.024368 | L\_D-transpeptidase\_>YbiS | 0 | 0 | 1 |
| | fig|642.770.peg.2129 | NODE\_2\_length\_947468\_cov\_91.192050 | Alpha-aspartyl\_dipeptidase\_Peptidase\_E\_(EC\_3.4.13.21) | 0 | 0 | 1 |
| | fig|642.770.peg.2153 | NODE\_2\_length\_947468\_cov\_91.192050 | Thermostable\_carboxypeptidase\_1\_(EC\_3.4.17.19) | 0 | 0 | 1 |
| | fig|642.770.peg.2266 | NODE\_2\_length\_947468\_cov\_91.192050 | Methionine\_aminopeptidase\_(EC\_3.4.11.18) | 0 | 0 | 1 |
| | fig|642.770.peg.2291 | NODE\_2\_length\_947468\_cov\_91.192050 | Peptidase\_M13\_family | 0 | 0 | 1 |
| | fig|642.770.peg.2456 | NODE\_2\_length\_947468\_cov\_91.192050 | γ-glutamyltranspeptidase\_(EC\_2.3.2.2)\_@\_Glutathione\_hydrolyase\_(EC\_3.4.19.13) | 0 | 0 | 1 |
| | fig|642.770.peg.2506 | NODE\_4\_length\_411610\_cov\_92.800192 | Xaa-Pro\_aminopeptidase\_(EC\_3.4.11.9) | 0 | 0 | 1 |
| | fig|642.770.peg.2556 | NODE\_4\_length\_411610\_cov\_92.800192 | Prolyl\_endopeptidase\_(EC\_3.4.21.26) | 0 | 0 | 1 |
| | fig|642.770.peg.4017 | NODE\_6\_length\_154685\_cov\_89.330397 | Oligoendopeptidase\_F-like\_protein | 0 | 0 | 1 |
| | fig|642.770.peg.4147 | NODE\_7\_length\_126480\_cov\_93.962673 | Bacterial\_leucyl\_aminopeptidase\_(EC\_3.4.11.10) | 0 | 0 | 1 |
| **DNA DIGESTION** | | | | | | |
| | fig|642.770.peg.760 | NODE\_1\_length\_1964260\_cov\_90.024368 | Extracellular\_and\_/or\_outer\_membrane\_deoxyribonuclease\_NucH/SO1066 | 0 | 0 | 1 |
| | fig|642.770.peg.1409 | NODE\_1\_length\_1964260\_cov\_90.024368 | UPT0294\_protein\_YafD\_(exo\_-\_and\_endo\_-\_nuclease\_family) | 0 | 0 | 1 |
| | fig|642.770.peg.1884 | NODE\_1\_length\_1964260\_cov\_90.024368 | DNA/RNA\_endonuclease\_G | 1 | 1 | 1 |
| | fig|642.770.peg.1925 | NODE\_1\_length\_1964260\_cov\_90.024368 | Extracellular\_and\_/or\_outer\_membrane\_deoxyribonuclease\_NucH/SO1066 | 0 | 0 | 1 |
| | fig|642.770.peg.2135 | NODE\_2\_length\_947468\_cov\_91.192050 | Succinyl-CoA\_synthetase\_\_alpha\_subunit | 0 | 0 | 1 |
| | fig|642.770.peg.274 | NODE\_1\_length\_1964260\_cov\_90.024368 | ABC\_transporter\_permease\_protein\_1\_(cluster\_5\_\_nickel\_/peptides/opines) | 4 | 6 | 3 |
| | fig|642.770.peg.275 | NODE\_1\_length\_1964260\_cov\_90.024368 | ABC\_transporter\_permease\_protein\_2\_(cluster\_5\_\_nickel\_/peptides/opines) | 3 | 5 | 3 |
| | fig|642.770.peg.1213 | NODE\_1\_length\_1964260\_cov\_90.024368 | Oligopeptide\_ABC\_transporter\_permease\_protein\_OppC\_(TC\_3.A.15.1) | 4 | 6 | 3 |
| | fig|642.770.peg.1214 | NODE\_1\_length\_1964260\_cov\_90.024368 | Oligopeptide\_ABC\_transporter\_permease\_protein\_OppB\_(TC\_3.A.15.1) | 4 | 6 | 3 |
| | fig|642.770.peg.1215 | NODE\_1\_length\_1964260\_cov\_90.024368 | Oligopeptide\_ABC\_transporter\_substrate-binding\_protein\_OppA\_(TC\_3.A.15.1) | 1 | 1 | 1 |
| **Peptide TRANSPORT into the cell** | | | | | | |
| | fig|642.770.peg.1819 | NODE\_1\_length\_1964260\_cov\_90.024368 | Dipeptide\_ABC\_transporter\_permease\_protein\_DppC\_(TC\_3.A.15.2) | 4 | 6 | 3 |
| | fig|642.770.peg.1820 | NODE\_1\_length\_1964260\_cov\_90.024368 | ABC\_transporter\_permease\_protein\_1\_(cluster\_5\_\_nickel\_/peptides/opines) | 4 | 6 | 3 |
| | fig|642.770.peg.1913 | NODE\_1\_length\_1964260\_cov\_90.024368 | ABC\_transporter\_permease\_protein\_2\_(cluster\_5\_\_nickel\_/peptides/opines) | 4 | 6 | 3 |
| | fig|642.770.peg.1914 | NODE\_1\_length\_1964260\_cov\_90.024368 | ABC\_transporter\_permease\_protein\_1\_(cluster\_5\_\_nickel\_/peptides/opines) | 4 | 6 | 3 |
Table A1. Cont.

| Putative Function | RAST Protein Id       | Contig Id                        | RAST Function                                             | TMHMM Inside | TMHMM Transmembrane | TMHMM Outside |
|-------------------|-----------------------|----------------------------------|-----------------------------------------------------------|--------------|---------------------|---------------|
| DNA TRANSPORT     | NODE_3_length_439005_cov_94.027299 | fig|642.770.peg.2922 NODE_3_length_439005_cov_94.027299 | Na_+_dependent_nucleoside_transporter_NupC | 5            | 9                   | 4             |
|                   | NODE_3_length_439005_cov_94.027299 | fig|642.770.peg.2967 NODE_3_length_439005_cov_94.027299 | Na_+_dependent_nucleoside_transporter_NupC | 4            | 8                   | 4             |
|                   | NODE_3_length_439005_cov_94.027299 | fig|642.770.peg.3112 NODE_3_length_439005_cov_94.027299 | Na_+_dependent_nucleoside_transporter_NupC | 4            | 8                   | 4             |
| DNA TRANSPORT     | NODE_7_length_126480_cov_93.962673 | fig|642.770.peg.4145 NODE_7_length_126480_cov_93.962673 | Predicted_nucleoside_ABC_transporter__permease_1_component | 6            | 10                  | 5             |
| DNA TRANSPORT     | NODE_7_length_126480_cov_93.962673 | fig|642.770.peg.4146 NODE_7_length_126480_cov_93.962673 | Predicted_nucleoside_ABC_transporter__permease_2_component | 4            | 7                   | 4             |
| DNA TRANSPORT     | NODE_13_length_22868_cov_91.969299 | fig|642.770.peg.177 NODE_13_length_22868_cov_91.969299 | DNA_uptake_protein | 0            | 0                   | 1             |
Appendix C

Appendix C describes the purity of the initial solution of T4 phages.

T4-phage particles labeled with $^{13}$C were produced on *Escherichia coli* B cells (DSM 613) grown in M9 minimal medium with $^{13}$C-glucose as the sole carbon source. The M9 medium was prepared using M9, Minimal Salts, 5× (Sigma-Aldrich) by adding MgSO$_4$ (Sigma-Aldrich) and CaCl$_2$ (Sigma-Aldrich) at final concentrations of 1 mM and D-Glucose at a final concentration of 10 g/L. Moreover, additional salts were added to favor phage adsorption (solution of CaCl$_2$ 0.5 M and MgCl$_2$ 1M, diluted 1 000 times in the culture medium). More precisely, starting from an *E. coli* stock of cells frozen in LB and glycerol, two successive overnight pre-cultures were grown in LB medium (LB broth, Fisher, Camas, WA, USA). Subsequently, 5 × 20 mL of M9 minimal medium containing D-Glucose-$^{13}$C$_6$ as the sole carbon source (10 g/L) were each inoculated with 20 µL of the second *E. coli* pre-culture; approximately 1500 T4-phage particles (DSM 4505, in PFU) were added. Finally, 20 µL of a solution containing 0.5 M CaCl$_2$ and 1 M MgCl$_2$ were also added in each case to favor phage adsorption.

After 30 h of incubation at 37 °C under 50 rpm agitation to allow the phages to replicate, the T4 phage particles were collected: the cultures were centrifuged for 15 min at $5000 \times g$ at 10 °C to eliminate the large cellular debris. The supernatants were collected and filtered through 0.22 µm pore-sized PES filters (Milipore, Burlington, MA, USA) to eliminate fine cellular debris. They were subsequently incubated overnight in 8% w/v PEG 6000 and 0.5 M NaCl solution at 4 °C to aggregate the viral particles with minimal losses. The phage aggregates were collected by centrifugation at $20,000 \times g$ for 30 min at 4 °C. The pellets were resuspended in SM buffer (100 mM NaCl, 8 mM MgSO$_4$, 50 mM Tris pH 7.5), and were centrifuged once more at $20,000 \times g$ for 4h at 4 °C. The viral particles were finally suspended in 1.4 mL of SM buffer and were stored at 4 °C before use.

To obtain the unlabeled T4 phage particles, the same procedure was used, except unlabeled glucose was employed in the M9 minimal medium.
and were centrifuged once more at 20,000×g for 4h at 4 °C. The viral particles were finally suspended in 1.4 mL of SM buffer and were stored at 4 °C before use. To obtain the unlabeled T4 phage particles, the same procedure was used, except unlabeled glucose was employed in the M9 minimal medium.

Figure A3. Purity of the bacteriophage preparation. We checked the purity of the bacteriophage preparation by means of electronic microscopy. Materials were directly adsorbed onto a carbon film membrane on a 300-mesh copper grid, stained with 1% uranyl acetate, dissolved in distilled water, and dried at room temperature. Grids were examined with a Hitachi HT7700 electron microscope operated at 80kV (Elexience—France), and the images were acquired with a charge-coupled device camera (AMT).

References
1. Bar-On, Y.M.; Phillips, R.; Milo, R. The biomass distribution on Earth. Proc. Natl. Acad. Sci. USA 2018, 115, 6506-6511. [CrossRef]
2. Comeau, A.M.; Krisch, H.M. The capsid of the T4 phage superfamily: The evolution, diversity, and structure of some of the most prevalent proteins in the biosphere. Mol. Biol. Evol. 2008, 25, 1321-1332. [CrossRef]
3. Jover, L.F.; Effler, T.C.; Buchan, A.; Wilhelm, S.W.; Weitz, J.S. The elemental composition of virus particles: Implications for marine biogeochemical cycles. Nat. Rev. Microbiol. 2014, 12, 519–528. [CrossRef] [PubMed]
4. Mondal, T.; Rouch, D.A.; Thurbon, N.; Smith, S.R.; Deighton, M.A. Factors affecting decay of Salmonella Birkenhead and coliphage MS2 during mesophilic anaerobic digestion and air drying of sewage sludge. J. Water Health 2015, 13, 459–472. [CrossRef] [PubMed]
5. Nasser, A.M.; Glozman, R.; Nitzan, Y. Contribution of microbial activity to virus reduction in saturated soil. Water Res. 2002, 36, 2589–2595. [CrossRef]
6. Gonzalez, J.M.; Suttle, C.A. Grazing by Marine Nanoflagellates on Viruses and Virus-Sized Particles—Ingestion and Digestion. Mar. Ecol. Prog. Ser. 1993, 94, 1–10. [CrossRef]
7. Brown, J.M.; Labonté, J.M.; Brown, J.; Record, N.R.; Poulton, N.J.; Sieracki, M.E.; Logares, R.; Stepanauskas, R. Single Cell Genomics Reveals Viruses Consumed by Marine Protists. Front. Microbiol. 2020, 11, 524828. [CrossRef]
8. Zemb, O.; Achard, C.S.; Hamelin, J.; De Almeida, M.-L.; Gabinaud, B.; Cauquil, L.; Verschuren, L.M.G.; Godon, J.-J. Absolute quantitation of microbes using 16S rRNA gene metabarcoding: A rapid normalization of relative abundances by quantitative PCR targeting a 16S rRNA gene spike-in standard. Microbiologyopen 2020, 9, e977. [CrossRef] [PubMed]
9. Magoc, T.; Salzberg, S.L. FLASH: Fast length adjustment of short reads to improve genome assemblies. Bioinformatics 2011, 27, 2957–2963. [CrossRef]
10. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. Nat. Methods 2016, 13, 581–583. [CrossRef]
11. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glockner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. Nucleic Acids Res. 2013, 41, D590–D596. [CrossRef]
12. Větrovský, T.; Baldrian, P. The Variability of the 16S rRNA Gene in Bacterial Genomes and Its Consequences for Bacterial Community Analyses. PloS ONE 2013, 8, e57923. [CrossRef] [PubMed]
13. Trousselier, M.; Bouvy, M.; Courties, C.; Dupuy, C. Variation of carbon content among bacterial species under starvation condition. Aquat. Microb. Ecol. 1997, 13, 113–119. [CrossRef]
14. Buckley, D.H.; Huangyutitham, V.; Hsu, S.F.; Nelson, T.A. Stable isotope probing with 15N achieved by disentangling the effects of genome G+C content and isotope enrichment on DNA density. *Appl. Environ. Microbiol.* **2007**, *73*, 3189–3195. [CrossRef] [PubMed]

15. Janda, J.M.; Abbott, S.L. The genus *Aeromonas*: Taxonomy, pathogenicity, and infection. *Clin. Microbiol. Rev.* **2010**, *23*, 35–73. [CrossRef] [PubMed]

16. Ye, L.; Zhang, T.; Wang, T.; Fang, Z. Microbial structures, functions, and metabolic pathways in wastewater treatment bioreactors revealed using high-throughput sequencing. *Environ. Sci. Technol.* **2012**, *46*, 13244–13252. [CrossRef] [PubMed]

17. Noonan, B.; Trust, T.J. The synthesis, secretion and role in virulence of the paracrystalline surface protein layers of *Aeromonas salmonicida* and *A. hydrophila*. *FEMS Microbiol. Lett.* **1997**, *154*, 1–7. [CrossRef] [PubMed]

18. Sleytr, U.B.; Schuster, B.; Egelseer, E.-M.; Pum, D. S-layers: Principles and applications. *FEMS Microbiol. Rev.* **2014**, *38*, 823–864. [CrossRef]

19. Islam, M.Z.; Fokine, A.; Mahalingam, M.; Zhang, Z.; Garcia-Doval, C.; van Raaij, M.J.; Rossmann, M.G.; Rao, V.B. Molecular anatomy of the receptor binding module of a bacteriophage long tail fiber. *PLoS Pathog.* **2019**, *15*, e1008193. [CrossRef]

20. Janda, J.M. Biochemical and exoenzymatic properties of *Aeromonas* species. *Diagn. Microbiol. Infect. Dis.* **1985**, *3*, 223–232. [CrossRef]

21. Seshadri, R.; Joseph, S.W.; Chopra, A.K.; Sha, J.; Shaw, J.; Graf, J.; Haft, D.; Wu, M.; Ren, Q.; Rosovitz, M.J.; et al. Genome Sequence of *Aeromonas hydrophila* ATCC 7966*: Jack of All Trades. *J. Bacteriol.* **2006**, *188*, 8272–8282. [CrossRef] [PubMed]