Peer Community Journal

Section: Microbiology

Research article

Published
2026-09-11

Cite as
Kelvin Gautier, Pauline Pattyn, Amani Briki, Audrey Soric and Romain Irague (2026) *A newly identified* Comamonas serinivorans *strain for PHA production from sterile-filtered dark-fermentation-derived volatile fatty acids: isolation, metabolic profiling and industrial prospects.*, Peer Community Journal, 6: e90.

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Peer-review
Peer reviewed and recommended by PCI Microbiology, https://doi.org/10.24072/pci.microbiol.100504

A newly identified *Comamonas serinivorans* strain for PHA production from sterile-filtered dark-fermentation-derived volatile fatty acids: isolation, metabolic profiling and industrial prospects.

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Volume **6** (2026), article e90

https://doi.org/10.24072/pcjournal.785

Abstract

Dark-fermentation for hydrogen production leaves a substantial fraction of substrate carbon unexploited, with a large fraction remaining as volatile fatty acids (VFAs), mainly acetate and butyrate. To improve process valorisation, this study investigates the microbial conversion of fermentation-derived VFAs into polyhydroxyalkanoates (PHAs). A PHA-producing strain was isolated from dairy wastewater sludge using a feast-famine enrichment strategy and identified as *Comamonas serinivorans* ATH based on 16S rRNA gene sequencing. The strain exhibited strict specialisation towards organic acids, efficiently assimilating VFAs but not carbohydrates. Under nutrient limitation, intracellular PHA accumulation reached up to 93% of cell dry weight. When cultivated in a sterile-filtered dark-fermentation stream, the strain consumed both acetate and butyrate and produced PHA with a yield of 0.54 g/g of consumed VFAs under non-optimised conditions. GC-MS analysis confirmed that *Comamonas serinivorans* ATH produces a polymer mainly composed of 3-hydroxybutyrate monomers. These findings demonstrate the feasibility of coupling hydrogen-producing dark-fermentation with PHA synthesis, supporting an integrated biorefinery approach for sustainable bioplastic production from waste-derived carbon streams.

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http://www.centre-mersenne.org/

e-ISSN 2804-3871

Introduction

Dark-fermentation is widely investigated for dihydrogen (H_2) production from agro-industrial waste streams. Anaerobic bacteria, particularly Clostridium spp., are among the most important and studied microorganisms involved in this biotransformation process. Recently, our group isolated the strain *Clostridium beijerinckii* C.sp.1.3 for its ability to convert lactose from dairy waste into H_2 (Mete et al., 2024). Although waste-to-hydrogen pathways are attractive from circular economy and carbon balance perspectives, techno-economic analyses indicate that feedstock valorisation alone does not ensure profitability. Costs are mainly driven by CAPEX and OPEX rather than substrate price, with reported production costs ranging from 3.2 to 48.96 $/kg of bio$H_2$, depending on process assumptions, highlighting persistent economic uncertainty (Teke et al., 2024).

A fundamental limitation of dark-fermentation lies in the mass distribution of the process. Mass balances typically show that less than 4% of the substrate is converted into H_2, while 50-60% remains as volatile fatty acids (VFAs), mainly acetate and butyrate. The remainder is incorporated into biomass or released as CO_2. Consequently, operating dark-fermentation solely for H_2 production exploits only a minor portion of the process potential. Improving economic viability therefore requires moving beyond a single-product perspective.

Volatile fatty acids are valuable short-chain organic acids with a wide range of industrial applications such as in food, chemical, cosmetic and pharmaceutical industries (Atasoy et al., 2018). Such versatility underpins the large global market for these acids, which represents a multi-billion-dollar industry that continues to grow steadily due to expanding demand across diverse industrial sectors (Saratale et al., 2021). Beyond these traditional applications, an alternative valorisation route for VFAs lies in microbial conversion to polyhydroxyalkanoates (PHAs), biodegradable polymers that offer a sustainable alternative to conventional plastics (Chai et al., 2021). PHAs can be biosynthesised from organic waste-derived VFAs, linking waste valorisation with bioplastic production. Recent reviews highlight progress in technical processes, metabolic pathways, and techno-economic considerations for implementing VFAs-to-PHA production at scale (Crutchik et al., 2020; Saratale et al., 2021; Saravanan et al., 2022; Sekoai et al., 2021; Sepúlveda & Seeger, 2025). The industrial relevance of this approach is reinforced by the rapid growth of the bioplastics market. Global production capacity currently exceeds 2 million tonnes per year and is projected to surpass 4 million tonnes by 2030 (European Bioplastics association, 2025). Within this market, PHAs currently account for only around 4.7% of total bioplastics production but are projected to reach 16.8% by 2030, making PHAs the only major bioplastics group expected to undergo a substantial increase in production capacity. However, production costs remain higher than those of conventional petrochemical plastics, making the use of low-cost, waste-derived substrates such as dark-fermentation effluents strategically important (Gundlapalli & Ganesan, 2025).

The present study focuses on the isolation and characterisation of a novel strain affiliated with *Comamonas serinivorans*, capable of efficiently assimilating acetate- and butyrate-rich streams derived from dark-fermentation. By demonstrating high intracellular PHA accumulation from a real, sterile-filtered, VFA-rich fermentation stream, this work highlights the complementarity between H_2-producing dark-fermentation and subsequent biopolymer synthesis, transforming a carbon-rich by-product stream into a valuable and sustainable material resource.

Materials and methods

PHA producer enrichment and isolation

A sequencing batch reactor (SBR) was used to enrich a PHA-producing mixed microbial culture. A glass reactor with a working volume of 200 mL, immersed in a water bath regulated at 30°C, was used for the culture. Enrichment was initiated by mixing activated sludge (inoculum), collected from a dairy treatment plant, with fresh culture medium in a 1:1 ratio. The SBR was

operated under a feast-famine regime. A total of 13 cycles were applied. Each cycle consisted of a 47h reaction phase during which the reactor was stirred (150 rpm) and aerated (0.1 L/min of filtered air). Neither pH nor dissolved oxygen was regulated. This phase was followed by a 1h rest phase, without agitation or aeration, to allow the flocculated PHA-producing strains to settle. At the end of the cycle, the top half of the reaction volume was withdrawn and replaced with the same volume of fresh culture medium to initiate a new enrichment cycle, with the flocculates acting as the inoculum for the following batch. The culture medium used in the study consisted of a filtered (0.2 µm) fermented broth (FFB), produced by dark-fermentation under conditions described previously (Mete et al., 2024) and using a mixture of cheese whey and wastewater as fermentation broth. After the last cycle, a small fraction of the enriched culture medium was spread on solid media in order to isolate PHA-accumulating strains. Selection was made on neutralised FFB medium supplemented with agar (15 g/L) and Nile red (0.5 mg/L). Nile red is a fluorescent dye that preferentially accumulates in intracellular hydrophobic inclusions, including PHA granules, enabling the detection of putative PHA-producing colonies through their fluorescence. After 72h at 30°C, fluorescent colonies (UV transilluminator – 312 nm) of different morphologies were picked and subcultured on fresh solid medium (Spiekermann et al., 1999). Pure colonies were transferred into 5 mL of sterile Nutrient Broth (NB) and incubated 24h at 30°C before being stored at -20°C in 20% (v/v) glycerol.

PCR amplification and 16S rDNA sequencing

Isolates were cultured in NB during 24h at 30°C. The cells were pelleted (5,000 rpm, 10min) and resuspended in sterile distilled water. Cells were disrupted by heat treatment (95°C, 5min) repeated twice. The mixtures were then centrifuged (10,000 rpm, 5min) and the supernatants were used as PCR templates for 16S rDNA gene amplification with the primers 27f (5'-AGAGTTTGATCMTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') and Phusion High-Fidelity DNA Polymerase (New England Biolabs, USA). PCR were performed as follows: denaturation at 98°C during 30s, 30 cycles of amplification at 98°C for 30s, 55°C for 30s and 72°C for 30s, a final elongation at 72°C for 1min. The PCR amplification products were purified and sequenced (Microsynth, Switzerland). Sequences were submitted to the NCBI database using BLAST tool (https://blast.ncbi.nlm.nih.gov/Blast.cgi) and EZbiocloud database (https://www.ezbiocloud.net/) for the identification at the species-level. Mega 11 was used for phylogenetic tree construction (Tamura et al., 2021).

Characterisation of *C. serinivorans* ATH

Optimal temperature, optimal pH, carbon sources and substrate toxicity were evaluated using M9 medium composed of (g/L): NH_4Cl (1), KH_2PO_4 (3), Na_2HPO_4 (6.8), NaCl (0.5), $CaCl_2$ (0.014), $MgSO_4$, $7H_2O$ (0.5), $FeCl_3$, $6H_2O$ (0.008), vitamins solution (2 mL/L), trace element solution (2 mL/L). The vitamins solution contained (g/L): thiamine-HCl (0.5) and biotin (0.5). The trace element solution contained (g/L): EDTA (25), $CoCl_2$,$6H_2O$ (0.9), $ZnSO_4$, $7H_2O$ (0.9), Na_2MoO_4, $2H_2O$ (0.2), H_3BO_3 (0.05), $MnSO_4$, $4H_2O$ (0.6), $CuCl_2$, $2H_2O$ (0.6). The medium was supplemented with 5 g/L of appropriate carbon source, depending of the experiment. Experiments were conducted in flasks with a working volume of 50 mL. Each condition was tested in triplicate.

Nutrients limitation experiments were performed under the same conditions, using butyric acid (10 g/L) as carbon source. The C:N and C:P ratios (molar ratios) were adjusted by varying the NH_4Cl, KH_2PO_4 and Na_2HPO_4 concentrations. Each condition was tested in triplicate.

For all experiments, pre-cultures were prepared by inoculating glycerol stocks into 5 mL of Nutrient Broth (NB), and incubating for 24h at 30°C under agitation (250 rpm). Culture flasks were then inoculated at an initial optical density (OD_{600nm}) of 0.05.

PHA production in dark-fermentation stream

Inoculum preparation and inoculations were performed as described above. Cultures were carried out in flasks containing 50 mL of FFB at an initial pH of 7, incubated at 35°C and agitated at 250 rpm. Growths were monitored during 52h.

PHA extraction

PHA extraction was performed according to Vu et al. with modifications (Vu et al., 2021). Briefly, the bacterial cells were harvested from the culture medium by centrifugation at 12,000 rpm for 10min. The resulting pellet was rinsed twice with chilled distilled water, resuspended in 15 mL of sodium hypochlorite (4%) and incubated at 30°C for 150min. The PHA was then precipitated by centrifugation at 12,000 rpm for 10min, the supernatant was removed and the pellet rinsed with absolute ethanol to eliminate residual cell debris. The precipitate was resuspended in 1 volume of chloroform and heated at 100°C during 10min to dissolve the PHA. After cooling to room temperature, 9 volumes of chilled methanol were added to precipitate the polymer. The recovered PHA was placed in an aluminium dish and dried in an oven at 70°C until constant weight was achieved.

Analytical methods

Cell growth was monitored by measuring optical density at 600 nm (OD_{600}) using an Orion™ AquaMate 7000 UV-Vis spectrophotometer (Thermo Scientific, USA).

Total biomass was determined gravimetrically as dry cell weight (DCW). Briefly, 10 mL of culture was centrifuged at 12,000 rpm for 10min. The supernatant was collected and stored for high-performance liquid chromatography (HPLC) analysis. The cell pellet was washed twice with chilled distilled water to remove residual medium components. Cells were then resuspended in 500 µL of distilled water, transferred onto pre-dried and pre-weighed aluminium dishes, and dried at 70°C until a constant weight was achieved. The mass of DCW was measured using an analytical balance (Mettler Toledo, Switzerland) with a readability of 0.005 mg.

Changes in culture medium composition were analysed by HPLC using a Nexera LC-40 system (Shimadzu, Japan) equipped with a refractive index detector and a Rezex™ ROA-Organic Acid H^+ (8%) column (Phenomenex, USA). The mobile phase consisted of 2.5 mM H_2SO_4, delivered at a flow rate of 1 mL/min. The column temperature was maintained at 85°C.

PHA quantification was performed directly from cell pellets following the method described by Juengert, Bresan, and Jendrossek (Juengert et al., 2018), with slight modifications. Briefly, cell pellets were obtained by centrifugation at 12,000 rpm for 10min. Pellets were resuspended in 1 mL of chloroform and 1 mL of methanol containing 15% (v/v) H_2SO_4. The mixtures were incubated at 100°C for 120min for methanolysis. After cooling to room temperature, 1 mL of deionised water and 1 mL of chloroform were added. The samples were vortexed and allowed to separate into phases. The organic phase (2 µL) was collected and analysed by gas chromatography-mass spectrometry (GC-MS). GC analysis was performed using a Clarus 680 (PerkinElmer, USA) equipped with an SLB-5ms column (30 m, 0.25 mm ID, 0.25 µm film thickness) (Supelco, USA) and a FID detector. The injector and detector temperatures were set at 250°C. The oven temperature sequence was as follows: 70°C during 2min, then increase at 235°C at a rate of 10°C/min, then to 300°C at a rate of 26°C/min. Helium was used as the carrier gas at a flow rate of 1.2 mL/min. Commercial poly(3-hydroxybutyric acid) (Sigma-Aldrich, USA) was used to establish the calibration curve. The monomeric composition of produced PHAs was determined with the Clarus 600S module with electron impact as the ionisation mode and an ion source temperature of 220°C. The NIST library (https://www.nist.gov/) was used for confirmation of the structural assignments.

Results and discussion

Isolation of a PHA-producing bacterium

Before initiating the isolation of PHA-producing bacteria, the first step consisted of producing the fermented broth containing the desired substrates. For this purpose, a mixed dairy waste stream was used as the fermentation medium and inoculated with strain *C. beijerinckii* c.sp.1.3 to initiate dark-fermentation (Mete et al., 2024). After 48h, the resulting broth mainly consisted of volatile fatty acids, predominantly butyric acid, followed by acetic and propionic acids, as well as unconsumed lactose (supplementary information). The fermented medium was then adjusted to pH 7 using NaOH (2M) and filtered to ensure sterilisation. This medium, named FFB, was subsequently used as the carbon source for the enrichment of PHA-producing bacteria.

Biological sludge collected from the wastewater treatment plant of an industrial dairy facility was used to inoculate the FFB medium. Thirteen repeated feast-famine cycles were applied to impose periods of carbon deprivation, selectively favouring microorganisms capable of accumulating intracellular carbon reserves. After this enrichment step, the resulting enriched microbial consortium was spread onto solid FFB medium. The presence of Nile red allowed the detection of colonies of strains capable of accumulating carbon reserves (Figure 1). A total of 13 fluorescent colonies were isolated and purified prior to identification by 16S rDNA sequencing. Several isolates were identified as duplicates. Consequently, only six unique taxa were isolated and belonged to the genera *Achromobacter*, *Comamonas*, *Pseudomonas* and *Stenotrophomonas*. All of these are Gram-negative bacteria commonly found in diverse environmental habitats and are known for their ability to produce PHA (Javaid et al., 2020; Saratale et al., 2021; Zakaria et al., 2008; Zhao et al., 2025). Considering its potential application in industrial biotechnology, the final selection was based on biosafety considerations. To minimise risks related to opportunistic pathogenicity and to facilitate future handling and scale-up, the study subsequently focused on the isolate M9PHA+2, belonging to the genus *Comamonas*. A neighbour-joining phylogenetic tree was constructed using 16S rDNA gene sequences from representative *Comamonas* species and revealed a close phylogenetic relationship between the isolate and *Comamonas serinivorans* DSM 26136 and *Comamonas serinivorans* SP-35 (Figure 2). This proximity was further supported by BLAST and EzBioCloud analyses, which showed that the 16S rDNA gene sequence of the isolate shared 99.19% sequence identity with *C. serinivorans* DSM 26136 and 99.04% identity with *C. serinivorans* SP-35. Based on these results, the isolate was designated *Comamonas serinivorans* ATH, and its 16S rDNA gene sequence was deposited in the GenBank database under accession number PX519101.1.

Selective carbon source utilisation of *C. serinivorans* ATH

The carbon source utilisation profile of *C. serinivorans* ATH was investigated in minimal M9 medium using a range of substrates as sole carbon sources (Table 1). No biomass formation was detected when carbohydrates were supplied, indicating the inability of the strain to assimilate sugars. This is consistent with genomic and physiological studies showing that species of the genus *Comamonas* lack key genes for hexose phosphorylation and complete sugar catabolic pathways (Liu et al., 2015; Wu et al., 2018).

In contrast, all tested organic acids supported cellular growth, with substantial differences observed among substrates. The highest cell dry weights were obtained with butyric acid (0.563 ± 0.025 g/L) and lactic acid (0.507 ± 0.015 g/L), whereas acetic, formic and propionic acids resulted in lower biomass formation. PHA accumulation was strongly substrate-dependent and occurred only when acetate, butyrate or lactate were used as carbon sources. The highest intracellular PHA content was observed with butyric acid, at 30.79 ± 0.68%. Acetic acid and lactic acid resulted in lower PHA accumulation, with similar polymer contents between 20 and 22%. These results align with previous findings showing that even-carbon VFAs are preferentially directed towards PHA synthesis due to the increased requirement for acetyl-CoA, a key precursor, whereas odd-carbon acids are metabolised later and may contribute less to polymer accumulation (Sekoai et al., 2022).

Accordingly, the PHA yields further reflected this trend, with butyric acid providing the highest conversion efficiency of 0.155 g $_{PHA}$/g $_{substrate\ consumed}$, approximately 67% higher than the observed yield using lactic and acetic acids.

Given that acetic and butyric acids are the major end-products of dark-fermentation, these results highlight and valorise the potential of the strain *C. serinivorans* ATH to specifically valorise dark-fermentation-derived VFAs into value-added bioplastics.

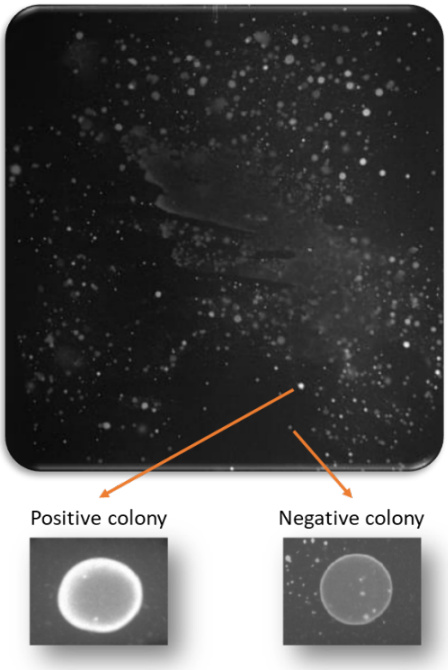

Figure 1 - Fluorescent Nile red staining of microbial colonies cultivated on solid FFB medium obtained from the enriched culture after 13 feast-famine cycles. Bright fluorescence indicates the accumulation of intracellular lipids or PHAs.

Influence of cultivation parameters on *C. serinivorans* ATH

The production of PHA by *C. serinivorans* ATH is expected to be strongly influenced by environmental parameters. Optimising these conditions is critical for maximising both biomass formation and polymer accumulation.

The effect of initial pH on the growth and PHA production of *C. serinivorans* ATH was assessed using butyric acid as the carbon source (Figure 3a). While pH had a moderate impact on the relative intracellular PHA content, its effect on biomass formation was more pronounced. The strain exhibited optimal and stable growth and PHA production over a pH range from 7 to 9, with biomass concentrations of 0.908 ± 0.041 g/L, 0.900 ± 0.043 g/L and 0.810 ± 0.050 g/L at pH 7, 8 and 9, respectively. At pH 10, performance decreased by approximately 20%, with a biomass of 0.698 ± 0.100 g/L. In contrast, at pH 6 the biomass formation was severely impaired with a 85% lower production compared with that at pH 7.

Biomass production was also strongly influenced by temperature (Figure 3b). Starting from a total biomass of 0.531 ± 0.072 g/L at 25°C, it increased to a maximum of 0.908 ± 0.041 g/L at 35°C. Above this temperature, biomass formation began to decline, with a reduction of approximately 28% at 40°C (0.653 ± 0.127 g/L) compared with the maximum. Higher temperatures severely impaired the strain's performance, with biomass decreasing to 0.289 ± 0.031 g/L at 45°C and almost complete inhibition at 50°C (0.164 ± 0.011 g/L, no detectable PHA). Notably, the PHA content remained relatively stable between 25 and 40°C, ranging from 40 to 45%.

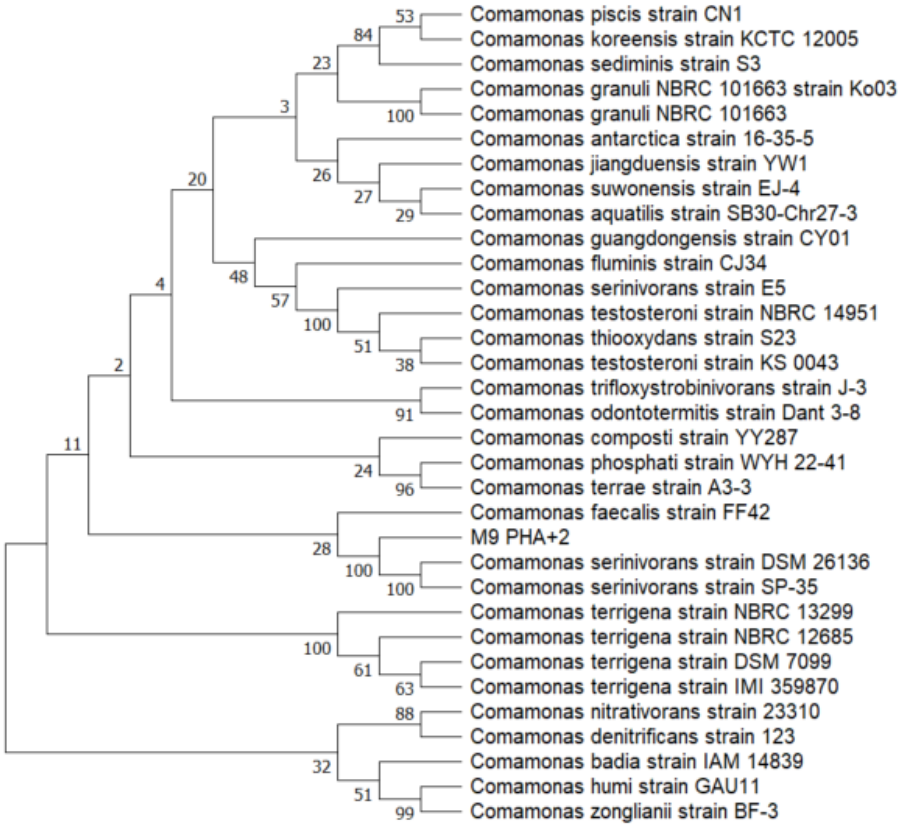

Figure 2 - Phylogenetic relationship of *Comamonas* species based on 16S rDNA sequences. The tree was constructed using the neighbour-joining method with 1,000 bootstraps replicates. Bootstrap value (%) are indicated at the nodes.

The effect of initial butyric acid concentration on the growth and PHA production of *C. serinivorans* ATH was also evaluated (Figure 3c). At a low substrate concentration of 2 g/L, the strain produced 0.768 g/L of biomass and 0.352 g/L of PHA, corresponding to a PHA content of 45.9%. Increasing the butyric acid concentration to 9 g/L slightly reduced total DCW to 0.610 g/L and PHA to 0.280 g/L, while the PHA content remained similar at 46.0%. At higher concentrations of 18 and 35 g/L, growth and PHA production were strongly inhibited, with biomass decreasing to 0.159 g/L and 0.132 g/L, and no detectable PHA.

Table 1 - Growth and PHA production on diverse carbon sources by the strain *C. serinivorans* ATH.

Carbon sources	DCW (g/L)	PHA (g/L)	PHA content (%)	yield ($g_{PHA}/g_{substrate\ consumed}$)***
Carbohydrates*	nd**	nd	-	-
Acetic acid	0.337 ± 0.006	0.071 ± 0.006	21.12 ± 1.08	0.090
Butyric acid	0.563 ± 0.025	0.173 ± 0.017	30.79 ± 0.68	0.155
Lactic acid	0.507 ± 0.015	0.103 ± 0.012	20.39 ± 0.76	0.096
Formic acid	0.310 ± 0.030	nd	0	0
Propionic acid	0.303 ± 0.020	nd	0	0

*Carbohydrates tested are glucose, galactose, fructose, lactose, sucrose and maltose.
**nd: not detected
***The PHA yields were calculated relative to consumed substrates

These results align with broader observations that pH, as well as the type and concentration of carboxylic acids, strongly influence the growth of PHA-producing strains and PHA biosynthesis. At

acidic pH, the proportion of undissociated acid increases, facilitating its passive diffusion across the cytoplasmic membrane (Guan & Liu, 2020). Once inside the cell, acid dissociation leads to cytoplasmic acidification, disruption of proton motive force, and increased energetic demand for pH homeostasis, ultimately impairing growth and metabolic activity. Recently, Leonhardt et al. systematically evaluated the effect of various carboxylic acids, including formic, acetic, propionic and butyric acids, on four PHA-producing strains (*Cupriavidus necator, Pseudomonas putida, Azohydromonas australica* and *Haloferax mediterranei*) (Leonhardt et al., 2025). Their results demonstrated relatively low IC_{50} values (<10 g/L) for growth inhibition in several cases, highlighting the sensitivity of these strains to elevated VFA concentrations. Moreover, toxicity was shown to increase with increasing carbon chain length and hydrophobicity of the acid, indicating a correlation between membrane permeability effects and inhibitory strength.

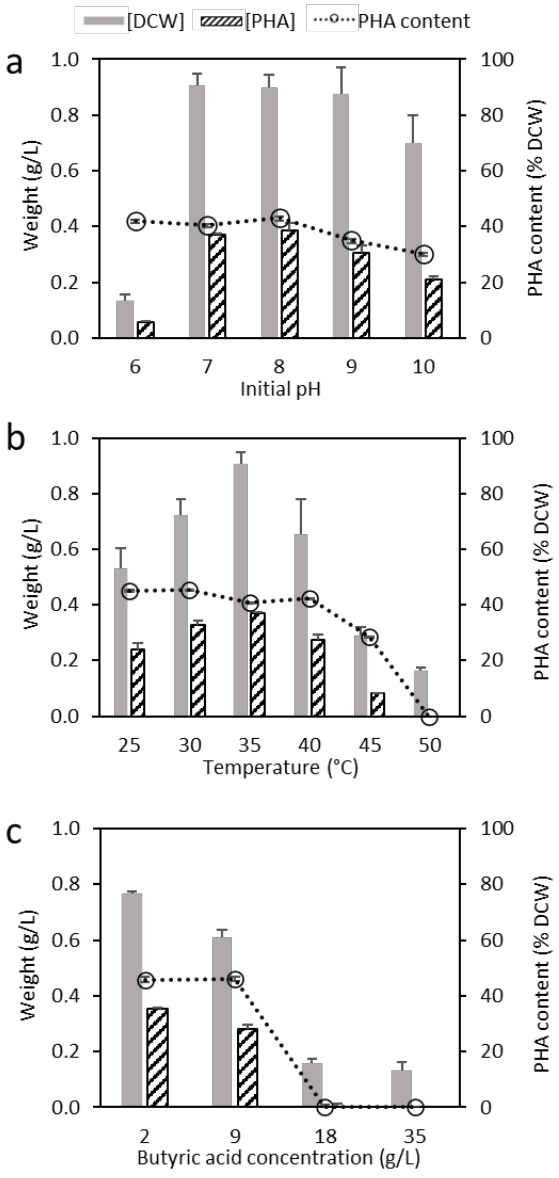

Figure 3 - Impact of pH (a), temperature (b) and substrate concentration (c) on biomass growth and PHA production by *C. serinivorans* ATH.

Effect of nutrient limitation on *C. serinivorans* ATH growth and PHA accumulation

The impact of nitrogen limitation was investigated by cultivating the strain in minimal M9 medium with butyric acid fixed at 10 g/L while varying NH_4Cl concentrations, with temperature and

pH maintained at their optimal values, namely 35°C and pH 8, within the optimal range identified above. Biomass formation and PHA accumulation were strongly influenced by the resulting C:N ratio (Figure 4a). At a C:N ratio of 1:1, biomass reached 0.237 ± 0.041 g/L, with a PHA concentration of 0.060 g/L, corresponding to a PHA content of 25.2% of DCW. Increasing the ratio to 10:1 resulted in higher biomass production (0.360 ± 0.028 g/L) and a marked increase in PHA content to 59.2% of DCW. A further increase in the C:N ratio, from 20:1 to 80:1, resulted in a slight increase in total biomass production, leading to a gradual decrease in the PHA accumulation to 45.9% of DCW.

The effect of phosphorus limitation was investigated by varying the KH_2PO_4 and Na_2HPO_4 concentrations in M9 medium while maintaining a fixed butyric acid concentration and optimal pH and temperature (Figure 4b). Total DCW and PHA accumulation were strongly dependent on the C:P ratio. As the C:P ratio increased from 4:1 to 86:1, total biomass concentration progressively decreased from 0.370 ± 0.026 g/L to 0.163 ± 0.055 g/L. Despite this reduction, the PHA concentration remained relatively stable, leading to a marked increase in intracellular PHA content from 42% to 84% of DCW. Maximum PHA accumulation, reaching 93% of DCW, was observed at a C:P ratio of 215:1. Beyond this ratio, PHA content declined sharply to values below 50%, although a partial recovery of biomass production was observed.

Nitrogen and phosphorus limitation are known to strongly influence carbon flux towards carbon storage at the expense of growth (Amadu et al., 2021), resulting, under certain conditions, in very high intracellular PHA contents exceeding 80-90% of DCW in strains such as *C. necator* and related species (Nagarajan et al., 2021). The maximum value of 93% of DCW observed in this study therefore places *C. serinivorans* ATH among highly efficient PHA accumulators.

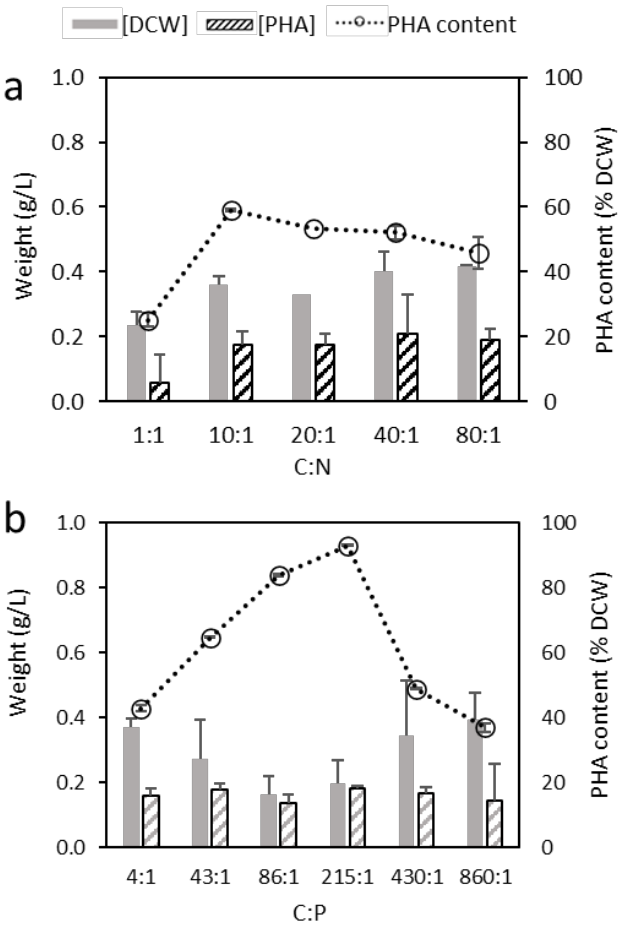

Figure 4 - Impact of nitrogen (a) and phosphorus (b) limitations on biomass growth and PHA production by *C. serinivorans* ATH.

PHA production from a sterile-filtered dark-fermentation stream

As the strain was isolated and selected with the perspective of valorising VFA-rich streams, *Comamonas serinivorans* ATH was cultivated in FFB neutralised to pH 7 (Figure 5). Cultivation was carried out in flasks without pH or aeration control and monitored for 52h. During cultivation, the pH increased from 7.0 to 8.6, reflecting the progressive consumption of carboxylic acids. The strain completely consumed acetic acid and 87% of butyric acid, while propionic acid was not assimilated. This substrate preference is consistent with previous studies reporting that acetate and butyrate are preferentially metabolised over propionate by efficient PHA-producing bacteria in VFA-rich streams, including *Pseudomonas oleovorans* (Aremu et al., 2021) and *Comamonas*-dominated mixed cultures (Zhang et al., 2023). As expected, lactose present in the FFB medium was not consumed, confirming the specialisation of the strain towards carboxylic acids rather than sugars.

Biomass production (DCW) increased steadily, reaching 0.74 and 1.18 g/L after 25 and 52h, respectively. PHA accumulation followed a similar trend, although the increase was less pronounced than biomass formation between 25 and 52h, with PHA concentrations of 0.475 g L^{-1} at 25h and 0.608 g L^{-1} at 52h. As a consequence, PHA content decreased from 65% to 51%, suggesting a transition from a storage-oriented metabolism towards biomass growth.

In comparison with other PHA-producing strains cultivated on VFA-rich substrates, *Comamonas serinivorans* ATH exhibited a particularly high PHA accumulation capacity under non-optimised and non-controlled conditions (Table 2). Its PHA content was comparable to that of *C. necator* grown on acetic acid (Khatami et al., 2022; Vu et al., 2021), but three times higher than that of *Paracoccus homiensis* cultivated on butyrate-rich streams (Szacherska et al., 2022). It should be noted, however, that these latter strains were cultivated in media with higher substrate concentrations, which are known to limit both growth and polymer accumulation. Moreover, the PHA yields, calculated relative to consumed VFAs, were 0.73 g PHA g^{-1} after 25 h and 0.54 g PHA g^{-1} after 52 h. These values are within the upper range reported for pure cultures grown on real VFA mixtures. However, the apparent biomass yields calculated from VFA consumption were approximately 1.14 and 1.05 g DCW g^{-1} of consumed VFAs, respectively. These values indicate that the measured biomass cannot be attributed solely to the consumed VFAs and suggest that other organic constituents of the FFB, such as proteins, may have also contributed to biomass formation.

These results confirm that *Comamonas serinivorans* ATH combines a strong preference for acetate and butyrate with a high PHA accumulation capacity, making it a promising candidate for the valorisation of fermentation-derived VFAs into PHA under simplified operational conditions. However, further improvements could likely be achieved through optimisation of operational parameters, such as aeration, nitrogen source, and substrate feeding strategies, to maximise both biomass growth and polymer yield.

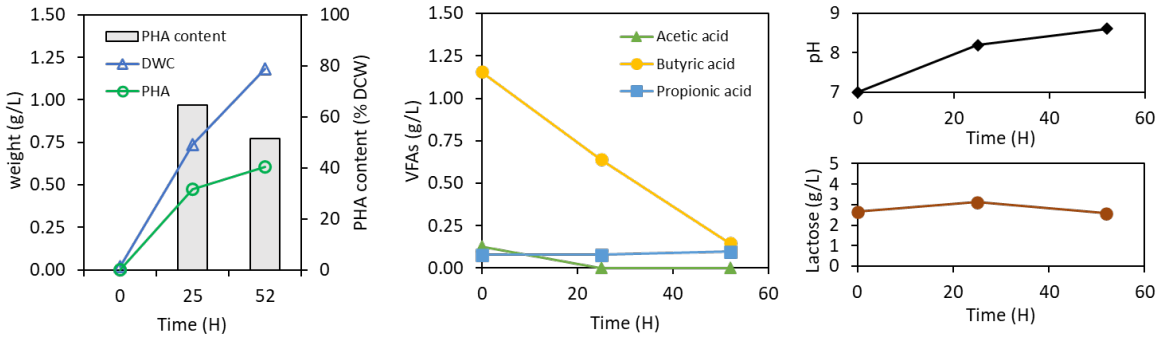

Figure 5 - Cultivation of *C. serinivorans* ATH on a VFAs-rich dark-fermentation stream.

Table 2 - Comparison of PHA production performances of different bacteria on a VFA-rich stream.

Microorganism	CAs type	Total CAs (g/L)	Produced DCW (g/L)	Produced PHA (g/L)	PHA content (% DCW)	Yield (g PHA/g substrates)	References
Bacillus megaterium ATCC 14945	Acetic, caproic, butyric, propionic and valeric acids	7	1.7	0.16	8.6	0.02	(Vu et al., 2021)
Paracoccus Homiensis	Butyric, lactic, caproic, acetic and valeric acids	~6.7	~ 2.4		16.7		(Szacherska et al., 2022)
Ralstonia eutropha KCTC 2658	Butyric, propionic ans acetic acids	5	1.8	0.82	46		(Khatami et al., 2022)
Pseudomonas oleovorans	Acetic, butyric and propionic acids	5	2.11	0.82	39		(Khatami et al., 2022)
Cupriavidus necator	Acetic, butyric and propionic acids	4 - 5	2.26	1.02	45.1	0.20	(Vu et al., 2022)
	Acetic, propionic, butyric and isovaleric acids	6 - 7			77.5	0.27	(Khatami et al., 2022)
Burkholderia capacia	Acetic, propionic, butyric and isovaleric acids	6 - 7			54.9	0.17	(Khatami et al., 2022)
Haloferax mediterranei	Butyric, acetic and propionic acids	2.27	4.9	0.64	13		(Urbina et al., 2024)
Comamonas serinivorans ATH	Butyric, acetic and propionic acids	1.4	1.18	0.61	51	0.54	This study

https://doi.org/10.24072/pcjournal.785

Structure of the synthesised PHA

The monomeric composition of the PHA produced by the strain *C. serinivorans* ATH was determined after acid-catalysed methanolysis of the purified polymer (Figure 6a), in the presence of sulphuric acid. The chromatogram of the commercial poly(3-hydroxybutyric acid) used as a reference exhibited a single major peak at 3.44 min (Figure 6b). Similarly, the PHA produced by *C. serinivorans* ATH exhibited a major peak at 3.42 min (Figure 6c), indicating close agreement in retention time with the standard. The associated spectra in both cases displayed the characteristic fragments at m/z 43 (base peak), 74 and 103, confirming the identity of the detected compounds as 3-hydroxybutyryl methyl ester. The absence of significant additional peaks in the chromatogram suggests a homogeneous monomeric composition. These results demonstrate that the polymer synthesised by *C. serinivorans* ATH is a poly(3-hydroxybutyrate), chemically comparable to the commercial standard.

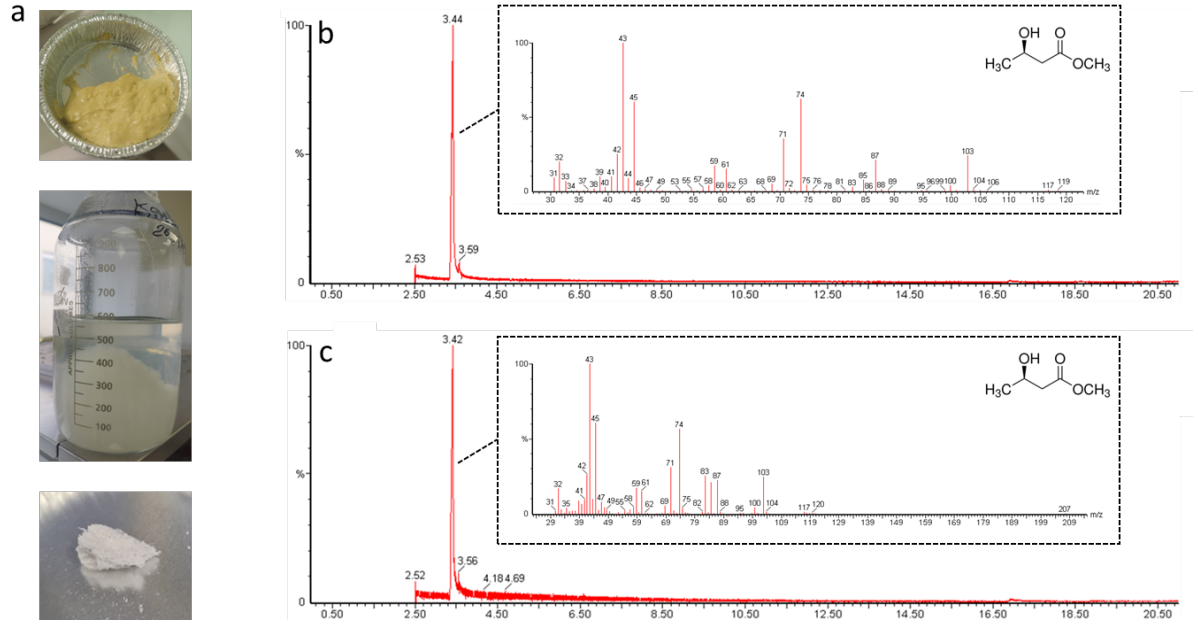

Figure 6 - PHA extraction (a) and comparison of the GC–MS profiles of the monomeric units of PHA produced by *C. serinivorans* ATH (b) with a commercial PHA standard (c).

Conclusion and Perspectives

This study describes the successful isolation and characterisation of *Comamonas serinivorans* ATH, a versatile PHA-producing bacterium with a metabolic specialisation towards VFAs as carbon sources. Using a targeted enrichment strategy based on feast–famine cycles, the strain was purified and identified as an industrially attractive candidate for PHA production from a sterile-filtered dark-fermentation-derived VFAs stream. To our knowledge, this is the first report demonstrating that a *C. serinivorans* species is capable of producing and accumulating PHA.

The metabolic specificity of *C. serinivorans* ATH for VFAs, combined with its broad tolerance to abiotic stresses, highlights its potential as a PHA-producing strain for the valorisation of fermentation-derived VFAs. Beyond demonstrating PHA accumulation under defined conditions, the ability of the strain to produce PHA from a real dark-fermentation-derived VFAs stream provides a relevant biological basis for further process development. The present study therefore establishes *C. serinivorans* ATH as a promising candidate for the biological valorisation of fermentation-derived VFAs, while its potential for direct process integration remains to be demonstrated.

Future work will focus on detailed characterisation of *C. serinivorans* ATH, alongside the implementation of continuous processes that integrate dark-fermentation and PHA production. An important consideration for process integration is the microbial status of the fermentation effluent. At an industrial scale, sterilisation could be considered a means of controlling the microbial load of the fermentation effluent prior to PHA production. However, its technical and economic feasibility at scale remains to be assessed, particularly in terms of operating costs. In the present study, the dark-fermentation broth was sterile-filtered through a 0.2 µm membrane prior to inoculation. Consequently, the ability of *C. serinivorans* ATH to establish and produce PHA in the presence of the indigenous microbial community of an untreated fermentation effluent remains to be demonstrated. This aspect will be particularly relevant for future bioaugmentation strategies, as microbial competition may affect strain establishment, substrate utilisation and PHA productivity. Future studies should therefore investigate the performance of *C. serinivorans* ATH in non-sterile fermentation effluents and evaluate appropriate strategies for microbial control.

Additionally, in-depth analyses of the physicochemical and mechanical properties of the PHA produced will be essential to fully assess its industrial applicability. Overall, the isolation of *C. serinivorans* ATH provides a promising platform for sustainable, waste-driven production of biodegradable plastics, bridging the gap between organic waste management and high-value bioproduct synthesis.

Credit authorship contribution statement

Gautier Kelvin: Writing – review & editing, Methodology, Investigation.
Pattyn Pauline: Methodology, Investigation.
Briki Amani: Writing – review & editing.
Soric Audrey: Conceptualization, Funding acquisition, Investigation, Project administration, Validation, Writing – review & editing.
Irague Romain: Conceptualisation, Funding acquisition, Investigation, Project administration, Resources, Validation, Writing – review & editing, Writing – original draft.

Acknowledgements

Preprint version 3 of this article has been peer-reviewed and recommended by PCI Microbiol (https://doi.org/10.24072/pci.microbiol.100504; Bouchez, 2026).

Funding

This research was supported by the CAP-H2 project funded by the Amidex foundation under the grant agreement AMIDEX 2023-02-D.

Conflict of interest disclosure

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data, script, code, and supplementary information availability

Supplementary table 1 can be found online on Zenodo (https://doi.org/10.5281/zenodo.22703471; Gautier et al., 2026).

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