Production of Polyhydroxyalkanoates and Extracellular Products Using *Pseudomonas Corrugata* and *P. Mediterranea*: A Review

Grazia Licciardello 1,*, Antonino F. Catara 2 and Vittoria Catara 3

1 Consiglio per la Ricerca in agricoltura e l’analisi dell’Economia Agraria-Centro di ricerca Olivicolatura, Frutticolture e Agrumicoltura (CREA), Corso Savoia 190, 95024 Acireale, Italy
2 Formerly, Science and Technologies Park of Sicily, ZI Blocco Palma I, Via V. Lancia 57, 95121 Catania, Italy; antoninocatara@virgilio.it
3 Dipartimento di Agricoltura, Alimentazione e Ambiente, Università degli studi di Catania, Via Santa Sofia 100, 95130 Catania, Italy; vcatara@unict.it
* Correspondence: grazia.licciardello@crea.gov.it

Received: 24 October 2019; Accepted: 12 November 2019; Published: 14 November 2019

**Abstract:** Some strains of *Pseudomonas corrugata* (*Pco*) and *P. Mediterranea* (*Pme*) efficiently synthesize medium-chain-length polyhydroxyalkanoates elastomers (mcl-PHA) and extracellular products on related and unrelated carbon sources. Yield and composition are dependent on the strain, carbon source, fermentation process, and any additives. Selected *Pco* strains produce amorphous and sticky mcl-PHA, whereas strains of *Pme* produce, on high grade and partially refined biodiesel glycerol, a distinctive filmable PHA, very different from the conventional microbial mcl-PHA, suitable for making blends with polylactide acid. However, the yields still need to be improved and production costs lowered. An integrated process has been developed to recover intracellular mcl-PHA and extracellular bioactive molecules. Transcriptional regulation studies during PHA production contribute to understanding the metabolic potential of *Pco* and *Pme* strains. Data available suggest that pha biosynthesis genes and their regulations will be helpful to develop new, integrated strategies for cost-effective production.

**Keywords:** medium-chain-length polyhydroxyalkanoate (mcl-PHA); alginate; biosurfactants; biopolymer; *Pseudomonas*; blends; film

1. Introduction

Polyhydroxyalkanoates (PHAs) are microbial polyesters synthesized by both Gram-negative and Gram-positive eubacteria, and an increasing number of archaea isolated from environmentally extreme habitats, to increase their survival and competition in environments where carbon and energy sources are limited, such as soil and rhizosphere [1–3].

Based on their repeat unit composition, the up to 150 different PHA structures identified so far [4] are classified mainly in two distinct groups: (i) short chain length (scl) PHAs where the repeat units are hydroxy fatty acids (HFAs) of 3–5 carbon chain length (C3–C5); and (ii) medium chain length (mcl) PHAs with repeat units of C6–14. In general, scl-PHAs are crystalline polymers with a fragile, rigid structure, whereas mcl-PHAs are amorphous thermoplastics, which have various degrees of crystallinity as well as elastomeric and adhesive properties [5]. Less common and least studied are long chain length (lcl) PHAs, constituted of monomers with more than 14 carbon atoms.

Thanks to two metabolic pathways based on the degradation of aliphatic carbon sources or *de novo* synthesis of fatty acids from unrelated carbon sources, *Pseudomonas* species included in the rRNA homology group I are among the most important producers of PHA [6–9]. Historically, fatty acids...
have been the preferred substrate for the microbial synthesis of mcl-PHA. Glucose, gluconate or ethanol, as well soy molasses [10], biodiesel co-product stream [11] and glycerol [12,13], have been successfully used. The fatty acyl composition of the substrate reflects the repeat unit composition of biopolymers [14]. Biodiesel glycerol has been recognized as a suitable and cost attractive substrate for PHA production, and therefore constitutes the main focus of this review [15,16].

Medium chain length-PHAs in *Pseudomonas* bacteria were first detected in *P. oleovorans* [17] and later in a variety of *Pseudomonas* [10]. *Pseudomonas*-PHAs are biodegradable, non-toxic and biocompatible and can be produced using a wide range of carbon sources. In fact, there has been considerable research exploring their potential in medical devices, foods, agriculture and consumer products [18,19]. Their elastic and flexibility properties improve the processability and mechanical properties of blends with other biodegradable polymers [20–22]. Of the various species tested worldwide, *P. aeruginosa*, *P. putida*, *P. resinovorans*, *P. mendocina*, and *P. chlororaphis* are the most extensively studied to clarify the metabolic processes of the production of PHA and to enhance the bioconversion efficiency [3,23].

This review focuses on the two taxonomically related Gram-negative rod ubiquitous bacteria, *P. corrugata* and the strictly related *P. mediterranea*, which cause disease on several crop species [24] and can produce an arsenal of secondary metabolites [25]. Among them, are biosurfactants (BSs) [26] as well as poly-mannuronic acid alginate [27], bioactive cyclic lipopeptides (CLPs), such as cormycin A and corpeptins [28–30], and a lipopeptide siderophore, corrugatin [31].

These bacteria produce different cellular mcl-PHAs and extracellular products, on waste fried edible oils, biodiesel glycerol and high-grade glycerol [13,32,33]. Selected *P. corrugata* strains produce intracellular mcl-PHA with a molecular weight of 120–150 kDa on waste edible oils, whereas strains of *P. mediterranea* generate a distinctive filmable PHA around 55–65 kDa on high-grade and partially refined biodiesel glycerol. Extracellular products, such as biosurfactants, exopolysaccharides (EPS, mostly alginate) and bioactive molecules, accumulate in the supernatant during the bioconversion process. Genome analysis of nine *P. corrugata* and *P. mediterranea* strains has helped to develop molecular and genetic investigations to enhance productivity [25].

### 2. Production of mcl-PHA and Extracellular Products

The first strain of *P. corrugata* investigated for its capacity to convert triacylglycerols to produce mcl-PHA was strain 388 [7,14,34]. The positive results led to the screening of different carbon sources of 56 strains of *P. corrugata* and 21 strains of its closely related *P. mediterranea* [9]. Flask-scale tests, carried out on related and unrelated carbon sources, have been reported [9,13,32–34].

Subsequently, some strains of *P. corrugata* producing lipase have been reported as being able to bioconvert waste exhausted fried edible oils, from a licensed collector, in mcl-PHAs [9,32]. One of these strains, namely *P. corrugata* A1 (DSM 18227) (hereafter *Pco A1*), obtained through culturing *P. corrugata* CFBP5454 in E* medium with triolein, helped to patent a fermentation process validated on a 5000 L fermenter [35]. This increased the productivity of the process from 2.90 g/L up to 26 g/L of dry cell weight with 38% of PHA [32,35].

To overcome the variable composition of licensed exhausted edible oils and the difficulty in collecting adequate stocks for industrial production, several sources of glycerol have been extensively tested to screen many strains of *P. corrugata* and *P. mediterranea*. Three of them, *Pco* 388, *Pco A1* and *Pme 9.1* (deposited as CFBP5447, hereafter *Pme 9.1*), have been selected to study the bioconversion processes exploiting commercial high-grade glycerol (±99%, pH 7) and biodiesel glycerol obtained from the transesterification of rapeseed oils (*Brassica cavenata* and *B. napus*). Crude biodiesel (15% glycerol), oil free (40%) and partially refined glycerol (87.5%) performed differently from commercial high grade glycerol in terms of yield, composition and properties of the mcl-PHA and extracellular products. It has also been highlighted that some apparently small differences in the carbon sources may have a large impact, and that the genetic and metabolic system of the strain are key to the bioconversion process [11–13,33].
Bioengineering 2019, 6, x FOR PEER REVIEW 4 of 13

Figure 1. Crude PHA film (A) and transparent PHA film obtained after floating a toluene solution on a water surface (B) achieved from Pseudomonas mediterranea 9.1 using refined glycerol as carbon source (Figure 1B courtesy of Copyright Elsevier from [13]).

The extracellular biosurfactants released by these strains during the bioconversion process showed their dependence on the carbon sources, and the highest yields were reached much later than the PHA. Pme 9.1 grown on crude glycerol (15%) obtained from Brassica spp. seed oil, was able to recover up to 14 g/L of surfactants with E24 (emulsification index) 54%, via chloroform:methanol (2:1) [36]. The highest accumulation occurred after 96–144 h. At the early stationary phase (48 h) P. mediterranea 9.1 yielded 6.9 g/L of partially purified EPS, 17-fold higher than in Pco A1 (0.39 g/L). PHA production was slightly higher in Pco A1 than in Pme 9.1 (respectively 0.92 g/L and 0.52 g/L) [37].
Table 1. Cell dry weight and raw PHA percentage obtained through bioconversion of different carbon sources by selected strains of *Pseudomonas mediterranea* and *P. corrugata*.

| Carbon Source | Grade | % V/V | Time (h) | *P. mediterranea 9.1* | *P. corrugata A1* | *P. corrugata 388* | References |
|---------------|-------|-------|----------|------------------------|-------------------|-------------------|-----------|
|               |       |       |          | CDW (g/L) | Raw PHA (%) | CDW (g/L) | Raw PHA (%) | CDW (g/L) | Raw PHA (%) |       |
| Glycerol      | 15%   | 1     | 72       | 3.4 | 50.2 | 4.7 | 50 | 4 | 28.7 | [33] |
|               | ≥99%  |       |          | 3   | 25.3 | 3.5 | 29.4 | 4.2 | 18.7 |       |
| Glycerol      | 15%   | 2     | 72       | 4.8 | 61.6 | 3.5 | 51.5 | 3.8 | 33.6 |       |
|               | ≥99%  |       |          | 3.2 | 26.1 | 3.4 | 30.2 | 3.6 | 15.7 |       |
| Glycerol      | 15%   | 5     | 72       | 4.2 | 38   | 4.1 | 48.5 | 3.2 | 32.1 |       |
|               | ≥99%  |       |          | 3.3 | 21.5 | 4.1 | 22.1 | 2.8 | 14.3 |       |
| Glycerol      | 87.5% | 2     | 48       | 3.1 | 16.5 | 3.3 | 18 |    |      |       |
|               | ≥99%  |       |          |      |       |      |    |    |      | [13] |
| Glycerol      | ≥99%  | 2     | 66       | 2.9 | 17.9 | 3.1 | 29.4 |    |      |       |
|               | ≥99%  | 2     | 66       | 3.6  | 38.8 |      |    |    |      | [38] |
| Glucose       | ≥99%  | 0.5   | 72       | 1.5 | 31.3 |    |    |    |      | [34] |
| Oleic acid    | ≥99%  |       |          | 1.6 | 61.8 |    |    |    |      |       |
| Glucose       | ≥99%  | 2     | 72       | 3.1  | 24   |    |    |    |      | [39] |
| Oleic acid    | ≥99%  |       |          | 1.3  | 2    |    |    |    |      |       |

1 time of cultivation; 2 this specific test was carried out with a modified strain of *P. mediterranea 9.1 VVCI1G.

3. Conversion Process and Recovery

In order to establish standard and suitable protocols to scale up the production of PHAs, many strategies have been investigated using batch and fed-batch processes in flasks and low-medium volume fermenters (3–30 L). Fed-batch fermentation has always been shown to be more productive than the batch mode, as reported for *Cupriavidus* sp. [40].

A fed-batch cultivation has also been used in a process of glycerol conversion by growing *P. mediterranea 9.1* in a substrate with 2% glycerol. The cultivation was conducted in a 30 L bioreactor, 30 °C, pH 7.0, and dissolved oxygen maintained at 20% saturation, using E* medium (pH 7.0) containing 5.8 g/L K₂HPO₄, 3.7 g/L KH₂PO₄, 10 mL/L MgSO₄ 0.1 M, supplemented with 1 mL/L of a microelement solution, with the addition of 2% glycerol (1% at the start, and 1% after 24 h) [13,33].

The biomass obtained has been routinely harvested by centrifugation, washed with saline solution and lyophilized. The extraction of the PHA using acetone [41] in an automatic Soxhlet was found to be more effective than chloroform extraction and less impactful for the environment. Treatments with mild alkaline solution [42] or maceration, attempted considering the potential use of mcl-PHA in the biomedical field, have yielded a lower recovery of products.

The analysis of PHA composition was carried out by gas chromatography/mass spectrometry (GC/MS) of the 3-hydroxymethylesters, after the removal of all the residual free glycerol [13]. Overall, different approaches have been evaluated to reduce the very high production costs by increasing the yield or by recovering both the PHA and extracellular products simultaneously from the fermentation process. The addition of either meat or yeast extracts at 0.1% to crude glycerol or glucose eliminated the prolonged lag-phase (5–12 h) [43].

Rizzo et al. [44] showed that adding 5 mM glutamine as a co-feeder significantly increased the biomass and PHA production, inducing the early expression of *phaC1* and *phaC2* genes. This was due to the improvement in the specific growth rate and cell metabolic activity, and to the enhanced uptake of the unrelated (glycerol and glucose) and related (sodium octanoate) carbon sources.

An integrated process for the bioconversion of crude biodiesel glycerol to simultaneously produce biosurfactants and PHAs by *Pme 9.1*, has also been established by applying a mathematical mechanistic model to define nutritional requirements, as well as pH and temperature, which mutually influenced PHA and BSs production within a narrow range of variation [43]. Surface response methodology analysis showed that, after 72 h, up to 1.1 g/L of crude PHA and 0.72 g/L of biosurfactants were recovered. On the other hand, the respective best single yields were obtained after 48 h for PHA (60% of CDW) and 96 h for BSs (0.8 g/L) [43].
4. Composition and Technological Properties of mcl-PHA

GC/MS profiles of mcl-PHA were largely affected by the carbon source and bacteria species. PHAs obtained on waste food oils have been found to be very different from those obtained on glycerol, and different types of glycerol produced different mcl-PHAs [13,32].

GC/MS chromatograms of mcl-PHAs obtained by *Pco A1* and *Pme 9.1* on crude glycerol revealed similar profiles and technological properties, whereas substantial differences were observed with respect to those obtained on partially refined biodiesel glycerol and high-grade glycerol. They showed monomeric units of side chains from C12 to C19 in length on crude glycerol (15% glycerol), and from C5 to C16 on refined glycerol (≥99%) (Table 2) [33,45]. Interestingly, mcl-PHA produced by *Pme 9.1* on high-grade glycerol was less sticky and produced a thin film (Figure 1A) [33]. These properties have been shown to be associated with differences at transcriptomic level [37], and in the genetic organization of *pha* gene locus which affects *pha* polymerase gene expression, PHA composition, and granule morphology [39].

Other experiments on *Pme 9.1* have been conducted in Erlenmeyer flasks containing 500 mL volumes of E* medium (pH 7.0) with 2% high grade glycerol or a partially refined glycerol (87.5%) obtained from a biodiesel process of *Brassica napus* [13]. The polyesters obtained on high grade commercial glycerol highlighted a structure composed of six monomers, indicative of elastic and flexibility properties: 3-hydroxyhexanoate (C6), 3-hydroxyoctanoate (C8), 3-hydroxydeca-noate (C10), 3-hydroxydodecanooate (C12), cis 3-hydroxydodec-5-enoate (C12:1Δ5), and cis 3-hydroxydodec-6-enoate (C12:1Δ6). The molecular weight (Mw) was 55,480 Da and polydispersity index (PDI = Mw/Mn) was 1.34 (Table 2). On the other hand, PHA obtained from glycerol 87.5% had a small variation in monomeric composition, a Mw of 63,200 Da, and a PDI of 1.38. Tsuge et al. [46] also observed that a higher glycerol concentration induced a considerable reduction in the molecular mass of PHA, caused by a termination of the PhaC polymerization activity. The NMR spectra and MALDI-TOF data were almost identical regardless of the glycerol grade, but different in intensity. The degradation temperature started at 230 °C, higher than the melting temperatures, with a volatilization rate of about −40%/min.

### Table 2. Molecular weight and monomer composition of PHAs obtained in different bioconversion processes of different carbon sources by *Pseudomonas corrugata* and *P. mediterranea*.

| Strain | Carbon Source | Grade | % V/V | Time (h) | Mw (kDa) | PDI | Molar Composition (mol %) | Reference |
|--------|---------------|-------|-------|----------|----------|-----|--------------------------|-----------|
|        |               |       |       |          | C6      | C8  | C10 | C12:0 | C12:1 | C12:0 | C14 | C14:1 |        |
| *Pme 9.1* | Waste fried oil | 80%   | 2     | 34       | 2        | 44  | 14  | 5     | 1     | 4     |      |        | Pappalardo et al., unpublished |
|         | Glycerol      | 40%   | 2     | 71       | 1        | 7   | 13  | 1     | 1     | 6     |      |        |
|         | Glycerol ≥99% | 2     | 48    | 60.8     | 4.2      | 17.0| 11  | 5.7   | -     | -     |      | [13]   |
|         | Glycerol 87.5% | 66    | 6     | 66.6     | 0.1      | 9.3 | 15  | 7.7   | -     | -     |      | [13]   |
| *Pme 9.1* | VVC1GI | Glycerol ≥99% | 2     | 66      | 0.9     | 13.5| 57.5| 12.8 | 11.8  | 3.7  |      | [37]   |
| *Pco A1* | Glucose | 0.5   | 72    | 115.0    | 2.4     | 2   | 14  | 11    | 17    | 0.4  | 3.6  | [47]   |
|         | Oleic acid   | 0.5   | 72    | 735.5    | 4.1     | 47  | 24.5| 16.5  |        |      |      | [34]   |
|         | Na octanoate | 0.5   | 72    |         | nd      | 2   | 19  | 5     | 11    | 2    | 9    | [34]   |
| *Pco 388* | Glucose | 0.5   | 72    | 114.8    | 1.8     | 7   | 82  | 11    |      |      |      | [47]   |
|         | Oleic acid   | 0.5   | 72    | nd      | 5       | 37  | 33  | 12    |      |      |      | [34]   |

1 time of cultivation.

Drop casting a toluene solution of polymers in Petri dishes resulted in quite different films, depending on the carbon source used to produce the PHA (Figure 1B). The PHA obtained on
high-grade glycerol produced an optically transparent film with a UV–vis absorption spectrum that was above the 800–350 nm range, comparable to the polyester film used for laser printer transparency. The mechanical properties (tensile strength, Young’s modulus and elongation at break of both PHAs) were not substantially affected by the different purities of the glycerol grade (87.5% and ≥99%). All these characteristics make the PHA obtained from *P. mediterranea* 9.1 on glycerol quite different to most mcl-PHAs produced from bacteria of the same phylogenetic group.

5. Evaluation of Mixed Blends and Coatings

The distinctive characteristics of a mcl-PHA obtained from *Pme* 9.1 grown on glycerol led to the investigation of the processability of blends with polylactide acid (PLA) to improve the mechanical and gas/vapors barrier properties of PLA [48]. Rheological tests indicated a significant increase in the elongation at break, while the elastic modulus was significantly lower only at higher contents of PHA. This suggests that the PHA macromolecules exert both a plasticization and lubricant action, which enable the PLA macromolecules subjected to solid deformation to slide more efficiently [48].

Preliminary investigation of blends of polyhydroxybutyrate (PHB) and a glycerol mcl-PHA obtained from *B. napus* oil showed an increase in crystallization temperature and a small increase in elongation at break, but at low concentrations of PHA (5%) the blend revealed some spaces between the two polymers.

Blends of mcl-PHA obtained from *Pco A1* on exhausted edible oils with Mater-Bi ZI01U/C polymers have poorly improved the processability of blends prepared by compression [22]. Soil mulching tests of paper sheets coated with blends based on PHA suggested some positive effects of coating. However, the expensive costs, as well the difficulty to obtain standardized exhausted edible oils as a carbon source, have discouraged further research [49,50].

6. *P. corrugata* and *P. mediterranea* PHA Locus

Genomic studies which investigated potential correlations between the phenotype and genotype of *Pco 388, Pco A1* and *Pme 9.1* have shown that, similarly to other *Pseudomonas*, the three strains have a class II PHA genetic system consisting of two synthase genes (*phaC1, phaC2*), separated by a gene coding for the depolymerization of PHA (*phaZ*) [8,9,23]. This genetic system allows *Pseudomonas* strains to utilize medium-chain-length (mcl) monomers (C6–C14), whereas class I, III and IV systems polymerize short-chain-length (scl) monomers (C3–C5) [6].

Sequence analysis of the pha locus revealed that the strains *Pco A1* (AY910767), *Pmed 9.1* (AY910768) and *Pco 388* (EF067339) share a high homology at nucleotide (93–95%) and amino acid levels (96–98%) [39,51,52]. An additional 121 bp in the *phaC1–phaZ* intergenic region containing a predicted strong hairpin structure were present in both strains 388 and A1 of *P. corrugata*, but not in *P. mediterranea* [39]. According to the authors, in *Pco A1* and 388 strains this additional sequence likely acts as a rho-independent terminator for the transcriptional terminator of *phaC1*, which would appear to be responsible for the slight variation in the PHA composition and granule organization [39].

Subsequently, genome analysis of strains *Pco A1* (ATKI01000000) and *Pme 9.1* (AUPB01000000) enabled the six genes of the entire pha locus to be studied (*phaC1, phaZ, phaC2, phaD, PhaF, PhaI*) [53,54]. Genome mining also identified gene coding for enzymes involved in β-oxidation (*fad*), fatty acid *de novo* synthesis (*fab*), and mcl-PHA precursor availability (*phaG and phaJ*) [53,54]. Pfam search domain and Blastp analysis on the glp operon, responsible for glycerol catabolism, revealed that both *P. corrugata* and *P. mediterranea* lack the *glpF* gene, coding for the glycerol uptake facilitator protein [53]. This condition had already been verified in all the *Pco* and *Pme* strains sequenced, which explains the prolonged lag growth phase observed during *P. mediterranea* 9.1 growth with glycerol as the sole carbon source [25,55].

7. Transcriptional Regulation during PHA Production

To improve the knowledge about PHA biosynthesis genes and their regulations, helpful to increase mcl-PHAs production and also to obtain new, tailor-made polymers [6], regulatory mechanisms during
PHA accumulation have been investigated in Pco A1, Pco 388 and Pme 9.1 through different gene expression studies and full transcriptome analysis.

7.1. Expression of phaC1 and phaC2 under Different Carbon Sources

Preliminary studies on the transcriptional levels of phaC1 and phaC2 genes in Pco 388 and Pco A1 showed an up-regulation of phaC1 in cultures with oleic acid as the sole carbon source (Table 3) [47]. On the other hand, both phaC1 and phaC2 were induced in cultures with glucose or sodium octanoate [47]. The significant correlation between PHA production and phaC1/phaC2 expression suggested at least two distinct networks for the regulation of the two PHA polymerases genes, and that a putative promoter(s) is likely present upstream of phaC2. In addition, the lack of polycistronic transcripts under any culture conditions indicated that phaC1 and phaC2 were not co-transcribed (Table 3) [47].

Table 3. Gene expression detected in P. corrugata and P. mediterranea strains during mcl-PHA biosynthesis on different carbon sources.

| Bacterial Strain | Carbon Source | % V/V | Time (h) | Detection Method | PhaC1 | PhaC2 | PhaI | Alg Genes | Operon | Reference |
|------------------|---------------|------|----------|------------------|-------|-------|------|-----------|--------|-----------|
| Pco 388          | Oleic acid    | 2    | 48       | Real-time PCR    | 1.2   | 1.4   | nt   | nt        | nt     |           |
| Pco 388 clone XI 32-1 | Oleic acid | 2    | 48       | Real-time PCR    | 6.6   | 4.7   | nt   | nt        | nt     | [39]      |
| Pco 388 clone XI 32-4 | Oleic acid | 0.5  | 48       | Real-time PCR    | 7.0   | 5.4   | nt   | nt        | nt     |           |
| Pco 388          | Glucose       | 2    | 48       | Real-time PCR    | 5.6   | No change | nt | nt        | nt     |           |
| Pco 388 clone XI 32-1 | Glucose | 2    | 48       | Real-time PCR    | 6.3   | No change | nt | nt        | nt     |           |
| Pco 388 clone XI 32-4 | Glucose | 0.5  | 48       | Real-time PCR    | 8.2   | No change | nt | nt        | nt     |           |
| Pco A1           | Oleic acid    | 0.5  | 48       | Real-time PCR    | 6.8   | 10.5  | No change | nt   | nt        | NO       | [47]      |
| Pco 388          | Glucose       | 2    | 48       | Real-time PCR    | 2.7   | 2     | No change | nt   | nt        | NO       |           |
| Pco A1           | Glucose       | 2    | 72       | Real-time PCR    | 6.2   | 3.5   | nt   | nt        | NT     | NO        |           |
| Pme 9.1          | Glycerol      | 2    | 24       | β-gal        | 420 U | 340 U | 7200 U | nt     | PhaC1ZC2D | PhaI      | [56]      |
| Pme 9.1 VVD (phaD-) | Glycerol | 2    | 48       | β-gal        | 140 U | 300 U | 545 U | nt     | PhaC1ZC2D | PhaI      |           |
| Pme 9.1          | Glycerol      | 2    | 48       | RNA-Seq      | No change | No change | No change | 5.53-2.32 | nt     |           |
| Pco A1           | Glycerol      | 2    | 48       | RNA-Seq      | No change | No change | No change | nt     |           |

1 The relative quantification was performed by comparing ∆Ct (i.e., Ct of the 16S rRNA housekeeping gene subtracted to the Ct of the target gene). The ∆Ct value of the control sample (time 0) was used as the calibrator and fold-activation was calculated by the expression: 2−∆∆Ct. 2 β-galactosidase activities detected by transcriptional fusion plasmids for phaC1, phaC2, and phaI promoter regions based on the pMP220 promoter probe vector and expressed as Miller units. 3 Pairwise comparison of mRNA levels analysis, using the Pme 9.1 sample as a reference (log2 fold change ≥ 2 and p-value ≤ 0.05).

Parallel studies showed that, in Pco 388, the phaC1–phaZ intergenic region plays an important but unclear role in the regulation of the carbon source-dependent expression of phaC1 and phaC2 genes [39]. Derivative mutants XI 32-1 and XI 32-4 of this strain (obtained by replacing the phaC1–phaZ intergenic region with a kanamycin resistance gene), showed a significant increase in phaC1 and phaC2 expression when grown for 48 h with oleic acid, but not with glucose. In addition, the wild type strain produced only a few large PHA inclusion bodies when grown with oleic acid, whereas the mutants showed numerous smaller PHA granules that line the periphery of the cells, as result of phasin activities [3,39]. A high content of the monounsaturated 3-hydroxydodecanoate as a repeat unit monomer was observed in the PHA of the mutant strains [39].

Diversely, the study of the promoter activity of pha genes in Pme 9.1 grown on high-grade glycerol, revealed that the upstream regions of phaC1 (PC1) and phaI genes (PI) are the most active [56]. PC1 is responsible for the phaC1ZC2D polycistronic unit transcription (Table 3). On the other hand, PI regulates the phaI operons, as confirmed by the presence of three and two putative rho independent terminators, respectively located downstream of phaD and phaF [56]. In turn, PI and PC1 are controlled
by PhaD, which acts as a transcriptional activator, as shown by the reduced promoter activities in the phaD- mutant [55]. Similar results were observed in P. putida KT2442 [23,57].

7.2. Transcriptome Analysis on Glycerol-Grown Strains

The transcriptional profiles of Pco A1 and Pme 9.1 growing on a substrate with 2% of high-grade glycerol under inorganic nutrient-limited conditions were investigated at the early stationary phase of the bioconversion into mcl-PHAs [37]. RNA-seq analysis revealed that in P. mediterranea, 175 genes were significantly upregulated and 217 downregulated, compared to P. corrugata. The genes responsible for stress response, central and peripheral metabolic routes and transcription factors involved in mcl-PHA biosynthesis, made up 39% of the genes differently transcribed by the two bacteria. Nonetheless, among the genes directly involved in PHA biosynthesis, slight differences were observed only in phaZ depolymerase and phaG transacylase genes (Table 3). Weak differences occurred in the expression levels of genes that are crucial for glycerol catabolism and pyruvate metabolism, transcriptionally downregulated, and fatty acid de novo biosynthesis pathways.

Interestingly, a significantly increased expression of 21 genes involved in alginate exopolysaccharide production was observed in Pme 9.1 compared to Pco A1 (Table 3), related to a 17-fold higher production of EPS (6.9 g/L compared to 0.39 g/L). A simultaneous production of PHA and alginate has been reported in some P. mendocina strains [58,59]. The increased EPS production, associated with the different transcriptome profiling between the two bacteria, suggests competition for the acetyl-CoA precursor amongst PHA and alginate metabolic pathways. Further studies on P. corrugata A1 showed that regulation of alginate production is controlled by quorum sensing and the RfaA regulator [60].

8. Genetically Modified Bacteria to Improve the Production of mcl-PHAs

Two different approaches have been made to evaluate the feasibility of improving the efficiency of the conversion by using genetically modified Escherichia coli and P. mediterranea.

Cloning of pha synthases genes of Pco A1 and Pme 9.1 in Escherichia coli, a well-known organism in the research on PHA biosynthesis, yielded 2%–4% of PHA/CDW on sodium decanoate [61].

In a second approach, additional copies of phaC1, phaG and phaI genes, cloned in two plasmids under the control of strong promoters, were transferred into Pme 9.1 [38]. When grown on high-grade glycerol, the modified Pme VVC1GI showed a higher cell fluorescence than WT, due to the presence of larger granules (Figure 2). It also showed a 40% increase in the cellular accumulation of crude PHA and a better PHA/CDW ratio (1.4 g/L, 38.8%), whereas the WT strain produced 1 g/L of PHA (23%) (Table 1). Biomass yield was 3.6 g/L in VVC1GI and 4.2 g/L in WT [38]. GC/MS analysis showed that the PHA structure was composed of six monomers, like PHA produced by the WT, although there were some differences in the ratio between C12 and C12:1 (12.8:11.8 in strain VVC1GI and 6:12 in WT) (Table 2).

Figure 2. Fluorescent granules of PHA after Nile red-staining of Pseudomonas mediterranea 9.1 wild-type strain (A) and VVC1GI recombinant strain (B) grown on high-grade glycerol (≥99%) as carbon source and limited nitrogen condition after 66 h of incubation.
9. Conclusions

Despite the fact that the PHAs market is still very small, the worldwide focus on the development of bio-polymers highlights that one day polyhydroxyalkanoates will replace some petroleum-based plastics. Pseudomonas species are a potential cell factory for their production [3]. Some strains of P. corrugata and its related P. mediterranea are able to convert different carbon sources and provide interesting mcl-PHA and co-products. They are naturally present in the soil and produce valuable extracellular co-products [37,43].

A manageable mcl-PHA film, unlike other mcl-PHAs reported to date, can be obtained by P. mediterranea 9.1. Although the yields are currently not particularly viable, their distinctive characteristics suggest a potential application as a softener in (bio) polymeric blends, for food packaging or medical devices. The unsaturated double bonds in the side chains could be used to enhance its properties and/or to help extend its applications to other biomaterials for food packaging or biomedicine [13].

P. mediterranea 9.1 also produces high-quality extracellular products (above all alginate) on a proper medium, which is very promising for high-level applications and which may orient further investigation towards an efficient co-production of cellular mcl-PHA and extracellular biosurfactants, EPS and other bioactive molecules [43]. These results and those available for other systems highlight the potential of such integrated microbial conversion processes [62]. Further top strategies are required to find solutions for the industrial production of such compounds and new ones.

Pioneering work on other PHA producers Pseudomonas, such as on expanding the number of inexpensive carbon sources [63], increasing the productivity [64,65] or making the PHA deposition extracellular [66], highlight the potential for successful future investments in this sector. In the meantime, given that robust strains are needed to reduce the high production costs, using genetic engineering and metabolic studies on these two bacteria should focus on developing over-producer strains of mcl-PHA, as well as the co-production of other valuable products, such as EPS and biosurfactants.

Author Contributions: The authors have several years of research experience on Pseudomonas corrugata and P. mediterranea and their bioconversion of waste oils and biodiesel co-products stream, mostly based on the long term projects “Biopolimeri” and “Polybioplast” (acknowledged below). All authors have contributed the writing and editing of the manuscript and approved the manuscript.

Funding: This paper has been prepared without any funding.

Acknowledgments: The results presented were partly generated by the projects MIUR PON 2000–2006 N.12842 “Biopolimeri” and MIUR PON 2007-2013N. 01 01377 “Polybioplast”, both co-funded by EU.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Wu, Q.; Sun, S.Q.; Yu, P.H.F.; Chen, A.X.Z.; Chen, G.Q. Environmental dependence of microbial synthesis of polyhydroxyalkanoates. Acta Polym. Sin. 2000, 6, 751–756.
2. Kadouri, D.; Jurkevitch, E.; Okon, Y.; Castro-Sowinski, S. Ecological and agricultural significance of bacterial polyhydroxyalkanoates. Crit. Rev. Microbiol. 2005, 31, 55–56. [CrossRef]
3. Mozejko-Ciesielska, J.; Szacherska, K.; Marciniak, P. Pseudomonas species as producers of eco-friendly polyhydroxyalkanoates. J. Polym. Environ. 2019, 27, 1151–1166. [CrossRef]
4. Chen, G.Q. A microbial polyhydroxyalkanoates (PHA) based bio- and materials industry. Chem. Soc. Rev. 2009, 38, 2434–2446. [CrossRef]
5. Zinn, M.; Witholt, B.; Egli, T. Occurrence, synthesis and medical application of bacterial polyhydroxyalkanoate. Adv. Drug Del. Rev. 2001, 53, 5–21. [CrossRef]
6. Mozejko-Ciesielska, J.; Kiewisz, R. Bacterial polyhydroxyalkanoates: Still fabulous? Microbiol. Res. 2016, 192, 271–282. [CrossRef] [PubMed]
7. Kessler, B.; Palleroni, N. Taxonomic implications of synthesis of poly-beta-hydroxybutyrate and other poly-beta-hydroxyalkanoates by aerobic pseudomonads. Int. J. Syst. Evol. Microbiol. 2000, 50, 711–713. [CrossRef]
8. Solaiman, D.K.Y.; Ashby, R.D.; Foglia, T.A. Rapid and specific identification of medium-chain-length poly(hydroxyalkanoate) synthase gene by polymerase chain reaction. *Appl. Microbiol. Biotechnol.* 2000, 53, 690–694. [CrossRef]

9. Solaiman, D.K.Y.; Catara, V.; Greco, S. Poly(hydroxyalkanoate) synthase genotype and PHA production of *Pseudomonas corrugata* and *P. mediterranea*. *J. Ind. Microbiol. Biotechnol.* 2005, 32, 75–82. [CrossRef]

10. Solaiman, D.K.Y.; Ashby, R.D.; Foglia, T.A.; Marmer, W.N. Conversion of agricultural feedstock and coproducts into poly(hydroxyalkanoates). *Appl. Microbiol. Biotechnol.* 2006, 71, 783–789. [CrossRef]

11. Ashby, R.D.; Solaiman, D.K.Y.; Foglia, T.A. Bacterial poly(hydroxyalkanoate) polymer production from the biodiesel co-product stream. *J. Polym. Environ.* 2004, 12, 105–112. [CrossRef]

12. Ashby, R.D.; Solaiman, D.K.Y.; Foglia, T.A. Synthesis of short-/medium-chain-length poly(hydroxyalkanoate) blends by mixed culture fermentation of glycerol. *Biomacromolecules* 2005, 6, 2106–2112. [CrossRef] [PubMed]

13. Pappalardo, F.; Fragalà, M.; Mineo, P.G.; Damigella, A.; Catara, A.F.; Palmeri, R.; Recsinña, A. Production of filmable medium-chain-length polyhydroxyalkanoates produced from glycerol by *P. mediterranea*. *Int. J. Biol. Macromol.* 2014, 65, 89–96. [CrossRef] [PubMed]

14. Ashby, R.D.; Foglia, T.A. Poly(hydroxyalkanoates) biosynthesis from triglyceride substrates. *Appl. Microbiol. Biotechnol.* 1998, 49, 431–437. [CrossRef] [PubMed]

15. Da Silva, G.P.; Mack, M.; Contiero, J. Glycerol: A promising and abundant carbon source for industrial microbiology. *Biotechnol. Adv.* 2009, 27, 30–39. [CrossRef]

16. Koller, M.; Marsalek, L. Principles of Glycerol-Based Polyhydroxyalkanoate Production. *Appl. Food Biotechnol.* 2015, 2, 3–10.

17. De Smet, M.J.; Eggingk, G.; Witholt, B.; Kingma, J.; Wynberg, H. Characterization of intracellular inclusions formed by *Pseudomonas oleovorans* during growth on octane. *J. Bacteriol.* 1983, 154, 870–878.

18. Panith, N.; Assavanig, A.; Lertsiri, S.; Berkgvist, M.; Surarit, R.; Niamsiri, N. Development of tunable biodegradable polyhydroxyalkanoates microspheres for controlled delivery of tetracycline for treating periodontal disease. *J. Appl. Polym. Sci.* 2016, 133, 44128–44140. [CrossRef]

19. Zhang, J.; Shishahskaya, E.I.; Volova, T.G.; da Silva, L.F.; Chen, G.Q. Polyhydroxyalkanoates (PHA) for therapeutic applications. *Mater. Sci. Eng. C* 2018, 86, 144–150. [CrossRef]

20. Basset, P.; Ching, K.Y.; Stolz, M.; Knowles, J.C.; Boccaccini, A.R.; Smith, C.; Locke, I.C.; Keshavarz, T.; Roy, I. Novel poly(3-hydroxyoctanoate)/poly(3-hydroxybutyrate) blends for medical applications. *React. Funct. Polym.* 2013, 73, 1340–1348. [CrossRef]

21. Takagi, Y.; Yasuda, R.; Yamaoka, M.; Yamane, T. Morphologies and mechanical properties of polylactide blends with medium chain length poly(3-hydroxyalkanoate) and chemically modified poly(3-hydroxyalkanoate). *J. Appl. Polym. Sci.* 2004, 93, 2363–2369. [CrossRef]

22. Scaffaro, R.; Dintcheva, N.T.; Marino, R.; La Mantia, F.P. Processing and properties of biopolymer/polyhydroxyalkanoates blends. *J. Polym. Environ.* 2012, 20, 267–272. [CrossRef]

23. Prieto, A.; Escapa, I.F.; Martinez, V.; Dinjaski, N.; Herencias, C.; de la Peña, F.; Tarazona, N.; Revelles, O. A holistic view of polyhydroxyalkanoate metabolism in *Pseudomonas putida*. *Environ. Microbiol.* 2016, 18, 341–357. [CrossRef] [PubMed]

24. Catara, V. *Pseudomonas corrugata*: Plant pathogen and/or biological resource? * Mol. Plant. Pathol.* 2007, 8, 233–244. [CrossRef]

25. Trantas, E.A.; Licciardello, G.; Almeida, N.F.; Witek, K.; Strano, C.P.; Duxbury, Z.; Ververidis, F.; Goumas, D.E.; Jones, J.D.G.; Guttman, D.S.; et al. Comparative genomic analysis of multiple strains of two unusual plant pathogens: *Pseudomonas corrugata* and *Pseudomonas mediterranea*. *Front. Microbiol.* 2015, 6, 811. [CrossRef] [PubMed]

26. Corsaro, N.M.; Pia, F.D.; Lanzetta, R.; Naldi, T.; Parrilli, M. Structure of Lipid A from *Pseudomonas corrugata* by electrospray ionisation quadrupole time-of-flight tandem mass spectrometry. *Rapid Commun. Mass Spectrom.* 2004, 18, 853–858. [CrossRef]

27. Fett, W.F.; Cescutti, P.; Wijey, C. Exopolysaccharides of the plant pathogens *Pseudomonas corrugata* and *Ps. fabesens* and the saprophyte *Ps. chlororaphis*. *J. Appl. Bacteriol.* 1996, 81, 181–187. [CrossRef]

28. Scaloni, A.; Dalla Serra, M.; Amodeo, P.; Mannina, L.; Vitale, R.M.; Segre, A.L.; Cruciani, O.; Lodovichetti, F.; Greco, M.L.; Fiore, A.; et al. Structure, conformation and biological activity of a novel lipodepsipeptide from *Pseudomonas corrugata*: Cormycin A. *Biochem. J.* 2004, 384, 25–36. [CrossRef]
29. Emanuele, M.C.; Scaloni, A.; Lavermicocca, P.; Jacobellis, N.S.; Camoni, L.; Di Giorgio, D.; Pucci, P.; Paci, M.; Segre, A.; Ballio, A. Corpeptins, new bioactive lipopeptidipeptides from cultures of Pseudomonas corrugata. *FEBS Lett.* 1998, 433, 317–320. [CrossRef]

30. Strano, C.P.; Bella, P.; Licciardello, G.; Fiore, A.; Lo Piero, A.R.; Fogliano, V.; Venturi, V.; Catara, V. *Pseudomonas corrugata crpCDE* is part of the cyclic lipopeptide corpeptin biosynthetic gene cluster and is involved in bacterial virulence in tomato and in hypersensitive response in *Nicotiana benthamiana*. *Mol. Plant Pathol.* 2014, 9. [CrossRef]

31. Risse, D.; Beiderbeck, H.; Taraz, K.; Budzikiewicz, H.; Gustine, D. Bacterial constituents part LXXVII. Corrugatin, a lipopeptide siderophore from *Pseudomonas corrugata*. *Z. Naturforsch.* C 1998, 53, 295–304.

32. Alicata, R.; Ballistreri, A.; Catara, V.; Conte, E.; Di Silvestro, S.; Ferreri, A.; Greco, S.; Guglielmino, S.; Impallomeni, G.; La Porta, S.; et al. Used cooking oils as renewable source for polyhydroxyalkanoates production. In Proceedings of the 3th European Symposium on Biopolymers CIB-CSIC, Madrid, Spain, 24–25 November 2005; p. 35.

33. Palmeri, R.; Pappalardo, F.; Fragalà, M.; Tomasello, M.; Damigella, A.; Catara, A.F. Polyhydroxyalkanoates (PHAs) production through conversion of glycerol by selected strains of *Pseudomonas mediterranea* and *Pseudomonas corrugata*. *Chem. Eng. Trans.* 2012, 27, 121–126.

34. Solaiman, D.K.Y.; Ashby, R.D.; Foglia, T.A. Physiological characterization and genetic engineering of *Pseudomonas corrugata* for medium-length polyhydroxyalkanoates synthesis from triacylglycerols. *Curr. Microbiol.* 2002, 44, 189–195. [CrossRef]

35. Bella, P.; Catara, A.; Catara, V.; Conte, E.; Di Silvestro, S.; Ferreri, A.; Greco, S.; Guglielmino, S.; Impirrizi, B.; Licciardello, G.; et al. Fermentation Process for the Production of Polyhydroxyalkanoates and the Digestion of Cooked Oils by Strains of *Pseudomonas* Producing Lipase. Italian Industrial Patent No. RM2005A00190, 20 April 2005.

36. Samadi, N.; Abadian, N.; Akhavan, A.; Fazeli, M.R.; Tahzibi, A.; Jamalifar, H. Biosurfactant production by the strain isolated from contaminated soil. *J. Biol. Sci.* 2007, 7, 1266–1269.

37. Licciardello, G.; Ferraro, R.; Russo, M.; Strozzi, F.; Catara, A.F.; Bella, P.; Catara, V. Transcriptome analysis of *Pseudomonas mediterranea* and *P. corrugata* plant pathogens during accumulation of medium-chain-length PHAs by glycerol bioconversion. *New Biotechnol.* 2017, 37, 39–47. [CrossRef] [PubMed]

38. Licciardello, G.; Russo, M.; Pappalardo, F.; Fragalà, M.; Catara, A. Genetically Modified Bacteria Producing Mcl-PHAs. Italian Industrial Patent No.15MG37I, 12 May 2015.

39. Solaiman, D.K.Y.; Ashby, R.D.; Licciardello, G.; Catara, V. Genetic organization of *pha* gene locus affects phaC expression, poly(hydroxyalkanoate) composition and granule morphology in *Pseudomonas corrugata*. *J. Ind. Microbiol. Biotechnol.* 2008, 35, 111–120. [CrossRef] [PubMed]

40. Shantini, K.; Yahya, A.R.; Amirul, A.A. Influence of feeding and controlled dissolved oxygen level on the production of poly(3-hydroxybutyrate-co-3-hydroxyvalerate) copolymer by *Cupriavidus* sp. USMAA2-4 and its characterization. *Appl. Biochem. Biotechnol.* 2015, 176, 1315–1334. [CrossRef]

41. Jiang, X.; Ramsay, J.A.; Ramsay, B.A. Acetone extraction of mcl-PHA from *Pseudomonas putida* KT2440. *J. Microb. Methods* 2006, 67, 212–219. [CrossRef]

42. Koller, M.; Niebelschütz, H.; Braunegg, G. Strategies for recovery and purification of poly[(R)-3-hydroxyalkanoates] (PHA) biopolymesters from surrounding biomass. *Eng. Life Sci.* 2013, 13, 549–562. [CrossRef]

43. Nicolò, M.S.; Franco, D.; Camarda, V.; Guccione, R.; Rizzo, M.G.; Fragalà, M.; Licciardello, G.; Catara, A.F.; Guglielmino, S.P. Integrated microbial process for bioconversion of crude glycerol from biodiesel into biosurfactants and PHAs. *Chem. Eng. Trans.* 2014, 38, 187–192.

44. Rizzo, M.G.; Chines, V.; Franco, D.; Nicolò, M.S.; Guglielmino, S.P. The role of glutamine in *Pseudomonas mediterranea* in biotechnological processes. *New Biotechnol.* 2017, 37, 144–151. [CrossRef] [PubMed]

45. Fragalà, M.; Palmeri, R.; Ferro, G.; Damigella, A.; Pappalardo, F.; Catara, A.F. Production of mcl-PHAs by *Pseudomonas mediterranea* conversion of biodiesel-glycerol. In Proceedings of the European Symposium on Biopolymers, Lisbon, Portugal, 7–9 October 2013.

46. Tsuge, T. Fundamental factors determining the molecular weight of polyhydroxyalkanoate during biosynthesis. *Polym. J.* 2016, 48, 1051–1057. [CrossRef]
47. Conte, E.; Catara, V.; Greco, S.; Russo, M.; Alicantà, R.; Strano, L.; Lombardo, A.; Di Silvestro, S.; Catara, A. Regulation of polyhydroxyalkanoate synthases (phaC1 and phaC2) gene expression in Pseudomonas corrugata. Appl. Microbiol. Biotechnol. 2005, 72, 1054–1062. [CrossRef] [PubMed]

48. Bottia, L.; Mistretta, M.C.; Palermo, S.; Fragalà, M.; Pappalardo, F. Characterization and processability of blends of poly lactide acid with a new biodegradable medium-chain-length polyhydroxyalkanoate. J. Polym. Environ. 2015, 23, 478–486. [CrossRef]

49. Cascone, G.; D’Emilio, A.; Buccellato, E.; Mazzarella, R. New biodegradable materials for greenhouse soil mulching. Acta Hortic. 2008, 801, 283–290. [CrossRef]

50. Salemi, F.; Lamagna, G.; Coco, V.; Barone, L.G. Preparation and characterization of biodegradable paper coated with blends based on PHA. Acta Hortic. 2008, 801, 203–210. [CrossRef]

51. Catara, V.; Bella, P.; Greco, S.; Licciardello, G.; Pitman, A.; Arnold, D.L. Cloning and sequencing of Pseudomonas corrugata polyhydroxyalkanoates biosynthesis genes. In Proceedings of the National Biotechnology Congress (CNB7), Catania, Italy, 8–10 September 2004; p. 199.

52. Bella, P.; Licciardello, G.; Lombardo, A.; Pitman, A.; Arnold, D.L.; Solaiman, D.K.Y.; Catara, V. Cloning of Pseudomonas mediterranea PHA locus. In Proceedings of the 4th European Symposium on Biopolymers, Kuşadası, Turkey, 2–4 October 2007; p. 131.

53. Licciardello, G.; Bella, P.; Devescovi, G.; Strano, C.P.; Catara, V. Draft genome sequence of Pseudomonas mediterranea strain CFBP 5447T, a producer of filmable medium-chain-length polyhydroxyalkanoates. Genome Announc. 2014, 2, e01260-14. [CrossRef]

54. Licciardello, G.; Jackson, R.W.; Bella, P.; Strano, C.P.; Catara, A.F.; Arnold, D.L.; Venturi, V.; Silby, M.W.; Catara, V. Draft genome sequence of Pseudomonas corrugata, a phytopathogenic bacterium with potential industrial applications. J. Biotechnol. 2014, 17, 65–66. [CrossRef]

55. Zachow, C.; Müller, H.; Laireiter, C.M.; Tilcher, R.; Berg, G. Complete genome sequence of Pseudomonas corrugata strain RMI-1-4, a stress protecting agent from the rhizosphere of an oilseed rape bait plant. Stand. Genomic Sci. 2017, 12, 66. [CrossRef]

56. Licciardello, G.; Devescovi, G.; Bella, P.; De Gregorio, C.; Catara, A.F.; Gugliemino, S.P.P.; Venturi, V.; Catara, V. Transcriptional analysis ofpha genes in Pseudomonas mediterranea CFBP 5447 grown on glycerol. Chem. Eng. Trans. 2014, 38, 289–294.

57. De Eugenio, L.I.; Escapa, I.F.; Morales, V.; Dinjaski, N.; Galán, B.; García, J.L.; Prieto, M.A. The turnover of medium-chain-length polyhydroxyalkanoates in Pseudomonas putida KT2442 and the fundamental role of PhaZ depolymerase for the metabolic balance. Environ. Microbiol. 2010, 12, 207–221. [CrossRef] [PubMed]

58. Guo, W.; Song, C.; Kong, M.; Geng, W.; Wang, Y.; Wang, S. Simultaneous production and characterization of medium-chain-length polyhydroxyalkanoates and alginate oligosaccharides by PhaZ depolymerase for the metabolic balance. Environ. Microbiol. 2018, 20, 521. [CrossRef]

59. Botta, L.; Mistretta, M.C.; Palermo, S.; Fragalà, M.; Pappalardo, F. Characterization and processability of blends of poly lactide acid with a new biodegradable medium-chain-length polyhydroxyalkanoate. J. Polym. Environ. 2015, 23, 478–486. [CrossRef]

60. Lombardò, A.; Bella, P.; Licciardello, G.; Palmeri, R.; Catara, V.; Catara, A. Poly(hydroxyalkanoate) synthase genes in Pseudomonads strains, isolation and heterologous expression. J. Biotechnol. 2010, 150, 420–421. [CrossRef]

61. Lombardò, A.; Bella, P.; Licciardello, G.; Palmeri, R.; Catara, V.; Catara, A. Poly(hydroxyalkanoate) synthase genes in Pseudomonads strains, isolation and heterologous expression. J. Biotechnol. 2010, 150, 420–421. [CrossRef]

62. Hori, K.; Marsudi, S.; Unno, H. Simultaneous production of polyhydroxyalkanoates and rhamnolipids by Pseudomonas aeruginosa. Biotechnol. Bioeng. 2002, 78, 699–707. [CrossRef]

63. Wang, Q.; Tappei, R.C.; Zhu, C.; Nomura, T.C. Development of a new strategy for production of medium-chain-length polyhydroxyalkanoates by recombinant Escherichia coli via inexpensive non fatty acid feedstocks. Appl. Environ. Microbiol. 2012, 78, 519–527. [CrossRef]

64. Lopez-Cortes, A.; Lanz-Landazuri, A.; García-Maldonado, J.Q. Screening and isolation of PHB-producing bacteria in a polluted marine microbial mat. Microb. Ecol. 2008, 56, 112–120. [CrossRef]
65. Luengo, J.M.; García, B.; Sandoval, A.; Naharro, G.; Olivera, E.R. Bioplastics from microorganisms. *Curr. Opin. Microbiol.* 2003, 6, 251–260. [CrossRef]

66. Sabirova, J.S.; Ferrer, M.; Lünsdorf, H.; Wray, V.; Kalscheuer, R.; Steinbüchel, A.; Timmis, K.N.; Golyshin, P.N. Mutation in a tesB-Like hydroxyacyl-Coenzyme a-specific thioesterase gene causes hyperproduction of extracellular polyhydroxyalkanoates by *Alcanivorax borkumensis* SK2. *J. Bacteriol.* 2006, 188, 8452–8459. [CrossRef]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).