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ORIGINAL PAPER

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Poly(hydroxyalkanoate) synthase genotype and PHA production of *Pseudomonas corrugata* and *P. mediterranea*

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Abstract A collection of *Pseudomonas corrugata* and *P. mediterranea* strains, two closely related species, was evaluated for the presence and variability of *pha* loci. Using PCR methods that specifically amplify segments of medium-chain-length poly(hydroxyalkanoate) (mcl-PHA) synthase genes, we demonstrated the presence of *phaC1* and *phaC2* in all *P. mediterranea* strains tested and in six out of 56 strains of *P. corrugata* screened. The remaining 50 strains of *P. corrugata* yielded only the *phaC2* subgene fragment on detection by a combined PCR-restriction endonuclease analysis method or a semi-nested PCR-amplification approach. A Southern hybridization study on a representative strain from this group, however, indicated the presence of the *phaC1* gene. Nucleic acid sequences of the subgene *phaC* fragments of the representative strains from the three groups showed an overall similarity ranging from 95% to 100%. The major repeat-unit monomers of the mcl-PHAs isolated from these selected strains are β-hydroxyoctanoate (33–47 mol%) and β-hydroxydecanoate (26–36 mol%). These results differentiate for the first time the strains of *P. corrugata* into two *pha*-distinguishable groups. This study also documents for the first time the production of mcl-PHA in *P. mediterranea*.

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Introduction

Poly(hydroxyalkanoates) (PHAs), synthesized by many bacteria, are biodegradable polymers of great potential for industrial and medical applications [21]. These microbial polymers are accumulated as inclusion bodies when nutrient supplies are imbalanced and are thus believed to play a role as a sink of carbon source and reducing equivalents.

Many pseudomonads belonging to rRNA-DNA homology group I produce medium-chain-length (mcl)-PHAs composed of hydroxyalkanoate repeat-unit monomers of six or more carbon atoms [24]. A type II PHA genetic system consisting of two synthase genes (*phaC1*, *phaC2*) separated by a gene coding for the depolymerization of PHA (*phaZ*) is commonly found in mcl-PHA-producing pseudomonads [11, 26]. Rapid and specific PCR-based methods have been developed for the screening of the *phaC1* and *phaC2* genes in microorganisms [22, 23]. Using these methods, *Pseudomonas corrugata* was identified as a mcl-PHA-producing organism for the first time [23]. By physiological study, Kessler and Palleroni [12] separately confirmed the synthesis of mcl-PHA in *P. corrugata*, an agriculturally important non-fluorescent species that belongs to the γ sub-class of the Proteobacteria in which species of the genus *Pseudomonas* sensu stricto within rRNA similarity group I are clustered. This bacterium is a soil inhabitant capable of multiplying on the root system, where it is a good competitor against other organisms. In addition to mcl-PHA, various *P. corrugata* strains also produce valuable microbial products, such as lipid A potentially useful as biosurfactants [6], exopolysaccharides [8], bioactive lipodepsipeptides such as cormycin A [20] and corpeptins [7], and a lipopeptide siderophore, corrugatin [17]. Strains of *P. corrugata* have been isolated both as a

plant pathogen inducing a syndrome known as tomato pith necrosis (observed also on pepper, chrysanthemum) and as an endophyte on symptomless plants and bare soil. *P. corrugata* is a very heterogeneous species, based on phenotypic and genetic studies. Recently, a taxonomic study indicated that two taxa were distinguishable within the species *P. corrugata*: *P. corrugata* (i.e., the taxon that includes the type strain) and *P. mediterranea* [5]. For a long time, *P. corrugata* was considered as a poly(*β*-hydroxybutyrate)-producing organism. Only recently was it demonstrated that this *Pseudomonas* species actually accumulates mcl-PHA [12, 23]. Results of gel permeation chromatography indicated that the weight-average and number-average molecular masses of the PHA of *P. corrugata* strain 388 were 735 ± 105 kDa and 181 ± 45 kDa, respectively [23]. Thus, the mcl-PHA of this organism appears to have a higher molecular weight than that of similar polymers produced by other pseudomonads.

The possibility of coproducing one of the valuable microbial products mentioned in the preceding paragraph with the mcl-PHA could help the partial recovery of the total production costs of the biopolymer. Furthermore, since PHA is suggested to play a role in bacterial cell survival [18], its metabolism may influence the resilience of the microorganism and indirectly contribute to its plant disease-causing potential. Consequently, we undertook a study to categorize the variability of the PHA synthase genotype of various strains of *P. corrugata* and *P. mediterranea* in our collection. The results provide a knowledge base potentially valuable for future attempts to select and develop these bacteria for the coproduction of mcl-PHA and a microbial coproduct, and for future study to probe how PHA-dependent microbial survival in soil under adverse conditions can influence the plant disease cycle through the availability of a viable inoculum.

Materials and methods

Bacterial strains, species attribution, and growth conditions

A total of 77 strains of *P. corrugata* and *P. mediterranea* were studied. These included ten *P. mediterranea* strains and 17 *P. corrugata* strains from the Collection Francaise des Bacteries Phytopathogenes (CFBP, Angers, France) previously used in a taxonomic study [5] and 50 strains of *P. corrugata* from the collection in the Dipartimento di Scienze e Tecnologie Fitosanitarie, Universitá degli Studi di Catania, Italy (those marked with IPVCT). The type strains of *P. corrugata* (three strains variously obtained as ATCC 29736^T, CFBP 2431^T, NCPPB 2445^T, *P. mediterranea* CFBP 5447^T) and a *P. corrugata* strain 388 previously used to study PHA production and the *pha* synthase genotype [23] were included in the analysis. Strains from long-term storage tubes (in nutrient broth containing 15% glycerol at −80°C) were streaked twice on nutrient dextrose agar (Difco, Detroit, Mich., USA) before they were used in PCR analysis. Strains that had not been previously included in the *P. mediterranea* taxonomic study [5] were first subjected to taxon identification. For this purpose, a multiplex PCR method employing two pairs of specific primers (i.e., PC5/1 with PC5/2 for *P. corrugata*, PC1/1 with PC1/2 for *P. mediterranea*) was used to obtain either the 1,100-bp DNA fragment or the 600-bp amplicon indicative of *P. corrugata* or *P. mediterranea*, respectively [5]. *P. corrugata* strain 388 was additionally phenotyped using Biotype 100 nutritional galleries (bioMérieux, La Balne les Grottes, France) to confirm its taxon.

For routine culture, cells were grown in Luria broth (LB; 1% w/v tryptone, 0.5% w/v yeast extract, 0.5% NaCl) and tryptic soy broth (Difco) at 30°C or 37°C with 200–250 rpm shaking. Solid media contained 1.2–1.5% agar.

PCR identification of mcl-PHA synthase genes

The PCR procedure with primers I-179L and I-179R [23] was used to assess the presence of genes coding for type II PHA synthases in strains included in this study. The primers were designed based on two highly conserved sequences found in the coding regions of the *Pseudomonas phaC1* and *phaC2* genes, thus allowing the concurrent amplification of their two subgene fragments, i.e., *dphaC1* and *dphaC2*, respectively. Selective amplification of the individual *dphaC1* and *dphaC2* fragments was performed using a semi-nested PCR procedure [22]. The PHA synthase genotype was distinguished either by performing *Pvu*II restriction endonuclease analysis (REA) on the mixed amplicons generated with I-179L and I-179R, or by using a semi-nested PCR protocol for selective amplification of the individual *dphaC1* and *dphaC2* fragments [22]. The PCR was performed using the native or recombinant *Taq* DNA polymerase (Invitrogen, Carlsbad, Calif., USA). A single bacterial colony lysed by heating (10 min at 95°C) in deionized water and a purified genomic DNA sample were variously used as template DNA for the PCR [23]. The PCR products and their REA fragments were variously analyzed by electrophoresis on 1.5% agarose gel or 2.0% NuSieve 3:1 agarose-gel in TBE (0.089 M Tris base, 0.089 M boric acid, 0.002 M Na-EDTA, pH ~8) or TAE (0.04 M Tris-acetate, 0.001 M Na-EDTA, pH ~8) buffer [19]. DNA fragments in gels were stained with ethidium bromide for visualization.

PCR cloning and sequencing

The subcloning and sequencing of the individual *dphaC1* and *dphaC2* subgene fragments of PHA synthase genes of *P. corrugata* strains IPVCT 2.2 and IPVCT 10.2 and *P. mediterranea* strains CFBP 5447^T, CFBP 5444,

IPVCT 3C, and IPVCT P1 were performed according to Solaiman et al. [23]. Specifically, these subgene fragments were cloned in *Escherichia coli* DH5α using a pT7Blue-3 vector linearized at the *Eco*RV site (Novagen, Madison, Wis., USA). The plasmids served as the double-stranded DNA template for sequencing reactions using an ABI Prism BigDye terminator cycle sequencing ready reaction kit (Applied Biosystems, Foster City, Calif., USA). In some cases, the individually amplified *dphaC1* and *dphaC2* fragments obtained from the semi-nested PCR experiments were sequenced without prior subcloning. In these cases, the PCR fragments were first purified using a MinElute PCR purification kit (Qiagen, Valencia, Calif., USA) prior to their use as the template in the sequencing reaction and the oligonuclotide I-179La or I-179Ra was used as the sequencing primer. The sequence data were collected and documented using an ABI Prism 310 genetic analyzer (Applied Biosystems). Analyses of the DNA sequences were performed using BLAST2 [28] and BLASTX [1] programs on the website of the National Center of Biotechnology Institute and commercially available software, e.g., Omiga (Oxford Molecular Group, Beaverton, Ore., USA) and CloneManager (Scientific & Educational Software, Durham, N.C., USA).

Southern hybridization

Genomic DNA was digested with restriction enzyme *Bam*HI, *Eco*RI, or *Hin*dIII. The restriction fragments were electrophoresed on an agarose gel in TAE buffer and transferred to a Nylon membrane by the Southern-blot protocol [19]. Hybridization probes were a mixture of *dphaC1* and *dphaC2* of *P. corrugata* 388 obtained by PCR using the I-179L with I-179R primer pair. The probes were labeled with digoxigenin (DIG) during the PCR procedure, using a DIG PCR probe synthesis kit (Roche Diagnostics, Monza, Italy). Hybridization and probe detection were performed using a DIG-high prime DNA labeling and detection starter kit (Roche Diagnostics) according to the manufacturer's suggested protocol.

PHA isolation and composition analysis

Seed cultures were prepared by inoculating LB medium (10 mL) in culture tubes with 0.2 mL of stock culture (previously stored in 15% glycerol at −80°C). The cultures were incubated for 24 h at 30°C with 200 rpm shaking. An aliquot (2.5 mL) of the seed culture was used to inoculate 250 mL of medium E* [3] supplemented with oleic acid (0.5% v/v, technical grade; Sigma Chemicals, St. Loius, Mo., USA). These experimental cultures (in 500-mL Erlenmeyer flasks) were grown for 48 h at 30°C with 200 rpm shaking. Cells were harvested by centrifugation, washed with ice-cooled water, and lyophilized to dryness. A portion of the lyophilized cells (approximately 20 mg) was subjected to acid-catalyzed methanolysis [27] and silylation [2]. The silylated methyl esters of 3-hydroxyalkanoate monomers were analyzed on a GC/MS, using the ion selection mode at *m*/*e* 175 [13].

Results and discussion

Bacterial collection screenings

We first screened the 77 strains of *Pseudomonas* spp used in this study by applying the multiplex PCR protocol described previously [4] to ascertain their identity as either *P. corrugata* or *P. mediterranea*. The results showed that the collection included 56 strains of *P. corrugata* (1,100-bp amplicon) and 21 strains of *P. mediterranea* (600-bp amplicon). We next applied the PCR method described by Solaiman et al. [23] to screen for the presence of mcl-PHA synthase genes in these strains. The results showed that PCR products (i.e., *dphaC1*, *dphaC2*) of about 500 bp were observed with all the strains tested, indicating the presence of type II PHA synthase gene(s) in these strains (data not shown). An earlier study showed that REA could provide a preliminary assessment regarding the sequence similarity of *dphaC2* fragments obtained from different *P. corrugata* strains [22]. We thus applied the REA protocol on the *dphaC1*/*dphaC2* PCR products of selected strains, using *Pvu*II as the restriction enzyme. The results showed two distinct digestion patterns (Fig. 1). The *P. corrugata* IPVCT 10.2 and IPVCT 2.2 samples exhibited a digestion pattern containing only a 0.54-kb band (Fig. 1, lanes 4, 5), whereas the *P. mediterranea* strains exhibited a pattern containing two fragments (0.54 kb, 0.45 kb;

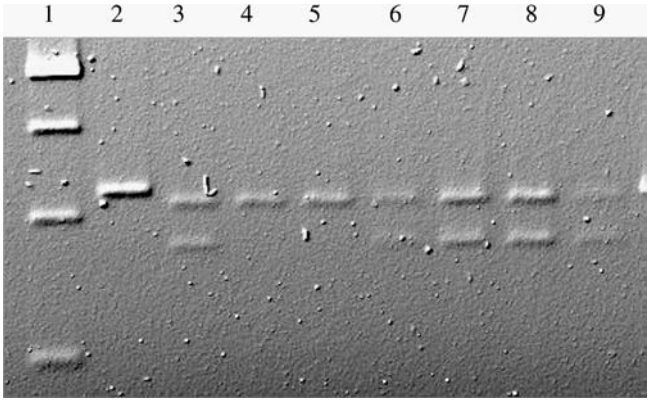

Fig. 1 *Pvu*II digestion of *dphaC1*/*dphaC2* of *P. corrugata* and *P. mediterranea*. Electrophoresis was performed on a 2% NuSieve 3:1 agarose gel in TBE buffer. *Lane 1* DNA size markers (1.0, 0.75, 0.5, 0.25 kb, from *top* to *bottom*), *lane 2* 0.56-kb fragment of λ-DNA/ *Hin*dIII digests, *lanes 3–9* *Pvu*II digests of *dphaC1*/*dphaC2* PCR products from *P. mediterranea* strains CFBP 5447^{T} (*lane 3*), IPVCT P1 (*lane 6*), CFBP 5458 (*lane 7*), CFBP 5444 (*lane 8*), IVIA 1.1.6 (*lane 9*), *P. corrugata* strains IPVCT 10.2 (*lane 4*), IPVCT 2.2 (*lane 5*). The sizes of the two *Pvu*II-digested fragments are 0.54 kb and 0.45 kb

Fig. 1, lanes 3, 6–9). These results suggest that the *dphaC1*/*dphaC2* PCR products of the five *P. mediterranea* strains tested contain two amplicons, of which one is amenable to *Pvu*II digestion, and that the PCR products of the two *P. corrugata* strains tested contain either one or two *Pvu*II-resistant *dphaC1*/*dphaC2* PCR fragments. The semi-nested PCR procedure [22] was used to clarify the presence of PCR-amplifiable *dphaC1* and *dphaC2* sequences in these test strains. The results showed that the five *P. mediterranea* test strains yielded amplifiable *dphaC1* and *dphaC2*, while the two strains of *P. corrugata* produced only the *dphaC2* fragment (data not shown). These observations suggest that the combined PCR-REA (*Pvu*II) method is a useful means to provide a preliminary indication of the presence or absence of *dphaC1* and *dphaC2*.

We proceeded to screen the set of 77 strains of *P. corrugata* and *P. mediterranea* using either the combined PCR-REA (*Pvu*II) method or the semi-nested PCR protocol. The results (Table 1) indicated the presence of both subgene fragments in all strains classified as *P. mediterranea* and in six out of 56 strains of *P. corrugata*. It appears that strains of *P. mediterranea* constitute a uniform group with respect to the sequences of the PHA synthase genes. While the majority of *P. corrugata* (i.e., 50 tested strains, including the type strains obtained as CFBP 2431^T, NCPPB 2445^T, ATCC 29736^T) lack an amplifiable *dphaC1*, six strains (i.e., CFBP 10532, CFBP 10058, CFBP 10900, 313, 388, 717) contain both the amplifiable *phaC* subgene fragments (Table 1). Of the six non-conforming *P. corrugata* strains, we selected strain 388 to further confirm its classification. The results from a study using the multiplex-PCR identification protocol and the biochemical discriminatory tests [5] confirmed that strain 388 is in fact *P. corrugata*. Solaiman [22] observed the failure of amplifying the *dphaC2* fragment of *P. oleovorans* NRRL B-778 due to a one-base variation at the 3′-terminal nucleotide of the I-179L binding site. Subsequent use of the primer pair with a degenerate nucleotide at the 3′ terminal sequence (i.e., I-179La with I-179Ra) alleviated the problem [22]. We thus repeated the PCR analysis using these degenerate primers on ten randomly selected strains of *P. corrugata* from the *dphaC1*-lacking group. The results did not change the original observation that an amplifiable *dphaC1* could not be detected (data not shown). We proceeded to carry out a Southern-blot hybridization study to verify the presence of the *phaC1* gene in the *P. corrugata* group with non-amplifiable *dphaC1*. An arbitrarily selected *P. corrugata* CFBP 5454 from the *dphaC1*-lacking group (Table 1), along with *P. mediterranea* CFBP 5447^T as a positive-control sample, was used for the Southern-blot hybridization study. Figure 2 shows that the *Hin*dIII-digested or *Bam*HI-digested genome of *P. corrugata* CFBP 5454 had a large restriction fragment that hybridized with the probes. The *Eco*RI-digested CFBP 5454 DNA, however, contained two restriction fragments that hybridized with the probes. These results suggested that *phaC1* and *phaC2* of *P. corrugata* CFBP 5454 reside in a single *Hin*dIII fragment or *Bam*HI fragment of the genome, but are on two separate *Eco*RI fragments of the DNA. The results also showed that the *phaC* genes of the positive-control sample, *P. mediterranea*, are co-located on a single *Eco*RI fragment or *Bam*HI fragment and two separate *Hin*dIII pieces of the genome. The occurrence of *phaC1* in *P. corrugata* CRBP 5454 (and by implication in the other strains of the *dphaC1*-lacking group)

Table 1 Screening for *dphaC1* and *dphaC2* in *P. corrugata* and *P. mediterranea*

Species[a]	Matrix of isolation	Geographic origin	Number of strains	*dphaC1*[b]	*dphaC2*[b]	Strain[c]
P. corrugata	Tomato	Italy	35[d]	–	+	
	Soil	Italy	4	–	+	
	Tomato	France	1	+	+	CFBP10532
	Tomato	Spain	6	–	+	
	Tomato	Spain	1	+	+	CFBP10900
	Tomato	South Africa	1	–	+	
	Tomato	USA	1	–	+	
	Alfalfa root	USA	2	+	+	313, 388
	Raspberry root	USA	1	+	+	717
	Tomato	Hungary	1	–	+	
	Tomato	UK	1[e]	–	+	
	Tomato	New Zealand	1	+	+	CFBP10058
	Tomato	Switzerland	1	–	+	
P. mediterranea	Tomato	Italy	18[f]	+	+	
	Tomato	France	1	+	+	
	Tomato	Spain	1	+	+	
	Pepper	Spain	1	+	+	

[a]Assessed or confirmed by multiplex PCR with specific primers
[b]Obtained by *Pvu*II digestion of *dphaC1*/*dphaC2* PCR fragment mix and/or semi-nested PCR
[c]Only *P. corrugata dphaC1*-positive strains are indicated
[d]One strain in this group is *P. corrugata* CFBP 5454 used in the Southern-blot study (Fig. 2)
[e]Type strain (^{T}) was tested using *P. corrugata* strains CFBP 2431^T, NCPPB 2445^T, and ATCC 29736^T
[f]One strain in this group is *P. mediterranea* CFBP 5447^T (type strain) used in the Southern-blot study (Fig. 2)

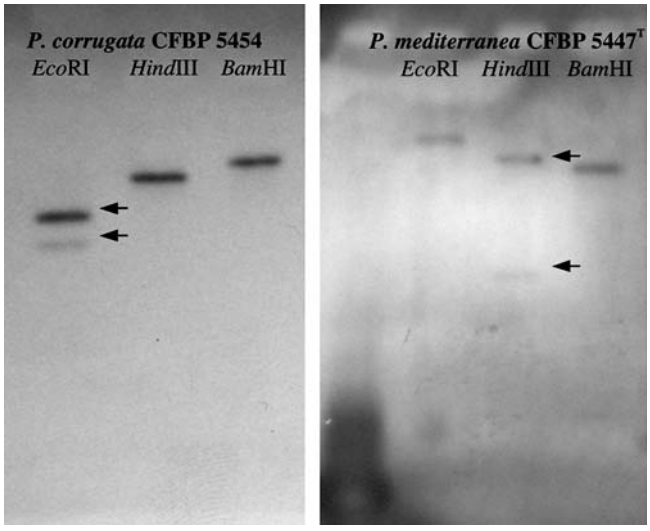

Fig. 2 Southern blot of total DNAs from *P. corrugata* CFBP 5454 and *P. mediterranea* CFBP 5447^T, using the mixture of DIG-labelled *dphaC1* and *dphaC2* probes prepared by PCR procedure. *Arrows* indicate the double-hybridization signals observed with DNA digested with the specified endonucleases

was separately confirmed by molecular cloning and sequence determination of the gene (Catara et al., unpublished data).

Sequence comparison of *dphaC1* and *dphaC2*

The identity of the cloned *dphaC1* and *dphaC2* was confirmed by sequence determination and subsequent alignment search against databases. Sequence analysis was performed on *P. mediterranea* strains CFBP 5444, CFBP 5447^T, and IPVCT P1 harboring both the *dphaC* subgene fragments and on *P. corrugata* strains IPVCT 10.2 and IPVCT 2.2 lacking an amplifiable *dphaC1*. Sequences obtained were subjected to BLAST2 analysis [28] against each other and the *P. corrugata* 388 *dphaC1* (gi 12659054) and *dphaC2* (gi 12659052) sequences. The results (Table 2) show that three *dphaC* sequence-similarity groups (*dphaC*-SSGs) are apparent. In this analysis, one *dphaC*-SSG consists of the three *P. mediterranea* strains. The second is constituted of *P. corrugata* IPVCT 10.2 and IPVCT 2.2, and the third group is represented by *P. corrugata* 388. This grouping pattern is consistent with the results found in Table 1, in which the *P. mediterranea* strains showed both the amplifiable *dphaC1* and *dphaC2*, the majority of *P. corrugata* strains (including IPVCT 10.2, IPVCT 2.2 in Table 2) yielded only an amplifiable *dphaC2*, and a small number of *P. corrugata* strains (including strain 388) exhibited both the *dphaC1* and *dphaC2* subgene fragments. Within each *dphaC*-SSG, there was no difference in PHA synthase genotype. Solaiman [22] reported that the respective *dphaC1* and *dphaC2* sequences of *P. corrugata* strains 313, 388, and 717 exhibited 100% nucleotide identity. The data in Table 2 further showed that, within a *dphaC* class, the nucleotide sequences of the subgene PCR fragments were highly similar to each other (95% nucleotide identity or better). These results attested to the extremely close phylogenetic relationship of *P. corrugata* and *P. mediterranea*.

The *dphaC* sequences from a species of each *dphaC*-SSG group were further subjected to BLASTX analysis (Table 3). Both the translated sequences of *dphaC1* and *dphaC2* of *P. mediterranea* CFBP 5447^T showed the highest amino acid (a.a.) sequence homology with the corresponding sequences of *P. corrugata* 388, *P. fluorescens* PfO-1, and *P. chlororaphis* (formerly *P. aureofaciens* [14]). An updated BLASTX analysis of the *dphaC1* fragment of *P. corrugata* 338 showed that the three highest a.a. homologous sequences were those of *P. fluorescens* PfO-1, *P. chlororaphis*, and *Pseudomonas* sp. 61-3. The translated sequence of *dphaC2* of *P. corrugata* 388, however, was most closely matched to those of *P. chlororaphis*, *P. fluorescens* PfO-1, and *Pseudomonas* sp. 61-3. The BLASTX results with the *dphaC2* sequence of the *dphaC1*-lacking *P. corrugata* IPVCT 10.2 showed the highest a.a. sequence homology to

Table 2 Sequence comparison of *dphaC1* and *dphaC2*

Strain	P. mediterranea			P. corrugata		
	CFBP 5447^T	IPVCT P1	CFBP 5444	IPVCT 10.2	IPVCT 2.2	388
Percent nucleotide identity of *dphaC1*[a]						
CFBP 5447^T	100	–	–			–
IPVCT P1	100	100	–			–
CFBP 5444	100	100	100			–
388	96	96	96			100
Percent nucleotide identity of *dphaC2*						
CFBP 5447^T	100	–	–	–	–	–
IPVCT P1	100	100	–	–	–	–
CFBP 5444	100	100	100	–	–	–
IPVCT 10.2	95	95	95	100	–	–
IPVCT 2.2	95	95	95	100	100	–
388	95	95	95	99	99	100

[a] *dphaC1* could not be detected with *P.corrugata* IPVCT 10.2 and IPVCT 2.2

Table 3 BLASTX analysis of *dphaC1* and *dphaC2*

Strain	Subgene PCR fragment	Homologous sequences (gi number; % identity)		
P. mediterranea CFBP 5447^T	*dphaC1*	*P.corrugata* 388 (gi 12659054; 98%)	*P.fluorescens* PfO-1 (gi 23059270; 95%)	*P.chlororaphis*[a] (gi 17402511; 96%)
	dphaC2	*P.corrugata* 388 (gi 12659052; 99%)	*P.fluorescens* PfO-1 (gi 23059268; 86%)	*P.chlororaphis*[a] (gi 17402513; 85%)
P.corrugata 388	*dphaC1*	*P.fluorescens* PfO-1 (gi 23059270; 94%)	*P.chlororaphis*[a] (gi 17402511; 94%)	*Pseudomonas* sp. 61–3 (gi 4062968; 92%)
	dphaC2	*P.chlororaphis*[a] (gi 17402513; 86%)	*P.fluorescens* PfO-1 (gi 23059268; 86%)	*Pseudomonas* sp. 61–3 (gi 4062970; 84%)
P.corrugata IPVCT 10.2	*dphaC1*[b]	–	–	–
	dphaC2	*P.corrugata* 388 (gi 12659052; 98%)	*P.chlororaphis*[a] (gi 17402513; 86%)	*P.fluorescens* PfO-1 (gi 23059268; 86%)

[a] Formerly *P. aureofaciens*

[b] *dphaC1* was not detected by the PCR protocols in this study

P. corrugata 388, *P. chlororaphis*, and *P. fluorescens* PfO-1. Based on the pattern of the homology-identity matches, the *phaC1* of *P. corrugata* 388 is highly related to that of *P. mediterranea*; and the *phaC2* of strain 388 is highly homologous to that of the *dphaC1*-lacking *P. corrugata* IPVCT 10.2.

Comparison of PHA composition

The repeat-unit compositions of the mcl-PHA synthesized by *P. mediterranea* CFBP 5447^T, *P. corrugata* IPVCT 10.2, and *P. corrugata* 388 were compared. Cells were grown in a chemically defined E* medium [3] using oleic acid as the sole carbon source. The results (Table 4) showed that the mcl-PHAs synthesized by these bacteria contain β-hydroxydecanoate (C_{10}), β-hydroxyoctanoate (C_8), and β-hydroxytetradecenoate ($C_{14:1}$) as their major repeat-unit monomers. Under these growth conditions, a substantial amount of β-hydroxydodecanoate (C_{12}) (8–10 mol%) was also incorporated into the polymer. Data in Table 4 also showed that the polymer of *P. corrugata* 388 contains a higher level of C_8-monomer and a lower level of C_{10}-monomer than that found in CFBP 5447^T and IPVCT 10.2. Although these differences in monomer composition are interesting, they are not significant enough to affect the physicochemical properties of the mcl-PHA. The basis for the compositional difference of PHA in these organisms most likely lies in subtle differences in their metabolic background, since *phaC1* and *phaC2* have not been conclusively and unequivocally shown to produce different PHA (see the following discussion). Genes encoding the enzymes involved in mcl-PHA formation and degradation have been described for a number of pseudomonads (see review in Ref. [25]). The two PHA polymerases encoded by the *phaC1* and *phaC2* genes were equally active in mediating PHA synthesis from fatty acids and glucose (see review in Ref. [15]). The fact that the *dphaC1* subgene fragment could not be PCR-amplified and cloned from the majority of the *P. corrugata* strains could be due to their *phaC1* genes having drastically different nucleotide sequences in the regions highly conserved for all other *Pseudomonas*. The repeat-unit composition of

Table 4 Repeat-unit composition of *P.mediterranea* and *P.corrugata*

Strain	Composition (mol%)[a]				
	C_6	C_8	C_{10}	C_{12}	$C_{14:1}$
P.mediterranea CFBP 5447^T	2	33	36	10	19
P.corrugata IPVCT 10.2	2	37	33	8	18
P.corrugata 388	3	47	26	8	16

[a] Values given are the means of two measurements with standard deviations $< 5\%$. C_6 β-Hydroxyhexanoate, C_8 β-hydroxyoctanoate, C_{10} β-hydroxydecanoate, C_{12} β-hydroxydodecanoate, $C_{14:1}$ β-hydroxytetradecenoate. Monomers detected at ≤ 1 mol% included β-hydroxybutanoate, β-hydroxydodecenoate, β-hydroxytetradecanoate and β-hydroxytetradecadienoate

the biopolymer isolated from a representative strain of this *dphaC1*-lacking *P. corrugata* group is similar to that of *P. mediterranea*, supporting the observations that any functional differences between the *phaC1* and *phaC2* of pseudomonads are not readily detected [10, 11, 16]. Hein et al. [9] used heterologous expression hosts to show that the *phaC1* gene of *P. mendocina* preferentially directs mcl-PHA synthesis from fatty-acid carbon sources, while the *phaC2* mediates the polymer synthesis using gluconate as substrate. Furthermore, *phaC1*and *phaC2* knockout mutants of *P. mendocina* showed that PHA production is mainly carried out by the gene product of *phaC1* [9]. The two *dphaC1*-SSGs of *P. corrugata* thus provide valuable systems for the study of the role of *phaC1* on substrate-dependent PHA synthesis.

In summary, the present work details an extensive PHA synthase genotyping of two closely related, agriculturally important *Pseudomonas* species. The discovery that the strains in these species have different *phaC* gene organizations lays down valuable groundwork for future study to select and develop strains of these organisms for the co-production of PHA and another value-added coproduct. Furthermore, the results also provide a valuable knowledge base for future study to probe the role of PHA-dependent survival of these agriculturally important bacteria in plant disease cycles.

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