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Regulation of polyhydroxyalkanoate biosynthesis in *Pseudomonas putida* and *Pseudomonas aeruginosa*

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Abstract

Regulation of medium-chain-length polyhydroxyalkanoate biosynthesis in *Pseudomonas aeruginosa* and *Pseudomonas putida* was studied conducting PHA accumulation experiments and transcriptional analysis of PHA biosynthesis genes with wild type strains and *rpoN*-negative mutants. In *P. putida* PHA accumulation was RpoN-independent, whereas in *P. aeruginosa* PHA accumulation was RpoN-dependent. Transcriptional analysis applying reverse transcriptase-polymerase chain reaction showed strong induction of *phaG*, encoding the transacylase, under nitrogen starvation in *P. putida* KT2440 and the respective *rpoN*-negative mutant, indicating an RpoN-independent regulation of *phaG*. No transcription of *phaG* and no PHA accumulation was detected in the *rpoN*-negative mutant of *P. aeruginosa* neither from gluconate nor from octanoate as carbon source. Alginate-overproducing mutant *P. aeruginosa* FRD1 showed strongly decreased PHA accumulation from gluconate but no difference in *phaC1* (encoding the PHA synthase) transcription, indicating that alginate biosynthesis competes with PHA biosynthesis regarding acetyl-CoA as precursor for both biopolymers. Transcription of *phaF* and *phaI-F* was nitrogen independent.

Keywords: *Pseudomonas aeruginosa*; PHA; Polyhydroxyalkanoate; Regulation; Alginate; *rpoN*; Nitrogen starvation

1. Introduction

The composition of medium-chain-length polyhydroxyalkanoates (PHA) accumulated by pseudomonads depends on the PHA synthases, the carbon source, and the metabolic routes involved [1–3]. In *Pseudomonas aeruginosa* two PHA synthase genes, *phaC1* and *phaC2*, which were separated by the PHA depolymerase encoding the *phaZ* gene, were identified and characterized [4]. These class II PHA synthases prefer 3-hydroxyacyl-CoA with a chain length of 6–14 carbon atoms as substrate [3]. Both PHA synthases have been purified and employed for in vitro synthesis of poly-3-hydroxydecanoate [3,5–7]. The PHA biosynthesis gene locus in *P. aeruginosa* also includes the *phaD* (encoding putative transcriptional regulator), *phaF* and *phaI* (unknown function) genes, whose homologues in *Pseudomonas oleovorans* are proposed to be PHA granule-associated structural proteins, whereas PhaF has been also considered as a negative regulator of PHA biosynthesis genes [8,9]. In *P. oleovorans* under PHA accumulating conditions PhaF is bound to PHA granules, and less PhaF is available for repression, which resulted in enhanced transcription of *phaC1* and *phaI* [9]. In addition *phaC1-lacZ* fusions in *P. oleovorans* indicated repression of *phaC1* transcription when gluconate was used, which does not enable PHA accumulation in *P. oleovorans*. In *Pseudomonas putida*, the genes involved in PHA metabolism are homologous to *P. aeruginosa* PHA synthesis genes and their genetic organisation is similar, but no DNA sequence similarity was found upstream of the respective *phaC1* gene (Fig. 1). Upstream of *phaC1* from *P. aeruginosa* sequence similarities to sigma 54 and 70 *Escherichia coli* consensus promotors as well as

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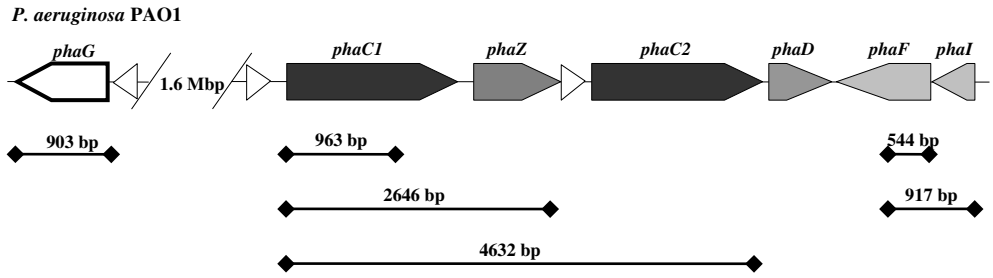

Fig. 1. Organization of genes relevant for PHA biosynthesis in *P. aeruginosa* PAO1. RT-PCR products for transcriptional analysis are indicated as bars. *phaC1 phaC2*, genes encoding PHA synthase; *phaZ*, encoding putative intracellular PHA depolymerase; *phaD*, encoding structural protein; *phaF*, encoding negative regulator/structural protein; *phaI*, gene encoding structural protein and *phaG*, encoding transacylase linking fatty acid biosynthesis with PHA biosynthesis. White triangles indicate the location of putative promoters.

upstream of *phaC2* a sigma 70 *E. coli* consensus promoter were found [4]. At least three different metabolic routes were found in pseudomonads for the synthesis of 3hydroxyacyl coenzyme A thioesters, which are the substrates of the PHA synthases [10–12]. (i) β-Oxidation is the main pathway when fatty acids are used as carbon source. (ii) Fatty acid de novo biosynthesis is the main route during growth on carbon sources which are metabolized to acetyl-CoA, like gluconate, acetate or ethanol. (iii) Chain elongation reaction, in which acetyl-CoA moieties are condensed to 3-hydroxyacyl-CoA, is involved in the PHA synthesis during growth on hexanoate. The transacylase PhaG from *P. putida* directly links fatty acid de novo biosynthesis with PHA biosynthesis by catalyzing the transfer of the (*R*)-3-hydroxydecanoyl moiety from the acyl carrier protein thioesters to CoA [13]. Transacylases have also been found in *P. aeruginosa* and in other pseudomonads accumulating PHA from non-related carbon sources [14–16]. Interestingly, *phaG* in P. oleovorans was not functional explaining the incapability to accumulate PHA from gluconate [15]. The transacylase-mediated biosynthesis pathway has already been established in non-PHA accumulating bacteria [17,18]. In *P. aeruginosa* and *P. putida* the *phaG* gene is not colocalized with the PHA gene cluster (Fig. 1).

Since (*R*)-3-hydroxyacyl-CoA thioesters serve as substrates for PHA synthases [3], intermediates of β-oxidation cannot be directly used for PHA biosynthesis. Therefore, enzymes such as the (*R*)-specific enoyl-CoA hydratase, hydroxyacyl-CoA epimerase, and 3ketoacyl-CoA reductase have been postulated to link the β-oxidation pathway with PHA biosynthesis [12,19,20]. Medium-chain length PHA biosynthesis employing the fatty acid β-oxidation has already been established in recombinant *Escherichia coli* and *Wautersia eutropha* [21–23]. The requirement of intact RpoN for PHA accumulation from gluconate provided evidence that RpoN might be involved in regulation of PHA biosynthesis genes in *P. aeruginosa* [4]. Moreover, evidence was obtained that heterologous *phaG* expression is induced under nitrogen limitation [17]. However, only a sigma 70 *E. coli* consensus promoter was identified upstream of *phaG* [11].

Overall, regulation of PHA biosynthesis genes in *P. aeruginosa* and *P. putida* has been only studied to a very limited extent [11]. This study should promote the understanding of PHA biosynthesis gene regulation particularly considering the role of RpoN, nitrogen availability and carbon source in PHA biosynthesis in two *Pseudomonas* species and shed light on the regulatory network involved in PHA biosynthesis by pseudomonads.

2. Materials and methods

2.1. Bacterial strains and growth conditions

The bacterial strains used in this study are listed in Table 1. *P. putida* and *P. aeruginosa* strains were grown at 30 and 37 °C, respectively, in mineral salts medium with either 0.05% or 0.2% (w/v) NH_4Cl as nitrogen source. For the glutamine-auxotrophic *P. aeruginosa* strain PAK-N1 half of the respective molar concentration of glutamine was used as nitrogen source. The cells were cultivated with either 15 $g\,l^{-1}$ sodium gluconate or 1 $g\,l^{-1}$ sodium octanoate. Tetracycline and kanamycin were used at final concentrations of 100 and 50 $\mu g\,ml^{-1}$, respectively. Growth was monitored photometrically with a Klett-Summerson photometer (Manostat) using Kodak filter No. 54 (520–580 nm).

2.2. Isolation of cellular DNA and RNA

Total cellular DNA of *Pseudomonas* strains was isolated [30]. Total RNA was isolated from 1×10^9 cells in the late logarithmic growth phase, which where cultivated in the presence of gluconate. Cells were lysed by lysozyme treatment and lysates were subjected to purification by the RNeasy RNA purification kit (Qiagen, Hilden, Germany). DNA-free total RNA was obtained

Table 1
Bacterial strains and oligonucleotides

Strains and oligonucleotides	Relevant characteristics	Reference
Pseudomonas putida		
KT2440	wild type	[24]
KT2440 *rpoN*	*rpoN*-negative mutant of *Pseudomonas putida* KT2440	[25]
GPp104	PHA-negative strain of *Pseudomonas putida* KT2440	[26]
Pseudomonas aeruginosa		
PAO1	Prototroph, Alg^-	ATCC 15692
PAK	Prototroph, Alg^-	[27]
PAK-N1	*rpoN*-negative mutant of *Pseudomonas aeruginosa* PAK, Tc^r	[28]
FRD1	Mucoid CF isolate	[29]

after on-column DNase I treatment and elution as described by the manufacturer. RNA was analyzed by agarose gel electrophoresis and the concentration was determined by measuring the absorbance at 260 nm in a spectrophotometer.

2.3. Transcription analysis with reverse transcriptase-PCR

To analyse transcripts of several genes involved in PHA biosynthesis and regulation in six different *Pseudomonas* strains, reverse transcriptase-PCR (RT-PCR) was applied using the following primers: Pp*phaG*N 5′-ATGAGGCCAGAAATCGCTGTACTTG-3′; Pp*phaG*C 5′-TCAGATGGCCAATGCATGCTGCCCC-3′; Pa*phaG*N 5′-ATGAGGCCGGAAACAGCCATCATCG-3′; Pa*phaG*C 5′-TTAGCTGGCGAGGGCGGCGAGG-GGG-3′; Pa*phaF*N 5′-AGAGT-CCAGTTGGATCGG-CG-3′; Pa*phaF*C 5′-GGTTTTCGCCGCGGTTTTTT-TC-3′; Pa*phaI*N 5′-GACCGTTTCCGAATCCACTT-CC-3′; Pa*phaC1*N 5′-AAACACGCTGAACCTGAA-TCCGG-3′; Pa*phaC1*C 5′-GCGACCTGGGTATTCA-GTTCGAAG-3′; Pa*phaZ*C 5′-TCAGCCGGTCTTGG-GCAGGAAC-3′; Pa*phaC2*C 5′-TCAGCGTATATGC-ACGTAGGTG-3′. One step RT-PCR (Qiagen, Hilden, Germany) was conducted according to the manufacturer's protocol, employing 0.5-5 ng RNA as template and the PCR conditions as described, applying from 29 to 33 cycles. For comparative analysis of the various strains under different cultivation conditions, the amount of RNA used as template and the number of cycles were kept constant. To analyse transcripts exceeding 2 kb, the enhanced avian HS RT-PCR kit (Sigma, St. Louis, MO, USA) was used. The applied two-step RT-PCR, which is especially designed for a variety of transcript sizes, was conducted according to the manufacturer's protocol, employing 10 ng RNA as template and 35 cycles in the PCR reaction. To exclude contaminating DNA that might have served as a template for PCR, isolated RNA was added in control experiments after inactivation of reverse transcriptase to the RT-PCR. The absence of PCR products in the control experiments indicated that the respective RT-PCR products were derived from RNA. When the enhanced avian HS RT-PCR kit was used for large transcripts, control experiments were performed, where RNA was replaced by DNA, which indicated the capability of the supplied Taq-DNA polymerase to amplify larger DNA fragments. RT-PCR experiments were conducted as duplicates.

2.4. Gas chromatographic analysis of PHA

PHA was qualitatively and quantitatively analyzed by gas chromatography (GC). Liquid cultures were centrifuged at 10,000*g* for 15 min, then the cells were washed twice in saline and lyophilized cell material was subjected to methanolysis in the presence of 15% (v/v) sulphuric acid. The resulting methyl esters of the constituent 3-hydroxyalkanoic acid were assayed by GC [31]. GC analysis was performed by injecting 3 μl of sample into a Agilent 6850 GC (Agilent Technologies, Inc., Palo Alto, CA, USA).

3. Results

3.1. The role of σ^{54} in PHA accumulation by P. putida and P. aeruginosa strains under various cultivation conditions

Since evidence was provided that RpoN(σ^{54}) might be involved in regulation of PHA biosynthesis genes [4,11] and RpoN is known to be involved in regulation of nitrogen metabolism as well as the fact that nitrogen limitation induces PHA biosynthesis, the role of RpoN in PHA biosynthesis was investigated. To evaluate the role of this alternative sigma factor RpoN in PHA biosynthesis in *P. putida* and *P. aeruginosa*, PHA accumulation experiments were performed with wild type strains and *rpoN*-negative mutants grown on medium containing either 15 $g\,l^{-1}$ gluconate or 3 $g\,l^{-1}$ octanoate (1 $g\,l^{-1}$ every 24 h) as carbon source and under nitrogen excess (2 $g\,l^{-1}$ NH_4Cl) or nitrogen limitation (0.5 $g\,l^{-1}$ NH_4Cl). Cells were grown with gluconate as carbon

source for 48h, whereas cells were grown with octanoate as carbon source for 72h. *P. putida* and *P. aeruginosa* strains were cultivated at 30 °C and 37 °C, respectively. Cultivation experiments were conducted as triplicates and mean values and standard deviation are provided. Since PhaG is involved in PHA biosynthesis from gluconate, its functional expression, which might be nitrogen-dependant, will be considered by using gluconate as carbon source under different nitrogen availability [11,17]. *P. putida* GPp104 a PHA-negative strain [26] did not accumulate PHA. The *P. putida rpoN*-negative mutant [25] and wild type *P. putida* did not accumulate PHA with gluconate as carbon source under nitrogen excess, but PHA contributed to $33 \pm 2\%$ cell dry mass under nitrogen limitation conditions. When octanoate was used as carbon source the *P. putida rpoN*-negative mutant and wild type *P. putida* were accumulating PHA contributing to $11 \pm 1\%$ cell dry mass under nitrogen excess, and to $47 \pm 3\%$ and $33 \pm 4\%$, respectively, when nitrogen was limiting. Thus PHA accumulation was affected by RpoN, because the respective mutant was accumulating about 40% more PHA than the wild type using octanoate as carbon source under nitrogen limitation conditions. The wild type *P. aeruginosa* strains PAO1 and PAK accumulated PHA to $1.6 \pm 2\%$ of cell dry mass when gluconate was used as carbon source under nitrogen excess, and to $16 \pm 2\%$ and $12 \pm 3\%$, respectively, when nitrogen was limiting. When octanoate was used as carbon source, the wild type strains were accumulating no PHA under nitrogen excess, but PHA contributing to $15 \pm 2\%$ and $24 \pm 5\%$ was accumulated, respectively, under nitrogen limiting conditions. Thus, wild type strains *P. aeruginosa* PAO1 and PAK showed similar capability with respect to PHA biosynthesis. The *rpoN*-negative strain *P. aeruginosa* PAK-N1 neither accumulated PHA with gluconate nor with octanoate as carbon source independent of nitrogen availability. The alginate overproducing strain *P. aeruginosa* FRD1 was chosen to investigate the competivity of the alginate and PHA biosynthesis with respect to PHA biosynthesis gene regulation. Strain FRD1 did not accumulate PHA under nitrogen excess conditions neither with gluconate nor with octanoate as sole carbon source. Under nitrogen limitation strain FRD1 accumulated PHA to $4 \pm 2\%$ and $27 \pm 4\%$ of cell dry mass, respectively. The composition of PHA accumulated by *P. putida* and *P. aeruginosa* wild type strains and mutants grown under various cultivation conditions showed no significant difference (data not shown).

3.2. Transcriptional analysis of transacylase gene phaG *in* P. putida

Transcription of the transacylase gene *phaG* in *P. putida* strain KT2440 and mutant *P. putida* KT2440 *rpoN* under various physiological conditions was determined with cells cultivated in medium containing gluconate as carbon source under nitrogen starvation or excess conditions. After isolation of total RNA from cells in the late logarithmic growth phase, RT-PCR was conducted with specific primers Pp*phaG*N and Pp*phaG*C, which resulted in a 888 bp RT-PCR product (Fig. 2). *PhaG* showed enhanced transcription under nitrogen starvation in strain KT2440 and mutant KT2440 *rpoN* (Fig. 2).

3.3. Transcriptional analysis of PHA biosynthesis genes in P. aeruginosa

Transcription of *phaG*, *phaC1*, *phaC1-Z*, *phaC1-C2*, *phaF* and *phaIF* in various *P. aeruginosa* strains under different physiological conditions was measured as *phaG* above. The specific primers Pa*phaG*N and Pa*phaG*C resulted in a 903 bp RT-PCR product (Fig. 1). *PhaG* transcription was enhanced under nitrogen starvation when compared with nitrogen excess conditions in wild type strains *P. aeruginosa* PAK and PAO1 (Fig. 2). In the *rpoN* negative mutant *P. aeruginosa* PAK-N1 and in the alginate overproducing strain FRD1 no or only weak transcription of *phaG* could be detected (Fig. 2).

To analyse whether or not differences in PHA accumulation are due to *phaC1* transcription, the transcription of the PHA synthase gene *phaC1* in wild type *P. aeruginosa* PAO1 and in alginate overproducing strain FRD1 was analyzed performing RT-PCR with primers Pa*phaC1*N and Pa*phaC1*C (Table 1), which resulted in a 963 bp fragment (Fig. 1). A *phaC1* transcript could be detected in both *P. aeruginosa* strains regardless of the nitrogen concentration in the medium (data not shown).

Since a sigma 70 *E. coli* consensus promoter sequence was found directly upstream *phaC2*, it was important to analyse whether *phaC1-phaZ* and *phaC1-phaZ-phaC2* are co-transcribed, RT-PCR with primer pairs Pa-

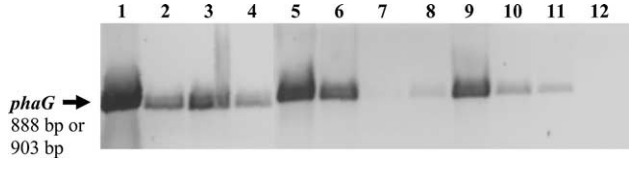

Fig. 2. RT-PCR analysis of *phaG* transcription in (lanes 1–4) wild type *P. putida* KT2440 and *rpoN* negative strain *P. putida* KT2440 *rpoN* and (lanes 5–12) various *P. aeruginosa* strains under different cultivation conditions with either 0.5 $g l^{-1}$ (odd-numbered lanes) or 2 $g l^{-1}$ (even-numbered lanes) NH_4Cl. RT-PCR product analysis was done by agarose gel electrophoresis. Gels were stained with ethidium bromide and a negative image is presented. The total RNA used as template for RT-PCR was isolated from cells in the late logarithmic growth phase grown in mineral medium, containing 1.5% (w/v) gluconate as carbon source. Lanes 1 and 2, *P. putida* KT2440; lanes 3 and 4, *P. putida* KT2440 *rpoN*; lanes 5 and 6, *P. aeruginosa* PAK; lanes 7 and 8, *P. aeruginosa* PAK-N1; lanes 9 and 10, *P. aeruginosa* PAO1; lanes 11 and 12, *P. aeruginosa* FRD1.

*phaC1*N/Pa*phaZ*C and Pa*phaC1*N/Pa*phaC2*C, were performed, which resulted in RT-PCR products of 2646 and 4632 bp, respectively (Fig. 1). To investigate whether any of the *P. aeruginosa* strains produces *phaC1-Z* or *phaC1-Z-C2* co-transcripts, a RT-PCR protocol was conducted with a mixture of total RNA from *P. aeruginosa* PAK, PAK-N1, PAO1 and FRD1 as template. Neither a *phaC1-Z* nor a *phaC1-Z-C2* transcript could be detected by RT-PCR (Fig. 3). Control reactions with genomic DNA from *P. aeruginosa* PAO1 as template for RT-PCR confirmed functionality of primers and RT-PCR kit (Fig. 3).

The *phaF* and *phaI* gene products are putative structural proteins, whereas PhaF is considered as a putative negative regulator. To investigate the transcription of *phaF* and co-transcription of *phaI-F* in wild type and in the alginate-overproducing strains *P. aeruginosa* PAO1 and FRD1, the primer pairs Pa*phaF* N/Pa*phaF*C and Pa*phaI*N/Pa*phaF*C were applied for RT-PCR, which resulted in 544 bp and 917 bp products, respectively (Fig. 1). In *P. aeruginosa* PAO1 and FRD1, a *phaF* transcript and a *phaI-phaF* co-transcript was detected at both high and low nitrogen in the medium (Fig. 4). In contrast to strain FRD1, strain PAO1 revealed an induction of *phaF* and *phaIF* transcription at low nitrogen (Fig. 4).

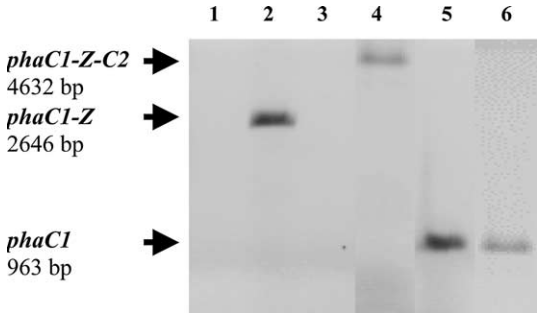

Fig. 3. RT-PCR analysis of *phaC1-Z* and *phaC1-Z-C2* transcription in *P. aeruginosa*. RT-PCR product analysis by agarose gel electrophoresis was done as in Fig. 2. The template RNA was isolated from cells grown in mineral medium, containing 15 $g l^{-1}$ gluconate as carbon source. Odd-numbered lanes, total RNA used as template for RT-PCR was a mixture of RNA isolated from *P. aeruginosa* strains PAK, PAK-N1, PAO1 and FRD1. Even-numbered lanes, genomic DNA isolated from *P. aeruginosa* PAO1 was used as template for RT-PCR as control of primer and RT-PCR kit functionality. Lanes 1 and 2, primer for *phaC1-Z* amplification were used; lanes 3 and 4, primer for *phaC1-Z-C2* amplification were used; lanes 5 and 6, primer for *phaC1* amplification were used.

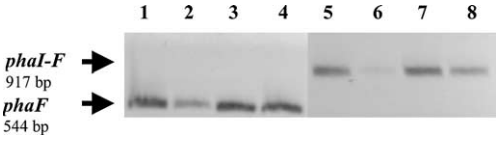

Fig. 4. RT-PCR comparison of (lanes 1-4) *phaF* and (lanes 5–8) *phaI-F* transcription in wild type *P. aeruginosa* PAO1 (lanes 1, 2, 5 and 6) and alginate overproducing strain FRD1 (lanes 3, 4, 7 and 8) as in Fig. 2.

4. Discussion

In this study, the role of RpoN (σ^{54}) an alternative sigma factor of RNA polymerase involved in growth phase dependent activation of promoters of various genes [32] was evaluated in PHA biosynthesis in *P. putida* and *P. aeruginosa.* Involvement of RpoN in the regulatory network of polyhydroxyalkanoate metabolism in *P. aeruginosa* had been suggested by the identification of a σ^{54}-dependent consensus promoter sequence upstream of *phaC1* gene [4]. Moreover PHA biosynthesis required an intact *rpoN* gene, when gluconate is used as carbon source [4]. Experiments showed a nitrogen-dependent and RpoN-dependent PHA accumulation in *P. aeruginosa* with either gluconate or octanoate as carbon source. The *rpoN* mutant *P. aeruginosa* PAK-N1 was not able to synthesize PHA under any condition tested. The alginate-overproducing strain *P. aeruginosa* FRD1 accumulated PHA comparable to wild type with octanoate as carbon source, whereas only 30% of wild type level PHA content was achieved with gluconate as carbon source. Therefore, it is possible that the biosynthesis pathways of PHA and alginate compete for the central metabolite acetyl-CoA. Gluconate has to be metabolized to acetyl-CoA, whereas fatty acids enter into the fatty acid β-oxidation pathway and the intermediate enoyl-CoA serves as precursor for PHA biosynthesis. Thus, under these conditions acetyl-CoA derived from gluconate serves mainly as precursor for alginate biosynthesis. No major differences with respect to transcription of PHA biosynthesis genes was obtained in the two strains, indicating that competitivity is restricted to the metabolic level. PHA accumulation in *P. putida* KT2440 appears to be more RpoN-independent when cultivated with octanoate as carbon source leading to about 40% more PHA accumulation in the *rpoN* mutant as compared to the wild type. In contrast to *P. aeruginosa*, PHA accumulation of about 11% cell dry mass was obtained even in excess of nitrogen. Interestingly, PHA biosynthesis in *P. putida* shows a stronger nitrogen-dependency when cultivated with gluconate as carbon source, indicating that genes which are involved in PHA biosynthesis from acetyl-CoA might be induced under nitrogen starvation. Consistently, RT-PCR analysis of *phaG* in *P. putida* indicated a strong induction under nitrogen starvation (Fig. 2).

Analysis of transcription of PHA biosynthesis genes in *P. putida* and *P. aeruginosa* was performed in order to elucidate the differences obtained in PHA content of cells grown under various cultivation conditions. In

P. aeruginosa inactivation of *rpoN* leads to an overexpression of several quorum-sensing-regulated virulence genes [33]. The question whether RpoN is involved in regulation of PHA biosynthesis, which starts in the late logarithmic growth phase [17], was investigated in this study.

In wild type strain KT2440 and the mutant KT2440 *rpoN*, evidence was obtained for strong induction of *phaG* transcription when cell were cultivated with gluconate as carbon source under nitrogen limiting conditions (Fig. 2). These data correspond to cultivation experiments, that showed no difference in PHA accumulation of *P. putida* wild type and *rpoN* negative mutant cultivated with gluconate. The *rpoN* negative mutant *P. aeruginosa* PAK-N1 did not accumulate PHA. These data indicated that in this mutant compared to wild type *P. aeruginosa* strains PAO1 and PAK the transcription of *phaG* is repressed (Fig. 2), which corresponds to decreased PHA contents, respectively. These data suggest that in *P. aeruginosa phaC1* is transcribed independently of PHA biosynthesis. A co-transcript of *phaC1*, *phaZ* and *phaC2*, or *phaC1* and *phaZ*, could not be detected in *P. aeruginosa* (Fig. 3) as had been previously proposed [4]. These data suggested a differential regulation of the PHA biosynthesis genes in *P. aeruginosa*. Furthermore, in *P. aeruginosa phaI* and *phaF* are co-transcribed as had been previously suggested for *P. oleovorans* [9].

In this study, information about the transcriptional regulation of PHA biosynthesis genes in two representative pseudomonads, which exert the PhaG-mediated PHA biosynthesis pathway enabling PHA biosynthesis from non-related carbon sources such as gluconate was presented for the first time. Similarities and differences in regulation of PHA biosynthesis in *P. putida* and *P. aeruginosa* particularly considering RpoN as well as nitrogen starvation and carbon source were revealed.

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