Regulation of polyphosphate kinase gene expression in *Acinetobacter baumannii* 252

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A strain of *Acinetobacter baumannii* cultured in butyric acid media was found to take up phosphate following a period of phosphate release. PCR was used to clone the polyphosphate kinase (*ppk*) gene from the strain. The promoter for the *ppk* gene was functional in the heterologous *Escherichia coli* host. Using RT-PCR, transcription of the *ppk* gene was found to be regulated by phosphate concentration.

Keywords: *Acinetobacter baumannii*, polyphosphate kinase, RT-PCR

INTRODUCTION

Polyphosphate is a linear polymer of several orthophosphate residues, linked by high-energy phosphoanhydride bonds. While possibly a precursor in prebiotic evolution, polyphosphate is now found in volcanic condensates, oceanic steam vents and in a wide variety of organisms (Kulaev, 1979). Its ubiquity suggests an essential physiological role; however no such function has yet been identified. Functions may depend on the organism and the physiological conditions as previously reviewed (Kornberg, 1995; Wood & Clark, 1988). Different functions which have been suggested include: (1) an energy or phosphate store, (2) a substitute for ATP in some reactions and in the chelation of ions (Keasling & Hupf, 1996), (3) a buffer for the maintenance of constant intracellular phosphate concentration, and (4) a role in DNA uptake in *Escherichia coli* cells (Castuma *et al.*, 1995; Reusch & Sadoff, 1988).

In *E. coli* there are at least 38 genes involved in phosphorus assimilation, which are organized as one regulon (the *pho* regulon) and are therefore subject to a common molecular control. Genes of the *pho* regulon are regulated by a two-component regulatory system composed of a response regulator, PhoB, and a sensor kinase, PhoR. Expression of the *pho* regulon is repressed when the extracellular phosphate concentration is in excess, and the genes of this regulon have a controlling promoter element called the *pho* box (Wanner, 1996). In *E. coli*, the enzyme polyphosphate kinase (Ppk) polymer-

izes the terminal phosphate of ATP into poly(P) in a freely reversible reaction:

There is, however, disagreement as to whether the gene responsible, *ppk*, is controlled by the *pho* regulon (Kato *et al.*, 1993a; Torriani-Gorini, 1994) or not (Wanner, 1996).

The polyphosphate kinase (*ppk*) gene has been cloned and sequenced in several species including *Acinetobacter* sp. strain ADP1, *Neisseria meningitidis*, *Klebsiella aerogenes* and *E. coli*, in which it was found to be located in the same operon as the gene for exopolyphosphatase (*ppx*) (Ahn & Kornberg, 1990; Akiyama *et al.*, 1992, 1993). Transcription of the *ppk* gene is induced by phosphate starvation in *Acinetobacter* sp. ADP1, whereby it is switched off in cells growing in conditions of phosphate abundance and switched on under phosphate-limiting conditions (Geißdörfer *et al.*, 1998). *K. aerogenes* has been known to exhibit extensive polyphosphate accumulation, particularly after being subjected to phosphate starvation (Harold, 1966).

Some strains of *Acinetobacter* that occur predominantly in wastewater are known to accumulate polyphosphate (Deinema *et al.*, 1980; Torein *et al.*, 1990) although they are no longer believed to be the principal bacteria involved in enhanced biological phosphate removal (EBPR) from waste streams (Mino *et al.*, 1998). The microbial removal of phosphate from wastewater is slow and improvements are required to support the industrial application of this physiological trait (Kato *et al.*, 1993b). Further knowledge about the genes involved in polyphosphate synthesis and their regulation in *Acinetobacter* species will aid in this task. Here, we report on the cloning and characterization of the *ppk*

Abbreviations: EBPR, enhanced biological phosphate removal; RACE, rapid amplification of cDNA ends.

The GenBank accession number for the sequence determined in this work is AF116175.

gene from *Acinetobacter baumannii* 252, isolated from EBPR-performing activated sludge and its transcriptional regulation by phosphate concentration.

METHODS

Bacterial strains and plasmids. The bacterial strains and plasmids used are shown in Table 1. *A. baumannii* 252 was isolated from activated sludge and identified using the API (bioMérieux) system for the identification of non-enteric Gram-negative organisms.

Media and growth conditions. *E. coli* and *A. baumannii* cells were routinely grown in LB, with 100 µg ampicillin ml⁻¹ added when culturing strains containing an ampicillin resistance plasmid. For polyphosphate kinase assays and growth studies, *A. baumannii* cultures were grown in media containing butyric acid as the sole carbon source, to which phosphate was added as required in the form of NaH₂PO₄ (van Veen *et al.*, 1993). For cultures of *A. baumannii*, high- and low-phosphate media were defined as containing 1 mM and 10 µM phosphate, respectively. For Miller assay studies in *E. coli*, LB was used as the high-phosphate medium (4 mM) and a phosphate-free medium was prepared containing (l⁻¹): NH₄Cl, 1 g; MgSO₄, 1.2 g; KCl, 0.75 g; Tris, 6 g; CaCl₂.2H₂O (1 M), 0.4 ml; glucose, 5 g; trace element solution (l⁻¹: MgSO₄, 3.0 g; MnSO₄, 0.5 g; NaCl, 1.0 g; FeSO₄.7H₂O, 0.1 g; CoCl₂, 0.1 g; ZnSO₄.H₂O, 0.01 g; CuSO₄, 0.1 g; H₃BO₃, 0.01 g; Na₂MoO₄.2H₂O, 0.01 g; AlK(SO₄)₂.12H₂O, 0.01 g; nitrilotriacetate, 1.5 g; pH 6.5), 2 ml; pH 7.2. For RNA extractions from *Acinetobacter* for RT-PCR studies, the above medium was used to which phosphate was added as required. To select for recombinant clones, 40 µg X-Gal ml⁻¹, 200 µg IPTG ml⁻¹ and 100 µg ampicillin ml⁻¹ were added to the media.

Chemicals and enzymes. Hexokinase, glucose-6-phosphate dehydrogenase, P¹,P⁵-di(adenosine-5') pentophosphate, ATP and NADP were purchased from Boehringer Mannheim. Synthetic polyphosphate in the form of Graham salt, which is a mixture of linear sodium polyphosphates (NaPO₃)_n, was prepared by heating NaPO₄ for 3 h in a furnace at 750 °C (where n ~ 10²) (van Groenestijn *et al.*, 1989).

PCR methods. The primer pair olm1/ojc8 (olm1: 5'-GAAT-TCTCAAGAACTGACAGGTATGG-3'; ojcs8: 5'-ATCCAG-

TCTGCACTTGAGCAATA-3') was used to amplify a 300 bp product from *A. baumannii*, which was cloned in pGEM-T. The resulting plasmid was named pJC11. Amplification was performed in a PTC-100 programmable thermal controller (MJ Research) using the following conditions: 95 °C for 5 min; 30 cycles of 95 °C for 1 min, 52 °C for 1 min and 72 °C for 1 min; and finally 72 °C for 10 min. The primer pair ojcs9/ojcs10 (ojcs9: 5'-TTCCCTGCTTTCGCAAGCACAAT-3'; ojcs10: 5'-TTGTGGGACGTTTCCTTGAACATA-3') was used for inverse-PCR on *Pst*I-digested and ligated genomic DNA along with buffer 3 from the Expand kit (Boehringer Mannheim) according to the manufacturer's instructions, with an elongation time of 260 s per cycle. The cloned product was named pJC13. The entire gene was subsequently obtained by PCR using the primer pair olm3/olm4 (olm3: 5'-TACG-CTATGATGATTGGTTTG-3'; olm4: 5'-TGTTTCATCAGCAGATTATGC-3'). The resulting cloned product was named pJC16. PCR amplification of the promoter region was carried out using the primer pair olm5/olm6 (olm5: 5'-AAGCTTGATTCAAGCAGCGGTATATG-3'; olm6: 5'-AAGCTTCCATCAGCCGTTTGTATTC-3'; *Hind*III sites underlined) and the resulting clone was named pJC20.

For RT-PCR, the reverse transcription reaction was first carried out on 1 µg RNA with 1 µl 10 mM Random Primers (Boehringer Mannheim), 1 µl 10 mM dNTPs (Boehringer Mannheim), 2 µl BSA (1 mg ml⁻¹), 4 µl 5 × buffer (Promega), 40 U RNasin and 200 U RT-AMV (Promega). The primer pair olm9/ojs13 (olm9: 5'-ATGGCGATTTTAATGGCGGTA-3'; ojs13: 5'-GTGCAACCTGTTCTCTGAA-3') was then used at an annealing temperature of 52 °C to carry out the PCR reaction on the cDNA, resulting in a 600 bp *ppk* gene product, which was sequenced in order to confirm its identity.

Physiology and growth studies. *E. coli* XL1 containing the construct pJC16 or pGEM-T (negative control) was grown in 10 ml LB broth overnight. The strains were subsequently subcultured into 500 ml LB broth to obtain a starting OD₅₈₀ of 0.08. Growth of the strains was monitored by measuring the optical density and plotting it against time. After 3 h, a 20 ml sample was removed from each flask and centrifuged for 5 min at 5000 g. IPTG (200 µl of a solution containing 200 mg ml⁻¹) was added to the remaining culture. Over the following 6 h,

Table 1. Bacterial strains and plasmids

Strain or plasmid	Relevant characteristics	Origin/reference
Strains		
<i>E. coli</i> XL1	Allows blue/white screening on X-Gal	Sambrook <i>et al.</i> (1989)
<i>E. coli</i> TG1	Allows blue/white screening on X-Gal	Sambrook <i>et al.</i> (1989)
<i>A. baumannii</i> 252	Activated sludge isolate	Primus (1998)
Plasmids		
pGEM-T	Cloning vector for PCR products, Ap ^R	Promega
Topo TA	Cloning vector for PCR products, Ap ^R	Invitrogen
pQF52	Vector containing promoterless <i>lacZ</i> gene, Ap ^R	McLean <i>et al.</i> (1997)
pJC11	pGEM-T containing 300 bp PCR product of <i>ppk</i> gene obtained with olm1/ojcs8	This work
pJC13	Inverse-PCR product of 3 kb in pGEM-T	This work
pJC16	Full-length <i>ppk</i> gene (2 kb) in pGEM-T	This work
pJC20	Promoter region (800 bp) obtained by PCR with olm5/olm6 in pQF52 <i>Hind</i> III site	This work

20 ml samples were removed at hourly intervals. After resuspension in 1 ml sonication buffer (0.1 mM EDTA, 50 mM Tris/HCl pH 7.0, 1 mM 2-mercaptoethanol), the cells were sonicated three times at 60 W for 15 s with 45 s intervals. The cell lysates were centrifuged at 10000 *g* for 3 min in a microfuge and the supernatant was stored at -20°C . The total protein content was determined using the Bradford (1976) assay.

Assays and nucleic acid isolation and manipulations. The assay for polyphosphate kinase activity described by van Groenstijn *et al.* (1989) was used. Specific activity values were expressed in $\text{nmol min}^{-1} (\text{mg protein})^{-1}$. Phosphate concentrations were determined according to a previously described method (Martin & Tolbert, 1983). The Miller (1972) assay was used for measuring β -galactosidase activity.

Genomic and plasmid DNA were prepared as previously described (Sambrook *et al.*, 1989). Alternatively, commercial miniprep kits (Qiagen) were used to obtain plasmid DNA for sequencing. For cloning experiments, either pGEM-T or the Topo TA cloning vectors were used. Restriction enzymes were purchased from Promega or Boehringer Mannheim and used according to the manufacturers' instructions. For detection of promoter activity, the promoter-probe vector pQF52 was used (McLean *et al.*, 1997). For transformations, *E. coli* cells were electroporated as previously described (Sambrook *et al.*, 1989). DNA sequence was determined by the dideoxy chain-termination method (Sanger *et al.*, 1977) using the DyeDeoxy Terminator Cycle Sequencing Ready Reaction kit with AmpliTaq DNA polymerase (Applied Biosystems) in a Gene Amp PCR system L400 (Perkin Elmer) and run on an automated DNA sequencer (model 373 STRETCH, Applied Biosystems). The sequencing strategy consisted of the initial use of universal M13 primers, followed by the design of primers using the newly acquired sequences. Sequence data were assembled and processed using the DNA STAR software package (DNA STAR, Madison, WI, USA). The BLAST algorithm was used to search DNA and protein databases for similarity.

RNA was prepared following the method outlined by Ausubel *et al.* (1987). Contaminating DNA was then digested with DNase according to the manufacturer's instructions (Boehringer Mannheim). Primer extension analysis was carried out as described by Engelke *et al.* (1992). The *fml* DNA Sequencing system (Promega) was used for the sequencing reaction to be run alongside the primer extension product. The sequence of the *ppk*-specific primer used was 5'-GGGG-AGGCTTGTAAGTAACTA-3'.

RESULTS

Phosphate uptake by growing cultures of *A. baumannii*

A. baumannii cultures were inoculated from high-phosphate overnight cultures into both high- and low-phosphate butyric acid media. An initial increase in the extracellular phosphate concentration was detected in the low-phosphate medium (Fig. 1a) but not in the high-phosphate medium (Fig. 1b). This increase could be explained as a release of phosphate by the cells due to the stress involved in growing under phosphate-limiting conditions. This initial release of phosphate by stressed cultures preceding phosphate uptake has been well-documented for poly(P)-accumulating *Acinetobacter* spp. (Ohtake *et al.*, 1985). The *A. baumannii* culture grown under low-phosphate conditions removed phos-

Fig. 1. Growth (□) and extracellular phosphate concentration (●) of *A. baumannii* cultures grown under low- (a) and high- (b) phosphate conditions.

Fig. 2. Specific activity of polyphosphate kinase from *A. baumannii* cells grown under low- (■) and high- (□) phosphate conditions.

phate from the media to undetectable levels within 3 h and this corresponded to maximal expression of polyphosphate kinase (Ppk) activity between 2 and 3 h for the low-phosphate culture as seen in Fig. 2.

Cloning of the *ppk* gene from *A. baumannii* strain 252

Alignments of the previously cloned *ppk* gene sequences of *Acinetobacter* sp. strain ADP1, *E. coli*, *Pseudomonas aeruginosa*, *K. aerogenes* and *N. meningitidis* were used to choose conserved regions to which PCR primers could be designed. The primers chosen (olm1/ojc8) gave rise to a 300 bp PCR fragment of the polyphosphate kinase gene, which was sequenced. Based on this sequence, primers (ojc9/ojc10) were then designed facing outwards from each other in order to amplify the rest of the gene using inverse-PCR. PCR with these primers on *Pst*I-digested and ligated genomic DNA led to the generation of a product of approximately 3 kb. This was cloned in pGEM-T and sequenced. Because of the nature of inverse-PCR, the resulting clone contains the gene divided into non-contiguous parts and so the gene was subsequently cloned in its entirety for expression studies using the primer pair olm3/olm4.

Sequence analysis of the *ppk* gene

The *ppk* gene was found to be 2079 bp in length and to have a GC content of 39 mol%, which is typical of *Acinetobacter* (Henriksen, 1976). The *ppk* gene encodes a putative protein of 692 amino acids, compared with 691 in *Acinetobacter* sp. strain ADP1, 688 in *E. coli* and 685 in both *N. meningitidis* and *K. aerogenes*. An identity of 77% was found at the nucleotide level and 82% at the amino acid level with strain ADP1. There was 33% identity between *A. baumannii* and both *K. aerogenes* and *E. coli* at the amino acid level and 50% between *A. baumannii* and *N. meningitidis*.

Induction of polyphosphate kinase enzyme activity in *E. coli* cells transformed with the cloned *ppk* gene

E. coli cells containing the cloned *ppk* gene on a plasmid (pJC16) under the control of the *lacZ* promoter or the vector alone (pGEM-T) (as a negative control), were grown for 3 h before the addition of IPTG. Two hours after induction, a 333-fold increase in Ppk activity [$100 \text{ nmol min}^{-1} (\text{mg protein})^{-1}$] was detected with the pJC16 cells relative to those containing the negative control pGEM-T ($0.3 \text{ nmol min}^{-1} \text{ mg}^{-1}$). This level of Ppk activity remained high at 5 h ($102 \text{ nmol min}^{-1} \text{ mg}^{-1}$) and at 7 h ($88 \text{ nmol min}^{-1} \text{ mg}^{-1}$) following induction. Detection of significantly higher levels of Ppk activity in *E. coli* transformed with pJC16 suggested that we had cloned a functional *ppk* gene.

Promoter studies

An 800 bp fragment including the start of the *ppk* gene and approximately 400 bp upstream of the coding region was cloned into the promoter-probe vector pQF52. The ligation mixture was electroporated into *E. coli* TG1 cells. Cells containing the resulting construct, named pJC20, were grown under conditions of phosphate limitation and phosphate excess and up to 11-fold more β -galactosidase activity was found under phosphate-

Fig. 3. Mapping the 5' start site of *ppk* mRNA by primer extension analysis. (a) RNA was prepared from exponentially grown cells under high- (lane 1) or low- (lane 2) phosphate conditions. Lanes A, C, G and T show the sequencing products obtained with the same primer. The arrow indicates the start site of transcription. (b) The sequence shown in (a) is underlined up to the start site, which is indicated in bold type. The translational start site ATG is shown in bold italics.

limiting conditions (148 Miller units) compared with those of phosphate excess (13 Miller units). This increased β -galactosidase activity was due to the presence of the *ppk* promoter fragment since cells containing the promoterless vector pQF52 assayed under similar conditions showed no induction of β -galactosidase under low-phosphate conditions (8 Miller units). Primer extension analysis was carried out in order to map the transcription start site. This was found to be within the fragment cloned in pJC20 (Fig. 3). In a further experiment, 5'-RACE (rapid amplification of cDNA ends) indicated the presence of a second transcriptional start site 222 bp upstream from this site, not contained within the pJC20 clone. This start site was also seen following primer extension studies under both high- and low-phosphate conditions (data not shown).

Induction of *ppk* transcription by phosphate starvation

RNA was isolated from *A. baumannii* cells grown under conditions of phosphate limitation and phosphate excess. Samples were taken in the exponential phase and again in the stationary phase. RT-PCR was then carried out using the primer pair olm9/ojs13 and 5 μ l aliquots of each reaction were electrophoresed on 1% agarose gels and the product band intensities within each experiment were visually compared after ethidium bromide staining. Transcripts were detected under low- but not under high-phosphate conditions for the exponential-phase cells. In stationary-phase cells from the high-phosphate culture, no transcripts were detected, while there were still detectable transcripts in the low-phosphate cells at

Fig. 4. (a) RT-PCR on exponential- and stationary-phase cells cultured under high- and low-phosphate conditions. Lanes 1 and 2, exponential-phase cells grown in high- and low-phosphate media, respectively; lanes 3 and 4, stationary-phase cells grown in high- and low-phosphate media, respectively. (b) RT-PCR products obtained from an *A. baumannii* culture grown under low-phosphate conditions with samples taken at 2, 4 and 6 h shown in lanes 1, 2 and 3, respectively. (c) RT-PCR products obtained from an *A. baumannii* culture initially grown under low-phosphate conditions and phosphate then added back into the culture after 2 h, with samples taken at 2, 4 and 6 h shown in lanes 1, 2 and 3, respectively.

the same time point (Fig. 4a). These results indicate that regulation of expression of the *ppk* gene in *A. baumannii* is due to phosphate limitation and not due to limitations in other nutrients. This confirms the results obtained for the *ppk* promoter in *E. coli*, which was selectively induced under low-phosphate growth conditions.

In a further experiment, two similar flasks of phosphate-free media were inoculated from overnight high-phosphate cultures. Samples were removed from one culture after 2, 4 and 6 h. For the other culture, the first sample was taken after 2 h, then phosphate was added back into the media at a final concentration of 1 mM. Subsequent samples were taken after 4 and 6 h. RNA was extracted from the six samples and RT-PCR was performed using the primer pair olm9/ojs13. The results of the RT-PCR showed transcripts at all three time points for the first culture, which had been maintained under low-phosphate conditions throughout (Fig. 4b). In the second culture, transcripts were detected at 2 h, but, following the introduction of phosphate to the media, a marked reduction in transcript levels was observed (Fig. 4c).

Samples were also taken to simultaneously measure polyphosphate kinase enzyme activity. The specific activity was found to be 26.4 and 29.6 nmol min⁻¹ (mg protein)⁻¹ at 2 and 4 h, respectively, for the low-

phosphate culture. However, following addition of phosphate to the second culture after 2 h when Ppk activity was 34.8 nmol min⁻¹ (mg protein)⁻¹, the activity dropped to 13.9 nmol min⁻¹ (mg protein)⁻¹.

DISCUSSION

In this study, we report on the cloning and characterization of the *ppk* gene from *A. baumannii* 252, which was isolated from an EBPR-performing activated sludge. The *ppk* gene encodes a putative protein with a high degree of similarity to previously described *ppk* genes. We demonstrate that transcription of *ppk* is regulated by phosphate concentration, and we show promoter activity from a cloned fragment upstream from the *ppk* gene.

The nature and properties of inorganic phosphate uptake/transport systems have been well-documented in *E. coli* (Rosenberg *et al.*, 1977; Willsky & Malamy, 1980). In *Acinetobacter johnsonii*, two phosphate transport systems have been characterized and they resemble those of *E. coli*. One is an inducible, high-affinity transport system and the other is a constitutive low-affinity transport system. Experiments carried out on *A. johnsonii* suggest the presence of a similar regulatory mechanism to the *pho* regulon in *E. coli* for reducing extracellular phosphate concentrations to micromolar levels (van Veen *et al.*, 1993).

To determine whether or not *A. baumannii* could take up phosphate when grown aerobically, its growth was studied under both high- and low-phosphate conditions and Ppk activity was measured. Growth studies showed an initial release of phosphate in the low-phosphate media. This phenomenon has previously been reported in both pure cultures of *Acinetobacter calcoaceticus* (Ohtake *et al.*, 1985) and EBPR-performing bacteria in activated sludge following an anaerobic period (Korste *et al.*, 1994). This initial release was followed by the uptake of phosphate from the media leading to undetectable extracellular levels within 3 h, which corresponded to maximal Ppk activity between 2 and 3 h for the low-phosphate culture.

In *E. coli*, the *ppk* gene is part of a two-gene operon together with exopolyphosphatase (*ppx*). The latter functions to degrade poly(P) granules to provide ATP to the *E. coli* cell (Akiyama *et al.*, 1993). However, to date this gene has not been identified in any *Acinetobacter* species. Even if present, it does not appear to be located in the same operon as the *ppk* gene in either *A. calcoaceticus* (Geißdörfer *et al.*, 1998) or in *A. baumannii* (data not shown).

We have shown that the region upstream of the *ppk* gene functions as a phosphate-regulated promoter in the heterologous *E. coli* host. Primer extension analysis with mRNA from cells grown in low-phosphate media located the transcription start site within the promoter region cloned in pJC20 (Fig. 3). Furthermore, 5'-RACE showed the presence of a second transcription start site upstream of the first one. This was seen in primer

extension analysis in both high- and low-phosphate-grown cells, suggesting that it is a constitutive promoter. A second constitutive promoter was also reported to be present in *Acinetobacter* sp. strain ADP1; however here it was located downstream from the inducible one (Geißdörfer *et al.*, 1998). The functioning of the *A. baumannii* *ppk* promoter in *E. coli* and its induction under low-phosphate conditions suggests that a common type of transcriptional control mechanism may be in operation in both organisms. In *E. coli*, PhoB is the transcriptional activator for Pho-regulated genes, and in addition PhoB has been shown to function as a transcriptional activator for Ppk activity in *K. aerogenes* (Kato *et al.*, 1993a). It is possible that a PhoB or a PhoB-like transcription factor is also present in *A. baumannii* which regulates transcription from the *ppk* promoter given that this promoter appears to be functional in *E. coli* where it is selectively induced (11-fold induction) under low-phosphate conditions. If a similar system is in operation in *A. baumannii*, then we might expect to see a *pho* box or *pho*-box-like sequence in the promoter region. However, no sequences similar to the *pho* box consensus sequence derived from *E. coli* and *K. aerogenes* phosphate-regulated promoters (Kato *et al.*, 1993b) appears to be present. Similarly, no *pho* boxes appear to be present in the promoter region of the *Acinetobacter* sp. strain ADP1 *ppk* gene (Geißdörfer *et al.*, 1998).

While no *phoB* or *phoR* genes have as yet been cloned from *Acinetobacter* species, it appears likely that genes with homology to *phoBR* do exist in this genus. For instance, in *Acinetobacter* strain RA3849, the promoter for the polyhydroxyalkanoic acid biosynthetic genes has been shown to be induced by phosphate starvation and a putative *pho* box was located in the promoter region (Schembri *et al.*, 1995).

Further proof for the regulation of *ppk* transcription in *A. baumannii* by phosphate was obtained using RT-PCR. *ppk* transcripts were detected in cells grown under phosphate-limiting conditions but not under conditions of phosphate excess. In stationary-phase cells, no transcripts were detected for the high-phosphate culture, indicating that expression is not induced in stationary phase but depends specifically on phosphate limitation.

ppk transcription in *A. baumannii* was repressed by the addition of phosphate back into the culture media (Fig. 4b and c) indicating an additional level of complexity with respect to the regulation of phosphorus metabolism in this strain. In *E. coli*, under conditions of excess environmental phosphate the PhoR protein sensor kinase cannot phosphorylate PhoB, leaving it in an inactive unphosphorylated form (Torriani-Gorini, 1994). If a similar system is in operation here, then PhoB would again appear a likely candidate in mediating such a repressive effect on *ppk* transcription. However, further genetic characterization of the *ppk* promoter region will be necessary to determine if this is in fact the case. The data presented here support the view that *phoBR*-like genes are likely to exist in *Acinetobacter*

species and that PhoB or a PhoB-homologue may be involved in mediating the transcriptional regulation of the *ppk* gene in *A. baumannii*.

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