The Unphosphorylated EIIA\textsuperscript{Ntr} Protein Represses the Synthesis of Alkylresorcinols in Azotobacter vinelandii

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Abstract

Upon encystment induction, Azotobacter vinelandii produces the phenolic lipids alkylresorcinols (ARs) that are structural components of the cysts. The enzymes responsible for the ARs synthesis are encoded in the \textit{arsABCD} operon, whose expression is activated by ArpR. The transcription of \textit{arpR} is initiated from an RpoS dependent promoter. The nitrogen-related phosphotransferase system (PTS\textsuperscript{Ntr}) is a global regulatory system present in Gram negative bacteria. It comprises the E\textsuperscript{Ntr}, NPr and EIIA\textsuperscript{Ntr} proteins encoded by \textit{ptsP}, \textit{ptsO} and \textit{ptsN} genes respectively. These proteins participate in a phosphoryl-group transfer from phosphoenolpyruvate to protein EIIA\textsuperscript{Ntr} via the phosphotransferases EIN\textsuperscript{Ntr} and NPr. In A. vinelandii, the non-phosphorylated form of EIIA\textsuperscript{Ntr} was previously shown to repress the synthesis of poly-ß-hydroxybutyrate. In this work, we show that PTS\textsuperscript{Ntr} also regulates the synthesis of ARs. In a strain that carries unphosphorylated EIIA\textsuperscript{Ntr}, the expression of \textit{arpR} was reduced, while synthesis of ARs and transcription of \textit{arsA} were almost abrogated. The expression of \textit{arpR} from an RpoS-independent promoter in this strain restored the ARs synthesis. Taken together these results indicate that unphosphorylated EIIA\textsuperscript{Ntr} negatively affects activation of \textit{arpR} transcription by RpoS.

Introduction

Azotobacter vinelandii is a soil bacterium that undergoes a differentiation process resulting in the formation of a desiccation resistant cyst. A mature cyst consists of a contracted cell, known as the central body, which is surrounded by a capsule made up of a laminated outer layer called the exine and an inner layer called the intine [1]. The polysaccharide alginate is a major component of the capsule layers. Other components of the cysts are the reserve polyester poly-ß-hydroxybutyrate (PHB), that is present in the central body forming large granules, and the phenolic lipids alkylresorcinols (ARs), which replace the membrane phospholipids in the cyst and...
are also components of the exine [2]. Encystment can be induced by transferring log-phase vegetative cells to Burk’s minimal medium with either n-butanol or β-hydroxybutyrate as the sole carbon source [3].

ARs play a structural role in the cyst, and strains carrying mutations in ARs biosynthetic genes produce cysts with a defective exine [4]. The arsABCD gene cluster encodes the enzymes that synthesize these lipids [5]. These genes are specifically expressed in encystment induction medium [4]. The transcriptional activator ArpR positively regulates transcription of the arsABCD operon, by direct binding to the arsA promoter region [6]. The mutational inactivation of rpoS impairs ARs synthesis [7] because this sigma factor is needed for the transcription of arpR [6].

The ptsP, ptsO and ptsN genes encode EIIA, NPr and EIINtr proteins, respectively, that are components of the nitrogen-related phosphotransferase system (PTSNtr), which is homologous to the carbohydrate transport PTS. The PTSNtr proteins participate in a phosphoryl transfer chain from phosphoenolpyruvate, where EIINtr appears to be the terminal phosphoryl acceptor [8]. The PTSNtr regulates a wide variety of processes in bacteria; in Legionella pneumophilia, a ptsP mutation, negatively affected its virulence in guinea pigs [9]; in Rhizobium species, the PTSNtr is associated to melamin synthesis, nitrogen fixation and regulation of ABC transport activation [10,11]. In Escherichia coli, the EIIA protein controls the potassium transport by interacting with the Trk transporter subunit TrkA and the sensor kinase KdpD (that controls the expression of high affinity potassium transporter system KdpFABC) [12,13]. The response of E. coli to phosphate starvation is also activated by EIIA due to an interaction with the sensor kinase PhoR [14].

In the A. vinelandii UW136, the non-phosphorylated form of EIINtr was shown to impair PHB production, by exerting a negative effect on expression of phbR, the gene encoding the transcriptional activator of the PHB biosynthetic operon phbBAC [15].

In this work we report the effect of mutations in the genes coding for the proteins of the PTSNtr on alkylresorcinol synthesis and show that the non-phosphorylated EIIA protein has a negative effect on the transcriptional activation of arpR by RpoS.

Materials and Methods
Bacterial strains, plasmids and growth conditions

Bacterial strains and plasmids used are listed in S1 Table. A. vinelandii was cultured at 30°C in Burk’s nitrogen-free salts medium [16] supplemented with 2% sucrose (BS) for vegetative growth or 0.2% n-butanol (BBOH) for encystment induction. For determination of β-glucuronidase activity of transcriptional phbR-gusA and phbB-gusA fusions, the cells were grown in peptone yeast medium supplemented with 2% sucrose (PY). Liquid cultures were carried out in 250-mL or 125-mL flasks containing 50 or 25 ml of medium, respectively, in a rotatory shaker at 200 rpm and 30°C. Inocula for all experiments were grown on BS, washed three times with sterile 10mM MgSO4, and transferred to BBOH medium.

E. coli strain DH5α was grown in Luria-Bertani medium (LB) at 37°C. Transformation of A. vinelandii were carried out as previously described [16].

Nucleic acid procedures

DNA purification and cloning procedures were carried out as previously described [17]. Total RNA extraction was performed as reported by Barry et al. [18]. DNA sequencing was done with a Perkin Elmer/Applied Biosystems DNA Sequencer. The sequences of oligonucleotides used in this work are described in the S2 Table.
Constructions of transcriptional and translational fusions of \textit{arpR} and \textit{arsA} with \textit{gusA} reporter

The pUMATc plasmid \cite{19} was digested with \textit{EcoRI} and \textit{HindIII} to clone the \textit{gusA} reporter gene obtained from pAHFUTs-Tc \cite{20}, resulting in the plasmid pUMATc-gusAT. The plasmids pUMATc-gusAT and pUMATc-gusAPT \cite{19}, unable to replicate in \textit{A. vinelandii} and used for transcriptional and translational fusions, respectively, were digested with \textit{SacI} and \textit{KpnI} restriction enzymes to remove the tetracycline cassette. The ends of the plasmids were made blunt by treatment with Klenow fragment and used for cloning a blunted \textit{MluI} gentamicin cassette obtained from pBSL98 \cite{21}. The new plasmids pLM2 and pLM3 (\textit{S1 Table}) were used to construct the transcriptional and translational fusions, respectively.

For the construction of transcriptional \textit{arsA-gusA} and \textit{arpR-gusA} fusions, DNA fragments of 1.0 and 0.99 Kb, containing the promoter region of \textit{arsA} and \textit{arpR}, respectively, were amplified using the primers \textit{FwarsA} and \textit{RvarsAtrans} and \textit{FwarpR} and \textit{RvarpRtrans} (\textit{S2 Table}). The fragments were gel-purified, digested with \textit{XbaI} and \textit{PstI} and ligated to \textit{XbaI-PstI} pLM2 vector to construct the plasmids pLM4 (\textit{arsA-gusA}) and pLM6 (\textit{arpR-gusA}). These plasmids were digested with \textit{NdeI} and \textit{ScaI}, respectively, and used to transform \textit{A. vinelandii} strains for the selection of transformants carrying transcriptional \textit{arsA-gusA} or \textit{arpR-gusA} fusions integrated into the \textit{melA} gene by a double recombination event. Digestion of the plasmids was carried out in order to avoid the selection of strains with plasmids integrated into the chromosome generated by single recombination events. The \textit{melA} gene has been previously used as a neutral site to introduce gene fusions \cite{19}. These strains are described in \textit{S1 Table}.

For the construction of translational \textit{arsA'-gusA} and \textit{arpR'-gusA} fusions, DNA fragments of 1.3 and 1.1 Kb (containing the promoter region, the 5' untranslated region and the first five codons of each gene) were amplified with \textit{FwarsA} and \textit{RvarsAtrad} and \textit{FwarpR} and \textit{RvarpR-trad} primers for \textit{arsA} and \textit{arpR}, respectively. The PCR products were purified, digested with \textit{XbaI} and \textit{PstI} enzymes and ligated to \textit{XbaI-PstI} pLM3 resulting in the plasmids pLM5 (\textit{arsA'-gusA}) and pLM7 (\textit{arpR'-gusA}). The plasmids pLM5 and pLM7 were digested with \textit{NdeI} and \textit{ScaI}, respectively, and used to transform \textit{A. vinelandii} strains for the selection of the transformants carrying translational \textit{arsA'-gusA} or \textit{arpR'-gusA} fusions described in \textit{S1 Table}. The presence of all fusions in the strains was confirmed by PCR analysis (data not shown).

Construction of plasmid pBpgyrA-\textit{arpR} to express \textit{arpR} from RpoS-independent promoter

First, we constructed the plasmid pJET-p\textit{gyrA} cloning a 0.3 Kb DNA fragment containing the promoter region of \textit{gyrA} gene (p\textit{gyrA}) into vector pJET1.2/blunt (Thermo Scientific). A DNA fragment of 1.0 Kb containing the encoding region of \textit{arpR} was amplified using the oligonucleotides \textit{arpRFw2} and \textit{arpRRv2} (\textit{S2 Table}) and cloned into pJET-p\textit{gyrA} downstream and the same direction of p\textit{gyrA}. The fusion p\textit{gyrA-arpR} was excised by digestion with BglII enzyme, gel purified, made blunt and cloned into Smal-digested plasmid pBRR1MCs-5 \cite{22}, resulting in the plasmid pBpgyrA-\textit{arpR}, which was transferred by conjugation into strain UW136::pALA8a.

Quantitative Real Time PCR (q-RT-PCR)

Expression levels of \textit{arsA} and \textit{arpR} was measured by qRT-PCR as previously reported \cite{15}. The primers used for the assays (\textit{S2 Table}) were as follows: \textit{arsA-RT-F} and \textit{arsA-RT-R} for \textit{arsA}, \textit{arpR-RT-F} and \textit{arpR-RT-R} for \textit{arpR}, and \textit{fw-gyrA} and \textit{rev-gyrA} for \textit{gyrA}. The level of \textit{gyrA} was used as internal control to normalize the results. All assays were performed in triplicate. The data was analyzed by the \(2^{-\Delta\Delta CT}\) method reported by Livak and Shmittgen \cite{23}.
Determination of alkylresorcinol production

The production of ARs was measured as previously described [24]. Briefly, the lipids were extracted with acetone for 1 h at room temperature. The acetone extract was removed, and a second extraction was done for 12 h at room temperature. The resulting extracts were mixed and used for spectrophotometric determination of alkylresorcinols by the use of Fast Blue B as previously described [24]. Orcinol was used as a standard. The protein content of the cells used for AR determination was measured by the method of Lowry et al [25].

Quantification of β-glucuronidase activity

The β-glucuronidase activity was measured as described previously [26] from encystment-induced cells in BBOH medium harvested to 72 hours of incubation. 1 U corresponds to 1 nmol of p-nitrophenyl-β-D-glucuronide hydrolyzed per minute per mg of protein.

Results

Effect of *ptsP*, *ptsO* and *ptsN* mutations on ARs synthesis

Strain UW136 is unable to produce alginate due to an insertion within the *algU* gene [27] therefore this strain is unable to produce genuine mature cysts, but under encystment induction medium produces ARs [4].

To determine if PTSNtr is involved in the regulation of the ARs synthesis, we analyzed ARs production in encystment-induced cells of *pts* mutants, by staining these lipids with dye Fast Blue B [4]. The *ptsN* mutant and the UW136 wild type strain developed a red color indicative of ARs synthesis, while the *ptsP* and *ptsO* mutants remained white (Fig. 1A). The quantification of ARs production in these strains confirmed the observed phenotype in plates; no ARs were detected in the *ptsP* and *ptsO* mutants, while the *ptsN* mutant presented a significant increase in ARs production relative to the UW136 strain (Fig. 1B). According to the phosphorylation cascade proposed for the PTSNtr [15] the *ptsP* and *ptsO* inactivations are expected to impair the phosphorylation of EIIANtr, therefore, the unphosphorylated form of EIIANtr could be involved in the negative effect observed on ARs synthesis. In agreement with this hypothesis, inactivation of *ptsN*, in the *ptsP* mutant background (*ptsP-ptsN* double mutant) restored the ARs synthesis (Fig. 1B).

Effects of PTSNtr mutations on *arsA* expression

In order to determine if PTSNtr affected ARs synthesis through an effect on *arsABCD* expression, transcriptional *arsA-gusA* and translational *arsA-´·´-gusA* gene fusions were used. The transcription and translation levels of *arsA* were determined by measuring β-glucuronidase activity in derivatives of the wild type UW136 strain and the *ptsP*, *ptsO*, *ptsN* and *ptsP-ptsN* mutants carrying the gene fusions (S1 Table). We observed that *ptsP* and *ptsO* inactivations caused a similar decrease in the β-glucuronidase activity of both fusions (Fig. 2A and 2B), while in the *ptsN* mutant the activity increased in the transcriptional and translational fusions by 27% and 60% respectively. In the double mutant *ptsP-ptsN*, the β-glucuronidase activity of transcriptional fusion was partially restored, while the translational fusion showed a similar level to that observed in the wild type strain (Fig. 2A and 2B).

The level of *arsA* transcripts in the *pts* mutants was also evaluated by qRT-PCR. Table 1 shows that the *arsA* mRNA levels in *ptsP* and *ptsO* mutants were very low when compared to those observed in the wild type strain. In contrast, the *arsA* mRNA level was higher in the *ptsN* mutant and the double mutant *ptsP-ptsN* than in the UW136 strain. These results support the hypothesis that the non-phosphorylated form EIIANtr negatively affects *arsA* expression at the transcriptional level.
The results shown above suggest that PTSNtr controls the ARs synthesis through the regulation of expression of \( \text{arsABCD} \). We recently reported that ArpR, a LysR-type regulator, directly activates the transcription of \( \text{arsABCD} \) \cite{6}. Therefore, the question of whether the PTSNtr affected the transcription of \( \text{arsA} \) through an effect on the \( \text{arpR} \) expression was raised.

To study the effects of \( \text{pts} \) mutations on \( \text{arpR} \) expression, we used the UW136, \( \text{ptsP} \), \( \text{ptsO} \), \( \text{ptsN} \) and \( \text{ptsP-ptsN} \) strains carrying transcriptional and translational fusions of \( \text{arpR} \) (S1 Table). Fig. 3A shows that transcription of \( \text{arpR} \), measured as \( \beta \)-glucuronidase activity, decreased about 40% in the \( \text{ptsP} \) and \( \text{ptsO} \) mutants relative to the wild type strain, while the \( \text{ptsN} \) inactivation had no effect on \( \text{arpR} \) transcription in the UW136, nor in the \( \text{ptsP} \) strains (Fig. 3A). The \( \beta \)-glucuronidase...
activity in the wild type and pts mutants carrying the translational arpR’-gusA fusion (Fig. 3B), showed that the ptsP and ptsO mutations reduced about 50% the translation of arpR, as compared to the UW136 strain. In contrast, in the ptsN and ptsP-ptsN mutants a significant increase in the β-glucuronidase activity, relative to UW136 and ptsP strains respectively, was observed (Fig. 3B).

Using qRT-PCR, we found that the ptsP and ptsO mutations diminished the arpR mRNA level, while the ptsN inactivation increased it, in both the wild type UW136 and ptsP strains (Table 1). These results suggest that the non-phosphorylated EIIA<sup>Ntr</sup> of the PTS<sup>Ntr</sup> negatively controls arpR expression at the transcriptional and posttranscriptional levels.
H68A mutation in the phosphorylation site of EIIANtr impairs the transcription of arpR

The results presented above imply that the unphosphorylated form of EIIANtr negatively controls the expression of arpR, affecting the transcription of arsA and, in turn, the synthesis of ARs. Thus, we tested the effect of a point mutation in ptsN (H68A), which produces a non-phosphorylatable EIIANtr, on ARs production and on transcription of arpR and arsA. For this experiment we used the strain UW136::pALA8a, which carries the ptsN-H68A mutation [15]. As shown in Fig. 4A, this strain showed a negative ARs production, similar to that observed in the ptsP and ptsO mutants (compare Figs. 1A and 4A). In contrast, the strain UW136::pALA7, which carries a wild type ptsN gene [15] presented a phenotype of ARs production, similar to UW136 wild type strain. As shown in Fig. 4B, the ptsN-H68A mutation almost abrogated the β-glucuronidase activity in the strain carrying the transcriptional arsA-gusA fusion, and reduced by 60% the activity of the arpR-gusA fusion relative to the strain UW136::pALA7. These results indicate that the unphosphorylated EIIANtr protein represses the transcription of arpR.

The negative effect of unphosphorylated EIIANtr H68A on arpR transcription is through RpoS

The data presented above indicate that transcription of arpR is negatively regulated by the unphosphorylated EIIANtr protein. Since arpR transcription is dependent on RpoS [6], we wanted to determine if the unphosphorylated EIIANtr affects arpR expression through this sigma factor. For this, we determined the capacity of AR synthesis in the strain UW136::pALA8a (expressing the unphosphorylatable EIIANtr) carrying the plasmid pBpgyrA-arpR, which expresses the arpR gene from an RpoS-independent promoter (gyrA promoter). As shown in the Fig. 5A, this strain was able to synthesize ARs in BBOH plates. In contrast, a negative phenotype of ARs production was shown by the strain UW136::pALA8a when was transformed with the empty plasmid pBBR1MCS-5. A similar effect was observed in BBOH liquid medium; the RpoS-independent expression of arpR increased the AR levels in the strain UW136::pALA8a (Fig. 5B). These results suggest that negative effect of unphosphorylated EIIANtr on arpR expression is due to a negative effect on its transcriptional activation by RpoS.

Discussion

The alkylresorcinols are exclusively synthesized during the encystment in *A. vinelandii*, since the expression of arsABCD operon is specifically activated in this condition [4]. Here, we
identified that PTS$^{\text{Ntr}}$ regulates the expression of ARs biosynthetic operon, through regulation of its transcriptional activator ArpR.

The PTS$^{\text{Ntr}}$ is present in many bacterial genus and controls diverse physiological processes through the phosphorylation state of EIIA$^{\text{Ntr}}$ [8]. Since mutations on ptsP or ptsO impair the phosphoryl-group transfer to EIIA$^{\text{Ntr}}$, we hypothesized that absence of ARs synthesis in ptsP and ptsO mutants was mainly due to the presence of the unphosphorylated EIIA$^{\text{Ntr}}$. This was confirmed by two approaches. First, the inactivation of ptsN was sufficient to restore the ARs
levels in the \textit{ptsP} mutant (Fig. 1B), and second, the strain that harbors an unphosphorylatable \textit{EIIA}^{Ntr} H68A (which presents a replacement on the phosphorylation site histidine by an alanine) showed a negative ARs production phenotype (Fig. 4A).

Unexpectedly, in the \textit{ptsP-ptsN} double mutant the ARs levels were lower than in the \textit{ptsN} mutant (Fig. 1B). Additionally, the \textit{ptsP} mutation produced a stronger negative effect on ARs production than the mutation producing an unphosphorylatable \textit{EIIA}^{Ntr} H68A protein (compare Figs. 1B and 5B), suggesting a secondary regulatory role of \textit{EINtr} and/or \textit{NPr} proteins on ARs synthesis independent of its role in the phosphorylation of \textit{EIIA}^{Ntr}. Additional experiments are necessary to validate this hypothesis.
The difference in ARs production between the *ptsN* and the *ptsP-ptsN* mutants could also be explained by the presence of an EIIA<sup>rr</sup> paralog that partially complements the *ptsN* mutation. However, a single *ptsN* gene was found in the *A. vinelandii* genome.

The transcription of *arsA* was reduced when EIIA<sup>rr</sup> was present in its unphosphorylated form (Fig. 4B). Recently, we reported that both *arsABCD* and *arpR* transcription are directly activated by ArpR and acetoacetyl Coenzyme A (acetoacetyl-CoA) as coinducer [6].

![Fig 5. Effect of arpR expression from RpoS-independent promoter in the strain that carries the nonphosphorylatable EIIA<sup>rr</sup> H68A protein.](image)

(A) Staining of ARs produced by UW136 and U136::pAL8a strains, transformed with plasmid PBpgrpA-arpR, carrying a constitutively expressed arpR gene or the empty plasmid pBBR1MCS-5 as negative control. (B) Quantification of ARs levels produced by the strains of the panel A. The data represent the mean of three independent experiments. Error bars, SD.
the unphosphorylated EIIANtr also reduced the arpR transcription (Fig. 4B), we concluded that the negative effect on arsABCD expression was due to a reduction of arpR expression. The negative effect of the EIIANtr on expression of arpR could be explained by a reduction of the acetoacetyl-CoA pool. However this does not seem to be the case, since the presence of 5 and 50 μM of acetoacetyl-CoA did not restore the AR synthesis in ptsP, ptsO and ptsN H68A mutants (S1 Fig.). In contrast, an increase of ARs production phenotype dependent of acetoacetyl-CoA concentration was observed in the strains UW136, ptsN and ptsP-ptsN (S1 Fig.).

EIIANtr has been shown to indirectly regulate the expression of several genes. For example, in E. coli, the interactions between EIIANtr and kinase sensors KdpD and PhoR, increase the phosphorylation of response regulators KdpE and PhoB, resulting in increased expression of kdpFABC and the pho regulon, respectively [13,14]. Another interesting example is present in Salmonella, where EIIANtr interacts with the SsrB response regulator, reducing the expression of Salmonella pathogenicity island 2 (SPI-2) [28]. Additionally, a relationship between EIIANtr and the activity of sigma factors RpoS and RpoD has been previously described in E. coli [29]. In the absence of EIIANtr (in a ptsN mutant), the potassium levels increase (by derepression of activity of K+ Trk transporter) resulting in preferential binding of the core RNA polymerase to RpoS instead of RpoD, and therefore, affecting the transcription of sigma regulons [29]. Here, we found that in A. vinelandii the negative effect of the unphosphorylated EIIANtr on arpR transcription is through RpoS, since the expression of arpR from an RpoS-independent promoter was sufficient to restore ARs synthesis in the presence of unphosphorylated EIIANtr (Figs. 5A and 5B). Further evidence supporting the participation of RpoS in the regulation exerted by EIIANtr includes previous results showing that transcription of phbR, the gene encoding the transcriptional activator of PHB, and transcription of promoter pB2 of phbBAC are also RpoS dependent [30,31] and repressed by unphosphorylated EIIANtr [15]. We carried out additional experiments to confirm the negative effect of unphosphorylated EIIANtr protein on the phbB and phbR RpoS-dependent promoters (S2A and S2B Fig.). Indeed, the β-glucuronidase activity of transcriptional phbR-gusA and phbB-gusA fusions is reduced in the ptsP mutant (S2A and S2B Fig.). The mechanism by which the nonphosphorylated EIIANtr affects the RpoS activity in A. vinelandii remains to be elucidated.

Nonphosphorylated EIIANtr also seems to control the expression of arpR at a posttranscriptional level since the ptsN mutation increased the activity of the translational arpR-gusA fusion in the wild type and ptsP strains (Fig. 3B). Additionally, mutations of ptsP and ptsO diminished about twofold the activity of the transcriptional arpR-gusA fusion (Fig. 3A), while the arpR mRNA levels, measured by qRT-PCR, were even lower in the ptsP and ptsO mutants (Table 1). A similar effect was shown on the expression of ilvBN in E. coli, where a ptsN mutation reduced about 50% the activity of a transcriptional ilvB-lacZ fusion, while the ilvB mRNA levels (detected by RT-PCR) were more drastically reduced [32]. The mechanism by which nonphosphorylated EIIANtr negatively affects the arpR expression at posttranscriptional level remains to be determined. However, as the translational arpR fusion contains the 5’ untranslated region of arpR mRNA (including the Shine-Dalgarno sequence), this mechanism could be related to a reduction of mRNA stability and/or to a blockage of translation.

In summary, a regulatory model for the control of ARs synthesis by PTSNtr is proposed (Fig. 6). The EIIANtr protein in its nonphosphorylated state inhibits the activation of the transcription of arpR by RpoS. The repression of arpR expression impairs the transcriptional activation of biosynthetic arsABCD operon. Additionally, EIIANtr negatively affects the arpR mRNA levels by an unknown mechanism. The elucidation of the molecular mechanisms that link PTSNtr with RpoS and posttranscriptional regulation of arpR will allow us understand the role of PTSNtr in A. vinelandii.
Supporting Information

S1 Fig. Effect of acetoacetyl-CoA on ARs synthesis in pts mutant strains of A. vinelandii. The strains were grown in BBOH medium in absence or presence of 5 and 50 μM acetoacetyl-CoA (coinducer) for 72 h at 30°C. (TIF)

S2 Fig. Effect of the ptsP mutation on transcription of RpoS-dependent phbR and phbB genes. β-glucuronidase activity of transcriptional phbR-gusA (A) and phbB-gusA (B) fusions in UW136 and ptsP strains. The cells were grown in PY solid medium for 48 h at 30°C. The data represent the mean of two independent experiments. Error bars, SD. (TIF)

S1 Table. Strains and plasmids used in this work. (DOCX)

S2 Table. Oligonucleotides used in this work. (DOCX)

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Author Contributions

Conceived and designed the experiments: GE LFMM. Performed the experiments: LFMM SM LBP YR. Analyzed the data: GE LFMM DS. Contributed reagents/materials/analysis tools: MC. Wrote the paper: GE LFMM DS.
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