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Gene *sco1417* encodes a positive regulator of the *de novo* biosynthesis of pyridoxal 5′-phosphate (vitamin B6) in *Streptomyces coelicolor* M145

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Abstract

Background Actinomycetes of the genus *Streptomyces* are renowned for their highly developed and diverse specialized metabolic pathways, and there is an extensive body of data on their specific and pleiotropic levels of regulation. Much less is known about routes leading to essential metabolites in this genus. In this work, we focused on elucidating the function of the highly conserved across *Streptomyces* gene *SCO1417* for GntR type regulator in the model strain *S. coelicolor* A3(2).

Results Combining the results of knockout and promoter probe experiments, we show that the gene *SCO1417* controls pyridoxal 5′-phosphate (PLP; vitamin B6) biosynthesis, and thus is a member of the PdxR group of the transcriptional regulators. The Sco1417 protein is a transcriptional repressor of its gene and an activator of the expression of the PLP synthase genes, *SCO1523* (*pdxS)* and *SCO1522* (*pdxT*). According to electrophoretic mobility shift assays, out of several tested B6 vitamers, only PLP served as a Sco1417 effector molecule. We also provide data on the location of the Sco1417 binding site within the promoter region of *pdxST*.

Conclusions Our work portrays for the first time an evidence-based picture of the genetic control of vitamin B6 biosynthesis in *S. coelicolor* M145. Given the high conservancy and synteny of *pdx* homologs in the other streptomycetes, we suggest that the described genetic circuit is a general feature for the entire genus.

Keywords Vitamin B6, PLP, Genes, Mutation, *Streptomyces coelicolor* A3(2), GntR type regulators, Essential metabolism

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Introduction

Species within the actinomycete genus *Streptomyces* are known for their sizable genomes harboring numerous biosynthetic gene clusters for specialized metabolism [1, 2]. There is much interest in studying these gene clusters and regulating their expression, as it will help to overproduce practically valuable small molecules and discover novel ones [3]. Specialized metabolism is intimately connected to the primary metabolic pathways serving as a source of energy, cofactors, and initial building blocks for small molecule biosynthesis [4–6]. A detailed knowledge of primary metabolism is therefore needed if one aims to obtain a comprehensive overview of the biosynthesis of specialized metabolites.

During the studies of genes for GntR family of transcriptional factors (TFs) in *Streptomyces* we revealed 12 TFs whose genetic determinants are invariably present in the genomes of this genus [7]. We denote these genes as "core GntRs" to underscore our suggestion about an essential role for these genes in *Streptomyces* physiology or morphogenesis. One core GntR gene in the model strain *S. coelicolor* A3(2), *SCO1417*, falls into the MocR subfamily of TFs. The MocR members are notable for their a two-domain architecture with an N-terminal helix-turn-helix motif (HTH), a hallmark of DNA-binding proteins, and an aspartate aminotransferase-like domain (AAT) at the C-terminus. The latter serves as an element to recognize pyridoxal phosphate (PLP; vitamin B6) and other amino compounds, and thus modulates the activity of HTH [8]. *SCO1417* is similar to the *pdxR* gene of *S. venezuelae* ISP5230, which has been shown to complement PLP auxotrophy in *S. lividans* TK24 [9]. In pairwise alignment, Sco1417 exhibits around 40% similarity to PdxR (cg0897) of *Corynebacterium glutamicum* ATCC13032, the TF involved in the upregulation of adjacent PLP synthase genes *pdxST* for *de novo* biosynthesis of PLP [10]. Hence, it is likely that Sco1417 is a member of a group of PdxR TFs that control the production of PLP from pentose and triose sugars (the *pdxST*-dependent pathway), as found in most eubacteria. However, the *SCO1417* gene is not physically linked to *pdxST* homologs, unlike the situation in *C. glutamicum*. The exact mechanism of action of Sco1417 and the scope of effector molecules it recognizes (e.g., different B6 vitamers – pyridoxine, pyridoxamine, pyridoxal, and their phosphates) remain unknown. We therefore carried out a set of experiments to clarify the function of *SCO1417*. Our data confirm the involvement of *SCO1417* in PLP biosynthesis as a positive regulator of expression of *pdxS* (*SCO1523*) and *pdxT* (*SCO1522*) homologs. We also provide information on the ligand preferences of the Sco1417 protein and the nucleotide sequence of its operator site. As *SCO1417* orthologs are omnipresent in *Streptomyces* genomes, it is reasonable to expect that *de novo* vitamin B6 biosynthesis in all streptomycetes will follow the logic described here for *S. coelicolor* A3(2). This fills one more gap in our understanding of essential metabolism of *Streptomyces*.

Materials and methods

Bacterial strains, plasmids and culture conditions

Bacterial strains and plasmids used in this work are listed in Table 1. *E. coli* strains were used as described in the Table 1 and respective references. The *Streptomyces* strains were grown at 28 °C and *E. coli* strains were grown at 37 °C in Luria-Bertani (LB) medium [11], unless otherwise stated. Soya flour agar medium SFM [12] was used to obtain sporulating *Streptomyces* lawns and to plate *Streptomyces* – *E. coli* conjugation mixtures. Vitamin B6 auxotrophic phenotype of generated mutants was screened on minimal medium MM [12]. Appropriate antibiotics were added to growth media where needed. PLP and hydrochlorides of pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) were purchased from Sigma, of highest possible purity.

For the gene transcription profiling, *S. coelicolor* wild type and *pdxR* null mutant were grown in TSB [12] medium for 36 h. Then, the cells were collected by centrifugation, washed three times with water, and inoculated in SMM [12] supplemented with 20 μM PL. The cells were then allowed to grow for another 36 h. To investigate the influence of PL and PLP on the transcriptional activity of the *pdxR* and *pdxST* promoters in *S. coelicolor* M145, strains were grown likewise in SMM with 20 μM PL and PLP. To study the auto-regulatory function of PdxR, strains were cultivated in TSB medium for 36 h.

Plasmid and strain construction

Routine molecular biology techniques (DNA isolation, transformation, intergeneric matings, PCR, etc.) were carried out as described in [11]. Molecular biology enzymes were used according to manufacturer instructions. The oligonucleotides used in this work are listed in Table 2. All plasmids were verified by DNA sequencing.

An *in-frame* deletion mutant, *S. coelicolor* Δ*pdxR*, was constructed using recombineering approach [12]. The cosmid St6D7, which carries the *pdxR* gene, was introduced into *E. coli* strain BW25113 pIJ790$^+$. The *aac(3)IV* cassette containing *oriT* was amplified from pIJ774 using primers pdxR_acc_f/pdxR_acc_r with specific homology extension to *pdxR*. The cassette was then electroporated into arabinose-induced BW25113 pIJ790$^+$ St6D7$^+$. The final construct, St6D7-*pdxR*::Am, was selected on plates supplemented with kanamycin and apramycin. The plasmid was conjugally transferred from *E. coli* ET12567 pUZ8002$^+$ into *S. coelicolor* M145. The double crossover mutant was selected for apramycin resistance and kanamycin sensitivity, and confirmed by PCR.

Table 1 Bacterial strains and plasmids used in this work

Strain or plasmid	Description	Source or reference
S. coelicolor M145	SCP1$^-$, SCP2– derivative of A3(2)	[11]
S. coelicolor pdxR::Am	*SCO1417* replaced with apramycin resistance gene (Am^r)	This work
S. coelicolor Δ*pdxR*	*pdxR*::Am strain with excised Am^r	This work
S. coelicolor Δ*pdxR* pIJ-pdxR$^+$	Δ*pdxR* complemented with *SCO1417*	This work
S. coelicolor Δ*pdxR* pIJ82$^+$	Δ*pdxR* carrying empty pIJ82	This work
S. coelicolor pdxST::Am	*SCO152-1523* replaced with Am^r	This work
S. coelicolor Δ*pdxST*	*pdxST*::Am strain with excised Am^r	This work
S. coelicolor Δ*pdxST* pSET-pdxST	Δ*pdxST* complemented with *SCO1522-1523*	This work
S. coelicolor Δ*pdxST* pSET152$^+$	Δ*pdxST* with empty vector	This work
S. coelicolor pGUS$^+$	M145 carrying pGUS	This work
S. coelicolor pGUS-pdxRp$^+$	M145 carrying pGUS-pdxRp	This work
S. coelicolor Δ*pdxR* pGUS-pdxRp$^+$	Δ*pdxR* carrying pGUS-pdxRp	This work
S. coelicolor pGUS-pdxSTp$^+$	M145 carrying pGUS-pdxSTp	This work
Escherichia coli DH5α	Routine cloning host	Life Technologies
E. coli ET12567 pUZ8002$^+$	Host for conjugative DNA transfer	[11]
E. coli BW25113 pIJ790$^+$	Host for recombineering experiments	[12]
E. coli BL21 (DE3) STAR pRARE	Strain for recombinant protein production. The pRARE plasmid was isolated from Rosetta™(DE3) (Novagen) and introduced into BL21 (DE3) STAR™ (Thermo Fisher Scientific)	This work
pIJ790	ts-plasmid carrying genes for λ-RED recombination, chloramphenicol resistant (Cm^r)	[12]
St6D7	Cosmid containing *pdxR* genomic region, kanamycin resistance (Km^r)	JIC
pIJ774	Carrying *oriT-acc(3)IV* flanked by *loxP*-sites, Am^r and ampicillin resistance (Ap^r)	[7]
pLERECJ	Carrying *acc(3)IV* flanked by *loxP*-sites, Am^r and Ap^r	Prof. A. Luzhetskyy
pUWLCre	Replicative plasmid (*rep*^{pIJ101}) harboring recombinase gene *cre* under *ermE*p	Prof. A. Luzhetskyy
pIJ82	Integrative φC31-based vector, hygromycin resistance (Hy^r)	[11]
pKC0702	pKC1139 derivative, Am^r replaced with Hy^r marker	Prof. A. Luzhetskyy
pSET152	Integrative φC31-based vector, Am^r	[11]
St6D7-*pdxR*::Am	Cosmid with inactivated *pdxR*; *pdxR*::*aac(3)IV* (Km^r and Am^r)	This work
pIJ-pdxR	pIJ82 harboring *pdxR* with promoter region; Hy^r	This work
pKC0702-pdxST	pKC0702 harboring *pdxST* with 2-kb flanking region, Hy^r	This work
pKC0702-pdxST::Am	*pdxST* knockout construct; *pdxST*::*aac(3)IV* (Hy^r and Am^r)	This work
pSET-pdxST	pSET152 harboring *pdxST* with promoter region, Am^r	This work
pGUS	The *gusA*-based reporter plasmid, Am^r	[13]
pGUS-pdxRp	pGUS with *pdxRp-gusA* fusion, Am^r	This work
pGUS-pdxSTp	pGUS with *pdxSTp-gusA* fusion, Am^r	This work
pET28a	Vector for histidine-tagged protein production, pET-system	Novagen
pET28a-pdxR	PdxR-6His protein production	This work

To inactivate *pdxST*, both genes along with flanking regions (approximately 2 kb long) were amplified from *S. coelicolor* chromosome with primers pdxST-f/pdxST-r. The PCR product was digested with HindIII and XbaI, purified, and ligated into the respective sites of pKC0702. The resulting plasmid pKC0702-pdxST was transformed into *E. coli* strain BW25113 pIJ790$^+$, thereby facilitating the replacement of *pdxST* with an apramycin resistance cassette. The *aac(3)IV* cassette with *pdxST*-specific extensions was amplified from pLERECJ using primers pdxST_acc_f/pdxST_acc_r. Subsequently, the plasmid pKC0702-pdxST::Am was introduced into *S. coelicolor* M145 by intergeneric conjugation with *E. coli* ET12567 pUZ8002$^+$, which harbored the respective construct. To generate single-crossover Am^r Hy^r mutants, initial exconjugants were incubated at 40 °C for 3 days (a nonpermissive temperature for the *pSG5* replication of pKC0702), and then screened for an apramycin resistance and hygromycin sensitivity (an indicative of vector loss and double crossover).

The apramycin resistance cassettes for *pdxR* and *pdxST* replacement are flanked with *loxP* sites. The marker genes were evicted from the genomes of *S. coelicolor pdxR*::Am and *S. coelicolor pdxST*::Am through the expression of a site-specific recombinase *cre* from a plasmid pUWLCre. The *pdxR* and *pdxST* knockouts and marker excisions were confirmed via PCR (primers pdxR_cmpl-f/r, pdxST_ cmpl-f/r).

The coding sequences of *pdxR* and *pdxST* along with the 300-/225-bp promoter regions were amplified

Table 2 Primers used in this work

Primer name	Sequence	Purpose/PCR product
pdxR_acc_f	GTCAACCATGGCCAATTTGGGGAAGGTGGACTGATTTCCATGATTCCGGGGATCCGTCGACC	To amplify *aac(3)IV* for *pdxR* replacement
pdxR_acc_r	GCGGGCGGAGGTCCCCCGGCGGCCCCGCCGGACGCGCCCTATGTAGGCTGGAGCTGCTTC	
pdxST_f	AATAAAAGCTTACAAGACGTCTGTCATGACA	To amplify *pdxST* with 3-kb flanks
pdxST_r	AATAATCTAGACCAGTTCACTTGATTCTCCT	
pdxST_acc_f	CGTTGCTGCACAACCCCCCTTCTCCCCAGTGAGGTACCCGTGGATATCTCTAGATACCG	To amplify *aac(3)IV* for *pdxST* replacement
pdxST_acc_r	CTTCGCGTAACCCATCTCCTGCAACGAACGCAGGAATCCTAAACAAAAGCTGGAGCTC	
pdxR-cmpl-f	ATTGAAAGGATCCCATCGACAGCCCCGTGTGCT	To clone *pdxR* for complementation
pdxR-cmpl-r	ATTGAAATCTAGAGCGCCCTAGGCCACGAACGT	
pdxST-cmpl-f	AATAAGGATCCCTGCGCGAGCACCGCAAGGT	To clone *pdxST* for complementation
pdxST-cmpl-r	AATAATCTAGAATCTGCCTGTCTCCTTCGCG	
Gus-R-f	TCTGAATCTAGATTCAGGACGATGCCCGCCAC	*pdxRp* for pGUS
Gus-R-r	TCTGAAGGTACCCCAAATTGGCCATGGTTGAC	
Gus-ST-f	TCTGAATCTAGAAGGAACTCGCCACCTCCGGC	*pdxSTp* for pGUS
Gus-ST-r	TCTGAAGGTACCACTGGGGAGAAGGGGGGTTG	
RT_1523_f	ACATGATCGAGGGCATCATC	To check the trans-cription of *pdxS*
RT_1523_r	ATCTCGTTCTTGATCTGGCG	
RThrdB_f	CGAGGACGAGGCGACCGAGGAG	Positive RT control (*hrdB*)
RThrdB_r	CAGCTTGTCCTCGGCGAACAGA	
SCO1417NcoI-f	AAAACCATGGCGCAGTGGACCTCGGCCGTG	*pdxR*-ORF for protein production
SCO1417BamHIhis-r	AAAGGATCCTCAGTGGTGGTGGTGGTGGTGGGCCACGAACGTCCGCG	
R-f	CTGGTGCAGCACGTTCCA	*pdxR* promoter for EMSAs
R-r	ACTGCGCCATGGAAATCAGT	
ST-f	CCGCCGCTTCCACAACGA	*pdxST* promoter for EMSA
ST-r	TGCTGGACACGGGTACCTCA	
OTR1-f	TGGGGTGGTGGTAGTGGACTGCCACATGACACCCTGTGGCTTCAGAA	To generate ds-oligonucleotides with predicted rPdxR binding sites (bs) in *pdxRp*
OTR1-r	TTCTGAAGCCACAGGGTGTCATGTGGCAGTCCACTACCACCACCCCA	
OTR2-f	TCAACCATGGCCAATTTGGGGAAGGTGGACTGATTT	
OTR2-r	AAATCAGTCCACCTTCCCCAAATTGGCCATGGTTGA	
OTST-f	ACGAAAACGATCCACCGCCTTCTCATTGGCCCTTGCTGTGGCCTGCT	To generate ds-oligonucleotides carrying putative rPdxR bs in *pdxSTp*
OTST-r	AGCAGGCCACAGCAAGGGCCAATGAGAAGGCGGTGGATCGTTTTCGT	
OTST2-f	CTTCTCATTGGCCCTTGCTGTGGCCTGCTC	
OTST2-r	GAGCAGGCCACAGCAAGGGCCAATGAGAAG	
OTR1a-f	TGGGGTGGTGGTAAAAAACTGCCACATGACACCCTGTGGCTTCAGAA	To generate ds-oligonucleotides with mutated rPdxR bs in *pdxRp*
OTR1a-r	TTCTGAAGCCACAGGGTGTCATGTGGCAGTTTTTTACCACCACCCCA	
OTR1b-f	TGGGGTGGTGGTAGTGGACTGCCACATGACACCCTAAAACTTCAGAA	
OTR1b-r	TTCTGAAGTTTTAGGGTGTCATGTGGCAGTCCACTACCACCACCCCA	
OTR1c-f	TGGGGTGGTGGTAAAAAACTGCCACATGACACCCTAAAACTTCAGAA	
OTR1c-r	TTCTGAAGTTTTAGGGTGTCATGTGGCAGTTTTTTACCACCACCCCA	
OTR2a-f	TCAACCATGGCCAATTTGGGGAAGAAAAACTGATTT	
OTR2a-r	AAATCAGTTTTTCTTCCCCAAATTGGCCATGGTTGA	
OTR2b-f	TCAACCAAAACCAATTTGGGGAAGGTGGACTGATTT	
OTR2b-r	AAATCAGTCCACCTTCCCCAAATTGGTTTTGGTTGA	
OTR2c-f	TCAACCAAAACCAATTTGGGGAAGAAAAACTGATTT	
OTR2c-r	AAATCAGTTTTTCTTCCCCAAATTGGTTTTGGTTGA	
OTST2a-f	CTTCTCATTGGCCCTTGCTAAAACCTGCTC	To generate ds-oligonucleotides carrying matated rPdxR bs in *pdxSTp*
OTST2a-r	GAGCAGGTTTTAGCAAGGGCCAATGAGAAG	
OTST2b-f	CTTCTCAAAAACCCTTGCTGTGGCCTGCTC	
OTST2b-r	GAGCAGGCCACAGCAAGGGTTTTTGAGAAG	
OTST2c-f	CTTCTCAAAAACCCTTGCTAAAACCTGCTC	
OTST2c-r	GAGCAGGTTTTAGCAAGGGTTTTTGAGAAG	
Pnat-f	CAGGTGATCGTTACGGAGCGTAGAGAACCTATGTCCCTGAGTGACGATACTGG	To generate unspecific oligonucleotide probe
Pnat-r	CCAGTATCGTCACTCAGGGACATAGGTTCTCTACGCTCCGTAACGATCACCTG	

from chromosomal DNA using primers pdxR-cmpl-f/r and pdxST-cmpl-f/r, respectively. The PCR products were digested with XbaI and BamHI and cloned into the respective sites of integrative plasmids pIJ82 and pSET152, resulting in pIJ-pdxR and pSET-pdxST.

The promoter regions of *pdxR* (*pdxRp*; 472 bp) and *pdxST* (*pdxSTp*; 500 bp) were amplified with primers Gus-R-f/Gus-R-r and Gus-ST-f/Gus-ST-r, respectively, and then digested and cloned into the XbaI and KpnI sites of the pGUS vector, giving pGUS-pdxRp and pGUS-pdxSTp.

RT-PCR

The RNA used for semiquantitative RT-PCR was isolated as described in [13]. Subsequently, 200 ng of cDNA were used as a template for the PCR analysis using primers listed in Table 2.

Analysis of β-glucuronidase activity

The β-glucuronidase activity of the strains carrying the reporter plasmid was assayed according to [14, 15].

Production and purification of His-tagged Sco1417 (rPdxR)

The coding sequence of *pdxR* was cloned into the pET28a expression vector (primers SCO1417NcoI-f and SCO1417BamHIhis-r) to give pET28a-pdxR. To produce the C-terminally hexahistidine-tagged PdxR protein (rPdxR), *E. coli* BL21 (DE3) STAR pRARE carrying pET28a-pdxR was grown in LB medium supplemented with chloramphenicol and kanamycin until an OD_{600} of 0.5 was reached. The culture was then induced with 0.25 mM IPTG (isopropyl thiogalactoside) and incubated for 18 h at 20°C. The cells were collected by centrifugation and resuspended in a lysis buffer (50 mM TrisHCl, 300 mM NaCl, 1 mM DTT, 5% glycerol, 20 mM imidazole, pH 7.5) containing a protease inhibitor cocktail (Roche). The lysis of the cells was achieved by two consecutive passages through a French press (American Instrument Corporation) at 1000 psi. The cell lysate was centrifuged at 16000 rpm for 30 min and the soluble fraction was applied to a Ni-NTA agarose resin (Qiagen), washed two times with a wash buffer (50 mM TrisHCl, 300 mM NaCl, 1 mM DTT, 5% glycerol, 50 mM imidazole, pH 7.5). The protein was eluted with increasing concentrations of imidazole (100–200 mM) and subsequently dialyzed against a storage buffer (50 mM TrisHCl, 300 mM NaCl, 1 mM DTT, 5% glycerol, pH 7.5). The concentration of rPdxR was determined by Bradford assay.

Electrophoretic mobility shift assay (EMSA) of DNA-protein complexes

The putative promoter regions *pdxRp* and *pdxSTp* were amplified from the *S. coelicolor* genome with the primers listed in Table 2. The PCR products were labelled at 5'-end with [γ-^{33}P]-ATP using T4 polynucleotide kinase. The labeled probes (20 fmol) were incubated with purified rPdxR in concentrations ranging from 0.05 to 1.00 μM, in binding buffer (20 mM Tris–HCl pH 7.9, 100 mM KCl, 10 mM $MgCl_2$, 1 mM EDTA, 1 mM DTT, 12.5% glycerol supplemented with 1 μg poly(dI-dC) and 4.5 μg BSA)) for 20 min at 30°C. The DNA-protein complexes were resolved on 8% nondenaturing polyacrylamide gels in 0.5xTBE buffer at 150 V. DNA bands were visualized by phosphor imaging on Typhoon FLA7000. For competition assays, an unlabelled DNA probe was added into the reaction mixture in 10- to 500-fold molar excess. Full-length unedited versions of all EMSA gels present in this work are given in Supplementary Materials, Figs. S7-S10.

To probe different B6 vitamers as potential ligands of rPdxR, they were incubated with the protein (50 nM) for 5 min at 30 °C prior to the addition of *pdxRp* or *pdxSTp* probes. The concentrations of vitamin B6 vitamers that were tested are indicated in the main text and in additional information Fig. S4.

To identify the putative binding sites of rPdxR within the *pdxR* and *pdxST* promoters, a number of double-stranded oligonucleotides R1, R2, ST, ST2 and their derivatives (mutated versions), were generated as described in [16] and used in EMSA.

Results

Regulatory and structural genes involved in PLP biosynthesis in *S. coelicolor* A3(2)

The putative translation product of the *SCO1417* gene is a typical TF of the MocR subfamily and harbors the all expected motifs involved in DNA and ligand recognition. In particular, when compared to the structurally studied MocR-type TF GabR involved in regulation of genes for γ-aminobutyric acid degradation in *Bacillus* [8], Sco1417 retains amino acid residues important for DNA binding (correspond to Arg43 and Ser152 within the HTH motif of GabR) and PLP recognition (Ser311, Lys312 and Arg319 within the AAT domain; Fig. S1). The *SCO1417* in A3(2) genome is flanked by genes that do not appear to be related to vitamin B6 metabolism (e.g. its closest neighbors encode membrane proteins of unknown function). Using the *pdxS* and *pdxT* genes of *C. glutamicum* as a queries in BLAST searches, we readily identified their orthologs, *SCO1523* and *SCO1522*, respectively, about a hundred genes away from *SCO1417*. We note here that disjoint location of *pdxR* and the genes it controls has been described for some bacteria, e.g. *Streptococcus* [17]. The coding sequences of the aforementioned genes are separated by a 12-bp gap, an indicative of their transcriptional coupling. BLASTP analysis revealed that *Streptomyces* genomes invariably encode orthologs of Sco1523 and Sco1522. Overall, our initial scrutiny of available data supports the hypothesis that Sco1417 is PdxR involved in

regulation of the expression of genes for the production of PLP from ribose 5-phosphate, glyceraldehyde 3-phosphate and glutamine (Fig. 1).

Inactivation of *SCO1417* leads to vitamin B6 auxotrophy and impaired transcription of the PLP synthase genes in *S. coelicolor*

To corroborate the regulatory function of Sco1417 (designated PdxR) in PLP biosynthesis, we constructed a knockout strain *S. coelicolor* Δ*pdxR*. First, the coding sequence of the gene on a chromosome of *S. coelicolor* M145 was replaced by an apramycin resistance cassette and further excised to generate a marker-free strain. In comparison to the wild type, the resulting mutant was unable to grow on minimal medium unless vitamin B6 vitamers were supplemented (PM, PN, PL or PLP were tested, Fig. 2A). *In trans* expression of *pdxR* gene from its own promoter in *S. coelicolor* Δ*pdxR* fully complemented the vitamin B6 auxotrophic phenotype, restoring the ability of the strain to grow on minimal medium.

Next, we wanted to confirm that *SCO1523 (pdxS)* and *SCO1522 (pdxT)* encode subunits of PdxST-complex that catalyzes *de novo* synthesis of PLP. For this purpose, *S. coelicolor* Δ*pdxST* mutant was generated and characterized by growth assay on minimal medium. As shown in Fig. 2B, the mutant did not grow on minimal medium without B6 vitamers, whereas the wild type and the complemented mutant grew abundantly. This concludes that *pdxST* encode a functional PLP synthase complex that is solely responsible for PLP production in *S. coelicolor* M145. In addition, utilization of different vitamers indicates a functional salvage pathway in the strain.

The inability of *S. coelicolor* Δ*pdxR* to grow on MM was the first evidence that PdxR is a positive regulator of *pdxST* transcription. To confirm this hypothesis, the transcriptional profile of *pdxST* genes was compared in M145 and Δ*pdxR* grown in MM supplemented with pyridoxal. As shown in Fig. 2C, no *pdxST* transcript was detected in the mutant compared to the wild-type strain. Altogether, our data show that Δ*pdxR* is indeed impaired in the gene encoding a TF involved in the regulation of PLP biosynthesis, and its absence abolishes the expression of *pdxST* genes.

Properties of Sco1417: ligand preferences and negative regulation of its own gene

Recombinant C-terminally 6His-tagged Sco1417 (rPdxR) was purified to homogeneity from *E. coli* (Fig. S2) and subjected to a series of EMSAs. First, rPdxR was shown to bind promoter regions of its own gene (*pdxRp)* and that of *pdxST* (*pdxSTp*; Fig. 3A, B and Fig. S3), directly confirming its role as a TF for these genes. Second, we

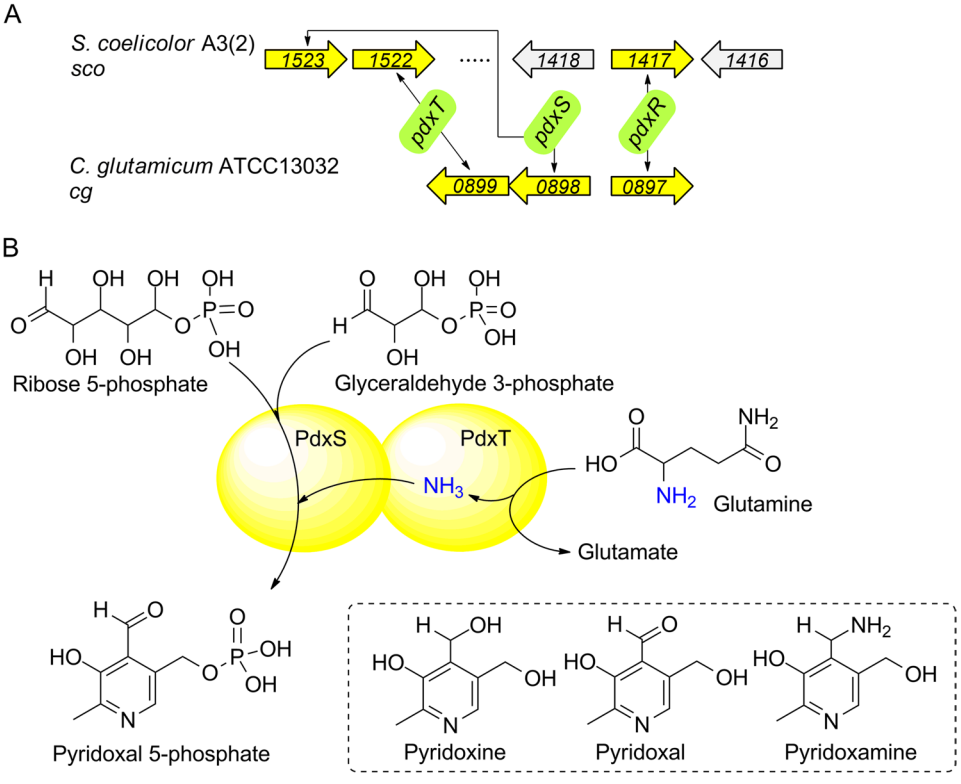

Fig. 1 PLP biosynthesis in *S. coelicolor* A3(2): genes (**A**) and the proposed pathway (**B**). The gene *SCO1417* (*pdxR*) is located separately (gap marked with dots) from the *pdxST* genes (*SCO1523*, *SCO1522*). Bidirectional arrows point to orthologous genes in *S. coelicolor* and *C. glutamicum*. Dashed rectangle (bottom of the figure) marks known (unphosphorylated) B6 vitamers. *SCO* genes colored in grey are likely not related to vitamin B6 metabolism

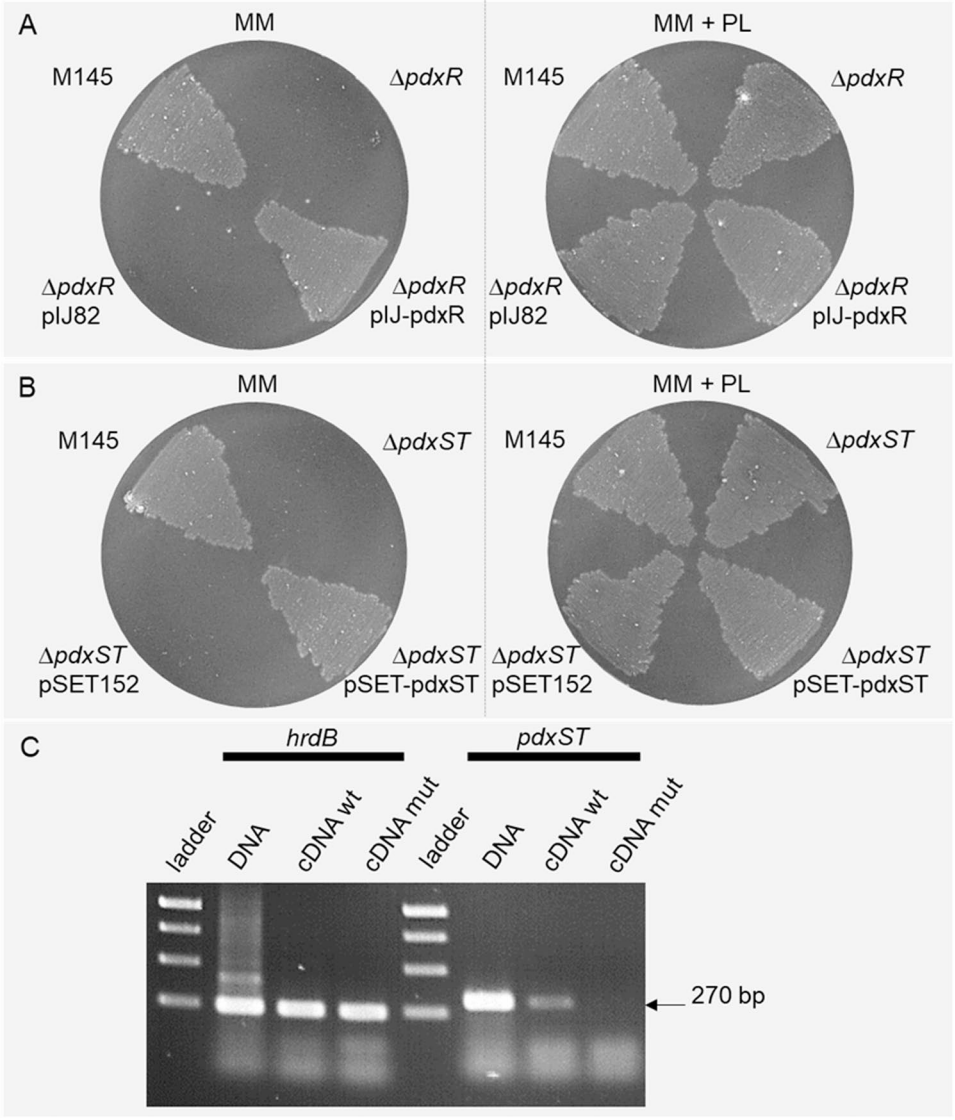

Fig. 2 The gene *SCO1417 (pdxR)* is involved in the regulation of the *de novo* biosynthesis of PLP in *S. coelicolor* M145. Growth of wild type (M145), *pdxR* (**A**) and *pdxST* (**B**) null mutants. Photos of the plates were taken after 120 h of growth on MM or MM supplemented with 10 μM pyridoxal. Introduction of an intact copy of *pdxR or pdxST* into the respective mutants restored their ability to grow on MM compared to mutants carrying empty vectors pIJ82 and pSET152. Knockout of *pdxR* abrogated the transcription of *pdxST* genes (**C**). The *hrdB* gene served as a positive control for sqRT-PCR. The cDNA wt, cDNA prepared from total RNA of M145; cDNA mut, cDNA of Δ*pdxR*; DNA – chromosomal DNA isolated from M145

revealed that, in vitro, only PLP was able to interact with rPdxR and prevent its binding to the promoter regions of *pdxR* and *pdxST* genes (Fig. 3C, D), while PL, PN and PM did not impact rPdxR-DNA interaction (Fig. S4).

To assess the biological consequence of the presence of vitamin B6 on the expression of *pdxR* and *pdxST*, we employed plasmids where the *gusA* reporter gene [14] was fused to either *pdxRp* or *pdxSTp*. The plasmids were introduced into M145 and glucuronidase (GusA) activity was measured in minimal medium in either the absence or presence of PL and PLP. Both vitamers had no influence on the transcription from the *pdxR* promoter, while their presence led to a strong (almost threefold) decrease in GusA activity from the *pdxST* promoter (Fig. 4A). The results suggest that PdxR represses the transcription of its own gene and upregulates *pdxST* genes. The MocR-like TFs are known to act as auto-repressors. To find an evidence of negative regulation of *pdxR* by PdxR, we compared the GusA activity of the *pdxRp-gusA* transcriptional fusion in Δ*pdxR* and parental strain M145. Indeed, the aforementioned reporter plasmid exhibited higher activity in the mutant than in the wild-type strain (Fig. 4B), in accord with auto-repressing function of PdxR.

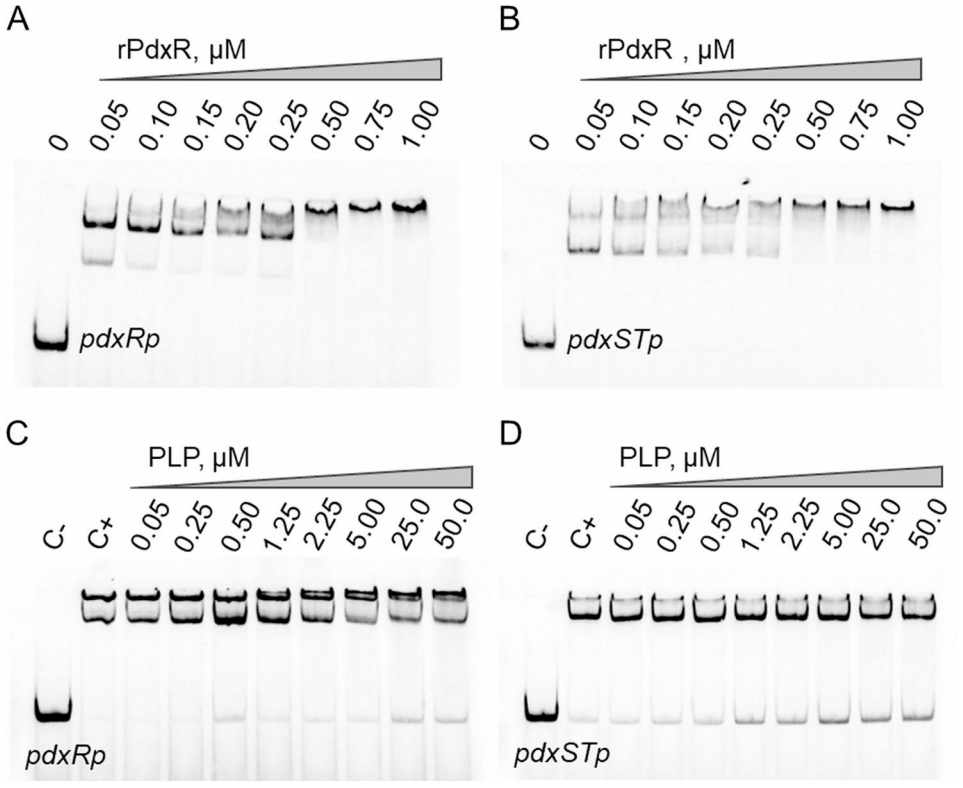

Fig. 3 EMSA confirms the interaction of rPdxR with the *pdxR* (**A**) and *pdxST* (**B**) promoter regions. The first lanes in gels A and B (marked as 0) correspond to free labeled DNA. PLP prevents the binding of rPdxR to *pdxRp* (**C**) and *pdxSTp* (**D**). Assay conditions: rPdxR (50 nM) was pre-incubated with increasing concentrations of PLP for 5 min; then, a labeled DNA probe was added, and reactions were run for 20 min. C^-, labeled DNA without rPdxR and PLP; C^+, DNA + rPdxR

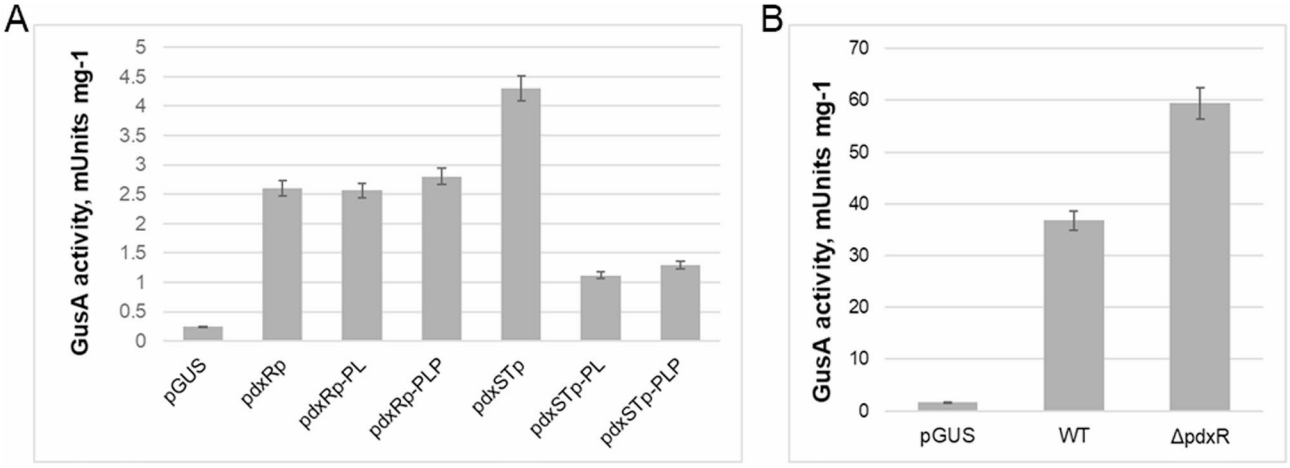

Fig. 4 PL and PLP act as in vivo effectors of PdxR which is a repressor of its own gene and an activator of *pdxST* genes. Glucuronidase (GusA) activity of cell lysates prepared from M145 strain grown in minimal medium for 36 h (**A**). Lanes: pGUS. M145 harboring empty *gusA* vector pGUS; pdxRp, M145 harboring *pdxRp-gusA* fusion in the absence of vitamers; pdxRp-PL, M145 harboring *pdxRp-gusA* fusion in the presence of PL; pdxRp-PLP, M145 harboring *pdxRp-gusA* fusion in the presence of PLP; pdxSpT, M145 harboring *pdxSTp-gusA* fusion in the absence of vitamers; pdxSTp-PL, M145 harboring *pdxSTp-gusA* fusion in the presence of PL; pdxSTp-PLP, M145 harboring *pdxSTp-gusA* fusion in the presence of PLP. Comparison of GusA activity of M145 (WT) and ΔpdxR strains harboring *pdxRp-gusA* fusion (**B**). Error bars, ±2SD (three independent biological replicates)

Mapping of the PdxR binding site within the *pdxR* and *pdxST* promoters

Multiple alignment of *pdxRp* with sequences found upstream of the *pdxR* orthologs in the other streptomycetes identified two adjacent regions, referred to as R1 and R2, carrying putative binding motifs of PdxR (Fig. S5). R2 is located immediately upstream of the *pdxR* start codon (Fig. 5A). The double-stranded oligomers

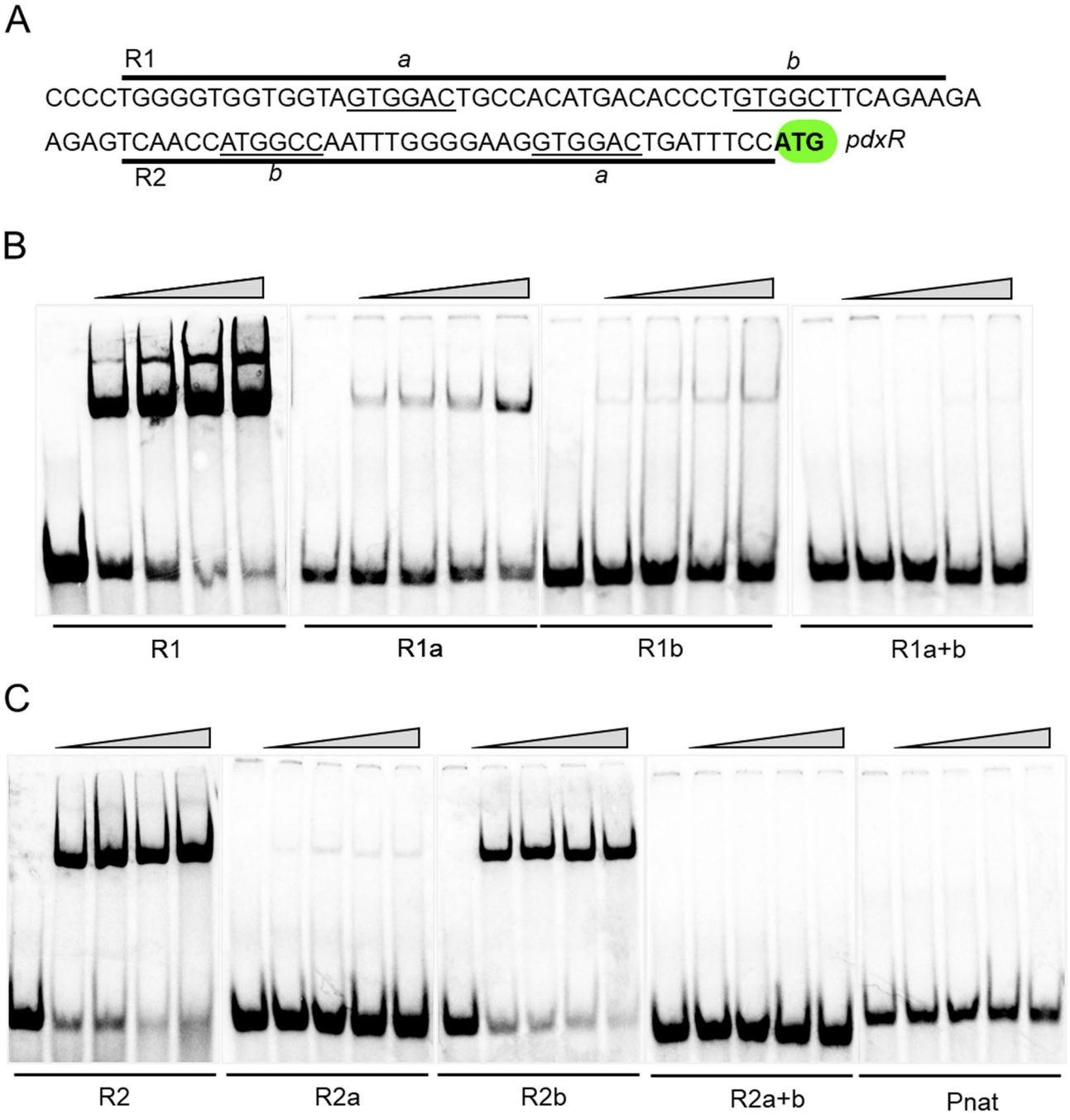

Fig. 5 The PdxR binding sites within the promoter of the *pdxR* gene. The nucleotide sequence of the promoter (**A**) comprised two conserved motifs, R1 and R2 (marked with bold line), each containing 6-nt imperfect repeats, underlined and marked with *a* and *b*. The start codon of *pdxR* is shown on green background. EMSA of rPdxR binding to the native and mutated (substituted with AAAAAA) versions of R1 (**B**) and R2 (**C**). Labels at the bottom of the gels: R1, native sequence, R1a, mutated repeat *a* of R1; R1b, mutated repeat *b* of R1; R1a + b – both *a* and *b* are mutated within R1. The versions of R2 are labeled analogously. Pnat, unspecific DNA (*ssfg_02181*). The first lane in each gel is free DNA, grey triangles mark increasing amounts of rPdxR (0.1, 0.25, 0.5 and 1.0 μM)

containing one of the two sequences were used in EMSA. Both R1 and R2 are bound by rPdxR (Fig. 5). Close examination of the R1 and R2 sequences led to the identification of conserved 6-nt direct repeats, the substitution of which by the hexa-A sequence perturbed rPdxR binding (Fig. 5A-C). Complete cessation of DNA binding was only observed when both repeats were mutated.

Analysis of the *pdxSTp* region also revealed a highly conserved sequence approximately 50 bp upstream of the *pdxS* start codon (Fig. S6), referred to as ST (Fig. 6A).

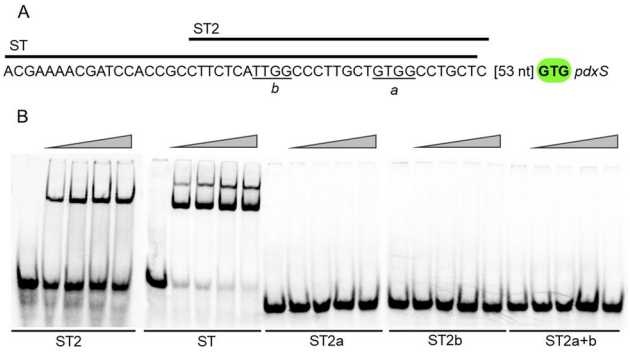

Fig. 6 The PdxR binding sites within the promoter of the *pdxS* gene. The nucleotide sequence of the promoter (**A**) contains the conserved motif ST, a subfragment of which, ST2 (marked with bold line) was further tested in a series of EMSAs. ST2 harbors 4-nt imperfect repeats, underlined and marked as *a* and *b*. The start codon of *pdxS* is shown on the green background. EMSA of rPdxR binding to ST2, ST and mutated (substituted with AAAAAA) versions of ST2 (**B**). Labels at the bottom of the gels: ST2a, mutated repeat *a* of ST2; ST2b, mutated repeat *b* of ST2; ST2a + b – both *a* and *b* are mutated within ST2. The first lane in each gel is free DNA, grey triangles mark increasing amounts of rPdxR (0.1, 0.25, 0.5 and 1.0 μM)

Both ST and its subfragment ST2 were bound by rPdxR, whereas the mutation to tetra-A either of the two 4-nt repeats within ST2 completely blocked rPdxR binding to DNA (Fig. 6B).

Discussion

This work provides experimental evidence that the gene *SCO1417* in *S. coelicolor* A3(2) encodes the PdxR protein involved in the regulation of the *de novo* biosynthesis of PLP. Similar to other PdxR-governed mechanisms investigated in Gram$^+$ bacteria, this transcriptional regulator controls the expression of genes *SCO1523-SCO1522* encoding subunits of the PLP synthase complex (PdxST), whose proposed biochemical functions are summarized in Fig. 1. Therefore, we propose the names *pdxR* and *pdxST* for *SCO1417* and *SCO1523-1522*, respectively, by analogy to the studied precedents.

Our current understanding of PdxR-controlled circuitry is summarized in Fig. 7 and detailed below. PdxR exerts a positive regulatory control on the *pdxST* transcription while negatively regulating the expression of its own gene in *S. coelicolor*. The differences in the biological effects of PdxR on the expression of *pdxST* and *pdxR*

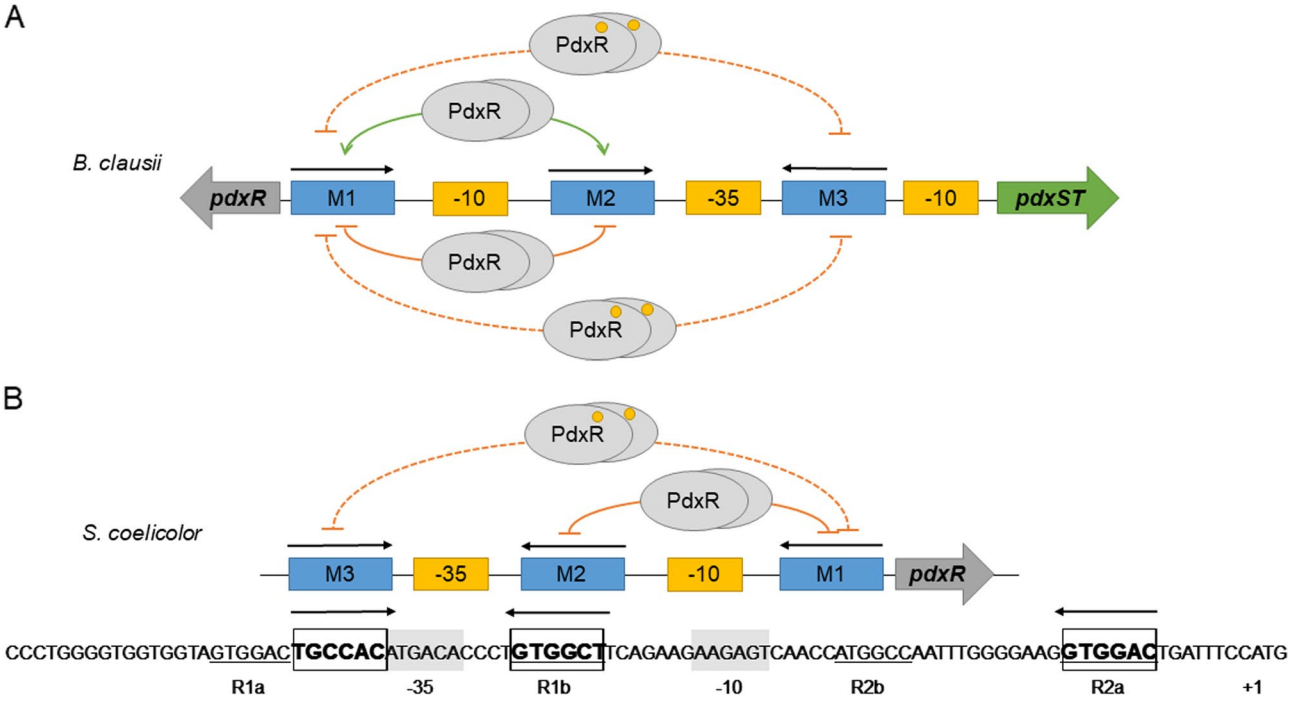

Fig. 7 Proposed regulatory mechanism governed by PdxR. In *B. clausii* (**A**), apo-PdxR preferably binds to the direct repeats (motif1 and motif2) exerting a positive and negative regulatory control on the transcription of the *pdxST* (green arrow lines) and *pdxR* (orange lines) genes, respectively. Holo-PdxR binds to the inverted motifs 1 and 3, and represses the expression of both genes (orange dashed lines). Similarly, in *S. coelicolor* (**B**), PdxR binds to the predicted DNA-binding sites (motifs 1–3) in the *pdxR* promoter region. Sequences (R1a, R1b, R2a and R2b) tested in EMSA are underlined, and the – 10 and – 35 RNA-polymerase binding hexamers in the nucleotide sequence of *pdxR*p are highlighted in grey. The predicted *pdxR* translational start codon (ATG) is indicated by + 1. Motifs 1 (M1) and 2 (M2) correspond to the boxed sequences R2a and R1b, whereas motif 3 (M3) represents the boxed TGCCAC inverted repeat. Apo-PdxR is shown as two grey ovals, holo-PdxR – as grey ovals with yellow dots

genes is likely underlined by the dissimilarity in the relative location of PdxR binding sites within the *pdxR* and *pdxST* promoters, as well as their sequence identity. The MocR-like regulators act as homodimers with a head-to-tail arrangement that allows them to interact with direct DNA repeats. However, the binding of the ligand alters their DNA-binding preferences towards inverted repeats. The PdxR binding sites, which were identified in the *pdxR-ST* intergeneric region of *C. glutamicum* and *B. clausii*, consist of two direct and one inverted repeats. Likewise, detailed analysis of the *pdxR* promoter identified at least four conserved imperfect direct hexanucleotide repeats RTGGVY possibly recognized by PdxR in *S. coelicolor*. According to the mutational analysis, two motifs R1b (GTGGCT) and R2a (GTGGAC) are indeed important for the DNA-binding activity of PdxR. An additional inverted repeat (motif 3) was identified ten nucleotides upstream of the motif 2, and might be required for binding by holo-PdxR. In the *pdxR* promoter region, the – 10 and – 35 regulatory sequences are located between PdxR recognition sites. Such an arrangement most likely prevents the binding of the RNA-polymerase to *pdxR*p irrespective of the interaction of PLP with PdxR, thus explaining no differences in the *gusA* expression from the promoter region of *pdxR*. Contrary to *C. glutamicum* and *B. clausii*, where the expression of *pdxR* and *pdxST* is governed by the binding of PdxR to the same motifs, in *S.coelicolor* the regulatory and the biosynthetic genes are transcribed from their own promoters. In contrast to *pdxR*p, in silico analysis of *pdxST*p did not revealed any prominent candidates for the PdxR recognition. Introduction of mutations into two conserved 4-bp sequences in *pdxST*p blocked PdxR binding in EMSA, indicating their importance for the interaction. However, additional experimental analyses are required to identify the regulatory sequences. We note here that the structure of the repeats recognized by PdxR from the probiotic bacterium *Bacillus clausii* [8] differs from the sites revealed in our study, suggesting diversity in the PdxR mechanism. Similarly, the role of PLP in the nuances of PdxR function in *S. coelicolor*, as recently reported for *B. clausii* PdxR [18], awaits further investigation.

Under in vitro conditions only PLP showed the ability to preclude PdxR from binding to DNA, while in vivo (promoter probe) tests showed that PL is also able to affect PdxR. We suggest that PL undergoes phosphorylation in the cells, and thus becomes a PdxR effector molecule. The feedback inhibition of the *pdxST* transcription tightly regulates levels of PLP, maintaining sufficient amounts of the cofactor in the cell pool but below its toxic concentrations. Indeed, vitamin B6 salvage pathways are well known for bacteria [19, 20], although their identity in *Streptomyces* remains unknown. Another interesting line of research that was left unaddressed in our study is the effect of the *pdxR* mutation on the specialized metabolism of *S. coelicolor*. The production of the two most prominent specialized metabolites of A3(2), the red pigment undecylprodigiosin and the deep blue actinorhodin, did not seem to be affected by the *pdxR* deletion when pyridoxal is abundant (data not shown). However, a rather narrow range of media was tested; it remains unknown how manipulations of vitamin B6 levels (in the Δ*pdxR* mutant) would impact specialized metabolism. Given the abundance of pyridoxal-dependent enzymes across tree of Life, some other important phenomena might be missed out [21]. We therefore invite further experimental scrutiny of the described here mutant in order to portray a comprehensive picture of the links between PLP biosynthesis and the other aspects of *Streptomyces* physiology.

Conclusions

Our results showed that the *SCO1417* gene of *S. coelicolor* M145 encodes the TF PdxR, which is involved in the positive regulation of the expression of the genes *SCO1523* (*pdxS*) and *SCO1522* (*pdxT*), homologs of enzymes catalyzing the *de novo* formation of PLP. PdxR negatively regulates the transcription of *pdxR*. PLP serves as an effector molecule for PdxR under in vitro and in vivo conditions, while PL was shown to affect *pdxST* expression under in vivo conditions. PdxR binding sites were mapped to short stretches of the *pdxR* and *pdxST* promoters and crucial repeat sequences were pinpointed by mutagenesis and EMSAs.

Abbreviations

PLP	Pyridoxal 5′–phosphate
TF	Transcriptional factor
PL	Pyridoxal
PN	Pyridoxine
PM	Pyridoxamine
HTH	Helix–turn–helix motif
AAT	Aspartate aminotransferase domain

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12866-025-04108-y.

Additional file 1: Fig. S1. Primary amino acid sequences of Sco1417 and GabR and their pairwise alignment. Fig. S2. Purification of the rPdxR protein. Fig. S3. Competition assay confirms the specificity of rPdxR binding to the promoters of *pdxR* (*pdxRp*) and *pdxST* (*pdxSTp*). Fig. S4. Pyridoxal (PL), pyridoxine (PN) and pyridoxamine (PM) were unable to prevent rPxdR binding to *pdxRp* and *pdxSTp*. Fig. S5. Multiple sequence alignment of *pdxRp* and its homologs found in the other streptomycetes. Fig. S6. Multiple sequence alignment of *pdxSTp* and its homologs found in the other streptomycetes. Fig. S7-S10. The full-length EMSA gels that are part of main Fig. 3, 5, 6 and supplementary Fig. S3, S4.

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Authors' contributions
O.T., K.F., A.M., A.B., W.W. and B.O. conceived and designed the experiments. O.T., R.M. and E.G. performed the experiments. B.O. and O.T. wrote the paper. All authors reviewed the manuscript.

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Data availability
All data are present in main text of the article and Additional file 1. Materials are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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