Arch Microbiol (2011) 193:399–405
DOI 10.1007/s00203-011-0685-x

ORIGINAL PAPER

Inducible expression of choline sulfatase and its regulator BetR in *Pseudomonas* sp. ATCC19151

Branko Jovcic · Vittorio Venturi · Ljubisa Topisirovic · Milan Kojic

Received: 31 August 2010 / Revised: 11 February 2011 / Accepted: 17 February 2011 / Published online: 3 March 2011

Abstract *Pseudomonas* sp. strain ATCC19151 is a natural isolate from sewage with the ability to degrade detergents. Genes encoding potential choline sulfatase (*betC*), substrate-binding ABC transporter protein (*betD*), sulfate transporter (*betE*), and divergent putative transcriptional regulator (*betR*) were cloned and characterized from strain ATCC19151. In silico analysis revealed that (1) the BetC protein belongs to alkPPc superfamily and shares CXPXR sequence with the cysteine sulfatases of group I, (2) BetR belongs to the LysR family of transcriptional regulators, (3) BetD is part of the PBPb superfamily of periplasmic and membrane-associated proteins, and (4) BetE is a permease and contains STAS domain. Insertional mutagenesis and genetic complementation show that *betC* gene encodes a functional choline sulfatase. Analysis of the *betC* (P_{betC}) and *betR* (P_{betR}) promoters revealed that they are inducible. BetR activates *betC* and *betR* transcription in the presence of choline sulfate, whilst in the absence of choline sulfate, BetR represses its own transcription. It was further established that BetR directly binds to *betC–betR* intergenic region in vitro, with higher affinity in the presence of choline sulfate as cofactor. Transcription of *betC* and *betR* was not induced in the presence of high concentration of NaCl.

Communicated by Jorge Membrillo-Hernandez.

B. Jovcic · L. Topisirovic · M. Kojic (✉)
Institute of Molecular Genetics and Genetic Engineering, Vojvode Stepe 444a, P.O. Box 23, 11010 Belgrade, Serbia
e-mail: mkojics@eunet.rs

V. Venturi
International Centre for Genetic Engineering and Biotechnology, AREA Science Park, Padriciano 99, 34149 Trieste, Italy

Keywords *Pseudomonas* · Choline sulfatase · Transcriptional regulation of gene expression · Osmoprotection

Introduction

Bacteria cope with the osmotic stress by accumulation of compatible solutes, compounds that balance turgor pressure. Compatible solutes can be accumulated to high intracellular concentrations without interfering with physiological processes. Glycine betaine for example is a widely used compatible solute, which can be synthesized de novo or accumulated from external sources (Miller and Wood 1996). Glycine betaine is commonly synthesized from choline, and several enzymatic systems have been reported to catalyze such a conversion. Two-step transformation of choline to betaine is mediated by means of subsequent betaine aldehyde dehydrogenase and choline dehydrogenase activities in *Escherichia coli* (Lamark et al. 1991), *Halomonas elongata* (Canovas et al. 2000) and *Sinorhizobium meliloti* (Pocard et al. 1997). Some bacteria and fungi can produce choline from choline-*O*-sulfate (C*O*S) by means of a choline sulfatase activity (Lucas et al. 1972; Osteras et al. 1998; Spencer et al. 1968; Takebe 1961). Thus far, the only cloned and characterized genes encoding a choline sulfatase (*betC*) are from *S. meliloti* and *Pseudomonas putida* KT2440 (Galvao et al. 2006; Osteras et al. 1998). The choline sulfatase gene from *S. meliloti* is co-transcribed with *betBA* genes, thereby forming a canonical operon mediating the conversion of C*O*S into betaine and therefore is involved in protection from osmotic stress. Genetic and biochemical approaches revealed that choline sulfatase gene from *P. putida* KT2440 is not clustered with *betBA* genes, but forms a cluster with two other genes

(*betDE* genes) encoding for a transport system, and its protein product function is uncoupled from osmoprotection (Galvao et al. 2006).

Strain *Pseudomonas* sp. ATCC19151 is able to degrade SDS and to utilize it as a carbon source (Hsu 1963, 1965) and could thus be used for the rapid removal of surfactants from the environment (Jovcic et al. 2010). Since bacteria in the environment are exposed to various stresses including osmotic stress, we have employed various genetic and molecular approaches to characterize expression and function of choline sulfatase from ATCC19151 strain. We reported a genetic arrangement of *bet* operon that endows *Pseudomonas* sp. ATCC19151 the ability to metabolize C*O*S.

Materials and methods

Bacterial strains and growth conditions

The bacterial strains used in this study were *Pseudomonas* sp. ATCC19151 from American Type Culture Collection (Hsu 1963, 1965), *E. coli* M15 and *E. coli* DH5α. Strain ATCC19151 was grown aerobically at 30°C in Luria broth medium (LB) or in M9 minimal medium containing choline sulfate (0.2%), betaine (0.2%) or choline (0.2%) as a carbon source. *E. coli* strains were grown aerobically at 37°C in Luria broth medium. Luria broth agar plates were prepared by adding 15 g of agar (Torlak, Belgrade, Serbia) to 1 l of LB medium.

A cosmid library construction and sequencing of choline sulfatase operon

Cosmid library of *Pseudomonas* sp. ATCC19151 genes was made by using "Gigapack III Gold Packaging Extract" (Stratagene, Amsterdam, Netherlands). Total DNA from ATCC19151 strain was partially digested with *Hin*dIII and cloned into pLAFR3 cosmid. Resulting constructs were transferred into *E. coli* and selected on tetracycline plates (20 μg/ml). Packaging efficiency was 5×10^3. A 1-Kbp fragment CS (CSF 5′-ATGAAGACCTCGCCGAACA-3′, CSR 5′-ATACCGAAGCGCCGATGC-3′, primers were generated based on *P. aeruginosa* PAO1 *betC* sequence) containing proximal part of choline sulfatase from ATCC19151 was amplified by PCR and was used for the selection of pLAFRCS cosmid carrying choline sulfatase operon by means of DNA–DNA hybridization. pLAFRCS was digested with *Kpn*I, and 3.5-kb *Kpn*I fragment was cloned into pBluescript digested with *Kpn*I (giving pBSSK3.5Kpn) and sequenced. pLAFRCS was then digested with *Eco*47III, and resulting fragments were hybridized with *Sal*I-*Kpn*I fragment from pBSSK3.5Kpn. A 3.5-Kbp *Eco*47III fragment derived from pLAFRCS hybridized with probe and was cloned into pBluescript digested with *Eco*RV (pBSSKE47III) and sequenced. Based on restriction pattern of 3.5-kb *Eco*47III fragment, pLAFRCS was treated with *Not*I, and obtained fragments were hybridized with *Eco*47III-*Not*I DNA fragment from pBSSKE47III. *Not*I fragment (5 kb) gave positive signal with probe and was cloned into *Not*I-digested pBluescript (giving pBSSKNotI5) and sequenced (constructs used for sequencing were pBSSKPstI300, pBSSKPstI1420, and pBSSKPstI1268). Sequencing was performed in Macrogen Inc. (Seoul, Korea) by using universal and custom-made primers.

Construction of transcriptional fusions and insertional mutants

Transcriptional fusions of *betC* and *betR* genes were constructed in the pMP220 promoter probe vector. DNA of *betC* and *betR* intergenic region was amplified (betIF 5′-GTGCGCTTATCCATACCG-3′, betIR 5′-GGGAAA TCTGCCATAAGAATC-3′) and cloned into pGEMT-Easy cloning vector giving pGEMbetI. The intergenic region was then subcloned from pGEMbetI to pBluescript by using *Eco*RI enzyme (resulting in pBSSKbetI) and subsequently transferred to pMP220 in direction of both *betC* (using *Kpn*I and *Xba*I) or *betR* promoter (using *Bam*HI/*Bgl*II and *Kpn*I), generating pMP220KXbetI and pMP220BKbetI constructs, respectively. To construct plasmids used in insertional mutagenesis of *betR* (pKNMbetR) and *betC* (pKNMbetC) genes, 702-bp *Sac*II-*Sal*I fragment of *betR* gene was cloned into pBluescript SK, as well as 834-bp PCR fragment of *betC* gene (primers: MbetCF 5′-CAAGATGCACTTCTGC GGC-3′, MbetCR 5′-ATGTAT TCGCCCAGCACC-3′) resulting in pBSSKMbetR and pB SSKMbetC constructs, respectively. Fragments for insertional mutagenesis were subcloned from pBSSKMbetR (*Sac*II-*Sal*I) and pBSSKMbetC (*Eco*RI) into pKNOCK suicide vector resulting in constructs designated pKNMbetR and pKNMbetR, respectively. The resulting constructs were transferred into strain *Pseudomonas* sp. ATCC19151 by electroporation as described by Smith and Iglewski (1989). Correct chromosomal insertion of the vectors was confirmed by DNA–DNA hybridization (data not shown). Complementation of *Pseudomonas* sp. ATCC 19151Δ*betC* for choline sulfate utilization was done by using pLAFRCS cosmid that carried complete choline sulfate operon (Table 1).

Choline sulfate synthesis

Choline-*O*-sulfate was prepared from choline chloride (Sigma, Taufkirchen, Germany) and sulfuric acid (Poch, Gliwice, Poland) according to Stevens and Vohra (1955).

Table 1 Plasmids used in this study

Plasmid	Relevant characteristics	Source or reference
pBluescript SK	Amp^r, ColE1 replicon	Stratagene
pBluescript KS	Amp^r, ColE1 replicon	Stratagene
pMP220	Promoter probe vector, IncP1, Tc^r	Spaink et al. (1987)
pKNOCK	Suicide vector used for gene inactivation, Km^r	Alexeyev (1999)
pQE32	Vector used for expression of BetR protein, Amp^r	Qiagen, Germany
pGEM-T Easy	PCR cloning vector, Amp^r	Promega, USA
pGEMCS	1-kb CS fragment cloned into pGEM-T Easy vector	This paper
pLAFRCS	Cosmid carrying choline sulfatase operon	This paper
pBSSK3.5Kpn	3.5-kb *Kpn*I fragment from pLAFRCS cloned into pBluescript SK digested with *Kpn*I	This paper
pBSSKE47III	3.5-kb *Eco*47III fragment from pLAFRCS cloned into pBluescript SK digested with *Eco*RV	This paper
pBSSKNotI5	5-kbp *Not*I fragment from pLAFRCS cloned into pBluescript SK digested with *Not*I	This paper
pBSSKPstI300	*Pst*I fragment 300 bp from pBSSKNotI5 cloned into pBluescript SK digested with *Pst*I	This paper
pBSSKPstI1420	*Pst*I fragment 1,420 bp from pBSSKNotI5 cloned into pBluescript SK digested with *Pst*I	This paper
pBSSKPstI1268	*Pst*I fragment 1,268 bp from pBSSKNotI5 cloned into pBluescript SK digested with *Pst*I	This paper
pBSSKbetR	PCR product of *betR* gene cloned into pBluescript SK digested with *Eco*RV	This paper
pBSSKMbetR	702-bp *Sac*II-*Sal*I fragment of *betR* gene cloned into pBluescript SK digested with *Sac*II-*Sal*I	This paper
pKNMbetR	*Sac*II-*Sal*I fragment from pBSSKMbetR cloned into pKNOCK (*Sac*II-*Sal*I)	This paper
pQEbetR	*Bam*HI-*Hin*dIII fragment from pBSSKbetR gene cloned into pQE32 (*Bam*HI-*Hin*dIII)	This paper
pGEMMbetC	834-bp PCR fragment for *betC* gene mutagenesis gene cloned into pGEM-T Easy	This paper
pBSSKMbetC	*Eco*RI fragment from pGEMMbetC cloned into pBluescript SK digested with *Eco*RI	This paper
pKNMbetC	*Eco*RI fragment from pBSSKMbetC cloned into pKNOCK (*Eco*RI)	This paper
pGEMbetI	PCR product of *betR* and *betC* gene intergenic region cloned into pGEM-T Easy	This paper
pBSSKbetI	*Eco*RI fragment from pGEMbetI cloned into pBluescript SK	This paper
pMP220KXbetI	*Kpn*I-*Xba*I fragment pBSSKbetI cloned into pMP220 (*Kpn*I-*Xba*I)	This paper
pMP220BKbetI	*Bam*HI-KpnI fragment pBSSKbetI cloned into pMP220 (*Bgl*II-*Kpn*I)	This paper

β-galactosidase assay and osmoprotection

Overnight cultures (100 µl) of *Pseudomonas* sp. ATCC19151 wild type, and *betR* and *betC* mutants harboring transcriptional fusions were resuspended in Z-buffer (60 mM $Na_2HPO_4 \cdot 7H_2O$, 40 mM $NaH_2PO_4 \cdot H_2O$, 10 mM KCl, 1 mM $MgSO_4 \cdot 7H_2O$) and then permeabilized by treatment with SDS and chloroform. Assays were conducted in Z-buffer containing 50 mM β-mercaptoethanol (Miller 1972). Activities (changes in optical density at 420 nm per min) were normalized to the actual cell density (OD_{600nm}) and were consistently compared to those of appropriate controls, which were assayed at the same time. For osmoprotection assays, β-galactosidase activity of transcriptional fusions was measured in the presence of increasing concentrations of NaCl. The experiments were conducted in triplicate, simultaneously. In order to eliminate readthrough activity from other promoters on pMP220, transformants with this plasmid were used as a control.

Electrophoretic mobility shift

DNA mobility shift assays with purified His6-BetR were performed with modified previously described procedure (Kojic et al. 2005). The *betR* gene was amplified (primers: betRF 5′-CGGATCCCAGATTTCCCTGAC-3′, betRR 5′-CGTTTAAGCTTAGATCGCGAT-3′) and cloned into pQE32 vector. His6-BetR protein was expressed and purified from *E. coli* M15 after IPTG induction. Fragment carrying intergenic region of *betC* and *betR* genes was removed from plasmid construct pBSSKbetI using *Bam*HI-*Kpn*I restriction enzymes and purified with Gel Extraction Kit (Qiagen, Hilden, Germany). Purified DNA was labeled at its *Bam*HI site with the Klenow fragment of DNA polymerase and CTP-11-biotin (Roche Diagnostics GmbH, Penzberg, Germany). Biotin-labeled DNA fragments and various quantities of purified His6-BetR (from 0 to 150 ng) were incubated for 30 min at room temperature in 10 µl reaction mixture containing 1× binding buffer (20 mM

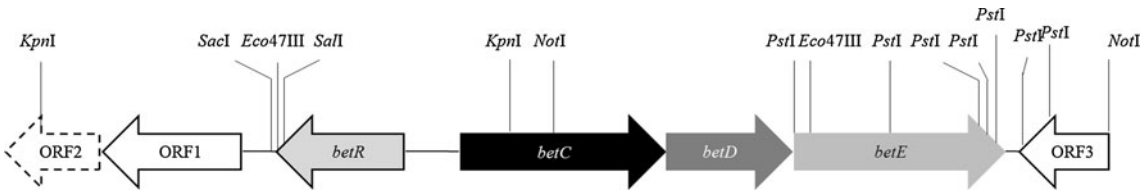

Fig. 1 Scheme of operon for C*O*S uptake and surrounding ORFs in *Pseudomonas* sp. ATCC1915. Incomplete ORF is presented with *dash line*. ORF 1—potential phospholipase C gene, ORF 2—potential shikimate dehydrogenase gene. ORF 3—hypothetical gene. Only relevant restriction sites used in this study are presented

HEPES–KOH pH 7.9, 20% v/v glycerol, 0.2 mM EDTA, 0.1 M KCl, 0.5 mM PMSF, 1 mM DTT), 10 μg of bovine serum albumin, 400 ng of salmon sperm DNA, and 1.5 mM $MgCl_2$. The choline sulfate was added to reactions as a coinducer (1, 10, 50, 100 mM). Samples were then loaded onto a non-denaturing 4.5% polyacrylamide 0.5× TBE (44.5 mM Tris, 44.5 mM boric acid, 0.5 mM EDTA) 3% v/v glycerol gel, which was prerun for 1 h at 110 V at room temperature. Afterward, the samples were run at 110 V. Sample transfer from the polyacrylamide gel to nylon membrane (SensiBlotPlus Nylon Membrane, MBI Fermentas, Lithuania) was performed by using semi-dry fastblot (Fastblot B43, Biometra, Goettingen, Germany) at 300 mA for 30 min in 1× TBE buffer. Biotin-labeled DNA fragments were detected with Biotin Luminescent Detection Kit (Roche Diagnostics GmbH, Penzberg, Germany).

Nucleotide accession number

The EMBL accession number for the choline sulfatase operon nucleotide sequence reported in this paper is FN646113.

Results

It was of interest to investigate the link between the osmotic stress response and the gene cluster putatively encoding glycine betaine—related functions in *Pseudomonas* sp. ATCC19151. To initiate these studies, the *bet* genes were identified and cloned. A pLAFR3 cosmid gene bank of strain ATCC19151 was constructed and screened by DNA–DNA hybridization with the CS probe, which was obtained by PCR and consisted of part of the choline sulfatase gene from ATCC19151. This led to the isolation of pLAFRCS, and the further DNA sequencing of *Kpn*I-*Not*I fragment (8,161 bp) from this cosmid (Fig. 1) was determined by subcloning and sequencing of restriction fragments as described in the Materials and methods section. Nucleotide sequence analysis of this fragment revealed the presence of ORFs encoding for choline sulfatase (*betC*), *betD* (encoding for periplasmic binding protein) and *betE* (encoding for sulfate permease containing STAS ATP-binding domain) as depicted in Fig. 1. The divergently orientated gene upstream of *betC* encodes a putative LysR family transcriptional regulator, named *betR*. *In silico* analysis revealed that BetC protein belongs to alkPPc superfamily of alkaline phosphatase homologs and shares CXPXR sequence (CAPSR) with the cysteine sulfatases belonging to group I of sulfatases, which are predominantly located in the cytoplasm. BetD belongs to protein PBPb superfamily of periplasmic and membrane-associated proteins, and BetE sulfate permease belongs to Xan_ur superfamily of permeases and contains STAS domain for binding and hydrolysis of the ATP. "DAS—Transmembrane Prediction" analysis (Cserzo et al. 1997) of the BetE amino acid sequence revealed 12 potential transmembrane segments. Genetic association of the predicted choline sulfatase (BetC) with presumptive periplasmic binding protein (BetD) and with a sulfate permease with ATP-binding function (BetE) suggested that the cluster could encode a system for quaternary nitrogenated sulfate compound choline-*O*-sulfate (C*O*S). Analyzed genes of *Pseudomonas* sp. ATCC19151 *bet* operon share the highest homology with the potential C*O*S utilization cluster from *Pseudomonas mendocina* ymp (*betC* 85%, *betR* 77%, *betD* 83%, and *betE* 77% of identical nucleotides).

Insertional mutagenesis of the *betC* and *betR* genes was performed by using pKNMbetC and pKNMbetR constructs, respectively. Resulting *Pseudomonas* sp. ATCC19151ΔbetC did not exhibit growth in the presence of choline sulfate as a sole carbon source, indicating that *betC* gene encodes functional choline sulfatase. When *Pseudomonas* sp. AT CC19151ΔbetC was complemented for choline sulfatase activity with the pLAFRCS cosmid carrying choline sulfatase operon, its growth rate in the presence of choline sulfate was restored to that of the wild type. The *Pseudomonas* sp. ATCC19151ΔbetR mutant could grow on choline sulfate albeit with a very slow growth rate. In order to determine whether the *betC* gene expression is regulated at the transcriptional level, transcriptional fusion pMP220KXbetI (P_{betC}) carrying the putative promoter of *betC* gene was introduced into ATCC19151 wild-type strain that was grown in the presence of 1 mM C*O*S, choline, betaine, citrate or both C*O*S and glucose. The data of *β*-galactosidase activity clearly show that P_{betC} promoter

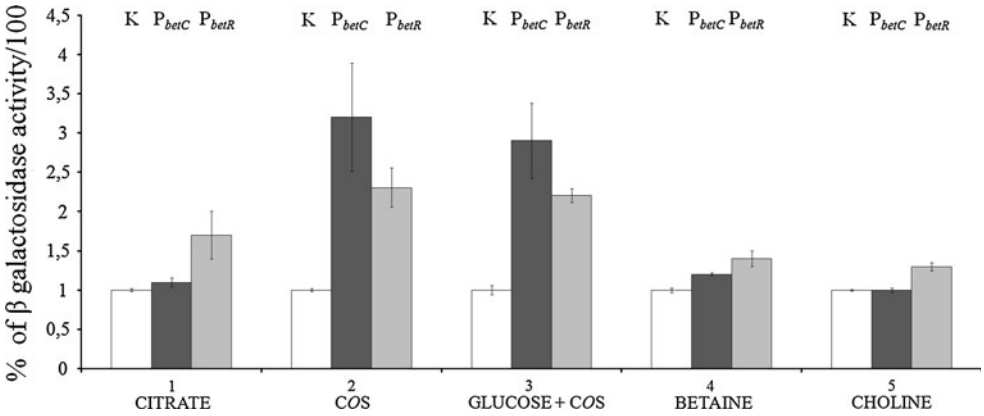

Fig. 2 Production of *β*-galactosidase activity using the *betC* and *betR* gene promoters cloned into pMP220 vector, analyzed in *Pseudomonas* sp. ATCC19151 in the presence of: 1. 0.2% citrate, 2. 0.2% choline sulfate (C*O*S), 3. 0.2% glucose + 0.2% C*O*S, 4. 0.2% betaine, 5. 0.2% choline. K—activity of pMP220 vector, P_{betC}—the *betC* gene promoter activity, P_{betR}—the *betR* gene promoter activity. Standard deviations are presented with *error bars*

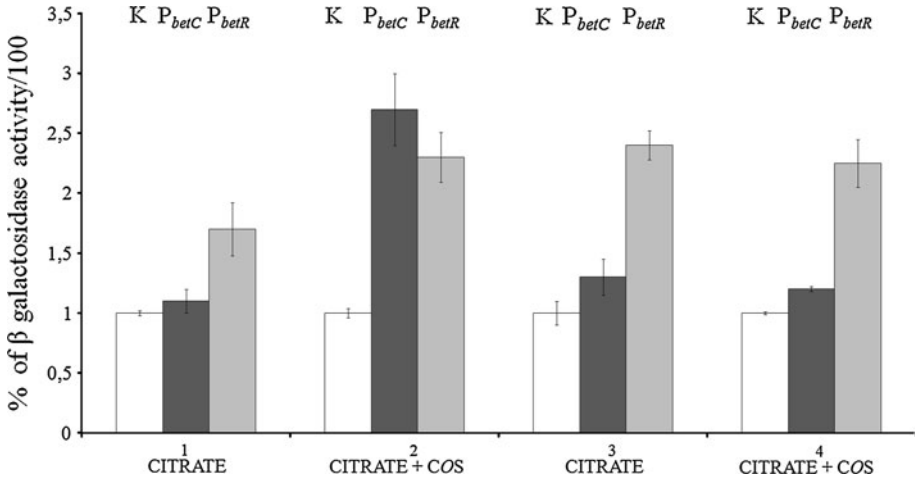

Fig. 3 Production of *β*-galactosidase activity using the *betC* and *betR* gene promoters cloned into pMP220 vector, analyzed in *Pseudomonas* sp. ATCC19151 (1 and 2) and *Pseudomonas* sp. ATCC19151ΔbetR (3 and 4) in the presence of citrate and combination of citrate and C*O*S. K—activity of pMP220 vector, P_{betC}—the *betC* gene promoter activity, P_{betR}—the *betR* gene promoter activity. Standard deviations are presented with *error bars*

was induced in the presence of C*O*S (Fig. 2). Increased *β*-galactosidase activity of the transcriptional fusion carrying P_{betC} in the presence of both C*O*S and glucose indicated that choline sulfatase expression did not display catabolic repression. Activity of the transcriptional fusion pMP220BKbetI (P_{betR}) carrying the putative *betR* gene promoter was also induced in the presence of C*O*S, and similarly, it was not repressed in the presence of glucose.

In contrast to what was observed in the wild-type genetic background, the activity of the promoter P_{betC} was independent of the presence of COS in the media, when measured in the ATCC19151_betR genetic background, while P_{betR} activity increased in ATCC19151ΔbetR even without induction (Fig. 3). This result suggests that BetR positively regulates expression of *betC* in the presence of C*O*S and is a negative autoregulator in the absence of C*O*S. In order to establish whether the BetR directly regulates *betC* and *betR* promoters, mobility shift assay with the *betC* and *betR* intergenic region was performed. DNA carrying intergenic region was retarded in the presence of BetR protein thus shown to bind BetR, and the shift was not observed in the presence of excess unlabeled fragment (Fig. 4). In addition, the amount of retarded complex increased when C*O*S was added as a coinducer. This result shows that BetR is able to

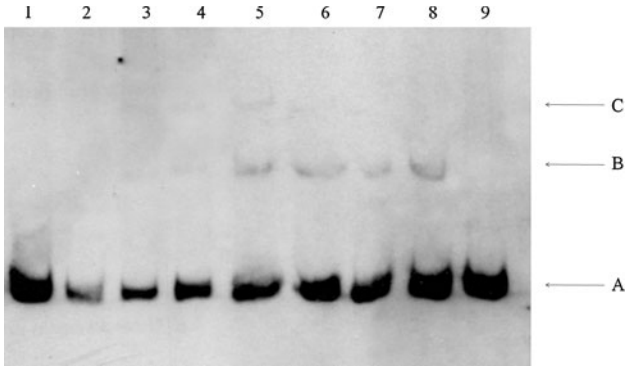

Fig. 4 Movement retardation of a biotin-labeled DNA fragment containing intergenic region of divergent *betC* and *betR* genes by purified His_6-BetR protein. The amounts of His_6-BetR protein used were 0, 50, 100, 150 ng (*lanes 1–4*, respectively). *Lanes 5* to *8*—binding of His_6-BetR (100 ng) to the *betC* and *betR* intergenic region in the presence of a choline sulfate. A 100-fold excess amount of the same unlabeled DNA fragment (*lane 9*). *A*—biotin-labeled DNA, *B*, *C*—DNA–His_6-BetR protein complexes

bind to the intergenic region between *betC* and *betR* and is supporting evidence that BetR may have a regulatory role in the expression of this locus.

To ascertain whether induction of *betC* and *betR* transcription is part of the osmotic stress response in

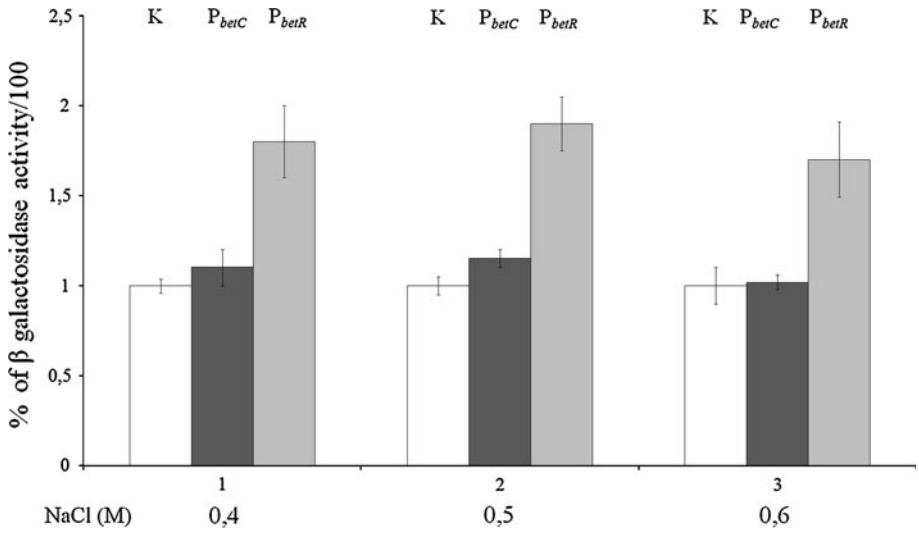

Fig. 5 Production of *β*-galactosidase activity using *betC* and *betR* gene promoters cloned into pMP220 vector, analyzed in *Pseudomonas* sp. ATCC19151 with increasing concentrations of NaCl. K—activity of pMP220 vector, P_{betC}—the *betC* gene promoter activity, P_{betR}—the *betR* gene promoter activity. Standard deviations are presented with *error bars*

Pseudomonas sp. ATCC19151, activity of P_{betC} and P_{betR} promoters in ATCC19151 wild-type strain in the presence of NaCl (0.4, 0.5, and 0.6 M) was analyzed. The results presented in Fig. 5 indicated that activities were similar regardless the presence of different concentrations of NaCl, indicating that choline sulfatase was most probably not involved in osmotic stress response.

Discussion

Choline-*O*-sulfate is widespread in the soil; thus, several transport systems for C*O*S are described in bacteria, including rhizosphere pseudomonads (Fitzgerald and Luschinski 1977; Hanson et al. 1994; Ikawa et al. 1972). It is known that organization of operons for the C*O*S utilization varies in the bacteria and has different physiological role in the cells (Galvao et al. 2006; Osteras et al. 1998).

We report here the identification and characterization of the *bet* genes from *Pseudomonas* sp. ATCC19151, and results showed that the operon for utilization of C*O*S has genetic organization similar to that in *P. putida* (Galvao et al. 2006). In addition, C*O*S utilization operon from *P. putida* is not involved in osmoprotection, as it was observed for the *bet* operon from strain ATCC19151.

Having in mind that putative periplasmic binding protein (PBP) with affinity for quaternary ammonium compounds (BetD) is associated with sulfate permease containing STAS ATP-binding domain (BetE), their involvement in transport of C*O*S is postulated. Coupling of the ATP hydrolysis and transport functions is not unusual and was described previously (Aravind and Koonin 2000). Since BetC from ATCC19151 is most similar to the alkPPc superfamily of alkaline phosphatases, it is assumed that this sulfatase could have phosphatase activity like choline sulfatase from *S. meliloti* where the phosphatase activity of the enzyme is 20% of its sulfatase activity (Osteras et al. 1998).

The function of choline sulfatase operons from bacteria has been intensively studied, but expression as well as the regulation of expression remains still unclear. In order to understand expression of choline sulfatase gene and the role of the *betR* regulator, we analyzed activities of the *betR* and *betC* gene promoter transcriptional fusions. Activities of *betC* (P_{betC}) and *betR* (P_{betR}) transcriptional fusions suggested that expression of choline sulfatase and *betR* is inducible and not catabolically repressed. For the first time, we confirmed that BetR directly binds to the *betC* and *betR* intergenic promoter region and positively regulates expression of *betC* gene in the presence of C*O*S as coinducer. This could be corroborated with gel retardation assay results where intensity of retarded DNA-BetR complex significantly increased in the presence of C*O*S as coinducer. Since the expression of *betR* gene was weakly repressed in the presence of BetR protein without C*O*S as coinducer, we propose that BetR negatively regulates its own expression in a C*O*S independent manner. LysR family transcriptional regulators usually undergo conformational change in the presence of inducer and consequentially change affinity to DNA sequences (Maddocks and Oyston 2008).

The absence of *betC* and *betR* transcriptional response during osmotic stress suggests that COS is most probably not linked to osmoprotection in *Pseudomonas* sp. ATCC19151, as reported for *P. putida* (Galvao et al. 2006). We therefore hypothesize that the most likely biological role of choline sulfate in strain ATCC19151 is to serve as a carbon or nitrogen source.

By analysis of the primary structure of the proteins identified here, it could be inferred that when C*O*S enters to periplasm, it binds to BetD protein, in *Pseudomonas* sp. ATCC19151. The resulting BetD-C*O*S complex then interacts

with BetE sulfate transporter of inner membrane that transports C*O*S to cytosol by using energy of ATP hydrolysis. C*O*S imported into cytosol interacts with BetR transcriptional regulator, which then changes its conformation and subsequently causes derepression of *betR* gene and activation of *betC*, enabling utilization of C*O*S as a carbon, nitrogen, or sulfur source.

Acknowledgments This work was funded by Ministry of Science and Technological Development, Republic of Serbia, Project No. 143036.

References

Alexeyev MF (1999) The pKNOCK series of broad-host-range mobilizable suicide vectors for gene knockout and targeted DNA insertion into the chromosome of Gram's negative bacteria. BioTechniques 26:824–828

Aravind L, Koonin EV (2000) The STAS domain—a link between anion transporters and antisigma-factor antagonists. Curr Biol 10:53–55

Canovas D, Vargas C, Kneip S, Moron MJ, Ventosa A, Bremer E, Nieto JJ (2000) Genes for the synthesis of the osmoprotectant glycine betaine from choline in the moderately halophilic bacterium *Halomonas elongata* DSM. Microbiology 146:455–463

Cserzo M, Wallin E, Simon I, von Heijne G, Elofsson A (1997) Prediction of transmembrane alpha-helices in procariotic membrane proteins: the dense alignment surface method. Prot Eng 10:673–676

Fitzgerald JW, Luschinski PC (1977) Further studies on the formation of choline sulfate by bacteria. Can J Microbiol 23:483–490

Galvao TC, de Lorenzo V, Canovas D (2006) Uncoupling of choline-*O*-sulphate utilization from osmoprotection in *Pseudomonas putida*. Mol Microbiol 62:1643–1654

Hanson AD, Rathinasabapathi B, Rivoal J, Burnet M, Dillon MO (1994) Osmoprotective compounds in the Plumbaginaceae: a natural experiment in metabolic engineering of stress tolerance. Proc Natl Acad Sci USA 91:306–310

Hsu YC (1963) Detergent (sodium lauryl sulfate) splitting enzyme from bacteria. Nature 200:1091–1092

Hsu YC (1965) Detergent-splitting enzyme from *Pseudomonas*. Nature 207:385–388

Ikawa M, Chakravarti A, Taylor RF (1972) Occurrence of choline in *Lactobacillus plantarum*. Can J Microbiol 18:1241–1245

Jovcic B, Venturi V, Davison J, Topisirovic L, Kojic M (2010) Regulation of the *sdsA* alkyl sulfatase of *Pseudomonas* sp. ATCC19151 and its involvement in degradation of anionic surfactants. J Appl Microbiol 109:1076–1083

Kojic M, Jovcic B, Vindigni A, Odreman F, Venturi V (2005) Novel target genes of PsrA transcriptional regulator of *Pseudomonas aeruginosa*. FEMS Microbiol Lett 246:175–181

Lamark T, Kaasen I, Eshoo MW, Falkenberg P, McDougall J, Storm AR (1991) DNA sequence and analysis of the *bet* genes encoding the osmoregulatory choline-glycine pathway of *Escherichia coli*. Mol Microbiol 5:1049–1064

Lucas JJ, Burchiel SW, Segel IH (1972) Choline sulfatase of *Pseudomonas aeruginosa*. Arch Biochem Biophys 153:664–672

Maddocks SE, Oyston PCF (2008) Structure and function of the LysR-type transcriptional regulator (LTTR) family proteins. Microbiology 154:3609–3623

Miller JH (1972) Experiments in molecular genetics. Cold Spring Harbor Laboratory, Cold Spring Harbor

Miller KJ, Wood JM (1996) Osmoadaptation by rhizosphere bacteria. Annu Rev Microbiol 50:101–136

Osteras M, Boncompagni E, Vincent N, Poggi MC, Le Rudulier D (1998) Presence of a gene encoding choline sulfatase in *Sinorhizobium meliloti* bet operon: choline-*O*-sulfatase is metabolized into glycine betaine. Proc Natl Acad Sci USA 95:11394–11399

Pocard JA, Vincent N, Boncompagni E, Smith LT, Poggi MC, Le Rudulier D (1997) Molecular characterization of the *bet* genes encoding glycine betaine synthesis in *Sinorhizobium meliloti* 102F34. Microbiology 143:1369–1379

Smith AW, Iglewski BH (1989) Transformation of *Pseudomonas aeruginosa* by electroporation. Nucl Acid Res 17:10509

Spaink HP, Okker RJH, Wijffelmann CA, Pees E, Lugtenberg BJJ (1987) Promoter in the nodulation region of the *Rhizobium leguminosarum* Sym plasmid pRL1JI. Plant Mol Biol 9:27–39

Spencer B, Hussey EC, Orsi BA, Scott JM (1968) Mechanism of choline-*O*-sulphate utilization in fungi. Biochem J 106:461–469

Stevens CM, Vohra P (1955) Occurrence of choline sulfate in *Penicillium chrysogenum*. J Am Chem Soc 77:4935–4936

Takebe I (1961) Isolation and characterization of a new enzyme choline sulfatase. J Biochem 50:245–255