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Moran GJ, Amii RN, Abrahamian FM, Talan DA (2005). Methicillinresistant Staphylococcus aureus in community-acquired skin infections. Emerg. Infect. Dis. 11: 928-930.

Pitout JDD, Church DL, Gregson DB, Chow BL, McCracken M, Mulvey M, Laupland KB (2007). Molecular epidemiology of CTXM-producing Escherichia coli in the Calgary Health Region: emergence of CTX-M-15-producing isolates. Antimicrob. Agents Chemother. 51: 1281-1286.

Pelczar JR, Harley JP, Klein DA (1993). Microbiology: Concepts and Applications. McGraw-Hill Inc., New York, pp. 591-603.

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*Full Length Research Paper*

# Isolation and sequence analysis of a putative MerR-type-transcriptional regulator and a multidrug efflux protein of *Bacillus circulans* ATCC 21588: As potential targets of therapeutics

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**Mercury-type transcriptional regulators (MerR-transcriptional regulator) and major facilitator superfamily (MFS) transporters usually form an important sensor-response transport system in many microorganisms. This system has been shown to be involved in the regulation and transport (efflux) of a wide and diverse array of secondary metabolites including antimicrobial agents, dyes, chemicals, metals and evem harmful oxygen radicals. Inhibition or inactivation of this transport system is considered a promising approach for controlling microbial resistance, and thus may become a promising target of therapeutics particularly for the clinically relevant pathogens. However, the genetic and proteomics of this system have not been fully studied. In this work, a DNA segment (1.926 kb) from *Bacillus circulans* ATCC 21588 harboring the two genes, *bciR and bciT* arranged in an operon was amplified using PCR, analyzed and submitted into the GenBank database (accession code, KR049081). A two open reading frames (ORFs), namely BciR and BciT were found to encode a putative MerR-transcriptional regulator (BciR; 153 aa) and a putative MFS transporter (BciT; 392 aa), respectively. Analysis of the conserved domains and modeled tertiary structures revealed that, BciR possesses an N-terminal H-T-H motive (HTH type) region with possible transcriptional related activity and a conserved metal binding site at the C-terminal end. BciT was likely an MFS protein with nine transmembrane helices. This is the first report about detection of a *bciR/bciT* operon that putatively encode a sensor-response transport system in *Bacillus circulans* ATCC 12588.**

**Key words:** MerR-type transcription regulator, multidrug efflux protein, major facilitator superfamily MFS, *Bacillus circulans* ATCC 21588.

## INTRODUCTION

Mercury regulatory (MerR) family transcription regulators have been shown to mediate responses to stress such as exposure to drugs, heavy metals, or harmful oxygen radicals in various microorganisms (Helmann et al., 1990 Baranova et al., 1999). Their regulations were elicited by reconfiguring the promoter elements of many transporter

proteins leading to suppression of the transcription process of the respective proteins (Helmann et al., 1989; Ahmed et al., 1995). A typical MerR regulator is comprised of two distinct domains that harbor the regulatory (effector-binding) site and the active (DNA-binding) site. Their N-terminal domains are homologous and contain a DNA-binding helix-turn-helix (HTH) motif, while the C-terminal domains are often dissimilar and bind specific co-activator molecules such as metal ions, drugs, and other organic substrates. In previous studies, it was confirmed that a MerR transcription regulator (BmrR) protein activates expression of a downstream multidrug efflux transporter *(bmr)* upon binding the transporter substrates (Ahmed et al., 1994; Zheleznova et al., 1999).

Bacterial transporters can be grouped based on energy sources. These groups are primary active transporters and the secondary transporters (Zhang et al., 2015). The primary transporters use energy generated by ATP hydrolysis while secondary transporters mainly rely on the electrochemical gradient across the cell membrane (Maloney, 1992; Floyd et al., 2010). The major facilitator super family (MFS) is a family of secondary transporters of usually transmembrane alpha-helices. The MFS transport diverse substrates, such as the ions, drugs, sugars, nucleosides, amino acids, small peptides, and other small molecules (Yan, 2013). Multi drug efflux functions of some MFS tranporters of many microorganisms have been studied (Wisedchaisri et al., 2014; Hinchliffe et al., 2014; Xu et al., 2014; Shilton, 2015). The MFS transporter was found in several pathogens such as *Escherichia coli*, *Klebsiella pneumoniae, Staphylococcus aureus*, *Bacillus cereus* and *Mycobacterium sp.* as integral membrane proteins involved in nonspecific antibiotic resistance to various antibacterial and anti-fungal agents (Changela et al., 2003; Floyd et al., 2010; Simm et al., 2012; Srinivasan et al., 2014; Zhang et al., 2015; Ogasawara et al., 2015). MFS transporters therefore, appear to contribute to intrinsic resistance to antibiotics in bacteria. Since, antibiotic resistance by the clinically relevant pathogens to the conventional antimicrobial agents are mediated by some MFS transporters, MFS transports become potential targets for the development of new antibacterial drugs (Saidijam et al., 2006). In a previous study, a MerR-type transcriptional regulator (Mta) was found to activate both bacillibactin secretion and an MFS transporter (YmfE) gene expression confirming involvement of both proteins in the bacillibactin biosynthetic pathway in *Bacillus subtilus* (Miethke et al., 2008). Therefore, molecular and 2008). Therefore, molecular and proteomic studies of efflux pumps, their substrates as well as their regulatory mechanisms may lead to the discovery of new therapeutics and pump inhibitors. A side from the use of inhibitors, photodynamic inactivation using the synergistic action of efflux pump inhibitors is an alternative (Wasaznik et al., 2009). *Bacillus circulans*, the Gram positive spore forming rod was shown to be a potential pathogen to both plants and humans. In plants, it causes rapid and destructive soft rot of the tissues of Date Palm (Leary et al 1986) and a case report identified this organism in the setting of fatal sepsis in an immunocompromised patient (Alebouyeh et al., 2011).

Resistance of *Bacillus* spores to ultraviolet light, disinfectants and some other sterilizing agents as well as resistant of clinical isolates to many prescribed antibiotics suggest new therapeutic agents are needed (Alebouyeh et al., 2011). This study focuses on the identification, phylogenetic and sequence analysis of a putative MerR-type-transcriptional regulator and a multidrug efflux protein of *B. circulans* ATCC 21588 that is expected to be involved in intrinsic resistance to commonly used antibiotics, chemicals, disinfectants and metals. This study is a first step in the quest for inhibitors of this regulatory/transport system.

## MATERIALS AND METHODS

### Bacterial strains, culture media

*B. circulans* ATCC 21588 was cultured on tryptic soy broth (TSB) or on solid or liquid LB culture medium at 37°C (Kieser et al., 2000).

### Extraction and manipulation of genomic DNA

Chromosomal DNA of *B. circulans* was prepared according to the method of Pospiech and Neumann (1995) with the following modifications. Strain inoculation was done in 25 ml TSB in 250 ml-volume flask and grown at 37°C on a shaker (250 rpm) for 24 h. The cells were then harvested by centrifugation at 13,000 rpm for 10 min and washed twice with 10.3% sucrose, resuspended in 20 ml of sodium chloride-EDTA-Tris (SET) buffer with 1.5 mg/ml lysozyme and incubated for 1 h at 37°C. About, 1/10 volume of 10% SDS and proteinase K (final concentration of 0.5 mg/ml) were added and incubated at 55°C for 1 - 2 h with frequent gentle inversion. About 1/3 volume of 5 M NaCl was added and an equal volume of phenol/chloroform was added and incubated at room temperature for 20 min with gentle inversion. The mixture was then centrifuged at 4,000 rpm for 10 min, and the aqueous phase was further extracted with an equal volume of chloroform/isoamyl alcohol (24:1), incubated at room temperature for 20 min with gentle inversion, and centrifuged at 10,000 rpm for 10 min. The DNA was precipitated by the addition of an equal volume of

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**Table 1**. Oligonucleotides used in this study.

| Primer | Target | Primer sequences [1] | Annealing temperature(°C), Annealing time (t) |
|---|---|---|---|
| **Designation** | **Gene** | | |
| PBciR-F | *bci*R | 5' ATGACACGGTTAAAAATTGATGATGTC 3' | 53°C, 45 s |
| PBciR-R | (0.5 kb) | 5' CTATTGTCCGGAAGACGGG 3' | |
| PBciT-F | *bciT* | 5' CAGCGGTGACCGGCCCGCTC 3' | 55°C, 1 min |
| PBciT-R | (1.1 kb) | 5' CGAGGTCATCGCGTCCCCCTGC 3' | |
| PBciR3' | bciR(5')/T(3') | 5' TCGTCGTCCGGCAAGACC 3' | 55°C, 1 min |
| PBciT5' | (0.8 kb) | 5' GCTGGATGGCCGACATATGAAGC 3' | |

isopropanol, centrifuged at 10,000 rpm for 5 min. DNA was then washed using 70% ice cold ethanol, dried and finally dissolved in 1000 µl TE buffer with RNase 100 µg/ml. Agarose gel electrophoresis was carried out essentially as described by Sambrook and Russell (2001) using 0.8% agarose gels containing 0.1 µg/ml ethidium bromide. DNA fragment size was determined by comparison to a conventional 1 Kb DNA ladder (Sigma-Aldrich co, Egypt).

**Polymerase chain reaction (PCR) and Recovery of DNA fragments from agarose gels**

Amplification of different probes by PCR was performed using 200 - 400 ng of the genomic DNA as a template and the selected primers for each probe (Table 1). PCR was performed in a Nyx-Technik Inc. Personal Cycler (ATC401, USA). Each assay (50 µl) consisted of 200 ng chromosomal DNA, 100 pmole of each appropriate primer, 0.2 mM dNTPs (Invitrogen, Karlsruhe, Germany), 3 mM $MgCl_2$, 10% DMSO to improve the denaturation of the template DNA and 2 U *Taq* DNA polymerase (Sigma, USA). PCR general conditions were: 98°C for 5 min; then 30 cycles [95°C for 1 min; annealing temperatures and time according to Table 1, 72°C for 1 min (normally 1 min for 1 kb)]; and 72°C for 5 min (ramping rate 1°C/s).

**DNA sequencing, assemble and detection of possible open reading frames (ORFs)**

The PCR products were purified using GeneJET™ purification kit at Sigma Scientific Services Company, Egypt. Afterwards, samples were sent for sequencing at GATC co, Germany using ABI 3730xl DNA sequencer. The PCR products were sequenced from both forward and reverse directions. The obtained sequence files were assembled into a final contig using Staden Package program version 3 (http://staden.sourceforge.net/) (Staden, 1996). The resulting contig was analyzed for ORFs using FramePlot2.3.2 (http://www0.nih.go.jp/~jun/cgi-bin/frameplot.pl) (Ishikawa and Hotta, 1999), annotated and submitted into the GenBank database. Restriction analysis of the final contig was carried out using RestrictionMapper version 3 (http://www.restrictionmapper.org).

**Nucleotide accession codes**

The nucleotide sequence reported in this study was submitted in the GenBank database under the accession code: KR049081

**Computer-assisted analysis of DNA sequences**

Multiple alignment and phylogeny analysis of the obtained ORFs were carried out using ClustalW2 (http://www.ebi.ac.uk/Tools/msa/clustalw2 (Thompson et al., 1994). Structure of proteins and conserved domain analysis were conducted using Basic Local Alignment Search Tool (NCBI): http://www.ncbi.nlm.nih.gov/Structure/index.shtml (Marchler-Bauer et al., 2015).

**Analysis and prediction of the tertiary structure of encoded proteins**

The putative tertiary structure of the obtained ORFs were predicted and analyzed using the Swiss-Model software (http://swissmodel.expasy.org; Arnold et al., 2006, Kiefer et al., 2009; Guex et al., 2009; Biasini et al, 2014). The QMEAN4 score of the predicted protein model was also calculated (Benkert et al., 2011). This was done to visualize the predicted conformation of the protein and the possible metal-binding residues which might have an effect on the enzyme activity.

## RESULTS

Sequence analysis of the DNA segment (final contig) was submitted into the GenBank database (Accession code, KR049081). As depicted in Figure 1, two complete open reading frames (ORFs) were detected and annotated BciR (153 aa) and BciT (392 aa) on the submitted DNA segment (1.926 kb) of *B. circulans* ATCC 12588. The *bciR* (462 bp) and *bciT* (1179 bp) genes were found to encode a predicted MerR family transcriptional regulator of 153 amino acids (aa) and a major facilitator transporter (1179 bp, 392 aa), respectively.

Both BciR and BciT were encoded by the parent DNA strand and BciT was located downstream of BciR. A possible strong ribosomal binding site (RBS) for each ORF was detected and annotated as 5'-AGGAG-3' located at position -7 from the predicted start codon (ATG) of BciR and 5'-GAAGGGG-3' located at position -12 from the predicted start codon (ATG) of BciT. Restriction analysis profile of the respective DNA segment using some selected restriction endonucleases is also illustrated (Figure 1).

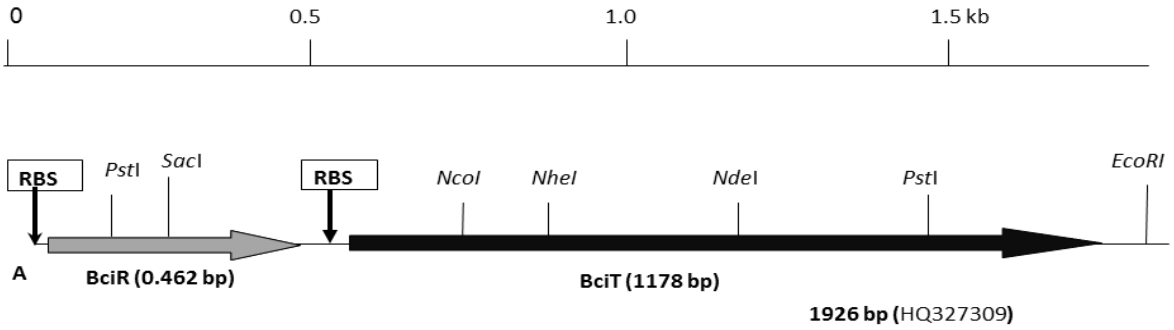


**Figure 1.** Restriction analysis profile of the DNA segment of *Bacillus circulans* ATCC 21588 submitted to GenBank database (accession code: KR049081) using some selected restriction endonucleases. BciR = predicted MerR family transcriptional regulator (462 bp, 153 aa); BciT = predicted major facilitator transporter (1179 bp, 392 aa). Arrows indicate direction of the oper reading frames (ORFs). RBS= possible ribosomal binding site.

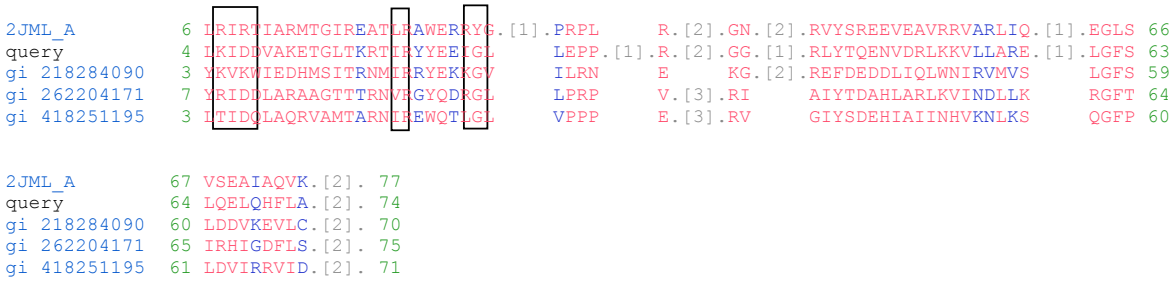


**Figure 2**. Multiple sequence alignment of the conserved domains of the transcriptional regulator protein of *Bacillus circulans* ATCC 21588 (BciR; query) and its homologs using http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi (Marchler-Bauer et al., 2015). The numbers indicate the position within the corresponding proteins: 2JML_A = a transcription regulator from the MerR superfamily of *Myxococcus Xanthus*, accession code (AC)= 2JML_A; gi 218284090 = hypothetical protein of *Eubacterium biforme* DSM 3989, AC= ZP_03489918; gi 262204171 = MerR family transcriptional regulator of *Gordonia bronchialis* DSM 43247, AC= YP_003275379; gi 418251195 = MerR family transcriptional regulator of *Mycobacterium abscessus* 47J26, AC= ZP_12877392. Amino acids within the indicated rectangules are required for DNA binding (DNA binding-residues).

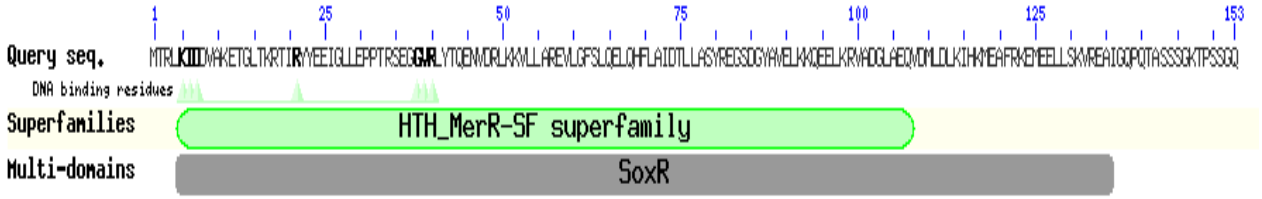


**Figure 3.** Putative conserved domain of BciR transcriptional regulator protein (http://blast.ncbi.nlm.nih.gov/Blast.cgi) HTH-MerR-SF = helix-turn-helix DNA binding domain of transcriptional metalo-regulatory protein superfamily; accession cl02600.SoxR= Predicted transcriptional regulators (COG0789).

## Multiple alignments and domain analysis of BciR (a predicted member of the MerR family transcriptional regulator) and homologous proteins

As shown in Figure 2, BciR showed more than 85, 83, 82, 80, 78.3% similarities in the amino acid sequences of homologous proteins encoding diverse MerR transcription regulators of *Myxococcus Xanthus*, accession code (AC= 2JML_A), *Eubacterium biforme* DSM 3989 (AC = ZP_03489918), Gordonia *bronchialis*

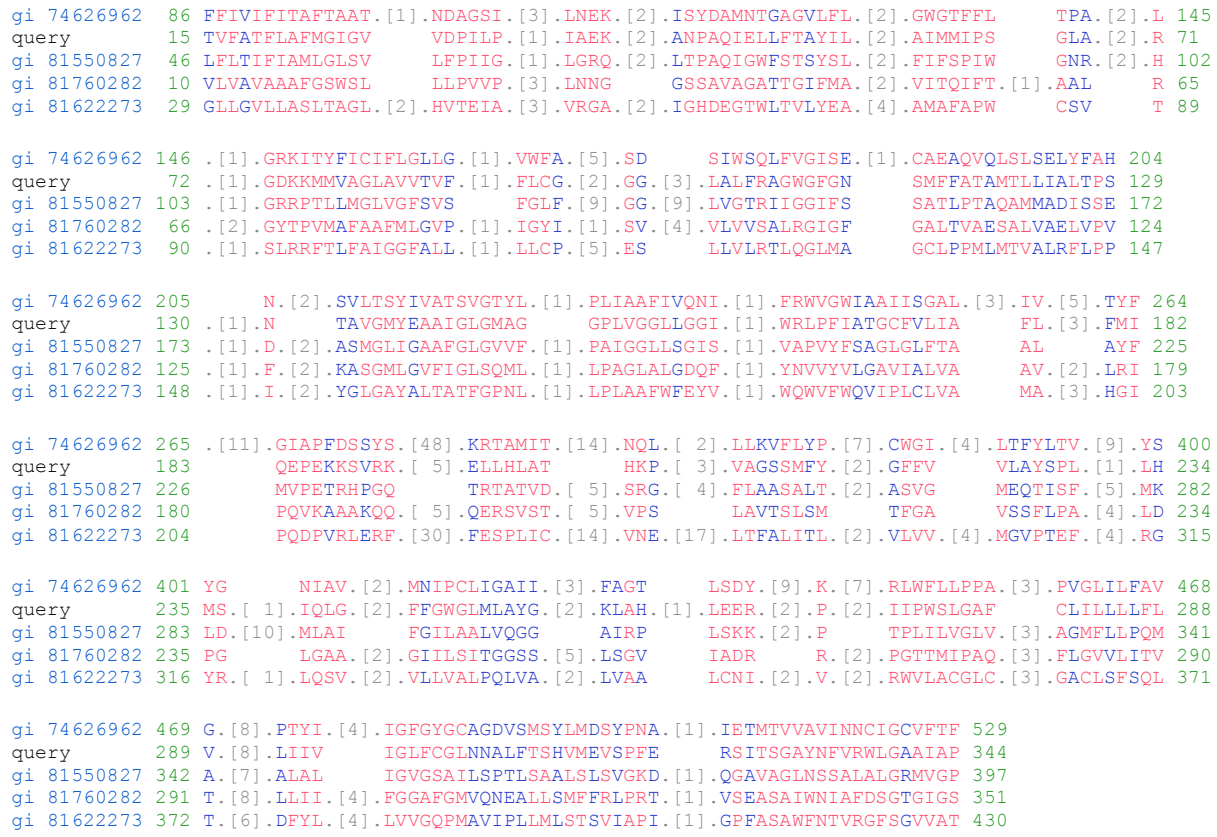


**Figure 4.** Multiple amino acid sequences of the conserved domains of the major facilitator transporter of *Bacillus circulans* ATCC 21588 (BciT; query) and its homologous using http://www.ncbi.nlm.nih.gov/Structure/cdd/cddsrv.cgi (Marchler-Bauer et al., 2015). The numbers indicate the position within the corresponding proteins: gi 74626962 = Transport protein of *Candida albicans*, accession code (AC)= O94019; gi 81550827 = Drug transport protein of *Deinococcus radiodurans*, AC= Q9RSF5; gi 81760282 = Permeases of the major facilitator superfamily of *Corynebacterium glutamicum*, AC= Q8NNT7; gi 81622273 = Probable major facilitator superfamily (MFS) transporter of *Pseudomonas aeruginosa*, AC= Q9I008.

DSM 43247 (AC= YP_003275379) and *Mycobacterium abscessus* 47J26 (AC= ZP_12877392), respectively. The N-terminal region of BciR and the respective homologous proteins were highly conserved (more than 95%) at the indicated catalytic sites. As depicted in Figures 2 and 3, the N-terminal domains of the BciR protein and its homologous proteins showed conservation of the amino acid moieties and the helix-turn-helix (H-T-H) motive required for DNA binding (DNA binding residues).

## Multiple alignment and domain analysis of BciT (predicted Major Facilitator Superfamily, MFS) and homologous proteins

As shown in Figure 4, BciT showed more than 83% sequence similarities to homologous proteins encoding diverse major facilitator transporters (multidrug efflux proteins) such as the multidrug transport protein of *Candida albicans* (AC, O94019), drug transport protein of *Deinococcus radiodurans* (AC, Q9RSF5), drug permeases of the major facilitator superfamily of *Corynebacterium glutamicum* (AC, Q8NNT7) and a probable major facilitator superfamily transporter of *Pseudomonas aeruginosa* (AC, Q9I008). As delineated in Figure 5, domain analysis of the BciT transporter protein revealed a putative conserved domain (specific hits) with the major facilitator superfamily, cd06174 (pfam07690).

## Phylogram analysis of BciR and BciT

A cladogram of BciR in relation to other MerR transcription proteins is shown in Figure 6. BciR of *B. circulans* clustered closely with two homlogous proteins of two *Paenbacillus* species (AC, WP-009673983.1 & AC, WP-042231219.1) with pairwise score ranging from

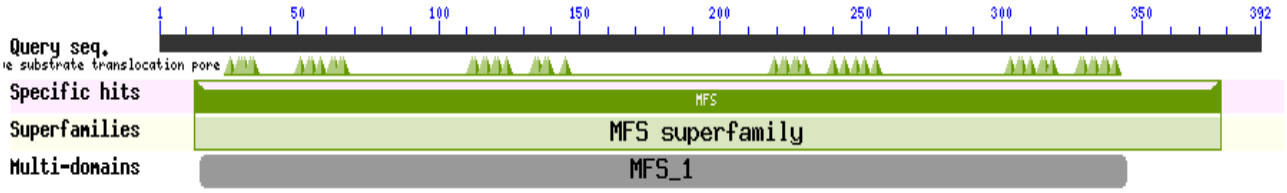


**Figure 5.** Putative conserved domain of BciT transporter protein (http://blast.ncbi.nlm.nih.gov/Blast.cgi). MFS= major facilitator superfamily; cd06174: MFS-1 = Major Facilitator Superfamily (pfam07690).

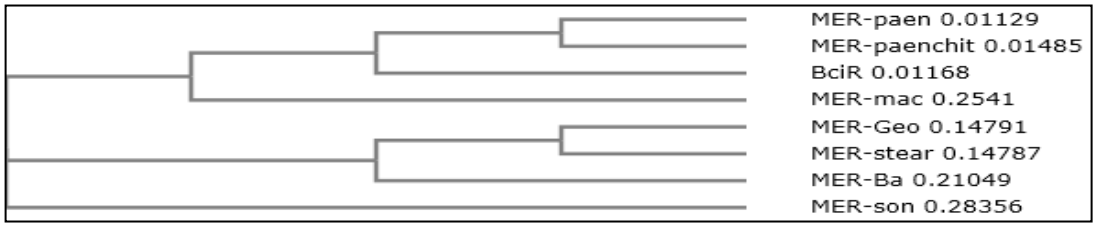


**Figure 6.** Cladogram of the phylogram analysis of BciR and its homologous proteins from the GenBank database. Mer-paen = MerR family transcriptional regulator of *Paenibacillus*, AC: WP_009673983.1; MER-Paenchit= MerR family transcriptional regulator of *Paenibacillus chitinolyticus*, AC: WP_042231219.1; MER-mac= MerR family transcriptional regulator of *Fictibacillus macauensis*, AC: WP_007202128; MER-Geo= MerR family transcriptional regulator of *Geobacillus* sp. JF8, Ac: WP_020958590.1; MER-Stear= MerR family transcriptional regulator of *Geobacillus stearothermophilus*, AC: WP_043903498.1; MER-Ba = MerR family transcriptional regulator of *Bacillus sp*. SJS, AC: WP_035412317.1;. MER-son= MerR family transcriptional regulator of *Bacillus sonorensis*, AC: WP_006637406.1. AC= Protein accession code within the GenBank database.

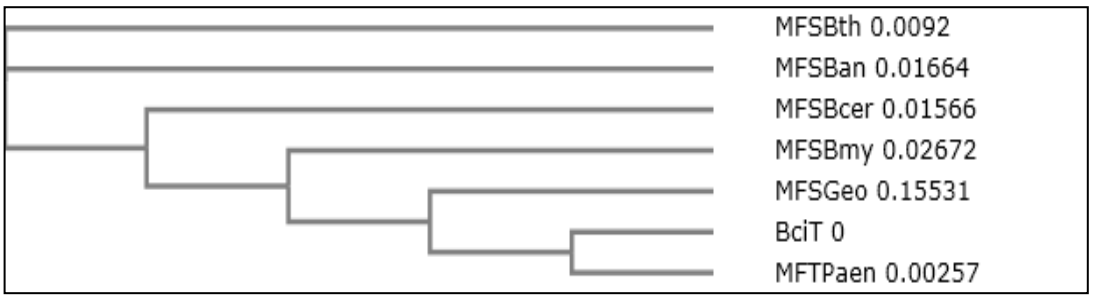


**Figure 7.** Cladogram of the phylogram analysis of BciT and its homologous proteins frm the GenBank database. MFSBth = major facilitator transporter of *Bacillus thuringiensis*, AC: WP_038413898.1; MFSBan= major facilitator transporter of *Bacillus anthracis*, AC: WP_003159546.1; MFSBcer = major facilitator transporter of *Bacillus cereus*, AC: WP_000444231.1; MFSBmy = major facilitator transporter of *Bacillus mycoides*, AC: WP_042981104.1; MFSGeo = major facilitator transporter of *Geobacillus stearothermophilus*, AC: WP_043903497.1; MFTPaen = major facilitator transporter of *Paenibacillus*, AC: WP_009673982.1. AC = Protein accession code within the GenBank database.

0.01129-0.01485 (MerR transcription proteins from other *Bacillus* species formed distinct clusters however, they were relatively related (pairwise scores ranged from 0.14791- 0.28356).

As depicted in Figure 7, a cladogram showing BciT in relation to other MFS transporter proteins showed, BciT of *Bacillus circulans* was clustered almost closely with a homologous protein of *Paenbacillus* species (AC, WP_009673982.1) of pairwise score that ranged from 0.0-0.00257. MFS transporter proteins from other *Bacillus* species were also closely related with pairwise scores that ranged from 0.0092- 0.01664.

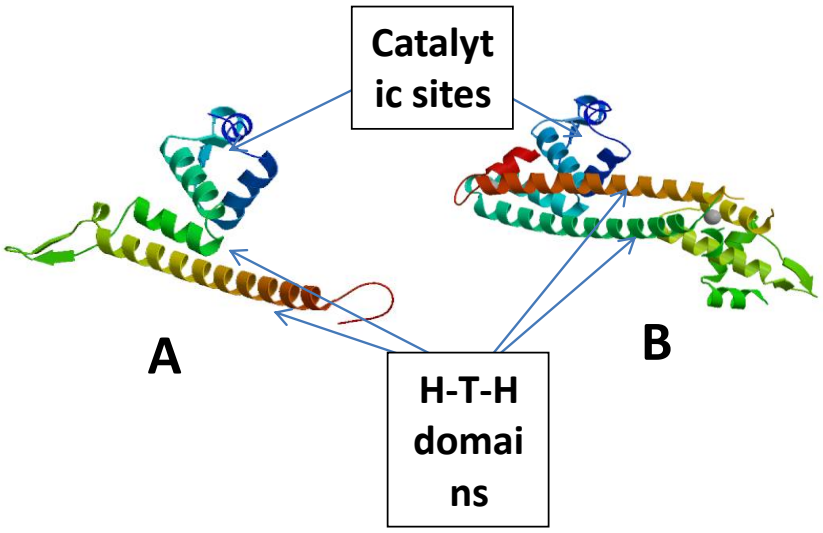


**Figure 8.** The predicted tertiary structure using SWISS-MODEL homology modeling report of: **A,** putative MerR family transcriptional regulator of *Bacillus circulans* ATCC 12588 (BciR), accession code GenBank KR049081 and **B**, MerR the transcriptional regulator family from *Bacillus cereus* crystal structure using X-RAY DIFFRACTION 2.67 Å. arrows show the suggested metal legends for binding (catalytic sites) and N-terminal Helix-Turn-Helix domains (H-T-H domains).

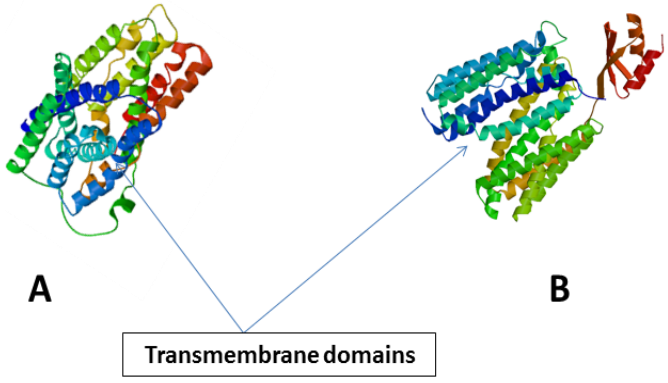


**Figure 9.** The predicted tertiary structure using SWISS-MODEL homology modeling report of: A; putative major facilitator superfamily transporter (MFS) of *Bacillus circulans* ATCC 12588 (BciT), accession code GenBank KR049081 and B; major facilitator superfamily transporter (YajR) Crystal structure using X-RAY DIFFRACTION 2.67 Å .YajR transporter suggests a transport mechanism based on the conserved motif A (Jiang, D. et al., 2013). Arrows indicate nine conserved transmembrane domains.

### Prediction of the tertiary structures of BciR and BciT proteins using SWISS-MODEL homology modeling report

As depicted in Figure 8, the three dimensional structure of BciR was predicated using a standard template model of a HTH-type transcriptional activator TIPA of *Bacillus cereus* (crystal structure using X-RAY DIFFRACTION 2.67Å). A model of BciR was built and revealed high degree of similarities to the template model as well as a conserved N-terminal helix-turn-helix domain (H-T-H domains) required for DNA binding in addition to a metal binding sites (catalytic sites) conserved at the C-terminal of the BciR protein.

The model showed also a QMEAN4 score of -1.08. As shown in Figure 9, the three dimensional structure of BciT was predicated using a standard template model of major facilitator superfamily transporter (YajR) crystal structure using x-ray diffraction 2.67 Å. A model of BciT was built and revealed conserved nine transmembraneous domains.

## DISCUSSION

With the increase of antibiotic-resistant pathogens, inadequate discovery of new antibiotics, as well as the extreme cost required for isolation of new antimicrobial agents, new approaches may be needed. Recently, with the extensive knowledge and profound use of gene manipulations worldwide, it became apparent that new approaches can be discovered. Transporter proteins particularly those involved in the transport/efflux of primary or secondary metabolites or those that regulate

their expression have been confirmed to play crucial roles in cell growth, spore formation, intrinsic and acquired resistance to various antimicrobial agents, chemical, dyes and even toxic oxygen radicals (Ahmed et al., 1995; Yan, 2013; Wisedchaisri et al., 2014; Hinchliffe et al., 2014; Xu et al., 2014; Shilton, 2015; Zhang et al., 2015). Inhibition or inactivation of this regulatory/transport transport system would be considered one of the promising approaches to control infection or even microbial resistance, and become a promising target of therapeutics particularly for the clinically relevant pathogens. Therefore, this study was concerned with the detection, sequence analysis of a model regulatory/transport system in a model of Gram positive, spore forming bacterium, *B. circulans*. Findings obtained from this study can be applied to those closely related pathogens such as *Bacillus anthracis*, *Bacillus cereus* and *Mycobacterium tuberculosis*. In this work various primers were designed based on the conserved amino acid sequences of some selected metalo-regulatory transcriptional regulators (MerR-Type) and major facilitator superfamily transporters (MFS-type) located in the GenBank database. The designed primers were used for amplification of the target sequences using chromosomal DNA of *B. circulans* ATCC 21588 as a PCR template. The PCR products obtained were analyzed using agarose gel electrophoresis, purified and sequenced in both forward and reverse directions. The obtained sequence files were assembled into a final consensus sequence (contig) of 1.926 kb using StadenPackage. The resulted consensus sequence was analyzed using the frameplot programme to detect the ORFs and was submitted into the GenBank database under accession code, KR049081.

Two complete open reading frames (ORFs) on the respective DNA segment (1.926 kb) of *Bacillus circulans* ATCC 12588 were detected and annotated BciR and BciT. The *bciR* (462 bp) and *bciT* (1179 bp) were found to encode a predicted MerR family transcriptional regulator (153 aa) and a major facilitator transporter (1179 bp, 392 aa), respectively. Both BciR and BciT were encoded by the parent DNA strand where BciT was located downstream BciR suggesting an operon of sensor/response. Analysis of the DNA segment harboring BciR and BciT ORFs showed a possible strong ribosomal binding site (RBS) for each ORF. The RBS (5'-AGGAG-3') of BciR was located at position-7 from the predicted start codon (ATG) of respective ORF while RBS of BciT (5'-GAAGGGG-3') located at position -12 from the predicted start codon (ATG) indicated that they are not coupled translated.

The first ORF (BciR; 153 aa) was found to encode a putative Metalo- transcriptional regulator (MerR-type) with aa identities to the multispecies Metalo- transcriptional regulator of: *Paenibacillus* sp. (97%, WP_009673983; 153 aa); *Paenibacillus chitinolyticus* (97%, WP_042231219; 153 aa); *Geobacillus* sp. JF8 (56%, WP_020958590; 142 aa); HTH-type transcriptional regulator of *Geobacillus* sp. GHH01 (64%, WP_015373901; 142 aa); and *Bacillus sonorensis* (50%, WP_006637406; 140 aa). Detection of the BciR showed a conserved domain with proteins encoding diverse MerR-type transcriptional regulators (MerR superfamily) of protein family COG0789 from wide varieties of microbial species such as N-Terminal domain of Cara Repressor *of Myxococcus xanthus* DK 1622 (AC, 2JML_A; Navarro-Aviles et al., 2007; 81 aa), *Eubacterium biforme* DSM 3989 (Ac, ZP_03489918; 211aa), *Gordonia bronchialis* DSM 43247 (AC, YP_003275379; Ivanova et al., 2010; 264 aa), and *Mycobacterium abscessus* 47J26 (AC, ZP_12877392; 246 aa). Multiple amino acid sequence alignment revealed presence of the amino acids moieties required for DNA binding (DNA binding residues) and located the N-terminal part of BciR as well as in the respective BciR homologous proteins (Marchler-Bauer et al., 2015). Moreover, the three dimensional structure of BciR was predicted via SWISS-MODEL Homology Modeling Report using a standard template model of MerR transcriptional regulator family of *Bacillus cereus* crystal structure. A model of BciR showed a QMEAN4 score of -1.08 as well as a conserved N-terminal Helix-Turn-Helix domain (H-T-H domains) required for DNA binding and metal binding sites (catalytic sites) conserved at the C-terminal (Ahmed et al., 1994; Zheleznova et al., 1999). Phylogenetic analaysis revealed that, BciR of *B. circulans* was clustered closely with two homlogous proteins of two *Paenbacillus* species (AC, WP-009673983.1 & AC, WP-042231219.1) of pairwise score that ranged from 0.01129-0.01485 (MerR transcription proteins from other *Bacillus* species formed distinct clusters however, they were relatively related (pairwise scores ranged from 0.14791- 0.28356).

The second ORF (BciT; 392 aa) was found to encode a putative major facilitator superfamily (MFS) with aa identities to the mutispecies major facilitator transporter of: *Paenibacillus* sp. (100%, WP_009673982); *Paenibacillus chitinolyticus* (99%, WP_042231220), *Geobacillus stearothermophilus* (66%, WP_043903497); *Bacillus cereus* (64%, WP_000444231); and *Bacillus thuringiensis* (64%, WP_000444219); *Bacillus mycoides* (64%, WP_042981104). Detection of the BciT showed that, it shared conserved domains with proteins encoding diverse major facilitator transporters (multidrug efflux proteins) of the protein family 07690 (pfam07690) from a wide varieties of microbial species such as *Candida albicans* (AC, O94019; Tait et al., 1997), *Deinococcus radiodurans* (AC, Q9RSF5; White et al., 1999),

*Corynebacterium glutamicum* (AC, Q8NNT7) and *Pseudomonas aeruginosa* (AC, Q9I008; Stover et al., 2000). The tertiary structure of BciT was predicted via SWISS-MODEL Homology Modeling Report using a standard template model of major facilitator superfamily transporter (YajR) crystal structure. A model of BciT revealed presence of nine transmembrane alpha helices and the majority of MFS proteins contained from 6-12 transmembrane alpha helices (TMs) connected by hydrophilic loop (Yan, 2013). BciT was predicted to be a MFS transporter protein in the form of monomer oligostate (Jiang et al., 2013). The BciT clustered almost closely with a homologous protein of *Paenbacillus* species (AC, WP_009673982.1) of pairwise score that ranged from 0.0-0.00257. MFS transporter proteins from other *Bacillus* species were also closely related with pairwise scores that ranged from 0.0092- 0.01664. The results obtained indicates that, MFS of *B. circulans* is closely related to those of *Paenbacillus* sp. than to those MFS of other *Bacillus sp*. Several studies showed presence of this regulatory/transport system where a MerR-type transcriptional regulator regulated a downstream multidrug-efflux transporter in *Bacillus subtilis* (Ahmed et al., 1994, 1995; Baranova et al., 1999). Other studies have confirmed the role of MerR-transcriptional regulator proteins in response to several metal ions conferring bacterial resistance to such toxic ions (Helmann et al., 1989, 1990) and to 2-nitroimidazole, the antifungal and antibacterial agent (Ogasawara et al., 2015). Zheleznova et al. (1999) revealed that up on drug (or toxin) binding to the transcription regulator, BmrR (MerR-type) of *Bacillus subtilis*, it activated expression of the multiple transporter (Bmr) that demonstrated an unusual ability to recognize multiple structurally dissimilar toxins. Therefore, BciR encodes a putative MerR-type transcriptional regulator that putatively regulate expression of the downstream located BciT. Both BciR and BciT that putatively encode a MFS-secondary transporter were shown to be arranged in an operon. This operon encodes proteins putatively involved in the assistance of transport across cytoplasmic or internal membranes of a variety of substrates. Therefore, inhibition or quenching the activity of this sensor/-response regulator system becomes a promising target of developing new therapeutics

## Conflict of interests

The authors did not declare any conflict of interest.

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