Characterization of a Putative Antimicrobial Peptide from an Antarctic Bacterium

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Abstract. The search for new antibiotics is a continuous effort since its discovery. Nevertheless, the speed of discovering novel antibiotics cannot match the speed of bacteria acquiring antibiotics resistant. Hence, the search efforts have broadened to include all compounds with antimicrobial activities. The toxin-antitoxin (TA) gene products are the potential antimicrobial compounds worth analyzing. The TA system consists of a set gene found either in the chromosome or plasmid, or both. At the moment, the toxin, a peptide from this system is known to kill some hosts that either encountered stress or have lost the plasmid carrying the TA genes. In a previous study, it was found that an Antarctic bacterium, Cryobacterium sp. SO1 chromosome harbored a putative antimicrobial peptide-coding gene similar to a class II TA, pemK gene. However, it is not clear whether this antimicrobial peptide has cross-species antimicrobial activity. Therefore, this work aims to determine whether this PemK protein has antimicrobial properties or not. The pemK_CryobacteriumSO1 gene was ligated to an arabinose-inducible promoter of the Topo pBAD plasmid and used to transform the Escherichia coli TOP10. The cloning of pemK inhibited the growth of the host E. coli TOP10 as the cells failed to grow. This indicated that PemK probably has a cross-species activity inhibited the growth of E. coli apart from its original host Cryobacterium sp. SO1.

1. Introduction

Routine used antibiotics or dosage can no longer kill some of the disease-causing pathogens [1, 2, 3] [4]. Efforts to look for new classes of antibiotics to treat multi-drug resistant pathogenic bacteria is an on-going process but with little success. Additionally, by introducing new antibiotics for treatment [4] has broadened the antibiotics resistant range among the pathogens [5]. As a result, more research is geared towards non-antibiotics antimicrobial compounds with the hope that antimicrobial peptides, for instance, will not extend the resistant capabilities of the superbugs [6]. The toxin peptide in the Toxin-Antitoxin (TA) system is believed to be a potential new alternative way to fight bacterial infections. The toxin from the TA system caused cell-death due to the loss of a plasmid bearing the toxin and antitoxin genes [7]. Recently, this system has been analyzed for potential as an alternative to antibiotics. The TA system is not only found in the plasmid [8] but also in the chromosome [9], [10]. It is known that the TA gene products are multifunctional, and plasmid stabilization is just one of its many functions. TA system is divided into several classes based on their mode of action. There are six known classes of the TA systems [11], [12]. TA system is a set of genes encoding toxin protein, and an antitoxin usually a peptide or an sRNA [10]. This system is partly responsible for the bacterial adaptation to stress or unfavorable growth conditions [13], [14], [15]. Plasmid loss or cell stress induced the TA system to stop the production of both toxin and antitoxin. As a result, there is an imbalance of toxin and antitoxin stoichiometry in the cell because of the longer lifespan of the toxin [16]. Hence, the toxin causes the
death of individual cells [17]. Recently, we have identified in *Cryobacterium* sp. genome a *pemK* gene coding a class II antimicrobial peptide. *pemK* gene is triggered when the cell encountered stresses such as antibiotics exposure, carbon and iron starvation, amino acid starvation and thymine starvation [18]. However, it is not clear whether the toxin encoded by the *pemK* gene target the host *per se* or other bacterial species as well. Hence, this project was conducted to determine whether PemK from *Cryobacterium* sp. is able to kill other bacterial species.

2. **Method**

2.1 **Culture**

*Cryobacterium* sp. SO1 was cultured in Luria-Bertani (LB, Difco, NJ, USA) medium at 20 °C with shaking at 200 rpm for 3 days. *E. coli* Top10 and BL21 were routinely cultured in Luria-Bertani broth or agar medium (LB, Difco, NJ, USA) at 37°C. Glucose or arabinose (Sigma-Aldrich, USA) were added to the medium when necessary.

2.2 **DNA extraction**

Genomic DNA of *Cryobacterium* sp. was extracted using the DNeasy Blood and Tissue® kit (QIAGEN, Hilden, Germany). *PemKpBAD* plasmid construct was isolated using the GeneJet Plasmid Miniprep Kit (Thermo Fisher Scientific, USA).

2.3 **Polymerase Chain Reaction (PCR)**

*pemK* gene was amplified using Forward primers 5’ATGCTGCGCGGTGAGATCC3’ and reverse primer, 5’TAGCTGCAGGTGAAGACGTA3’. The amplicon was ligated to the pBAD TOPO vector according to the manufacturer’s instructions. Another set of *pemK* primer, Forward primer, 5’GGCCATGGTGCGCGGTGAGATCC3’ and reverse primer, 5’TGCCATGCGCTATAGCTG CAGGTGAAGAC3’ with a NcoI restriction site added were used to amplify the gene that was cloned into the pET21 plasmid.

2.4 **Restriction digestion and ligation.**

*pemK* gene in the chromosome of *Cryobacterium* sp. SO1 was amplified using PCR and was ligated to an arabinose-inducible promoter of the Topo pBAD plasmid to create plasmid *PemKpBAD* according to the manufacturer’s instructions. Subsequently, it was used to transform the *E. coli* TOP10 (Invitrogen, Netherlands). Transformants were picked and used for plasmid miniprep. The plasmid was digested with restriction enzyme PmeI (New England BioLabs, US) and NcoI (New England BioLabs, US) to check for the presence of the *pemK* gene. Gel electrophoresis was performed on a 0.7% agarose gel. A control plasmid, ORF5pBAD containing a gene, *orf5* with no antimicrobial effect was prepared by cloning the *orf5* from *Pedobacter* sp. BG5 into Topo pBAD plasmid using a similar protocol.

2.5 **Transformation and transformant selection**

Transformation *E. coli* TOP10 was performed according to the manufacturer’s instruction (Invitrogen, Netherlands). LB plate supplemented with 100µg/ml ampicillin and 0.2% of glucose to repress the expression from plasmid pBAD. Selected colonies were checked for insert using PCR with primers targeting the *pemK* gene.
3. Results

There was no clone containing the pemKpBAD or ORF5pBAD plasmid that grew on the LB plates containing glucose containing ampicillin (Figures 1(a) and (b)). In order to test why the E. coli TOP10 transformant did not grow, plasmids pemKpBAD and ORF5pBAD were used to transform E. coli BL21. The result showed no colony for E. coli BL21 carrying the pemKpBAD (Figure 1(c)), but E. coli BL21 carrying the control plasmid, ORF5pBAD formed colonies (Figure 1(d)).

Since the E. coli BL21 was probably inhibited by the pemK CryobacSO1 gene product, Saccharomyces cerevisiae was used as the host. pemK CryobacSO1 gene was cloned into a yeast expression system. Shuttle E.coli–S. cerevisiae plasmid, PemKpYeast carrying the pemK CryobacSO1 gene was constructed and used to transform E. coli TOP10 (Figures 2 (a) and (b). The yeast plasmid PemKpYeast was confirmed to carry the pemK gene fused to a yeast GAL10. This was confirmed by colony PCR targeting the pemK CryobacSO1 gene (Figure 3).

Figure 1: Plates of E. coli (Top10) transformed with: (a) plasmid PemKpBAD on LB medium containing ampicillin and 0.2% glucose and (b) plasmid ORF5pBAD on LB medium containing ampicillin and 0.2% glucose. Plates of E. coli (E. coli (BL21) transformed with: (c) plasmid PemKpBAD in LB medium containing ampicillin and 0.2% glucose and (d) plasmid ORF5pBAD in LB with 100µg/ml ampicillin and 0.2% glucose; e) Plates of untransformed E. coli in LB medium with ampicillin and 0.2% glucose (negative control).

Figure 2: a) E. coli TOP10 carrying the shuttle plasmid. PemKpYeast (transformants of yeast plasmid with its restriction sites treated without Antarctic phosphatase treatment prior to the ligation step) on LB medium containing ampicillin. (b) E. coli (TOP10) carrying PemKpYeast (transformants of yeast plasmid with its restriction sites treated with Antarctic phosphatase treatment prior to the ligation step) on LB medium containing ampicillin. (c) E. coli TOP10 on LB medium without ampicillin (positive control) and (d) Untransformed E. coli TOP10 recombinant plasmid in LB media containing ampicillin (negative control).
4. Discussion

Promoter for gene expression in plasmid pBAD is an arabinose-inducible promoter. It is repressed when glucose is added to the growth medium. This promoter was chosen for this work because it is repressed the expression of the gene that was harmful to the host. Nevertheless, the results of this work showed that plasmid, pemKpBAD used to transform E.coli TOP10 failed to produce any colony on the agar plate. This showed that the E.coli TOP10 used was probably not viable, since there was also no growth when it was transformed with the control plasmid, ORF5pBAD.

Transformation of E.coli BL21 using plasmids, pemKpBAD, and ORF5pBAD yielded results. There was no colonies formation by cells transformed with plasmid, pemKpBAD. However, E.coli BL21 transformed with the control plasmid, ORF5pBAD formed colonies. This indicated that the pemK CryobacSO1 gene was being expressed despite the presence of glucose in the medium. As a result, the E.coli BL21 was killed, while the control E.coli BL21 cells survived. It is not clear at the moment whether the promoter expressing the pemK CryobacSO1 gene was leaky or there were other reasons for this.

Hence, pemK expression in a yeast system was performed. PemK is known to target and kills bacteria, and hence is not likely to affect yeast, S. cerevisiae [19]. pemK CryobacSO1 expressed from a yeast GAL10 promoter was successfully cloned onto a yeast plasmid, and used to transform E.coli. There were many E.coli colonies formed on the LB plates containing ampicillin. This showed that the yeast GAL10 promoter did not function and the pemK gene silenced in E.coli, and thus host survived, and formed colonies on the agar plate. In the future, an experiment will be performed to transform S. cerevisiae using plasmid PemKpYeast extracted from E.coli. Subsequently, the gene product of pemK CryobacSO1 from S. cerevisiae will be purified and its antimicrobial activity will be assayed using tester bacterial strains.

5. Conclusion

It is concluded that the pemK CryobacSO1 gene product seemed to have an antimicrobial effect on E. coli apart from Cryobacterium sp. SO1.

6. References

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Acknowledgment

The funding support from the Yayasan Penyelidikan Antartika Sultan Mizan (YPASM) under the YPASM Polar Research Grant Scheme (GL00163-AK-2016) is gratefully acknowledged.