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Short Communication

Characterisation of a cryptic plasmid from an Antarctic bacterium *Pedobacter cryoconitis* strain BG5

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ABSTRACT

A cryptic plasmid, pMWHK1 recovered from an Antarctic bacterium *Pedobacter cryoconitis* BG5 was sequenced and characterised. The plasmid is a circular 6206 bp molecule with eight putative open reading frames designated as *orf1*, *orf2*, *orf3*, *orf4*, *orf5*, *orf6*, *orf7* and *orf8*. All the putative open reading frames of pMWHK1 are found to be actively transcribed. Proteins encoded by *orf2* and *orf4* are predicted to be responsible for the mobilization and replication of the plasmid respectively. *orf4* shares 55% and 61% identities with the theta-type Rep proteins from two strains of *Riemerella anatipestifer*. This suggests that pMWHK1 could be a member of the theta-type replicating plasmid. The origin of replication is located within the AT-rich region upstream of *orf4*. *orf5* and *orf6* encode bacterial toxin-antitoxin proteins predicted to maintain plasmid stability. *orf3* encodes an entry exclusion protein that is hypothetically involved in reducing the frequency of DNA transfer through conjugation. *orf1*, *orf7* and *orf8* encode proteins with unknown functions. Plasmid, pMWHK1 is stably maintained in *P. cryoconitis* BG5 at 20 °C.

1. Introduction

Plasmids are extra-chromosomal genetic elements which contain genes that confer special traits such as antibiotic resistance and metal tolerance to host cells (del Solar et al., 1998). These genetic elements are commonly found in bacteria isolated from marine, estuarine and freshwater environments (Burton et al., 1982; Schutt, 1988). Similarly, bacteria isolated from various habitats in the extremely low temperature regions of the Antarctica are also found to carry plasmids (Kobori et al., 1984). Members of the *Pedobacter* genus are diverse and produce a wide range of secondary metabolites such as antibiotic and metal resistant compounds. They also exhibit xenobiotic catabolism as well as causing infections in mammals. Some of these properties are conferred by genes found in their plasmids (Poirel et al., 2001; El-Sayed et al., 2003; Ma et al., 2006).

An Antarctic bacterium, *Pedobacter cryoconitis* strain BG5 was isolated in 2007 from soil collected at the vicinity of the Belen Lake (S62° 13′ 45.4″ W58° 58′ 53.9″), King George Island, Antarctica during the 43rd Scientific Antarctic Expedition organized by the Instituto Antártico Chileno (INACH) (Wong et al., 2011). This psychrophilic bacterium harbors a plasmid with an approximate size of 6.2 kb that is designated as pMWHK1. *P. cryoconitis* BG5 grows optimally at 20 °C. Nonetheless its growth tolerates temperatures ranging from 1 to 25 °C. *P. cryoconitis* BG5 inhibits the growth Gram-negative and -positive food pathogens such as *Escherichia coli*, *Salmonella* spp., *Klebsiella pneumonia*, *Enterococcus cloacae*, *Vibrio parahaemolyticus* and *Bacillus cereus* (Wong et al., 2011). Additionally, it displays multiple resistant towards antibiotics such as ampicillin, ceftazidime, chloramphenicol, gentamicin, kanamycin, streptomycin, polymyxin B sulfate, novobiocin and

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vancomycin (Wong et al., 2011). The metabolic abilities of *P. cryoconitis* BG5 described above suggested that the plasmid it harbors probably confers some of these properties. In this paper, we describe the properties of pMWHK1. This plasmid is anticipated to have major role as a cloning vector for temperature-sensitive proteins.

2. Materials and methods

2.1. Bacterial strains, media and growth conditions

P. cryoconitis BG5 described in Wong et al., (2011) was routinely grown in tryptic soy broth (Sigma) at 20 °C with shaking at 200 rpm. *E. coli* TOP10 (Invitrogen) was grown in Luria–Bertani broth at 37 °C with shaking at 200 rpm. Bacto-agar (1.5%) and ampicillin (50 μg/ml) were added when necessary.

2.2. Cloning, sequencing and DNA manipulations

Plasmid DNA was extracted and purified using QIAprep Miniprep kit (Qiagen) according to the manufacturer's manual. Plasmid, pMWHK1 was partially digested with *Hin*dIII (New England Biolabs) generating several DNA fragments and were resolved in a 0.7% agarose gel. The fragments were then cloned in the pUC19 plasmid and delivered into *E. coli* TOP10 (Invitrogen) through heat shock transformation. Fragments of pMWHK1 ligated in the pUC19 plasmid were sequenced using the Applied Biosystems AB3100 Sequencer. Primer-walking was employed to determine DNA sequences between the gaps of the respective contigs. Both forward and reverse fragments of pMWHK1 were sequenced, assembled and analyzed. The assembly and analysis of the DNA fragments were performed with Lasergene from DNASTAR.

2.3. Sequence analysis and annotations

Open reading frames (ORFs) were deduced and annotated using an interpolated Markov models with GLIMMER (Salzberg et al., 1998; Delcher et al., 1999). DNA sequences were compared to those in the GenBank using nucleotide BLAST. Amino acid sequences were analyzed against information of proteins deposited in the Conserved Domain database (CDD), Pfam and Swiss-Prot using PSI-BLAST (Altschul et al., 1997). The theoretical isoelectric point was determined by ExPASy. The complete sequence of pMWHK1 was deposited to GenBank.

2.4. Gene expression analysis

Real-time reverse transcriptase (RT) PCR using iScript One-Step RT-PCR kit with SYBR green (Bio-Rad) was conducted to assess putative open reading frames (ORFs) in pMWHK1. Total RNA was isolated from an exponentially growing culture of *P. cryoconitis* BG5 using RNeasy mini prep kit (Qiagen). The total RNA was stabilized with RNAprotect Bacteria Reagent (Qiagen). To eliminate DNA contaminants, DNase I was added during total RNA preparation. Eight sets of primers targeting the individual eight ORFs of pMWHK1 were used in real-time RT-PCR (Table 1). The primer pair, RP70F and RP70R targeting the Sigma factor 70 was used as a control for positive amplification. The absence of DNA contaminant was confirmed by a real time RT-PCR run using primers NCF and NCR that target the non-coding region of pMWHK1. Real-time RT-PCR was performed using Bio-Rad iCycler IQ™ Multicolor Real-Time Detection System (Bio-Rad).

2.5. Plasmid stability assay

Segregational stability of pMWHK1 was determined by evaluating the presence of plasmid-free cells on tryptic soy agar. *P. cryoconitis* strain BG5 and control strain, *Pedobacter* BG22 (without plasmid) were cultured in 50 ml tryptic soy broth at 20 °C with shaking at 200 rpm. Over the course of 10 days, successive serial dilution transfers were performed by inoculating 50 ml fresh tryptic soy broth with 50 μl of 1/1000 diluted overnight culture. The optical density at 600 nm was measured prior and post overnight growth. The number of generations was calculated using the algorithm described by Zaleski et al. (2006). Fifty random colony forming units were assessed through diagnostic PCR targeting the *orf6* of pMWHK1. The diagnostic PCR mixture contains 20 ng DNA, 0.2 μM of each primer, 0.2 mM of each dNTP, 2.5 mM $MgCl_2$ and 1.25 U Taq Polymerase (Promega). The PCR was performed at 96 °C for 2 min, followed by 30 cycles of 96 °C for 30 s, 52 °C for 30 s, 72 °C for 30 s with a final extension step of 72 °C for 10 min.

Table 1
The PCR primers used for RT-PCR assay.

Genes/putative open reading frames	Primers	Amplicon size (bp)
Sigma factor 70 (+ve control)	RP70F 5′-AATCCACCATACTGCATAAGAGA-3′ RP70R 5′-ATCACCGCAGAAGAAGAAGTAA-3′	250
Non-coding region (−ve control)	NCF 5′-AGCCTGATCGGCTGACTTTC-3′ NCR 5′-GGCAATAGCGAAGCGCTTTG-3′	223
orf1	G1F 5′-CCAGGACAAAGCCAATGAAT-3′ G1R 5′-TGCGGTAGGCATAGCGTATT-3′	227
orf2 (Mobilization protein)	G2F 5′-GATCGGAAGACCGTTCAAAA-3′ G2R 5′-TCCCCATCCATACCTACTCG-3′	215
orf3 (Entry exclusion protein)	G3F 5′-AAATTACCTATTCGCCTCATACC-3′ G3R 5′-TTAGGAGTAACATGGCTTTTTGA-3′	224
orf4 (Replication protein)	G4F 5′-CTGTTCAGGTGCTTTTCCTTT-3′ G4R 5′-GGCTGTTCCTTATTTCTTTGACT-3′	201
orf5 (PemI/antitoxin protein)	G5F 5′-GAATAATACTTCCCTCAATGCTT-3′ G5R 5′-GTCCATTCCTCTTTTTCAAACTC-3′	206
orf6 (PemK/toxin protein)	G6F 5′-CGGATGAAATGAATGAATTTTTA-3′ G6R 5′-TCCGAGCCCTATCTATTACTTTT-3′	150
orf7	G7F 5′-GACTTAGATCCATCAAGACAAGG- 3′ G7R 5′-GGTGCTAACTTTTCAAGTAATG-3′	227
orf8	G8F 5′-TCGACTATTTCAAATTGCTTGGT-3′ G8R 5′-AGGCAAAGTGTTCGTTACCC-3′	198