ARTICLE

Sequence analysis of the complete catechol 1,2-dioxygenase gene in *Acinetobacter baumannii* strain OS1

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The catechol 1,2-dioxygenase gene (*catA)* codes for an enzyme that is responsible for the cleavage of aromatic hydrocarbons present in oil-polluted environments. This research focused on the amplification and sequence analysis of the *catA* gene from *Acinetobacter baumannii* strain OS1, a strain isolated from oil sludge that exhibited significant bunker oil degradation. PCR using four primers designed to amplify different regions of the strain OS1 *catA* gene, produced the expected size amplicons of about 641, 643, and 1,122 base pairs. Sequence analysis of partial 641-bp and 643-bp *catA* gene fragments and the 1,122-bp complete *catA* gene cloned into pCR™2.1-TOPO® vector, revealed the complete sequence of the *Acinetobacter baumannii* strain OS1 *catA* gene (GenBank Accession No. KF038386) with 921 nucleotides that are expected to translate into a protein with 306 amino acids. The OS1 *catA* gene exhibits 99% sequence similarity to the *catA* gene previously reported in the database for strains AB307 0294, AB0057, and AYE, and 98% similarity with strain SDF of *Acinetobacter baumannii*. The corresponding amino acid sequence of the catechol 1,2-dioxygenase in strain OS1 is the same for all strains previously studied, except strain SDF with four amino acid differences. The sequence analysis of the complete *catA* gene from *A. baumannii* strain OS1 will pave the way for further studies including the expression of the gene in an appropriate non-pathogenic host cell for possible use in addressing petroleum-derived hydrocarbon pollution.

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INTRODUCTION

Cases of oil spills have been reported to threaten plant and animal species in different parts of the world (Atlas and Philp 2005, Rogowska and Namiesnik 2010, Trevors and Saier 2010, McGenity et al. 2012). In the Philippines, some of the reported cases of oil spills were the bunker oil spills in Guimaras, Negros Islands (Toms 2006), Semirara, Antique (Magramo 2007), and Sarangani Bay, Mindanao (Zonio 2011). Oil spills are considered petroleum-derived contaminants and usually contain aromatics, paraffins, naphthenes, and non-hydrocarbon compounds (Clark et al. 1990). One of the possible solutions that have been recommended for these types of contaminants is the use of microorganisms to break down such chemical compounds (Bento et al. 2005, Sathiya Moorthi et al. 2008).

Acinetobacter baumannii is a Gram negative bacterium commonly reported as an opportunistic human pathogen implicated in epidemic pneumonia, urinary tract infection, and meningitis (Fournier et al. 2006, Magnet et al. 2001). This pathogen could survive even in hostile environments because of the enzymes that allow it to use different carbon and energy sources (Wendt et al. 1997, Abbo et al. 2005). Examples of these enzymes are alkane 1-monooxygenase and cathecol 1,2-dioxygenase. These two enzymes have been implicated in the bioremediation of petroleum-derived pollutants because of the nature of their substrates for enzyme activities that are alkanes and aromatic hydrocarbon -– the major components of the oil contaminants (Funhoff et al. 2006). *Acinetobacter baumannii*, which produces both enzymes, however, is s a human pathogen that exhibits multiple antibiotic resistance (Cunha 2013, Maragakis and Peri 2008). Because the use of this pathogenic bacterium for bioremediation and enzyme synthesis is not recom-

KEYWORDS
Acinetobacter baumannii, catechol 1,2-dioxygenase, *catA*

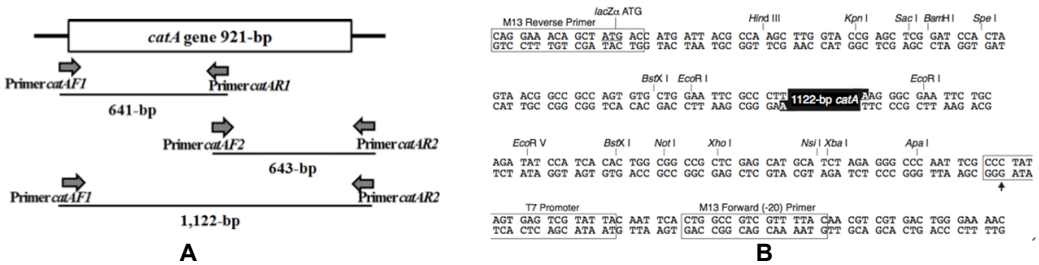

Figure 1. Schematic diagram of catA gene-targeted amplification using 3 primer pairs(A) and complete catA gene cloning into pCR™2.1-TOPO® vector (B).

mended, the isolation and sequencing of the complete gene for the enzymes involved in oil degradation, like catechol 1,2-dioxygenase, are necessary for the transfer and expression of these genes in a non-pathogenic host bacterium.

In an earlier study (Hedreyda and Sarmago 2014), the complete alkane 1-monooxygenase gene (*alkM*) was sequenced from *Acinetobacter baumannii* strain OS1. This strain was isolated from the hostile environment of oil sludge and was reported to exhibit significant bunker oil degradation in an assay using a minimal agar plate with bunker oil overlay. In this study, experiments were conducted to design PCR primers for detecting and amplifying different regions of the catechol 1,2-dioxygenase gene (*catA*) in the same bacterium strain OS1. The amplicons of *catA*-targeted PCR representing partial gene fragments and the complete gene cloned into TOPO vector were sequenced and analyzed, resulting in the complete sequence of the *catA* gene from strain OS1 of *Acinetobacter baumannii*.

MATERIALS AND METHODS

Isolation and growth of *Acetobacter baumannii* strain OS1

Acinetobacter baumannii strain OS1 was isolated in an earlier study from an oil sludge sample of Petron Corporation refinery in Manila, Philippines (Hedreyda and Sarmago 2014) and was identified based on *16s rRNA* gene sequence analysis.

The isolate was maintained in Luria-Bertani agar. Genomic DNA extraction was done using the Nucleospin Tissue kit (Clonetech Laboratories, Inc., Palo Alto, CA, USA) following manufacturer's protocol.

Design of *catA* gene-targeted primers and detection of the gene

Four primers ((*catAF1:* 5'-CCTTAGAGCATGAGGTAACGTG-3', *catAR1:* 5'-ACGGTAACTGACACTGATGG-3', *catAF2:* 5'-CCATCTGCATCGGTGA-3', and *catAR2:* 5'- GGTAACAAG-GAACGTGGCGGC-3') were designed based on *catA* sequences of *Acinetobacter baumannii* strain AB307-0294 (GenBank Accession No. CP001172) in order to detect and amplify different regions of the *A. baumannii* strain OS1 *catA* gene. Optimized conditions for PCR using three primer pairs and *A. baumannii* strain OS1 DNA template were determined through gradient PCR. PCR profiles were visualized under UV after agarose gel electrophoresis (1% agarose) and ethidium bromide (0.0025%) staining. Amplicons were photographed using an Alpha DigiDoc Pro (Alpha Innotech, USA).

Cloning and sequencing of *A. baumanii* strain OS1 *catA* gene

In addition to the amplification of partial *cat*A gene fragments, the complete *catA* gene amplified using primer set *catAF1/catAR2* (Fig.1) was also cloned into pCR™2.1-TOPO® vector following manufacturer's procedure (TOPO® TA Cloning® Kit, Life Technologies-Thermo Fisher Scientific Inc., 81 Wyman Street, Waltham MA USA 02451). Amplicons and the cloned complete *catA* gene were submitted for sequencing to 1st BASE (BASE Life Sciences Holdings, Singapore) and sequences were compared to confirm consistency of sequence results. Strain OS1 complete *catA* sequence was compared to sequences of the gene reported for the species in the GenBank database using Multalin (http://multalin.toulouse.inra.fr/multalin/). The amino acid sequence based on nucleotide sequence was analyzed using CLC Sequence Viewer (CLC Bio–Qiagen, 24 School Street, 6th Fl, Boston, MA 02108 USA).

RESULTS AND DISCUSSION

Design of *catA* gene-targeted primers

A schematic diagram (Fig.1A) illustrates the relative annealing sites of each primer and the size of expected amplicons from PCR using each primer pair, if the *catA* gene is present in strain OS1 DNA template.

Detection and cloning of the *catA* gene in *A. baumannii* strain OS1

The optimized PCR conditions using primer pairs *catAF1/catAR1, catAF2/catAR2*, and *catAF2/catAR2* were as follows: 30 cycles of denaturation for 5 min at 94°C, annealing for 0.5 min at 58°C (except for primers *catAF2/catAR2* at 62°C), extension for 1 min at 72°C, and a final extension for 10 min at 72°C. PCR using the optimized parameters produced expected-size amplicons of about 641-bp with primer pair *catAF1/catAR1*, a 643-bp fragment with primer pair *catAF2/catAR2*, and a 1,122-bp amplicon (containing the complete *catA* gene) from primer pair *catAF1*/catA*R2* (Fig 2). The amplicon containing the complete gene was also successfully cloned into the pCR™2.1-TOPO® vector for sequencing.

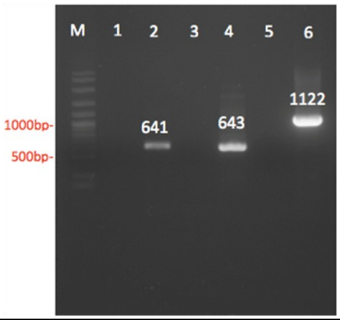

Figure 2. Amplicons of PCR using *catA*-targeted primers and *A. baumannii* strain OS1 DNA template. Lanes 1, 3, and 5, are result of PCR with no template DNA; lanes 2, 4, and 6 are result of PCR using primer pairs *catAF1/catAR1, catAF2/catAR2,* and *catAF1/catAR2,* respectively. M is the 100-bp Vivantis DNA ladder (CA, USA).

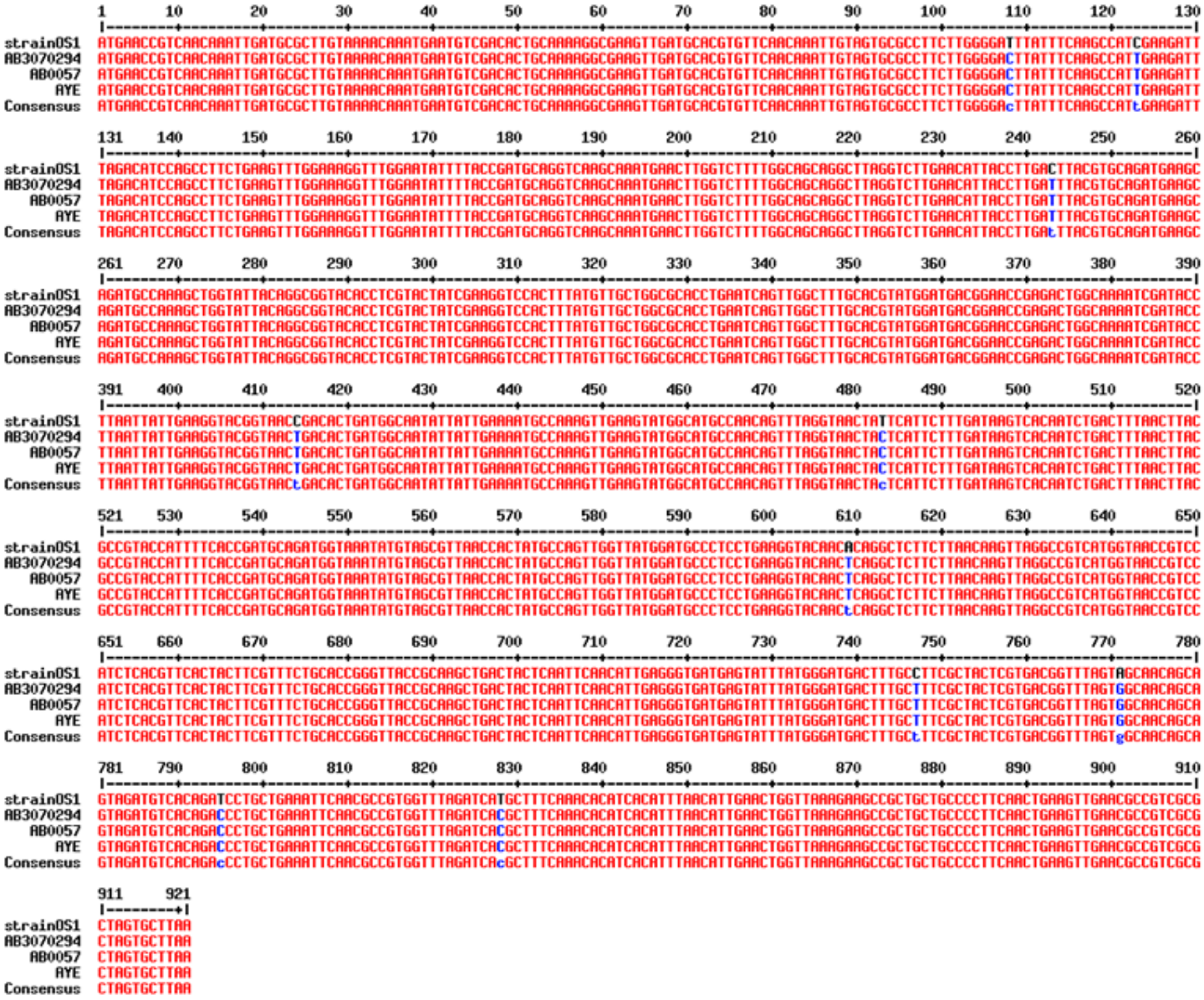

Figure 3. Alignment of *catA* gene sequences showing 10 nucleotide variation in *catA* of *Acinetobacter baumannii* strain OS1 with strains AB307-0294, AB0057, and AYE.

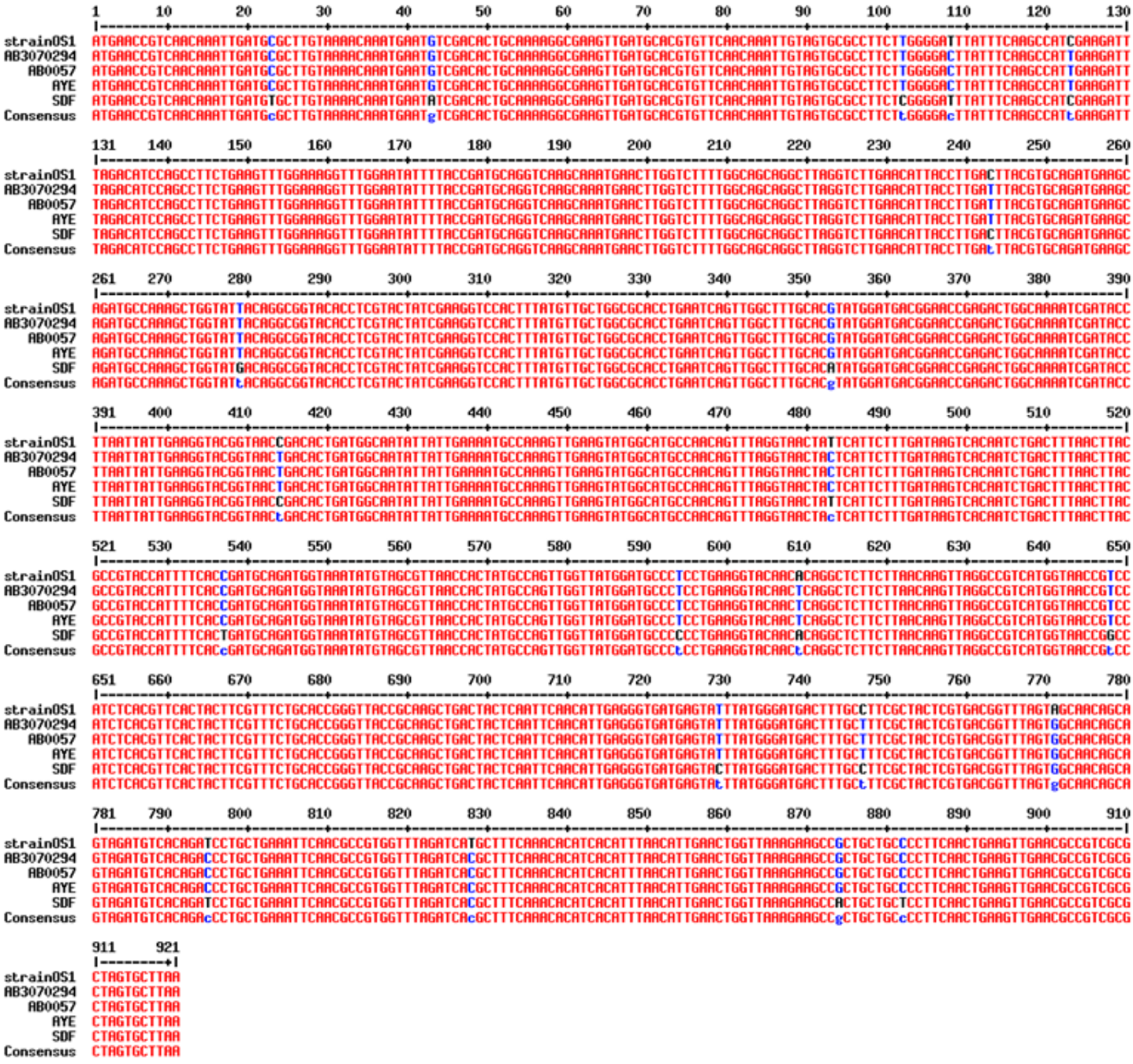

Figure 4. Alignment of *catA* gene sequences showing nucleotide variation of the gene in *Acinetobacter baumannii* SDF from the other strains.

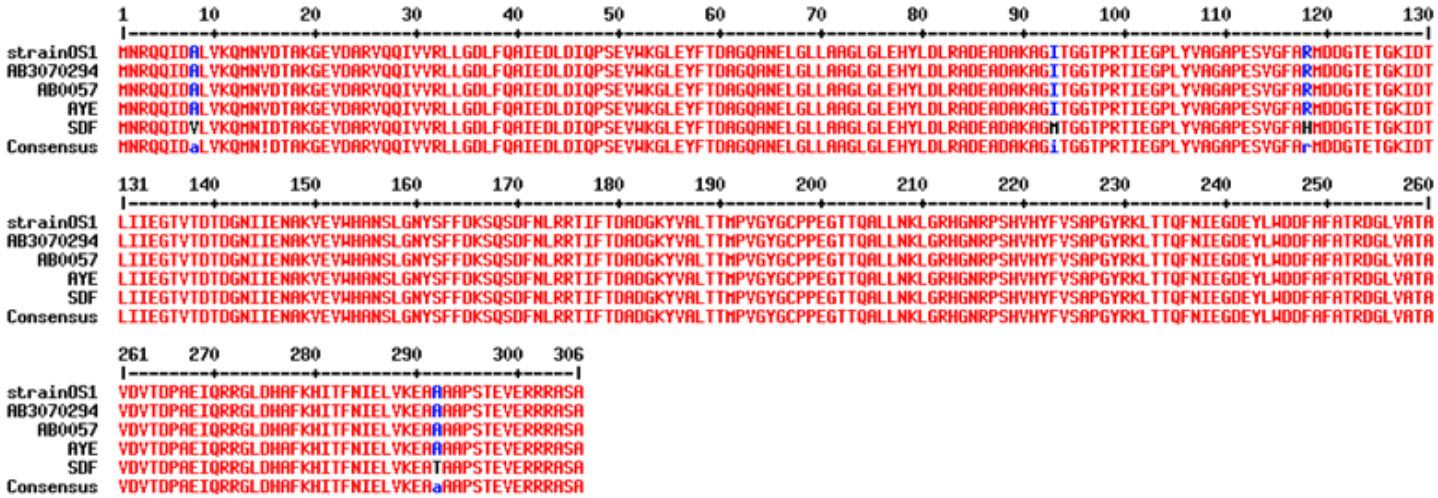

Figure 5. Alignment of amino acid sequence translated from the *catA* genes of *A. baumannii* strains OS1, AB307-0294, AB0057, AYE, and SDF.

Sequence analysis of *A. baumanii* strain OS1 *catA* gene fragments

Direct sequencing of the 641-bp, 643-bp, and 1,122-bp amplicons from PCR using optimized parameters, three primer pairs (*catAF1*/*catAR1, catAF2*/*catAR2,* and *catAF1*/catA*R2,* respectively) and strain OS1 DNA template, provided the sequences that resulted in the complete sequence of the *catA* gene in *A. baumannii* strain OS1 (GenBank Accession No. KF038386). The sequence was consistent with the sequence data obtained from the complete *catA* gene cloned in Topo Cloning vector (Fig. 1B).

BLAST and Multalin alignment programs showed that strain OS1 *catA* gene exhibits about 99% sequence similarity to the catechol 1,2-dioxygenase gene reported in the database for *Acinetobacter baumannii* strain AB307 0294 (GenBank Accession No. CP001172), strain AB0057 (GenBank Accession No. CP001182), and strain AYE (GenBank Accession No. NC010410), and 98% similarity with strain SDF (GenBank Accession No. NC010400). Sequencing showed a 10-nucleotide variation from the *catA* gene sequence reported for 3 strains (AB307-0294, AB0057, and AYE), while a 19-nucleotide difference was observed between *Acinetobacter baumannii* OS1 and strain SDF (Fig. 3). Alignment of expected amino acid sequences based on *catA* gene sequences in all strains showed no amino acid sequence variation among strains OS1, AB307-0294, AB0057, and AYE (Fig. 4). Four of the 19 nucleotide differences between *Acinetobacter baumannii* strain SDF and strain OS1 are expected to result in a 4-amino acid variation between strain SDF and all other strains (AB307-0294, AB0057, and AYE) including OS1 (Fig. 5). These 4-amino acid variations between strain SDF and the other strains include an alanine to valine change (A to V) at position 8, threonine to methionine (T to M) at position 93, arginine to histidine (R to H) at position 118, and alanine to threonine (A to T) at position 292.

Strain OS1 of *Acinetobacter baumannii*, which exhibited visually significant bunker oil utilization in both minimal liquid and solid media (Hedreyda and Sarmago 2014), is a good source of hydrocarbon-degrading enzymes, alkane 1-monooxygenase (*alkM*) and catechol 1,2-dioxygenase (*catA*), which have been reported to be produced by the species (Benedek et al. 2011). Alkane 1-monooxygenase is one of the essential enzymes for growth of *Acinetobacter* on alkanes as sole carbon source (Ratajczak et al. 1998). Catechol 1,2-dioxygenase is a key enzyme in the degradation of aromatic hydrocarbon (Guzik et al. 2011). *Acinetobacter baumannii,* however, is a human pathogen (Fournier et al. 2006, Magnet et al. 2001) and is not suitable for direct use in bioremediation. It is for this reason that the gene for catechol 1,2-dioxygenase was amplified and sequenced from strain OS1. Isolation and sequence data of the genes are necessary to achieve the ultimate goal of cloning the genes into expression vectors and having them expressed in suitable non-pathogenic bacterial host cells. A non-pathogenic bacterium that expresses the genes could be used in enzyme production for possible use in bioremediation.

This study reports the design of four primers and the use of combinations of these primers to produce 3 primer pairs, *catAF1/catAR1, catAF2/catAR2,* and *catAF1/catAR2*, targeting the 5' region, 3' end, and the complete *catA* gene fragment, respectively. Using primer pairs and optimized PCR conditions obtained in this study, the expected-size *catA* gene fragments were amplified from the OS1 DNA template (Fig.2). These *catA*-targeted primer pairs and the optimized PCR conditions for amplification are valuable for future *catA* gene detection experiments to evaluate the presence of the *catA* gene in other bacteria, or in performing a metagenomic detection approach using environmental samples.

Nucleotide sequences obtained through sequencing of amplicons from *catA* gene-targeted PCR provided the basis for reporting the sequence of complete *catA* gene in *A. baumannii* strain OS1 (Genbank Accession No. KF038386). The sequences from amplicons containing incomplete fragments of the *catA* gene were consistent with the sequence data from the cloned 1,122-bp complete gene, which served as an additional confirmatory step for OS1 *catA* gene sequence data. From nucleotide sequence analysis, corresponding amino acid sequences were obtained which could provide insights into protein function, including function relevant to bioremediation.

The 10-nucleotide difference between *catA* in strain OS1 and *catA* in strains AB307-0294, AB0057, and AYE is not expected to translate into any change in amino acid sequence (Fig. 4) and theoretically not expected to result in altered protein function. A 19-nucleotide variation was observed between strain SDF and strain OS1, which is expected to translate into four amino acid differences (Fig. 5). These 4-amino acid variations between strain SDF and other strains are not within the active site of the catechol 1,2-dioxygenase, which was described to contain Tyr 200, His 226, Tyr 164, and His 224 (Vetting and Ohlendorf 2000, Di Nardo et al. 2004). It is not known if these amino acid changes in catechol 1,2-dioxygenase of strain SDF affect enzyme function, or if they are relevant to the observation that strain SDF isolated from body louse is avirulent while the other strains are (Vallenet et al. 2008). The effect of amino acid differences on protein structure and the ability of strain SDF to degrade petroleum-derived hydrocarbon could be the subject of further investigation.

CONCLUSION

Based on the complete *catA* gene sequence, strain OS1 is expected to produce catechol 1,2-dioxygenase with the same 306 amino acid sequence as those of the three strains, AB307-0294, AB0057, and AYE. A 4-amino acid difference is expected between the catechol 1,2-dioxygenase from strain OS1 and that from the avirulent strain SDF. The effect of these amino acid substitutions on enzyme function and on strain virulence could be the subject of future research.

The primer pair *catAF1/catAR2* from this study could amplify the complete OS1 *catA* gene that will pave the way for the cloning of the gene from strain OS1 into an expression vector, which could later be introduced into a non-pathogenic bacterial host. Expression of the gene in the non-pathogenic bacterium would allow production of catechol 1,2-dioxygenase for use in addressing problems of oil pollution.

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CONFLICTS OF INTEREST

There is no conflict of interest among authors, institutions, and individuals mentioned above in the conduct of this study and the preparation and submission of this manuscript.

CONTRIBUTIONS OF INDIVIDUAL AUTHORS

The research was conceptualized by Dr. Hedreyda while Ms. A. Mittsu G. Sarmago conducted experiments. The manuscript was prepared by Dr. Hedreyda and Ms Sarmago.

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