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CLONING AND EXPRESSION OF *pigC* GENE IN *ESCHERICHIA COLI*

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SUMMARY

Prodigiosin (Pg), which is particularly of interest because of anticancer and antimicrobial activities, can be produced through the PigC-catalyzed condensation reaction of 4-methoxy-2, 2'-bipyrrole-5-carboxyaldehyde (MBC) and 2-methyl-3-amylpyrrole (MAP). Therefore, the PigC protein plays an important role in prodigiosin biosynthetic pathway. However, studies related to PigC protein have not been carried out in Vietnam yet. In this work, the *pigC* gene was cloned and expressed in *Escherichia coli* DH10B and BL21 (DE3), respectively. Using PCR and universal primers, we amplified a fragment of 3 kb covering entire coding region of the *pigC* gene from *Serratia* sp. strain M5. The *pigC* gene was inserted into pJET1.2 vector, and then transformed into *E. coli* DH10B. The sequence of a recombinant vector pJET1.2/pigC was evaluated by using whole colony PCR amplification. Sequence alignment results revealed that the obtained *pigC* gene possesses 71.5% and 75.4% of nucleotide identity in comparison with two strains, *Serratia* 39006 and *Serratia* sp. AS9 published in GenBank with their respective accession numbers of AJ833001 and CP002773. The recombinant vector pJET1.2/pigC was used to reamplify *pigC*, and the acquired amplicon was inserted into pET22b vector at the site of *Hind*III and *Xho*I. The clone *E. coli* BL21 (DE3) containing recombinant vector pET22b/pigC was expressed in the auto-induced medium. The presence of PigC protein in the lysate was identified as a 100 kDa band through Western Blot analysis using anti his-tag antibody. Afterward, the PigC protein was purified by Ni-NTA column, and its expression level was quantified through SDS-PAGE analysis. The results of our study provide a potential material for producing prodigiosin from recombinant protein in Vietnam.

Keywords: *MAP, MBC*, *pigC*, *prodigiosin*, *Vietnam*

INTRODUCTION

Prodigiosin (Pg), a red pigment belonging to the prodiginine group, is a tripyrrolic secondary metabolite isolated from *Serratia marcescens*. Recently, prodigiosin and its related compounds have been particularly of interest because of their biological activities (Darshan, Manonmani 2015). The selective anti-cancer property of prodigiosin and its analogues were well-demonstrated on many cancer cell lines (Diaz-Ruiz *et al.,* 2001; Montaner *et al.,* 2000; Tomás *et al.,* 2003) as well as *in vivo* tests (Wang *et al.,* 2016; Yamamoto *et al.,* 1999; Zhang *et al.,* 2005). In addition, prodigiosin and its derivatives also have antiparasitic and antimicrobial activities (Rahul *et al.,* 2015; Suryawanshi *et al.,* 2017). In Vietnam, there were a few studies interested in *S. marcescens* and prodigiosin. Nguyen (2015) extracted and evaluated antibiotic of prodigiosin against *Bacillus subtilis* and *Staphylococcus aureus* from *S. marcescens* strain M10. The anti-insect property against *Spodoptera litura* of *S. marcescens* and prodigiosin was also detected (Nguyen, Nguyen, 2015). However, the opportunistic human pathogen *S. marcescens* species is harmful to human health, making it unsuitable for drug production by fermentation process (Domröse *et al.,* 2015; Liu *et al.,* 2017). Therefore, genetic engineering could create promising strategy for Pg production.

A biosynthetic gene cluster consisting 14-15 genes was identified to be responsible for Pg production in *Serratia* (Harris *et al.,* 2004). Among these, a membrane located enzyme named PigC, with an approximate molecular weight of mass 100 kDa, plays a crucial role in the final step to produce prodigiosin

(Chawrai *et al.,* 2008; Harris *et al.,* 2004; Williamson *et al.,* 2005). This enzyme is involved in the enzyme-based condensation reaction of 4-methoxy-2, 2'-bipyrrole-5-carboxyaldehyde (MBC) with 2-methyl-3-amylpyrrole (MAP). Previous reports indicated that recombinant PigC protein is capable of using a wide range of substrate to synthesize prodigiosin and its derivatives applied in pharmaceutical industry (Chawrai *et al.,* 2012; Chawrai *et al.,* 2008; Klein *et al.,* 2017; You *et al.,* 2018a; You *et al.,* 2018b). In 2017, Liu and colleagues selected a mutant which could produce up to 12 fold increase in prodigiosin production when compared to the wild-type strains (Liu *et al.,* 2017). In addition, prodigiosin biosynthesis gene cluster of *S. marcescens* was successfully transformed into *Pseudomonas putida,* leading to produce recombinant prodigiosin with 94 mg/Liter (Domröse *et al.,* 2015). Therefore, it paves the way for applying recombinant PigC protein in pharmaceutical manufacturing. However, studying on PigC has not yet been performed in Vietnam. The aim of this study was to clone and express the recombinant PigC protein in *E. coli* in order to provide a potential material for producing large-scale prodigiosin.

MATERIALS AND METHODS

Materials

Serratia sp. M5 strain was provided by Enzyme Biotechnology laboratory, Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST). *E. coli* DH10B (Invitrogen) and *E. coli* BL21 (DE3) strains (Novagen) were used as cloning and expression hosts, respectively. Vector pJET1.2 was utilized as cloning one and pET22b (Novagen) as expression vector. *E. coli* and *Serratia* sp. strains were respectively grown in media containing 1% NaCl, 1% tryptone, 0.5% yeast extract (Merck, Germany) at 37°C and 30°C.

Methods

Primer design for amplification of pigC gene

Because of highly variable sequences of *pigC* among strains available in Genbank, complete sequences of Pg clusters from different strains available in Genbank were downloaded and aligned. The consensus sequences being 300 nucleotides away from the *pigC* gene were chosen to design primers. After cloning, the obtained sequence of the region covering the *pigC* gene was used to design expression primers. The primer sequences were listed in Table 1.

Total DNA extraction and sequence amplification

Total DNA was extracted from *S. macescence* using GeneJET Genomic purification kit according to the manufacturer's instruction. The *pigC* gene was amplified with *pigC* universal pair of primers (Table 1) using the following program: 98^{o}C for 30 sec; 40 cycles of 98^{o}C 10 sec, 58^{o}C for 15 sec, 72^{o}C for 1 min 30 sec, and a final cycle of 72^{o}C for 5 min.

The targeted band was purified via GeneJET gel extraction kit according to the vendor's manual. This candidate sequence was then ligated into pJET1.2 blunt vector and transformed into *E. coli* DH10B chemically competent cells as described before. Several colonies were selected for further examination using plasmid extraction and sequencing by pJET1.2 specific primers (Table 1).

PigC encoded gene was reamplified from colonies containing recombinant vector using the primers pigC_HindIII_F and pigC_XhoI_R (Table 1). The amplified product was digested with *Hind*III and *Xho*I (ThermoFisher Scientific, USA) and ligated into the vector pET22b. The ligated product was transformed into *E. coli* DH10B strain (Sambrook *et al.,* 2001). Some clones were further examined using PCR, plasmid extraction, enzyme digestion and sequencing by T7 primers (Table 1).

Table 1. Primer pairs used in this study.

Primer names	Primer sequences
PigC_universal_F	5' TATTCAYTTCGCYAATCAGGACA 3'
PigC_universal_R	5' AAATTCGGYCACKAYAMAGCC 3'
PigC_HindIII.F	5' AAAAAAAAGCTTAATCCTACCCTGGTGGTTGA 3'
PigC_XhoI.R	5' AAAAAACTCGAGGCCATCGGCACGTTCTC 3'
pJET1.2F	5' CGACTCACTATAGGGAGAGCGGC 3'
pJET1.2R	5' AAGAACATCGATTTTCCATGGCAG 3'
T7 Promoter	5' TAATACGACTCACTATAGGG 3'
T7 Terminator	5' GCTAGTTATTGCTCAGCGG 3'

Expression of pigC in E. coli BL21 (DE3) and protein purification

The recombinant vector with the correct sequence was transformed into BL21 (DE3) and expressed using auto-induced medium. In brief, overnight culture was inoculated into auto-induced medium containing tryptone 10 g/l, yeast extract 5 g/l, NaCl 5 g/l, KH_2PO_4 6.8 g/l, Na_2HPO_4 7.1 g/l, $(NH_4)_2SO_4$ 3.3g/l, $MgSO_4$ 2 mM, glucose 0.05%, glycerol 0.5%, and lactose 0.2% w/v). Firstly, the culture was incubated at 37°C/200 rpm for 3 hours. The temperature was then decreased to 16°C and the culture was incubated for 16 hours for protein expression. Afterwards, the bacteria were pelleted by centrifugation at 6,000 g for 10 min. The pellet was solubilized in phosphate buffered saline (PBS, pH 7.4) plus 8M urea (Sigma-Aldrich, USA) and sonicated for 1 min to release proteins from lysate. Subsequently, the soluble extract was centrifuged at 13,000 g for 30 min at 4°C and the supernatant was transferred to a new tube.

The solution containing the targeted protein was supplemented with lysis and column equilibration buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole and 8M urea) at a ratio of 1:1 and then incubated with HisPur™ Ni-NTA Resin (Thermo Fisher Scientific, USA) at 4°C. After 2 hours, the flow through was removed and the protein-bound resin in Ni-NTA column was washed thoroughly three times with wash buffer containing 50 mM NaH_2PO_4, 300 mM NaCl, 50 mM imidazole (Sigma-Aldrich, USA) and 8M urea. The recombinant proteins were achieved by eluting with elution buffer including the same ingredients with wash buffer but high concentration of imidazole (500 mM) from the Ni-NTA column and stored at -20°C. The expression and purification level were quantified by SDS-PAGE and Western blot analyses.

RESULTS AND DISCUSSION

Cloning *pigC* region from *Serratia* sp. strain M5

Electrophoresis result showed a shaped band with approximately expected size of 3000 bps representing *pigC* region sequences (Figure 1A). After purification, this obtained band was inserted into pJET1.2 vector and transformed into *E. coli* DH10B strain. Five clones were randomly selected for plasmid extraction. Result indicated that two clones contained the recombinant pJET1.2/insert (Figure 1B). The clones were further confirmed the presence of the insert by PCR method using a pair of pJET1.2 primers (Table 1), and an obviously single band of around 3 kb corresponding to *pigC* was observed in these clones (Figure 1C). Additionally, Sanger sequencing these PCR products indicated that *pigC* region was successfully amplified and inserted into vector pJET1.2.

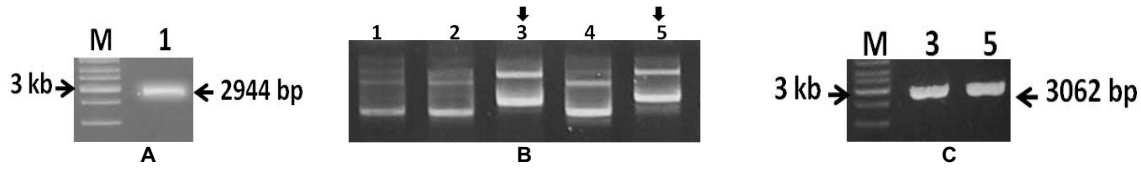


Figure 1. (A) Electrophoresis of *pig*C amplification. M: Marker 1 kb (ThermoFisher Scientific), 1: PCR product of *pig*C region; (B) Plasmid extraction. M: Marker 1kb (Thermo Fisher Scientific), 1, 2, 3, 4, 5: Plasmid extraction from colonies 1, 2, 3, 4, 5, respectively. The arrows indicate recombinant vectors selected for further experiments; (C) PCR amplification using pJET1.2 specific primers. 3, 5: PCR products obtained from the respective recombinant plasmid number 3 and 5.

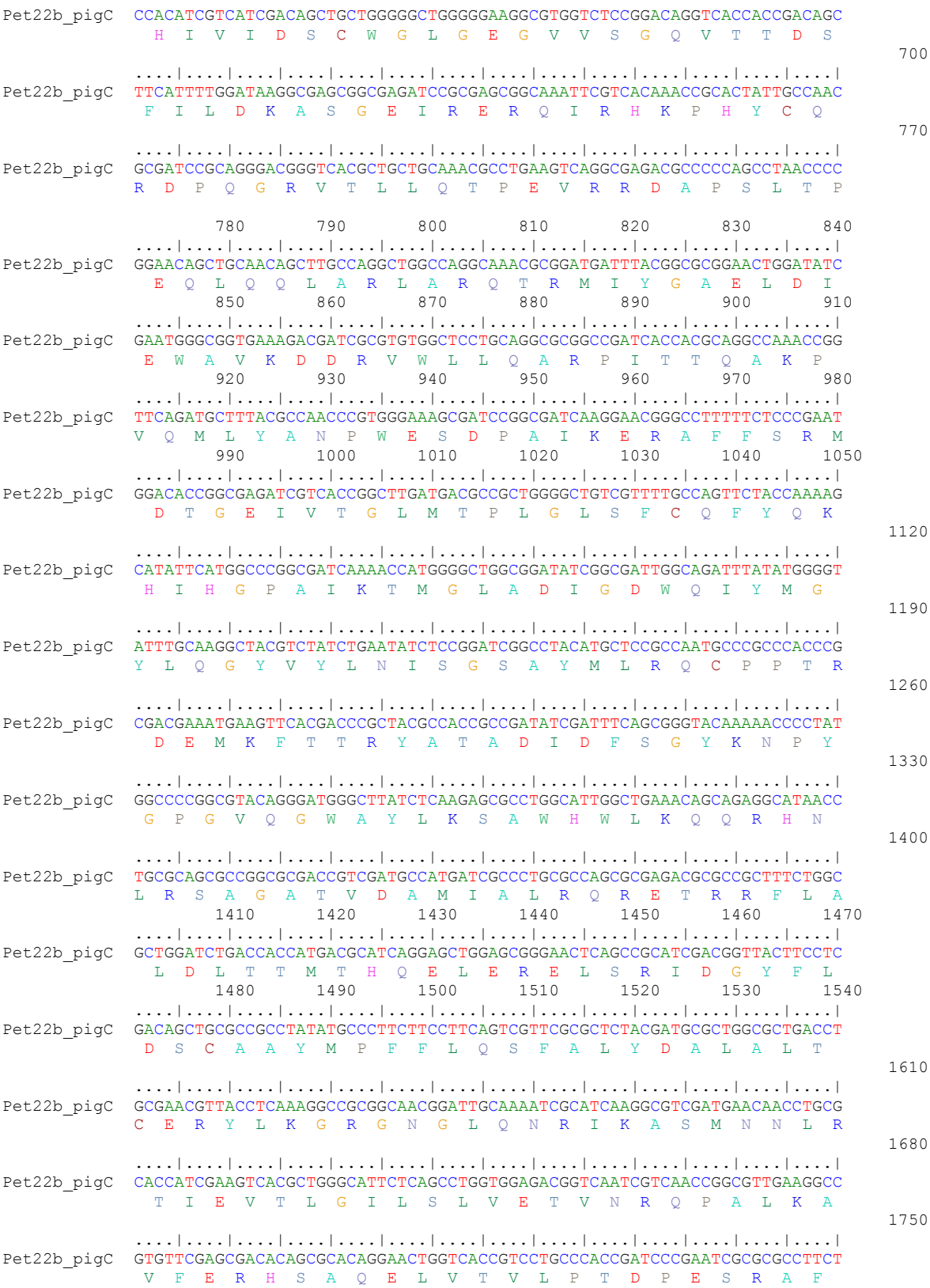
Sequence alignment indicated that the *pigC* sequence of *Serratia* sp. strain M5 isolated in Vietnam has over 98% identity at the nucleotide level when compared with *pigC* of other strain *S. marcescens* WW4 from GenBank. However, it should be noted that the difference of *pigC* gene between *Serratia* sp. M5 strain and the well-known strains *S. marcescens* 39006 as well as *Serratia* sp. AS9 was up to 25 – 30% (Table 2). Previous reports observed that the proportion of similarity between *Serratia* strain 39006 and *S. marcescens* Sma was 75.6% (Harris *et al.,* 2004). The author's hypothesis was that *Serratia* 39006 strain was atypical *S. marcescens* strain with significant differences in genome characteristics. The similar reason could be used to explain the difference between *pigC* of M5 strain and that of strains AS9, AS12 or AS13 of *S. plymuthica* (Neupane *et al.,* 2012a; Neupane *et al.,* 2012b; Neupane *et al.,* 2012c). In addition, *pigC* clusters like Sma strain were observed in 6 pigmented strains belonging to *S. marcescens* (Harris *et al.,* 2004).

PigC expression in *E. coli* BL21 (DE3)

Complete sequence of *pigC* was amplified from recombinant vector pJET1.2/pigC and inserted into vector pET22b. After cloning, the final product was transformed into DH10B as described above. The recombinant vector was further examined by Sanger sequencing. As a result, there was no stop mutation detected in this construction, and an amino acid substitution at the position K265R was revealed when aligned with the *pigC* of *S. marcescens* WW4 (Figure 2). On the other hand, deduced amino acid sequence comparison showed some differences scattered throughout the protein sequence when compared to those of other strains *Serratia* sp. 39006 and AS9 (Table 2).

Table 2. Nucleotide (lower triangle) and amino acid (upper triangle) identification matrix of *pigC* from *Serratia* sp. M5 strain in comparison to reference database from GenBank.

No	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	HQ833702S.marcescens jx1		99.5%	99.5%	99.2%	98.9%	98.9%	99.2%	98.9%	79.1%	79.1%	79.1%	74.8%	99.7%	99.6%
2	CP005927 Serratia sp. FS14	99.5%		99.5%	99.2%	98.9%	98.9%	99.2%	98.9%	79.2%	79.2%	79.2%	75.1%	99.5%	99.4%
3	CP021984 S.marcescens S2I7	99.4%	99.6%		99.4%	99.2%	99.2%	99.2%	98.9%	79.3%	79.3%	79.3%	74.9%	99.7%	99.6%
4	CP013046 S.marcescens B3R3	99.3%	99.4%	99.5%		99.7%	99.1%	99.1%	98.8%	79.3%	79.3%	79.3%	75.0%	99.2%	99.1%
5	CP016032 S.marcescens U36365	99.2%	99.2%	99.4%	99.8%		98.8%	98.8%	98.6%	79.2%	79.2%	79.2%	75.0%	98.9%	98.8%
6	CP016948 Serratia sp. YD25	98.8%	98.8%	98.9%	98.9%	98.8%		99.1%	98.8%	79.1%	79.1%	79.1%	74.9%	98.9%	98.8%
7	AJ833002 S.marcescens	98.8%	98.8%	98.8%	98.8%	98.7%	98.6%		99.7%	79.4%	79.4%	79.4%	75.4%	99.2%	99.1%
8	CP018927 S.marcescens UMH8	98.8%	98.7%	98.8%	98.7%	98.6%	98.5%	99.7%		79.4%	79.4%	79.4%	75.3%	98.9%	98.8%
9	CP002775 Serratia sp. AS13	75.3%	75.3%	75.4%	75.3%	75.2%	75.1%	75.3%	75.2%		100.0%	100.0%	79.7%	79.1%	79.1%
10	CP002774 Serratia sp. AS12	75.3%	75.3%	75.4%	75.3%	75.2%	75.1%	75.3%	75.2%	100.0%		100.0%	79.7%	79.1%	79.1%
11	CP002773 S.plymuthica AS9	75.3%	75.3%	75.4%	75.3%	75.2%	75.1%	75.3%	75.2%	100.0%	100.0%		79.7%	79.1%	79.1%
12	AJ833001 Serratia 39006	71.6%	71.6%	71.5%	71.5%	71.5%	71.4%	71.4%	71.4%	76.7%	76.7%	76.7%		74.8%	74.8%
13	CP003959 S.marcescens WW4	99.5%	99.6%	99.8%	99.3%	99.2%	98.9%	98.9%	98.8%	75.3%	75.3%	75.3%	71.5%		99.8%
14	M5	99.6%	99.5%	99.7%	99.3%	99.2%	98.9%	98.9%	98.8%	75.3%	75.3%	75.3%	71.6%	99.8%	


Pet22b_pigC CCACATCGTCATCGACAGCTGCTGGGGGCTGGGGGAAGGCGTGGTCTCCGGACAGGTCACCACCGACAGC
H I V I D S C W G L G E G V V S G Q V T T D S
700
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC TTCATTTTGGATAAGGCGAGCGGCGAGATCCGCGAGCGGCAAATTCGTCACAAACCGCACTATTGCCAAC
F I L D K A S G E I R E R Q I R H K P H Y C Q
770
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Pet22b_pigC GCGATCCGCAGGGACGGGTCACGCTGCTGCAAACGCCTGAAGTCAGGCGAGACGCCCCCAGCCTAACCCC
R D P Q G R V T L L Q T P E V R R D A P S L T P
780 790 800 810 820 830 840
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Pet22b_pigC GGAACAGCTGCAACAGCTTGCCAGGCTGGCCAGGCAAACGCGGATGATTTACGGCGCGGAACTGGATATC
E Q L Q Q L A R L A R Q T R M I Y G A E L D I
850 860 870 880 890 900 910
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC GAATGGGCGGTGAAAGACGATCGCGTGTGGCTCCTGCAGGCGCGGCCGATCACCACGCAGGCCAAACCGG
E W A V K D D R V W L L Q A R P I T T Q A K P
920 930 940 950 960 970 980
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC TTCAGATGCTTTACGCCAACCCGTGGGAAAGCGATCCGGCGATCAAGGAACGGGCCTTTTTCTCCCGAAT
V Q M L Y A N P W E S D P A I K E R A F F S R M
990 1000 1010 1020 1030 1040 1050
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC GGACACCGGCGAGATCGTCACCGGCTTGATGACGCCGCTGGGGCTGTCGTTTTGCCAGTTCTACCAAAAG
D T G E I V T G L M T P L G L S F C Q F Y Q K
1120
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC CATATTCATGGCCCGGCGATCAAAACCATGGGGCTGGCGGATATCGGCGATTGGCAGATTTATATGGGGT
H I H G P A I K T M G L A D I G D W Q I Y M G
1190
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Pet22b_pigC ATTTGCAAGGCTACGTCTATCTGAATATCTCCGGATCGGCCTACATGCTCCGCCAATGCCCGCCCACCCG
Y L Q G Y V Y L N I S G S A Y M L R Q C P P T R
1260
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Pet22b_pigC CGACGAAATGAAGTTCACGACCCGCTACGCCACCGCCGATATCGATTTCAGCGGGTACAAAAACCCCTAT
D E M K F T T R Y A T A D I D F S G Y K N P Y
1330
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Pet22b_pigC GGCCCCGGCGTACAGGGATGGGCTTATCTCAAGAGCGCCTGGCATTGGCTGAAACAGCAGAGGCATAACC
G P G V Q G W A Y L K S A W H W L K Q Q R H N
1400
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC TGCGCAGCGCCGGCGCGACCGTCGATGCCATGATCGCCCTGCGCCAGCGCGAGACGCGCCGCTTTCTGGC
L R S A G A T V D A M I A L R Q R E T R R F L A
1410 1420 1430 1440 1450 1460 1470
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC GCTGGATCTGACCACCATGACGCATCAGGAGCTGGAGCGGGAACTCAGCCGCATCGACGGTTACTTCCTC
L D L T T M T H Q E L E R E L S R I D G Y F L
1480 1490 1500 1510 1520 1530 1540
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Pet22b_pigC GACAGCTGCGCCGCCTATATGCCCTTCTTCCTTCAGTCGTTCGCGCTCTACGATGCGCTGGCGCTGACCT
D S C A A Y M P F F L Q S F A L Y D A L A L T
1610
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC GCGAACGTTACCTCAAAGGCCGCGGCAACGGATTGCAAAATCGCATCAAGGCGTCGATGAACAACCTGCG
C E R Y L K G R G N G L Q N R I K A S M N N L R
1680
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC CACCATCGAAGTCACGCTGGGCATTCTCAGCCTGGTGGAGACGGTCAATCGTCAACCGGCGTTGAAGGCC
T I E V T L G I L S L V E T V N R Q P A L K A
1750
....|....|....|....|....|....|....|....|....|....|....|....|....|....|....|
Pet22b_pigC GTGTTCGAGCGACACAGCGCACAGGAACTGGTCACCGTCCTGCCCACCGATCCCGAATCGCGCGCCTTCT
V F E R H S A Q E L V T V L P T D P E S R A F

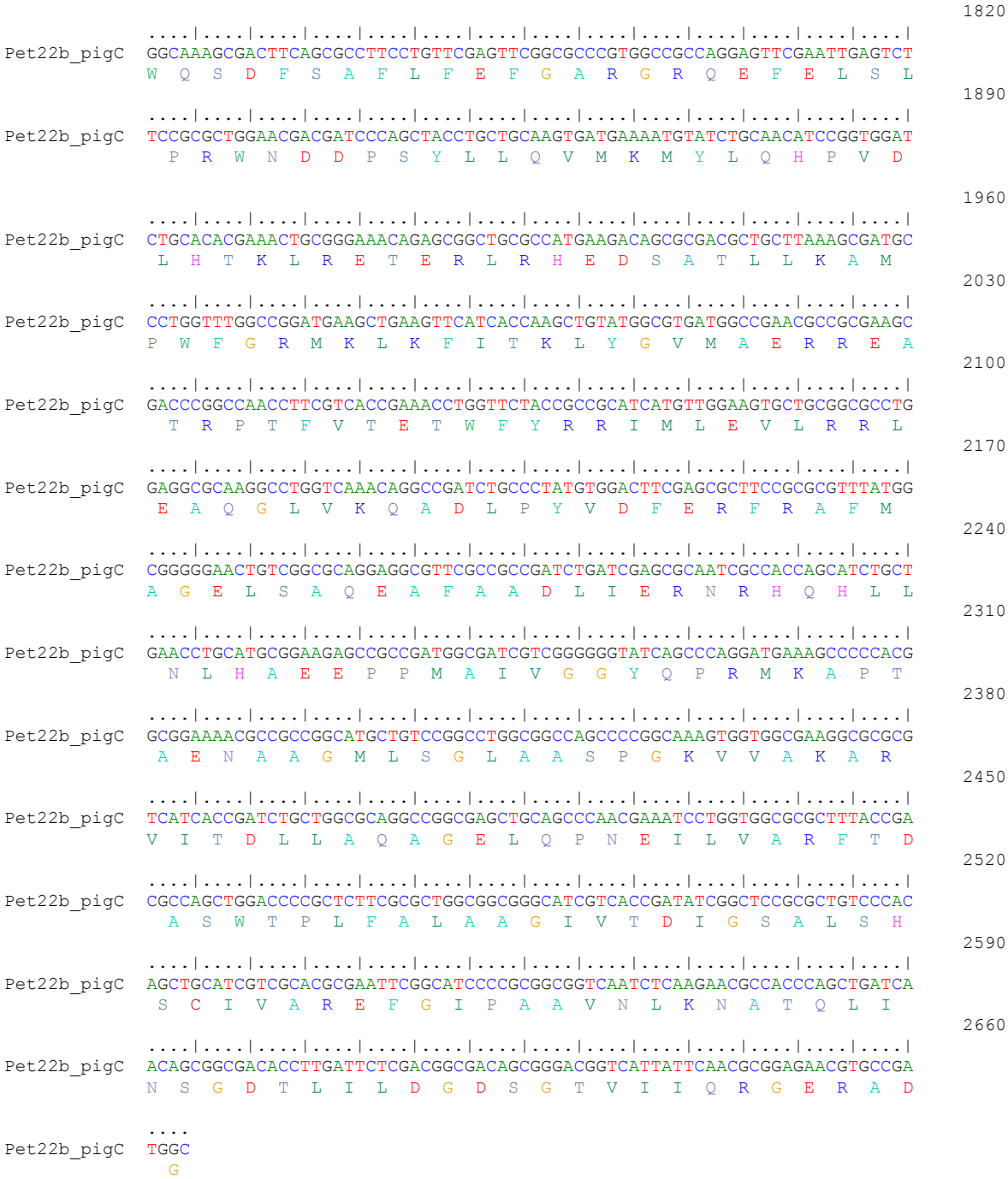

Figure 2. Nucleotide sequence and deduced amino acid sequence of pET22b/pigC.

Recombinant vector pET22b/*pig*C was initially expressed in *E. coli* BL21 (DE3) in LB medium supplemented 100 µg/ml ampicilline and 1 mM IPTG at 37°C/6 hours or at 16°C/16 hours. However, the expression of *pigC* under this condition was really weak (data not shown). Previous reports indicated that maximum production of PigC was achieved using optimized auto-induced medium (You *et al.,* 2018b), which was therefore used for expressing the targeted pigC protein. As a result, a

sharp band of approximately 100 kDa was observed in Western blot using anti his-tag antibody (Figure 3A).

In order to purify the PigC protein for further study, the phase of recombinant PigC under expression conditions was investigated. Results indicated that recombinant PigC mostly accumulated in the pellet fraction (data not shown). Furthermore, it should be mentioned that PigC protein was demonstrated as a membrane binding protein *in vivo* and *in vitro* with full activity observed in pellets (Chawrai *et al.,* 2012; You *et al.,* 2018a). In these studies, therefore, a majority of recombinant PigC was in the insoluble fraction. In addition, enzymatic reaction indicated that enzyme activity was maintained in this fraction whereas no activity was identified in the supernatant. For all above the reasons, we further purified PigC protein under the denaturing condition using Ni-NTA resin. As expected, we achieved a purified precise band with the size corresponding to PigC protein (Figure 3B). This purified protein can be refolded and applied for examining the characteristics of recombinant PigC protein as well as producing prodigiosin.

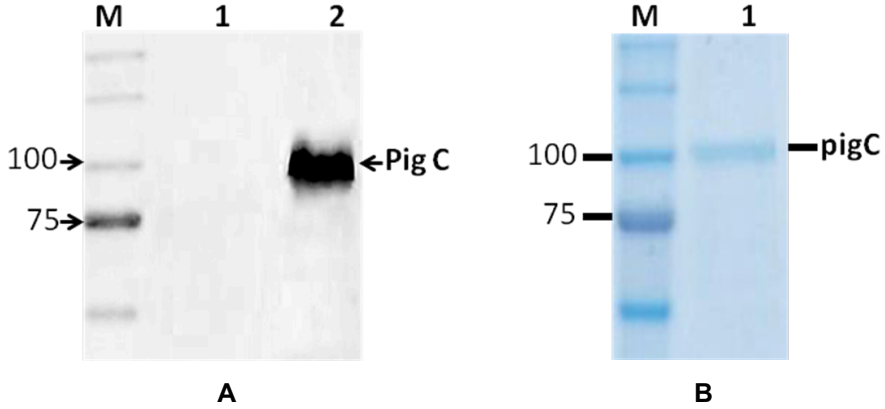

Figure 3. (A) Western Blot result of expression of recombinant PigC protein in *E. coli*. M: marker gangnam stain (Intron), 1: *E. coli* BL21 (DE3) strain containing vector pET22b, 2: *E. coli* BL21 (DE3) strain containing recombinant vector pET22b_LacP_*pig*C; (B) Purification of PigC protein. M: marker Gangnam stain (Intron), 1: Purified PigC.

CONCLUSION

In the present study, the *pigC* sequence from *Serratia* sp. M5 isolated in Vietnam was successfully cloned into pJET1.2 vector. This gene, 2667 bps in length, encoded a protein of 888 amino acids. Sequence comparison indicated the obtained sequence of the *pigC* gene was highly similar to that of *S. marcescens* WW4, sharing over 98% of their DNA sequence. Meanwhile, it was 25 – 30% different from those of *Serratia* sp. 39006 and AS9 strains. The *pigC* gene was then inserted into pET22b and expressed in *E. coli* BL21 (DE3) using auto-induced medium. The targeted PigC, being a 100 kDa protein in SDS-PAGE as well as in Western blot analyses, was purified by Ni-NTA column. The prodigiosin production using the purified recombinant pigC is being examined in the further study.

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TÁCH DÒNG VÀ BIỂU HIỆN GEN *pigC* TRONG *ESCHERICHIA COLI*

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TÓM TẮT

Prodigiosin (Pg) có hoạt tính kháng ung thư hoặc kháng vi sinh vật được tổng hợp từ phản ứng ngưng kết 4-methoxy-2, 2'-bipyrrole-5-carboxyaldehyde (MBC) và 2-methyl-3-amylpyrrole (MAP) dưới sự xúc tác của enzyme PigC. Mặc dù PigC đóng vai trò quan trọng trong quá trình tổng hợp prodigiosin tuy nhiên chưa có nghiên cứu nào về protein này được thực hiện ở Việt Nam. Trong nghiên cứu này, chúng tôi đã tách dòng và biểu hiện protein PigC ở *Escherichia coli.* Sử dụng cặp mồi chung cho vùng gen *pigC*, đoạn gen khoảng 3kb chứa *pigC* được khuếch đại thành công từ chủng *Serratia* sp. chủng M5. Kết quả so sánh trình tự cho thấy, trình tự *pigC* có kích thước 2667 bp của chủng M5 phân lập tại Việt Nam tương đồng 98% với trình tự của các chủng *S. marcescens* khác, tuy nhiên khác biệt tới 30% khi so với chủng *S. marcescens* 39006 và AS9 với mã số tương ứng AJ833001 và CP002773. Trình tự gen mã hoá protein PigC với kích thước 2664 bp sau khi được nhân lên sử dụng cặp mồi có chứa vị trí nhận biết enzyme cắt *Hind*III và *Xho*I được đưa vào vector pET22b tạo thành vector tái tổ hợp có gắn đuôi His và biểu hiện ở *E. coli* sử dụng môi trường tự cảm ứng. Kết quả điện di cho thấy protein PigC đã được biểu hiện thành công với kích thước ~100 kDa và được kiểm tra bằng kĩ thuật Western Blot sử dụng kháng thể kháng 6 histidine. Kết quả này tạo nguồn nguyên liệu khởi đầu cho việc nghiên cứu tạo prodigiosin từ protein tái tổ hợp ở Việt Nam.

Từ khoá: *MAP, MBC*, *pigC*, *prodigiosin*, *Việt Nam*