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CLONING AND EXPRESSION OF *pigI* GENE IN *Escherichia coli* BL21 (DE3)

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ABSTRACT

Prodigiosin (Pg), a secondary metabolite with anticancer and antimicrobial activities, can be produced in *Serratia marcescens* bacteria through the condensation reaction of 4-methoxy-2, 2'-bipyrrole-5-carboxyaldehyde (MBC) and 2-methyl-3-amylpyrrole (MAP). Among these, the MBC synthetic pathway is started by the conversion of L-proline to L-proline-AMP before this complex is covalently attached to PigG. This reaction is catalyzed by an L-prolyl-AMP ligase named *PigI*. Therefore, *PigI* protein plays an important role in the prodigiosin biosynthetic pathway. However, studies related to *PigI* protein have not been carried out in Vietnam yet. In this work, the *pigI* gene was cloned and expressed in *Escherichia coli* DH10B and BL21 (DE3), respectively. Sequence alignment results revealed that the obtained *pigI* gene is 99.7% identical to the four strains, CP027798, CP027796, CP021984 and CP003959. This recombinant vector pJET1.2/pigI was used to reamplify *pigI*, and the acquired amplicon was inserted into pET22b vector at the site of *Hin*dIII and *Xho*I. The clone *E. coli* BL21 (DE3) containing the recombinant vector pET22b/pigI was expressed in an auto-induced medium. The presence of *PigI* protein in the lysate was identified due to a 53 kDa band through Western Blot analysis using an anti-his-tag antibody. The results of our study provide a potential method for producing prodigiosin from recombinant protein in Vietnam.

Keywords: MAP, MBC, *pigI*, prodigiosin, Vietnam.

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INTRODUCTION

Prodigiosin (Pg), a secondary metabolite produced from *Serratia marcescens*, is of high interest because of its anti-cancer activities (Diaz-Ruiz et al., 2001; Hong et al., 2014; Montaner et al., 2000; Prabhu et al., 2016). Its potential as an anti-cancer agent against various cancer cell lines but not normal cell lines was well demonstrated (Francisco et al., 2007; Sumathi et al., 2014). Furthermore, its antiparasitic and antimicrobial effects were indicated (Lapenda et al., 2015; Rahul et al., 2015). Therefore, many studies have focused on the biosynthesis of prodigiosin and the gene cluster involved in this pathway with the goal of improving its production (Domröse et al., 2015; Harris et al., 2004; Klein et al., 2017; Williamson et al., 2005).

The biosynthesis pathway for prodigiosin is demonstrated as a pathway in which 2-methyl-3-amylpyrrole (MAP) and 4-methoxy-2,2'-bipyrrole-5-carboxylaldehyde (MBC) are independently synthesized and then condensed to prodigiosin by PigC (Harris et al., 2004). Among these, the MBC synthetic pathway is started by the conversion of L-proline to L-proline-AMP before this complex is covalently attached to PigG. This reaction is catalysed by an L-prolyl-AMP ligase named *PigI*. Although the function of *PigI* is well-known, its enzymatic characteristics are not clear.

Until now, in Vietnam, there have been a few studies on prodigiosin, mainly on its antibiotic and anti-insect characterizations (Nguyen & Nguyen, 2015; Thanh & Quyen, 2015). In addition, preliminary crystallographic data of *PigI* was reported (Han et al., 2014). In particularly, the *PigI* encoded gene of *S. marcesence* strain FS14 was cloned and expressed in *E. coli* strain C43 (DE3). After purification, *PigI* underwent methylation and crystallization. Results indicated that *PigI* belonged to the space group P1 with a diffraction resolution of 2.0 A^{o} resolution and unit-cell parameters a = 51.2, b = 62.8, c = 91.3 A^{o}, α = 105.1, β-90.1, γ = 92.2^{o}. Other results revealed the specific function of *PigI* in MBC synthesis pathway. In studies on its expression and catalytic function, *PigI* of *Serratia* strain 39006 has been found to reversibly catalyze L-Proline to L-Proline-AMP, using ATP and releasing PPi. After that, *PigI* tethers L-Proline-AMP to phosphopantetheinylated-PigG which is further metabolized to other steps in MBC synthesis. However, it should be noted that the differences between the Pig cluster of *Serratia* 39006 strain and other *S. marcescence* strains were quite high (Harris et al., 2004). In addition, studies on *PigI* have not yet been carried out in Vietnam. This study aimed to clone and express the recombinant *PigI* in *E. coli*. The result of this study is the initial step for revealing the characteristics of *PigI*.

MATERIAL AND METHOD

Materials

Serratia sp. strain M5 was provided by Enzyme Biotechnology laboratory, Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (IGR). *E. coli* strain DH10b (Invitrogen) and *E. coli* strain BL21 (DE3) (Novagen) were used as cloning and expression hosts, respectively. pJET1.2 and pET22b (Novagen) were used as cloning and expression vectors, respectively. *E. coli* and *Serratia* sp. strains were grown in a medium containing 1% NaCl, 1% tryptone, 0.5% yeast extract (Merck, Germany) at 37 ^{o}C and 30 ^{o}C, respectively.

Methods

Primer design for amplification of pigI gene

The primer pairs for specific amplification *pigI* were designed based on the *pigI* sequence from Genbank with accession number AJ833002.

Table 1. Primer pairs used in this study

Primer names	Primer sequences
PigI_F	5’ ATGGCAACCTTCATTTCACCGAT 3’
PigI_R	5’ CTACGCGCATTCACCTCGGACAA 3’
PigI_HindIII.F	5’ AAAAAAAGCTTGCAACCTTCATTTCACCGAT 3’
PigI_XhoI.R	5’ AAAAACTCGAGCGCGCATTCACCTCGGACAA 3’
pJET1.2F	5’ CGACTCACTATAGGGAGAGCGGC 3’
pJET1.2R	5’ AAGAACATCGATTTTCCATGGCAG 3’
T7 Promoter	5’ TAATACGACTCACTATAGGG 3’
T7 Terminator	5’ GCTAGTTATTGCTCAGCGG 3’

Total DNA extraction and sequence amplification

Total DNA was extracted from *S. macescens* using a GeneJET Genomic purification kit according to the manufacturer’s instructions. *PigI* gene was amplified by *PigI*_F and *PigI*_R primers as follows: 98 ºC for 30 sec; 40 cycles of 98 ºC 10 sec, 58 ºC for 15 sec, 72 ºC for 30 sec, final cycle of 72 ºC for 5 min.

The target band was purified via a GeneJET Gel extraction kit before double digestion with the mentioned enzyme according to the vendor’s manual. This candidate sequence was then ligated into pJET1.2 blunt vector and transformed to *E. coli* DH10B chemical competence cell. Several colonies were selected for further examination using plasmid extraction and sequencing by pJET1.2 specific primers (Table 1).

PigI encoded gene was reamplified from suitable clones using specific primers PigI_HindIII.F and PigI_XhoI.R (Table 1). The amplified product was digested with *Hin*dIII and *Xho*I (ThermoFisher Scientific, USA) and ligated into the vector pET22b. The ligated product was transformed into *E. coli* strain DH10b (Sambrook et al., 2001). Some clones were further examined using PCR, plasmid extraction, enzyme digestion and sequencing by T7 primers (Table 1).

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

The recombinant vector with the modified sequence was transformed into BL21 (DE3) *E. coli* and recombinant protein was expressed. The overnight culture was inoculated into an LB medium containing peptone 10 g/L, yeast extract 5 g/L, NaCl 5 g/L. Firstly, the culture was incubated at 37 ºC/200 rpm to reach an OD of 0.6−0.8 and IPTG was added to the final concentration of 1 mM or 0.5 mM. The culture was incubated for 3 hours for protein expression at 37 ºC or 25 ºC. Afterwards, bacteria were pelleted by centrifuging at 6,000 x g for 10 min. The pellet was solubilized in phosphate-buffered saline (PBS, pH 8) (Sigma-Aldrich, USA) and sonicated for 1 min to release proteins from the lysate. Subsequently, the soluble extract was centrifuged at 13,000 x g for 30 min in a refrigerated centrifuge and the supernatant was transferred to a new tube. The inclusion body was solubilized in an equal volume of PBS plus 8M Urea, centrifuged at 13,000 x g for 30 min in a refrigerated centrifuge to remove the insoluble materials. The expression level of recombinant *PigI* was examined by SDS-PAGE and Western blot analysis.

RESULT AND DISCUSSION

Cloning *pigI* region from Serratia marcescens strain M5

Using a pair of *pigI* primers (Table 1), a band of approximately 1500 bp was observed from the amplified product obtained from *Serratia* sp. SM after electrophoresis on 0.8% agarose gel. This product was then purified by a gel extraction kit according to the manufacturer's instructions (Fig. 1A).

The purified *pigI* amplicon was ligated with pJET1.2 vector. Afterwards, the recombinant vector was transformed into *E. coli* DH10B competent cells. To precisely select the recombinant vectors carrying the insert *pigI*, transformed *E. coli* clones on LB medium containing ampicillin were screened. Several colonies were cultured and used to extract plasmids. These plasmid products would serve as templates for amplification using specific primers to examine the presence of the *pigI* gene (Table 1). The electrophoresis results of amplification products from six colonies showed that they all carried recombinant vectors containing the insert *pigI* as expected (Fig. 1B).

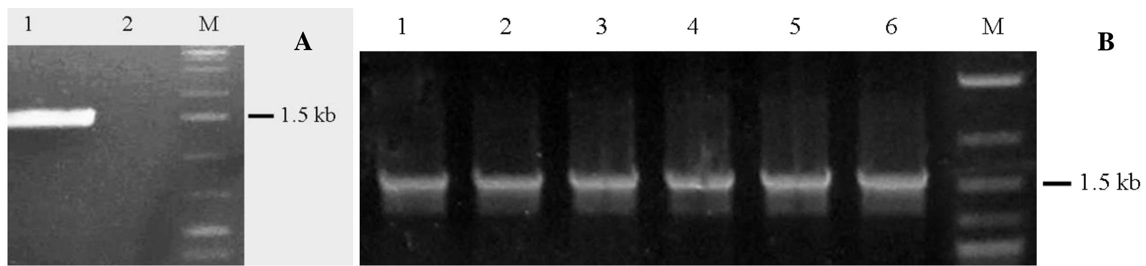

Figure 1. **A**. The amplification of *pigI* gene from *S. marcescens* strain M5. 1: PCR product of *pigI* region, 2: Negative control, M: Marker 1 kb plus (ThermoFisher Scientific); **B**. PCR amplification of *pigI* region using colonies. 1-6: PCR products obtained from six colonies, M: Marker 1 kb plus (NEB)

To accurately confirm which colonies carried recombinant vectors containing the *pigI* gene, all six recombinant vectors were sequenced. Analyzing the data revealed that all six *pigI* sequences of these recombinant vectors matched each other with 100% identity and their sizes were confirmed to be 1476 bp, same as theoretical calculation.

In comparison with the sequence of *pigI* of *S. marcescens* WW4 (accession no. CP003959), there were differences in four positions including 335A>G, 507A>G, 1286A>G, and 1464C>T. Additionally, alignment of the *pigI* gene in our strain against *pigI* sequences published on GenBank (CP027798, CP027796, CP021984, CP003959, CP005927, CP016032, CP013046, CP016948, CP031316, AP019009, CP027300, CP018927 and AJ833002) using BioEdit showed a high level of similarity to reference strains. The highest resemblance between our interest gene and those of *S. marcescens* strains CP027798, CP027796, CP021984 and CP003959 were 99.7% while the lowest similarity was 97.6% when compared with that of the *S. marcescens* AJ833002 strain (Table 2).

Table 2. The similarity between the *pigI* gene of *S. marcescens* strain with those of reference strains

No	Strain	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1	CP027798_*S. marcescens*_ KS10	ID	100.0%	99.6%	99.6%	99.6%	99.2%	99.1%	98.2%	98.0%	98.0%	97.8%	97.8%	97.5%	99.7%
2	CP027796_*S. marcescens* _EL1	100.0%	ID	99.6%	99.6%	99.6%	99.2%	99.1%	98.2%	98.0%	98.0%	97.8%	97.8%	97.5%	99.7%
3	CP021984_*S. marcescens* _S2I7	99.6%	99.6%	ID	99.7%	99.4%	99.1%	99.1%	98.2%	98.0%	97.9%	97.7%	97.7%	97.4%	99.7%
4	CP003959_*S. marcescens* _WW4	99.6%	99.6%	99.7%	ID	99.5%	99.3%	99.2%	98.3%	98.1%	98.0%	97.8%	97.8%	97.6%	99.7%
5	CP005927_*Serratia* sp._FS14	99.6%	99.6%	99.4%	99.5%	ID	99.1%	99.1%	98.2%	98.0%	97.9%	97.7%	97.7%	97.4%	99.5%
6	CP016032_*S. marcescens* _U36365	99.2%	99.2%	99.1%	99.3%	99.1%	ID	99.9%	98.6%	98.4%	98.3%	98.1%	98.1%	97.8%	99.1%
7	CP013046_*S. marcescens* _B3R3	99.1%	99.1%	99.1%	99.2%	99.1%	99.9%	ID	98.5%	98.3%	98.2%	98.0%	98.0%	97.8%	99.1%
8	CP016948_*Serratia* sp._YD25	98.2%	98.2%	98.2%	98.3%	98.2%	98.6%	98.5%	ID	98.0%	98.0%	97.9%	97.9%	97.7%	98.2%
9	CP031316_*S. marcescens* _N4-5	98.0%	98.0%	98.0%	98.1%	98.0%	98.4%	98.3%	98.0%	ID	99.7%	99.5%	99.3%	98.9%	98.1%
10	AP019009_*S. marcescens* _AS1	98.0%	98.0%	97.9%	98.0%	97.9%	98.3%	98.2%	98.0%	99.7%	ID	99.4%	99.3%	98.9%	98.0%
11	CP027300_*S. marcescens* _SGAir0764	97.8%	97.8%	97.7%	97.8%	97.7%	98.1%	98.0%	97.9%	99.5%	99.4%	ID	99.2%	98.8%	97.8%
12	CP018927_*S. marcescens* _UMH8	97.8%	97.8%	97.7%	97.8%	97.7%	98.1%	98.0%	97.9%	99.3%	99.3%	99.2%	ID	99.3%	97.8%
13	AJ833002_*S. marcescens _pigI*	97.5%	97.5%	97.4%	97.6%	97.4%	97.8%	97.8%	97.7%	98.9%	98.9%	98.8%	99.3%	ID	97.6%
14	pJET1.2_*pigI _Serratia* sp._SM	99.7%	99.7%	99.7%	99.7%	99.5%	99.1%	99.1%	98.2%	98.1%	98.0%	97.8%	97.8%	97.6%	ID

Expression of *pigI* in *E. coli* strain BL21 (DE3)

The complete sequence of *pigI* was amplified from recombinant vector pJET1.2/*pigI* and digested with two restriction enzymes *Xho*I and *Hin*dIII, then ligated into the multicloning site of expression vector pET22b. PET22b_pigI plasmid for expressing His-tagged protein was successfully constructed. Transformation of recombinant vectors into *E. coli* BL21 competent cells exhibited an abundance of single colonies. The presence of the insert in the expression vector was confirmed by comparing the different sizes of plasmid DNA extracted from recombinant *E. coli* colonies. It was further verified by digesting purified plasmid DNA with a couple of appropriate restriction enzymes. Analysis results indicated that the vector backbone and the insert are of the expected sizes, approximately 5.5 kb and 1.5 kb, respectively (Fig. 2). Six plasmids containing *pigI* gene numbered pET22b_pigI_SM1 to pET22b_pigI_SM6 were sequenced.

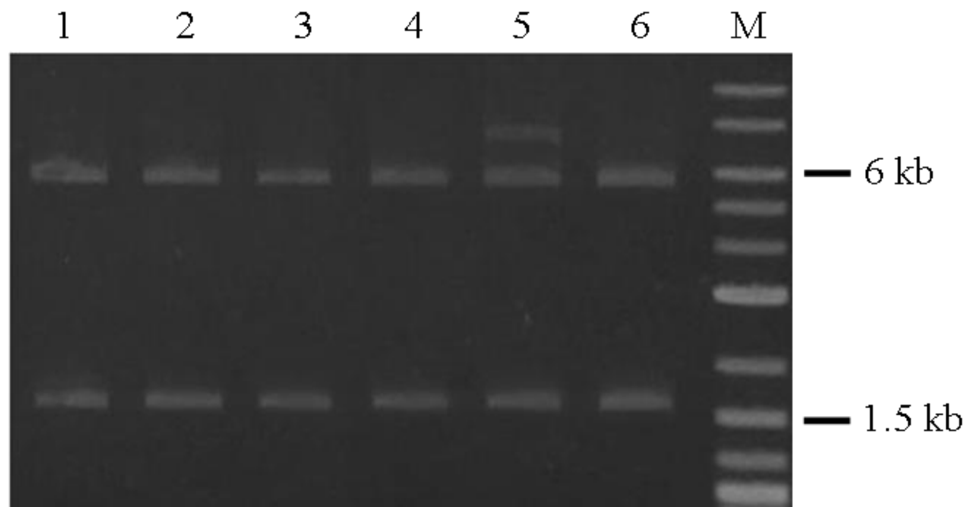

Figure 2. Electrophoresis results of digested plasmids on 0.8% agarose gel.
1-6: Six plasmids digested with *Xho*I and *Hin*dIII, M: Marker 1kb plus (NEB)

The result of the sequencing of six recombinant vectors pET22b_pigI_SM1 - SM6 showed that the *pigI* sequences of all six vectors were identical with a size of 1476 bp. Moreover, the open reading frame was confirmed to encode the *PigI* protein, including 491 amino acid residues and a stop codon. When compared with the *pigI* sequence of the *Serratia* sp. WW4 strain, there was only a substitution at position 727A > G (p. Asn232Ser) on the sequence of the *pigI* from *Serratia* sp. SM, leading to asparagine replaced by serine (Fig. 3).

In order to examine the expression of *pigI* in the *Escherichia coli* system, we investigated combinations of two temperatures and different IPTG concentrations. It is revealed that the target protein was expressed in all conditions with its molecular weight (MW) ca. of about 53 kDa. However, a majority of recombinant *PigI* was insoluble at 37 °C. Meanwhile, at 25 °C, they were obtained in soluble form (Fig. 4). As for different IPTG concentrations, there were no significant differences in expression level of the target protein. Therefore, we concluded that the optimal condition for recombinant PigI protein expression was obtained using the concentration of induction 0.5 mM at 25 °C.

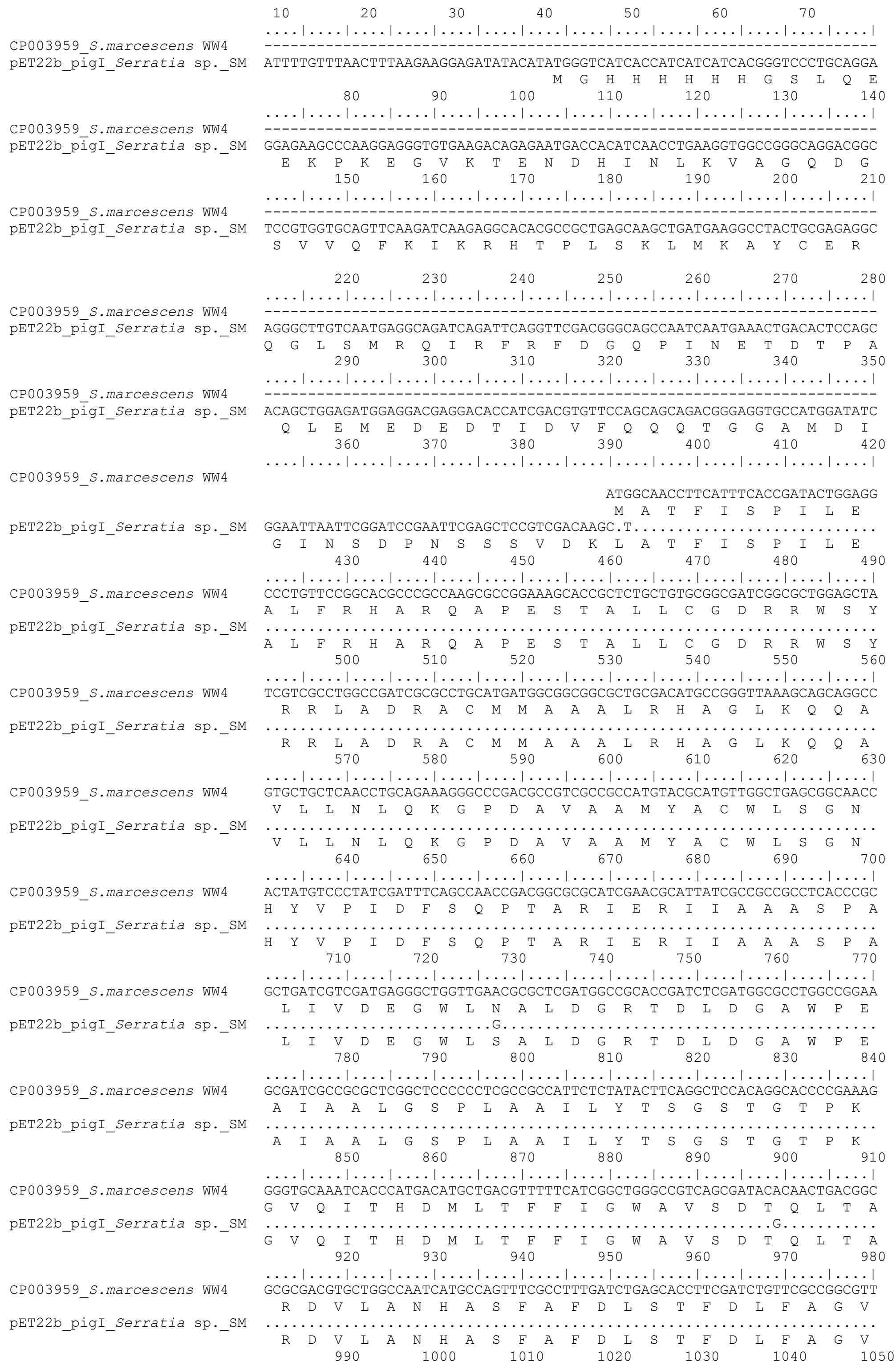

Figure 3. Comparing *pigI* gene of the recombinant vector with that of the reference strain *S. marcescens* WW4

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                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      TGCGCCGGCGCCGCGACCTGGATCGTGCGCGAAGATGAACAGAAAGATTGCCAGGCTCTCGTTCGCGGAT
                                C  A  G  A  A  T  W  I  V  R  E  D  E  Q  K  D  C  Q  A  L  V  R  G
pET22b_pigI_Serratia sp._SM    ......................................................................
                                C  A  G  A  A  T  W  I  V  R  E  D  E  Q  K  D  C  Q  A  L  V  R  G
                                       1060      1070      1080      1090      1100      1110      1120
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      TGCAAACGCACGGCGTCACTCTGTGGTACAGCGTGCCTTCGATTTTAGCCATGCTGGAAAAGAGCGCGTT
                               L  Q  T  H  G  V  T  L  W  Y  S  V  P  S  I  L  A  M  L  E  K  S  A  L
pET22b_pigI_Serratia sp._SM    ......................................................................
                               L  Q  T  H  G  V  T  L  W  Y  S  V  P  S  I  L  A  M  L  E  K  S  A  L
                                       1130      1140      1150      1160      1170      1180      1190
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      GCTGGCGCCATCGACGGTGAAAACGCTGCGCCAGGTCACGTTCGCCGGTGAGCCTTATCCGGCCGCCGCG
                                 L  A  P  S  T  V  K  T  L  R  Q  V  T  F  A  G  E  P  Y  P  A  A  A
pET22b_pigI_Serratia sp._SM    ......................................................................
                                 L  A  P  S  T  V  K  T  L  R  Q  V  T  F  A  G  E  P  Y  P  A  A  A
                                       1200      1210      1220      1230      1240      1250      1260
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      CTGAGGCGTCTGGTTGCGCATCTGCCGGCCCGGTGCCGCGTCAGCAACTGGTATGGCCCGACGGAAACCA
                                L  R  R  L  V  A  H  L  P  A  R  C  R  V  S  N  W  Y  G  P  T  E  T
pET22b_pigI_Serratia sp._SM    ......................................................................
                                L  R  R  L  V  A  H  L  P  A  R  C  R  V  S  N  W  Y  G  P  T  E  T
                                       1270      1280      1290      1300      1310      1320      1330
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      ATGTCTGCACCGCCTACGCGCTGGATCGAACGCAGTTGGCGACGCTGGAGCAGATCCCCATCGGCCATCC
                               N  V  C  T  A  Y  A  L  D  R  T  Q  L  A  T  L  E  Q  I  P  I  G  H  P
pET22b_pigI_Serratia sp._SM    ......................................................................
                               N  V  C  T  A  Y  A  L  D  R  T  Q  L  A  T  L  E  Q  I  P  I  G  H  P
                                       1340      1350      1360      1370      1380      1390      1400
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      GTTGCCCGGCTTGACGGCGCAGTTGGTCGATGAACAGGGGCGCCTGCAGCCGATTGACGGTACGCCGGGC
                                 L  P  G  L  T  A  Q  L  V  D  E  Q  G  R  L  Q  P  I  D  G  T  P  G
pET22b_pigI_Serratia sp._SM    ......................................................................
                                 L  P  G  L  T  A  Q  L  V  D  E  Q  G  R  L  Q  P  I  D  G  T  P  G
                                       1410      1420      1430      1440      1450      1460      1470
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      CGACGCGGAGAATTGCTGATCGGCGGGCCGTGCGTGACCCCCGGCTACAGCAACGTCGCCTCATCGCGGC
                                R  R  G  E  L  L  I  G  G  P  C  V  T  P  G  Y  S  N  V  A  S  S  R
pET22b_pigI_Serratia sp._SM    ......................................................................
                                R  R  G  E  L  L  I  G  G  P  C  V  T  P  G  Y  S  N  V  A  S  S  R
                                       1480      1490      1500      1510      1520      1530      1540
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      AAGCGCAATGGCATCCGCGCCAATGTCACGCGACCGGCGACTGGGTGGAGACGACGGCGAATGGGCTGGT
                               Q  A  Q  W  H  P  R  Q  C  H  A  T  G  D  W  V  E  T  T  A  N  G  L  V
pET22b_pigI_Serratia sp._SM    ......................................................................
                               Q  A  Q  W  H  P  R  Q  C  H  A  T  G  D  W  V  E  T  T  A  N  G  L  V
                                       1550      1560      1570      1580      1590      1600      1610
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      CTACCGCGGCCGGCTGGACGACATGGTGAAAATCAACGGCTACCGGGTGGAATTGGGTGAGATTGAATCG
                                 Y  R  G  R  L  D  D  M  V  K  I  N  G  Y  R  V  E  L  G  E  I  E  S
pET22b_pigI_Serratia sp._SM    ......................................................................
                                 Y  R  G  R  L  D  D  M  V  K  I  N  G  Y  R  V  E  L  G  E  I  E  S
                                       1620      1630      1640      1650      1660      1670      1680
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      ATCCTTCATCACCATCCCTCGGTCAGCCAGGCCGCACTGTATGTCGAGCTCGGCGAGCTGAAGCAAAAAC
                                I  L  H  H  H  P  S  V  S  Q  A  A  L  Y  V  E  L  G  E  L  K  Q  K
pET22b_pigI_Serratia sp._SM    ...................................................................G..
                                I  L  H  H  H  P  S  V  S  Q  A  A  L  Y  V  E  L  G  E  L  K  Q  R
                                       1690      1700      1710      1720      1730      1740      1750
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      TGATCGCCGTGATCACCCTCCATCCCGGCGCGCTCCGCCCCAACCTGCTGGAGCTCAAGCAGTTCCTGCA
                               L  I  A  V  I  T  L  H  P  G  A  L  R  P  N  L  L  E  L  K  Q  F  L  Q
pET22b_pigI_Serratia sp._SM    ......................................................................
                               L  I  A  V  I  T  L  H  P  G  A  L  R  P  N  L  L  E  L  K  Q  F  L  Q
                                       1760      1770      1780      1790      1800      1810      1820
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      GCCCAGGCTGCCCGCCTATATGCTCCCCAGCCAACTGGTGGTCGCCGACAGTCTGCCCACCAATGCCAAC
                                 P  R  L  P  A  Y  M  L  P  S  Q  L  V  V  A  D  S  L  P  T  N  A  N
pET22b_pigI_Serratia sp._SM    ......................................................................
                                 P  R  L  P  A  Y  M  L  P  S  Q  L  V  V  A  D  S  L  P  T  N  A  N
                                       1830      1840      1850      1860      1870      1880      1890
                               ....|....|....|....|....|....|....|....|....|....|....|....|....|....|
CP003959_S.marcescens WW4      GGCAAGGTGGACAGAGGACGTTTGTCCGAGGTGAACGCGCGA------------
                                G  K  V  D  R  G  R  L  S  E  V  N  A  R
pET22b_pigI_Serratia sp._SM    ...................................T......CTCGAGCACCACCACCACCACCACTGAG
                                G  K  V  D  R  G  R  L  S  E  V  N  A  R  L  E  H  H  H  H  H  H  *
                                       1900
                               ....|....|...
CP003959_S.marcescens WW4      -------------
pET22b_pigI_Serratia sp._SM    ATCCGGCTGCTAA
```

Figure 3. Comparing *pigI* gene of the recombinant vector with that of the reference strain *S. marcescens* WW4 (next)

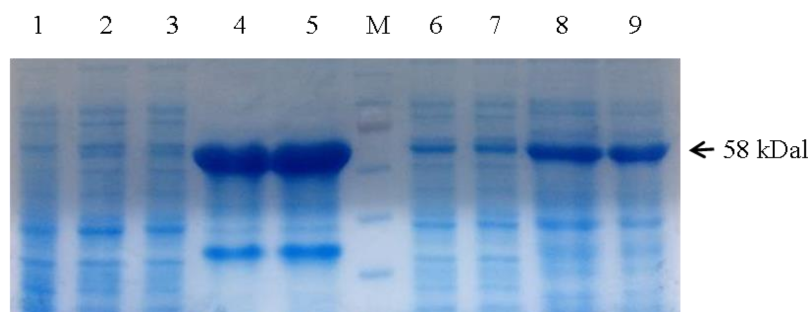

Figure 4. SDS-PAGE gel analysis of expression of recombinant *PigI* protein. 1: Proteins of empty vector; 2-3: *PigI* insoluble phase induced by 0.5 mM and 1mM IPTG, respectively, at 37 ºC; 4–5: *PigI* in insoluble fraction induced by 0.5 mM and 1 mM IPTG, respectively, at 37ºC; 6–7: *PigI* insoluble phase induced by 0.5 mM and 1 mM IPTG, respectively, at 25 ºC; 8–9: *PigI* in insoluble fraction induced by 0.5 mM and 1 mM IPTG, respectively, at 25 ºC; M: Gangnam-STAIN™ prestained protein molecular weight marker (Intron)

CONCLUSION

In this study, the *pigI* sequence with 1476 bps encoding a protein of 492 amino acids from *Serratia marcescens* strain M5 isolated in Vietnam was successfully cloned and expressed into pJET1.2 cloning vector and *E. coli* BL21 (DE3), respectively. Sequence comparison indicated the sequence of the *pigI* gene was highly similar to those of other *Serratia marcescens*. The protein *PigI* with 53 kDa protein was determined by SDS-PAGE and Western blot analysis. Prodigiosin production using the purified recombinant *PigI* should be examined in further studies.

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