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' : bipyrole-5-carboxyaldehyde (MBC) and 2-methyl-3-aminopyrrole (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 HindIII and Xhol. 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 tripyrrole 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'-bipyrole-5-carboxaldehyde (MBC) with 2-methyl-3-aminopyrrole (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 <i>S. marcescens</i> was successfully transformed into <i>Pseudomonas putida</i>, 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 <i>E. coli</i> in order to provide a potential material for producing large-scale prodigiosin.

MATERIALS AND METHODS

Materials

<i>Serratia sp.</i> M5 strain was provided by Enzyme Biotechnology laboratory, Institute of Biotechnology (IBT), Vietnam Academy of Science and Technology (VAST). <i>E. coli</i> DH10B (Invitrogen) and <i>E. coli</i> 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. <i>E. coli</i> and <i>Serratia</i> 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 <i>S. marcescens</i> 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°C for 30 sec; 40 cycles of 98°C 10 sec, 58°C for 15 sec, 72°C for 1 min 30 sec, and a final cycle of 72°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 <i>E. coli</i> 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 remarified from colonies containing recombinant vector using the primers pigC_HindIII_F and pigC_Xhol_R (Table 1). The amplified product was digested with HindIII and <i>XhoI</i> (ThermoFisher Scientific, USA) and ligated into the vector pET22b. The ligated product was transformed into <i>E. coli</i> DH10B strain (Sambrook et al., 2001). Some clones were further examined using PCR, plasmid extraction, enzyme digestion and sequencing by T7 primers (Table 1).

| Primer names        | Primer sequences                  |
|---------------------|----------------------------------|
| PigC_universal_F    | 5’TATTCAAYTTCCGCTAACAGGACA3’     |
| PigC_universal_R    | 5’AAATTCGGYCACKAYAMAGCC3’        |
| PigC_HindIII_F      | 5’AAAAAAAAAGCTTAATCTCTACGGAAGG3’|
| PigC_Xhol.R         | 5’AAAAAATCGAGGCATCGGAGGCGGCGC3’ |
| pJET1.2F            | 5’CGACCTACTATAGGGAGAGCGG3’       |
| pJET1.2R            | 5’AGAACATCGATTCTTTGCGGAGG3’     |
| T7 Promoter         | 5’TATACGTACTCATATAGGG3’         |
| T7 Terminator       | 5’GCTAGTTATATGTCAGCGG3’         |
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, KH₂PO₄ 6.8 g/l, Na₂HPO₄ 7.1 g/l, (NH₄)₂SO₄ 3.3g/l, MgSO₄ 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 NaH₂PO₄, 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 NaH₂PO₄, 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](image)

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 pET22h. 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  | HOf33702S.*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. F814    | 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* S217    | 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* U366S   | 99.2%| 99.2%| 99.4%| 98.8%| 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%| 98.9%| 98.8%| 79.1%| 79.1%| 79.1%| 74.9%| 98.9%| 98.8%|     |
| 7  | AJ833002 *S.marcescens* UHi8    | 98.8%| 98.7%| 98.8%| 98.7%| 98.6%| 98.7%| 98.6%| 99.7%| 79.4%| 79.4%| 79.4%| 75.3%| 98.9%| 98.8%|     |
| 8  | CP002775 *Serratia* sp. AS13    | 75.3%| 75.3%| 75.4%| 75.3%| 75.4%| 75.3%| 75.4%| 75.2%| 100.0%| 100.0%| 97.9%| 79.1%| 79.1%|     |
| 9  | CP002774 *Serratia* sp. AS12    | 75.3%| 75.3%| 75.4%| 75.3%| 75.4%| 75.3%| 75.4%| 75.2%| 100.0%| 100.0%| 97.9%| 79.1%| 79.1%|     |
| 10 | CP002773 *S.plymuthica* AS9     | 75.3%| 75.3%| 75.4%| 75.3%| 75.4%| 75.3%| 75.4%| 75.2%| 100.0%| 100.0%| 97.9%| 79.1%| 79.1%|     |
| 11 | CP013301 *Serratia* 39006      | 76.1%| 76.1%| 76.5%| 76.1%| 76.5%| 76.1%| 76.5%| 76.2%| 76.7%| 76.7%| 76.7%| 74.8%| 74.8%|     |
| 12 | 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%| 98.9%| 98.8%|     |
| 13 | 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%| 98.8%|     |

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Do Minh Trung et al.
**Journal of Biotechnology** 16(4): 757-765, 2018

| Pet22b_pigC | CCACATCGTCGTAGCAGCAGCTGCGGCGGAAAGCGGTCTCCGAGAGGTCCATCCAGACAGC |
|-------------|-----------------------------------------------------------------|
|             | HVIDSCWGLGEGVVSVQVTTDSD                                          |
| Pet22b_pigC | TTTGTATTGGAATGACGGGACGGGACGGGAGATCGCCGCTACCGCATCCACAGCAGCTGAC |
|             | FLDKASGEIERRQIRHKPHYQCQ                                           |
| Pet22b_pigC | CTACGGGACCCGGAGGCACGGGACGAGGATCCGGCGGAGATCCCGCGCAGCGCTAGACAGC |
|             | RDPGRVTLLQUPERDRAPSLTP                                             |
|             | 780  790  800  810  820  830  840                                 |
| Pet22b_pigC | GGAGTGGTGAAAGAGATCTGGGTGCTCCGTGCCAGGCACGGCAGCAGCTGCTAGACAGC |
|             | EWAHYKODRVWLLQAPFRPITTCAKP                                         |
|             | 890  900  910                                                     |
| Pet22b_pigC | TTACGATCTTTAAGCACCGAAGACGATCCGGGACGGCAGCTACCGCTAGACAGCAGCTGAGAT |
|             | VQMLYANMWDSPAIKERAPPSTEM                                         |
|             | 1090  1090  1090  1090  1100  1110  1120                         |
| Pet22b_pigC | GACACCGGCAGATTCACGGCCTTCGAGCCGGTGCTTTGCGAGCTTACACAAAGGAGTGGGTT |
|             | HINGPAIKTMGLADIDGWQIYM                                             |
|             | 1190                                                             |
| Pet22b_pigC | ATTTGGACAGCTACGGGACGCACGGGACGGGACGACGATCCGGGACGGGACGGGACGGGAG |
|             | YLQGYVYLNISSCGSAYMLRQCPPTR                                         |
|             | 1260                                                             |
| Pet22b_pigC | GGCCCGGCGATACGGGATGGGATACGGGATACGGGATACGGGATACGGGATACGGGATACGGG |
|             | DEMKFTTRYATATATIDDFGSYRNPY                                         |
|             | 1330                                                             |
| Pet22b_pigC | TGGCCACGGCGCGCCGGACGCTAGGATGACGGCAGCGCAGCTACGGGATACGGGATACGGGAG |
|             | LRSAGATTVIALMRQETRFLA                                              |
|             | 1470                                                             |
| Pet22b_pigC | GCTGAGATCGACACCGACACCGACACCGACACCGACACCGACACCGACACCGACACCGACAC |
|             | LDDLTTTMTHQELERELSRIDGYFL                                         |
|             | 1540                                                             |
| Pet22b_pigC | GACGCTGCCGCTCCTTTCTTCTTCGCTGTCTCGACGTCGACGCTGCTCGGGCTGCTGCTT    |
|             | DSCAAAYMPFPLQSFALYDALALT                                           |
|             | 1610                                                             |
| Pet22b_pigC | GCAAACTCTAACAGGCGGACGGGACGAGGATCCGCAACGGAGGATCCGCAACGGAGGATCCGCA |
|             | CERYLKGORNGGLQNRKASMNLR                                             |
| Pet22b_pigC | CACATCGAGCTACGGGACGCTACGGGACGCTACGGGACGCTACGGGACGCTACGGGACGCTAC |
|             | TIEVLGLSLEVTRQPAK                                                 |
|             | 1750                                                             |
| Pet22b_pigC | CTCAGGCAACGCAACGGGACGCTACGGGACGCTACGGGACGCTACGGGACGCTACGGGACGCTAC |
|             | VFERHASAOELVTVLPTDPESRAF                                           |
|             | 761                                                              |
Recombinant vector pET22b/pigC was initially expressed in E. coli BL21 (DE3) in LB medium supplemented 100 μg/ml ampicillin 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.

**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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**REFERENCES**

Chawrai SR, Williamson NR, Mahendiran T, Salmond GP, Leeper FJ (2012) Characterisation of PigC and HapC, the prodigiosin synthetases from *Serratia* sp. and *Hahella chejuensis* with potential for biocatalytic production of anticancer agents. Chem Sci 3(2): 447-454.

Chawrai SR, Williamson NR, Salmond GP, Leeper FJ (2008) Chemoenzymatic synthesis of prodigiosin analogues-exploring the substrate specificity of PigC. Chem Commun (16): 1862-1864.

Darshan N, Manonmani H (2015) Prodigiosin and its
potential applications. J Food Sci Technol 52(9): 5393-5407.

Diaz-Ruiz C, Montaner B, Perez-Tomas R (2001) Prodigiosin induces cell death and morphological changes indicative of apoptosis in gastric cancer cell line HGT-1. Histol Histopathol 16(2): 415-421.

Domröse A, Klein AS, Hage-Hülsmann J, Thies S, Svensson V, Classen T, Pietruszka J, Jaeger K-E, Drepper T, Loechschke A (2015) Efficient recombinant production of prodigiosin in Pseudomonas putida. Front Microbiol 6: 972.

Harris AK, Williamson NR, Slater H, Cox A, Abbasi S, Foulds I, Simonsen HT, Leeper FJ, Salmond GP (2004) The Serratia gene cluster encoding biosynthesis of the red antibiotic, prodigiosin, shows species-and strain-dependent genome context variation. Microbiology 150(11): 3547-3560.

Klein AS, Domröse A, Bongen P, Brass HU, Classen T, Loechschke A, Drepper T, Laraia L, Sievers S, Jaeger K-E (2017) New prodigiosin derivatives obtained by mutasynthesis in Pseudomonas putida. ACS Synth Biol 6(9): 1757-1765.

Liu P, Zhu H, Zheng G, Jiang W, Lu Y (2017) Metabolic engineering of Streptomyces coelicolor for enhanced prodigiosins (RED) production. Sci China Life Sci 60(9): 948-957.

Montaner B, Navarro S, Piqué M, Vilaseca M, Martinell M, Giralt E, Gil J, Pérez Montaner B, Navarro S, Piqué M, Vilaseca M, Martinell M, Giralt E, Gil J, Pérez-Tomás R (2000) Prodigiosin from the supernatant of Serratia marcescens induces apoptosis in haematoepietic cancer cell lines. Br J Pharmacol 131(3): 585-593.

Neupane S, Finlay RD, Alstrom S, Goodwin L, Kyprides NC, Lucas S, Lapidus A, Bruce D, Pitluck S, Peters L, Ovchinnikova G, Cherkov O, Han J, Han C, Tapia R, Detter JC, Land M, Hauser L, Cheng JF, Ivanova N, Pagani I, Klenk HP, Woyke T, Hogberg N (2012a) Complete genome sequence of Serratia plymuthica strain AS12. Stand Genomic Sci 6(2): 165-173.

Neupane S, Finlay RD, Alstrom S, Goodwin L, Alström S, Lucas S, Land M, Han J, Lapidus A, Cheng J-F, Bruce D, Pitluck S, Peters L, Ovchinnikova G, Held B, Han C, Detter JC, Tapia R, Hauser L, Ivanova N, Pagani I, Woyke T, Klenk H-P, Hogberg N (2012) Complete genome sequence of the plant-associated Serratia plymuthica strain AS13. Stand Genomic Sci 7(1): 22-30.

Nguyen HH, Nguyen HAK (2015) Bioefficacy of Serratia marcescens isolated from entomopathogenic nematodes (EPN) and their secondary metabolite prodigiosin against Spodoptera litura. Science and Technology Development 18 (2): 5-15.

Nguyen SLT, Le DQ (2015) Purification and antibacterioactivity of anticaner agent prodigiosin from Serratia marcescens M10. Academia Journal of Biology 37: 210-216.

Rahul S, Chandrashekhar P, Hemant B, Bipinchandra S, Mourgay E, Guerrler P, Satish P (2015) In vitro antiparasitic activity of microbial pigments and their combination with phytosynthetised metal nanoparticles. Parasitol Int 64(5): 353-356.

Sambrook J, Russell DW, Russell DW (2001) Molecular Cloning. A Laboratory Manual, 3rd ed Cold Spring Harbor Laboratory, Cold Spring Harbor, NY.

Suryawanshi RK, Patil CD, Koli SH, Hallsworth JE, Patil SV (2017) Antimicrobial activity of prodigiosin is attributable to plasma-membrane damage. Nat Prod Res 31(5): 572-577.

Tomas P, Ricardo E, Montaner B (2003) Effects of the proapoptotic drug prodigiosin on cell cycle-related proteins in Jurkat T cells. Histol Histopathol 18(2): 379-385.

Wang Z, Li B, Zhou L, Yu S, Su Z, Song J, Sun Q, Sha O, Wang X, Jiang W (2016) Prodigiosin inhibits Wnt/β-catenin signaling and exerts anticancer activity in breast cancer cells. Proc Natl Acad Sci USA 113(46): 13150-13155.

Williamson NR, Simonsen HT, Ahmed RA, Goldet G, Slater H, Woodley L, Leeper FJ, Salmond GP (2005) Biosynthesis of the red antibiotic, prodigiosin, in Serratia: identification of a novel 2-methyl-3-n-amyly-pyrrole (MAP) assembly pathway, definition of the terminal condensing enzyme, and implications for undecylprodigiosin biosynthesis in Streptomyces. Mol Microbiol 56(4): 971-989.

Yamamoto C, Takehito H, Kuno K, Yamamoto D, Tsukuba A, Kamata K, Hirata H, Yamamoto A, Kana H, Seki T (1999) Cycloprodigiosin hydrochloride, a new H+/Cl− symporter, induces apoptosis in human and rat hepatocellular cancer cell lines in vitro and inhibits the growth of hepatocellular carcinoma xenografts in nude mice. Hepatology 30(4): 894-902.

You Z, Liu X, Zhang S, Wang Y (2018a) Characterization of a prodigiosin synthetase PigC from Serratia marcescens jx-1 and its application in prodigiosin analogue synthesis. Biochem Eng J 134: 1-11.

You Z, Zhang S, Liu X, Wang Y (2018b) Enhancement of prodigiosin synthetase (PigC) production from recombinant Escherichia coli through optimization of induction strategy and media. Prep Biochem Biotechnol 48(3): 226-233.

Zhang J, Shen Y, Liu J, Wei D (2005) Antimetastatic effect of prodigiosin through inhibition of tumor invasion. Biochem Pharmacol 69(3): 407-414.
TÁCH ĐỒNG 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 phân ứng ngưng kết 4-methoxy-2, 2’-bipyrrrole-5-carboxyaldehyde (MBC) và 2-methyl-3-amylypyrrrole (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. Sự dụng cắp mới chúng cho vùng gen pigC, đoạn gen khoảng 3kb chứa pigC được khử khác đai thành công từ chúng Serratia sp. chúng M5. Kết quả sơ sánh trình tự cho thấy, trình tự pigC có kích thước 2667 bp của chúng M5 phân lập tại Việt Nam tương đồng 98% với trình tự của các chúng S. marcescens khác, tuy nhiên khác biệt tối 30% khi so với chúng S. marcescens 39006 và AS9 với mã số tương ứng AJ833001 và CP002773. Trình tự gen mà hoà protein PigC với kích thước 2664 bp sau khi được nhân lên sử dụng cắp mới có chứa vị trí nhánh protein enzyme cắt HindIII và XhoI được đưa vào vector pET22b tạo thành vector t'ai t'o hop có gắn đầu 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 kỹ 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 t'ai t'o hop ở Việt Nam.

Từ khóa: MAP, MBC, pigC, prodigiosin, Việt Nam