

Sequence Analysis of Putative *potB*, *potC*, and *potD* Genes from *Serratia rubidae*

SONY SUHANDONO*, RASI FITRIA, AND ERNAWATI ARIFIN GIRI RACHMAN

*School of Life Sciences and Technology,
Institut Teknologi Bandung, Jalan Ganesa 10, Bandung 40132, Indonesia*

Amplification of putative *potBCD* genes from *Serratia rubidae* was conducted by PCR using a pair of primers FERC and RERC. A fragment with ~1800 bp size was ligated to pGEM-T Easy vector then cloned to competent *Escherichia coli* DH5a. The recombinant plasmid was sequenced using SP6, T7 and two internal primers, FPC and RPC. A sequence similarity search and analysis was performed with the BLASTN program. The sequence was found to have 83% similarity to *potABCD* genes from *E. coli*. Those genes encoded a spermidine-preferential uptake system that consists of four kinds of protein: PotA is a membrane-associated ATPase, PotB and PotC are transmembrane proteins that form channels, and PotD is a periplasmic substrate-binding protein. Alignment analysis showed that the isolated clone consisted of *potB* (partial), *potC* (full length) and *potD* (partial). The sequences for *potBCD* genes from *S. rubidae* are not available in the NCBI database. Furthermore, we have submitted this sequence, *potBCD* from *S. rubidae*, on GeneBank with Acc. number FJ447342.

Key words: *Serratia rubidae*, spermidine-preferential uptake system, PotABCD, polyamine

Serratia is a prominent opportunistic pathogen (Holt *et al.* 1994) that infects plants and animals, including human. The bacteria belongs to Gram negative bacteria group (Holt *et al.* 1994). Ten species are presently known to belong in the genus *Serratia*. These species are *S. marcescens*, *S. liquefaciens*, *S. proteamaculans*, *S. grimesii*, *S. plymuthica*, *S. rubidae*, *S. odorifera*, *S. ficaria*, *S. entomophila*, and *S. fonticola* (Grimont and Grimont 2006).

Serratia rubidae has been isolated from coconuts, vegetables and fresh cheese as a dairy product (Malcata 1998). In the previous research, *S. rubidae* was found in human clinical specimens such as blood, bile (Ewing *et al.* 1973), urine (Menezes *et al.* 2004), respiratory tract (sputum) (Johnson and Ellner 1974), and feces (Farmer *et al.* 1985).

Serratia rubidae may be responsible for nosocomial infection in particular sepsis (Stock *et al.* 2003), colangitis, septicemia and urine infection (Menezes *et al.* 2004). It was shown to be an invasive pathogen (Ursua *et al.* 1996). Hospital infection due to *S. rubidae* is regarded to be associated with the consumption of contaminated coconuts or vegetable salads (Stock *et al.* 2003) and the bacteria may also be carried by hospital gowns (Pilonetto *et al.* 2004). *Serratia* produces multiple enterotoxic factors that may be significant in the understanding of its pathology (Singh *et al.* 1996). In addition, pathogenicity factors found in *Serratia* strains are the formation of fimbriae, the production of potent siderophores, the presence of cell wall antigens, the ability to resist the bactericidal action of serum and the production of proteases (Grimont and Grimont 2006).

In the recent studies, it was reported that some pathogenic bacteria had genes with homology to a polyamine transporter (Pot) operon in *Escherichia coli*. Proteins that are encoded by those genes have been implicated in the pathogenesis of bacteria, such as pneumococcal infection (Ware *et al.* 2006). When a

pathogen invades host cells, it must adapt quickly to a new environment to multiply and evade the host immune system. Polyamines would be actively involved in those cellular processes. Polyamines (spermidine, spermine, putrescine and cadaverine) are small polycationic compounds present in all living organisms (Tabor and Tabor 1985). They are essential for normal cell growth due to their role in cell proliferation and differentiation (Igarashi and Kashiwagi 1999). The polyamine content in cells is maintained by biosynthesis, degradation and transport from the environment (Igarashi and Kashiwagi 1999).

In *E. coli*, the genes for three different polyamine transport systems have been cloned and characterized (Kashiwagi *et al.* 1990). Two uptake systems (spermidine-preferential or PotABCD and putrescine-specific or PotFGHI) were ABC (ATP binding cassette) transporters (Kashiwagi *et al.* 2002). The third transport system, catalyzed by PotE, mediates both the uptake and the excretion of putrescine (Kashiwagi *et al.* 1992). Although polyamine transport systems may play an important role in pathogenesis and immunity of pathogenic bacteria, polyamine uptake in *S. rubidae* has not been well studied. In this study, we isolated three genes on *potABCD* operon from *S. rubidae*, namely *potB*, *potC* and *potD*.

Spermidine-preferential uptake systems specifically bind either spermidine or putrescine, with a higher affinity for spermidine (Kashiwagi *et al.* 1996). This polyamine transport system consists of four kinds of protein: PotA, PotB, PotC and PotD (Kashiwagi *et al.* 1990). PotA is a membrane-associated ATPase, PotB and PotC are integral membrane proteins that form a polyamine-specific transport channel and PotD is a surface-associated, polyamine-binding protein (Ware *et al.* 2006). The calculated molecular weights for these proteins were 43 026, 31 060, 29 109 and 38 865 Da, respectively (Furuchi *et al.* 1991). The PotD affinity for putrescine is ten-fold lower than spermidine (Igarashi and Kashiwagi 1999). Immunization with recombinant PotD on spermidine-preferential uptake system was proven to induce a vigorous antibody response in mice

*Corresponding author, Phone/Fax: +62-22-2511575;
Email: sony@sith.itb.ac.id

against pneumococcal infection (Shah and Swiatlo 2006). The purpose of this research is to isolate the *potABCD* operon from *S. rubidae*.

MATERIALS AND METHODS

Bacterial Strain. The strain used in this research was isolated from degraded vegetables and identified by The Microbiology Laboratory, School of Life Sciences and Technology, Institut Teknologi Bandung.

Genomic and Gene Isolation. Chromosomal DNA from *S. rubidae* was isolated from cells harvested at their early stationary growth phase. The isolation protocol follows that of Bronke *et al.* (2001). The *potABCD* operon was amplified by PCR with genomic DNA from *S. rubidae* serving as a template. A pair of primers FERC (5'-ATGGTGAAGCAGGCTGTTT-3') and RERC (5'-ATAAATGCCCGACTGCCA-3') were used in this reaction. The reaction included 25 cycles with denaturation temperature of 95°C, annealing temperature of 49°C and elongation temperature of 72°C. PCR product with ~1800 bp size was purified using Geneaid Gel/PCR-DNA-Fragment-Extraction Kit.

Cloning and Transformation. The purified PCR product was ligated to pGEM-T Easy vector (Promega) using the standard protocol. Furthermore, the vector was transformed into competent *E. coli* DH5 α cells with *heat shock* method (Sambrook *et al.* 1989). The transformants were plated onto LB medium that contained ampicillin, IPTG and X-Gal and then incubated overnight at 37°C.

Plasmid Isolation. A single white colony (putative clone with DNA insert) was picked and inoculated to LB medium supplemented with 100 $\mu\text{g mL}^{-1}$ ampicillin as a selection antibiotic. The bacterial culture was incubated overnight (16 hours) at 37°C with shaking at 250 rpm. Plasmid isolation was performed using GeneJET Plasmid Miniprep Kit (Fermentas) as described in the product manual. The existence of insert DNA was confirmed by cutting the plasmid with *EcoRI* as it was one of the restriction enzyme that cut the pGEM-T Easy vector at the multiple cloning sites.

Sequencing. The DNA plasmid containing the insert was sequenced by Macrogen, Inc. Korea using SP6 primer, T7 primer, and two internal primers, FPC and RPC. Nucleic acid and deduced asam amino sequences were analyzed by BiOEDIT and ClustalW programs. Domain prediction of putatif PotB protein was performed by ScanProsite, online pogram from www.expasay.ch/tools.

RESULTS

The genomic DNA samples of *S. rubidae*, as a template in the PCR reaction, were successfully extracted. Their concentration ranged from 2-6.8 $\mu\text{g mL}^{-1}$, and the DNA-protein ratios were around 1.75-1.79. Fig 1 (left) showed some DNA fragments amplified by the PCR method as given in the Materials and Methods.

The purified fragment (size ~1800 bp) was ligated to pGEM-T Easy as a cloning vector. Blue-white colony

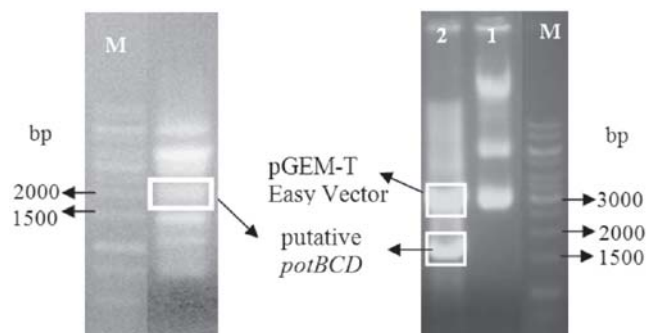


Fig 1 (Left) PCR reaction products. Putative *potBCD* were sized approximately 1800 bp; (Right) Line 1: Recombinant plasmid uncut. Line 2: Recombinant plasmid cut by *EcoRI*. M: marker (1 kb DNA ladder).

screening was performed by employing IPTG and X-Gal within the LB-medium. We also used ampicillin as selective antibiotic for the *E. coli* transformant. A single white colony, which is the putative clone with the DNA insert, was then inoculated into fresh LB-medium for the next stage. Confirmation of DNA insertion was conducted by cutting the plasmid using *EcoRI*. Line 2 at the electrophoregram (Fig 1, right) showed 2 fragments of DNA, the upper fragment was pGEM-T Easy vector, while the fragment with smaller molecular mass (lower fragment) was the DNA insert. Sequencing using Automatic DNA sequencer showed that the DNA insert was a 1868 bp (Fig 2).

DISCUSSION

Sequence analysis revealed that length of the fragment was 1868 bp (Fig 2). The *potB*, *potC* and *potD* was also found in *E. coli* (Blattner *et al.* 1997; Kashiwagi *et al.* 2002). The sequence had 83% similarity to *potA*, *potB*, *potC* and *potD* genes from *E. coli* (Acc. number M64519.1) with expectation value 0 and 1% (34/1870) gaps. Alignment analysis showed that the isolated clone consisted of *potB* (partial), *potC* (full length) and *potD* (partial). The *potB*, *potC*, and *potD* genes were located at 14-594, 590-1382 and 1378-1860 bp on the nucleotide sequence, respectively. The open reading frames for those genes partially overlapped.

We obtained the amino acid sequence of these genes using in-silico translation of the nucleotide sequence by BioEdit software. The amino acid sequence analysis was performed by BLASTP program. Putative PotB from *Serratia rubidae* had 95% similarity to spermidine/putrescine ABC transporter membrane protein (PotB) from *Citrobacteri koseri* ATCC BAA-895 (Acc. number ABV13055.1). Putative PotC had 96% similarity to spermidine/putrescine ABC transporter membrane protein (PotC) *Shigella dysenteriae* Sd197 (Acc. number ABB 62123.1). In addition, putative PotD had 89% similarity to spermidine/putrescine ABC transporter periplasmic substrate-binding protein (PotD) from *Klebsiella pneumoniae* subsp. *pneumoniae* MGH (Acc. number ABR 76562.1).

PotB and PotC are the membrane components of the ABC transporter (Igarashi 1999). Domain prediction of putatif PotB protein was performed by ScanProsite, an

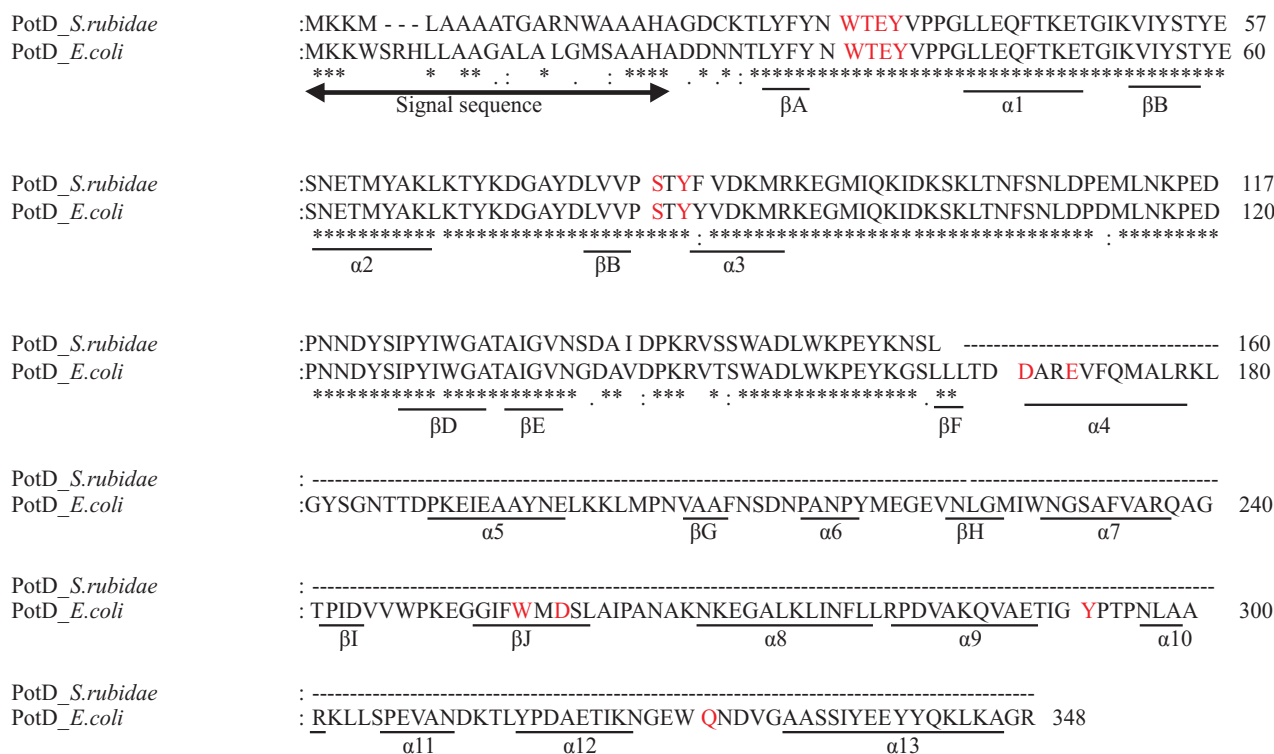


Fig 5 Sequence alignment between putative PotD protein from *S. rubidae* and PotD from *E. coli* Acc. number M 64519.1 Based on Sugiyama *et al.* (1996) the active residue which binds to spermidine are indicated by grey boxes. Consensus symbols; identical residues: "*", conserved substitutions: ".", semi-conserved substitutions: ":".

partial *potD*. We have submitted this sequence, *potBCD* from *S. rubidae*, on GeneBank with Acc. number FJ447342.

ACKNOWLEDGEMENT

We thank Sri Harjati Suhardi from SITH ITB and Puspita Lisdiyanti from Puslit Bioteknologi LIPI for critical comments on the manuscript. We also appreciate Tati Kristianti and M Bahrelfi Belaffiff for their helpful comments and advice. This study was funded by Indonesia agency for agricultural research and development.

REFERENCES

Blattner FR, Plunkett G III, Bloch CA, Perna NT, Burland V, Riley M, Collado-Vides J, Glasner JD, Rode CK, Mayhew GF, Gregor J, Davis NW, Kirkpatrick HA, Goeden MA, Rose DJ, Mau B, Shao Y. 1997. *Science* 277:1453-74.

Bronke F, Hajirezaei M, Sonnewald U. 2001. Cloning and characterization of the gene cluster for palatinose metabolism from the phytopathogenic bacterium *Erwinia rhapsontici*. *J Bacteriol* 183:2425-30.

Ewing WH, Davis BR, Fife MA, Lessel EF. 1973. Biochemical characterization of *Serratia liquefaciens* (Grimes and Hennerty) Bascomb *et al.* (Formerly *Serratia* and *Enterobacter liquefaciens*) and *rubidaea* (Stapp) comb. nov. designation of type and neotype strains. *Int J Sys Bacteriol* 23:217-25.

Farmer III BB, Davis BR, Hickman-Brenner FW, Mc Whorter A, Huntleycarter GP, Asbury MA, Riddle C, Wathen-Grady HG, Elias C, Fanning GR, Steigerwalt AG, O'hara CM, Morris GK, Smith PB, Brenner DJ. 1985. Biochemical identification of new species and biogroups of *Enterobacteriaceae* isolated from clinical specimens. *J Clin Microbiol* 21:46-76.

Furuchi T, Kashiwagi K, Kobayashi H, Igarashi K. 1991. Characteristics of the gene for a spermidine and putrescine transport system that maps at 15 min on the *Escherichia coli* Chromosome. *J Biol Chem* 266:20928-33.

Grimont F, Grimont PAD. 2006. The genus *Serratia*. *Prokaryotes* 6:219-44.

Holt GH, Krieg NR, Sneath PHA, Staley JT, Williams ST. 1994. *Bergey's Manual of Determinative Bacteriology*, 9th ed. Baltimore: Williams & Wilkins. p 175-287.

Igarashi K, Kashiwagi K. 1999. Polyamine transport in bacteria and yeast. *Biochem J* 344:633-42.

Johnson E, Ellner PD. 1974. Distribution of *Serratia* species in clinical specimens. *Appl Microbiol* 28:513-4.

Kashiwagi K, Hosokawa N, Furuchi T, Kobayashi H, Sasakawa C, Yoshikawa M, Igarashi K. 1990. Isolation of polyamine transport-deficient mutants of *Escherichia coli* and cloning of the genes for polyamine transport proteins. *J Biol Chem* 265:20893-7.

Kashiwagi K, Innami A, Zenda R, Tomitori H, Igarashi K. 2002. The ATPase activity and the functional domain of PotA, a component of the spermidine-preferential uptake system in *Escherichia coli*. *J Biol Chem* 277:24212-9.

Kashiwagi K, Miyamoto S, Suzuki F, Furuchi T, Kobayashi H, Igarashi K. 1992. Excretion of putrescine by the putrescine ornithine antiporter encoded by the *potE* gene of *Escherichia coli*. *PNAS USA* 89:4529-33.

Kashiwagi K, Pistocchi R, Shibuya S, Sugiyama S, Morikawa K, Igarashi K. 1996. Spermidine-preferential uptake system in *Escherichia coli*, identification of amino acids involved in polyamine binding in PotD protein. *J Biol Chem* 271:12205-8.

Malcata FX. 1998. Critical issues affecting the future of dairy industry, individual contributions in the scope of a global approach. *J Dairy Sci* 82:1595-611.

Menezes EA, Cezafar FC, Andrade MSS, Rocha MVAP, Cunha FA. 2004. Frequency of *Serratia* sp. in urine infections of intern patients in the Santa Casa de Misericórdia in Fortaleza. *Revista Sociedade Brasileira Med Trop* 37:70-71.

Pilonetto M, Edvaldo, Rosa EAR, Brofman PRS, Baggio D, Calvário F, Schelp C, Nascimento A, Messias-Reason I. 2004. Hospital gowns as a vehicle for bacterial dissemination in an intensive care unit. *Braz J Infect Dis* 8:206-10.

Sambrook J, Fritsch EF, Maniatis T. 1989. *Molecular cloning: a laboratory manual*. New Jersey: Cold Spring Harbor Laboratory Pr.

- Shah P, Swiatlo E. 2006. Immunization with polyamine transport protein PotD protects mice against systemic infection with *Streptococcus pneumoniae*. *Infect Immun* 74:5888-92.
- Singh BR, Singh Y, Tiwari AK. 1996. Characterization of virulence factors of *Serratia* strains isolated from foods. *Int J Food Microbiol* 34:259-66.
- Stock I, Burak S, Sherwood KJ, Gröger T, Wiedemann B. 2003. Natural antimicrobial susceptibilities of strains of 'unusual' *Serratia* species: *S. ficaria*, *S. fonticola*, *S. odorifera*, *S. plymuthica* and *S. rubidaea*. *J Antimicrob Chemother* 51:865-85.
- Sugiyama S, Matsuo Y, Maenaka K, Vassylyev, Matsushima M, Kashiwagi K, Igarashi K, Morikawa K. 1996. The 1.8-Å X-ray structure of the *Escherichia coli* PotD protein complexed with spermidine and the mechanism of polyamine binding. *Prot Sci* 5:1984-90.
- Tabor CW, Tabor H. 1985. Polyamines in microorganisms. *Microbiol Rev* 49:81-99.
- Ursua PR, Unzaga MJ, Melero P, Iturburu I, Ezpeleta C, Cisterna R. 1996. *Serratia rubidaea* as an invasive pathogen. *J Clin Microbiol* 34:216-7.
- Ware D, Jiang Y, Lin W, Swiatlo E. 2006. Involvement of *potD* in *Streptococcus pneumoniae* polyamine transport and pathogenesis. *Infect Immun* 74:352-61.