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

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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* DH5α. 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

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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'-ATGGTGGAAGCAGGCTGTTT-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* DH5α cells with *heat shock* method (Sambrook *et al*. 1989). The transformans 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 µg 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 *Eco*RI 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 µg $µL^{-1}$, 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

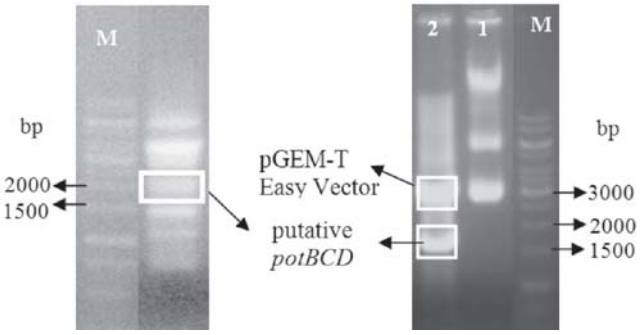

Fig 1 (Left) PCR reaction products. Putative *potBCD* were sized approximately 1800 bp; (Right) Line1: Recombinant plasmid uncut. Line 2: Recombinant plasmid cut by *Eco*RI. M: marker (1 kb DNA ladder).

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 *Eco*RI. 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

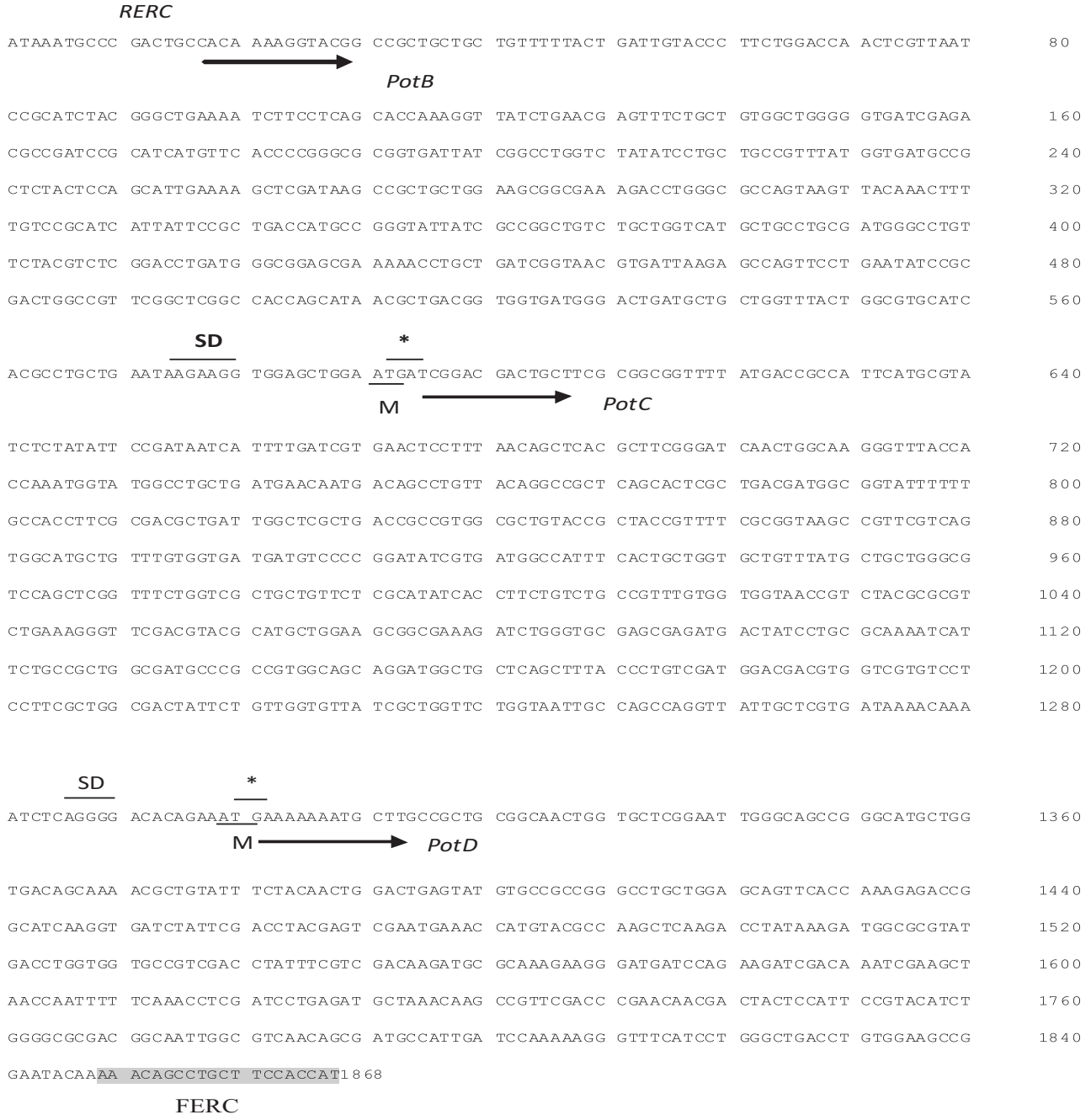

Fig 2 Nucleotide sequence of *potB*, *potC* and *potD* genes from *S. rubidae*. SD denotes putative Shine- Delgarno sequence. Codon start indicated by "M" and codon stop indicated by "*". Grey boxes indicate a pair of primers.

online program available at www.expasay.ch/tools. It was ascertained that the mentioned protein belonged to the ABC-transporter-integral-membrane type-1 domain. In addition, putative PotC protein was analyzed by ScanProsite and InterProScan from EMBL. Putative PotC from *S. rubidae* belonged to the ABC transporter integral membrane type-1 domain. In *E. coli*, both proteins (PotB and PotC) are membrane proteins that form a transport channel on the spermidine-preferential uptake system. Protein domain prediction for PotD could not be undertaken because the sequence obtained was not adequate to be analyzed with previous program.

The amino acid sequence of each of the genes was aligned with the amino acid sequence of PotB, PotC and PotD from *E. coli* using ClustalW. As mentioned above, in *E. coli* PotB and PotC are membrane proteins. The prediction of six transmembrane segments was in line with the finding of Furuchi *et al.* (1991). The alignment analysis results (Fig 3) indicate that fragments of putative PotB from *S. rubidae* has only four transmembrane segments (TM 3-TM 6). It had 95% similarity to PotB protein from *E. coli*. It appears that the putative *potB* was not the complete isolate, only its carboxyl terminus.

Fig 4 shows the alignment analysis between putative PotC from *S. rubidae* and PotC from *E. coli*. Both sequences had 94 % similarity according to the alignment score. The putative PotC from *S. rubidae* lacks one amino acid compared with its PotC counterpart from *E. coli*.

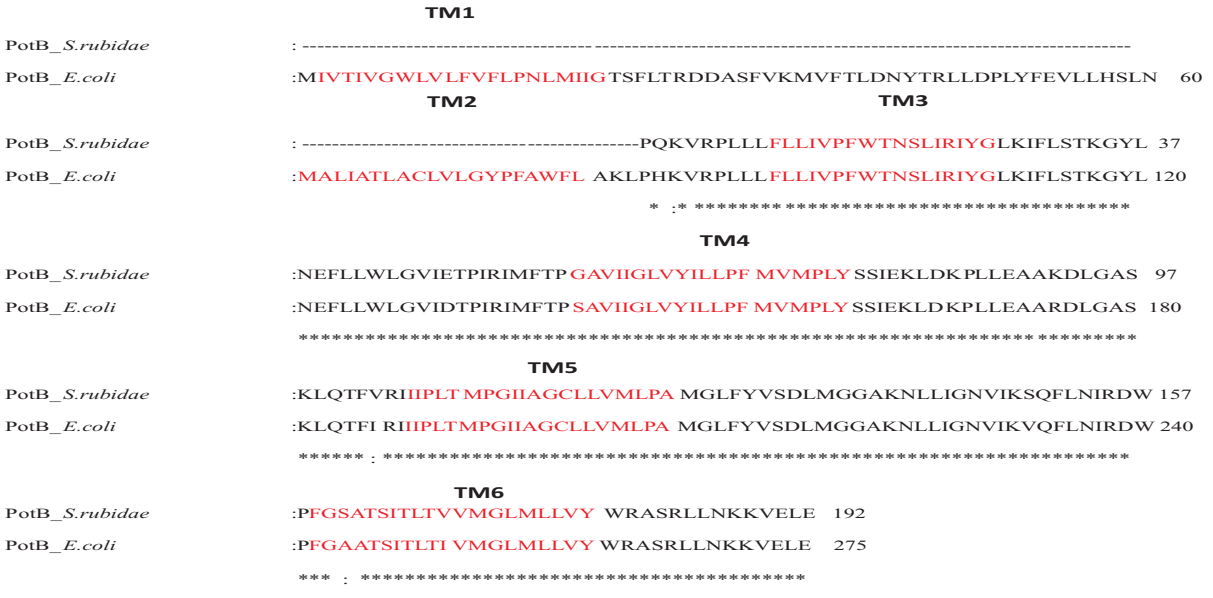

Fig 3 Sequence alignment of putative PotB protein from *S. rubidae* and PotB from *E. coli* Acc Number M 64519.1 which were aligned using the Clustal W algorithm. Grey boxes indicate the six predicted transmembrane segments (TM1 to TM6). Consensus symbols; identical residues: "*", conserved substitutions: ":", semi-conserved substitutions: ".".

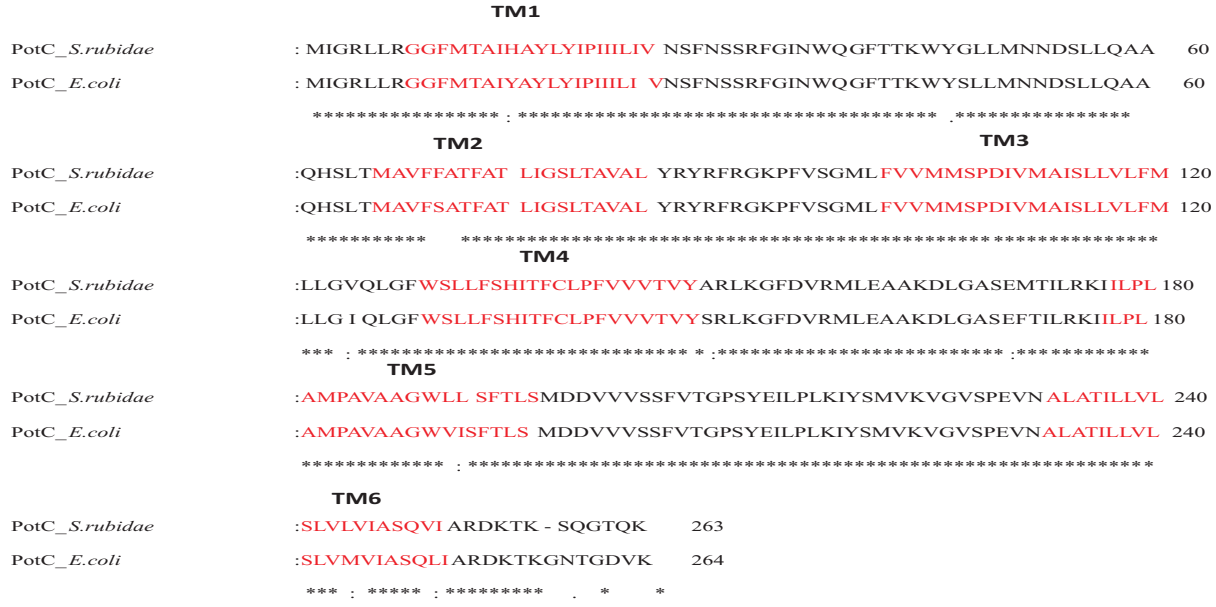

Fig 4 Sequence alignment of putative PotC protein from *S. rubidae* and PotC from *E. coli* Acc number M64519.1 using the Clustal W algorithm. Grey boxes indicate the six predicted transmembrane segments (TM 1 to TM 6). Consensus symbols; identical residues: "*", conserved substitutions: ":", semi-conserved substitutions: ".".

Alignment analysis revealed that fragments of putative PotD from *S. rubidae* had 86% similarity to PotD protein from *E. coli*. Fourteen residues of the PotD protein (Trp 34, Thr 35, Glu 36, Tyr 37, Ser 83, Tyr 85, Asp 168, Glu 171, Ser 211, Trp 229, Trp 255, Asp 257, Tyr 293 and Gln 327) in *E. coli* are involved in spermidine binding. Six of them (Trp 34, Thr 35, Glu 36, Tyr 37, Ser 83 and Tyr 85) were found in the putative PotD from *S. rubidae* (shown in grey boxes, Fig 5). It is predicted that the isolated putative *potD* gene represents only its amino terminus domain. The PotD protein and the periplasmic proteins consist of repeated β-α-β units. The isolated putative *potD* had five β units and three α units (Fig 5).

In *E. coli,* the *potA* gene is located in at the upstream region of *potABCD* operon, followed by *potB*, *potC* and *potD* genes. The size of *potABCD* operon from *E. coli* is approximately 4300 bp (Furuchi *et al.* 1991), thus gene walking experiment should be conducted in the future to identify the full length of *potABCD* operon from *S. rubidae*.

We conclude that the isolated *potBCD* genes from *S. rubidae* consisted of partial *potB*, full length *potC* and

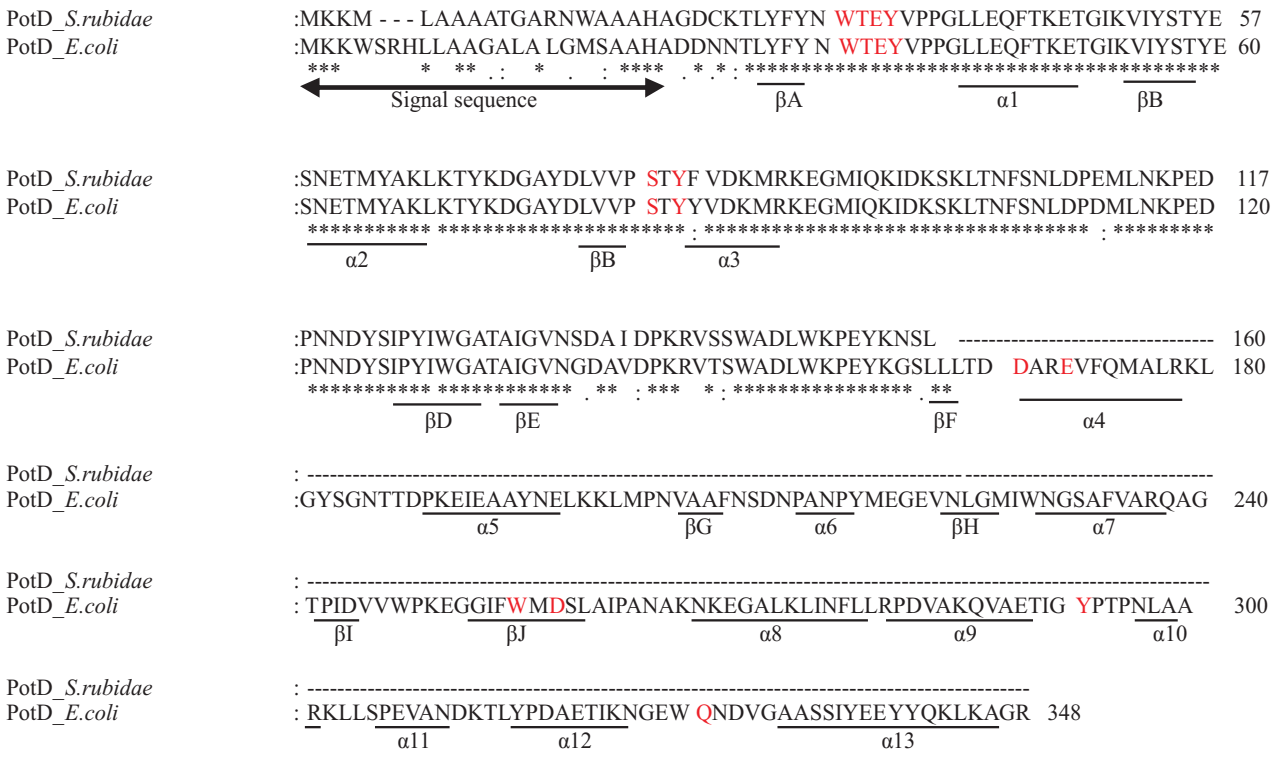

Fig 5 Sequence alignment between putative PotD protein from *S. rubidae* and PotD from *E. coli* Acc. number M 64519.1 Based on Sugiyama *et al.* (1996) the active residue which binds to spermidine are indicated by grey boxes. Consensus symbols; identical residues: "*", conserved substitutions: ":",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

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

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