

## Characterization of the Sugar Utilization Gene *polS* from *Ralstonia solanacearum*

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### ABSTRACT

The gene coding for sorbitol dehydrogenase (*polS*) from *Ralstonia solanacearum* strain TO 264 biovar 3 was cloned, sequenced, and compared to homologous sequences from the other bacteria. The result showed that the sorbitol dehydrogenase gene from *R. solanacearum* strain TO264 displayed the highest similarity to that of *R. solanacearum* GMI1000 with 99.6% amino acid similarity and the least similarity to sequence of *Pseudomonas syringae* pv. *syringae* with 56% amino acid similarity. Phylogenetic analysis of *polS* showed the *Burkholderia cepacia* sequence to join as sister to the *R. solanacearum* TO264 pair. Analysis of the deduced amino acid sequence revealed homology to enzymes of the short-chain dehydrogenase/reductase protein family. The eight amino acid residues were conserved in most of these proteins. These residues included the almost invariant tyrosine (Y) and lysine (K) residues of consensus sequence Y-X-X-X-K, which were essential for catalysis and were located in the active site in C-terminal whereas the glycine (G) residues of the G-X-X-X-G-X-G segment were characteristic of the NAD<sup>+</sup> binding domain in the N-terminal region. The 771 bp of *polS* gene from *R. solanacearum* TO264 was subcloned into expression pGEX-2T vector. The *polS* ORF encoded a protein consisting 256 amino acid residues with estimated molecular mass of 27 kDa by SDS-PAGE analysis.

**Key words:** *Ralstonia solanacearum*, sorbitol dehydrogenase, *polS*, sugar utilization

### INTRODUCTION

*Ralstonia solanacearum*, formerly known as *Pseudomonas solanacearum*, is the causal agent of bacterial wilt on several hundred-plant species in over 50 families in the tropical and subtropical regions around the world (Hayward, 1991). Being soil borne, *R. solanacearum* normally infects the roots and

systemically moves through the xylem. It causes wilting symptoms that are often lethal (Vasse *et al.*, 1995). Most of host plants are dicotyledious in the Solanaceae family which includes tomato, potato, tobacco, eggplant and pepper. Bacterial wilt is also found in monocotyledious plants such as banana, bird of paradise, *Heliconia* spp., ginger, and related plants of Zingiberaceae family (Hayward, 1994). In addition, various woody,

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perennial plants of economic importance have been reported as host (Hayward, 2000). Based on their ability to utilize disaccharides (i.e., lactose, maltose and cellobiose) and hexose alcohols (i.e., mannitol, sorbitol and dulcitol) as carbon source, *R. solanacearum* is divided into four biovars (Hayward, 1964). Biovar 1 is unable to oxidize disaccharide and hexose alcohol whereas biovar 3 is capable of oxidizing both disaccharides and hexose alcohols. Biovar 4 utilizes only hexose alcohol but Biovar 2 utilizes only disaccharides. In Thailand, Biovar 3 and 4 have been reported to destroy several economically important crops such as tomato, potato, ginger, pepper, tobacco and sesame, as well as marigold and exported ornamental plants such as Patumma, *Curcuma alismatifolia* Gagnep. (Thaveechai *et al.*, 2000). Understanding genes involving in sugar utilization in *R. solanacearum*, it may provide insight into factors controlling host specificity and thus suggesting new methods of disease control. We therefore cloned, sequenced, and expressed the sorbitol gene (*polS*) from *R. solanacearum* strain TO264 Biovar 3 for further investigation and mutation analysis.

Sorbitol dehydrogenase (SDH), also known as glucitol, polyol, or L-iditol dehydrogenase (EC.1.1.1.14), catalyzes the oxidation of sorbitol to fructose with NAD as a cofactor. The activity of SDH has been detected in various bacteria including *E. coli* (Lengeler, 1975), *Rhodobacter sphaeroides* (Schauder *et al.*, 1995), *B. subtilis* (Ng *et al.*, 1992), *Pseudomonas* sp. (Schneider and Giffhorn, 1991) and *Pseudomonas cepacia* (Allenza *et al.*, 1982). In this study, the cloning, sequencing, and expression of sorbitol dehydrogenase gene (*polS*) in *E. coli* were described. Its deduced amino acid sequence (GeneBank accession number AY946241) was compared to the *polS* gene from the other sources.

## MATERIALS AND METHODS

**Bacterial strain, plasmids and growth condition.** *R. solanacearum* TO264 was isolated from wilted tomato in this laboratory and grown at 28-30°C on TZC and TTC medium. Biovar was identified using mineral medium supplemented with peptone, agar, and a pH indicator (Hayward, 1964). For extraction of genomic DNA, *R. solanacearum* was cultured in liquid YP broth. *E. coli* DH5 $\alpha$  which was used as competent cell for transformation was grown on LB supplemented with an appropriated antibiotic at 37°C with agitation.

**Recombinant DNA techniques.** Plasmid DNA was purified by the standard alkaline-lysis method (Birmboim and Doly, 1979). *E. coli* cells were transformed according to the standard heat-shocked method (Hanahan, 1983). Isolation of chromosomal DNA of *R. solanacearum* followed the puregene kit (Invitrogen Inc., Minneapolis, MN) as described by the manufacturer. Standard methods for restriction analysis, ligation, and Southern blotting have been described (Sambrook *et al.*, 1989). DNA labeling was performed with digoxigenin-11-dUTP and the signal was detected with chemiluminescence according to the supplier's recommendations.

**Expression of *polS* in plasmid pGEX-2T.** The *polS* gene was amplified using two sets of specific primers. For amplification of 600 bp fragment of the *polS* gene, the forward primer used was sorSF (5'GGC ACG CTC GCG CAA CTC ATC GA3') and the reverse primer was sorSR (5'GAT TAC ATC ACC G CT CAG ACG CT3'). For amplification of 800 bp fragment, the forward primer used was polSF (5'GTC AAG GGA TCC GTT GCA GGA CAA GGT CGC GAT CCT3' where the underline indicated the addition on *Bam* HI site) and the reverse primer was polSR (5'CTA CAT GAA TTC GCT CAT CCA GTT GCC GCC GTC3' where the underline indicated the addition

on *EcoRI* site). DNA amplification of *polS* gene was performed in the final volume of 50  $\mu$ l containing 10 mM Tris-HCl (pH 8.3), 50 mM KCl, 1.5 mM  $MgCl_2$ , 0.2 mM each deoxynucleoside triphosphate, 50 pmole of each primer, 2.5 u of Taq DNA polymerase (Promega), and 50 ng of genomic DNA from *R. solanacearum* strain TO264. The condition used in PCR amplification was 5 min of denaturation at 94°C, followed by 30 cycles of amplification with denaturation at 94°C for 45 s, annealing at 65°C for 30 s, and DNA elongation at 72°C for 30 s and a final elongation for 7 min at 72°C using thermocycler (Perkin-Elmer/Applied Biosystem). The 600 bp or 800 bp PCR products obtained were separated by 0.8 % agarose gel electrophoresis in TBE buffer and visualized under UV-transilluminator. The approximate 800 bp fragment containing the complete coding sequence of *polS* was subsequently cloned in pGEM-T. Clone containing pGEM with *polS* insert, pGEM-*polS* was isolated and double digested with *Bam*HI and *Eco*RI. The recombinant expression plasmid, pGEX-*polS*, was constructed by ligation of the *Bam*HI- and *Eco*RI-digested *polS* gene into plasmid pGEX-2T (Amersham), which pre-digested with the same restriction enzymes.

#### Sequence assembly and analysis.

Sequences were analyzed by Lasergene 4.03 software package (DNASTAR, USA, WI; <http://www.dnastar.com/>). *Seqman* was used to assemble contigs and *MegAlign* was used to compare sequences and to assess their similarity. Phylogenetic trees was constructed using Clustal W (Thompson *et al.*, 1994).

#### Expression of recombinant SDH.

*E. coli* DH5 $\alpha$  culture harboring pGEX-*polS* was inoculated in 10 ml of LB broth containing ampicillin (100  $\mu$ g/ml) and shaken at 37°C. After 2 h of cell growth designated as T<sub>0</sub>, the expression of the enzyme was induced by addition of IPTG to cell culture at the final concentration of 1 mM. 2, 4, and 6 h after induction, designated as T<sub>2</sub>, T<sub>4</sub>,

T<sub>6</sub>, respectively, cells were harvested by centrifugation. All cultures collected from different time intervals were harvested for cell pellet to determine optimum time for induction.

#### Protein analysis by SDS-PAGE.

Protein was analyzed on 15% (w/v) sodium dodecyl sulfate (SDS)-PAGE (Laemmli, 1970) and stained with Coomassie brilliant blue R 250 (LABCHEM; Cat. 3195-25G). Protein samples were prepared by mixing 50  $\mu$ l of cell suspension with 25  $\mu$ l of 4x sample solubilizing buffer. The mixtures were boiled for 10 min and cooled on ice. An aliquot of 12  $\mu$ l of cell extracts were applied to the gel and compared to protein marker from Biolab (New England, UK).

### RESULTS AND DISCUSSION

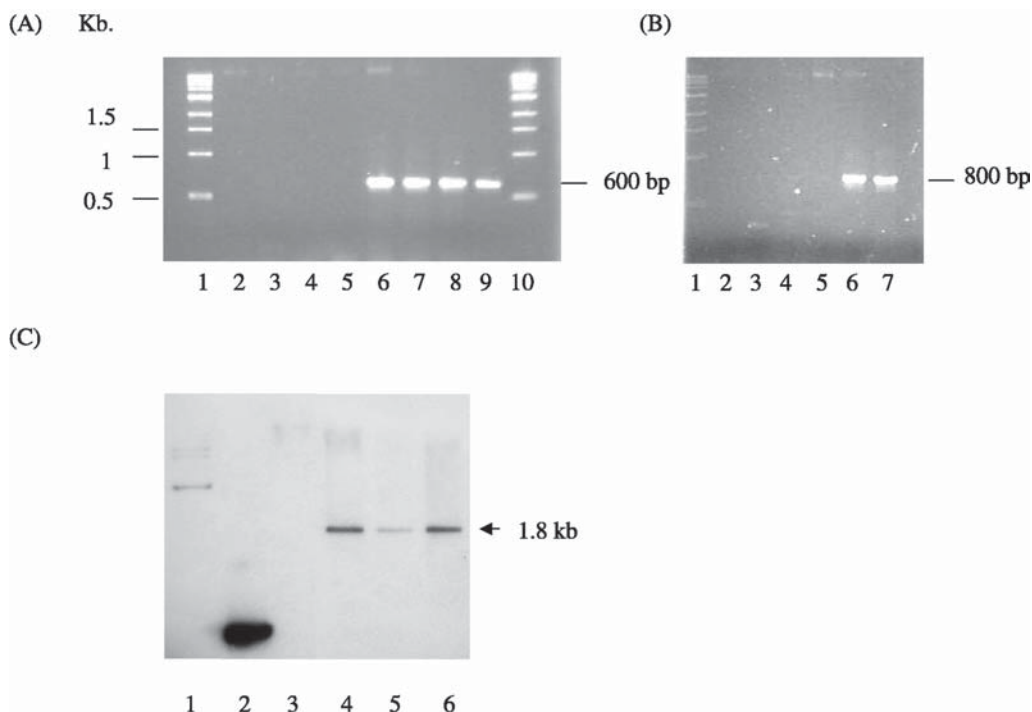
Using sorSF and sorSR as primers and genomic DNA as template, PCR amplification resulted in a single band with an approximate size of 600 bp (Figure 1A). This 600 bp PCR product was observed in Biovar 3 and 4 which were able to oxidize sorbitol as a sole carbon source (Figure 1A; lanes 6-7 and 8-9) but not in Biovar 1 and 2 (Figure 1A; lanes 2-3 and 4-5) which were unable to oxidize sorbitol. To confirm that *polS* gene was present in Biovar 3 and 4, Southern blot analysis of DNA isolated from all biovars and partially digested with *Cla*I was carried out. Hybridization DNA isolated from Biovar 3 and 4 to the sorS probe showed a 1.8 kb fragment corresponded to the endogenous *polS* gene (Figure 1C; lanes 4-6). However, this fragment was not observed in Biovar 1 (Figure 1C; lane 3). To further confirm that Biovar 3 and 4 contained *polS* gene, the activity of sorbitol dehydrogenase was determined in all biovars. When crude extract from all biovars were assayed for sorbitol dehydrogenase activity, no activity was detected in Biovar 1 and 2 (data not shown). Therefore, the results from PCR amplification, Southern blot analysis, and measuring sorbitol dehydrogenase

all indicated that Biovar 3 and 4 contained *polS* gene. Biovar classification of 3 and 4 could utilize sorbitol as carbon source. Therefore, it was presumed that Biovar 1 and 2 did not possess sorbitol utilizing gene. Furthermore, no PCR product was observed when polSF was used as forward primer and polSR was used as reverse primer in the amplification of the DNA isolated from Biovar 1 and 2 (Figure 1B; lanes 2-3 and lanes 4-5, respectively).

To determine the nucleotide sequence, the 800 bp fragments of *polS* gene from *R. solanacearum* strain TO264 was cloned and sequenced. The obtained 771 nucleotide sequence

(GeneBank accession no. AY946241) and deduced amino acid sequence are shown in Figure 2. Being differed from the other *polS* genes, the nucleotides sequence of the *polS* gene contained an alternative initiator TTG (rather than ATG). The ORF of *polS* encodes a protein consisting of 256 amino acid residues with predicted molecular mass of 27,314 Da.

When sorbitol dehydrogenase (SDH) was overexpressed as a glutathione-S-transferase (GST) fusion protein in *E. coli*, the molecular mass of this fusion protein, GST-SDH, was estimated to be approximately 52,000 Da (25,000 Da GST + 27,000 Da SDH) by SDS-PAGE (Figure 5). The



**Figure 1** (A) Gel electrophoresis of the 600 bp fragment amplified by sorSF and sorSR primer : lane 1; 1 kb ladder marker, lanes 2-3; Biovar 1, lanes 4-5; Biovar 2, lanes 6-7; Biovar 3, lanes 8-9; biovar 4. (B) Electrophoresis of 800 bp fragment amplified by polSF and polSR primer: lane 1; 1 kb ladder marker, lanes 2-3; Biovar 1, lanes 4-5; Biovar 2, lane 6; Biovar 3 (strain TO264), lane 7; Biovar 4 (strain PB41-4). (C) Southern blot analysis between sorS probe and DNA extracted from *R. solanacearum* and partially digested with *Cla*I : lane 1; 1 kb ladder marker, lane 2; sorS probe, lanes 3-6; *R. solanacearum* strain FC 325 (biovar 1), TO264 (biovar 3), Pe109 (biovar 3) and PB41-4 (biovar 4), respectively.

molecular mass of the polypeptide was in good agreement with the molecular mass obtained by SDS-PAGE (27,000 Da). This result was consistent with the molecular mass (29,000 Da) which had been determined for one subunit of purified SDH from *R. sphaeroides* M22. SDH is a homodimeric enzyme with a subunit molecular mass of 29,000 Da. Although the enzyme is active on sorbitol and galactitol, sorbitol is demonstrated to be a better substrate (Schauder *et al.*, 1995). The *smoS* gene encoding for SDH from *R. sphaeroides* Si4 was reported to locate 55 nucleotides upstream from the mannitol dehydrogenase gene (*mtlK*) (Stein *et al.*, 1997). It consisted of 256 amino acid residues with predicted molecular mass of 27,012 Da. The *smoS* was subcloned into the over expressing vector pET-24a and the overproduced in *E. coli* BL21 (DE3). The yield of enzyme obtained using pET expression vector was approximately 270-fold higher than that of the native host, *R. sphaeroides* (Stein *et al.*, 1997). Unfortunately, it was unable

to subclone the *polS* into pQE81L (Qiagen), T4 based promoter. Because this gene was expressed efficiently as a GST fusion protein with the pGEX-2T vector, the reason why it expressed inefficiently with pQE81L vector was unclear.

Sequence comparison analysis revealed significant homologies between the deduced amino acid sequences of *polS* and proteins of the short chain alcohol dehydrogenase/ reductase family (SDR) (Jornvall *et al.*, 1995). SDR protein is a very large family of enzymes, most of which are known to be NAD- or NADP-dependent oxidoreductases. The first member of this family to be characterized was *Drosophila* alcohol dehydrogenase. This family commonly is referred to as the 'insect-type' or 'short-chain' alcohol dehydrogenase (Persson *et al.*, 1991). Most member of this family are proteins of about 250 to 300 amino acid residues. The overall identity between *polS* gene and SDR protein was about 30% (data not shown). The similarity of *polS* gene to SDR protein also extended to the predicted

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      L  Q  D  K  V  A  I  L  T  G  A  A  S  G  I  G  E  A  V  A  Q  R  Y  L  E
1  TTGCAGGACAAGGTCGCGATCCTGACAGGGGCAGCCAGCGGCATCGGCGAAGCGGTCGCGCAACGCTATCTGGAA
      A  G  A  R  C  V  L  V  D  L  K  P  A  G  G  T  L  A  Q  L  I  E  T  H  P
76  GCGGGCGCAGCTGCGTGCCTGCTCGATTTGAAACCCGCGGGCGGCACGCTCGCGCAACTCATCGAAACGCATCCC
      D  R  A  F  A  L  S  A  D  V  T  K  R  D  D  I  E  R  I  V  S  A  A  V  E
151  GACCGTGCCTTCGCGCTTCGCCGACGTCACGAAACGTGACGATATCGAGCGCATCGTCTCGGTCGCGGTCGAG
      R  F  G  G  I  D  I  L  F  N  N  A  A  A  F  D  M  R  P  L  D  E  A  W
226  CGTTTCGGCGGCATCGACATCCTGTTCAACAACGCGGCCGCTTCGACATGCGTCCGTTGCTCGACGAAGCCTGG
      E  V  F  D  R  L  F  A  V  N  V  K  G  M  F  F  L  M  Q  A  V  A  Q  R  M
301  GAGGTGTTTCGACCGGCTGTTTCGCGGTCAACGTGAAGGGCATGTTCTTTCTGATGCAGGCGGTTGCACAGCGGATG
      A  A  Q  G  R  G  G  K  I  I  N  M  A  S  Q  A  G  R  R  G  E  A  L  V  S
376  GCGGCGCAGGGACGCGCGGCAAGATCATCAACATGGCTTCGCGAGGCCGCGCGCGCGGCGAGGCGCTGGTGTCTG
      H  Y  C  A  T  K  A  A  V  I  S  Y  T  Q  S  A  A  L  A  L  A  P  Y  K  I
451  CACTATTGCGCGACGAAGGCGGCGGTTCATCAGCTATACGACGTCGGCTGCACTGGCGCTCGCGCCGTACAAGATC
      N  V  N  G  I  A  P  G  V  V  D  T  P  M  W  E  Q  V  D  A  L  F  A  R  Y
526  AACGTGAACGGGATCGCGCGGGCGTTCGTCGACACGCCGATGTGGGAGCAGGTCGACGCGCTGTTTCGCGCGCTAT
      E  N  R  P  L  G  E  K  K  R  L  V  G  E  A  V  P  L  G  R  M  G  V  P  A
601  GAGAATCGCCCACTCGGTCGAGAAGAAGCGTCTGGTTCGGTGAGGCGGTACCGCTCGGCGCGATGGGGGTGCCGGCT
      D  L  T  G  A  A  L  F  L  A  S  T  D  A  D  Y  I  T  A  Q  T  L  N  V  D
676  GACCTGACGGGCGCGCTGTTTCGCGCTCGACCGACGCTGATTACATCACCGCTCAGACGCTGAACGCTCGAC
      G  G  N  W  M  S  *
751  GGCGCAACTGGATGAGCTGA

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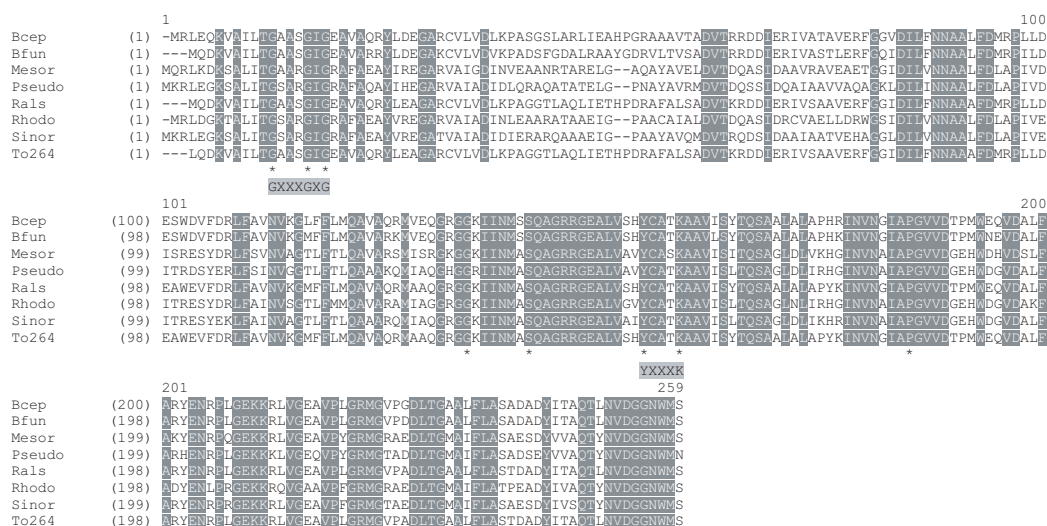
**Figure 2** Partial nucleotide and deduced amino acid sequence of sorbitol dehydrogenase (*polS*).

The two sets of primers, *polSF-polSR* and *sorSF-sorSR*, were marked with underline and dotted-line, respectively. A total of 771 bp and 256 amino acid residues were submitted to Genbank (AY946241).

secondary structures, which predicted locations of helices and sheets being remarkably similar among these enzymes (Ghosh *et al.*, 1994). Although SDR enzymes typically showed overall amino acid residue identities between 15 to 30%, the two regions (GGGG and YKSP) were highly conserved among these proteins (Figure 3). Significantly, half of the conserved residues were glycines, which was typical of distantly related proteins with a conserved fold (Jornvall *et al.*, 1995). The highly conserved glycine-rich region was shown to involve in coenzyme binding and is located at N-terminus. The invariant glycine residues (G) of the G-X-X-X-G-X-G segment was characteristic of the coenzyme binding fold in dehydrogenases. The invariant tyrosine (Y) and lysine residues (K) of the consensus sequence Y-X-X-X-K were demonstrated to be functionally

important for catalysis (Chen *et al.*, 1993) and were located in C-terminal (Ghosh *et al.*, 1995). As with other dehydrogenase, the deduced amino acid sequence of *polS* gene also contained glycine box, GAASGIG, at positions 13 to 19. Structural prediction using NCBI conserved domain search (CDD. V2.04) (Marchler-Bauer and Bryant, 2004) suggested that the N-terminal region around the glycine box processed an  $\beta\alpha\beta$  structure and that 3D structure of *polS* was similar to SDR protein family.

This protein family includes a large number of highly diverse enzymes, most of which have homodimeric structures like SDH and do not require metals for catalytic activity (Jornvall *et al.*, 1995). The SDH of *R. sphaeroides* differed from that of *B. subtilis* which was a tetrameric zinc metallo-enzyme with MW of 150,000 (Ng *et al.*,



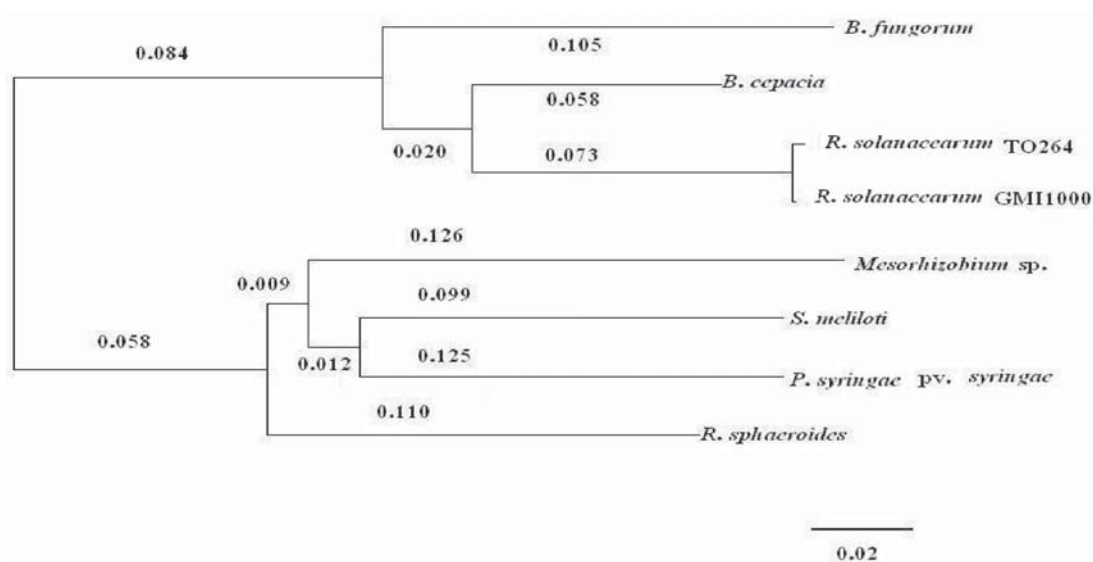
**Figure 3** Multiple sequence alignment of the SDR proteins. Sources of sorbitol dehydrogenase protein sequence from Bcep: *Burkholderia cepacia* R1808 (NZ\_AAEH01000123), Bfun: *Burkholderia fungorum* LB400 (NZ\_AAAJ03000006), Mesor: *Mesorhizobium* sp. BNC1 (NZ\_AAED01000002), Pseudo: *Pseudomonas syringae* pv. *syringae* B728a (NZ\_AABP02000006), Rals: *Ralstonia solanacearum* GMI1000 (NC\_003295), Rhodo: *Rhodobacter sphaeroides* (Q59787), Sinor: *Sinorhizobium meliloti* 1021 (NC\_003047) and TO264: *Ralstonia solanacearum* TO264. Conserved amino acids of SDR protein are marked with asterisk. The solid block denoted identical amino acids. The two conserved NAD<sup>+</sup> binding domain and catalytic domain are shaded with gray block GXXXGXXG and YXXXX.



1992) and *Cephalosporium chrysogenum* which was a 10 subunit enzyme with MW 3,000,000 (Birken and Pisano, 1976). However, the *R. sphaeroides* SDH resembles the SDH from *Pseudomonas* sp. (MW 65,000) with respect to size, subunit composition, the absence of a metal requirement and some kinetic properties (Schneider and Giffhorn, 1991). The mammalian SDHs generally require zinc as cofactor for catalytic activity and share a high degree of structural similarity (Karlsson and Hoog, 1993). However, microbial SDHs are much more diverse. On the basis of sequence data, mammalian SDHs are assigned to the group of zinc-containing medium-chain alcohol dehydrogenases (Jornvall *et al.*, 1987). Only the SDH of *B. subtilis* (Ng *et al.*, 1992) and *Saccharomyces cerevisiae* (Sarthy *et al.*, 1994) are included in the same enzyme family and share 36 and 42% homology with the sequences of the mammalian SDHs, respectively. However, other microbial polyol dehydrogenases including *polS* of *R. solanacearum* are classified on the basis of sequence data as members of the short chain alcohol dehydrogenase family (SDR)

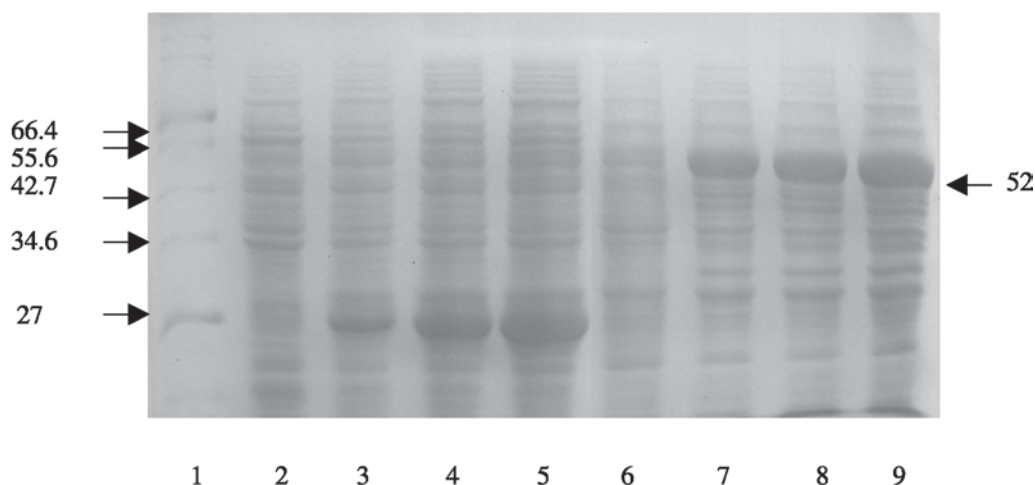
which comprises a group of relatively small enzymes exhibiting no metal requirements (Persson *et al.*, 1991). This enzyme family includes RDH from *Enterobacter aerogenes* (Dothie *et al.*, 1985), arabinitol dehydrogenase from *Candida albicans* (Wong *et al.*, 1993) and D-glucitol-6-phosphate dehydrogenase from *Klebsiella pneumoniae* (EMBL accession no. S23835).

Nucleotide and amino acid sequences alignment showed that *R. solanacearum* strain TO264 was closely related to *R. solanacearum* strain GMI1000, *B. ceapacia* and *B. fungorum* at 99%, 90%, 85% similarity, respectively, but was distantly related from *P. syringae* pv. *syringae*, *R. sphaeroides*, *Sinorhizobium meliloti* and *Mesorhizobium* sp. (60% similarity) (Table 1). A phylogenetic analysis indicated *polS* to join the *Burkholderia cepacia* sequence as sister to the *R. solanacearum* pair as shown in Figure 4. This result clearly concluded that *R. solanacearum* was closely related to *B. ceapacia* and *B. fungorum* by comparing with sorbitol dehydrogenase gene sequence. Insertion-duplication mutation at



**Figure 4** Neighbor joining phylogenetic tree of sorbitol dehydrogenase from seven bacteria. Clustal W program was used to construct neighbor joining phylogenetic trees.

kDa



**Figure 5** SDS-PAGE of crude extract from *E. coli* harboring pGEX-2T and pGEX-*polS*, respectively: lane 1; MW marker, lanes 2-5; crude extract of pGEX-2T at T<sub>0</sub>, T<sub>2</sub>, T<sub>4</sub>, T<sub>6</sub>, lanes 6-9; crude extract of pGEX-*polS* at T<sub>0</sub>, T<sub>2</sub>, T<sub>4</sub>, T<sub>6</sub>.

**Table 1** Nucleotide and amino acid sequence similarity of SDH and its homologues among seven bacterial species.

Percent nucleotide sequence identity									
1	2	3	4	5	6	7	8		Bacteria
	84.8	76.0	56.0	55.8	99.6	59.0	57.1	1	<i>R. solanacearum</i> TO264
89.1		80.0	59.3	57.6	85.2	61.7	59.4	2	<i>B. ceapacia</i>
83.7	86.4		55.9	52.1	76.4	56.2	56.7	3	<i>B. fungorum</i>
57.6	58.5	58.4		71.4	56.3	72.6	73.5	4	<i>Mesorhizobium</i> sp.
56.0	57.0	56.0	79.8		55.5	70.3	75.8	5	<i>P. syringae</i> pv. <i>syringae</i>
99.6	89.1	83.7	57.6	56.0		59.5	57.3	6	<i>R. solanacearum</i> GMI1000
57.6	58.4	58.4	77.4	76.7	57.6		75.1	7	<i>R. sphearoides</i>
58.0	60.5	59.1	79.1	83.7	58.0	80.5		8	<i>S. meliloti</i>
Percent amino acid sequence identity									

specific *polS* gene has been currently underway in our laboratory to study factors controlling host specificity.

### CONCLUSION

In this study, sorbitol dehydrogenase gene (*polS*) GeneBank accession no. AY946241

identified from *R. solanacearum* Biovar 3 strain TO264 encoded protein consisting of 256 amino acid residues with estimated molecular weight of 27 kDa. This gene processed sorbitol utilizing enzyme in Biovar 3 and 4 but not Biovar 1 and 2. *R. solanacearum* was closely related to *B. ceapacia* and *B. fungorum* by comparing with sorbitol dehydrogenase gene sequence. Analysis



of the *polS* deduced amino acid sequence revealed homology to enzymes of the short-chain dehydrogenase/reductase protein family. The eight amino acid residues consisting of four glycines (G), serine (S), lysine (K), tyrosine (Y), and proline (P) were conserved in most of these proteins. The tyrosine (Y) and lysine (K) residues of consensus sequence Y-X-X-X-K were essential for catalysis in C-terminal whereas the glycine (G) residues of the G-X-X-X-G-X-G segment were characteristic of the NAD<sup>+</sup> binding domain in the N-terminal region.

### ACKNOWLEDGEMENTS

We wish to thank Asst. Prof. Somchai Pornbanlualap Department of Biochemistry, Kasetsart University for proofreading this paper and the Center for Agricultural Biotechnology (CAB) and Prince of Songkla University, Surat Thani Campus for providing a doctoral scholarship.

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