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## N-terminal 112 amino acid residues are not required for the sialyltransferase activity of *Photobacterium damsela* $\alpha$ 2,6-sialyltransferase

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### Abstract

**Photobacterium damsela**— $\alpha$ 2,6-sialyltransferase was cloned as N- and C- His-tagged fusion proteins with different lengths (16–497 aa or 113–497 aa). Expression and activity assays indicated that the N-terminal 112 amino acid residues of the protein were not required for its  $\alpha$ 2,6-sialyltransferase activity. Among four truncated forms tested, N-His-tagged  $\Delta$ 15Pd2,6ST(N) containing 16–497 amino acid residues had the highest expression level. Similar to the  $\Delta$ 15Pd2,6ST(N), the shorter  $\Delta$ 112Pd2,6ST(N) was active in a wide pH range of 7.5–10.0. A divalent metal ion was not required for the sialyltransferase activity, and the addition of EDTA and dithiothreitol did not affect the activity significantly.

### Keywords

Carbohydrate; Expression; *Photobacterium damsela*; Sialic acid Sialyltransferase

### Introduction

Sialyltransferases (EC 2.4.99.-) are key enzymes in the biosynthesis of sialoglycoconjugates (sialic acid-containing glycoconjugates) (Harduin-Lepers *et al.* 1995). They catalyze the reaction that transfers a sialic acid residue from its activated sugar nucleotide donor cytidine 5'-monophosphate sialic acid (CMP-sialic acid) to an acceptor, usually a structure with a galactose, *N*-acetylgalactosamine (GalNAc) or another sialic acid at the non-reducing terminus.

*Photobacterium damsela*  $\alpha$ 2,6-sialyltransferase (Pd2,6ST or sialyltransferase 0160) (GenBank accession number: BAA25316) was the first bacterial  $\alpha$  2,6-sialyltransferase that has ever been cloned and expressed in *E. coli* (Yamamoto *et al.* 1998). The flexible donor and acceptor substrate specificities of the enzyme were described and the enzyme was applied in the synthesis of  $\alpha$ 2,6-linked sialosides and glycopeptides (Yamamoto *et al.* 1998a; Yu *et al.* 2006; Yamamoto *et al.* 1998b; Kajihara *et al.* 1996; Teo *et al.* 2005; Yamamoto *et al.* 1996; Endo *et al.* 2001). Pd2,6ST shares amino acid sequence homology with a multifunctional *Pasteurella multocida* sialyltransferase PmST1 encoded by gene *Pm0188* (GenBank accession numbers: AAY89061, AAK02272) (Yu *et al.* 2005), a *Haemophilus ducreyi*  $\alpha$ 2,3-sialyltransferase encoded by gene *Hd0053* (GenBank accession number: AAP95068) (Li *et al.* 2007), a *Photobacterium phosphoreum*  $\alpha$ 2,3-sialyltransferase encoded by gene *pst3-467* (GenBank accession number: BAF63530) (Tsukamoto *et al.* 2007), and a *Photobacterium leiognathi*  $\alpha$ 2,6-sialyltransferase (Yamamoto *et al.* 2007). These proteins do not share sequence

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homology with any other reported bacterial or mammalian glycosyltransferases. They have been classified into glycosyltransferase family GT80 on the Carbohydrate-Active enZyme database (CAZy – <http://www.cazy.org/index.html>) (Coutinho *et al.* 2003; Campbell *et al.* 1997).

Unlike other sialyltransferases in the GT80 family, Pd2,6ST has an extra C-terminal domain (D498 to D675) showing high homology to the bacterial phosphate transport system regulatory protein PhoU. This PhoU domain has been proven to be unrelated to the sialyltransferase activity of Pd2,6ST (Yamamoto *et al.* 1998a; Yu *et al.* 2006). The lipobox-containing N-terminal 15 amino acids deleted in the purified native Pd2,6ST enzyme (Tsukamoto *et al.* 2007) may function as a signal sequence. This is similar to the *Photobacterium phosphoreum*  $\alpha$ 2,3-sialyltransferase, for which a lipobox signal sequence was also identified (Tsukamoto *et al.* 2007).

Compared to other sialyltransferases in the GT80 family, Pd2,6ST has an extra N-terminal domain (C16 to P112). By cloning and characterizing four constructs of Pd2,6ST fusion proteins differing in terms of the length of the protein and the position of the Histidine tag, we show here that the N-terminal 112 amino acid residues are not required for the sialyltransferase activity of Pd2,6ST. This finding will help the structural characterization of this important enzyme by NMR and crystal structure studies.

## Material and methods

### Chemicals and reagents

Restriction enzymes and T4 DNA ligase were purchased from Promega and New England Biolabs. *E. coli* competent cell DH5 $\alpha$  and BL21(DE3) were purchased from Invitrogen. *Pasteurella damsela* (ATCC #33539)  $\alpha$ 2,6-sialyltransferase gene in a TOPO vector was a kind gift from Dr. Ajit Varki at the University of California – San Diego.

### Cloning

Using the plasmid of *Photobacterium damsela* (ATCC # 33539)  $\alpha$ 2,6-sialyltransferase gene in a TOPO vector as the template for polymerase chain reactions (PCR), four different constructs in pET15b or pET22b(+) vector were obtained for expressing N- or C- His<sub>6</sub>-tagged fusion proteins. Forward primer 5' GATCCATATGTGTAATAGTGACAATACCAGC 3' (*Nde*I restriction site is underlined) was used to clone 15Pd2,6ST(N) in pET15b vector and 15Pd2,6ST(C) in pET22b(+) vector. Forward primer 5' GATCCATATGACGCTAGAAGTTTACATCGATC 3' (*Nde*I restriction site is underlined) was used to clone  $\Delta$ 112Pd2,6ST(N) in pET15b vector and  $\Delta$ 112Pd2,6ST(C) in pET22b(+) vector. Reverse primer 5' CGCGGATCCTTAAGCCCAGAACAGAACATC 3' (*Bam*HI restriction site is underlined) was used to clone both  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N) in pET15b vector, and reverse primer 5' CGCGGATCCTTAGTGATGATGATGATGATGAGCCC *AGAACAGAACATCTTTTTC* 3' (*Bam*HI restriction site is underlined, codons encoding hexahistidine are in italics) was used to clone both  $\Delta$ 15Pd2,6ST(C) and  $\Delta$ 112Pd2,6ST(C) in pET22b(+) vector. PCR reactions were performed in a 50  $\mu$ l reaction mixture using Herculase enhanced DNA polymerase (Stratagene) according to manufacturer's instruction. PCR product was purified and digested with *Nde*I and *Bam*HI restriction enzymes. The purified and digested PCR product was ligated with predigested pET15b or pET22b(+) vector and transformed into *E. coli* DH5 $\alpha$  electrocompetent cells. Selected clones were grown for minipreps and characterization by restriction mapping. DNA sequencing was performed by Davis Sequencing Facility at the University of California-Davis.

## Expression

Positive plasmids were selected and transformed into BL21 (DE3) chemical competent cells. Plasmid-bearing *E. coli* strain was cultured in LB rich medium (10 g l<sup>-1</sup> tryptone, 5 g l<sup>-1</sup> yeast extract, and 10 g l<sup>-1</sup> NaCl) supplemented with ampicillin (100 µg ml<sup>-1</sup>). Overexpression of target proteins was achieved by inducing the *E. coli* culture with 0.1 mM of isopropyl-1-thio-β-D-galactopyranoside (IPTG) when the OD<sub>600 nm</sub> of the culture reached 0.8–1.0, followed by incubating the culture at 20 °C for 24 h with vigorous shaking at 250 rpm in a C25KC incubator shaker (New Brunswick Scientific, Edison, NJ).

## Purification of Pd2,6ST by affinity chromatography

His<sub>6</sub>-tagged target proteins were purified from the cell lysate using ÄKTA FPLC system (GE Healthcare Life Sciences). To obtain the cell lysate, cells were harvested by centrifugation at 4000 × g for 2 hrs. Cell pellets were then re-suspended in 20 ml of lysis buffer (Tris-HCl, pH 8.0, 100 mM, 0.1% Triton X-100) containing lysozyme (100 µg ml<sup>-1</sup>) and DNaseI (3 µg ml<sup>-1</sup>). After incubating the mixture at 37 °C for 50 min with vigorous shaking, the cell lysate was collected by centrifugation at 12,000 × g for 30 min. The supernatant (lysate) was applied to a HisTrap™ FF 5 ml column (GE Healthcare). The column was then washed with 10 volumes of binding buffer (5 mM imidazole, 0.5 M NaCl, 20 mM Tris-HCl, pH 7.5), 15 volumes of washing buffer (30 – 50 mM imidazole, 0.5 NaCl, 20 mM Tris-HCl, pH 7.5), followed by 8 volumes of elute buffer (200 mM imidazole, 0.5 NaCl, 20 mM Tris-HCl, pH 7.5). The fractions containing the purified enzyme were collected, dialyzed against Tris-HCl buffer (20 mM, pH 7.5) containing 10% glycerol, and stored at 4 °C.

## Quantification of purified protein

Protein concentration was determined in a 96-well plate using Bicinchoninic acid (BCA) Protein Assay Kit (Pierce Biotechnology, Rockford, IL) with bovine serum albumin as a protein standard. The absorbance of each sample was measured at 562 nm using a Bio-Tek EL311SX plate reader.

## pH profile by HPLC

Typical enzymatic assays were performed in a 20 µl reaction mixture containing a buffer (200 mM) with a pH in the range of 5.0–11.0, CMP-Neu5Ac (1 mM), 4-methylumbelliferyl β-D-lactoside (LacMU) (1 mM), and Δ15Pd2,6ST(N) (240 ng) or Δ112Pd2,6ST(N) (285 ng). Reactions were allowed to proceed for 30 min at 37 °C and were quenched by adding ice-cold 12% acetonitrile to make 60-fold dilutions. The samples were then kept on ice until an aliquot of 8 µl was injected and analyzed by a Shimadzu LC-2010A system equipped with a membrane online degasser, a temperature control unit and a fluorescence detector. A reverse phase Premier C18 column (250 × 4.6 mm I.D., 5 µm particle size, Shimadzu) protected with a C18 guard column cartridge was used. The mobile phase was 12% acetonitrile. The fluorescent compounds LacMU and Neu5Acα2,6LacMU were detected by excitation at 325 nm and emission at 372 nm (Kajihara *et al.* 1999; Kajihara *et al.* 2004).

## Results and discussion

### Cloning, expression and purification of four constructs of Pd2,6ST

The N-terminal 15 amino acids and the C-terminal domain containing amino acid residues D498–D675 of Pd2,6ST have been shown to be unrelated to its α2,6-sialyltransferase activity (Yu *et al.* 2006). Amino acid sequence alignments of the five reported sialyltransferase members in the GT80 family indicate that compared to the multifunctional *Pasteurella multocida* sialyltransferase encoded by *Pm0188* (Yu *et al.* 2005), the *Haemophilus ducreyi* α2,3-sialyltransferase encoded by gene *Hd0053* (Li *et al.* 2007), and the *Photobacterium*

*photobacterium*  $\alpha$ 2,3-sialyltransferase encoded by gene *pst3-467* (Tsukamoto *et al.* 2007), Pd2,6ST has an extra domain containing amino acid residues C16-P112. A similar domain is also found in the *Photobacterium leiognathi*  $\alpha$ 2,6-sialyltransferase (Yamamoto *et al.* 2007). In order to determine whether this extra domain in Pd2,6ST is related to its sialyltransferase activity, four different Pd2,6ST constructs including  $\Delta$ 15Pd2,6ST(N),  $\Delta$ 15Pd2,6ST(C),  $\Delta$ 112Pd2,6ST(N), and  $\Delta$ 112Pd2,6ST(C) were cloned. They differ from each other in terms of the length of the protein and the position of the Histidine tag (at N- or C-terminus). As shown in Fig. 1, all four Pd2,6ST proteins could be expressed in large quantity (> 60% of the whole cell extract) in *E. coli* after IPTG induction (lanes AI). While both  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 15Pd2,6ST(C) having an N-terminal 15 amino acid residues deletion were quite soluble, only minimal amounts of the targeted proteins were present in the soluble portion of the cell extracts (cell lysate, lanes L in Fig. 1) of  $\Delta$ 112Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(C) having an N-terminal 112 amino acid residues deletion. A single protein band in the purified fractions (lanes PP in Fig. 1) indicated that onestep affinity column purification using a HisTrap™ FF 5 ml column in ÄKTA FPLC system was efficient for all four constructs.

As a result, up to 36 mg of purified  $\Delta$ 15Pd2,6ST(N) could be routinely obtained from one liter of *E. coli* culture (Table 1). The expression level (24 mg per liter culture) of soluble  $\Delta$ 15Pd2,6ST(C) was 33% less than that of the  $\Delta$ 15Pd2,6ST(N). The amounts of soluble  $\Delta$ 112Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(C) that could be purified from one liter *E. coli* cell culture were about 20 fold less than the ones missing only the first 15 amino acid residues. These data indicated that the amino acid residues C16-P112 of Pd2,6ST were important for the solubility of the recombinant proteins. The position of the Histidine-tag also affected, to some extent, the solubility of the resulted fusion protein.

### Kinetics of $\Delta$ 15Pd2,6ST(N) and $\Delta$ 112Pd2,6ST(N)

In order to determine whether deleting the amino acid residues C16-P112 of Pd2,6ST affects the kinetic parameters of the recombinant proteins, kinetic studies were carried out for  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N). The results are listed in Table 2. The apparent  $K_M$  values of both donor CMP-Neu5Ac and acceptor LacMU for  $\Delta$ 112Pd2,6ST(N) are similar to those of  $\Delta$ 15Pd2,6ST(N). The  $K_M$  values of CMP-Neu5Ac for  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N) (both are 0.9 mM) are in the same magnitude as the  $K_M$  of Pd2,6ST 0160 purified from *Photobacterium damsela* (0.32 mM) (Yamamoto *et al.* 1996). They are about 100-fold lower than the  $K_M$  of rat liver  $\alpha$ 2,6-sialyltransferase (7.59  $\mu$ M) (Angata *et al.* 1998). The  $K_M$  values of LacMU for  $\Delta$ 15Pd2,6ST(N) (0.8 mM) and  $\Delta$ 112Pd2,6ST(N) (0.6 mM) are about one magnitude less than the  $K_M$  of lactose (6.82 mM) and *N*-acetyllactosaminide (8.95 mM) for Pd2,6ST 0160 (Yamamoto *et al.* 1996). The  $k_{cat}$  values of both CMP-Neu5Ac and LacMU for  $\Delta$ 15Pd2,6ST(N) are about 2-fold of those for  $\Delta$ 112Pd2,6ST(N), leading to about 2-fold increase in  $k_{cat}/K_M$  values for  $\Delta$ 15Pd2,6ST(N). Taken together, these results indicate that the amino acid residues C16-P112 of Pd2,6ST do not affect the binding affinity of the protein to the substrates, but slightly affect the turnover rate of the enzyme.

The native function of the amino acid sequence C16-P112 in marine bacterium *Photobacterium damsela* is currently unknown. Pd2,6ST itself may be involved in the biosynthesis of  $\alpha$ 2,6-linked sialosides on the surface of the bacterium. Indeed, glycans containing  $\alpha$ 2,6-linked sialic acid have been detected on the surface of *Photobacterium leiognathi* JT-SHIZ-145 using SSA lectin staining (Yamamoto *et al.* 2007). More detailed glycobiology studies are yet to be carried out in order to fully understand the biological functions of these bacterial sialyltransferases.

### pH profile of $\Delta$ 15Pd2,6ST(N) and $\Delta$ 112Pd2,6ST(N)

As shown in Fig. 2, the pH profiles of  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N) are very similar. Both enzymes are active in a wide pH range varying from 7.5 to 10.0 with about 5% variation

in the production of Neu5Ac $\alpha$ 2,6LacMU. The activities of both enzymes decline quickly when pH goes below 6.5. The activities drop to about 5% when the pH is at 5.0 (Fig. 2). This property is different from previous reports which found optimal pH values of 5.0 and 6.0 for Pd2,6ST 0160 (Yamamoto *et al.* 1996) and a truncated recombinant enzyme (Teo *et al.* 2005), respectively.

### Effects of metal ions, reducing agent DTT, and chelating agent EDTA

A Tris-HCl buffer of pH 8.5 was chosen for the assays to compare the effects of metal ions Mg<sup>2+</sup> and Mn<sup>2+</sup>, reducing agent DTT, and chelating agent EDTA on the activity of  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N). As shown in Table 3, the presence of various concentrations of metal ion Mg<sup>2+</sup> did not affect the  $\alpha$ 2,6-sialyltransferase activity of either  $\Delta$ 15Pd2,6ST(N) or  $\Delta$ 112Pd2,6ST(N). The addition of 5 mM chelating agent EDTA did not affect the activity significantly, indicating a metal ion was not required for the activity of the enzymes. However, addition of 10 mM of Mn<sup>2+</sup> ion decreased the activity of  $\Delta$ 15Pd2,6ST(N) to 60% but did not affect the activity of  $\Delta$ 112Pd2,6ST(N). Increasing the concentration of Mn<sup>2+</sup> to 20 mM caused the decrease of the activity of both  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N). The detrimental effect of Mn<sup>2+</sup> ion was similar to that reported for PmST1 (Yu *et al.* 2005).

There are three cysteine residues (C16, C60, and C89) in the amino acid sequence C16-P112 of Pd2,6ST. The addition of DTT up to 5 mM did not affect the sialyltransferase activity of both  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N), indicating that disulfide bond formation in  $\Delta$ 15Pd2,6ST(N) was unlikely.

In conclusion, we show here that the N-terminal 112 amino acid residues in Pd2,6ST are not required for the  $\alpha$ 2,6-sialyltransferase activity, but affect the solubility of the recombinant protein expressed in *E. coli*.

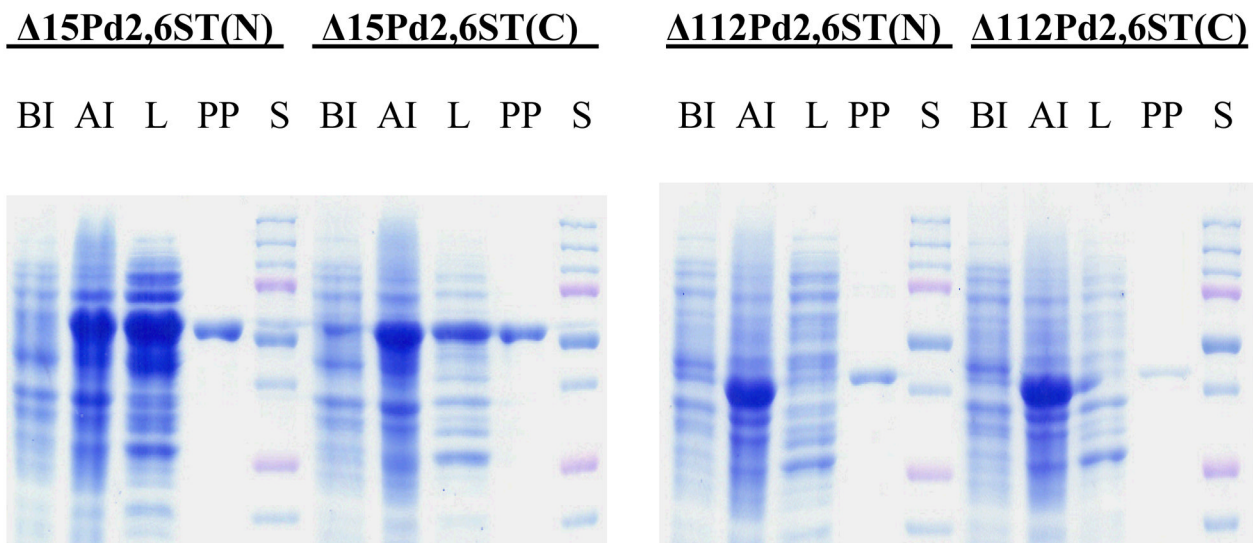
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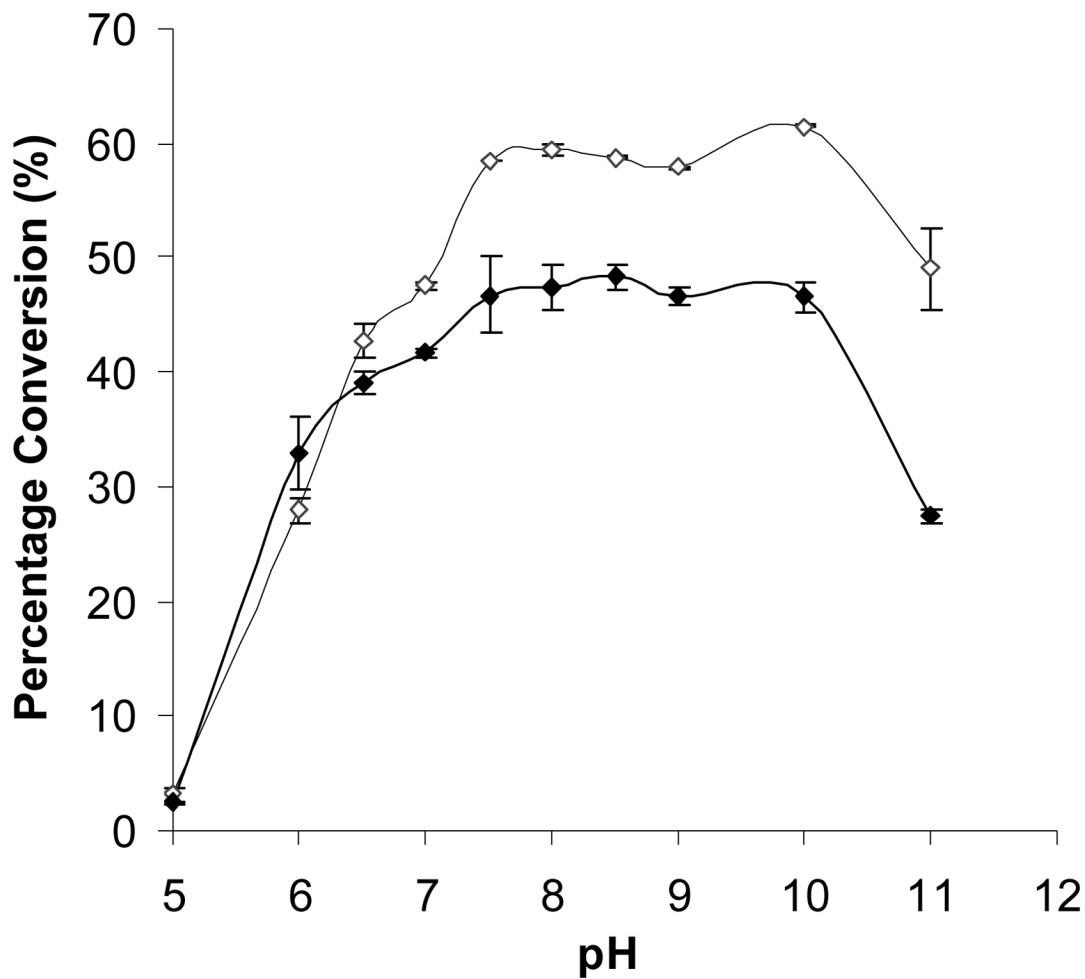
### References

- Angata T, Matsuda T, Kitajima K. Synthesis of neoglycoconjugates containing deaminated neuraminic acid (KDN) using rat liver  $\alpha$ 2,6-sialyltransferase. *Glycobiology* 1998;8:277–284. [PubMed: 9451037]
- Campbell JA, Davies GJ, Bulone V, Henrissat B. A classification of nucleotide-diphospho-sugar glycosyltransferases based on amino acid sequence similarities. *Biochem J* 1997;326:929–939. [PubMed: 9334165]
- Coutinho PM, Deleury E, Davies GJ, Henrissat B. An evolving hierarchical family classification for glycosyltransferases. *J Mol Biol* 2003;328:307–317. [PubMed: 12691742]
- Endo T, Koizumi S, Tabata K, Kakita S, Ozaki A. Large-scale production of the carbohydrate portion of the sialyl-Tn epitope, alpha-Neup5Ac-(2→6)-D-GalpNAc, through bacterial coupling. *Carbohydr Res* 2001;330:439–443. [PubMed: 11269395]
- Harduin-Lepers A, Recchi MA, Delannoy P. 1994, the year of sialyltransferases. *Glycobiology* 1995;5:741–758. [PubMed: 8720072]
- Kajihara Y, Yamamoto T, Nagae H, Nakashizuka M, Sakakibara T, Terada I. A novel alpha-2,6-sialyltransferase: transfer of sialic acid to fucosyl and sialyl trisaccharides. *J Org Chem* 1996;61:8632–8635.
- Kajihara Y, Akai S, Nakagawa T, Sato R, Ebata T, Kodama H, Sato K. Enzymatic synthesis of Kdn oligosaccharides by a bacterial alpha-(2→6)-sialyltransferase. *Carbohydr Res* 1999;315:137–141. [PubMed: 10385976]

- Kajihara Y, Kamiyama D, Yamamoto N, Sakakibara T, Izumi M, Hashimoto H. Synthesis of 2-[(2-pyridyl)amino]ethyl beta-D-lactosaminide and evaluation of its acceptor ability for sialyltransferase: a comparison with 4-methylumbelliferyl and dansyl beta-D-lactosaminide. *Carbohydr Res* 2004;339:1545–1550. [PubMed: 15178399]
- Li Y, Sun M, Huang S, Yu H, Chokhawala HA, Thon V, Chen X. The Hd0053 gene of *Haemophilus ducreyi* encodes an alpha2,3-sialyltransferase. *Biochem Biophys Res Commun* 2007;361:555–560. [PubMed: 17662691]
- Teo C, Hwang T, Chen P, Hung C, Gao H, Chang L, Lin C. Synthesis of sialyl TN glycopeptides - enzymatic sialylation by alpha2,6-sialyltransferase from *Photobacterium damsela*. *Adv Synth Catal* 2005;347:967–972.
- Tsukamoto H, Takakura Y, Yamamoto T. Purification, cloning, and expression of an alpha/beta-galactoside alpha-2,3-sialyltransferase from a luminous marine bacterium, *Photobacterium phosphoreum*. *J Biol Chem* 2007;282:29794–29802. [PubMed: 17702755]
- Yamamoto T, Hamada Y, Ichikawa M, Kajiwara H, Mine T, Tsukamoto H, Takakura Y. A beta-galactoside alpha2,6-sialyltransferase produced by a marine bacterium, *Photobacterium leiognathi* JT-SHIZ-145, is active at pH 8. *Glycobiology*. 200710.1093/glycob/cwm086(Advance Access published on August 17, 2007)
- Yamamoto T, Nagae H, Kajihara Y, Terada I. Mass production of bacterial alpha 2,6-sialyltransferase and enzymatic syntheses of sialyloligosaccharides. *Biosci Biotechnol Biochem* 1998b;62:210–214. [PubMed: 9532777]
- Yamamoto T, Nakashizuka M, Kodama H, Kajihara Y, Terada I. Purification and characterization of a marine bacterial beta-galactoside alpha 2,6-sialyltransferase from *Photobacterium damsela* JT0160. *J Biochem (Tokyo)* 1996;120:104–110. [PubMed: 8864851]
- Yamamoto T, Nakashizuka M, Terada I. Cloning and expression of a marine bacterial beta-galactoside alpha2,6-sialyltransferase gene from *Photobacterium damsela* JT0160. *J Biochem (Tokyo)* 1998a; 123:94–100. [PubMed: 9504414]
- Yu H, Chokhawala H, Karpel R, Wu B, Zhang J, Zhang Y, Jia Q, Chen X. A multifunctional *Pasteurella multocida* sialyltransferase: a powerful tool for the synthesis of sialoside libraries. *J Am Chem Soc* 2005;127:17618–17619. [PubMed: 16351087]
- Yu H, Huang S, Chokhawala H, Sun M, Zheng H, Chen X. Highly efficient chemoenzymatic synthesis of naturally occurring and non-natural alpha-2,6-linked sialosides: a *P. damsela* alpha-2,6-sialyltransferase with extremely flexible donor-substrate specificity. *Angew Chem Int Ed Engl* 2006;45:3938–3944. [PubMed: 16721893]



**Fig. 1.** Sodium dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE) analyses for four different Pd2,6ST constructs. Lanes: S, Bio-Rad Precision Plus Protein Standards (10–250 kDa); BI, whole cell extract before induction; AI, whole cell extract after induction; L, lysate; PP, HisTrap<sup>™</sup> FF 5 ml column purified protein. SDS-PAGE was performed in 10% Tris–glycine gels using Bio-Rad Mini-protein III cell gel electrophoresis unit (Bio-Rad, Hercules, CA) at DC = 150 V. Gels were stained with Coomassie Blue R-250.



**Fig. 2.** The pH profile of  $\Delta 15\text{Pd}2,6\text{ST}(\text{N})$  and  $\Delta 112\text{Pd}2,6\text{ST}(\text{N})$  (100% conversion was defined as the formation of 1 mM Neu5Ac $\alpha$ 2,6LacMU). Activity was measured in duplicate at indicated pH at 37 °C for 30 min. Buffers (200 mM) used were: MES, pH 5.0–6.0; HEPES, pH 7.0; Tris-HCl, pH 7.5–9.0; and CAPS, pH 10.0–11.0. Symbols and amounts of enzymes used:  $\Delta 15\text{Pd}2,6\text{ST}(\text{N})$  ( $\diamond$ , unfilled diamond), 240 ng;  $\Delta 112\text{Pd}2,6\text{ST}(\text{N})$  ( $\blacklozenge$ , filled diamond), 285 ng.



**Table 1**  
Expression levels of purified recombinant Pd2,6STs from *E. coli*

Enzymes	$\Delta 15\text{Pd2,6ST(N)}$	$\Delta 15\text{Pd2,6ST(C)}$	$\Delta 112\text{Pd2,6ST(N)}$	$\Delta 112\text{Pd2,6ST(C)}$
Amino acid residues	16 – 497 aa	16 – 497 aa	113 – 497 aa	113 – 497 aa
His-tag position	N-His tag	C-His tag	N-His tag	C-His tag
Expression level (mg l <sup>-1</sup> )	36.0	24.4	1.6	1.1

**Table 2**Apparent kinetic parameters of Pd2,6STs<sup>a</sup>

Enzymes	$\Delta 15\text{Pd}2,6\text{ST(N)}$		$\Delta 112\text{Pd}2,6\text{ST(N)}$	
Substrates	CMP-Neu5Ac	LacMU	CMP-Neu5Ac	LacMU
$K_M$ (mM)	$0.9 \pm 0.2$	$0.8 \pm 0.1$	$0.9 \pm 0.3$	$0.6 \pm 0.1$
$V_{\max}$ (mMs <sup>-1</sup> )	$(4.6 \pm 0.4) \times 10^{-4}$	$(4.0 \pm 0.2) \times 10^{-4}$	$(4.2 \pm 0.3) \times 10^{-4}$	$(3.4 \pm 0.2) \times 10^{-4}$
$k_{\text{cat}}$ (s <sup>-1</sup> )	$2.7 \pm 0.2$	$2.3 \pm 0.1$	$1.4 \pm 0.1$	$1.1 \pm 0.1$
$k_{\text{cat}}/K_M$ (s <sup>-1</sup> mM <sup>-1</sup> )	2.9	3.0	1.6	1.9

<sup>a</sup> Assays were performed in duplicate in a reaction mixture of 20  $\mu\text{l}$  containing Tris-HCl buffer (100 mM, pH 8.5) using varied concentrations (0.1, 0.25, 0.4, 1, 2, and 4 mM) of LacMU and a fixed concentration (1 mM) of CMP-Neu5Ac, or a fixed concentration (1 mM) of LacMU and varied concentrations (0.1, 0.25, 0.4, 1, 2, and 4 mM) of CMP-Neu5Ac. The amount of  $\Delta 15\text{Pd}2,6\text{ST(N)}$  used was 190 ng, and the amount of  $\Delta 112\text{Pd}2,6\text{ST(N)}$  used was 285 ng. Reactions were allowed to proceed for 30 min at 37 °C. Apparent kinetic parameters were obtained by fitting the data (the average values of duplicate assay results) into the Michaelis-Menten equation using Grafit 5.0.

**Table 3**Effects of metal ions, EDTA and DTT on the  $\alpha$ 2,6-sialyltransferase activity of  $\Delta$ 15Pd2,6ST(N) and  $\Delta$ 112Pd2,6ST(N)<sup>a</sup>

Reactions	$\Delta$ 15Pd2,6ST(N)	$\Delta$ 112Pd2,6ST(N)
Control	50%	43%
MgCl <sub>2</sub>	48%	43–44%
5 mM MnCl <sub>2</sub>	44%	39%
10 mM MnCl <sub>2</sub>	28%	38%
20 mM MnCl <sub>2</sub>	15%	21%
DTT	51–52%	45–46%
5 mM EDTA	43%	35%

<sup>a</sup>The numbers in the table represent the percentage conversions (Standard Deviation  $\leq$  1.4%). The activity was determined in duplicate at 37 °C for 30 min (100% conversion was defined as the formation of 1 mM Neu5Ac $\alpha$ 2,6LacMU). Different concentrations (5 mM, 10 mM, and 20 mM) of MgCl<sub>2</sub> or MnCl<sub>2</sub>, 5 mM EDTA, and various concentrations (0.2 mM, 1 mM, and 5 mM) of DTT were used in a reaction mixture of 20 $\mu$ l containing Tris-HCl buffer (pH 8.5, 100 mM), CMP-Neu5Ac (1 mM), LacMU (1 mM), and  $\Delta$ 15Pd2,6ST(N) (190 ng) or  $\Delta$ 112Pd2,6ST(N) (285ng). Reaction in the absence of DTT, EDTA, or metal ion was used as a control.