

Expanded View Figures

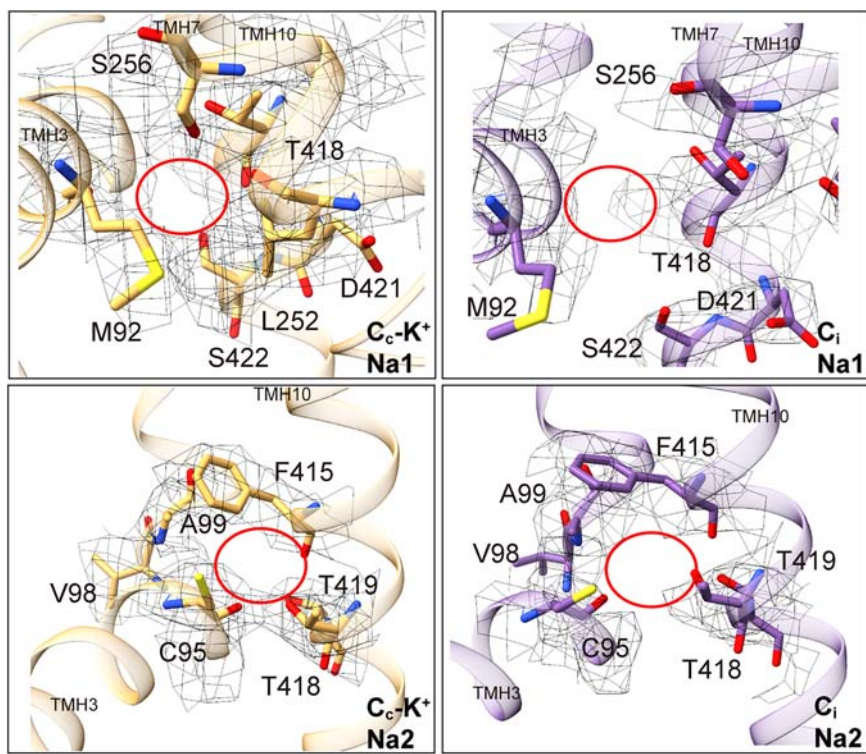


Figure EV1. Loss of cryo-EM densities at the putative Na1 and Na2 sites in the C_c -K⁺ and C_i structures determined in Na⁺-free buffer.

The gray mesh shows the local cryo-EM density of the residues involved in sodium binding, contoured at 4 RMSD. The densities corresponding to Na1 and Na2, which are clearly observed in the C_c and C_cS states, are absent under Na⁺-free conditions.

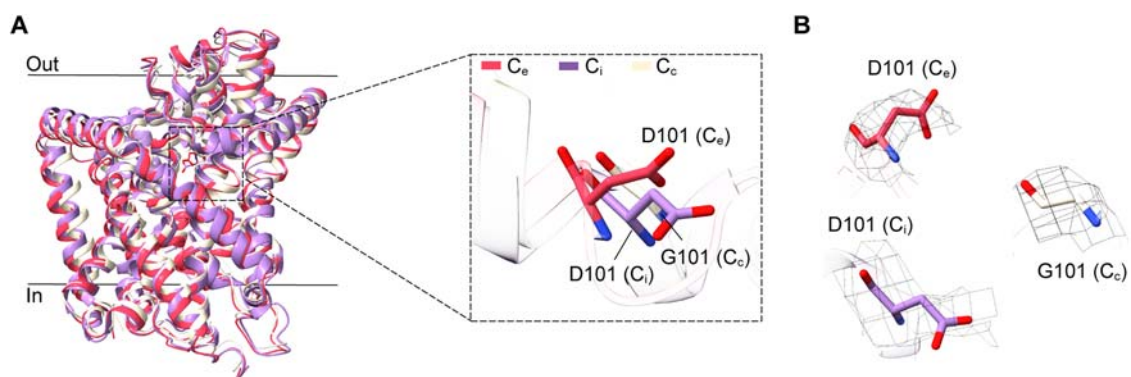


Figure EV2. Structural comparison of residue 101 in DddT among the C_c , C_e , and C_i states.

(A) Structural differences of residue 101 between the C_c , C_e , and C_i states. The C_c , C_e , and C_i states are colored in beige, red, and purple, respectively. (B) Cryo-EM densities surrounding Asp101 (C_e), Asp101 (C_i), and Gly101 (C_c), contoured at 6 RMSD, 4 RMSD, and 8 RMSD, respectively.

		Na1 binding site DddT			Na2 binding site DddT			Na1 binding site BetP	
		252	256	418 421 422	95	98 99	415 418 419	197	201
★	DddT <i>Psychrobacter</i> sp. D2	: GLKLLSD	ATTFDSA		FCAGVAS	IFLATTF		GILGAAG	
★	DddT <i>Halomonas</i> sp. HTNK1	: GIRILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
★	BccT5 <i>V. vulnificus</i> YJ016	: GIKVLN	ATTFDSI		FCGGIGA	IFTATTF		GLLGGA	
	DddT <i>Marinomonas</i> sp. MWYL1	: GIKILSE	VTSADSA		FSAGIGI	TYFVTTA		GTVFGLA	
	DddT <i>Pseudomonas</i> sp. J465	: GIRMLSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	DddT <i>Psychrobacter</i> sp. J466	: GVKWLSN	VTLDSSA		FGAGIGV	TYLVTTT		ATILGVA	
★	BccT2 <i>V. parahaemolyticus</i> RIMD2210633	: GVKRLSE	ITSSDSG		FAAGMGI	VFFITSS		ATVFGLA	
★	BccT3 <i>V. cholerae</i> N16961	: GVKVLN	ITSSDSG		FAAGMGI	VFFITSS		ATLFGLA	
	WP_142056479.1 <i>Pseudonocardia kunmingensis</i>	: GIQWLSN	VSGADAA		FSAGMGI	IFFVSGA		ATLFGSA	
	WP_285937307.1 <i>Actinomadura xylanilytica</i>	: GIQWLSN	VSGADAA		FSAGMGI	IFFVSGA		ATLFGSA	
	WP_092224259.1 <i>Desulforhopalus singaporensis</i>	: GIRILSL	VTTSDSA		FSAGFGL	VFGVTTT		STLGGNG	
	WP_066084478.1 <i>Hydrogenophaga crassostreae</i>	: GIKILSD	ATTLDSA		FCGGIGI	IFLATTL		GIVGGVA	
	WP_088916717.1 <i>Granulosicoccus antarcticus</i>	: GIRILSD	ATTLDSA		FCGGIGI	IFLATTL		GIVGGVG	
	WP_095133227.1 <i>Anaeromicrobium sediminis</i>	: GVKLSE	VTTSDSG		FSAGIGI	LEFVTTT		SVLFGLA	
	WP_108133289.1 <i>Halomonas</i>	: GIKILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
	WP_110674477.1 <i>Salinicola</i>	: GIRILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
	WP_118711689.1 <i>Enterocloster aldenensis</i>	: GKKTLSN	ASSLDSA		ALAALAS	GFVASSL		CIIGAAG	
	WP_191237274.1 <i>Cobetia</i>	: GIKILSD	ATTLDS		FTCGMGI	IFLATTL		GIVGGVG	
	WP_191237276.1 <i>Cobetia pacifica</i>	: GIKILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
	WP_169641146.1 <i>Roseobacter ponti</i>	: GIKILSD	ATTLDSA		FCGGIGI	IFLATTL		GIVGGVG	
	WP_186674364.1 <i>Pseudomonas oryzicola</i>	: GIKILSD	ATTLDSA		FCGGIGI	VFLATTL		GIVGGVG	
	WP_215375185.1 <i>Pseudomonas boanensis</i>	: GIKILSD	ATTLDSA		FCGGIGI	VFLATTL		GIVGGVG	
	WP_225346819.1 <i>Cobetia amphilecti</i>	: GIKILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
	WP_279833044.1 <i>Cobetia litoralis</i>	: GIKILSD	ATTLDS		FAGGIGI	IFLATTL		GIVGGVG	
	WP_074750954.1 <i>Pseudomonas abietaniphila</i>	: GIKLLSD	ATTLDSA		FCGGIGI	VFLATTL		GIVGGVG	
	WP_085879181.1 <i>Roseisalinus antarcticus</i>	: GIKILSD	ATTLDSA		FCGGIGI	IFLATTL		GIVGGVG	
	WP_013794832.1 <i>Marinomonas posidonica</i>	: GIKILSE	VTSADSA		FSAGIGI	TYFVTTA		GTVFGLA	
	WP_016855432.1 <i>Halomonas smyrnensis</i>	: GIKWLSN	VTSADSA		FATGMGI	LEFVTTA		STVIGLS	
	WP_002538432.1 <i>Grimontia indica</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_016417475.1 <i>Halomonas anticariensis</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_022951303.1 <i>Leucothrix mucor</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATVLGVA	
	WP_027258061.1 <i>Leisingera aquimarina</i>	: GVKYLSN	VTSADSG		FGAGLGV	TELVTSA		ATILGVS	
	WP_024089211.1 <i>Leisingera methylohalidivorans</i>	: GVKYLSN	VTSADSG		FGAGLGV	TELVTSA		ATILGVS	
	WP_028024020.1 <i>Enterovibrio calviensis</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_055463442.1 <i>Marinomonas fungiae</i>	: GINMLSQ	VTAADSG		FGCGTGA	VFFVTAA		GTIFGLA	
	WP_067385204.1 <i>Marinobacterium aestuarii</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_071472804.1 <i>Oceanisphaera psychrotolerans</i>	: GIKVLSE	VTTSDSS		FSAGIGI	SWFVTSS		GCVFGLA	
	WP_094199895.1 <i>Oceanimonas</i>	: GVKRLSE	VTTSDSG		FAAGMGI	VFFVTSS		ATLFGLA	
	WP_108385793.1 <i>Yoonia sediminilitoris</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_116473943.1 <i>Zobellia maritima</i>	: GIKWLSN	VTSADSA		FATGMGI	LEFVTTA		STVIGLS	
	WP_109822491.1 <i>Leucothrix arctica</i>	: GIKWLSN	VTTADSA		FGAGIGV	TYLVTTA		ATILGVA	
	WP_097805652.1 <i>Pelagimonas varians</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_066598641.1 <i>Celeribacter halophilus</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_113872885.1 <i>Marinomonas aquiplantarum</i>	: GIKILSE	VTSADSA		FSAGIGI	TYFVTTA		GTVFGLA	
	WP_115467766.1 <i>Marinomonas piezotolerans</i>	: GINMLSQ	VTAADSG		FGCGTGA	VFFVTAA		GTIFGLA	
	WP_113918092.1 <i>Marinomonas rhizomae</i>	: GIKILSE	VTSADSA		FSAGIGI	TYFVTTA		GTVFGLA	
	WP_129495376.1 <i>Enterovibrio baiaou</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_136464757.1 <i>Aliishimia ponticola</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_136665970.1 <i>Pseudomonas leptonychotis</i>	: GIRMLSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_146786251.1 <i>Colwellia demingiae</i>	: GIKILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_153718230.1 <i>Spiribacter salilacus</i>	: GLKWLSL	ITSSDSG		FSAGMGI	TYFITSS		ATLFGLA	
	WP_159963523.1 <i>Profundibacterium mesophilum</i>	: GVKVLSE	VTTSDSG		FAAGMGI	VFFVTSS		ATLFGLA	
	WP_183475125.1 <i>Limimarinicola variabilis</i>	: GIKWLSN	VTTADSA		FGAGIGV	TELVTTA		ATVLGVS	
	WP_189641528.1 <i>Amylibacter ulvae</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_191161141.1 <i>Bacillus</i> sp. IB182487	: GIKYLSN	VTSADSA		FSAGMGI	TEFVTSA		ATIFGLA	
	WP_191600050.1 <i>Marinomonas algicola</i>	: GINMLSQ	VTAADSG		FGCGTGA	VFFVTAA		GTIFGLA	
	WP_213639020.1 <i>Pseudomonas lalucatii</i>	: GVKWLSN	VTSADSA		FGAGIGV	TELVTSA		ATLLGLA	
	WP_213641632.1 <i>Pseudomonas lalucatii</i>	: GIKILSE	VTTSDSG		FSAGIGI	SWFVTSS		GCVFGLA	
	WP_218451483.1 <i>Roseobacteraceae</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_222223896.1 <i>Marinobacterium arenosum</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_253963426.1 <i>Aurantimonas marianensis</i>	: GVKRLSE	VTTSDSG		FAAGMGI	VFFVTSS		ATLFGLA	
	WP_264210529.1 <i>Leisingera</i> sp. BMJM1	: GVKYLSN	VTSADSG		FGAGLGV	TELVTSA		ATILGVS	
	WP_282351393.1 <i>Pontibacterium granulatum</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_067033200.1 <i>Marinomonas gallaica</i>	: GINMLSQ	VTAADSG		FGCGTGA	VFFVTAA		GTIFGLA	
	WP_090240933.1 <i>Pseudomonas guineae</i>	: GIRMLSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_092082295.1 <i>Poseidonocella sedimentorum</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_093992248.1 <i>Flavimaricola marinus</i>	: GIKWLSN	VTTADSA		FGAGIGI	TYLVTTA		ATILGVA	
	WP_163390697.1 <i>Enterovibrio norvegicus</i>	: GIRILSE	VTSADSA		FGAGIGI	TYFVTTA		GTVFGLA	
	WP_189582206.1 <i>Psychrobacter glaciei</i>	: GVKWLSN	VTTLDSSA		FGAGIGV	TYLVTTT		ATILGVA	

◀ **Figure EV3. Multi-sequence alignment of the experimentally ratified DddT proteins and 61 identified DddT homologues whose genes are linked to the DMSP lyase genes DddD/DddX.**

Key residues around the sodium-binding site are marked out. Based on the hydrophilicity and hydrophobicity of key residues at the Na⁺-binding site of BetP, sequences are divided into three categories. In the gray box, there is one hydrophilic residue and one hydrophobic residue. In the blue box, both residues are hydrophilic, and in the pink box, both residues are hydrophobic. The functional DddTs are marked with ★. Hydrophilic residues are colored green, and hydrophobic residues are colored orange. Source data are available online for this figure.

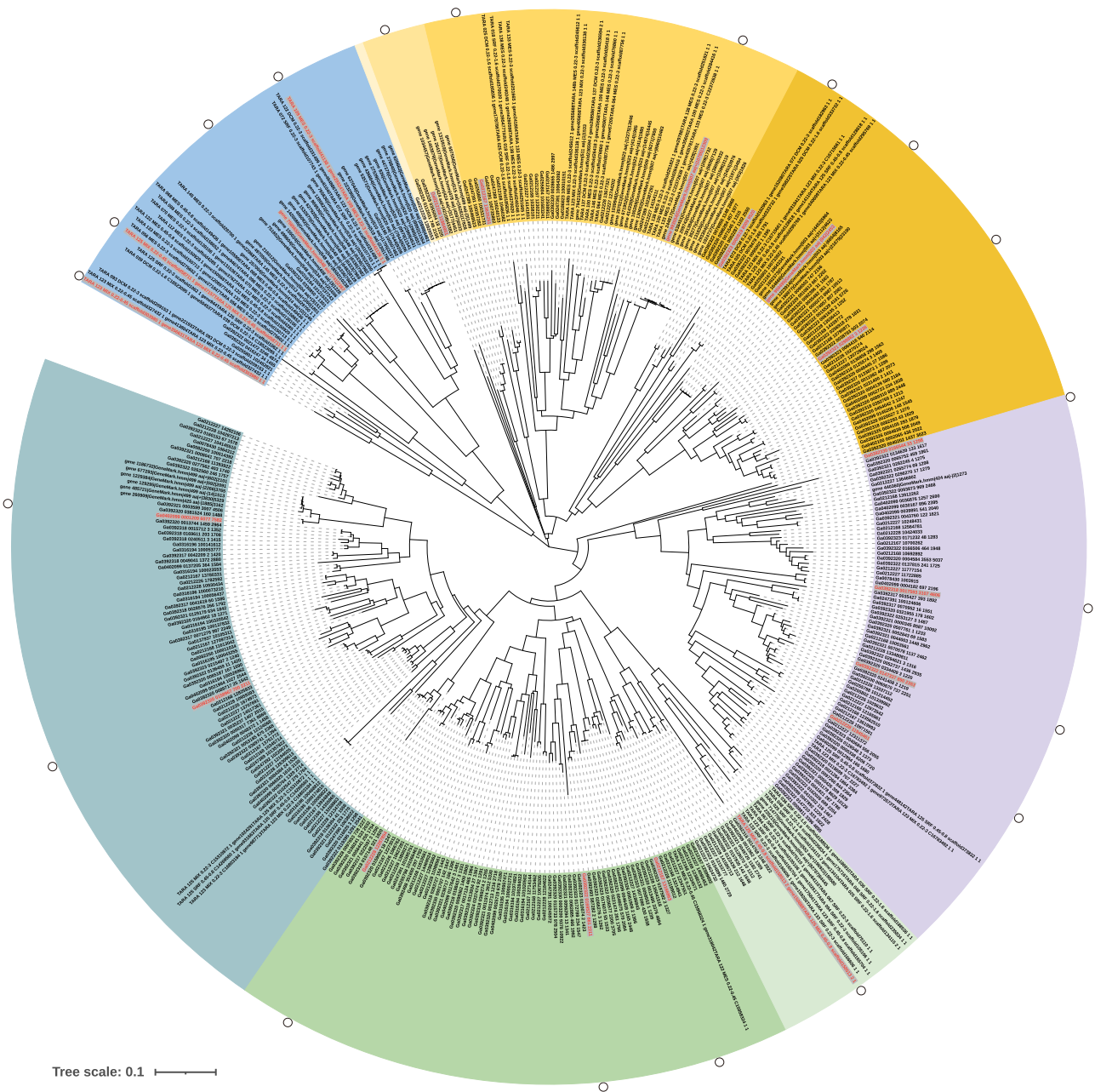


Figure EV4. Phylogenetic analysis of selected 411 DddT homologues from metagenome data.

Branches with different colors represent distinct clades in the phylogenetic tree. The sequences highlighted by shading and shown in red within the circle correspond to 20 randomly selected sequences from different branches of the phylogenetic tree, which were used for the multiple sequence alignment in Fig. 6C. Source data are available online for this figure.