

Journal: Applied Microbiology and Biotechnology

Degradation mechanism of difructose dianhydride III in *Blautia* species

Running Title: Characterization of *Blautia* DFA-IIIase

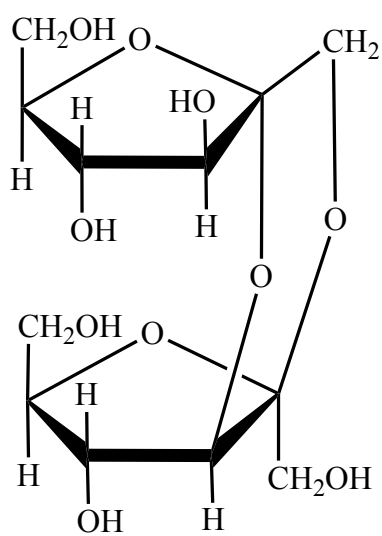
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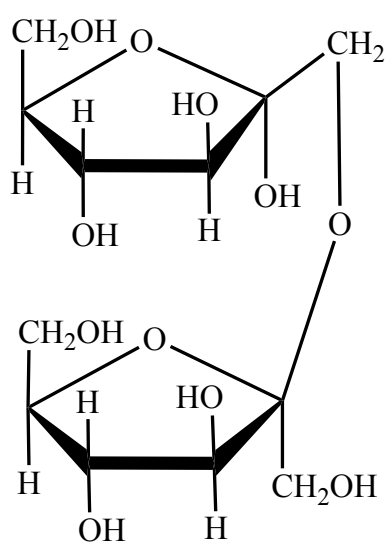
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DFA-III



inulobiose

Fig. S1. The chemical structures of DFA-III and inulobiose.

1	ATGAAATACCTGCTGCCGACCGCTGCTGCTGGTCTGCTGCTCCTCGCTGCCAGCCGGCGATGGCCATGGCAGGAAAAATTATTACGAT	90
	M K Y L L P T A A A G L L L L A A Q P A M A M A G K N Y Y D	
91	GTAACAGAATGGAACGTGGGCGATCCGTATAAAGACATCGGCGAGGTGATCAACAGCATGCTGGCTGACATCAAAGCAGGCAAACTGAC	180
	V T E W N V G D P Y K D I G E V I N S M L A D I K S R Q T D	
181	AGTAATGTTGAGGAGGTGGCAAGCCAGGTGCGGTTATCTACATTCGAGCGGCACTATCATCTGCGCACCCAGGTGGTTATAGACATC	270
	S N V E E G G K P G A V I Y I P S G D Y H L R T Q V V I D I	
271	TCCTATCTAAAGATCATGGGTGCCGGTCACGGTTTTGTTTCTAGCTCGATTCTGTACAATCTGCCGAAGAGTGAGTGGGAAGATTGCAC	360
	S Y L K I M G A G H G F V S S S I R Y N L P K S E W E D L H	
361	GAGGTTTGGCTGGCGGTAGCCGCATCCTGGTGGACCTTCCCGAAAGATGCGGACGGTGAAGCTGCGGGTGCGGCGTTTTACGTCGAG	450
	E V W P G G S R I L V D L S P K D A D G E A A G A A F Y V E	
451	CGCTCCGGCAACCCGCGTATCTCCAGCGTGGAGTTCGAGAACTTCTGTATTGATGGTTTGCACCTTCGCGGACGACGGCAGCGGTGAAGAC	540
	R S G N P R I S S V E F E N F C I D G L H F A D D G S G K N	
541	GACCCAGAAAAACGTACGTTAACGGCAAGACCGGTATTACGTCGCGCTGCGCAAGATTCTTTCTGATTACCGGCATGGGCTTCGTG	630
	D P E N T Y V N G K T G I Y V A S A Q D S F R I T G M G F V	
631	TATCTGGAACATGGTGTATCATCTACCACGCCGATGCCCTGAGCGTTCACGATAATTTTCATCGCTGAATGTGGTAACTGCATCGAACTG	720
	Y L E H G V I I Y H A D A L S V H D N F I A E C G N C I E L	
721	CGTGGTTGGGTCAGGCAAGCAAAATCACTGACAACCTGATGGGTGCGGGCTATCGTGGTTACAGCGTATTCGCGCAGAATTTTGGTGA	810
	R G W G Q A S K I T D N L M G A G Y R G Y S V F A Q N F G G	
811	TTGCTGATTACGTCCAACAATATCTTCCGAGAGGCACAGCTGCGTGCACCTTGATAAAGTTGCGGTTCCGTGATTACGGGCAATCGT	900
	L L I T S N N I F P R G T S C V H F D K V A R S V I T G N R	
901	TTTCATAGCTTTTACCGGGTGCCTGGTGGTTTTGAGGGTAACTGCAGCGAGAACCACGTCAGCTCCAATCACTTCCTGCGTGATCATGAA	990
	F H S F Y P G A L V F E G N C S E N T V S S N H F L R D H E	
991	CCGTGGGCACCGATGCTGAAAAACGATAACGGTCTCGACGATCGTTATGGCTTGTTACGCATCTCTGGCAATCACAATTCTGTCATTGCT	1080
	P W A P M L K N D N G L D D R Y G L L R I S G N H N S V I A	
1081	AATCATATTTCTGAAACCATTCATCGTCAGAGCATTAAGCCGGCTGGCGCAAAACCGGTGATCATTATGTCGTGTCAGGCAAGGGCAAC	1170
	N H I S E T I H R Q S I K P A G A K P V I I H V V S G K G N	
1171	TATATCTCCGACAACCATTTGTTGGCCACCACGGAAGCAGCGGATGCTCAAACCGGTGCCAGCGATAGTTGCTTCAGCGCTCAAGTGGAT	1260
	Y I S D N H I V A T <u>T E A A D A Q T G A S D S C F S A Q V D</u>	
	Lid region	
1261	GCGCTGACCGCAACGACAAGCTGGCAGTTCTGGACGTGACCACCGTTTTGGTAGCCAAGAAAGCTTCCAGAACACGATTCTGGATTCT	1350
	<u>A L T A T D K</u> L A V L D V T T V L V A K E S F Q N T I L D S	
1351	GGTTCGGAAGAGCAGGTTGTGCTTGACCGCGGTGTAATGCATTCCGCGCAACCCCGGTGCCGGGAGGCGTTCTCGAGCACCACCACCAC	1440
	G S E E Q V V L D R R V N A F R A T P V P G G V L E H H H H	
1441	CACCACTGA	1449
	H H *	

Fig. S2. The nucleotide sequence and deduced amino acid sequence of *BpDFA*-IIIase with a C-terminal His-tag. The predicted lid region is indicated by red color with underline.

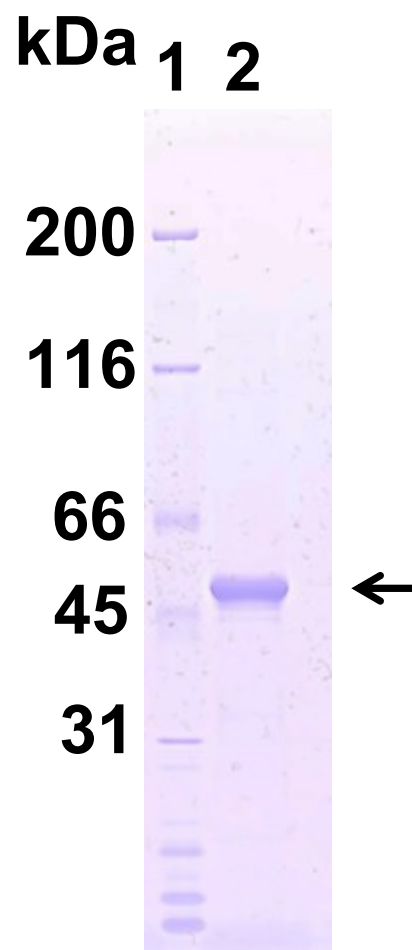


Fig. S3. SDS-PAGE of purified *BpDFA-IIIase*. Lane 1, protein size maker and lane 2, *BpDFA-IIIase*. Proteins and markers are stained with Coomassie Blue.

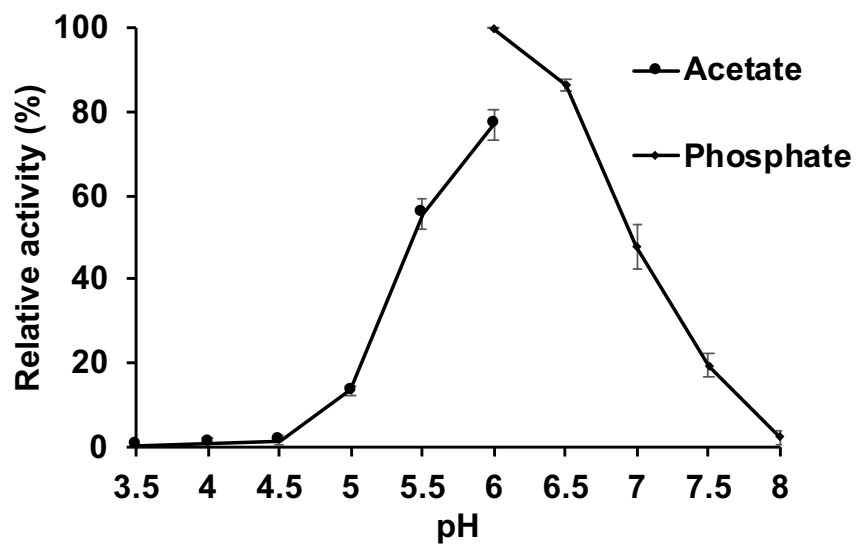
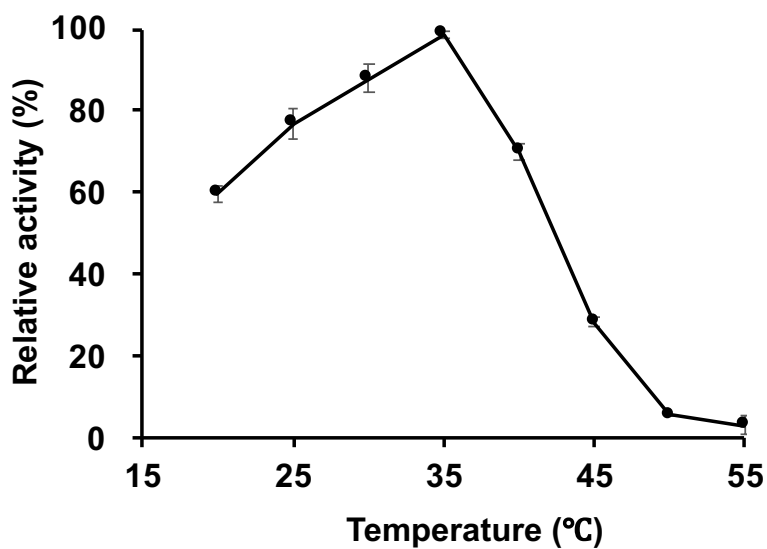
A**B**

Fig. S4. Effect of pH and temperature on *BpDFA*-IIIase activity. **(A)** Relative activity was evaluated at 37°C and different pH values. **(B)** Relative activity was evaluated at 20°C–55°C in sodium phosphate buffer (pH 6.0). Relative activity was expressed as a percentage of maximal enzyme activity. Values are means of three replicates $\pm$ standard deviation.