

Supplemental information for:

Exploration of three *Dyadobacter fermentans* enzymes uncovers molecular activity determinants in CE15

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Supplementary tables

Table S1. Primers used for the cloning of *D. fermentans* CE15 genes, and for the mutated variants of *DfCE15A* and *DfCE15B*.

Gene	Primer	5'-3' sequence
<i>DfCE15A</i>	<i>DfCE15Af</i>	CTTCCAGGGCCATAGTCAGAATTATGATGAATCCAAAAGTGCCGC
	<i>DfCE15Ar</i>	TGGTGGTGCTCGAGTCTAAAATTGTTTAGCGAAACGGATGAATTTATC
<i>DfCE15B</i>	<i>DfCE15Bf</i>	CTTCCAGGGCCATAGTCAACAAGCCGCGAATCGACCG
	<i>DfCE15Br</i>	TGGTGGTGCTCGAGTCTACAAATACTTTTGCATGTAGCGTATGAAGAG
<i>DfCE15C</i>	<i>DfCE15Cf</i>	CTTCCAGGGCCATAGTCAAGATGTGCAGCGACTACCCG
	<i>DfCE15Cr</i>	TGGTGGTGCTCGAGTCTATTGCAAAATGTAAATCCGAGAAGTTGAGAAT
<i>DfCE15A-3v</i>	<i>DfCE15A-N142F/R143Af</i>	ATCAATTTGCTGGGAAGGAC
	<i>DfCE15A-N142F/R143Ar</i>	CTTCCCAGCGAAATTGATCAG
	<i>DfCE15A-G242Lf</i>	TCGCGCCTTGGTAAACTTC
	<i>DfCE15A-G242Lr</i>	TTTACCAAGGCGCGAATGCC
<i>DfCE15B-Q318W</i>	<i>DfCE15B-Q318Wf</i>	TTGTATTGGTTTGCGCCCAAT
	<i>DfCE15B-Q318Wr</i>	CGCAAACCAATACAAGTACCG

Table S2. Genomic neighbourhood of *DfCE15A*. The closest characterized homolog for a neighbouring gene was identified through either primary sequence analysis using BLAST (Altschul et al. 1990) or structural analysis through Foldseek (van Kempen et al. 2023) from the AlphaFold2 (Varadi et al. 2022) predicted structure found on Uniprot (Consortium 2023). *DfCE15A* is highlighted in green and sequences of a cluster of 3 or more putatively annotated genes associated with a specific function are additionally highlighted.

Start	End	Direction	New locus tag	Old locus tag	NCBI Accession	Closest characterized homolog	% Seq Identity	% Query coverage	Citation	Name or Putative annotation	Putative cluster annotation
3686925	3687737	-	DFER_RS14895	Dfer_3014	WP_015812478.1	LpxA a UDP-N-acetylglucosamine acyltransferase from <i>Bacteroides fragilis</i> 9343	49	94	PMID: 25945572	UDP-GlcNAc acyltransferase	Possible lipid A biosynthesis & modification cluster
3687734	3689125	-	DFER_RS14900	Dfer_3015	WP_015812479.1	Dual domain - LpxC - UDP-(3-O-((R)-3-hydroxymyristoyl))-N-acetylglucosamine deacetylase from <i>Yersinia enterocolitica</i> & acyl-carrier-protein] dehydratase FabZ from <i>E. coli</i>	38	63	PMID: 21171638	CE11 - Deacetylase & Dehydratase carrier protein	
3689174	3690214	-	DFER_RS14905	Dfer_3016	WP_015812480.1	N-Acyltransferase of lipid A biosynthesis	37	97	PMID: 17360522	UDP-N-acyltransferase	
3690296	3691528	-	DFER_RS14910	Dfer_3017	WP_229206007.1	Phosphohydrolase (BT4208) from <i>Bacteroides thetaiotaomicron</i> VPI-5482	51	98	Unpublished	dNTPase	
3691649	3691722	+	DFER_RS14915	Dfer_R0009	tRNA	tRNA	-	-	-	tRNA	
3691822	3692013	+	DFER_RS14920	Dfer_3018	WP_015812482.1	Cold shock protein E from <i>Salmonella typhimurium</i>	49	96	PMID: 20054119	Cold shock protein	
3692138	3693040	+	DFER_RS14925	Dfer_3019	WP_015812483.1	Predicted lipid A biosynthesis acyltransferase	-	-	-	Lipid A biosynthesis acyltransferase	
3693050	3694216	-	DFER_RS14930	Dfer_3020	WP_015812484.1	Sialidase from <i>Micromonospora viridifaciens</i>	33	88	PMID: 8591030	GH33 (sialidase?)	
3694227	3695732	-	DFER_RS14935	Dfer_3021	WP_015812485.1	<i>Xanthomonas</i> xyloglucan acetylsterase	34	92	https://doi.org/10.1038/s41467-021-24277-4	CE20 (sialic acid acetyl est?)	
3695729	3698380	-	DFER_RS14940	Dfer_3022	WP_015812486.1	Sialic acid symporter from <i>Proteus mirabilis</i> with a separate kelch-type beta propeller domain	37	54	PMID: 29717135	Sugar transporter	
3698380	3699321	-	DFER_RS14945	Dfer_3023	WP_015812487.1	N-acetylneuraminate lyase from <i>Alivibrio salmonicida</i>	38	90	PMID: 31185017	N-acetylneuraminate lyase - class I aldolase	
3699352	3700926	-	DFER_RS14950	Dfer_3024	WP_015812488.1	SusD homolog from <i>Bacteroides fragilis</i> NCTC 9343	10	90	NA	SusC/D - Sugar binding TonB receptor	
3700931	3704317	-	DFER_RS14955	Dfer_3025	WP_015812489.1	SusC homolog from <i>Bacteroides thetaiotaomicron</i> VPI-5482	27	90	PMID: 28077872	SusC/D - Sugar binding TonB receptor	
3704501	3705202	+	DFER_RS14960	Dfer_3026	WP_015812490.1	Uxu operon regulator from <i>Haemophilus influenzae</i> Rd KW20 - FadR/GntR type	32	83	P44487.1	Transcriptional regulator	
3705243	3705932	-	DFER_RS14965	Dfer_3027	WP_229206008.1	2-Hydroxy-6-oxo-6-phenylhexa-2,4-dienoate hydrolase (BphD homolog) from <i>Rhodococcus jostii</i> RHA1	23	88	https://doi.org/10.2183/pjab.73.154	BphD type hydrolase - alpha/beta-hydrolase	
3706236	3706931	+	DFER_RS14970	Dfer_3028	WP_187293386.1	Predicted bacterial Ig-like domain containing protein	-	-	-	-	
3706966	3707937	-	DFER_RS14975	Dfer_3029	WP_015812493.1	Predicted peptidase S74 containing domain	-	-	-	-	
3708192	3708371	-	DFER_RS29920	Dfer_3030	WP_015812494.1	None - unknown (59 aa)	-	-	-	-	
3708611	3709012	+	DFER_RS14990	Dfer_3031	WP_015812495.1	None - unknown (133 aa)	-	-	-	-	
3709009	3709236	-	DFER_RS14995	Dfer_3032	WP_015812496.1	None - unknown (75 aa)	-	-	-	-	
3709360	3710535	-	DFER_RS15000	Dfer_3033	WP_229206009.1	Glucuronoyl esterase from <i>Opitutus terrae</i> OtCE15A	39	94	PMID: 30083226	<i>DfCE15A</i>	
3710721	3711095	+	DFER_RS15005	Dfer_3034	WP_041735155.1	None - unknown (124 aa)	-	-	-	-	
3711190	3711723	+	DFER_RS15010	Dfer_3035	WP_015812499.1	RNA polymerase sigma factor SigM <i>Mycobacterium tuberculosis</i>	25	81	H8F2P4.1		
3711723	3713324	+	DFER_RS15015	Dfer_3036	WP_015812500.1	Predicted Outer membrane protein beta-barrel domain-containing protein	-	-	-	-	
3713349	3715559	-	DFER_RS15020	Dfer_3037	WP_015812501.1	SusC homolog from <i>Bacteroides thetaiotaomicron</i> VPI-5482	10	90	PMID: 28077872	SusC/D - sugar binding TonB receptor	
3715889	3716446	-	DFER_RS15025	Dfer_3038	WP_041735158.1	None - unknown (169 aa)	-	-	-	-	
3716623	3716961	+	DFER_RS15030	Dfer_3039	WP_015812503.1	None - Cupin domain (112 aa)	-	-	-	-	
3716986	3717729	-	DFER_RS15035	Dfer_3040	WP_015812504.1	Short-chain dehydrogenase/reductase FabG from <i>Bacillus sp.</i>	42	98	PMID: 24212572	Dehydrogenase	
3717952	3719268	+	DFER_RS15040	Dfer_3041	WP_041735160.1	GH158 - endo-laminarinase from beta1,3)-glucan utilization locus of <i>Bacteroides uniformis</i>	26	91	PMID: 32265336	GH	Sugar metabolism
3719265	3722207	+	DFER_RS15045	Dfer_3042	WP_015812506.1	GT2 - cellulose synthase from <i>Cereibacter sphaeroides</i> + a GH domain similar to GH158	30	28	PMID: 26958837	GT + GH	
3722201	3723877	-	DFER_RS15050	Dfer_3043	WP_015812507.1	Predicted 2 domains: Nt= ??? (probably another GH) & Ct = GH26 (beta-mannanase)	-	20	PMID: 15987675	??? + GH	
3724066	3724398	-	DFER_RS15055	Dfer_3044	WP_015812508.1	None - unknown (110 aa)	-	-	-	-	
3724610	3725785	-	DFER_RS15060	Dfer_3045	WP_015812509.1	Glutathionylspermidine synthetase/amidase from <i>Escherichia coli</i> K-12	37	91	PMID: 36745518	Ligase	
3725832	3726443	-	DFER_RS15065	Dfer_3046	WP_015812510.1	None - unknown (203 aa)	-	-	-	-	
3726626	3727912	-	DFER_RS15070	Dfer_3047	WP_015812511.1	Aminotransferase PvdN from <i>Pseudomonas aeruginosa</i>	24	98	PMID: 27139833	Transferase/esterase	
3727984	3728742	-	DFER_RS15075	Dfer_3048	WP_015812512.1	Lysostaphin-type metalloendopeptidase from <i>S. aureus</i>	30	34	PMID: 16269153	Peptidase/esterase	
3728904	3729875	+	DFER_RS15080	Dfer_3049	WP_015812513.1	4-Hydroxy-2,3-trans-nonenal reductase from <i>Bacillus halodurans</i> (or 1-deoxyxylulose-5-phosphate synthase)	30	95	PMID: 16242712	Oxidoreductase	
3730103	3731326	-	DFER_RS15085	Dfer_3050	WP_015810681.1	Putative transposase y4r1 from <i>Sinorhizobium fredii</i> NGR234	28	65	P55643.1	Transposase	
3731421	3731831	-	DFER_RS15090	Dfer_3051	WP_015812514.1	None - unknown (136 aa)	-	-	-	-	
3732017	3734278	+	DFER_RS15095	Dfer_3052	WP_015812515.1	SusC homolog from <i>Bacteroides thetaiotaomicron</i> VPI-5482	12	90	PMID: 28077872	SusC/D - sugar binding TonB receptor	Sugar metabolism
3734425	3734733	+	DFER_RS15100	Dfer_3053	WP_015812516.1	CBM20-like from a GH13 Rv1326c of <i>Mycobacterium tuberculosis</i>	67	20	PMID: 20444687	Sugar binding/acting	
3734865	3735785	-	DFER_RS15105	Dfer_3054	WP_015812517.1	3-Keto-α-D-gulosides epimerase from <i>Escherichia coli</i> K-12 MG1655	16	95	PMID: 19255464	Sugar epimerase	
3735867	3738569	-	DFER_RS15110	Dfer_3055	WP_229206010.1	PQQ-dependent glucose dehydrogenase from <i>Acinetobacter calcoaceticus</i>	14	50	no ref	Sugar dehydrogenase	

Table S3. Genomic neighbourhood of *DfCE15B*. The closest characterized homolog for a neighbouring gene was identified through either primary sequence analysis using BLAST (Altschul et al. 1990) or structural analysis through Foldseek (van Kempen et al. 2023) from the AlphaFold2 (Varadi et al. 2022) predicted structure found on Uniprot (Consortium 2023). *DfCE15B* is highlighted in green and sequences of a cluster of 3 or more putatively annotated genes associated with a specific function are additionally highlighted.

Start	End	Direction	New locus tag	Old locus tag	NCBI Accession	Closest characterized homolog	% Seq Identity	% Query coverage	Citation	Name or Putative annotation	Putative cluster annotation
3801959	3802300	+	DFER_RS15400	Dfer_3112	WP_015812575.1	Metallochaperone <i>Staphylococcus aureus</i>	24	50	PDB: 6FF2	Metal chaperone	
3802319	3803716	+	DFER_RS15405	Dfer_3113	WP_015812576.1	OmpF transporter from <i>E. coli</i>	9	80	PMID: 29038444	Transporter	
3803767	3804510	+	DFER_RS15410	Dfer_3114	WP_015812577.1	Methyltransferase from <i>Streptomyces caelestis</i>	17	90	PMID: 24699640	Methyltransferase	
3804511	3804969	-	DFER_RS15415	Dfer_3115	WP_015812578.1	Predicted transporter - DUF6691	-	-	-	Transporter	
3805072	3805647	-	DFER_RS15420	Dfer_3116	WP_015812579.1	Predicted sulfur transporter	-	-	-	Transporter	
3806056	3806265	-	DFER_RS15430	Dfer_3118	WP_015812580.1	None - unknown (69 aa)	-	-	-	-	
3806314	3807705	-	DFER_RS15435	Dfer_3119	WP_015812581.1	Protein with beta-lactamase and rhodanese domains from <i>Alcyclobacillus acidocaldarius</i>	35	98	PDB: 3TP9	Metallo hydrolase	
3807930	3809315	-	DFER_RS15440	Dfer_3120	WP_015812582.1	Multidrug and Toxin Compound Extrusion (MATE) transporter from <i>Vibrio cholerae</i> O1	22	86	PMID: 20861838	Transporter	
3809655	3810890	+	DFER_RS15445	Dfer_3121	WP_015812583.1	GTPase HFIX from <i>Escherichia coli</i>	40	87	PMID: 26458047	GTPase	
3810959	3811030	+	DFER_RS15450	Dfer_R0010	tRNA	tRNA	-	-	-	-	
3811321	3812429	+	DFER_RS15460	Dfer_3123	WP_222837289.1	Transposase from <i>E. coli</i>	12	90	PMID: 35654042	Transposase	
3812599	3813690	+	DFER_RS15465	Dfer_3124	WP_015812584.1	None - unknown (363 aa)	-	-	-	-	
3813891	3814100	+	DFER_RS15470	Dfer_3125	WP_015812585.1	None - unknown (69 aa)	-	-	-	-	
3814185	3815477	+	DFER_RS15475	Dfer_3126	WP_015812586.1	Glucuronoyl esterase from <i>Opitutus terrae</i> OtCE15A	36	100	PMID: 30083226	<i>DfCE15B</i>	Sugar metabolism
3815760	3816980	+	DFER_RS15480	Dfer_3127	WP_015812587.1	Myo-inositol 1-phosphate synthase from <i>Mycobacterium tuberculosis</i>	42	89	PMID: 12005437	Inositol 1-phosphate synthase	
3817008	3817775	-	DFER_RS15485	Dfer_3128	WP_015812588.1	Predicted DeoR type regulator	-	-	-	Transcription regulator	
3818116	3819381	+	DFER_RS15490	Dfer_3129	WP_015812589.1	Predicted MFS transporter	-	-	-	MFS Transporter	
3819447	3820451	+	DFER_RS15495	Dfer_3130	WP_015812590.1	Human 3-phosphoglycerate dehydrogenase	31	90	PMID: 29262655	2-hydroxy acid dehydrogenase	
3820463	3821908	+	DFER_RS15500	Dfer_3131	WP_015812591.1	L-fucose kinase from <i>Streptococcus Pneumoniae</i>	25	67	PMID: 24333485	Sugar kinase	
3821942	3823399	+	DFER_RS15505	Dfer_3132	WP_015812592.1	Sugar kinases from <i>Synechococcus elongatus</i> PCC7942	30	87	PMID: 27223615	Sugar kinase	
3823402	3824004	+	DFER_RS15510	Dfer_3133	WP_015812593.1	Phosphoserine phosphatase from <i>Hydrogenobacter thermophilus</i> TK-6	32	98	PMID: 23479726	Phosphatase/Phosphoglycerate mutase	
3824018	3824956	+	DFER_RS15515	Dfer_3134	WP_015812594.1	Glycerophosphodiester phosphodiesterase from <i>Parabacteroides distasonis</i> ATCC 8503	50	89	PDB: 3MZ2	Phosphodiesterase	
3825141	3825494	+	DFER_RS15520	Dfer_3135	WP_015812595.1	Predicted PadR type transcriptional regulator	-	-	-	Transcription regulator	
3825498	3825998	+	DFER_RS30555		WP_229206013.1	Predicted ABC transporter associated protein	-	-	-	Transporter	
3825898	3828159	+	DFER_RS15525	Dfer_3136	WP_229206014.1	Predicted ABC transporter	-	-	-	Transporter	
3828309	3829505	+	DFER_RS15530	Dfer_3137	WP_015812596.1	L, D-transpeptidase from <i>E. coli</i>	37	56	PMID: 31015395	L, D-transpeptidase	
3829478	3830245	-	DFER_RS15535	Dfer_3138	WP_015812597.1	Predicted murein L, D-transpeptidase	-	-	-	L, D-transpeptidase	
3830388	3831245	-	DFER_RS15540	Dfer_3139	WP_015812598.1	Prephenate dehydrogenase from <i>Aquifex aeolicus</i>	38	88	PMID: 19279014	Dehydrogenase	
3831274	3832431	-	DFER_RS15545	Dfer_3140	WP_015812599.1	Pyridoxal 5-phosphate (PLP)-dependent stereospecific transaminase from <i>Bacillus subtilis</i>	38	95	PMID: 32134000	Transaminase	

Table S4. Genomic neighbourhood of *DfCE15C*. The closest characterized homolog for a neighbouring gene was identified through either primary sequence analysis using BLAST (Altschul et al. 1990) or structural analysis through Foldseek (van Kempen et al. 2023) from the AlphaFold2 (Varadi et al. 2022) predicted structure found on Uniprot (Consortium 2023). *DfCE15C* is highlighted in green and sequences of a cluster of 3 or more putatively annotated genes associated with a specific function are additionally highlighted.

Start	End	Direction	New locus tag	Old locus tag	NCBI Accession	Closest characterized homolog	% Seq Identity	% Query coverage	Citation	Name or Putative annotation	Putative cluster annotation
6841666	6841789	-	DFER_RS28420	Dfer_5694	WP_015815123.1	2-Keto-myo-inositol dehydratase from <i>Clostridium tetani</i> E88	55	99	Q898E6.1	Sugar dehydratase	Possible uronate to amino acid synthesis cluster
6841789	6843654	-	DFER_RS28425	Dfer_5695	WP_015815124.1	Acetohydroxyacid synthase from <i>Arabidopsis thaliana</i>	27	77	PMID: 35690625	Acetohydroxyacid synthase	
6843693	6844634	-	DFER_RS28430	Dfer_5696	WP_015815125.1	Fructose-1,6-bisphosphate (FBP) aldolase from <i>Thermus aquaticus</i>	42	98	PMID: 14699122	Class II aldolase	
6844643	6846253	-	DFER_RS28435	Dfer_5697	WP_015815126.1	Sodium/galactose transporter from <i>Vibrio parahaemolyticus</i>	48	99	PMID: 21131949	Sugar transporter	
6846421	6847449	-	DFER_RS28440	Dfer_5698	WP_015815127.1	Myo-inositol 2-dehydrogenase from <i>Thermotoga maritima</i> MSB8	51	97	Q9WYP5.1	Sugar dehydrogenase	
6847477	6848943	-	DFER_RS28445	Dfer_5699	WP_015815128.1	Methylmalonate semialdehyde dehydrogenase from <i>Pseudomonas</i> sp. AAC	41	98	PMID: 28045390	Sugar dehydrogenase	
6848957	6849964	-	DFER_RS28450	Dfer_5700	WP_015815129.1	Fructokinase from <i>Halothermothrix orenii</i> H 168	25	97	PMID: 20493950	Sugar kinase	
6850215	6851078	-	DFER_RS28455	Dfer_5701	WP_015815130.1	Uncharacterized 5-deoxy-glucuronate isomerase from <i>Geobacillus kaustophilus</i> HTA426	39	88	Q5KYR2.1	Kdul/IolB uronate isomerase	Sugar metabolism
6851205	6852236	+	DFER_RS28460	Dfer_5702	WP_015815131.1	Catabolite control protein A (CcpA) from <i>Bacillus megaterium</i>	32	97	PMID: 17500051	Transcription regulator	
6852350	6854977	+	DFER_RS28465	Dfer_5703	WP_143828846.1	None - unknown (875 aa)	-	-	-	-	
6855047	6856741	-	DFER_RS28470	Dfer_5704	WP_015815133.1	SusD homolog from <i>Bacteroides thetaiotaomicron</i> VPI-5482	26	94	PMID: 28077872	SusC/D - sugar binding TonB-dependent receptor	
6856752	6859787	-	DFER_RS28475	Dfer_5705	WP_015815134.1	SusC homolog from <i>Bacteroides thetaiotaomicron</i> VPI-5482	31	90	PMID: 28077872	SusC/D - sugar binding TonB-dependent receptor	
6860250	6860483	-	DFER_RS28480	Dfer_5706	WP_015815135.1	None - short sequence (74 aa)	-	-	-	-	
6860487	6860951	-	DFER_RS28485	Dfer_5707	WP_015815136.1	None - unknown (154 aa)	-	-	-	-	
6860996	6863305	-	DFER_RS28490	Dfer_5708	WP_310586751.1	ABC transporter from <i>E. coli</i> K-12 - uncharacterized	27	95	P77504.1	ABC Transporter	
6863315	6863539	-	DFER_RS30800		WP_310586752.1	None - short sequence (74 aa)	-	-	-	-	
6863553	6864227	-	DFER_RS28495	Dfer_5709	WP_015815137.1	ABC transporter from <i>Streptococcus pneumoniae</i> R6	49	98	PMID: 29335499	ABC Transporter	
6864404	6865099	+	DFER_RS28500	Dfer_5710	WP_041735644.1	Lysophospholipase A (TesaA) from <i>Pseudomonas aeruginosa</i>	42	80	PMID: 23874889	Esterase	
6865135	6865686	-	DFER_RS28505	Dfer_5711	WP_015815139.1	None - unknown (183 aa) - DUF456 protein	-	-	-	-	
6865762	6865926	-	DFER_RS29990	Dfer_5712	WP_015815140.1	None - short sequence (54 aa) - DUF5670 protein	-	-	-	-	
6866152	6866970	+	DFER_RS28510	Dfer_5713	WP_015815141.1	Feruloyl esterase Fae1A from <i>Bacteroides intestinalis</i>	27	92	PMID: 28669823	Feruloyl esterase	
6866995	6868242	+	DFER_RS28515	Dfer_5714	WP_015815142.1	Glucuronoyl esterase from <i>Opitutus terrae</i> DtCE15A	45	93	PMID: 30083226	<i>DfCE15C</i>	
6868278	6869531	+	DFER_RS28520	Dfer_5715	WP_015815143.1	L-fucose-proton symporter from <i>Escherichia coli</i> K-12	38	93	PMID: 20877283	MFS transporter	
6869843	6870742	+	DFER_RS28525	Dfer_5716	WP_015815144.1	GlcNAc kinase from <i>Plesiomonas shigelloides</i>	35	98	10.1101/2021.09.30.462564	Sugar kinase	
6870891	6872087	+	DFER_RS28530	Dfer_5717	WP_015815145.1	None - unknown (398 aa) - COG3503	-	-	-	-	
6872118	6873545	+	DFER_RS28535	Dfer_5718	WP_015815146.1	None - alpha/beta hydrolase fold predicted (2 domains predicted, other is beta-sandwich)	-	-	-	Esterase (likely AcEst of Fae)	
6873549	6873965	-	DFER_RS28540	Dfer_5719	WP_015815147.1	None - unknown (138 aa)	-	-	-	-	
6873987	6874511	-	DFER_RS30650	Dfer_5720	WP_015815148.1	None - unknown (174 aa)	-	-	-	-	
6874465	6876093	-	DFER_RS28545	Dfer_5721	WP_229206131.1	Predicted to have GH10-like fold	-	-	-	GH10	
6876210	6877340	+	DFER_RS28550	Dfer_5722	WP_041735648.1	Predicted to have GH10-like fold	-	-	-	GH10	
6877356	6878492	-	DFER_RS28555	Dfer_5723	WP_015815151.1	Uncharacterized MFS-type transporter from <i>Yersinia pestis</i>	48	94	Q7CH99.1	MFS transporter	
6878630	6880351	-	DFER_RS28560	Dfer_5724	WP_015815152.1	<i>Burkholderia cepacia</i> FAD glucose dehydrogenase (Chain A)	24	78	PMID: 36473944	Sugar dehydrogenase	
6880427	6880957	-	DFER_RS28565	Dfer_5725	WP_015815153.1	Predicted gluconate 2-dehydrogenase subunit 3	-	-	-	Sugar dehydrogenase	
6881534	6882583	+	DFER_RS28570	Dfer_5726	WP_015815154.1	Aldo-keto reductase from <i>Rhizobium leguminosarum</i>	40	93	PMID: 36042239	Reductase	
6882607	6883245	-	DFER_RS28575	Dfer_5727	WP_015815155.1	Response regulator from <i>Deinococcus radiodurans</i>	40	28	PMID: 34285211	Transcription regulator	
6883992	6885932	+	DFER_RS28580	Dfer_5728	WP_015815156.1	Predicted GH (unk)-CBM32-Ig-Ig	-	-	-	GH	
6886039	6886536	+	DFER_RS28585	Dfer_5729	WP_015815157.1	None - unknown (165 aa)	-	-	-	-	
6886608	6886802	+	DFER_RS28590	Dfer_5730	WP_015815158.1	None - unknown (64 aa)	-	-	-	-	

Table S5. Matrix of sequence identity of selected CE15 members. Representative CE15 members from fungi (fuchsia) and bacteria (blue) shown alongside the members from *D. fermentans* (green).

	<i>Cu</i> GE	<i>St</i> GE2	<i>Df</i> CE15A	<i>Df</i> CE15B	<i>Df</i> CE15C	<i>Or</i> CE15A	<i>Su</i> CE15C
<i>Cu</i> GE	100.0	48.8	24.2	23.6	27.2	25.8	23.6
<i>St</i> GE2	48.8	100.0	26.3	25.7	27.1	24.6	24.1
<i>Df</i> CE15A	24.2	26.3	100.0	38.3	34.9	40.6	38.2
<i>Df</i> CE15B	23.6	25.7	38.3	100.0	35.6	37.6	41.9
<i>Df</i> CE15C	27.2	27.1	34.9	35.6	100.0	44.9	42.3
<i>Or</i> CE15A	25.8	24.6	40.6	37.6	44.9	100.0	48.6
<i>Su</i> CE15C	23.6	24.1	38.2	41.9	42.3	48.6	100.0

Supplementary figures

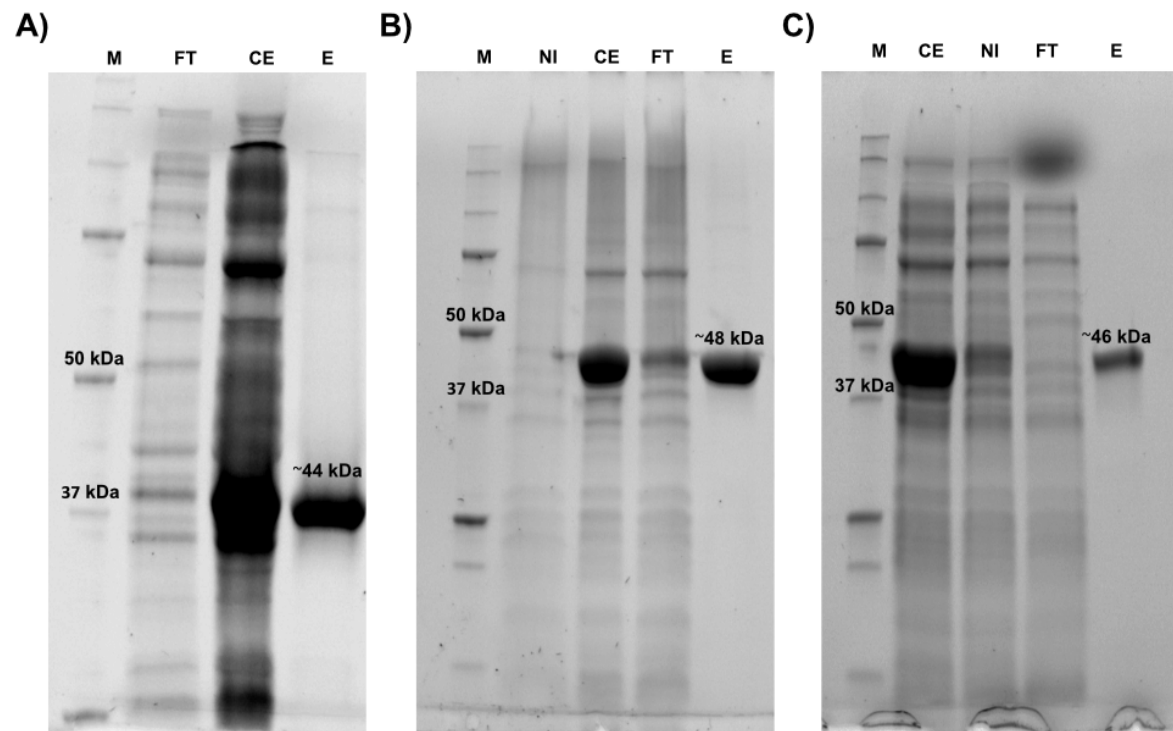


Fig. S1: SDS-PAGE of enzyme purification. SDS-PAGE gel images of the purification of A) *DfCE15A*, B) *DfCE15B*, and C) *DfCE15C*. Gel images contain a protein marker (M, Bio-rad Precision Plus Protein unstained standard), crude extract of induced cells (CE), crude extract of uninduced cells (NI), collected flow-through from the IMAC column loading (FT), and eluted fraction after the IMAC purification (E).

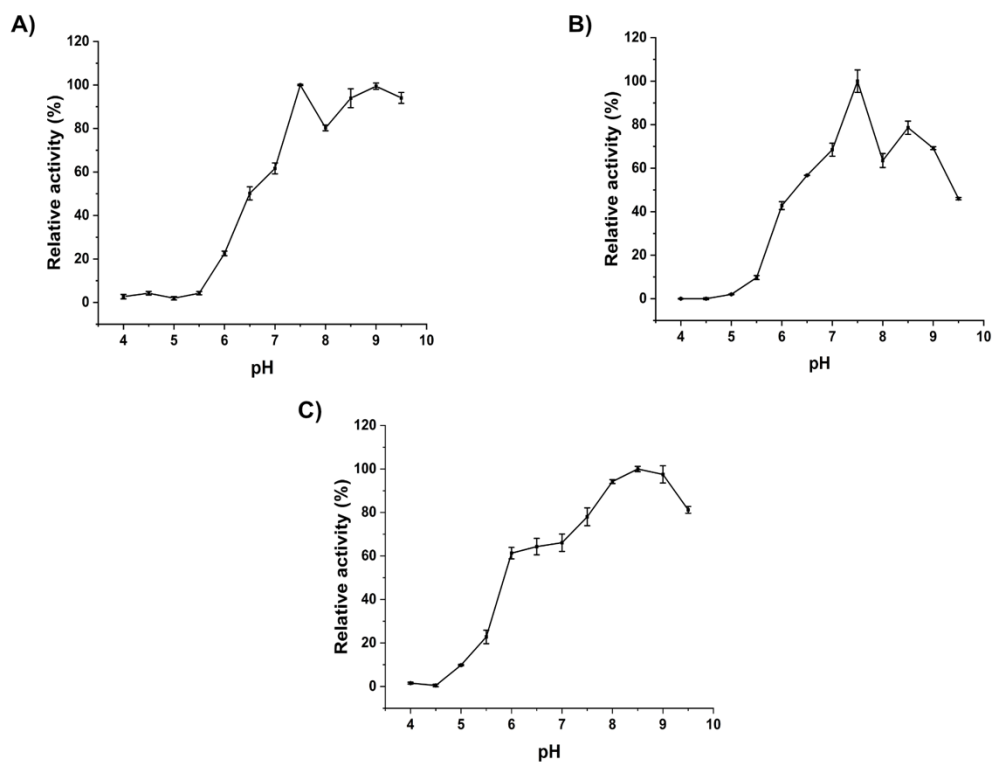


Fig. S2: Dependency of pH on BnzGlcA hydrolysis catalyzed by *DfCE15A* (A), *DfCE15B* (B) and *DfCE15C* (C). Specific activity with 2 mM BnzGlcA at different pH values was measured in three-component buffer as described in the methods. Error bars represent standard errors taken from duplicate measurements with the activity resulting in the highest activity for each enzyme taken as 100%. The rate of ester autohydrolysis increases with increasing pH values, and above pH 9.5 the high rate of autohydrolysis makes accurate determination of the enzymatic cleavage rate unreliable.

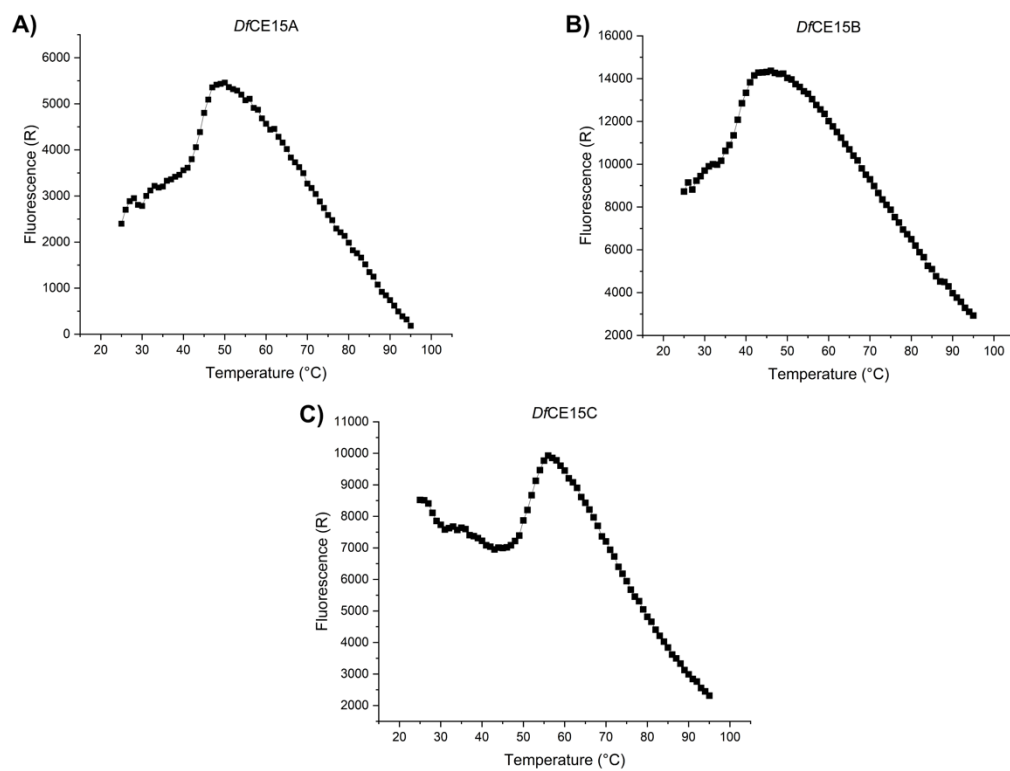


Fig. S3: Thermal shift plots of *DfCE15s*. Assay mixtures contained 5-10 μ M of proteins with the temperature increasing by 1 $^{\circ}$ C/min and changes in fluorescence were quantified relative to a no protein control.

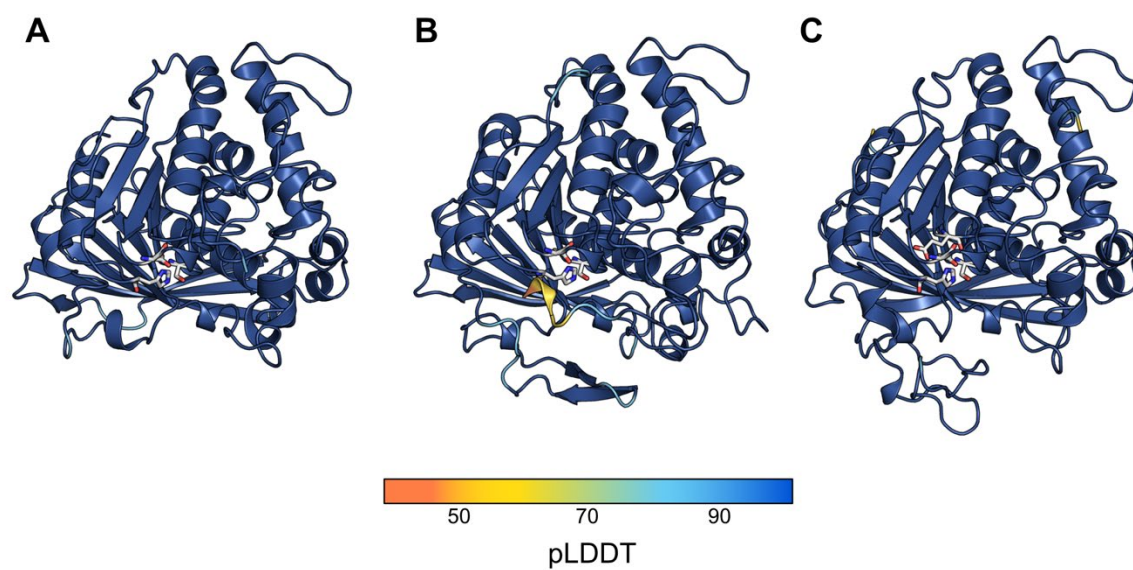


Fig. S4: The overall predicted structures of *DfCE15A* (A), *DfCE15B* (B) and *DfCE15C* (C), coloured relative to the pLDDT confidence values in the colour bar. The predictions are made using AlphaFold (Varadi et al. 2022). Note that all models have a very high confidence score.

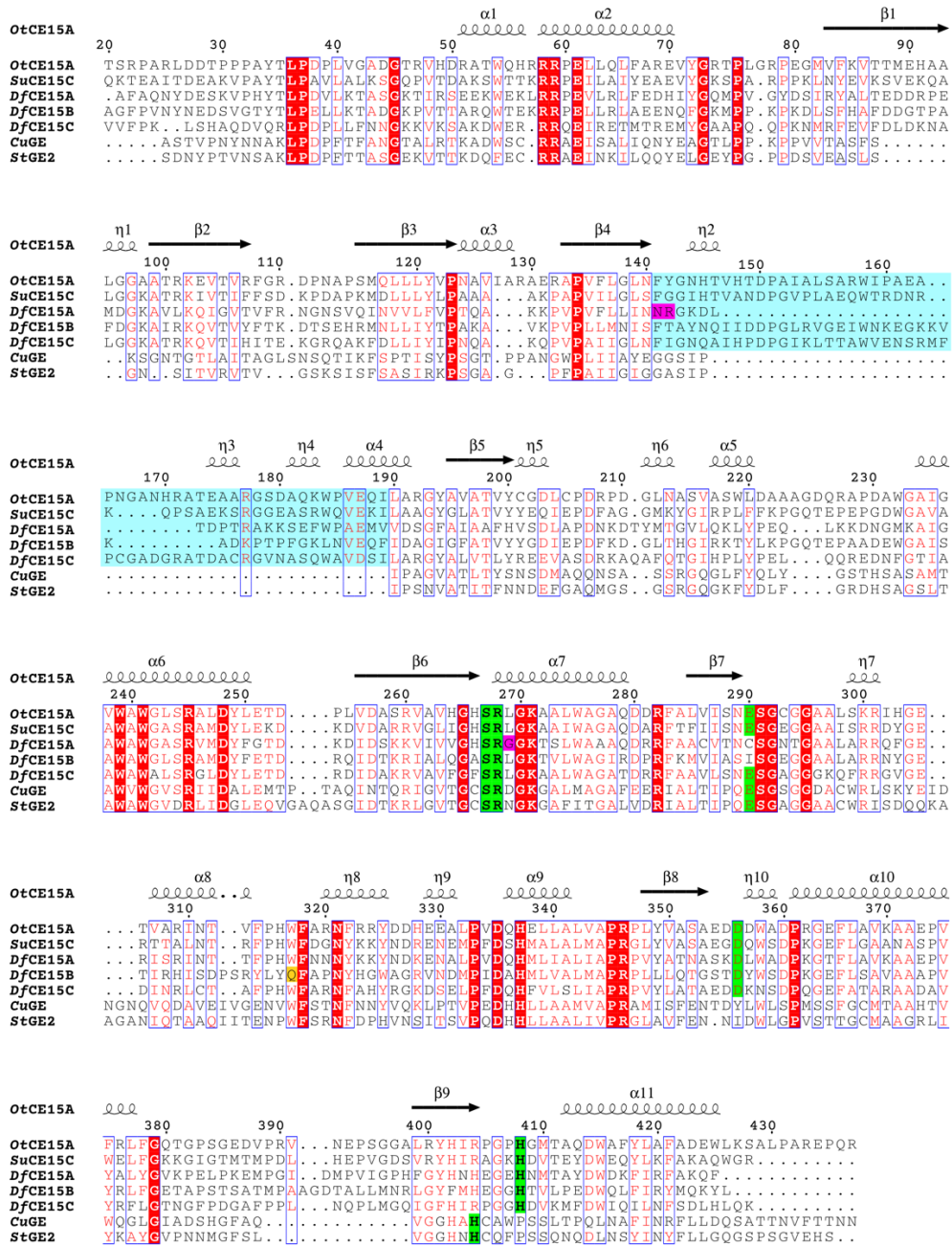


Fig. S5: Multiple sequence alignment of the *DfCE15* enzymes and selected previously characterized CE15 members. The alignment contains sequences of characterized enzymes from both fungi (*C. unicolor*, CuGE; and *S. thermophile* StGE2) and bacteria (*O. terrae*, OtCE15A; and *S. usitatus*, SuCE15A). The residue numbering and secondary structural elements above the alignment are from OtCE15A (PDB: 6GS0). Residues of the catalytic triad and oxyanion stabilizing arginine are shaded in green. Note that, while both bacterial and fungal catalytic histidine residues align structurally, they do not align by primary sequence alignment. Further, fungal CE15 enzyme catalytic acidic residues are found at the position equivalent to residue 290 in OtCE15A, while in bacterial enzymes the acid is found at the position equivalent to residue 356 in OtCE15A, and some bacteria contain both. "Region 2", found in bacterial members is highlighted in cyan. Residues targeted for substitution are highlighted in magenta for DfCE15A and orange for DfCE15B.

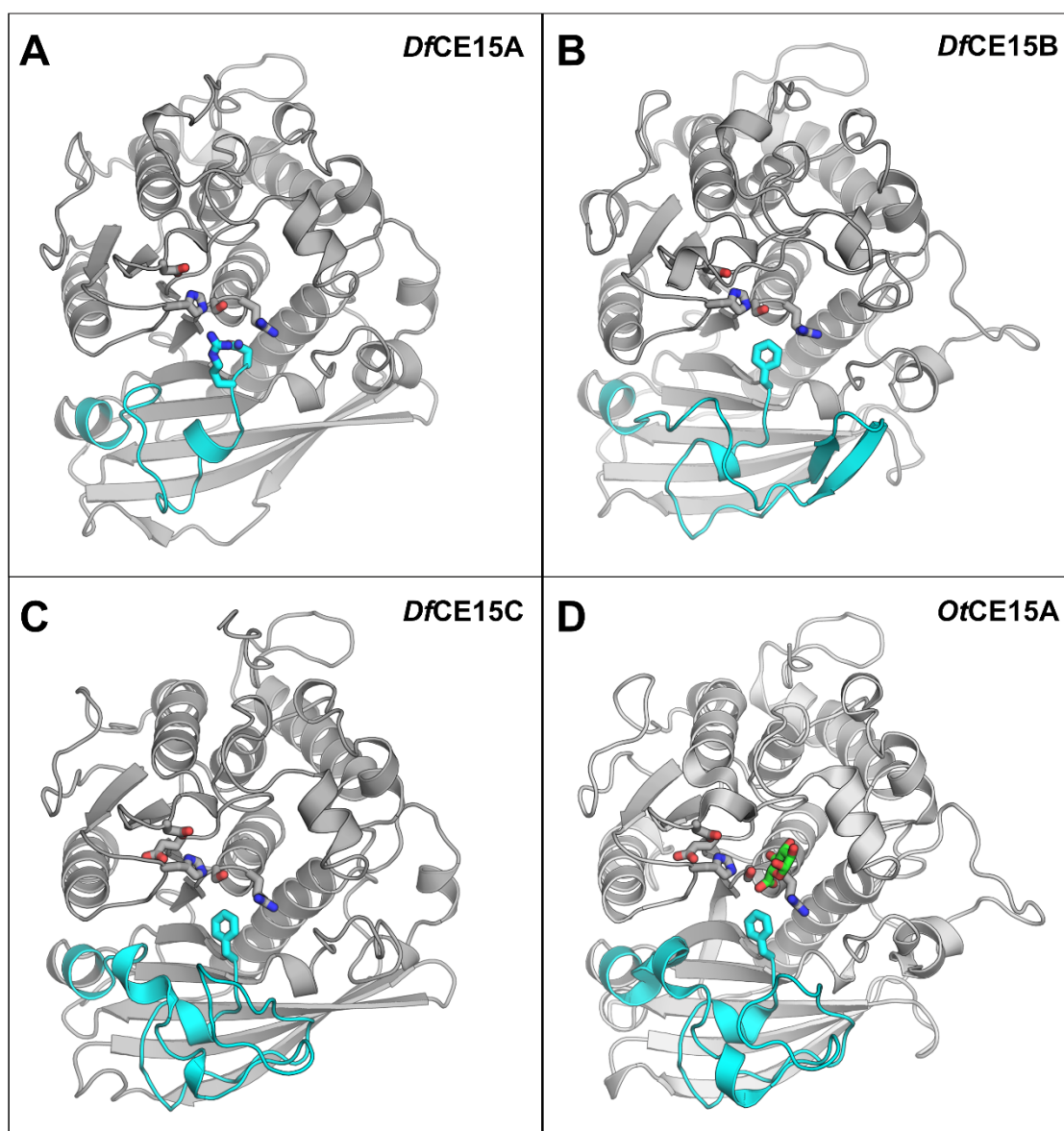


Fig. S6: Comparison of the overall folds of the *DfCE15* protein models. The predicted models of *DfCE15A* (A), *DfCE15B* (B), *DfCE15C* (C) and the experimentally determined model of *OtCE15A* with the bound glucuronate molecule shown as green sticks (D; PDB accession: 6SYR). The Region 2 in each structure is coloured in cyan. The figure was made using PyMOL 2.5.

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