

Engineered Mors1 enzyme from Antarctic bacteria *Moraxella* TA144 for enhanced thermal stability and activity for polyethylene terephthalate degradation.

Supporting Information

Table S1. Composition of different media used in this study

Medium	Composition	Application
Low salt LB medium	Tryptone -10 g/L Yeast extract - 5 g/L NaCl– 5 g/L	Growth of <i>E. coli</i>
YPD	Yeast extract - 10 g/L Peptone – 20 g/L Dextrose –20 g/L	Growth of <i>P. pastoris</i>
BMGY	Yeast extract - 10 g/L Peptone – 20 g/L Yeast Nitrogen Base – 13.4 g/L Biotin – 0.0004 g/L Glycerol – 10 g/L Phosphate Buffer (pH 6) – 100 mL	Primary medium for expression for recombinant <i>P. pastoris</i>
BMMY	Yeast extract - 10 g/L Peptone – 20 g/L Yeast Nitrogen Base – 13.4 g/L Biotin – 0.0004 g/L Methanol – 5 g/L Phosphate Buffer (pH 6) – 100 mL	Induction of recombinant <i>P. pastoris</i> for protein expression

Table S2. Nucleotide and protein sequences for truncated Mors1^{WT/MUT}

Name	DNA/Protein sequence
Mors1 ^{WT} (NT)	ATGTTTCATCATGATTAAGAAATCTGAATTGGCTAAGGCTATCATCGT TACTGGTGCTTTGGTTTTCTCTATTCCAACCTTGGCTGAAGTTACTT TGCTCTGAGACTACTGTTTCTTCTATTAAATCTGAGGCTACTGTTTCT TCTACTAAGAAAGCTTTGCCAGCTACTCCTTCTGATTGTATTGCTGA TTCTAAGATTACTGCTGTTGCTTTGTCTGATACTAGAGATAACGGTC CATTTTCTATCAGAACTAAGAGAATCTCTAGACAATCTGCTAAAGG TTTCGGTGGTGGTACTATTCAATACCCTACTAACGCTTCTGGTTGTG GTTTGTGTTGGGTGCTATTGCTGTTGTTCCAGGTTACGTTTCTTACGAA AATTCTATTAAGTGGTGGGGTCCTAGATTGGCTTCTTGGGGTTCGT TGTTATTACTATTAACACTAATTCTATCTACGATGATCCAGATTCTAG AGCTGCTCAATTGAACGCTGCTTTGGATAATATGATTGCTGATGATA CTGTTGGTTCTATGATTGATCCTAAAAGATTGGGTGCTATTGGTTGG TCTATGGGTGGTGGTGGTGGTCTTTGAAGTTGGCTACTGAAAGATCTA CTGTTAGAGCTATTATGCCATTGGCTCCTTACCATGATAAGTCTTATG GAGAGGTAAAACTCCAACCTTTGGTTATTGCTTGTGAAGATGATAG AATTGCTGAGACTAAGAAATACGCTAACGCTTTCTACAAGAACGCT ATCGGTCCTAAGATGAAGGTTGAAGTTAACAATGGTTCTCACTTTT

	GTCCATCTTACAGATTCAATGAGATCTTGTTGTCTAAGCCTGGTATT GCTTGGATGCAAAGATACATCAACAACGATACTAGATTTCGATAAGT TCTTGTGTGCTAACGAGAACTACTCTAAGTCTCCAAGAATCTCTGC TTACGATTATAAGGATTGTCCTGTCGACCATCATCATCATCATCATTG A
Mors1 ^{MUT} (NT)	ATGTTTCATCATGATTAAGAAATCTGAATTGGCTAAGGCTATCATCGT TACTGGTGCTTTGGTTTTCTCTATTCCAACCTTGGCTGAAGTTACTT TGTCTGAGACTACTGTTTCTTCTATTAAATCTGAGGCTACTGTTTCT TCTACTAAGAAAGCTTTGCCAGCTACTCCTTCTGATTGTATTGCTGA TTCTAAGATTACTGCTGTTGCTTTGTCTGATACTAGAGATAACGGTC CATTTTCTATCAGAACTAAGAGAATCTCTAGACAATCTGCTATTGGT TTCGGTGGTGGTACTATTCAATTACCCTACTAACGCTTCTGGTTGTGG TTTGTGGGTGCTATTGCTGTTGTTCCAGGTTACGTTTCTTACGAAA ATTCTATTAAGTGGTGGGGTCTAGATTGGCTTCTTGGGGTTTCGTT GTTATTACTATTAACACTAATTCTATCTACGATGATCCAGATTCTAGA GCTGCTCAATTGAACGCTGCTTTGGATAATATGATTGCTGATGATAC TGTTGGTTCTATGATTGATCCTAAAAGATTGGGTGCTATTGGTTGGT CTATGGGTGGTGGTGGTGGTCTTTGAAGTTGGCTACTGAAAGATCTAC TGTTAGAGCTATTATGCCATTGGCTCCTTACCATGATAAGTCTTATG GAATTGTTAAACTCCAACCTTTGGTTATTGCTTGTGAAGATGATTC ATTGCTGAGACTAAGAAATACGCTAACGCTTTCTACAAGAACGCTA TCGGTCCTAAGATGAAGGTTGAAGTTAACAATGGTTCTCACTTTTG TCCATCTTACAGATTCAATGAGATCTTGTTGTCTAAGCCTGGTATTG CTTGGATGCAAAGATACATCAACAACGATACTAGATTTCGATAAGTT CTTGTGTGCTAACGAGAACTACTCTAAGTCTCCAAGAATCTCTGCT TACGATTATAAGGATTGTCCTGTCGACCATCATCATCATCATCATTG A
Mors1 ^{WT} (PR)	MFIMIKKSELAKAIIVTGALVFSIPTLAEVTLSETTVSSIKSEATVSSTK KALPATPSDCIADSKITAVALS DTRDNGPFSIRTKRISRQSAKGFGGGTI HYPTNASGCGLLGAIIVPGYVSYENSIKWWGPRLASWGFVVITINT NSIYDDPDSRAAQLNAALDNMIADDTVGSMIDPKRLGAIGWSMGGG GALKLATERSTVRAIMPLAPYHDKSYGEVKTPTLVIACEDDRIAETKK YANAFYKNAIGPKMKVEVNNGSHFCPSYRFNEILLSKPGIAWMQRYI NNDTRFDKFLCANENYSKSPRISAYDYKDCPVDHHHHHHH*
Mors1 ^{MUT} (PR)	MFIMIKKSELAKAIIVTGALVFSIPTLAEVTLSETTVSSIKSEATVSSTK KALPATPSDCIADSKITAVALS DTRDNGPFSIRTKRISRQSAIGFGGGTI HYPTNASGCGLLGAIIVPGYVSYENSIKWWGPRLASWGFVVITINT NSIYDDPDSRAAQLNAALDNMIADDTVGSMIDPKRLGAIGWSMGGG GALKLATERSTVRAIMPLAPYHDKSYGIVKTPTLVIACEDDFIAETKK YANAFYKNAIGPKMKVEVNNGSHFCPSYRFNEILLSKPGIAWMQRYI NNDTRFDKFLCANENYSKSPRISAYDYKDCPVDHHHHHHH*

Table S3. Experimental design for enhanced PET degradation using Mors1^{MUT} with independent variables, experimental and response values.

Run	Independent Variables					Response Values
	Amount of enzyme (U)	Reaction time (Days)	pH	Temperature (°C)	Agitation (RPM)	PET degradation (%)
1	12	8	5	20	40	40
2	21	12	7	37	65	74
3	21	12	7	25	5.5	79
4	21	2.5	7	25	65	56
5	12	16	5	30	40	54
6	12	8	9	20	40	49
7	21	12	7	25	65	97
8	12	8	5	20	90	40
9	30	16	9	20	40	92
10	12	16	5	20	40	49
11	30	16	9	20	90	86
12	21	12	7	25	65	97
13	21	12	7	25	65	97
14	12	8	9	30	40	48
15	12	16	9	30	90	51
16	21	12	7	25	65	97
17	12	16	5	30	90	53
18	30	16	5	30	90	86
19	30	8	5	20	90	73
20	30	8	5	30	90	77
21	30	16	5	30	40	84
22	30	8	9	20	40	82
23	21	12	7	25	65	97
24	21	12	2.2	25	65	31
25	30	16	5	20	90	80
26	21	21.5	7	25	65	90
27	30	16	9	30	40	91
28	30	8	9	30	40	80
29	21	12	7	25	65	97

30	30	16	5	20	40	80
31	12	16	9	20	40	57
32	21	12	7	25	65	97
33	21	12	7	25	65	97
34	42	12	7	25	65	92
35	12	8	9	20	90	43
36	21	12	11	25	65	67
37	21	12	7	13	65	65
38	0	12	7	25	65	0
39	30	8	5	20	40	70
40	12	16	9	20	90	48
41	30	8	5	30	40	72
42	12	16	9	30	40	58
43	30	8	9	20	90	80
44	12	16	5	20	90	46
45	12	8	9	30	90	45
46	30	8	9	30	90	80
47	12	8	5	30	90	46
48	12	8	5	30	40	44
49	30	16	9	30	90	88
50	21	12	7	22	124	70

A1. Protein expression analysis using SDS-PAGE

Protein samples were prepared by mixing 30 μ L of the sample with 10 μ L of 4X SDS loading buffer, followed by heating at 95°C for 5 minutes to ensure denaturation. Gel plates were assembled according to the manufacturer's guidelines, and a 12% resolving gel was prepared using the composition listed in Table S4. The acrylamide solution was poured between the glass plates, leaving space for the stacking gel. Once polymerized, the stacking gel was prepared as per Table A3, poured over the resolving gel, and a comb was inserted.

After solidification, the gel was mounted into the electrophoresis apparatus, and 10X Tris-Glycine buffer was added to both reservoirs. Protein samples were further prepared by mixing 10 μ L of the sample with 7 μ L of loading dye, followed by heating at 95°C for 5 minutes. After electrophoresis, the gel was carefully removed and stained with Coomassie Brilliant Blue R-250. The staining solution was prepared by dissolving 0.3 g of Coomassie Brilliant Blue R-250 in 200 mL of a 5:4:1 mixture of water, methanol, and glacial acetic acid. The gel was incubated

in the staining solution at room temperature for 4 hours, followed by destaining in the same solvent mixture for approximately 6 hours.

Table S4. Resolving and stacking gel composition

Component	Volume (mL) for Resolving gel	Volume (mL) for Stacking gel
H ₂ O	1.6	1.4
30% Acrylamide	2.0	0.34
1.5 M Tris (pH 8)	1.3	0.25
10% SDS	0.05	0.02
10% APS	0.05	0.02
TEMED	0.002	0.002
Total Volume	5	2