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Research article

Functional characterizations of polyethylene terephthalate-degrading cutinase-like enzyme Cut190 mutants using bis(2-hydroxyethyl) terephthalate as the model substrate

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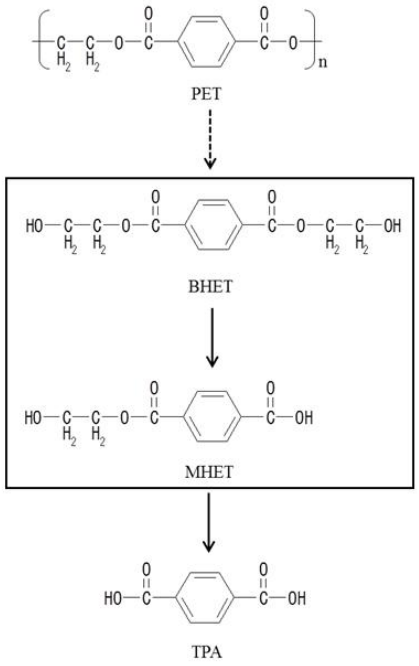
Supplementary material

x=4, y=2, 4

poly(butylene succinate-*co*-adipate) (PBSA)

p-nitrophenyl butyrate (pNPB)



Formula of PET degradation reaction

Figure S1. Structures of substrates and formula of PET degradation reaction. Hydrolysis of BHET by Cut190 was framed by solid line.

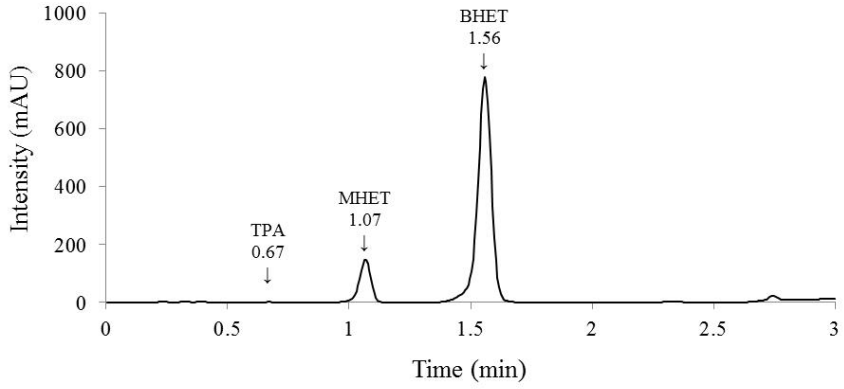


Figure S2. HPLC chromatograph of BHET degradation with Cut190*. The reactions were performed at pH 7.5 at 37 ºC in the presence of 5 mM Ca^{2+} for 2 h, and analyzed as described in Materials and methods.

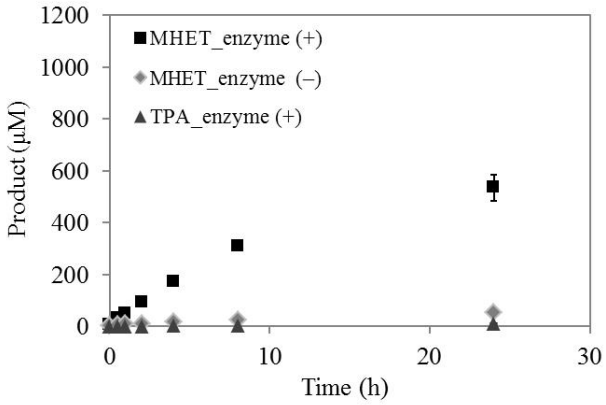


Figure S3. Time course of hydrolysis by Cut190*. The reactions were performed at 37 ºC in the presence of 5 mM Ca^{2+}. Hydrolysis of BHET (1 mM) was measured at 0.5, 1, 2, 4, 8, and 24 h. Black squares and triangles were MHET and TPA amounts in the reaction mixtures with enzyme, and grey diamonds were MHET amounts in the reaction mixtures without enzyme. Data are shown with the standard deviation of triplicate measurements.

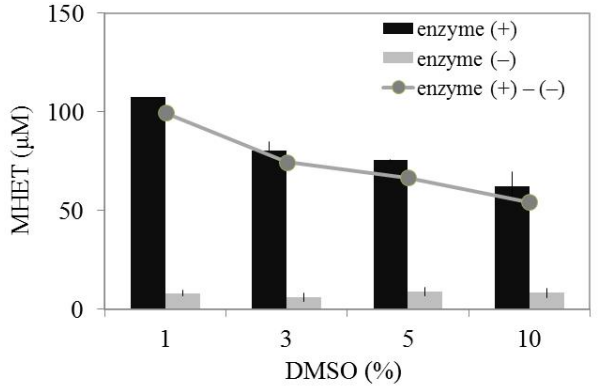


Figure S4. Effect of DMSO concentration on Cut190* activity. Black and grey bars were MHET amounts in the reaction mixtures with and without enzyme, respectively. Grey circles were MHET amounts subtracted without enzyme from with enzyme to indicate net enzyme activities. Data are shown with the standard deviation of triplicate measurements.

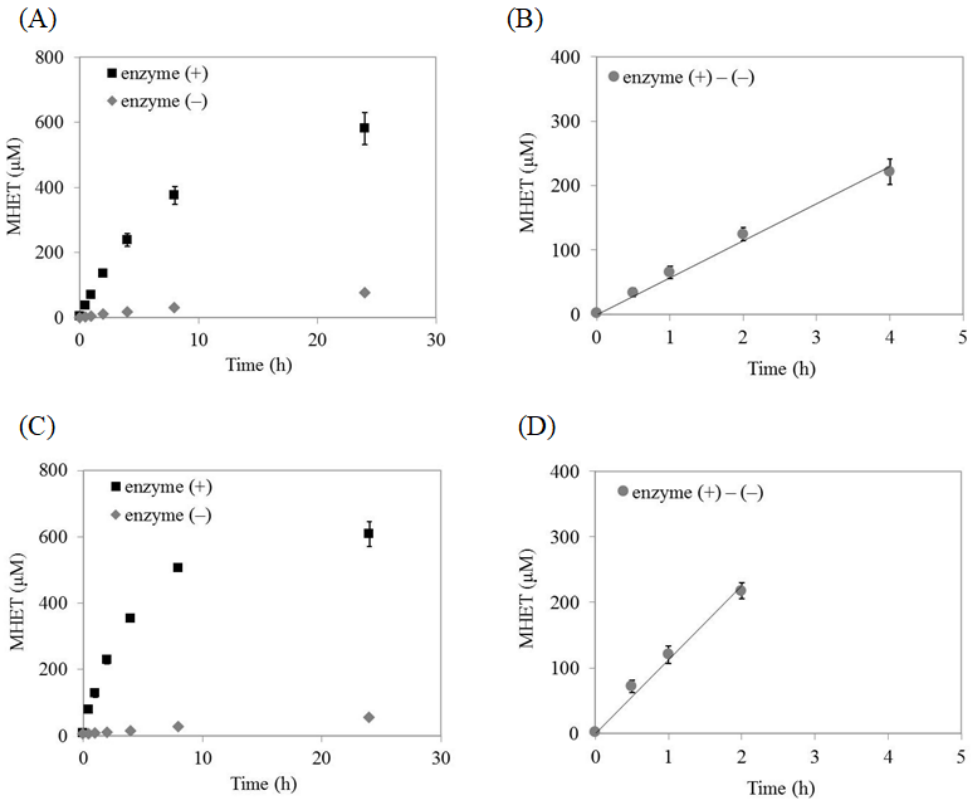


Figure S5. Time course of hydrolysis by Cut190* (A, B) and Cut190*Q138A/D250C-E296C (C, D) in the presence of 25 mM Ca^{2+}. Black squares and grey diamonds in A and C were MHET amounts in the reaction mixtures with and without enzyme, respectively. Grey circles in B and D were MHET amounts subtracted without enzyme from with enzyme to indicate net enzyme activities. Data are shown with the standard deviation of triplicate measurements.