ENZYMATIC DEGRADATION OF POLYETHYLENE TEREPHTHALATE

BACKGROUND OF THE INVENTION

[0001] The use of polyethylene terephthalate (PET) is widespread and ubiquitous. As such, PET is a major source of environmental pollution globally. Physical recycling is possible but underused in many settings. Enzymatic degradation has been explored in the last decades but has proven challenging.

[0002] Provided herein are variant enzymes for use in the enzymatic degradation of PET.

SUMMARY

[0002a] In one aspect, the present disclosure relates to a composition comprising a variant Bhr-PETase as compared to SEQ ID NO:1, wherein said variant comprises at least one amino acid substitution compared to SEQ ID NO: 1 selected from the group consisting of S27L, L90Y, D203R and F92L, wherein said variant Bhr-PETase has at least 90% identity to SEQ ID NO:1 and has PETase activity and exhibits increased PETase activity compared to SEQ ID NO:1.

[0002b] In another aspect, the present disclosure relates to a nucleic acid encoding the variant Bhr-PETase enzyme as disclosed herein. In another aspect, the present disclosure relates to an expression vector comprising the nucleic acid as disclosed herein. In another aspect, the present disclosure relates to a host cell comprising the expression vector as disclosed herein.

[0002c] In another aspect, the present disclosure relates to a method of making a variant Bhr-PETase enzyme comprising culturing the host cell as disclosed herein under conditions wherein said variant Bhr-PETase enzyme is produced, and recovering said variant Bhr-PETase enzyme.

[0002d] In another aspect, the present disclosure relates to a method of degrading PET, comprising contacting the PET with the variant Bhr-PETase enzyme as disclosed herein.

[0003] In one example, the present disclosure relates to a composition comprising a variant Bhr-PETase as compared to SEQ ID NO:1, wherein said variant comprises at least one amino acid substitution compared to SEQ ID NO: 1 at an amino acid position(s) selected from the group consisting of 27, 1, 2, 5, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 30, 32, 33, 34, 40, 46, 48, 49, 53, 54, 55, 56, 57, 60, 62, 68, 70, 72, 74, 77, 82, 83, 85, 87, 88, 90, 92, 97, 98, 101, 102, 105, 108, 109, 110, 113, 114, 117, 119, 121, 122, 125, 127, 135, 136, 138, 139, 140, 142, 143, 145, 147, 149, 150, 153, 156, 157, 158, 160, 161, 162, 163, 164, 167, 170, 173, 174, 177, 179, 181, 182, 184, 185, 189, 190, 193, 194, 195, 198, 200, 203, 204, 206, 208, 209, 211, 212, 213, 216, 217, 218, 219, 221, 222, 223, 225, 227, 228, 229, 231, 236, 237, 241, 242, 243, 246, 249, 250, 251, 252, 253, 254, 255, 258, 8, 31, 38, 95, 126, 137, 165, 169, 172, 191, 192 and 197, wherein said variant Bhr-PETase has at least 85% identity to SEQ ID NO:1 and has PETase activity. In another example, the present disclosure relates to a composition comprising a variant Bhr-PETase as compared to SEQ ID NO:1, wherein said variant comprises a amino acid substitution at position 27 and at least one further amino acid substitution compared to SEQ ID NO: 1 at an amino acid position(s) selected from the group consisting of 1, 2, 5, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 30, 32, 33, 34, 40, 46, 48, 49, 53, 54, 55, 56, 57, 60, 62, 68, 70, 72, 74, 77, 82, 83, 85, 87, 88, 90, 92, 97, 98, 101, 102, 105, 108, 109, 110, 113, 114, 117, 119, 121, 122, 125, 127, 135, 136, 138, 139, 140, 142, 143, 145, 147, 149, 150, 153, 156, 157, 158, 160, 161, 162, 163, 164, 167, 170,

173, 174, 177, 179, 181, 182, 184, 185, 189, 190, 193, 194, 195, 198, 200, 203, 204, 206, 208, 209, 211, 212, 213, 216, 217, 218, 219, 221, 222, 223, 225, 227, 228, 229, 231, 236, 237, 241, 242, 243, 246, 249, 250, 251, 252, 253, 254, 255, 258, 8, 31, 38, 95, 126, 137, 165, 169, 172, 191, 192 and 197, wherein said variant Bhr-PETase has at least 85% identity to SEQ ID NO:1 and has PETase activity. In another example, the present disclosure relates to a composition comprising a variant Bhr-PETase as compared to SEQ ID NO:1, wherein said variant comprises at least one amino acid substitution compared to SEQ ID NO: 1 at an amino acid position(s) selected from the group consisting of 27, 1, 2, 5, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 30, 32, 33, 34, 40, 46, 48, 49, 53, 54, 55, 56, 57, 60, 62, 68, 70, 72, 74, 77, 82, 83, 85, 87, 88, 90, 92, 97, 98, 101, 102, 105, 108, 109, 110, 113, 114, 117, 119, 121, 122, 125, 127, 135, 136, 138, 139, 140, 142, 143, 145, 147, 149, 150, 153, 156, 157, 158, 160, 161, 162, 163, 164, 167, 170, 173, 174, 177, 179, 181, 182, 184, 185, 189, 190, 193, 194, 195, 198, 200, 203, 204, 206, 208, 209, 211, 212, 213, 216, 217, 218, 219, 221, 222, 223, 225, 227, 228, 229, 231, 236, 237, 241, 242, 243, 246, 249, 250, 251, 252, 253, 254, 255, 258, 8, 31, 38, 95, 126, 137, 165, 169, 172, 191, 192 and 197, wherein said variant Bhr-PETase has at least 85% identity to SEQ ID NO:1 and has PETase activity equal to or greater than wild type Bhr-PETase, SEQ ID NO:1. In some examples, said amino acid substitution above is at an amino acid position(s) selected from the group consisting of 27, 1, 2, 5, 9, 12, 13, 14, 15, 16, 17, 18, 20, 21, 22, 23, 24, 25, 26, 30, 32, 33, 34, 40, 46, 48, 49, 53, 54, 55, 56, 57, 60, 62, 68, 70, 72, 74, 77, 82, 83, 85, 87, 88, 90, 92, 97, 98, 101, 102, 105, 108, 109, 110, 113, 114, 117, 119, 121, 122, 125, 127, 135, 136, 138, 139, 140, 142, 143, 145, 147, 149, 150, 153, 156, 157, 158, 160, 161, 162, 163, 164, 167, 170, 173, 174, 177, 179, 181, 182, 184, 185, 189, 190, 193, 194, 195, 198, 200, 203, 204, 206, 208, 209, 211, 212, 213, 216, 217, 218, 219, 221, 222, 223, 225, 227, 228, 229, 231, 236, 237, 241, 242, 243, 246, 249, 250, 251, 252, 253, 254, 255 and 258. In some examples, said amino acid substitution above is at an amino acid position(s) selected from the group consisting of 27, 2, 17, 20, 21, 23, 24, 40, 46, 49, 55, 57, 77, 83, 97, 101, 102, 109, 110, 114, 117, 135, 136, 139, 140, 142, 143, 149, 161, 163, 164, 167, 184, 185, 195, 222, 227, 228, 229, 249, 250 and 251. In some examples, said amino acid substitution above is at an amino acid position(s) selected from the group consisting of 8, 126, 137, 165, 169, 172, 191, 192 and 197.

[0004] In some examples, said amino acid substitution is selected from the group consisting of S27L, S27F, S27H, S27T, S27W, S1A, S1G, S1M, S1R, N2E, N2F, N2L, N2R,

R251E, R251I, R251K, R251L, R251Q, R251T, R251V, S252T, N253S, N253Y, N254R, R255E, R255G, R255L, R255M, R255S, R255V, R255W, R255Y, Q258P, P8T, L31M, G38D, S95N, V126I, L137M, V165I, I169C, I169L, I169V, A172T, L191F, L191V, P192A, K197L, K197R, K197T, K197V and K197Y. In some examples, said amino acid substitution is selected from the group consisting of S27L, S27F, S27H, S27T, S27W, S1A, S1G, S1M, S1R, N2R, Q5E, N9A, N9E, N9S, R12K, S13L, S13R, A14K, A14S, L15I, T16E, T17A, T17C, T17G, T17H, T17K, T17L, T17Q, T17S, D18R, P20T, F21W, S22A, S22I, S22K, S22P, S22R, S22V, V23L, V23T, A24S, A24V, T25A, T25F, T25H, T25Q, T25R, T25V, Y26K, Y26L, Y26T, R30K, S32K, S32M, S32Q, S32Y, V33G, V33Q, V33T, S34R, V40T, G46L, G46S, T48N, T48S, L49G, G53A, I54V, A55L, A55V, M56I, M56L, S57I, S57M, S57V, Y60A, Y60H, Y60I, A62T, A68F, L70M, R72P, L74W, H77Q, I82F, I82L, I82M, V83T, N85D, N87F, N87H, N87I, N87K, N87L, N87M, N87R, N87V, N87W, N87Y, S88K, S88T, L90F, L90K, L90Y, F92G, F92I, F92K, F92L, F92N, F92Q, F92V, F92Y, A97F, A97G, S98A, S98D, S98E, S98L, S98M, S98N, S98Q, S98T, S98V, S101A, S101D, S101F, S101H, S101K, S101L, S101M, S101N, S101Q, S101R, S101V, S101W, A102V, N105D, R108C, R108E, R108H, R108K, R108N, R108P, R108Q, R108S, R108T, R108V, T109F, T109K, T109L, T109N, T109R, S110G, S110H, S110R, S113A, S113K, S113N, S113P, S113Q, S113R, S113T, S113Y, A114K, A114L, A114S, A114V, A117G, A117N, A117Q, A117S, L119I, L119M, A121S, N122A, N122E, N122H, N122P, N122R, N122S, A125S, A127M, A127S, A127V, A135G, T136A, T136M, T136S, T136V, R138E, R138L, I139A, I139T, S140A, Q142L, Q142W, I143N, I143R, T145S, K147F, K147G, K147N, K147Q, G149A, G149C, G149S, G149V, V150I, V150L, T153L, H156N, T157A, T157G, D158E, D158I, D158K, D158L, T160K, T160Q, T160R, T160S, T160V, F161W, N162E, N162H, N162P, N162R, T163S, P164S, Q167T, Q167V, V170L, E173R, A174K, A174R, V177A, P179Q, S181A, S181C, S181R, Q182T, A184S, I185L, I185Y, Q189I, Q189L, Q189V, N190S, S193E, S193F, S193H, S193K, S193N, S193P, S193T, S193V, T194G, T194S, T195F, V198A, V200L, D203N, D203R, D203V, N204A, N204K, N204R, N204S, T206G, T206K, T206L, T206P, T206R, F208G, F208L, F208R, F208T, A209V, N211F, N211I, N211L, N211M, N211V, S212F, S212L, S212M, P213N, P213R, A216L, A216P, A216S, A216V, I217S, S218A, V219F, V219I, V219K, V219L, V219R, T221S, I222L, S223A, S223C, M225L, L227R, W228F, V229C, V229I, N231L, N231Q, N231S, R236C, R236E, R236K, R236Q, Q237R, N241P, V242T, N243P, A246D, A246K, A246S, A246T, D249T, F250I, F250Y, R251I, R251K, R251V, S252T, N253Y, N254R, R255E, R255G, R255L, R255M, R255S, R255V, R255W, R255Y and Q258P. In some

examples, said amino acid substitution is selected from the group consisting of S27L, S27F, S27H, S27T, S27W, N2R, T17A, T17C, T17G, T17H, T17K, T17L, T17Q, T17S, P20T, F21W, V23L, V23T, A24S, A24V, V40T, G46L, G46S, L49G, A55L, A55V, S57I, S57M, S57V, H77Q, V83T, A97F, A97G, S101A, S101D, S101F, S101H, S101K, S101L, S101M, S101N, S101Q, S101R, S101V, S101W, A102V, T109F, T109K, T109L, T109N, T109R, S110G, S110H, S110R, A114K, A114L, A114S, A114V, A117G, A117N, A117Q, A117S, A135G, T136A, T136M, T136S, T136V, I139A, I139T, S140A, Q142L, Q142W, I143N, I143R, G149A, G149C, G149S, G149V, F161W, T163S, P164S, Q167T, Q167V, A184S, I185L, I185Y, T195F, I222L, L227R, W228F, V229C, V229I, D249T, F250I, F250Y, R251I, R251K and R251V. In some examples, said amino acid substitution is selected from the group consisting of N2E, N2F, N2L, N2S, P8T, T17I, T17M, T17N, T17R, P20D, P20E, P20I, P20Q, F21Y, A24D, A24G, A24H, A24N, A24T, Y26C, L31M, G38D, G46E, G46N, G46R, A55C, A55I, A55M, A55T, S57C, S57E, S57F, S57L, S57T, V83I, V83L, N87Q, S95N, A97C, A97E, A97L, A97P, A97Q, A97S, A97T, A97V, S101C, S101Y, T109A, T109G, T109Y, S110D, S110K, S110N, A117F, A117L, A117T, A117Y, V126I, L137M, Q142D, Q142E, Q142H, G149D, G149N, G149T, F161V, T163I, P164E, P164H, P164N, P164R, P164T, V165I, Q167I, I169C, I169L, I169V, A172T, A184C, A184G, I185A, I185E, I185G, I185Q, I185R, I185S, L191F, L191V, P192A, K197L, K197R, K197T, K197V, K197Y, A216T, V229L, R236H, D249I, D249M, D249N, D249S, F250L, F250V, R251A, R251E, R251L, R251Q, R251T and N253S.

[0005] In some examples, said variant Bhr-PETase enzyme has one or more amino acid substitutions at one of said positions, two of said positions, three of said positions, four of said positions, five of said positions, six of said positions, seven of said positions, eight of said positions, nine of said positions, ten of said positions, eleven of said positions, twelve of said positions, thirteen of said positions, fourteen of said positions, fifteen of said positions, sixteen of said positions, seventeen of said positions, eighteen of said positions, nineteen of said positions or twenty of said positions.

[0006] In some examples, said variant Bhr-PETase comprises a set of amino acid substitutions selected from the group consisting of A102V/T136M, A14S/L15I/S22A/F92Q/I143R/Q167T/V219K, D18R/G46S/M56I/R108T/A127M/R138E/I139A/N190S/L227R/W228F/R236E, D18R/I139A/F161W/W228F, D18R/I54V/I82F/N105D/A127M/A184S/S218A/V219K/M225L/N241P/N243P,

S27L/P20T/A117Q/I222L/V229C, S27L/T17Q/V23T/A24V/I222L/V229I, S27L/N2F/D249N/F250L, S27L/V23T/Q142L/I222L/V229C, S27L/P20E/A24D/D249S/F250V, S27L/A24D/S110K, S27L/T109R/I222L/V229I, S27L/P20E/F21Y/K197T, S27L/T17A/V23L/S101M/T109K/S110R/A114V/A117Q/T136V/V229I, S27L/P20T/F21W/Q142W/I222L/V229I, S27L/F21W/I222L/V229I, S27L/P20T/V23T/A24V/T109L/S110R/I222L/V229I, S27L/A24D/L191F/R251Q, S27L/A24D/T136S/F250V, S27L/P20T/F21W/T109L/T136A/Q142W/I222L/V229C, S27L/N2E/I169L/F250L/R251Q, S27L/N2S/A24N/G46E/A55L/Q142D/V229C/R251L, S27L/N2E/P20E/F250V/R251L, S27L/N2F/P20E, S27L/A24V/V229C, S27L/P20D/F21Y/G46N/S110K/T163I/Q167V, S27L/P20T/T109K/A114K/Q142W/I222L/V229C, S27L/T17Q/S101D/T136A/I222L/V229I, S27L/F21W/T109R/A117N/T136V/Q142W/I222L/V229C, S27L/A24D/T109L/K197L, S27L/T17Q/Q142L/I222L/V229I, S27L/T17A/A24V/S101D/S110R/T136A, S27L/P20T/F21W/T109R/A117Q/T136V/Q142L/I222L/V229I, S27L/P20T/F21W/T109K/A117N/I222L/V229C, S27L/P20T/S101M/I222L/V229I, S27L/N2S/A24D/S101Y/P164E/V165I/I185E, S27L/T17N/A117S/V229L/R251A, S27L/S101W/A114V/T136V/V229C, S27L/P20D/G46N/A55C/S110N/T163I/A184S, S27L/A24H/G46E, S27L/T17H/S101Q/T136V/Q142L/V229C and S27L/V23T/A24V/I222L/V229C. In some examples, said variant Bhr-PETase comprises a set of amino acid substitutions selected from the group consisting of A102V/T136M, A14S/L15I/S22A/F92Q/I143R/Q167T/V219K, D18R/G46S/M56I/R108T/A127M/R138E/I139A/N190S/L227R/W228F/R236E, D18R/I139A/F161W/W228F, D18R/I54V/I82F/N105D/A127M/A184S/S218A/V219K/M225L/N241P/N243P, D18R/I54V/I82F/R108T/V150I/N253Y/N254R, D18R/I54V/M56I/R138E/I139A/T194S/D203N/V219K/F250I, D18R/M56I/I139A/L227R/W228F, D18R/M56I/R138E/I139A/N190S/D203N/M225L, D18R/M56I/R72P/H77Q/F92Q/A127M/V150I/A184S/D203N/N254R, D18R/N85D/R108T/L119I/N190S/T194S, D18R/V40T/I139A/D203N, D18R/V40T/I82F/S101L/N105D/L119I/H156N/F161W/N190S/T194S/D203N, D18R/V40T/M56I/R108T/R138E/H156N, G53A/R108T/Q167T/A184S/T194S/N243P, I143R/Q167T/V198A/W228F, I82F/R108T/L119I/V150I/F161W/Q167T/T194S/D203N, L15I/D18R/R108T/R138E/I139A/F161W/A184S/N190S/D203N,

T17A/S27T/T48S/A135G/S140A, T17A/S27T/T48S/I82L,
T17A/S27T/T48S/I82L/L90F/F92Y/Q167V/S252T,
T17A/S27T/T48S/I82L/L90F/F92Y/S140A/T145S,
T17A/S27T/T48S/I82L/L90F/F92Y/S140A/T145S/P213N,
T17A/S27T/T48S/I82L/L90F/F92Y/S252T, T17A/S27T/T48S/I82L/L90F/P213N/S252T,
T17A/S27T/T48S/I82L/L90F/Q167V, T17A/S27T/T48S/I82L/L90F/Q167V/P213N/S252T,
T17A/S27T/T48S/I82L/P213N/S252T, T17A/S27T/T48S/L90F/F92Y,
T17A/S27T/T48S/P213N, T17A/S27T/T48S/T145S/Q167V, T17A/T48S/I82L,
T17A/T48S/I82L/F92Y/Q167V/S252T, T17A/T48S/I82L/L90F/A135G/S140A/Q167V,
T17A/T48S/P213N, T17K/A125S and V177A/A216L. In some examples, said variant Bhr-PETase comprises a set of amino acid substitutions selected from the group consisting of A102V/T136M, A14S/L15I/S22A/F92Q/I143R/Q167T/V219K,
D18R/G46S/M56I/R108T/A127M/R138E/I139A/N190S/L227R/W228F/R236E,
D18R/I139A/F161W/W228F,
D18R/I54V/I82F/N105D/A127M/A184S/S218A/V219K/M225L/N241P/N243P,
D18R/I54V/M56I/R138E/I139A/T194S/D203N/V219K/F250I,
D18R/M56I/I139A/L227R/W228F, D18R/M56I/R138E/I139A/N190S/D203N/M225L,
D18R/M56I/R72P/H77Q/F92Q/A127M/V150I/A184S/D203N/N254R,
D18R/V40T/I139A/D203N,
D18R/V40T/I82F/S101L/N105D/L119I/H156N/F161W/N190S/T194S/D203N,
D18R/V40T/M56I/R108T/R138E/H156N, G53A/R108T/Q167T/A184S/T194S/N243P,
I143R/Q167T/V198A/W228F, I82F/R108T/L119I/V150I/F161W/Q167T/T194S/D203N,
L15I/D18R/R108T/R138E/I139A/F161W/A184S/N190S/D203N,
M56I/I82F/R108T/I139A/F161W/T194S/D203N/N241P, M56I/S88T/R108T/N190S/L227R,
N9A/S22A/G46S/M56I/R72P/F92Q/L119I/T221S/M225L, N9A/S22A/Q167T,
N9A/V40T/L49G/I54V/R108T/V150I/D203N/T221S/M225L,
Q5E/N9A/M56I/F92Q/R108T/L119I/Q167T/N253Y, Q5E/S22A/R72P/H77Q/F92Q,
R108T/L119I/K147Q/F161W/A184S/D203N, S101F/T136A,
S22A/A24S/G46S/V150I/Q167T, S22A/A97G/V150I/D203N/M225L/R236E,
S22A/G46S/S101L/F161W/D203N/R236E,
S22A/L49G/M56I/V83T/S101L/R108T/L119I/A127M/T194S, S27T/I82L/P213N,
S27T/T48S/I82L/F92Y/S252T, S27T/T48S/I82L/L90F/A135G/S140A/I143N/T145S/P213N,
S27T/T48S/I82L/S140A/I143N/G149A,
S88T/R108T/V150I/A184S/D203N/T221S/M225L/N243P,

T16E/D18R/G53A/I54V/R72P/L119I/A127M/Q167T/V200L,
T16E/D18R/M56I/A127M/R138E/T194S/V200L/M225L,
T16E/D18R/M56I/K147Q/Q167T/A184S/D203N,
T16E/D18R/M56I/N85D/S88T/R108T/F161W/Q167T,
T16E/D18R/R108T/K147Q/Q167T/A184S/N190S/T194S,
T16E/D18R/S22A/M56I/N85D/L119I/A184S,
T16E/D18R/S22A/V40T/A97G/S101L/L119I/A127M/Q167T/D203N,
T16E/D18R/S88T/R108T/W228F,
T16E/D18R/V40T/M56I/I82F/R108T/L119I/F161W/L227R/Q258P,
T16E/D18R/V40T/S88T/L119I/A127M/V150I/D203N,
T16E/D18R/Y26T/S88T/S101L/H156N/V200L, T17A/I82L/F92Y/P213N,
T17A/I82L/L90F/F92Y/A135G/S140A/I143N/G149A/Q167V,
T17A/I82L/L90F/F92Y/Q167V, T17A/I82L/S140A/I143N/Q167V/P213N/S252T,
T17A/Q167V, T17A/S27T, T17A/S27T/I82L, T17A/S27T/I82L/G149A/Q167V/P213N,
T17A/S27T/I82L/L90F/F92Y/Q167V, T17A/S27T/I82L/P213N,
T17A/S27T/I82L/P213N/S252T, T17A/S27T/I82L/S252T,
T17A/S27T/I82L/T145S/G149A/P213N,
T17A/S27T/L90F/A135G/S140A/Q167V/P213N/S252T,
T17A/S27T/L90F/F92Y/A135G/Q167V/S252T, T17A/S27T/L90F/F92Y/P213N,
T17A/S27T/T48S, T17A/S27T/T48S/A135G/Q167V/P213N,
T17A/S27T/T48S/A135G/S140A, T17A/S27T/T48S/I82L,
T17A/S27T/T48S/I82L/L90F/F92Y/Q167V/S252T,
T17A/S27T/T48S/I82L/L90F/F92Y/S140A/T145S,
T17A/S27T/T48S/I82L/L90F/F92Y/S140A/T145S/P213N,
T17A/S27T/T48S/I82L/L90F/F92Y/S252T, T17A/S27T/T48S/I82L/L90F/P213N/S252T,
T17A/S27T/T48S/I82L/L90F/Q167V, T17A/S27T/T48S/I82L/L90F/Q167V/P213N/S252T,
T17A/S27T/T48S/I82L/P213N/S252T, T17A/S27T/T48S/L90F/F92Y,
T17A/S27T/T48S/P213N, T17A/S27T/T48S/T145S/Q167V, T17A/T48S/I82L,
T17A/T48S/I82L/F92Y/Q167V/S252T, T17A/T48S/I82L/L90F/A135G/S140A/Q167V,
T17A/T48S/P213N, T17K/A125S and V177A/A216L. In some examples, said variant Bhr-PETase comprises a set of amino acid substitutions selected from the group consisting of L90F/F92G/D158L/A174R/D203V, V23L/A24V/S32K/N87F/F92Y/A125S/T136A, P20T/L90Y/F92L/S101M/D158E/A174K/I222L, D18R/S32K/Y60H/L90F, P20T/F21W/A24V/L90Y/F92L/T109K/A125S/T136A,

[0007] In another example, the present disclosure relates to a nucleic acid encoding the variant Bhr-PETase enzyme of any one of the preceding claims. In another example, the present disclosure relates to an expression vector comprising the nucleic acid. In another example, the present disclosure relates to a host cell comprising the expression vector. In some examples, the cell is bacteria, yeast or fungi.

[0008] In another example, the present disclosure relates to a method of making a variant Bhr-PETase enzyme comprising culturing the host cell described herein under conditions wherein said variant Bhr-PETase enzyme is produced, and recovering said variant Bhr-PETase enzyme.

[0009] In another example, the present disclosure relates to a method of pretreating PET, comprising a mechanical pretreatment, a thermo-mechanical pretreatment, and/or a chemical pretreatment of the PET prior to an enzymatic degradation of the PET. In some examples, the mechanical pretreatment comprises grinding of the PET into particles. In some examples, the thermo-mechanical pretreatment comprises extruding the PET at a temperature configured to amorphize and reduce crystallinity of the PET. In some examples, the chemical pretreatment comprises contacting the PET with an ionic liquid, strong acid, base, or solvent configured to reduce crystallinity or to change a surface structure of the PET.

[0010] In another example, the present disclosure relates to a method of degrading PET, comprising contacting the PET with the variant Bhr-PETase enzyme described herein. In some examples, the method further comprises pretreating the PET according to the method described herein. In some examples, the method degrades the PET in a mixed plastics composition. In some examples, the plastics composition comprises analog of PET, PET-like or PET substitute derived biologically or chemically. In some examples, the plastics composition comprises at least one selected from the group consisting of Polybutylene terephthalate (PBT), Polycabonate (PC), Polycaprolactone (PCL), Polyethylene Furanoate (PEF), and High Density Polyethylene (HDPE). In some examples, the method excludes sorting plastics to select PET from a mixture of plastics.

BRIEF DESCRIPTION OF THE DRAWINGS

[0011] Figure 1 depicts the sequence of an exemplary wild type Bhr-PETase (also called G1P Bhr-PETase herein; SEQ ID NO:1).

[0012] Figures 2A-2I depicts the sequence alignment of the exemplary wild type Bhr-PETase (SEQ ID NO:1), wild type Lcc-PETase (SEQ ID NO:4), wild type Is-PETase (SEQ ID NO:6) and 20 exemplary homologs of Bhr-PETase. Figure also discloses SEQ ID NOS 8-34, respectively, in order of appearance.

[0013] Figure 3 depicts %Sequence identity of the exemplary wild type Bhr-PETase (SEQ ID NO:1), wild type Lcc-PETase (SEQ ID NO:4), wild type Is-PETase (SEQ ID NO:6) and 20 exemplary homologs of Bhr-PETase w.r.t. wild type Bhr-PETase.

[0014] Figure 4 depicts the comparison of thermostability between three wild type enzymes Lcc-PETase, Bhr-PETase and Is-PETase.

[0015] Figure 5 depicts the comparison of Bhr-PETase and Lcc-PETase on amorphous PET at 65°C (Fig 5 graph A) and at 72°C (Fig 5 graph B).

[0016] Figure 6 depicts the comparison of Bhr-PETase and Lcc-PETase on > 40% crystalline PET at 65°C (Fig 6 graph A) and at 72°C (Fig 6 graph B).

[0017] Figures 7A–D show the Bhr-PETase G1 variants with improved total activity and thermostability over Bhr-PETase G1P (wild type Bhr-PETase). A Bhr-PETase G1 variant with amino acid substitution S27L exhibited 1.80 fold improved total activity and 3.36 fold improved thermostability over Bhr-PETase G1P and therefore selected as Bhr-PETase G2P.

[0018] Figures 8A–B show the Bhr-PETase G1 variants with improved thermostability over Bhr-PETase G1P (wild type Bhr-PETase).

[0019] Figures 9A–I show the Bhr-PETase G1 variants with improved total activity over Bhr-PETase G1P (wild type Bhr-PETase). Figures 9J–AA show the Bhr-PETase G2 variants with improved total activity over Bhr-PETase G2P (Bhr-PETase G1P with amino acid substitution S27L).

[0020] Figures 10A–B display particular variants of Bhr-PETase by position that demonstrate beneficial properties in total activity, and/or thermostability as displayed in Figures 7A–D, Figures 8A–B and Figures 9A–AA.

CLAIMS

1. A composition comprising a variant Bhr-PETase as compared to SEQ ID NO:1, wherein said variant comprises at least one amino acid substitution compared to SEQ ID NO: 1 selected from the group consisting of S27L, L90Y, D203R and F92L, wherein said variant Bhr-PETase has at least 90% identity to SEQ ID NO:1 and has PETase activity and exhibits increased PETase activity compared to SEQ ID NO:1.

2. The composition according to claim 1, wherein said variant Bhr-PETase further comprises one or more amino acid substitutions selected from the group consisting of S1A, S1G, N2E, N2F, N2L, N2R, N2S, N9E, N9S, R12K, S13R, A14K, T17A, T17C, T17G, T17H, T17I, T17K, T17L, T17M, T17N, T17Q, T17R, T17S, D18R, P20D, P20E, P20I, P20Q, P20T, F21W, F21Y, S22K, S22P, S22R, S22V, V23L, V23T, A24D, A24G, A24H, A24N, A24T, A24V, T25A, T25F, T25Q, Y26C, Y26K, Y26L, Y26T, S32K, S32M, S32Q, S34R, G46E, G46N, G46R, G46S, T48N, T48S, A55C, A55I, A55L, A55M, A55T, A55V, S57C, S57E, S57F, S57I, S57L, S57M, S57T, S57V, Y60A, Y60H, Y60I, I82F, I82L, I82M, V83I, V83L, N87F, N87H, N87I, N87K, N87L, N87M, N87Q, N87V, N87Y, L90F, F92G, F92Y, A97C, A97E, A97F, A97L, A97P, A97Q, A97S, A97T, A97V, S98A, S98E, S98L, S98M, S98N, S98T, S98V, S101A, S101C, S101D, S101H, S101K, S101M, S101N, S101Q, S101R, S101W, S101Y, R108C, R108H, R108K, R108Q, R108S, R108T, T109A, T109G, T109K, T109L, T109R, T109Y, S110D, S110K, S110N, S110R, S113K, S113N, S113R, S113Y, A114K, A114V, A117F, A117L, A117N, A117Q, A117S, A117T, A117Y, L119M, N122A, N122E, N122R, N122S, A125S, A127S, T136A, T136S, T136V, I139T, Q142D, Q142E, Q142H, Q142L, Q142W, G149A, G149C, G149D, G149N, G149S, G149T, D158E, D158I, D158L, T160K, T160Q, T160R, T160S, T160V, F161V, F161W, T163I, P164E, P164H, P164N, P164R, P164S, P164T, Q167I, Q167T, Q167V, E173R, A174K, A174R, V177A, S181C, S181R, A184C, A184G, A184S, I185A, I185E, I185G, I185Q, I185R, I185S, I185Y, Q189L, Q189V, S193H, S193K, S193N, S193P, D203V, N204K, N204R, T206G, N211F, N211I, N211L, N211M, N211V, S212F, S212L, S212M, A216P, A216T, V219I, V219K, V219L, I222L, S223A, V229C, V229I, V229L, N231S, R236C, R236H, R236Q, D249I, D249M, D249N, D249S, D249T, F250L, F250V, R251A, R251E, R251L, R251Q, R251T, R251V, S252T, N253S, R255L, R255M, P8T, L31M, G38D, S95N, V126I, L137M, V165I, I169C, I169L, I169V, A172T, L191F, L191V, P192A, K197L, K197R, K197T, K197V and K197Y.

3. The composition according to any one of the preceding claims, wherein said variant Bhr-PETase enzyme has one or more amino acid substitutions at one of said positions, two of said positions, three of said positions, four of said positions, five of said positions, six of said positions, seven of said positions, eight of said positions, nine of said positions, ten of said positions, eleven of said positions, twelve of said positions, thirteen of said positions, fourteen of said positions, fifteen of said positions, sixteen of said positions, seventeen of said positions, eighteen of said positions, nineteen of said positions or twenty of said positions.

4. The composition according to any one of the preceding claims, wherein said variant Bhr-PETase comprises a set of amino acid substitutions selected from the group consisting of
P20T/L90Y/F92L/S101M/D158E/A174K/I222L,
P20T/F21W/A24V/L90Y/F92L/T109K/A125S/T136A,
Y60H/S101A/A114K/A117Q/D203R/I222L,
S32Q/F92G/S101Q/A114K/Q142W/D158I/D203R/I222L,
T17G/L90F/F92L/T109R/A114K/D158E/D203V/I222L,
R12K/S32K/F92L/T136A/D158E/A174R, S32K/L90F/F92L/T109K/A114K/Q142W,
V23T/S32Q/N87I/L90F/F92Y/S101N/A114V/Q142W/A174R/D203R/I222L/V229I,
R12K/S32K/L90Y/F92L/T136A/A174K/D203V/I222L,
R12K/A14K/V23T/L90Y/F92G/S101A/D203R, T17G/S32M/L90Y/F92Y/D203R/A216P,
S32Q/F92L/A114V/A125S,
P20T/F21W/S32M/L90Y/F92G/A114K/A125S/T136V/D158E/A174K/D203V/I222L,
R12K/F21W/S32K/F92L/T109K/A125S/T136V/D158E/D203R,
T17Q/L90Y/F92L/A174K/D203V/I222L, F21W/F92L/S101A/A117Q/D203R,
T17S/D18R/L90Y/F92G/A114V/A117Q/D203R,
L90Y/F92L/S101A/S110R/A125S/Q142W/A174R/A216P,
R12K/V23L/A24V/F92G/T136V/D203R, A24V/S32Q/F92L/T136V,
S32K/L90Y/F92G/A114K/D203V,
S32M/L90F/F92G/A114V/A117N/A125S/A174K/D203R,
R12K/T17A/V23L/A24V/L90F/F92G/S101N/A125S/Q142W/D158I/D203R,
T17C/L90Y/F92L/S101N/T109L/S110R/A125S/A174K/D203R,
T17C/L90Y/F92L/A125S/T136V/D158E/A174K,
L90Y/F92L/S110R/A125S/D158E/D203V,
T17Q/L90Y/F92L/T109L/T136A/D158E/A174R/D203V/V229C,
R12K/V23T/A24V/S32Q/F92G/D158L/A174R/D203R,

T17S/V23L/S32Q/L90F/F92L/A114K/A117N/A125S/T136V/D158E/D203V/V229I,
P20T/F21W/S32M/L90Y/F92G/T109L/S110R,
A14K/S32M/L90Y/S101R/A114V/T136A/A216P,
P20T/F21W/L90Y/F92G/A125S/A174K/V229I,
V23L/S32K/L90F/F92L/S101R/T109R/T136V/I222L, A24V/F92L/Q142L/A216P,
R12K/A14K/F92G/S110R/A117N/A125S/A174R/D203R,
T17Q/A24V/N87M/F92G/A114K/A117N/D158E/D203R/I222L,
R12K/P20T/D158E/D203R/I222L, S32K/L90Y/F92Y/S101A,
V23T/N87I/F92G/A125S/T136A/D158L/D203R, A24T/L90F/F92L/A125S/T136A/D203V,
R12K/A14K/F92G/T109R/T136A/Q142L/D158E/A174K/D203R/I222L,
D18R/S32Q/N87H/L90Y/F92G/S101K/Q142L/D158I/A174R,
T17A/F21W/S32K/N87F/L90F/F92Y/S101Q/T136A/D203R,
T17Q/L90Y/F92G/S101N/T109L/S110R/T136V/Q142L/D203V/A216P,
S32Q/L90Y/F92L/T109K/A125S/T136V, P20T/L90Y/T109K/T136A/D158I/D203R,
N2R/S32Q/L90Y/F92Y/T109K/D203V,
T17H/A24V/S32Q/L90Y/F92L/S101H/A114K/D158I/A174R/D203V,
P20T/F92Y/S101K/A125S/D158E/D203R, R12K/A14K/P20T/F21W/S32M/F92L,
R12K/A14K/V23T/A24V/L90F/F92L/S101W/T136A/D203V,
T17L/S32K/F92L/T109L/A114V/T136V/D158L/R236H,
P20T/F21W/S32Q/N87F/F92L/D158E/D203V,
V23T/A24V/L90Y/F92G/A125S/T136V/D203V,
P20T/S32K/L90Y/F92G/S101N/T109L/S110R/A125S/A174K/D203R/A216P,
V23L/L90F/F92L/T136A/D203R/I222L,
R12K/A14K/A24V/N87L/L90Y/A125S/T136V/A216P, P20T/F21W/L90Y/A125S,
N2R/L90Y/A114K/A117Q/D203V/V229I, F21W/S32K/L90Y/A125S/D158E/A216P/V229I,
S27L/T136V, S27L/P8T/T17Q/F21W/S101A/T136V/Q142L, S27L/N2R/T17L,
S27L/V23T/A24V/T136A/Q142W, S27L/F21W/T136V,
S27L/T17Q/T109K/A114K/T136V/V229I, S27L/T17Q, S27L/T136V/I222L/V229C,
S27L/T17A/A114V/A117N/T136A, S27L/N2R/T136A, S27L/N2R/T17A,
S27L/T17C/S101A/T136V, S27L/P20T/T136A, S27L/T136V/Q142W/V229I,
S27L/T109K/T136V/I222L/V229I, S27L/T17L/S101H/A117N/Q142L/I222L/V229I,
S27L/S101Q/T109L/A117N/T136A/Q142L, S27L/T109K/S110R/S193N/S252T/R255M,
S27L/S22R/Y26K/R236Q,
S27L/L90F/F92L/S98E/S113Y/A114K/T136A/D158E/S181R/T206G/S212M/V219I,

S27L/N9S/S22P/T48N/L90Y/F92G/T109K/S110R/T136A/Q189V/N211F/R236Q,
S27L/T17A/Y26L/T48N/I82M/S101D/R236Q, S27L/Y26T/S101D/D158E/V219I/S252T,
S27L/S13R/S98E/T136V/S181R/T206G/V229I/N231S,
S27L/F21W/F92L/S98N/S193P/I222L,
S27L/S1G/Y26K/L90Y/S113Y/A114V/T136A/Q189V/N204R/N211L/R236Q,
S27L/S22K/I82L/L90Y/F92G/R108S/A117N/D158E/S193N,
S27L/S1G/N9S/T48S/L90Y/S98T/S101A/S113N/A114K/L119M/S193N/T206G/S252T,
S27L/N9E/R12K/S22P/V23L/T160R/D203R/I222L,
S27L/N9E/R12K/S22P/V23T/T48S/S98E/R108S/T160S/Q189V/T206G/S212L/V229I,
S27L/S1A/N2R/N9S/T48N/L90F/F92L/D203V/S223A,
S27L/N9E/R12K/V23T/I82M/L90Y/F92L/T136V/N204K/N231S/R255M,
S27L/S1G/N9S/S22V/I82F/L90Y/F92L/A117N/L119M/Q142L/T206G/S212L/S223A,
S27L/N9S/Y60H/R108C/S193P/V219L,
S27L/N9E/S22K/S32M/L90F/F92G/R108T/L119M/Q189V/I222L/S223A/R236Q,
S27L/T17Q/F92G/S98N/Q142L/Q189L/R236C,
S27L/N9E/R12K/Q142W/T160Q/T206G/S212M,
S27L/Y26T/S113N/A114V/T136A/D158E/D203R/N211F/R236Q/S252T/R255M,
S27L/S1A/T17H/S22V/L90Y/F92G/S98E/A114V/T136A,
S27L/N9E/T48S/I82M/L90Y/F92L/N122A/A127S/T160S/A174R/T206G/S212L/R255M,
S27L/N9S/S22P/V23L/L90Y/F92G/A125S/T160S/N204R/I222L/S223A/R236Q,
S27L/N9E/S22P/T48S/L90Y/F92L/T109R/E173R/A174K/D203V/S223A,
S27L/R12K/Y26K/T48N/I82L/L90F/A125S/N211I/A216P,
S27L/N9S/R12K/F92Y/T109K/S110R/T160K/Q189L/S223A/S252T/R255M,
S27L/S1A/N2R/N9E/R12K/S32K/N87F/R108T/N211I/V219K/R255M,
S27L/L90F/F92G/N122A/T136A/T160V/E173R/D203R/S252T,
S27L/L90Y/F92G/S98T/T109L/D158I/S193K/V219K/R236Q/S252T,
S27L/N9E/R12K/T48N/L90F/S98L/R108Q/A117Q/T136A/T206G/S212F/V219I/V229I/R25
5L, S27L/S1A/S22V/N87K/R108K/N122E/D158E/S193P/V219I/R255M,
S27L/F21W/Y26T/S34R/R108H/L119M/N211M/R236C/S252T,
S27L/S1A/N9E/R12K/V23T/T48S/N87H/S101Q/Q189L/N211M/V219I/R236Q/R255M,
S27L/N9S/T25Q/L90Y/S101D/T136A/Q142L/Q189L/R236Q,
S27L/A14K/I82F/F92G/S98E/R108C/A117N/L119M/Q189V/T206G/I222L/S223A,
S27L/T17C/Y26T/N87V/R108C/N122E/T136V/S193P/S252T/R255M,
S27L/L90Y/F92G/T109L/N122S/R255M, S27L/R12K/L90Y/T160V/A174R/R236Q,

S27L/N9E/R12K/T48N/L90Y/F92G/S98T/S113R/N122R/A127S/T136A/A174K/N204K/S212L,
S27L/V23T/L90Y/F92G/S98E/T109L/A125S/T160V/A174K/S181C/S193K/T206G/S212M/R255M, S27L/A24V/F92G/S101D/A114V/A117N/A125S/T136V/D203R,
S27L/R12K/S32Q/S101Q, S27L/L90F/F92G/T136V/A174R/D203R,
S27L/P20T/S32K/L90Y/D203V, S27L/N2R/A14K/T17S/L90Y/T109K/T136V/A216P,
S27L/N2R/V23T/A24V/S32M/L90Y/F92G,
S27L/A14K/L90Y/F92L/S101D/A117N/D158I/D203R,
S27L/F21W/N87H/A114V/A117N/T136V,
S27L/N2R/V23T/A24V/N87M/F92L/S101K/A125S/T136A,
S27L/L90Y/F92L/S101N/T109R/S110R/Q142W, S27L/S32M/F92G/A216P,
S27L/F21W/L90Y/F92L/A114K/A117N/T136A/D203V,
S27L/N2R/V23T/S32M/N87M/F92L/T136A, S27L/R12K/L90F/F92G/S101A/D203V,
S27L/A24V/L90F/F92G, S27L/R12K/F92L/S101A/A125S/T136A/D203R,
S27L/R12K/V23L/L90F/F92L/A114K/T136A/D158E/D203V/I222L, S27L/L90F/F92G,
S27L/A14K/P20T/S32Q/L90Y/A125S/D203V,
S27L/T17L/L90F/F92G/A125S/A174R/D203V,
S27L/N2R/N87K/A114K/A117Q/T136V/D203V, S27L/N2R/A114K/T136V/D203V,
S27L/N2R/T17A/T136V/A216P, S27L/N2R/R12K/N87F/T136V/D158E/A174R,
S27L/D203R, S27L/R12K/T17Q/T136V/D203V/A216P, S27L/N87F/T109L/Q142L,
S27L/N2R/T17Q/A24V/A114K/A117Q/T136A/D158E/A174K/D203V/I222L,
S27L/N2R/P20T/F21W/N87M/T109R/A117N/A125S/T136V/D203V/I222L,
S27L/V23T/A24V/T136A/D158E/A174K/D203V/I222L,
S27L/R12K/V23L/A114K/T136A/D158I/D203V/I222L,
S27L/N87Y/T136V/D158E/D203V, S27L/T109K/S110R/D203R,
S27L/N2R/T17L/A125S/T136A/D158E, S27L/A24V/D158E/A174K/A216P,
S27L/T17G/V23L/N87L/A117Q/A125S/T136V/D158E/V229C,
S27L/V23L/N87L/T109R/A114K/A117N/A125S/T136V/Q142L,
S27L/N87Y/S101A/A114V/A117Q/T136V/A174K/A216P/I222L,
S27L/R12K/T109R/Q142L, S27L/D203V, S27L/T17G/N87F/S101A/D203V,
S27L/T17L/N87M/Q142L/D203V, S27L/N2R/R12K/S101D/A117N/T136A/D203V,
S27L/R12K/P20T/F21W/A114K/Q142W/D203R,
S27L/N2R/T17G/T109L/S110R/A114K/A117Q/T136V/Q142L/D203R, S27L/N2R,
S27L/S32K/L90F/F92G/S101M/A114V/A117N/A125S/T136A/D158E/D203V,

S27L/R12K/T17A/F21W/S32Q/Y60H/D203R, S27L/R12K/F92L/S110R/T136V/I222L,
S27L/A14K/V23T/A24V/L90Y/F92G/A114V/A117Q/Q142L/A174R/I222L,
S27L/F21W/S32Q/L90Y/S101D/T109K/A125S/T136A/A174K,
S27L/V23T/L90Y/F92G/T136A/D203V/A216P,
S27L/T17S/L90Y/F92G/A117N/T136A/D158L,
S27L/A14K/V23L/A24V/F92L/Q142W/D203V, S27L/T17L/L90F/F92G/S101K/A174K,
S27L/L90F/F92L/A125S/T136V/A174K/D203V/A216P/I222L,
S27L/R12K/P20T/S32M/L90F/F92G/S101M/A114V/D203V,
S27L/T17A/S32Q/L90F/F92L/T136V/Q142W/A174K/D203R,
S27L/R12K/S32M/L90F/F92G/S101W/A114V/D158I/I222L,
S27L/P20T/S32M/L90Y/F92L/A114V/A117N/I222L,
S27L/T17C/L90Y/F92G/T136A/A174K/D203V,
S27L/S32Q/N87Y/F92G/T109K/A114K/A117N/T136V/D203V/V229C,
S27L/F21W/L90F/F92G/T136V/A174R, S27L/P20T/N87Y/T136A/D203V/I222L,
S27L/A24V/L90Y/F92L/D158I/A174K/D203R/I222L,
S27L/F21W/S32Q/L90Y/S110R/A114K/T136V/D203R/A216P/V229I,
S27L/N87H/F92G/D203R, S27L/R12K/S32K/Y60A/A125S/T136V/D158L,
S27L/V23T/A24V/S32Q/L90F/F92L/T136A, S27L/F92L/A114V/D158E/D203R,
S27L/S32Q/L90Y/D203V/V229I, S27L/V23T/S32Q/Y60H/T109K/T136V/A216P/I222L,
S27L/A14K/A24V/L90F/F92G/T109K/T136V/D158L/A174R/D203V/I222L/V229I,
S27L/R12K/N87F/A174K, S27L/P20T/S32Q/Y60H/T109K/A114V/T136A/A174K,
S27L/V23T/A24V/Y60A/A125S/A174R/A216P, S27L/F92Y/D158L/I222L,
S27L/V23L/A24V/L90Y/F92G/D203V/I222L,
S27L/V23T/A24V/S32Q/L90F/F92G/S101H/T136A/A174K/D203V/V229C,
S27L/S32K/N87H/A125S, S27L/P20T/F21W/F92G/A114V,
S27L/T17L/V23L/A24V/L90Y/F92L/A117N/T136V/L137M/Q142L,
S27L/P20T/L90Y/D203R, S27L/S110R/Q142L/D158I/D203R/V229I,
S27L/P20T/S32Q/L90F/F92L/T109K/S110R/A125S/D203V,
S27L/V23T/A24V/L90F/F92G/D158E/D203R, S27L/F92G/T136A/A174R/D203V/V229C,
S27L/P20T/L90Y/F92G/T109R/S110R/A125S/D203R,
S27L/A14K/L90Y/F92L/D158L/A174R/D203V,
S27L/F21W/S32Q/L90Y/F92G/T109L/S110R/A117N/D158I/A174K/D203V,
S27L/R12K/A114K/A117N/D158E/D203V,
S27L/T17S/N87H/T109R/A114K/A117N/T136V/Q142W/D203V, S27L/N2R/N87Y,

S27L/T17S/V23T/A24V/S32Q/Y60H/N87K/T136A/D203R,
S27L/P20T/F92Y/T109K/D158E/D203V,
S27L/S32K/N87M/F92L/S101M/T109L/S110R/D203V,
S27L/L90Y/F92G/T136A/D158E/D203V/A216P/V229I, S27L/A14K/S32M/F92L/A125S,
S27L/L90Y/F92G/T136V/D158L/D203V,
S27L/A24V/L90Y/F92G/S101A/T109L/S110R/A117Q/A174R/D203R,
S27L/P20T/S101D/A114V/T136V/Q142W/D158E/D203R/A216P,
S27L/N87Y/F92G/S101Q/A114K/A117N/T136A/D158L/D203R/A216P,
S27L/V23T/A24V/N87Y/L90Y/F92L/S101D/T109R/A174K/D203R/A216P,
S27L/N2R/P20T/F21W/L90F/F92Y, S27L/L90Y/F92G/D158I/D203V/V229C,
S27L/S32Q/F92G/Q142W/I222L, S27L/N2R/R12K/S32M/N87Q/L90F/F92L/I222L,
S27L/R12K/S32K/F92L/A114V/A117Q/T136A/D158E/A216P, S27L/L90Y/F92G,
S27L/R12K/F21W/S32M/F92G/T136V/Q142L/D203R,
S27L/R12K/A14K/L90Y/F92L/A125S/A174K, S27L/A24V/A114V/A117Q/T136V/D203V,
S27L/R12K/T17Q/V23L/S32M/L90Y/F92G/S101D/T136V/I222L,
S27L/V23T/A24V/S32Q/F92L/S101N/T109L/S110R/D203R,
S27L/P20T/Y60H/N87K/T136A/A174R/D203V,
S27L/P20T/F21W/L90Y/F92L/T136A/A174R/D203V/V229C,
S27L/T17G/F21W/S32K/L90F/F92G/A125S/T136A/A174K,
S27L/T17G/N87Y/T136A/D203V/A216P,
S27L/F21W/L90F/F92G/S101D/A117N/T136A/A174K/D203V,
S27L/R12K/A14K/A24V/L90Y/D203V, S27L/N2R/L90F/F92G/S101M/Q142L/D158L,
S27L/N2R/T17C/A24V/N87K/A174K/A216P, S27L/N87M/Q142L/I222L,
S27L/F92L/S98T/R108C/A117Q/A127S/Q142L/Q189V/A216P/R236Q,
S27L/S22K/F92L/R108H/A127S/T136A/N211I,
S27L/F21W/T48S/L90Y/F92L/T109L/A127S/T136V/N204K/S223A,
S27L/T17L/L90Y/F92L/S98M/A174K/D203V/N211M/S212L/S223A,
S27L/N87K/S98M/N211M, S27L/N9E/S22P/L90Y/N204K,
S27L/S32M/T48S/F92G/A127S/Q142W,
S27L/T17S/S22K/L90F/F92L/D158E/D203V/V219I/S252T,
S27L/L90F/F92L/A127S/D203V, S27L/T25F/L90Y/F92G/N204R/S223A/N231S/R236C,
S27L/F21W/S22V/T48S/L90F/F92L,
S27L/S1A/S98T/S113K/A114V/L119M/A127S/Q142W/S193K/V219K/S252T/R255L,
S27L/A24V/I82L/L90F/F92G/T109L/L119M/A127S/E173R/N204K,

S27L/T17C/Y60H/L90F/A127S/E173R/A174R/N204K,
S27L/L90F/F92L/Q142W/D203V/S223A, S27L/P20Q/L90Y/Q142L/S223A,
S27L/N9S/Y60A/A216P/R236Q/R255M,
S27L/F92G/R108C/S110R/A117Q/T136A/N211M,
S27L/S22P/Y60H/S98A/S113K/A114K/T136V/Q189L/S193H,
S27L/V23L/S98A/Q142L/N211M,
S27L/V23L/Y26L/L31M/T48S/F92G/A174R/N204K/S252T,
S27L/S13R/A14K/Y26T/T136A/S181R/N211M/S212M,
S27L/F21W/I82F/L90Y/F92G/A127S/N211M, S27L/P20T/Y60H/S98L/T136A/R255L,
S27L/P20T/S34R/N87M/D158E/S252T, S27L/T25A/L90F/F92G/Q189L/S193P/V229I,
S27L/V219L/I222L,
S27L/N9E/T48S/L90F/F92L/S98N/R108H/S110R/S113Y/N211V/S212L/S252T,
S27L/L90F/F92Y/S113R/V219I/R255L, S27L/F21W/S22K/T48S/F92G/T109R/A127S,
S27L/S1G/A14K/P20T/S32K/T48S/L90Y/F92L/T109L/T160R/V219L/I222L/S252T/R255L,
S27L/L90F/F92G/D158E/T160R/S193N/N204K/S223A,
S27L/A14K/Y26T/F92L/Q142L/S212L,
S27L/S22R/L90Y/F92G/V126I/A127S/A174K/N204K,
S27L/P20T/T25A/L90F/F92L/A117N/L119M/S252T/R255L,
S27L/T17A/A24V/T48S/L90F/F92G/T109R/A127S/T160K/D203R/S223A,
S27L/F21W/T48S/T109L/A127S/V219I/R255L,
S27L/A14K/S22P/T48S/L90F/F92L/T109L/A127S/Q142L/A174R/N204K/S223A,
S27L/T136A/S212M/N231S, S27L/F21W/L90F/F92G/A127S/S223A,
S27L/T48S/L90Y/F92L/R108S/S110R/A127S/T136V/E173R/S223A,
S27L/T48S/F92G/S98E/Q142L/Q189V/S193P/N204R/S223A,
S27L/T17S/L90F/F92Y/S101K/A127S/T136V/N204R/N211I/V229I/N231S,
S27L/N9E/F21W/T48S/L90Y/F92L/T109R/N204K/R236Q,
S27L/N9E/S22R/T48S/F92L/S101N/A127S/E173R/N204R,
S27L/S22K/N87F/A117Q/S181C/N204K/N211M/V229I/N231S/R236Q,
S27L/N9S/T48S/L90F/F92G/S101N/A127S/N204K/A216P/S252T/R255M,
S27L/I82L/S193H/N211M/S212F/S223A,
S27L/A14K/P20T/T48S/L90F/F92G/S98N/R108C/T136A/N204K/N211L/S212L/R236Q/S2
52T, S27L/S13R/T17L/T25Q/L90F/F92G/R108C/A127S/T160R/I222L/R255L,
S27L/T48S/L90Y/F92G/T109L/S193N,
S27L/S22V/T48S/L90Y/F92L/S98E/A127S/T136A/N204K/A216P/S223A,

S27L/V23L/N87H/F92L/R108C/S110R/N122A/T160S/Q189L/S193P/N204K/S223A/R255L
, S27L/F92G/A127S/A174R/S223A/V229I/N231S/R236C,
S27L/S1G/L90Y/A127S/E173R/S223A, S27L/F92Y/S98N/Q142W,
S27L/P20T/S32M/S34R/T48S/L90Y/F92L/R108K/A127S/Q142W/A174R/N204K/V219I/S2
52T, S27L/F92G/A127S/Q189V/S193N/S223A,
S27L/S34R/F92G/S110R/D158L/D203V/R255M,
S27L/N9E/F21W/T48S/F92G/T109L/A127S/R236C,
S27L/S1A/S22P/T48S/L90Y/F92G/A127S/E173R/A174R/N204K/S223A,
S27L/S22K/T48S/T160S/N204K,
S27L/F21W/T48S/L90Y/F92G/S98V/R108C/A127S/N211L/S212M/N231S/R236C,
S27L/V23L/A24V/A114K/T136A/I222L/V229I,
S27L/P20T/S101H/T109R/A114V/T136A/Q142L/I222L/V229I,
S27L/V23L/S101H/T109K/A114K/A117N/T136V/Q142L/I222L/V229I,
S27L/T17L/F21W/S101H/T109R/A114V/T136A/Q142L/I222L/V229I,
S27L/T17S/T136V/Q142L/I222L/V229C,
S27L/T17C/S22P/T48S/L90F/F92G/A127S/A174R/P192A/S193H/N204K/S223A/V229I/N2
31S/R236Q/R255L, S27L/N9E/T48N/N87H/F92L/A174K/Q189L/S193K/N204R/V219I,
S27L/A14K/V23L/Y26L/L90F/F92G/S98E/S113K/A114K/T136A/V229I/N231S,
S27L/L90Y/F92L/S101D/A117N/Q142L/S193N,
S27L/S22K/T25A/L90Y/Q142L/E173R/N204K/S223A,
S27L/P20T/L90Y/T109L/A127S/A174K/V229C,
S27L/S22V/L90Y/F92L/A117Q/R236C/R255L,
S27L/V23L/A24G/Y26T/I82F/L90Y/F92G/R108H/T136A/D158L/T160K/D203V,
S27L/A14K/S22K/F92G/A127S/N204K/V229I,
S27L/S22V/L90F/F92G/A127S/A174R/A216P/R236C,
S27L/V23L/Y60A/S98T/T109L/A127S/T136V/T160Q/N204K/S223A/R255L,
S27L/S13R/L90Y/S98L/T160V/Q189L/S193P/A216P/S252T/N253S/R255L,
S27L/S22R/I82M/F92L/T109L/A127S/Q142W/Q189L/S223A,
S27L/S1G/Y60A/L90F/F92L/N122E/T136A/N204K/S223A/N231S,
S27L/S32K/F92G/S98L/R108Q/S110R/R255L,
S27L/L90Y/F92G/L119M/A127S/A174R/S252T, S27L/L90F/S110R/S181R/N211F,
S27L/A14K/S22P/Y60H/S110R/N122S/D158I/N204K/S223A/R255M,
S27L/F92L/S98V/T109L/N122A/E173R/R255L, S27L/S22R/N87L/F92G/R236C,
S27L/S32M/S34R/I82L/F92G/N204K/S223A,

S27L/A24V/T25Q/Y60I/A127S/A174K/N204K/V219I/S223A/S252T/R255M,
S27L/S98V/T136A/N211F, S27L/N9S/L90F/F92L/A127S/N204R/S212M,
S27L/S22R/T48S/F92G/R108H/S110R/A127S/E173R/N204K/S223A/R236Q,
S27L/A14K/L90Y/F92G/A127S, S27L/A14K/T48S/F92G/E173R/N204R/S223A/N231S,
S27L/L90Y/T109R/S113N/V219L/I222L, S27L/N9S/T48S/L90Y/T136A/T206G/S223A,
S27L/S22K/T48N/L90F/F92G/N204K/S223A/V229C/N231S,
S27L/S22P/T48S/L90F/F92G/A127S/A174R/N204R/S223A,
S27L/S1G/P20T/S32K/S34R/T48S/N87F/S98A/A127S/T136A,
S27L/F21W/S22R/S98E/Q142L/T160V/S223A/S252T/R255L,
S27L/P20T/T48S/F92Y/R108K/S110R/A127S/V219L/S223A/V229I/R236Q,
S27L/Y26T/L90Y/F92L/A127S/V219L/S252T/R255L,
S27L/N9S/F21W/T48S/F92L/A127S/D203V/S212F,
S27L/F21W/Y60H/S98E/L119M/D158E/R236Q,
S27L/F21W/S22R/L90F/F92L/N122S/Q142W/R236Q,
S27L/F21W/T48S/L90F/F92G/T109K,
S27L/L90Y/F92L/T109R/A127S/E173R/N204K/S223A,
S27L/N9S/F21W/T48S/L90Y/F92G/A127S/N204K/V219I/R255M,
S27L/N9E/I82F/L90Y/F92L/S95N/T109L/A127S/A174R/S223A,
S27L/L90F/F92L/S98L/N204K/A216P, S27L/A24V/T48S/A117Q/N211M/S212M/S223A,
S27L/L90Y/F92G/T109L/A127S/N204R/S223A, S27L/N2R/S32Q/N87K/A125S/V219L,
S27L/S22P/T48S/L90F/F92L/S193N/V219L/S223A, S27L/T25Q/L119M/N211M/R236Q,
S27L/L90Y/F92G/N122S/A127S/E173R/A216P/R236Q,
S27L/P20T/S32K/L90F/F92G/N122E/D158E/T160S/Q189L/S223A/S252T,
S27L/N9S/F21W/T48S/N87H/L90F/F92L/S113N/V229C,
S27L/T25Q/I82L/L90F/F92G/S98L/S113R/A114V/T136A/S181R,
S27L/P20T/R108Q/S110R/D203V/N211I,
S27L/S1G/N2R/P20T/Y26T/S32M/T48S/L90Y/F92L/A127S/N204R/V219L,
S27L/A14K/P20T/T48N/L90F/F92G/A117Q/A127S,
S27L/N9S/T48N/L90Y/F92L/S98M/T109K/A127S/N204R/N211I/S212L/V219I,
S27L/F21W/S22R/T48S/L90F/F92L/R108Q/A174K/N204K/S212F/S223A,
S27L/S22R/Y60H/S98T/T136V/S193K/R236Q,
S27L/S22K/Y26K/N87Y/L90Y/F92G/T109R/A127S/S252T/R255L,
S27L/L90F/F92L/T109L, S27L/F21W/F92L/N204K/S252T/R255L,
S27L/V23L/L90Y/F92G/A127S/S193K/N204R/V219I/R236C/R255L,

S27L/F21W/L90Y/F92G/A114V/N122S/A127S/N204K/S223A/S252T,
S27L/P20T/Y60A/T109L/Q189L/S212L, S27L/S22V/T48S/L90Y/F92G/N204K,
S27L/S22V/S32K/T48S/I82F/F92G/A127S/N204K/S223A, S27L/A55L, S27L/A97V,
S27L/F250L, S27L/T109G, S27L/G38D, S27L/A97S, S27L/A55V/A216T, S27L/P20D,
S27L/A55V, S27L/T109Y, S27L/V165I, S27L/A184G, S27L/A97E, S27L/A184S,
S27L/A97F, S27L/A97T, S27L/K197Y, S27L/A55I, S27L/A97P, S27L/A55M, S27L/P20E,
S27L/A117L, S27L/T109L, S27L/K197T, S27L/T136S, S27L/A97L, S27L/T109A,
S27L/P20I, S27L/L191V, S27L/A184C, S27L/A97Q, S27L/F250V, S27L/K197V,
S27L/A117S, S27L/K197R, S27L/T109K, S27L/A55C, S27L/N2S/V177A, S27L/Q142D,
S27L/G149C, S27L/F21Y, S27L/G149A, S27L/P164E, S27L/Y26C, S27L/T17N,
S27L/S57M, S27L/D249I, S27L/T17S, S27L/P164T, S27L/Q142L, S27L/D249N,
S27L/I185R, S27L/V83L, S27L/G149S, S27L/A24D, S27L/R251V, S27L/V83I,
S27L/S110N, S27L/T17M, S27L/F161W, S27L/G46E, S27L/D249T, S27L/T17I,
S27L/S57C, S27L/S57T, S27L/S57L, S27L/Q167T, S27L/S57E, S27L/T17K, S27L/G149N,
S27L/Q142E, S27L/S57F, S27L/T17A, S27L/P164H, S27L/T17R, S27L/G149T,
S27L/R251T, S27L/S101Y, S27L/S57V, S27L/A24T, S27L/I185E, S27L/G46N,
S27L/P164N, S27L/P164S, S27L/Q167V, S27L/F161V, S27L/S57I, S27L/G149D,
S27L/Q167I, S27L/I185Q, S27L/N2L/T17I/T136S/P164H/D249M,
S27L/P20E/F21Y/I139T/P164H/K197T/R251E, S27L/A114V/A117N/T136A/I222L/V229I,
S27L/P20T/A24V/S101Q/T109R/A117Q/I222L/V229I, S27L/S110D/A184S/K197T,
S27L/T136S/D249T, S27L/F21Y/A184S, S27L/N2F/P20E/S110D/A184S/L191V/R251V,
S27L/T17G/V23T/A24V/T109L/A117Q/T136A/I222L/V229I,
S27L/P20D/F21Y/A55T/F250L/R251V, S27L/T109R/A117N/I222L/V229C,
S27L/F21Y/A55T/T109A/A184S, S27L/V23L/A24V/S101K/A117N/I222L/V229C,
S27L/S110K/A117Y/P164R, S27L/I139T/Q142E/I169V, S27L/A24H/R251V,
S27L/F21W/A114V/T136A/I222L/V229I, S27L/N2S/A55V/I185Q/R251E,
S27L/N2F/S101Y/Q142L/G149T/Q167I/I169C/V229L, S27L/N2F/S101C/A117F/P164S,
S27L/Q142H/I185S/R251A, S27L/F21W/T109R/A117N/T136V/I222L/V229I,
S27L/G46R/T109K/T136S/D249T, S27L/N2E/G46N/A55V/I185Y/D249I,
S27L/V83L/F250V/R251E, S27L/F21W, S27L/S57C/R251E,
S27L/V23L/A114V/A117N/T136A/I222L/V229I, S27L/A24N,
S27L/T17A/P20T/S101Q/T109K/S110R/T136A/I222L/V229C, S27L/P164T/V165I/R251E,
S27L/F21Y/G46E/A117T/T136S/G149C/R251Q,
S27L/G46N/I139T/Q142E/P164T/I185A/V229L/F250V, S27L/V23L/T136A/I222L/V229I,

S27L/V23T/A24V/T109K/A114K/A117Q/T136A/I222L/V229C, S27L/F250L/R251Q,
S27L/P20E/F21Y/S57T/P164N/A184C/V229C, S27L/P20T/V23T/A24V/I222L/V229I,
S27L/V83L/A97S/A184C/D249S, S27L/P20T/S101H/A114V/A117N/I222L/V229I,
S27L/N2E/R251L, S27L/P20E/F21Y/S110D/A117S, S27L/T17A/V23T/A24V/T136A,
S27L/P20D/S57E/S101C, S27L/N2S/V83L/A184S,
S27L/T17S/S101N/T109K/A117N/T136V/Q142L/I222L/V229C,
S27L/P20D/A55T/A117Y/K197R, S27L/P20T/I222L/V229C, S27L/T17I/A24D/S57I,
S27L/N2F, S27L/T109K/A117N/T136V/I222L/V229C, S27L/N2L/A24T,
S27L/N2F/P20I/F21Y/R251Q, S27L/N2F/G46E, S27L/T136V/Q142W/I222L/V229I,
S27L/K197R/R251E, S27L/T17S/R251T, S27L/P20I/F21Y/A117F/I185G/D249N,
S27L/T17L/S101M/T109K/S110R/A117Q/I222L/V229C,
S27L/T17Q/T136A/I222L/V229C, S27L/V229I, S27L/T17A/F21W/S101Q/I222L/V229C,
S27L/T17S/F21W/Q142L, S27L/T17L/T109L/S110R/A117Q/I222L/V229I,
S27L/F21W/T136A, S27L/P20E/F21Y/G46E/V83L/A97Q,
S27L/P20T/F21W/T136A/Q142L/I222L/V229I, S27L/T17G/F21W/A117N/I222L/V229I,
S27L/F21W/T109K/S110R/T136A/V229I, S27L/T17C/T109K/A117N/T136A/I222L/V229I,
S27L/P20T/T109L/S110R/A117N/I222L/V229I, S27L/A24N/P164S/K197V/R251T,
S27L/V23L/A117Q, S27L/A55V/V83L/A117T, S27L/P20D/G46S/A55I/K197T,
S27L/A117Y, S27L/F21W/S101M/I222L/V229C,
S27L/N2L/T17N/S57C/S110D/A117L/P164N/I185G/V229L/R251T,
S27L/N2E/G46S/V83L/T136S/A172T/V229C/D249N,
S27L/P20T/T109R/A114K/A117Q/T136V/I222L/V229C,
S27L/V23L/A24V/T136A/Q142W/I222L/V229I, S27L/N2L,
S27L/T17A/F21W/T109R/S110R/I222L/V229I,
S27L/T17C/A114K/A117N/T136V/I222L/V229I, S27L/V23L/I222L/V229C,
S27L/S101N/A117Q/I222L, S27L/N2E/P20I/T109L/L191V/K197L/V229C,
S27L/P20T/F21W/Q142W/I222L/V229C, S27L/N2L/G46E/T109K/F161V,
S27L/T17S/V229I, S27L/T17Q/T136A/I222L/V229I, S27L/N2L/A172T,
S27L/P20D/F21Y/F250V, S27L/N2L/A24H/A55C/V229L, S27L/N2L/P20E/F21Y/V229L,
S27L/V23T/T109L/A114V/T136A/Q142L/I222L/V229C, S27L/P20T/F21W/I222L/V229C,
S27L/T17H/F21W/T136V/Q142W/I222L/V229I, S27L/G46E/A55I/Q142E,
S27L/N2F/R251Q, S27L/F21W/Q142L/I222L/V229C, S27L/I222L/V229I,
S27L/P20T/S101M/A114V/A117N/Q142W/I222L/V229C,
S27L/T17Q/A24V/S101W/T136A, S27L/N2S/P20D/A97C/A117F/F250L/R251E,

S27L/S101M/A114V/A117Q/T136A, S27L/T163I, S27L/S101Q/Q142L/I222L/V229I, S27L/F21W/I222L/V229C, S27L/S101M/T136A/I222L/V229I, S27L/F21W/A24V, S27L/A24D/K197L, S27L/T17H/S110K, S27L/P20T/F21W/V229I, S27L/T17G/P20T/F21W/A117Q/I222L/V229C, S27L/F21W/A24V/A114V/A117Q/Q142W/I222L/V229C, S27L/P20T/A117Q/I222L/V229C, S27L/T17Q/V23T/A24V/I222L/V229I, S27L/N2F/D249N/F250L, S27L/V23T/Q142L/I222L/V229C, S27L/P20E/A24D/D249S/F250V, S27L/A24D/S110K, S27L/T109R/I222L/V229I, S27L/P20E/F21Y/K197T, S27L/T17A/V23L/S101M/T109K/S110R/A114V/A117Q/T136V/V229I, S27L/P20T/F21W/Q142W/I222L/V229I, S27L/F21W/I222L/V229I, S27L/P20T/V23T/A24V/T109L/S110R/I222L/V229I, S27L/A24D/L191F/R251Q, S27L/A24D/T136S/F250V, S27L/P20T/F21W/T109L/T136A/Q142W/I222L/V229C, S27L/N2E/I169L/F250L/R251Q, S27L/N2S/A24N/G46E/A55L/Q142D/V229C/R251L, S27L/N2E/P20E/F250V/R251L, S27L/N2F/P20E, S27L/A24V/V229C, S27L/P20D/F21Y/G46N/S110K/T163I/Q167V, S27L/P20T/T109K/A114K/Q142W/I222L/V229C, S27L/T17Q/S101D/T136A/I222L/V229I, S27L/F21W/T109R/A117N/T136V/Q142W/I222L/V229C, S27L/A24D/T109L/K197L, S27L/T17Q/Q142L/I222L/V229I, S27L/T17A/A24V/S101D/S110R/T136A, S27L/P20T/F21W/T109R/A117Q/T136V/Q142L/I222L/V229I, S27L/P20T/F21W/T109K/A117N/I222L/V229C, S27L/P20T/S101M/I222L/V229I, S27L/N2S/A24D/S101Y/P164E/V165I/I185E, S27L/T17N/A117S/V229L/R251A, S27L/S101W/A114V/T136V/V229C, S27L/P20D/G46N/A55C/S110N/T163I/A184S, S27L/A24H/G46E, S27L/T17H/S101Q/T136V/Q142L/V229C and S27L/V23T/A24V/I222L/V229C.

5. A nucleic acid encoding the variant Bhr-PETase enzyme of any one of the preceding claims.

6. An expression vector comprising the nucleic acid of claim 5.

7. A host cell comprising the expression vector of claim 6.

8. The host cell according to claim 7, wherein the cell is bacteria, yeast or fungi.

9. A method of making a variant Bhr-PETase enzyme comprising culturing the host cell of claim 7 or 8 under conditions wherein said variant Bhr-PETase enzyme is produced, and recovering said variant Bhr-PETase enzyme.

10. A method of degrading PET, comprising contacting the PET with the variant Bhr-PETase enzyme of any one of claims 1-4.

11. The method according to claim 10, further comprising pretreating PET, comprising a mechanical pretreatment, a thermo-mechanical pretreatment, and/or a chemical pretreatment of the PET prior to an enzymatic degradation of the PET.

12. The method according to claim 11, wherein the mechanical pretreatment comprises grinding of the PET into particles.

13. The method according to claim 11, wherein the thermo-mechanical pretreatment comprises extruding the PET at a temperature configured to amorphize and reduce crystallinity of the PET.

14. The method according to claim 11, wherein the chemical pretreatment comprises contacting the PET with an ionic liquid, strong acid, base, or solvent configured to reduce crystallinity or to change a surface structure of the PET.

15. The method according to claim 10 or 11, wherein the method degrades the PET in a mixed plastics composition.

16. The method according to claim 15, wherein the plastics composition comprises analog of PET, PET-like or PET substitute derived biologically or chemically.

17. The method according to claim 15, wherein the plastics composition comprises at least one selected from the group consisting of Polybutylene terephthalate (PBT), Polycabonate (PC), Polycaprolactone (PCL), Polyethylene Furanoate (PEF), and High Density Polyethylene (HDPE).

18. The method according to any one of claims 10-17, wherein the method excludes sorting plastics to select PET from a mixture of plastics.