

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.

BRIEF SUMMARY OF THE INVENTION

[0003] 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 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 aspect, 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 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 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 embodiments, 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 embodiments, 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 embodiments, 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 embodiments, said amino acid substitution is selected from the group consisting of S27L, S27F, S27H, S27T, S27W, S1A, S1G, S1M, S1R, N2E, N2F, N2L, N2R,

N2S, Q5E, N9A, N9E, N9S, R12K, S13L, S13R, A14K, A14S, L15I, T16E, T17A, T17C, T17G, T17H, T17I, T17K, T17L, T17M, T17N, T17Q, T17R, T17S, D18R, P20D, P20E, P20I, P20Q, P20T, F21W, F21Y, S22A, S22I, S22K, S22P, S22R, S22V, V23L, V23T, A24D, A24G, A24H, A24N, A24S, A24T, A24V, T25A, T25F, T25H, T25Q, T25R, T25V, Y26C, Y26K, Y26L, Y26T, R30K, S32K, S32M, S32Q, S32Y, V33G, V33Q, V33T, S34R, V40T, G46E, G46L, G46N, G46R, G46S, T48N, T48S, L49G, G53A, I54V, A55C, A55I, A55L, A55M, A55T, A55V, M56I, M56L, S57C, S57E, S57F, S57I, S57L, S57M, S57T, S57V, Y60A, Y60H, Y60I, A62T, A68F, L70M, R72P, L74W, H77Q, I82F, I82L, I82M, V83I, V83L, V83T, N85D, N87F, N87H, N87I, N87K, N87L, N87M, N87Q, N87R, N87V, N87W, N87Y, S88K, S88T, L90F, L90K, L90Y, F92G, F92I, F92K, F92L, F92N, F92Q, F92V, F92Y, A97C, A97E, A97F, A97G, A97L, A97P, A97Q, A97S, A97T, A97V, S98A, S98D, S98E, S98L, S98M, S98N, S98Q, S98T, S98V, S101A, S101C, S101D, S101F, S101H, S101K, S101L, S101M, S101N, S101Q, S101R, S101V, S101W, S101Y, A102V, N105D, R108C, R108E, R108H, R108K, R108N, R108P, R108Q, R108S, R108T, R108V, T109A, T109F, T109G, T109K, T109L, T109N, T109R, T109Y, S110D, S110G, S110H, S110K, S110N, S110R, S113A, S113K, S113N, S113P, S113Q, S113R, S113T, S113Y, A114K, A114L, A114S, A114V, A117F, A117G, A117L, A117N, A117Q, A117S, A117T, A117Y, L119I, L119M, A121S, N122A, N122E, N122H, N122P, N122R, N122S, A125S, A127M, A127S, A127V, A135G, T136A, T136M, T136S, T136V, R138E, R138L, I139A, I139T, S140A, Q142D, Q142E, Q142H, Q142L, Q142W, I143N, I143R, T145S, K147F, K147G, K147N, K147Q, G149A, G149C, G149D, G149N, G149S, G149T, G149V, V150I, V150L, T153L, H156N, T157A, T157G, D158E, D158I, D158K, D158L, T160K, T160Q, T160R, T160S, T160V, F161V, F161W, N162E, N162H, N162P, N162R, T163I, T163S, P164E, P164H, P164N, P164R, P164S, P164T, Q167I, Q167T, Q167V, V170L, E173R, A174K, A174R, V177A, P179Q, S181A, S181C, S181R, Q182T, A184C, A184G, A184S, I185A, I185E, I185G, I185L, I185Q, I185R, I185S, 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, A216T, A216V, I217S, S218A, V219F, V219I, V219K, V219L, V219R, T221S, I222L, S223A, S223C, M225L, L227R, W228F, V229C, V229I, V229L, N231L, N231Q, N231S, R236C, R236E, R236H, R236K, R236Q, Q237R, N241P, V242T, N243P, A246D, A246K, A246S, A246T, D249I, D249M, D249N, D249S, D249T, F250I, F250L, F250V, F250Y, R251A,

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 embodiments, 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

embodiments, 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 embodiments, 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 embodiments, 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 embodiments, 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,
M56I/I82F/R108T/I139A/F161W/T194S/D203N/N241P, M56I/S88T/R108T/N190S/L227R,
N204A/Q237R, N9A/D18R/M56I/N85D/L119I/N254R,
N9A/S22A/G46S/M56I/R72P/F92Q/L119I/T221S/M225L, N9A/S22A/Q167T,
N9A/V40T/L49G/I54V/R108T/V150I/D203N/T221S/M225L, N9S/T25H,
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/I54V/R72P/F92Q/V150I/V200L,
S22A/L49G/M56I/V83T/S101L/R108T/L119I/A127M/T194S, S22I/Y26K,
S27T/I82L/P213N, S27T/T48S/I82L/F92Y/S252T,
S27T/T48S/I82L/L90F/A135G/S140A/I143N/T145S/P213N,
S27T/T48S/I82L/S140A/I143N/G149A, S32Y/A62T,
S88T/R108T/V150I/A184S/D203N/T221S/M225L/N243P, S98Q/A209V,
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/M56I/V150I/S218A/V219K,
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, V177A/A216L, 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,
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, L90F/F92G/T109K/A125S/A174K,
S32K/L90F/F92L/T109K/A114K/Q142W,
V23T/S32Q/N87I/L90F/F92Y/S101N/A114V/Q142W/A174R/D203R/I222L/V229I,
S32Q/F92G/A114V/D203V, 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,

T17A/S32M/Y60H/Q142L/D203V/I222L,
R12K/Y60H/L90F/S101R/A114V/A117N/D158E,
T17S/D18R/L90Y/F92G/A114V/A117Q/D203R,
L90Y/F92L/S101A/S110R/A125S/Q142W/A174R/A216P, S32M/S110R/A125S/D158E,
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,
P20T/F21W/Y60I/S101A/T109K/A174K,
T17C/L90Y/F92L/S101N/T109L/S110R/A125S/A174K/D203R,
T17Q/L90F/F92G/S101M/A117Q/T136A/D158I/A174R,
V23L/L90F/F92G/A125S/D203V/I222L, T17C/L90Y/F92L/A125S/T136V/D158E/A174K,
V23L/S32Q/Y60I/A117Q/T136A/A174K/V229C, V23T/L90F/F92Y/D203V,
R12K/A24V/Y60H/A174R, T17G/P20T/Y60I/F92G/D203V,
L90Y/F92L/S110R/A125S/D158E/D203V,
T17Q/L90Y/F92L/T109L/T136A/D158E/A174R/D203V/V229C,
R12K/V23T/A24V/S32Q/F92G/D158L/A174R/D203R,
S32M/N87Y/L90F/F92G/A174K/D203V,
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,
F92G/A125S/T136A/D158E/A216P, N87R/F92G/A117N/D203V/A216P,
R12K/A14K/F92G/S110R/A117N/A125S/A174R/D203R,
S32Q/N87F/L90F/F92G/S101M/A114V/A117Q/D203V,
T17Q/A24V/N87M/F92G/A114K/A117N/D158E/D203R/I222L,
N2R/S32Q/L90F/F92G/Q142L/D203V,
A14K/S32Q/L90F/F92G/A114K/A117N/A174R/D203V/A216P/I222L,
R12K/P20T/D158E/D203R/I222L, N2R/A14K/T17A/L90F/F92G/S101H/A174R/D203V,
T17G/A24V/L90F/F92G/Q142W, S32K/L90Y/F92Y/S101A,
V23T/N87I/F92G/A125S/T136A/D158L/D203R, T17S/N87L/V229I,
A24T/L90F/F92L/A125S/T136A/D203V,
R12K/A14K/F92G/T109R/T136A/Q142L/D158E/A174K/D203R/I222L,

A24V/S32Q/Y60A/T136A, D18R/S32Q/N87H/L90Y/F92G/S101K/Q142L/D158I/A174R,
T17A/F21W/S32K/N87F/L90F/F92Y/S101Q/T136A/D203R,
A24V/L90F/F92G/A114V/A117N/T136V/Q142L/D203V/V229C,
T17Q/L90Y/F92G/S101N/T109L/S110R/T136V/Q142L/D203V/A216P,
S32Q/L90Y/F92L/T109K/A125S/T136V,
Y60H/N87K/A114K/A117Q/D158E/A174K/A216P,
P20T/L90Y/T109K/T136A/D158I/D203R, T17L/Y60A/D203V,
N2R/S32Q/L90Y/F92Y/T109K/D203V,
T17H/A24V/S32Q/L90Y/F92L/S101H/A114K/D158I/A174R/D203V,
N2R/S32M/F92Y/T109K/S110R/I222L, 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,
F21W/L90F/F92G/S101A/A114V/A125S/T136V/Q142L/D203V/A216P,
P20T/F21W/S32K/F92G/A125S/V229I, 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/Parent, 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,
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S27L/R12K/V23L/L90F/F92L/A114K/T136A/D158E/D203V/I222L, S27L/L90F/F92G,
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S27L/T17L/V23L/A24V/L90Y/F92L/A117N/T136V/L137M/Q142L,
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S27L/T17S/V23T/A24V/S32Q/Y60H/N87K/T136A/D203R,
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S27L/L90Y/F92G/T136V/D158L/D203V,

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S27L/S32Q/F92G/Q142W/I222L, S27L/N2R/R12K/S32M/N87Q/L90F/F92L/I222L,
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S27L/T17G/F21W/S32K/L90F/F92G/A125S/T136A/A174K,
S27L/T17G/N87Y/T136A/D203V/A216P,
S27L/F21W/L90F/F92G/S101D/A117N/T136A/A174K/D203V,
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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,
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S27L/T17S/S22K/L90F/F92L/D158E/D203V/V219I/S252T,
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S27L/S1A/S98T/S113K/A114V/L119M/A127S/Q142W/S193K/V219K/S252T/R255L,
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S27L/N9S/Y60A/A216P/R236Q/R255M,
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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. In some embodiments, 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,

M56I/I82F/R108T/I139A/F161W/T194S/D203N/N241P, M56I/S88T/R108T/N190S/L227R,
N204A/Q237R, N9A/D18R/M56I/N85D/L119I/N254R,
N9A/S22A/G46S/M56I/R72P/F92Q/L119I/T221S/M225L, N9A/S22A/Q167T,
N9A/V40T/L49G/I54V/R108T/V150I/D203N/T221S/M225L, N9S/T25H,
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/I54V/R72P/F92Q/V150I/V200L,
S22A/L49G/M56I/V83T/S101L/R108T/L119I/A127M/T194S, S22I/Y26K,
S27T/I82L/P213N, S27T/T48S/I82L/F92Y/S252T,
S27T/T48S/I82L/L90F/A135G/S140A/I143N/T145S/P213N,
S27T/T48S/I82L/S140A/I143N/G149A, S32Y/A62T,
S88T/R108T/V150I/A184S/D203N/T221S/M225L/N243P, S98Q/A209V,
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/M56I/V150I/S218A/V219K,
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 embodiments, 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 embodiments, 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,

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, L90F/F92G/T109K/A125S/A174K,
S32K/L90F/F92L/T109K/A114K/Q142W,
V23T/S32Q/N87I/L90F/F92Y/S101N/A114V/Q142W/A174R/D203R/I222L/V229I,
S32Q/F92G/A114V/D203V, 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,
T17A/S32M/Y60H/Q142L/D203V/I222L,
R12K/Y60H/L90F/S101R/A114V/A117N/D158E,
T17S/D18R/L90Y/F92G/A114V/A117Q/D203R,
L90Y/F92L/S101A/S110R/A125S/Q142W/A174R/A216P, S32M/S110R/A125S/D158E,
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,
P20T/F21W/Y60I/S101A/T109K/A174K,
T17C/L90Y/F92L/S101N/T109L/S110R/A125S/A174K/D203R,
T17Q/L90F/F92G/S101M/A117Q/T136A/D158I/A174R,
V23L/L90F/F92G/A125S/D203V/I222L, T17C/L90Y/F92L/A125S/T136V/D158E/A174K,
V23L/S32Q/Y60I/A117Q/T136A/A174K/V229C, V23T/L90F/F92Y/D203V,
R12K/A24V/Y60H/A174R, T17G/P20T/Y60I/F92G/D203V,
L90Y/F92L/S110R/A125S/D158E/D203V,
T17Q/L90Y/F92L/T109L/T136A/D158E/A174R/D203V/V229C,
R12K/V23T/A24V/S32Q/F92G/D158L/A174R/D203R,
S32M/N87Y/L90F/F92G/A174K/D203V,
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,
F92G/A125S/T136A/D158E/A216P, N87R/F92G/A117N/D203V/A216P,
R12K/A14K/F92G/S110R/A117N/A125S/A174R/D203R,
S32Q/N87F/L90F/F92G/S101M/A114V/A117Q/D203V,
T17Q/A24V/N87M/F92G/A114K/A117N/D158E/D203R/I222L,
N2R/S32Q/L90F/F92G/Q142L/D203V,
A14K/S32Q/L90F/F92G/A114K/A117N/A174R/D203V/A216P/I222L,
R12K/P20T/D158E/D203R/I222L, N2R/A14K/T17A/L90F/F92G/S101H/A174R/D203V,
T17G/A24V/L90F/F92G/Q142W, S32K/L90Y/F92Y/S101A,
V23T/N87I/F92G/A125S/T136A/D158L/D203R, T17S/N87L/V229I,
A24T/L90F/F92L/A125S/T136A/D203V,
R12K/A14K/F92G/T109R/T136A/Q142L/D158E/A174K/D203R/I222L,
A24V/S32Q/Y60A/T136A, D18R/S32Q/N87H/L90Y/F92G/S101K/Q142L/D158I/A174R,
T17A/F21W/S32K/N87F/L90F/F92Y/S101Q/T136A/D203R,
A24V/L90F/F92G/A114V/A117N/T136V/Q142L/D203V/V229C,
T17Q/L90Y/F92G/S101N/T109L/S110R/T136V/Q142L/D203V/A216P,
S32Q/L90Y/F92L/T109K/A125S/T136V,
Y60H/N87K/A114K/A117Q/D158E/A174K/A216P,
P20T/L90Y/T109K/T136A/D158I/D203R, T17L/Y60A/D203V,
N2R/S32Q/L90Y/F92Y/T109K/D203V,
T17H/A24V/S32Q/L90Y/F92L/S101H/A114K/D158I/A174R/D203V,
N2R/S32M/F92Y/T109K/S110R/I222L, 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,
F21W/L90F/F92G/S101A/A114V/A125S/T136V/Q142L/D203V/A216P,
P20T/F21W/S32K/F92G/A125S/V229I, 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/Parent, 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,
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S27L/S13R/S98E/T136V/S181R/T206G/V229I/N231S,
S27L/F21W/F92L/S98N/S193P/I222L,
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S27L/N9E/R12K/S22P/V23T/T48S/S98E/R108S/T160S/Q189V/T206G/S212L/V229I,
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S27L/S1G/N9S/S22V/I82F/L90Y/F92L/A117N/L119M/Q142L/T206G/S212L/S223A,
S27L/N9S/Y60H/R108C/S193P/V219L,
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S27L/N9E/R12K/T48N/L90F/S98L/R108Q/A117Q/T136A/T206G/S212F/V219I/V229I/R25

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R255M, S27L/A24V/F92G/S101D/A114V/A117N/A125S/T136V/D203R,
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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.

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

[0008] In another aspect, 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 aspect, 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 embodiments, the mechanical pretreatment comprises grinding of the PET into particles. In some embodiments, the thermo-mechanical pretreatment comprises extruding the PET at a temperature configured to amorphize and reduce crystallinity of the PET. In some embodiments, 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 aspect, the present disclosure relates to a method of degrading PET, comprising contacting the PET with the variant Bhr-PETase enzyme described herein. In some embodiments, the method further comprises pretreating the PET according to the method described herein. In some embodiments, the method degrades the PET in a mixed plastics composition. In some embodiments, the plastics composition comprises analog of PET, PET-like or PET substitute derived biologically or chemically. In some embodiments, the plastics composition comprises at least one selected from the group consisting of Polybutylene terephthalate (PBT), Polycarbonate (PC), Polycaprolactone (PCL), Polyethylene Furanoate (PEF), and High Density Polyethylene (HDPE). In some embodiments, 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–AA 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.

[0021] Figures 11A–B depict the wild type sequences of Bhr-PETase, Lcc-PETase, and Is-PETase, as well as starting nucleic acids and codon optimized sequences for expression in Bacteria, Yeast and Fungi.

DETAILED DESCRIPTION OF THE INVENTION

A. Introduction.

[0022] The present invention is directed to enzymes that will hydrolyze polyethylene terephthalate (PET). PET is a polyester polymer created by the combination of two monomers: modified ethylene glycol and purified terephthalic acid. While plastics such as PET find literally thousands of uses in modern society, PET, is essentially non-degradable. As such, plastic pollution has contaminated the entire planet, which poses a number of significant issues for the planet and human health. PET can be recycled; however this still does not prevent major amounts of PET from being dumped into landfill and/or the ocean.

[0023] Plastics including PET are remarkably resistant to enzymatic degradation. There are two categories of PET hydrolases: (i) PET-modifying enzymes that limit the degradation only at the surface of PET without visible change by electron microscope observations and (ii) PET-degrading enzymes or PETases that can significantly degrade the inner block of PET (e.g., by at least 10%) with visible change by electron microscope observations. To date, a plethora of PET-modifying enzymes have been reported but they may not significantly degrade the body of PET and may not be applicable for biorecycling of PET. As is known in the art, there are a few enzyme types that show limited ability to degrade the inner block of PET. The first reported enzyme able to act on ester bonds of PET polymers was a cutinase from *Thermobifida fusca* in 2005. Subsequent work identified additional enzymes, including a PET-hydrolyzing enzyme from *Ideonella sakaiensis*, Is-PETase, in 2016 and a leaf branch compost cutinase (Lcc-PETase) in 2012. While these PETases show activity at ambient temperatures, these enzymes are not particularly thermostable and do not show robust PET degradation.

[0024] PET exists both as an amorphous and as a semi-crystalline material. Chain mobility may be increased in the amorphous phase around PET's glass transition temperature T_g (around 70°C), which allows better access to ester linkages and hence faster degradation.

The reaction temperature around T_g may be controlled to achieve efficient enzymatic PET degradation. In addition, the physical aging process of PET at around 70°C may convert the mobile amorphous fraction to recalcitrant microstructures which hinders further enzymatic hydrolysis of PET. Therefore, a thermostable and thermoactive PETase is desirable to allow the degradation reaction to occur at around glass transition temperature and overcome the competing physical aging process.

[0025] As shown in Figures 4, 5 and 6, although wild type Bhr-PETase is a close homolog of Lcc-PETase, sharing 94% sequence identity between the two enzymes, it is found to be more thermostable and thermoactive than Lcc-PETase, as well as Is-PETase,. Thermostability and thermostability are key factors for efficient PET degradation. The present disclosure relates to variants of a PETase from the bacterium HR29 (Bhr-PETase) that have been engineered to exhibit even greater activity and thermostability. The outstanding thermostability and high PET hydrolyzing thermoactivity of Bhr-PETase variants render them great potentials for further analysis and industrial applications.

B. Definitions

[0026] By "modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence or an alteration to a moiety chemically linked to a protein. For example, a modification may be an altered carbohydrate or PEG structure attached to a protein. By "amino acid modification" herein is meant an amino acid substitution, insertion, and/or deletion in a polypeptide sequence. For clarity, unless otherwise noted, the amino acid modification is always to an amino acid coded for by DNA, e.g. the 20 amino acids that have codons in DNA and RNA.

[0027] By "amino acid substitution" or "substitution" herein is meant the replacement of an amino acid at a particular position in a parent polypeptide sequence with a different amino acid. In particular, in some embodiments, the substitution is to an amino acid that is not naturally occurring at the particular position, either not naturally occurring within the organism or in any organism. For example, the substitution S27L refers to a variant polypeptide, in this case a PETase, in which the serine (S) at position 27 is replaced with leucine L. For clarity, a protein which has been engineered to change the nucleic acid coding sequence but not change the starting amino acid (for example exchanging CGG (encoding arginine) to CGA (still encoding arginine) to increase host organism expression levels) is not

an “amino acid substitution”; that is, despite the creation of a new gene encoding the same protein, if the protein has the same amino acid at the particular position that it started with, it is not an amino acid substitution.

[0028] By "amino acid insertion" or "insertion" as used herein is meant the addition of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, -233E or 233E designates an insertion of glutamic acid after position 233 and before position 234. Additionally, -233ADE or A233ADE designates an insertion of AlaAspGlu after position 233 and before position 234.

[0029] By "amino acid deletion" or "deletion" as used herein is meant the removal of an amino acid sequence at a particular position in a parent polypeptide sequence. For example, F250- or F250#, F250() or F250del designates a deletion of glutamic acid at position 250. Additionally, FRS250- or FRS250# designates a deletion of the sequence PheArgSer that begins at position 250.

[0030] By "parent polypeptide" as used herein is meant a starting polypeptide that is subsequently modified to generate a variant. The parent polypeptide may be a naturally occurring polypeptide, or a variant or engineered version of a naturally occurring polypeptide. Parent polypeptide may refer to the polypeptide itself, compositions that comprise the parent polypeptide, or the amino acid sequence that encodes it. In the present case, some embodiments utilize an exemplary wild-type Bhr-PETase (also called G1P Bhr-PETase; SEQ ID NO: 1; sequence shown in Figure 1) as the parent polypeptide.

[0031] By "variant protein" or "protein variant", or "variant" as used herein is meant a protein that differs from that of a parent protein by virtue of at least one amino acid modification. Protein variant may refer to the protein itself, a composition comprising the protein, or the amino sequence that encodes it. Preferably, the protein variant has at least one amino acid modification compared to the parent protein, e.g. from about one to about seventy amino acid modifications, and preferably from about one to about five amino acid modifications compared to the parent. As described below, in some embodiments the parent polypeptide is a wild type sequence, for example the exemplary wild type Bhr-PETase designated “G1P” herein. As further discussed below, the protein variant sequence herein will preferably possess at least about 80, 81, 82, 83, 84, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity with a parent protein sequence, preferably at least about 90% identity, and preferably at least about 95-98-99% identity. Variant protein can refer to the

variant protein itself, compositions comprising the protein variant, or the DNA sequence that encodes it. Thus, by “variant PETase” herein is meant a novel PETase that has at least one amino acid modification in the amino acid sequence as compared to a parent PETase enzyme. Unless otherwise noted or as will be obvious from the context, the variant PETases of the invention generally are compared to the wild type GIP sequence. Additionally, unless otherwise noted, the variant PETases of the invention are enzymatically active, that is, there is detectable PETase activity using the PETase assay described in Example 9.

[0032] As used herein, “protein” herein is meant at least two covalently attached amino acids, which includes proteins, polypeptides, oligopeptides and peptides. The peptidyl group generally comprise naturally occurring amino acids and peptide bonds. In addition, polypeptides may include synthetic derivatization of one or more side chains or termini, glycosylation, PEGylation, circular permutation, cyclization, linkers to other molecules, fusion to proteins or protein domains, and addition of peptide tags or labels.

[0033] By “residue” as used herein is meant a position in a protein and its associated amino acid identity. For example, Serine 27 (also referred to as Ser27 or S27) is a residue at position 27 in the GIP parental enzyme.

[0034] By “non-naturally occurring modification” as used herein is meant an amino acid modification that is not found in the parent (e.g. GIP) enzyme in nature.

[0035] By “amino acid” and “amino acid identity” as used herein is meant one of the 20 naturally occurring amino acids that are coded for by DNA and RNA.

[0036] By “position” as used herein is meant a location in the sequence of a protein. In general, the position number (which is more fully discussed below) is relative to the first amino acid of the mature PETase sequence, e.g. excluding the signal peptide.

[0037] By “PETase” herein is meant a protein with PETase activity. By “PETase activity” herein is meant that in the absence of MHTase, the enzyme catalyzes the hydrolysis of PET to mono(hydroxyethyl)terephthalate (MHET) as the major product. In the presence of MHTase, which is the case of Example 9, MHTase will further convert MHET to terephthalic acid (TPA) and ethylene glycol (EG) as the major products of the enzymatic reaction. Enzymes having detectable PETase activity in the assay outlined below and in Example 9 are considered PETases herein. The PETase activity may be measured as PETase total activity and/or PETase thermostability as described herein.

[0038] By "identity" in reference to two sequences herein is meant that the same amino acid is at the same position considering the alignment. The degree of identity between an amino acid sequence of the present invention ("invention sequence") and the parent amino acid sequence referred to in the claims (e.g. for G1P, SEQ ID NO:1) is calculated as the number of exact matches in an alignment of the two sequences, divided by the length of the "invention sequence," or the length of the SEQ ID NO:1, whichever is the shortest. The result is expressed in percent identity as calculated below.

[0039] For purposes of the present invention, the mature polypeptide disclosed in SEQ ID NO:1 is used to determine the corresponding amino acid residue in another PETase of the present invention. The amino acid sequence of another PETase is aligned with the mature polypeptide disclosed in SEQ ID NO:1, and based on the alignment, the amino acid position number corresponding to any amino acid residue in the mature polypeptide disclosed in SEQ ID NO:1 is determined using the Needleman-Wunsch algorithm (Needleman and Wunsch, 1970, J. Mol. Biol. 48: 443-453) as implemented in the Needle program of the EMBOSS package (EMBOSS: The European Molecular Biology Open Software Suite, Rice et al., 2000, Trends Genet. 16: 276-277), preferably version 5.0.0 or later. The parameters used are gap open penalty of 10, gap extension penalty of 0.5, and the EBLOSUM62 (EMBOSS version of BLOSUM62) substitution matrix.

[0040] Identification of the corresponding amino acid residue in another PETase can be determined by an alignment of multiple polypeptide sequences using several computer programs including, but not limited to, MUSCLE (multiple sequence comparison by log-expectation; version 3.5 or later; Edgar, 2004, Nucleic Acids Research 32: 1792-1797), MAFFT (version 6.857 or later; Katoh and Kuma, 2002, Nucleic Acids Research 30: 3059-3066; Katoh et al. , 2005, Nucleic Acids Research 33: 511-518; Katoh and Toh, 2007, Bioinformatics 23: 372-374; Katoh et al., 2009, Methods in Molecular Biology 537: 39-64; Katoh and Toh, 2010, Bioinformatics 26: 1899-1900), EMBOSS EMMA employing ClustalW (1.83 or later; Thompson et al., 1994, Nucleic Acids Research 22: 4673-4680), and EMBL-EBI employing Clustal Omega (Sievers and Higgins, 2014, Methods Mol Biol. 2014;1079:105-16), using their respective default parameters.

[0041] When the other enzyme has diverged from the polypeptide of SEQ ID NO:1 such that traditional sequence-based comparison fails to detect their relationship (Lindahl and Elofsson, 2000, J. Mol. Biol. 295: 613-615), other pairwise sequence comparison algorithms can be used. Greater sensitivity in sequence-based searching can be attained using search

programs that utilize probabilistic representations of polypeptide families (profiles) to search databases. For example, the PSI-BLAST program generates profiles through an iterative database search process and is capable of detecting remote homologs (Atschul et al., 1997, *Nucleic Acids Res.* 25: 3389-3402). Even greater sensitivity can be achieved if the family or superfamily for the polypeptide has one or more representatives in the protein structure databases. Programs such as GenTHREADER (Jones, 1999, *J. Mol. Biol.* 287: 797-815; McGuffin and Jones, 2003, *Bioinformatics* 19: 874-881) utilize information from a variety of sources (PSI-BLAST, secondary structure prediction, structural alignment profiles, and solvation potentials) as input to a neural network that predicts the structural fold for a query sequence. Similarly, the method of Gough et al., 2000, *J. Mol. Biol.* 313: 903-919, can be used to align a sequence of unknown structure with the superfamily models present in the SCOP database. These alignments can in turn be used to generate homology models for the polypeptide, and such models can be assessed for accuracy using a variety of tools developed for that purpose.

[0042] For proteins of known structure, several tools and resources are available for retrieving and generating structural alignments. For example the SCOP superfamilies of proteins have been structurally aligned, and those alignments are accessible and downloadable. Two or more protein structures can be aligned using a variety of algorithms such as the distance alignment matrix (Holm and Sander, 1998, *Proteins* 33: 88-96) or combinatorial extension (Shindyalov and Bourne, 1998, *Protein Engineering* 11 : 739-747), and implementation of these algorithms can additionally be utilized to query structure databases with a structure of interest in order to discover possible structural homologs (e.g., Holm and Park, 2000, *Bioinformatics* 16: 566-567).

[0043] In describing the variants of the present invention, the nomenclature described below is adapted for ease of reference. The standardly accepted IUPAC single letter or three letter amino acid abbreviation is employed.

[0044] For an amino acid substitution, the following nomenclature is used herein: Original amino acid, position, substituted amino acid. Accordingly, the substitution of serine at position 27 with leucine is designated as “Ser27Leu” or “S27L”. Multiple mutations are separated by forward slash marks (“/”), e.g., “A102V/T136M”, representing substitutions at positions 102 and 136, respectively.

Abbreviation	1 letter	Amino acid	Abbreviation	1 letter	Amino acid name
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Ala	A	Alanine	Leu	L	Leucine
Arg	R	Arginine	Lys	K	Lysine
Asn	N	Asparagine	Met	M	Methionine
Asp	D	Aspartic acid	Phe	F	Phenylalanine
Cys	C	Cysteine	Pro	P	Proline
Gln	Q	Glutamine	Ser	S	Serine
Glu	E	Glutamic acid	Thr	T	Threonine
Gly	G	Glycine	Trp	W	Tryptophan
His	H	Histidine	Tyr	Y	Tyrosine
Ile	I	Isoleucine	Val	V	Valine

[0045] By “isolated” in the context of a PETase herein is meant that the polypeptide is devoid of other proteins. In a particular embodiment the PETase of the invention is isolated. The term “isolated” as used herein refers to a polypeptide which is at least 20% pure, preferably at least 40% pure, more preferably at least 60% pure, even more preferably at least 80% pure, most preferably at least 90% pure, and even most preferably at least 95 to 98% pure, as determined by SDS-PAGE. In particular, it is preferred that the polypeptides are in “essentially pure form”, i.e., that the polypeptide preparation is essentially free of other polypeptide material with which it is natively associated. This can be accomplished, for example, by preparing the polypeptide by means of well-known recombinant methods or by classical purification methods.

[0046] By “recombinant enzyme” herein is meant that the enzyme is produced by recombinant techniques and that nucleic acid encoding the enzyme of the invention is operably linked to at least one exogenous (e.g. not native to the parent PETase) sequence, including, for examples, promoters, terminators, signal sequences, etc., as are more fully outlined below.

[0047] The term “nucleic acid construct” refers to a nucleic acid molecule, either single-stranded or double-stranded, which is isolated from a naturally occurring gene or is modified to contain segments of nucleic acids in a manner that would not otherwise exist in nature or which is synthetic, and which comprises one or more control sequences.

[0048] The term “operably linked” refers to a configuration in which a control sequence is placed at an appropriate position relative to the coding sequence of a polynucleotide such that the control sequence directs expression of the coding sequence.

[0049] As used herein, the term “about” means modifying, for example, lengths of nucleotide sequences, degrees of errors, dimensions, the quantity of an ingredient in a composition, concentrations, volumes, process temperature, process time, yields, flow rates, pressures, and like values, and ranges thereof, refers to variation in the numerical quantity that may occur, for example, through typical measuring and handling procedures used for making compounds, compositions, concentrates or use formulations; through inadvertent error in these procedures; through differences in the manufacture, source, or purity of starting materials or ingredients used to carry out the methods; and like considerations. The term “about” also encompasses amounts that differ due to aging of, for example, a composition, formulation, or cell culture with a particular initial concentration or mixture, and amounts that differ due to mixing or processing a composition or formulation with a particular initial concentration or mixture. Whether modified by the term “about” the claims appended hereto include equivalents to these quantities. The term “about” further may refer to a range of values that are similar to the stated reference value. In certain embodiments, the term “about” refers to a range of values that fall within 10, 9, 8, 7, 6, 5, 4, 3, 2, 1 percent or less of the stated reference value.

C. PETases of the Invention

[0050] Accordingly, the present invention provides variant PETases with improved enzymatic activity that can be used in a variety of applications, most notably in the degradation of plastics made from PET.

[0051] In general, the variant PETases of the invention have modified, improved biochemical properties as compared to the wild type Bhr-PETase, “G1P” (i.e. “Generation 1 Parent”), SEQ ID NO:1 herein, as shown in Figure 1. The variant PETases of the invention can also have modified, improved biochemical properties as compared to the “G2P” (i.e. “Generation 2 Parent”), which has amino acid substitution S27L. The biochemical properties of the variant PETases that can be improved herein include, but are not limited to, thermostability, thermoactivity, specific activity, and production.

[0052] The variant Bhr-PETases of the invention have one or more improved properties as compared to G1P or G2P. By “improved” herein is meant a desirable change of at least one biochemical property. “Improved function” can be measured as a percentage increase or decrease of a particular activity, or as a “fold” change, with increases of desirable properties

(e.g. activity or thermostability). That is, a variant Bhr-PETase may have a 10% increase in thermostability or a 10% increase in PETase activity, as compared to G1P or G2P. In general, percentage changes are used to describe changes in biochemical activity of less than 100%, and fold-changes are used to describe changes in biochemical activity of greater than 100% (as compared to the parental enzyme, in many cases G1P or G2P). In the present invention, percentage changes (usually increases) of biochemical activity of at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98% and 99% can be accomplished. In the present invention, a “fold increase” (or decrease) is measured as compared to the starting or parent enzyme. For example, as shown in the Figures 9A-AA, the variant T17A/S27T/T48S/I82L/L90F/Q167V/P213N/S252T has 1.6 fold increase in specific activity as compared to G1P: this is calculated by [(activity of variant)/(activity of parent)]. In many embodiments, the improvement is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0053] In general, improvements are measured as compared to the G1P or G2P enzyme using a Bhr-PETase activity assay, under conditions that challenge the variant Bhr-PETase against the G1P or G2P enzyme.

1. Total Activity Increases

[0054] The present invention provides variant Bhr-PETases that have total activity equal to or greater than the total activity of G1P (the wild type Bhr-PETase of SEQ ID NO:1) or G2P (G1P with amino acid substitution S27L). The “total activity” herein may be determined by monitoring the production of TPA (terephthalic acid) during the PET depolymerization reaction at an elevated temperature such as 65°C, quantified using a colorimetric assay or HPLC as described in Example 9. Any improvement in total activity may be due to the improvement of thermoactivity, specific activity, and/or production of the variant PETase.

[0055] In many embodiments, the variant Bhr-PETases have improved total activity that is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0056] In general, improvements are measured as compared to the G1P or G2P enzyme using a Bhr-PETase activity assay, under conditions that challenge the variant Bhr-PETase against the G1P or G2P enzyme.

(i) Thermoactivity Increases

[0057] In one aspect, the variant Bhr-PETases may have increased thermoactivity. The “thermoactivity” herein may be determined by monitoring the production of TPA (terephthalic acid), MHET (mono(hydroxyethyl)terephthalate) and BHET (bis(2-hydroxyl)terephthalate) during the PET depolymerization reaction at an elevated temperature such as 65°C, quantified in mg of equivalent TPA generated per h per mg of enzyme ($\text{mg}_{\text{TPAeq}} \cdot \text{h}^{-1} \text{mg}_{\text{enzyme}}^{-1}$). Herein “Equivalent TPA” is calculated by a sum of TPA, MHET converted to TPA, BHET converted to TPA, and any other measurable oligomers converted to TPA. Thus, PETases that have increased activity per milligram of enzyme as compared to G1P (the wild type Bhr-PETase of SEQ ID NO:1) or G2P (G1P with amino acid substitution S27L) may show an improved thermoactivity.

[0058] In many embodiments, the variant Bhr-PETases have improved thermoactivity that is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0059] In general, improvements are measured as compared to the G1P or G2P enzyme using a Bhr-PETase activity assay, under conditions that challenge the variant Bhr-PETase against the G1P or G2P enzyme.

(ii) Specific Activity Increases

[0060] In another aspect, the variant Bhr-PETases may have increased specific activity. The “specific activity” herein may be determined by monitoring the production of TPA (terephthalic acid), MHET (mono(hydroxyethyl)terephthalate) and BHET (bis(2-hydroxyl)terephthalate) during the PET depolymerization reaction at the optimal operating temperature of Bhr-PETases, quantified in mg of equivalent TPA generated per h per mg of enzyme ($\text{mg}_{\text{TPAeq}} \cdot \text{h}^{-1} \text{mg}_{\text{enzyme}}^{-1}$). Thus, PETases that have increased activity per milligram of enzyme as compared to G1P (the wild type Bhr-PETase of SEQ ID NO:1) or G2P (G1P with amino acid substitution S27L) may show an improved specific activity.

[0061] In many embodiments, the variant Bhr-PETases have improved specific activity that is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher. In some embodiments, the variant Bhr-PETases have increased equivalent TPA generated per h per mg of enzyme that is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0062] In general, improvements are measured as compared to the G1P or G2P enzyme, under conditions that challenge the variant Bhr-PETase against the G1P or G2P enzyme.

(iii) Production Increases

[0063] In one aspect, the variant Bhr-PETases may have increased production. The “production” herein may be determined by monitoring the protein titer of Bhr-PETase in g/L. Thus, PETases that have increased quantity per liter of enzyme supernatant as compared to G1P (the wild type Bhr-PETase of SEQ ID NO:1) or G2P (G1P with amino acid substitution S27L) may show an improved production.

[0064] In many embodiments, the variant Bhr-PETases have improved thermoactivity that is at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0065] In general, improvements are measured as compared to the G1P or G2P enzyme using a Bhr-PETase activity assay, under conditions that challenge the variant Bhr-PETase against the G1P or G2P enzyme.

2. Thermostability

[0066] Additionally, as will be appreciated by those in the art, it can be desirable to run PET degradation at around the glass transition temperature. Amorphous PET domains will increase the mobility at around the glass transition temperature (around 67-72°C), making it more accessible for enzyme hydrolysis. At higher temperatures above the transition temperature, the PET substrate will re-crystallize over time, which is not favorable for PET degradation. Therefore, for example, about 65-72°C may be considered an optimal temperature range for PET degradation.

[0067] Accordingly, in many embodiments, the variant Bhr-PETases have improved thermostability. “Thermostability” in this context means that the variant enzymes are more stable than G1P (the wild type Bhr-PETase of SEQ ID NO:1) or G2P (G1P with amino acid substitution S27L) under the same thermal challenge conditions, that is, the activity of the variant is higher than that of the G1P or G2P enzyme under identical conditions (generally using an assay as outlined herein and as shown in Example 9).

[0068] In one embodiment, the variant Bhr-PETases are more stable than the G1P or G2P enzyme when exposed to temperatures of about 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84 and/or 85°C for a period of time, for example ranging from

about 0.5, 1, 2, 3, 4, 5, or 6 hour to about 5, 6, 7, 8, 9, 10 hours or longer, depending on the ultimate conditions for the use of the variant Bhr-PETase. In some embodiments, the variant Bhr-PETases are more stable than the G1P or G2P enzyme when exposed to temperatures preferably from about 65°C to 85°C for at least about 0.5 hour, preferably from about 65°C to 72°C for at least about 1 hour, preferably at least 65°C for at least about 1 hour, preferably at least 70°C for at least about 1.5 hour.

[0069] Accordingly, in many embodiments, the variant Bhr-PETases are more thermostable than the G1P or G2P enzyme by at least 1.1 fold, 1.2 fold, 1.3 fold, 1.4 fold, 1.5 fold, 1.6 fold, 1.7 fold, 1.8 fold, 1.9 fold, 2 fold, 3 fold, 4 fold, 5 fold, 6 fold, 7 fold, 8 fold, 9 fold, or 10 fold or higher.

[0070] In general, improvements are measured as compared to the G1P or G2P enzyme using a Bhr-PETase activity assay, under conditions that measure the variant Bhr-PETase or the G1P or G2P enzyme activity with and without thermal treatment.

3. PETase Assays

[0071] There are several PETase activity assays that can be used to determine activity, as generally outlined in Examples 6 and 9 for PET film-based assay, as well as in Example 5 for BHET-based assay. PETase activity can also be monitored by pNPB (p-Nitrophenyl Butyrate) assay. In pNPB assay, surrogate substrate p-nitrophenylbutyrate may be hydrolyzed by PETase to p-nitrophenol and butyric acid. The release of p-nitrophenol directly correlates to PETase activity and can be determined spectrophotometrically at 405 nm, for example.

4. Bhr-PETases

[0072] The present invention provides a number of specific variant Bhr-PETases with improved activity and/or thermostability for use in the degradation of PET.

[0073] In some embodiments, the variant Bhr-PETase has one or more amino acid substitutions at a position (relative to G1P, SEQ ID NO:1) 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. In some embodiments, the variant Bhr-PETase has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions at a position (relative to G1P, SEQ ID NO:1) selected from the same group.

[0074] In some embodiments, the variant Bhr-PETase has one more amino acid substitutions selected from the group consisting of S27L, S27F, S27H, S27T, S27W, S1A, S1G, S1M, S1R, N2E, N2F, N2L, N2R, N2S, Q5E, N9A, N9E, N9S, R12K, S13L, S13R, A14K, A14S, L15I, T16E, T17A, T17C, T17G, T17H, T17I, T17K, T17L, T17M, T17N, T17Q, T17R, T17S, D18R, P20D, P20E, P20I, P20Q, P20T, F21W, F21Y, S22A, S22I, S22K, S22P, S22R, S22V, V23L, V23T, A24D, A24G, A24H, A24N, A24S, A24T, A24V, T25A, T25F, T25H, T25Q, T25R, T25V, Y26C, Y26K, Y26L, Y26T, R30K, S32K, S32M, S32Q, S32Y, V33G, V33Q, V33T, S34R, V40T, G46E, G46L, G46N, G46R, G46S, T48N, T48S, L49G, G53A, I54V, A55C, A55I, A55L, A55M, A55T, A55V, M56I, M56L, S57C, S57E, S57F, S57I, S57L, S57M, S57T, S57V, Y60A, Y60H, Y60I, A62T, A68F, L70M, R72P, L74W, H77Q, I82F, I82L, I82M, V83I, V83L, V83T, N85D, N87F, N87H, N87I, N87K, N87L, N87M, N87Q, N87R, N87V, N87W, N87Y, S88K, S88T, L90F, L90K, L90Y, F92G, F92I, F92K, F92L, F92N, F92Q, F92V, F92Y, A97C, A97E, A97F, A97G, A97L, A97P, A97Q, A97S, A97T, A97V, S98A, S98D, S98E, S98L, S98M, S98N, S98Q, S98T, S98V, S101A, S101C, S101D, S101F, S101H, S101K, S101L, S101M, S101N, S101Q, S101R, S101V, S101W, S101Y, A102V, N105D, R108C, R108E, R108H, R108K, R108N, R108P, R108Q, R108S, R108T, R108V, T109A, T109F, T109G, T109K, T109L, T109N, T109R, T109Y, S110D, S110G, S110H, S110K, S110N, S110R, S113A, S113K, S113N, S113P, S113Q, S113R, S113T, S113Y, A114K, A114L, A114S, A114V, A117F, A117G, A117L, A117N, A117Q, A117S, A117T, A117Y, L119I, L119M, A121S, N122A, N122E, N122H, N122P, N122R, N122S, A125S, A127M, A127S, A127V, A135G, T136A, T136M, T136S, T136V, R138E, R138L, I139A, I139T, S140A, Q142D, Q142E, Q142H, Q142L, Q142W, I143N, I143R, T145S, K147F, K147G, K147N, K147Q, G149A, G149C, G149D, G149N, G149S, G149T, G149V, V150I, V150L, T153L, H156N, T157A, T157G, D158E, D158I, D158K, D158L, T160K, T160Q, T160R, T160S, T160V, F161V, F161W, N162E, N162H, N162P, N162R, T163I, T163S, P164E, P164H, P164N, P164R, P164S, P164T, Q167I, Q167T, Q167V, V170L, E173R, A174K, A174R, V177A, P179Q, S181A, S181C, S181R, Q182T, A184C, A184G, A184S, I185A, I185E, I185G, I185L, I185Q, I185R, I185S, 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, A216T, A216V, I217S, S218A, V219F, V219I, V219K, V219L, V219R, T221S, I222L, S223A, S223C, M225L, L227R, W228F, V229C, V229I, V229L, N231L, N231Q, N231S, R236C, R236E, R236H, R236K, R236Q, Q237R, N241P, V242T, N243P, A246D, A246K, A246S, A246T, D249I, D249M, D249N, D249S, D249T, F250I, F250L, F250V, F250Y, R251A, 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 embodiments, the variant Bhr-PETase has 1, 2, 3, 4, 5, 6, 7, 8, 9, 10 or more amino acid substitutions selected from the same group.

[0075] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 27 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S27L. In some embodiments, the amino acid substitution is S27F. In some embodiments, the amino acid substitution is S27H. In some embodiments, the amino acid substitution is S27T. In some embodiments, the amino acid substitution is S27W.

[0076] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 1 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S1A. In some embodiments, the amino acid substitution is S1G. In some embodiments, the amino acid substitution is S1M. In some embodiments, the amino acid substitution is S1R.

[0077] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 2 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N2E. In some embodiments, the amino acid substitution is N2F. In some embodiments, the amino acid substitution is N2L. In some embodiments, the amino acid substitution is N2R. In some embodiments, the amino acid substitution is N2S.

[0078] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 5 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Q5E.

[0079] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 9 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N9A. In some embodiments, the amino acid substitution is N9E. In some embodiments, the amino acid substitution is N9S.

[0080] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 12 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is R12K.

[0081] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 13 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S13L. In some embodiments, the amino acid substitution is S13R.

[0082] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 14 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A14K. In some embodiments, the amino acid substitution is A14S.

[0083] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 15 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L15I.

[0084] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 16 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T16E.

[0085] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 17 of SEQ ID NO:1. In some embodiments, the substitution is with any

other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is T17A. In some embodiments, the amino acid substitution is T17C. In some embodiments, the amino acid substitution is T17G. In some embodiments, the amino acid substitution is T17H. In some embodiments, the amino acid substitution is T17I. In some embodiments, the amino acid substitution is T17K. In some embodiments, the amino acid substitution is T17L. In some embodiments, the amino acid substitution is T17M. In some embodiments, the amino acid substitution is T17N. In some embodiments, the amino acid substitution is T17Q. In some embodiments, the amino acid substitution is T17R. In some embodiments, the amino acid substitution is T17S.

[0086] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the aspartic acid at position 18 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is D18R.

[0087] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the proline at position 20 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P20D. In some embodiments, the amino acid substitution is P20E. In some embodiments, the amino acid substitution is P20I. In some embodiments, the amino acid substitution is P20Q. In some embodiments, the amino acid substitution is P20T.

[0088] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the phenylalanine at position 21 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline,

alanine, isoleucine, leucine, methionine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is F21W. In some embodiments, the amino acid substitution is F21Y.

[0089] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 22 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is S22A. In some embodiments, the amino acid substitution is S22I. In some embodiments, the amino acid substitution is S22K. In some embodiments, the amino acid substitution is S22P. In some embodiments, the amino acid substitution is S22R. In some embodiments, the amino acid substitution is S22V.

[0090] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 23 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V23L. In some embodiments, the amino acid substitution is V23T.

[0091] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 24 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A24D. In some embodiments, the amino acid substitution is A24G. In some embodiments, the amino acid substitution is A24H. In some embodiments, the amino acid substitution is A24N. In some embodiments, the amino acid substitution is A24S. In some embodiments, the amino acid substitution is A24T. In some embodiments, the amino acid substitution is A24V.

[0092] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 25 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, lysine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T25A. In some embodiments, the amino acid substitution is T25F. In some embodiments, the amino acid substitution is T25H. In some embodiments, the amino acid substitution is T25Q. In some embodiments, the amino acid substitution is T25R. In some embodiments, the amino acid substitution is T25V.

[0093] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the tyrosine at position 26 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and valine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is Y26C. In some embodiments, the amino acid substitution is Y26K. In some embodiments, the amino acid substitution is Y26L. In some embodiments, the amino acid substitution is Y26T.

[0094] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 30 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is R30K.

[0095] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 32 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to

steric effects). In some embodiments, the amino acid substitution is S32K. In some embodiments, the amino acid substitution is S32M. In some embodiments, the amino acid substitution is S32Q. In some embodiments, the amino acid substitution is S32Y.

[0096] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 33 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V33G. In some embodiments, the amino acid substitution is V33Q. In some embodiments, the amino acid substitution is V33T.

[0097] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 34 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S34R.

[0098] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 40 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V40T.

[0099] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glycine at position 46 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to

steric effects). In some embodiments, the amino acid substitution is G46E. In some embodiments, the amino acid substitution is G46L. In some embodiments, the amino acid substitution is G46N. In some embodiments, the amino acid substitution is G46R. In some embodiments, the amino acid substitution is G46S.

[0100] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 48 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T48N. In some embodiments, the amino acid substitution is T48S.

[0101] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 49 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L49G.

[0102] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glycine at position 53 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is G53A.

[0103] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 54 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some

embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I54V.

[0104] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 55 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is A55C. In some embodiments, the amino acid substitution is A55I. In some embodiments, the amino acid substitution is A55L. In some embodiments, the amino acid substitution is A55M. In some embodiments, the amino acid substitution is A55T. In some embodiments, the amino acid substitution is A55V.

[0105] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the methionine at position 56 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is M56I. In some embodiments, the amino acid substitution is M56L.

[0106] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 57 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is S57C. In some embodiments, the amino acid substitution is S57E. In some embodiments, the amino acid substitution is S57F. In some embodiments, the amino acid substitution is S57I. In some embodiments, the amino acid substitution is S57L. In some embodiments, the amino acid substitution is S57M. In some embodiments, the amino acid substitution is S57T. In some embodiments, the amino acid substitution is S57V.

[0107] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the tyrosine at position 60 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and valine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Y60A. In some embodiments, the amino acid substitution is Y60H. In some embodiments, the amino acid substitution is Y60I.

[0108] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 62 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A62T.

[0109] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 68 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A68F.

[0110] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 70 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L70M.

[0111] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 72 of SEQ ID NO:1. In some embodiments, the substitution is with any

other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is R72P.

[0112] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 74 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L74W.

[0113] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the histidine at position 77 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is H77Q.

[0114] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 82 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I82F. In some embodiments, the amino acid substitution is I82L. In some embodiments, the amino acid substitution is I82M.

[0115] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 83 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline,

alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V83I. In some embodiments, the amino acid substitution is V83L. In some embodiments, the amino acid substitution is V83T.

[0116] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 85 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N85D.

[0117] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 87 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N87F. In some embodiments, the amino acid substitution is N87H. In some embodiments, the amino acid substitution is N87I. In some embodiments, the amino acid substitution is N87K. In some embodiments, the amino acid substitution is N87L. In some embodiments, the amino acid substitution is N87M. In some embodiments, the amino acid substitution is N87Q. In some embodiments, the amino acid substitution is N87R. In some embodiments, the amino acid substitution is N87V. In some embodiments, the amino acid substitution is N87W. In some embodiments, the amino acid substitution is N87Y.

[0118] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 88 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to

steric effects). In some embodiments, the amino acid substitution is S88K. In some embodiments, the amino acid substitution is S88T.

[0119] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 90 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L90F. In some embodiments, the amino acid substitution is L90K. In some embodiments, the amino acid substitution is L90Y.

[0120] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the phenylalanine at position 92 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is F92G. In some embodiments, the amino acid substitution is F92I. In some embodiments, the amino acid substitution is F92K. In some embodiments, the amino acid substitution is F92L. In some embodiments, the amino acid substitution is F92N. In some embodiments, the amino acid substitution is F92Q. In some embodiments, the amino acid substitution is F92V. In some embodiments, the amino acid substitution is F92Y.

[0121] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 97 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine. In some embodiments, the amino acid substitution is A97C. In some embodiments, the amino acid substitution is A97E. In some embodiments, the amino acid substitution is A97F. In some embodiments, the amino acid substitution is A97G. In some embodiments, the amino acid substitution is A97L. In some embodiments, the amino acid substitution is A97P. In some embodiments, the amino acid substitution is A97Q. In some embodiments, the amino acid

substitution is A97S. In some embodiments, the amino acid substitution is A97T. In some embodiments, the amino acid substitution is A97V.

[0122] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 98 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S98A. In some embodiments, the amino acid substitution is S98D. In some embodiments, the amino acid substitution is S98E. In some embodiments, the amino acid substitution is S98L. In some embodiments, the amino acid substitution is S98M. In some embodiments, the amino acid substitution is S98N. In some embodiments, the amino acid substitution is S98Q. In some embodiments, the amino acid substitution is S98T. In some embodiments, the amino acid substitution is S98V.

[0123] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 101 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is S101A. In some embodiments, the amino acid substitution is S101C. In some embodiments, the amino acid substitution is S101D. In some embodiments, the amino acid substitution is S101F. In some embodiments, the amino acid substitution is S101H. In some embodiments, the amino acid substitution is S101K. In some embodiments, the amino acid substitution is S101L. In some embodiments, the amino acid substitution is S101M. In some embodiments, the amino acid substitution is S101N. In some embodiments, the amino acid substitution is S101Q. In some embodiments, the amino acid substitution is S101R. In some embodiments, the amino acid substitution is S101V. In some embodiments, the amino acid substitution is S101W. In some embodiments, the amino acid substitution is S101Y.

[0124] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 102 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine,

asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A102V.

[0125] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 105 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N105D.

[0126] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 108 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine. In some embodiments, the amino acid substitution is R108C. In some embodiments, the amino acid substitution is R108E. In some embodiments, the amino acid substitution is R108H. In some embodiments, the amino acid substitution is R108K. In some embodiments, the amino acid substitution is R108N. In some embodiments, the amino acid substitution is R108P. In some embodiments, the amino acid substitution is R108Q. In some embodiments, the amino acid substitution is R108S. In some embodiments, the amino acid substitution is R108T. In some embodiments, the amino acid substitution is R108V.

[0127] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 109 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T109A. In some embodiments, the amino acid substitution is T109F. In some embodiments, the amino acid substitution is T109G. In some embodiments, the amino acid substitution is T109K. In some embodiments, the amino acid substitution is T109L. In some embodiments, the amino acid

substitution is T109N. In some embodiments, the amino acid substitution is T109R. In some embodiments, the amino acid substitution is T109Y.

[0128] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 110 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S110D. In some embodiments, the amino acid substitution is S110G. In some embodiments, the amino acid substitution is S110H. In some embodiments, the amino acid substitution is S110K. In some embodiments, the amino acid substitution is S110N. In some embodiments, the amino acid substitution is S110R.

[0129] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 113 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is S113A. In some embodiments, the amino acid substitution is S113K. In some embodiments, the amino acid substitution is S113N. In some embodiments, the amino acid substitution is S113P. In some embodiments, the amino acid substitution is S113Q. In some embodiments, the amino acid substitution is S113R, S113T. In some embodiments, the amino acid substitution is S113Y.

[0130] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 114 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A114K. In some embodiments, the amino acid substitution is A114L. In some embodiments, the amino acid substitution is A114S. In some embodiments, the amino acid substitution is A114V.

[0131] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 117 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A117F. In some embodiments, the amino acid substitution is A117G. In some embodiments, the amino acid substitution is A117L. In some embodiments, the amino acid substitution is A117N. In some embodiments, the amino acid substitution is A117Q. In some embodiments, the amino acid substitution is A117S. In some embodiments, the amino acid substitution is A117T. In some embodiments, the amino acid substitution is A117Y.

[0132] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 119 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L119I. In some embodiments, the amino acid substitution is L119M.

[0133] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 121 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A121S.

[0134] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 122 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some

embodiments, the amino acid substitution is N122A. In some embodiments, the amino acid substitution is N122E. In some embodiments, the amino acid substitution is N122H. In some embodiments, the amino acid substitution is N122P. In some embodiments, the amino acid substitution is N122R. In some embodiments, the amino acid substitution is N122S.

[0135] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 125 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A125S.

[0136] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 127 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A127M. In some embodiments, the amino acid substitution is A127S. In some embodiments, the amino acid substitution is A127V.

[0137] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 135 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A135G.

[0138] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 136 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some

embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T136A. In some embodiments, the amino acid substitution is T136M. In some embodiments, the amino acid substitution is T136S. In some embodiments, the amino acid substitution is T136V.

[0139] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 138 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is R138E. In some embodiments, the amino acid substitution is R138L.

[0140] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 139 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I139A. In some embodiments, the amino acid substitution is I139T.

[0141] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 140 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S140A.

[0142] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 142 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some

embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Q142D. In some embodiments, the amino acid substitution is Q142E. In some embodiments, the amino acid substitution is Q142H. In some embodiments, the amino acid substitution is Q142L. In some embodiments, the amino acid substitution is Q142W.

[0143] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 143 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I143N. In some embodiments, the amino acid substitution is I143R.

[0144] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 145 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T145S.

[0145] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the lysine at position 147 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is K147F. In some embodiments, the amino acid substitution is K147G. In some embodiments, the amino acid substitution is K147N. In some embodiments, the amino acid substitution is K147Q.

[0146] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glycine at position 149 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine,

asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is G149A. In some embodiments, the amino acid substitution is G149C. In some embodiments, the amino acid substitution is G149D. In some embodiments, the amino acid substitution is G149N. In some embodiments, the amino acid substitution is G149S. In some embodiments, the amino acid substitution is G149T. In some embodiments, the amino acid substitution is G149V.

[0147] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 150 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V150I. In some embodiments, the amino acid substitution is V150L.

[0148] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 153 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T153L.

[0149] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the histidine at position 156 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is H156N.

[0150] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 157 of SEQ ID NO:1. In some embodiments, the substitution is with

any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T157A. In some embodiments, the amino acid substitution is T157G.

[0151] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the aspartic acid at position 158 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is D158E. In some embodiments, the amino acid substitution is D158I. In some embodiments, the amino acid substitution is D158K. In some embodiments, the amino acid substitution is D158L.

[0152] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 160 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T160K. In some embodiments, the amino acid substitution is T160Q. In some embodiments, the amino acid substitution is T160R. In some embodiments, the amino acid substitution is T160S. In some embodiments, the amino acid substitution is T160V.

[0153] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the phenylalanine at position 161 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is F161V. In some embodiments, the amino acid substitution is F161W.

[0154] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 162 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is N162E. In some embodiments, the amino acid substitution is N162H. In some embodiments, the amino acid substitution is N162P. In some embodiments, the amino acid substitution is N162R.

[0155] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 163 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T163I. In some embodiments, the amino acid substitution is T163S.

[0156] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the proline at position 164 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P164E. In some embodiments, the amino acid substitution is P164H. In some embodiments, the amino acid substitution is P164N. In some embodiments, the amino acid substitution is P164R. In some embodiments, the amino acid substitution is P164S. In some embodiments, the amino acid substitution is P164T.

[0157] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 167 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to

steric effects). In some embodiments, the amino acid substitution is Q167I. In some embodiments, the amino acid substitution is Q167T. In some embodiments, the amino acid substitution is Q167V.

[0158] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 170 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V170L.

[0159] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamic acid at position 173 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is E173R.

[0160] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 174 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A174K. In some embodiments, the amino acid substitution is A174R.

[0161] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 177 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V177A.

[0162] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the proline at position 179 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P179Q.

[0163] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 181 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is S181A. In some embodiments, the amino acid substitution is S181C. In some embodiments, the amino acid substitution is S181R.

[0164] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 182 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Q182T.

[0165] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 184 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is A184C. In some embodiments, the amino acid substitution is A184G. In some embodiments, the amino acid substitution is A184S.

[0166] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 185 of SEQ ID NO:1. In some embodiments, the substitution is with

any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I185A. In some embodiments, the amino acid substitution is I185E. In some embodiments, the amino acid substitution is I185G. In some embodiments, the amino acid substitution is I185L. In some embodiments, the amino acid substitution is I185Q. In some embodiments, the amino acid substitution is I185R. In some embodiments, the amino acid substitution is I185S. In some embodiments, the amino acid substitution is I185Y.

[0167] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 189 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Q189I. In some embodiments, the amino acid substitution is Q189L. In some embodiments, the amino acid substitution is Q189V.

[0168] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 190 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N190S.

[0169] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 193 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is S193E. In some embodiments, the amino acid

substitution is S193F. In some embodiments, the amino acid substitution is S193H. In some embodiments, the amino acid substitution is S193K. In some embodiments, the amino acid substitution is S193N. In some embodiments, the amino acid substitution is S193P. In some embodiments, the amino acid substitution is S193T. In some embodiments, the amino acid substitution is S193V.

[0170] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 194 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T194G. In some embodiments, the amino acid substitution is T194S.

[0171] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 195 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T195F.

[0172] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 198 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V198A.

[0173] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 200 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some

embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V200L.

[0174] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the aspartic acid at position 203 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is D203N. In some embodiments, the amino acid substitution is D203R. In some embodiments, the amino acid substitution is D203V.

[0175] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 204 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N204A. In some embodiments, the amino acid substitution is N204K. In some embodiments, the amino acid substitution is N204R. In some embodiments, the amino acid substitution is N204S.

[0176] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 206 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is T206G. In some embodiments, the amino acid substitution is T206K. In some embodiments, the amino acid substitution is T206L. In some embodiments, the amino acid substitution is T206P. In some embodiments, the amino acid substitution is T206R.

[0177] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the phenylalanine at position 208 of SEQ ID NO:1. In some embodiments, the substitution is

with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is F208G. In some embodiments, the amino acid substitution is F208L. In some embodiments, the amino acid substitution is F208R. In some embodiments, the amino acid substitution is F208T.

[0178] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 209 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A209V.

[0179] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 211 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N211F. In some embodiments, the amino acid substitution is N211I. In some embodiments, the amino acid substitution is N211L. In some embodiments, the amino acid substitution is N211M. In some embodiments, the amino acid substitution is N211V.

[0180] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 212 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S212F. In some embodiments, the amino acid substitution is S212L. In some embodiments, the amino acid substitution is S212M.

[0181] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the proline at position 213 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P213N. In some embodiments, the amino acid substitution is P213R.

[0182] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 216 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is A216L. In some embodiments, the amino acid substitution is A216P. In some embodiments, the amino acid substitution is A216S. In some embodiments, the amino acid substitution is A216T. In some embodiments, the amino acid substitution is A216V.

[0183] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 217 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I217S.

[0184] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 218 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S218A.

[0185] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 219 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V219F. In some embodiments, the amino acid substitution is V219I. In some embodiments, the amino acid substitution is V219K. In some embodiments, the amino acid substitution is V219L. In some embodiments, the amino acid substitution is V219R.

[0186] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the threonine at position 221 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is T221S.

[0187] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the isoleucine at position 222 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is I222L.

[0188] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 223 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is S223A. In some embodiments, the amino acid substitution is S223C.

[0189] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the methionine at position 225 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is M225L.

[0190] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the leucine at position 227 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L227R.

[0191] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the tryptophan at position 228 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is W228F.

[0192] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 229 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is V229C. In some embodiments, the amino acid substitution is V229I. In some embodiments, the amino acid substitution is V229L.

[0193] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 231 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine,

lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N231L. In some embodiments, the amino acid substitution is N231Q. In some embodiments, the amino acid substitution is N231S.

[0194] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 236 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is R236C. In some embodiments, the amino acid substitution is R236E. In some embodiments, the amino acid substitution is R236H. In some embodiments, the amino acid substitution is R236K. In some embodiments, the amino acid substitution is R236Q.

[0195] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 237 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is Q237R.

[0196] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 241 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is N241P.

[0197] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the valine at position 242 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine,

asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V242T.

[0198] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 243 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is N243P.

[0199] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the alanine at position 246 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A246D. In some embodiments, the amino acid substitution is A246K. In some embodiments, the amino acid substitution is A246S. In some embodiments, the amino acid substitution is A246T.

[0200] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the aspartic acid at position 249 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is D249I. In some embodiments, the amino acid substitution is D249M. In some embodiments, the amino acid substitution is D249N. In some embodiments, the amino acid substitution is D249S. In some embodiments, the amino acid substitution is D249T.

[0201] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the phenylalanine at position 250 of SEQ ID NO:1. In some embodiments, the substitution is

with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is F250I. In some embodiments, the amino acid substitution is F250L. In some embodiments, the amino acid substitution is F250V. In some embodiments, the amino acid substitution is F250Y.

[0202] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 251 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is R251A. In some embodiments, the amino acid substitution is R251E. In some embodiments, the amino acid substitution is R251I. In some embodiments, the amino acid substitution is R251K. In some embodiments, the amino acid substitution is R251L. In some embodiments, the amino acid substitution is R251Q. In some embodiments, the amino acid substitution is R251T. In some embodiments, the amino acid substitution is R251V.

[0203] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the serine at position 252 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely threonine, glutamine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S252T.

[0204] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 253 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to

steric effects). In some embodiments, the amino acid substitution is N253S. In some embodiments, the amino acid substitution is N253Y.

[0205] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the asparagine at position 254 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is N254R.

[0206] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 255 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is R255E. In some embodiments, the amino acid substitution is R255G. In some embodiments, the amino acid substitution is R255L. In some embodiments, the amino acid substitution is R255M. In some embodiments, the amino acid substitution is R255S. In some embodiments, the amino acid substitution is R255V. In some embodiments, the amino acid substitution is R255W. In some embodiments, the amino acid substitution is R255Y.

[0207] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the glutamine at position 258 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, asparagine, lysine, arginine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is Q258P.

[0208] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 8 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine,

isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P8T.

[0209] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 31 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L31M.

[0210] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 38 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is G38D.

[0211] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 95 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is S95N.

[0212] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 126 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V126I.

[0213] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 137 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L137M.

[0214] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 165 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is V165I.

[0215] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 169 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing proline (due to steric effects). In some embodiments, the amino acid substitution is I169C. In some embodiments, the amino acid substitution is I169L. In some embodiments, the amino acid substitution is I169V.

[0216] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 172 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is A172T.

[0217] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 191 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine,

asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is L191F. In some embodiments, the amino acid substitution is L191V.

[0218] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 192 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation). In some embodiments, the amino acid substitution is P192A.

[0219] In some embodiments, the variant Bhr-PETase has an amino acid substitution of the arginine at position 197 of SEQ ID NO:1. In some embodiments, the substitution is with any other of the 19 naturally occurring amino acids, namely serine, threonine, glutamine, asparagine, lysine, histidine, glutamic acid, aspartic acid, cysteine, glycine, proline, alanine, isoleucine, leucine, methionine, phenylalanine, tryptophan, valine and tyrosine, with some embodiments not utilizing cysteine (due to possible disulfide formation) or proline (due to steric effects). In some embodiments, the amino acid substitution is K197L. In some embodiments, the amino acid substitution is K197R. In some embodiments, the amino acid substitution is K197T. In some embodiments, the amino acid substitution is K197V. In some embodiments, the amino acid substitution is K197Y.

[0220] In some embodiments, the variant Bhr-PETase enzyme has one or more amino acid substitutions at 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29 or 30 of the positions of SEQ ID NO: 1 as described above.

D. Nucleic Acids of the Invention

[0221] The present invention additionally provides nucleic acids encoding the variant Bhr-PETases of the invention. As will be appreciated by those in the art, due to the degeneracy of the genetic code, an extremely large number of nucleic acids may be made, all of which encode the variant Bhr-PETases of the present invention. Thus, having identified a particular amino acid sequence, those skilled in the art could make any number of different nucleic

acids, by simply modifying the sequence of one or more codons in a way which does not change the amino acid sequence of the protein. Thus, providing the amino acid sequence allows the generation of a very large number of different nucleic acid sequences encoding the proteins.

[0222] In some embodiments, specific variant Bhr-PETases are encoded by specific nucleic acid sequences, as are listed in SEQ ID NOs 2 and 3. In some embodiments, specific variant Bhr-PETases are encoded by a nucleic acid sequence having at least about 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99 or 100% identity to SEQ ID NOs 2 and 3.

[0223] As is known in the art, the nucleic acids encoding the components of the invention can be incorporated into expression vectors as is known in the art, and depending on the host cells used to produce the heterodimeric antibodies of the invention. Generally the nucleic acids are operably linked to any number of regulatory elements (promoters, origin of replication, selectable markers, ribosomal binding sites, inducers, etc.). The expression vectors can be extra-chromosomal or integrating vectors.

[0224] The nucleic acids and/or expression vectors of the invention are then transformed into any number of different types of host cells as is well known in the art, including mammalian, bacterial, yeast, insect and/or fungal cells, with bacteria, yeast and fungi finding use in many embodiments.

1. Preparation of Variants

[0225] The nucleic acids encoding the variant Bhr-PETases of the invention can be prepared using any mutagenesis procedure known in the art, such as site-directed mutagenesis and synthetic gene construction as are well known in the art.

[0226] Synthetic gene construction entails *in vitro* synthesis of a designed polynucleotide molecule to encode a polypeptide of interest. Gene synthesis can be performed utilizing a number of techniques, such as the multiplex microchip-based technology described by Tian *et al.* (2004, *Nature* 432: 1050-1054) and similar technologies wherein oligonucleotides are synthesized and assembled upon photo-programmable microfluidic chips. A preferred technique is GenScript®.

2. Regulatory sequences

[0227] The present invention also relates to nucleic acid constructs comprising a polynucleotide encoding a variant of the present invention operably linked to one or more control sequences that direct the expression of the coding sequence in a suitable host cell under conditions compatible with the control sequences.

[0228] The polynucleotide may be manipulated in a variety of ways to provide for expression of a variant. Manipulation of the polynucleotide prior to its insertion into a vector may be desirable or necessary depending on the expression vector. The techniques for modifying polynucleotides utilizing recombinant DNA methods are well known in the art.

[0229] The control sequence may be a promoter, a polynucleotide which is recognized by a host cell for expression of the polynucleotide. The promoter contains transcriptional control sequences that mediate the expression of the variant. The promoter may be any polynucleotide that shows transcriptional activity in the host cell including mutant, truncated, and hybrid promoters, and may be obtained from genes encoding extracellular or intracellular polypeptides either homologous or heterologous to the host cell.

[0230] Promoters for bacteria, yeast and fungi are well known in the art. Exemplary operons for expression in lactic acid bacteria include *S. thermophilus* lactose operons or *L. lactis* lac ABCDFEGX operons, which have been successfully used to induce foreign gene expression in hosts (eg, Simons et al., 1993, J. Bact. 175: 5186-5175; see Mollet et al., 1993, J. Bact. 175: 4315-4324). Non-limiting additional examples of constitutive promoters for bacteria include lac promoter, trp promoter, tac promoter, T7 promoter, erm promoter, tip promoter, nit promoter, and Sp6 promoter. Exemplary promoters for yeasts include, but not limited to, AOX1 promoter, ADH promoter, PH05 promoter, gal10 promoter, PKG promoter and GAP promoter. Other useful promoters for yeast host cells are described by Romanos et al., 1992, Yeast 8: 423-488. Moreover, examples of useful promoters for fungi vectors include, but not limit to, *Aspergillus niger* GLA promoter, *Aspergillus nidulans* GPD promoter, those derived from *Aspergillus nidulans* glycolytic genes, such as the adh3 promoter (McKnight et al., EMBO J. 4:2093-2099, 1985).

3. Codon Optimization

[0231] While the wild-type coding sequence of Bhr-PETase is shown in SEQ ID NO:1, those of skill in the art will recognize that for heterologous expression, codon optimization can be done to increase expression in any particular host organism. Codon optimization can be employed with any of the variant Bhr-PETase polypeptides of the present invention, in order

to optimize expression in the host cell employed. Such methods are well known in the art and described in, for example, WO 2007/142954. In heterologous expression systems, optimization steps can improve the ability of the host to produce the desired variant Bhr-PETase polypeptides. Protein expression is governed by a host of factors including those that affect transcription, mRNA processing, and stability and initiation of translation. The polynucleotide optimization steps can include steps to improve the ability of the host to produce the foreign protein as well as steps to assist the researcher in efficiently designing expression constructs. Optimization strategies can include, for example, the modification of translation initiation regions, alteration of mRNA structural elements, and the use of different codon biases. The following paragraphs discuss potential problems that may result in reduced heterologous protein expression, and techniques that may overcome these problems.

[0232] In some embodiments, reduced heterologous protein expression results from a rare codon-induced translational pause. A rare codon-induced translational pause includes the presence of codons in the polynucleotide of interest that are rarely used in the host organism can have a negative effect on protein translation due to their scarcity in the available tRNA pool. One method of improving optimal translation in the host organism includes performing includes performing codon optimization which can result in rare host codons being modified in the synthetic polynucleotide sequence.

[0233] In some embodiments, reduced heterologous protein expression results from by alternate translational initiation. Alternate translational initiation can include a synthetic polynucleotide sequence inadvertently containing motifs capable of functioning as a ribosome binding site (RBS). These sites can result in initiating translation of a truncated protein from a gene-internal site. One method of reducing the possibility of producing a truncated protein, which can be difficult to remove during purification, includes modifying putative internal RBS sequences from an optimized polynucleotide sequence.

[0234] In some embodiments, reduced heterologous protein expression occurs through repeat-induced polymerase slippage. Repeat-induced polymerase slippage involves nucleotide sequence repeats that have been shown to cause slippage or stuttering of DNA polymerase which can result in frameshift mutations. Such repeats can also cause slippage of RNA polymerase. In an organism with a high G+C content bias, there can be a higher degree of repeats composed of G or C nucleotide repeats. Therefore, one method of reducing the possibility of inducing RNA polymerase slippage includes altering extended repeats of G or C nucleotides.

[0235] In some embodiments, reduced heterologous protein expression occurs through interfering secondary structures. Secondary structures can sequester the RBS sequence or initiation codon and have been correlated to a reduction in protein expression. Stemloop structures can also be involved in transcriptional pausing and attenuation. An optimized polynucleotide sequence can contain minimal secondary structures in the RBS and gene coding regions of the nucleotide sequence to allow for improved transcription and translation.

[0236] In some embodiments, restriction sites can affect heterologous protein expression. By modifying restriction sites that could interfere with subsequent sub-cloning of transcription units into host expression vectors a polynucleotide sequence can be optimized.

[0237] Optimizing a DNA sequence can negatively or positively affect gene expression or protein production. For example, modifying a less-common codon with a more common codon may affect the half-life of the mRNA or alter its structure by introducing a secondary structure that interferes with translation of the message. It may therefore be necessary, in certain instances, to alter the optimized message.

[0238] All or a portion of a gene can be optimized. In some embodiments, the desired modulation of expression is achieved by optimizing essentially the entire gene. In other embodiments, the desired modulation will be achieved by optimizing part but not all of the gene.

[0239] The codon usage of any coding sequence can be adjusted to achieve a desired property, for example high levels of expression in a specific cell type. The starting point for such an optimization may be a coding sequence with 100% common codons, or a coding sequence which contains a mixture of common and non-common codons.

[0240] Two or more candidate sequences that differ in their codon usage can be generated and tested to determine if they possess the desired property. Candidate sequences can be evaluated by using a computer to search for the presence of regulatory elements, such as silencers or enhancers, and to search for the presence of regions of coding sequence which could be converted into such regulatory elements by an alteration in codon usage. Additional criteria can include enrichment for particular nucleotides, *e.g.*, A, C, G or U, codon bias for a particular amino acid, or the presence or absence of particular mRNA secondary or tertiary structure. Adjustment to the candidate sequence can be made based on a number of such criteria.

[0241] Promising candidate sequences are constructed and then evaluated experimentally. Multiple candidates may be evaluated independently of each other, or the process can be iterative, either by using the most promising candidate as a new starting point, or by combining regions of two or more candidates to produce a novel hybrid. Further rounds of modification and evaluation can be included.

[0242] Modifying the codon usage of a candidate sequence can result in the creation or destruction of either a positive or negative element. In general, a positive element refers to any element whose alteration or removal from the candidate sequence could result in a decrease in expression of the therapeutic protein, or whose creation could result in an increase in expression of a therapeutic protein. For example, a positive element can include an enhancer, a promoter, a downstream promoter element, a DNA binding site for a positive regulator (*e.g.*, a transcriptional activator), or a sequence responsible for imparting or modifying an mRNA secondary or tertiary structure. A negative element refers to any element whose alteration or removal from the candidate sequence could result in an increase in expression of the therapeutic protein, or whose creation would result in a decrease in expression of the therapeutic protein. A negative element includes a silencer, a DNA binding site for a negative regulator (*e.g.*, a transcriptional repressor), a transcriptional pause site, or a sequence that is responsible for imparting or modifying an mRNA secondary or tertiary structure. In general, a negative element arises more frequently than a positive element. Thus, any change in codon usage that results in an increase in protein expression is more likely to have arisen from the destruction of a negative element rather than the creation of a positive element. In addition, alteration of the candidate sequence is more likely to destroy a positive element than create a positive element. In some embodiments, a candidate sequence is chosen and modified so as to increase the production of a therapeutic protein. The candidate sequence can be modified, *e.g.*, by sequentially altering the codons or by randomly altering the codons in the candidate sequence. A modified candidate sequence is then evaluated by determining the level of expression of the resulting therapeutic protein or by evaluating another parameter, *e.g.*, a parameter correlated to the level of expression. A candidate sequence which produces an increased level of a therapeutic protein as compared to an unaltered candidate sequence is chosen.

[0243] In some embodiments, one or a group of codons can be modified, *e.g.*, without reference to protein or message structure and tested. Alternatively, one or more codons can be chosen on a message-level property, *e.g.*, location in a region of predetermined, *e.g.*, high

or low GC content, location in a region having a structure such as an enhancer or silencer, location in a region that can be modified to introduce a structure such as an enhancer or silencer, location in a region having, or predicted to have, secondary or tertiary structure, *e.g.*, intra-chain pairing, inter-chain pairing, location in a region lacking, or predicted to lack, secondary or tertiary structure, *e.g.*, intra-chain or inter-chain pairing. A particular modified region is chosen if it produces the desired result.

[0244] Methods which systematically generate candidate sequences are useful. For example, one or a group, *e.g.*, a contiguous block of codons, at various positions of a synthetic nucleic acid sequence can be modified with common codons (or with non common codons, if for example, the starting sequence has been optimized) and the resulting sequence evaluated. Candidates can be generated by optimizing (or de-optimizing) a given “window” of codons in the sequence to generate a first candidate, and then moving the window to a new position in the sequence, and optimizing (or de-optimizing) the codons in the new position under the window to provide a second candidate. Candidates can be evaluated by determining the level of expression they provide, or by evaluating another parameter, *e.g.*, a parameter correlated to the level of expression. Some parameters can be evaluated by inspection or computationally, *e.g.*, the possession or lack thereof of high or low GC content; a sequence element such as an enhancer or silencer; secondary or tertiary structure, *e.g.*, intra-chain or inter-chain pairing.

[0245] In some embodiments, the optimized nucleic acid sequence can express the variant Bhr-PETase polypeptide of the invention, at a level which is at least about 110%, 150%, 200%, 500%, 1,000%, 5,000% or even 10,000% of that expressed by nucleic acid sequence that has not been optimized.

[0246] Starting with the amino acid sequence of a variant Bhr-PETase, a candidate DNA sequence can be designed. During the design of the synthetic DNA sequence, the frequency of codon usage can be compared to the codon usage of the host expression organism and rare host codons can be modified in the synthetic sequence. Additionally, the synthetic candidate DNA sequence can be modified in order to remove undesirable enzyme restriction sites and add or alter any desired signal sequences, linkers or untranslated regions. The synthetic DNA sequence can be analyzed for the presence of secondary structure that may interfere with the translation process, such as G/C repeats and stem-loop structures. Before the candidate DNA sequence is synthesized, the optimized sequence design can be checked to verify that the sequence correctly encodes the desired amino acid sequence. Finally, the candidate DNA sequence can be synthesized using DNA synthesis techniques, such as those known in the art.

[0247] In some embodiments, the general codon usage in a host organism, such as any of those described herein, can be utilized to optimize the expression of the heterologous polynucleotide sequence in the host organism. The percentage and distribution of codons that rarely would be considered as preferred for a particular amino acid in the host expression system can be evaluated. Values of 5% and 10% usage can be used as cutoff values for the determination of rare codons.

4. Host Cells and Production Strains

[0248] In one aspect, the present disclosure relates to an expression vector comprising the nucleic acid encoding the variant Bhr-PETase described herein. In another aspect, the present disclosure also relates to a host cell comprising the expression vector. In some embodiments, the host cell is bacteria. In some embodiments, the host cell is yeast. In some embodiments, the host cell is fungi. In some embodiments, the host cell may be bacteria, including but not limited to *E. coli*, *Bacillus*. In some embodiments, the host cell may be yeast, including but not limited to *Saccharomyces cerevisiae*, *Pichia*. In some embodiments, the host cell may be fungi, including but not limited to *A. niger*, *T. reesei*, or *Myceliophthora thermophila*.

[0249] The expression vector may be any of integration vectors which are to be integrated into genome or autonomously replicating plasmids in the selected host. In one embodiment, the vector can be stably maintained in the introduced cell, with the variant Bhr-PETase gene supported thereon in a fit state for expression. The expression vector may be selected to be suitable for the specific host cells to which the vector is introduced. Specific examples available for use include but not limit to pBR322, pACYC184, pUC18, pKK223-2, pHSG398 (Takara Bio Inc.), pTrcHis (Invitrogen Corporation) and pET11a (Stratagene Corporation) in the case where *Escherichia coli* is used as a host; pBBR122 (Mobiotech) and pBHR1 (Mobiotech) for the other gram-negative bacteria; pHW1520 (Mobiotech) and pHY300PLK (Takara Bio Inc.) for *Bacillus*; pSH19 (Herai et al., Proc. Natl. Acad. Sci., 101, 14031-14035, 2004), pIJ702 (John Innes Centre), pIJ943 (John Innes Centre), pIJ8600 (John Innes Centre), pIJ602 (John Innes Centre), 1, pTip-vectors (Nakashima et al., Appl. Environ. Microbiol., 70, 5557-5568, 2004), pTYM19 (Onaka et al., J. Antibiot., 56, 950-956, 2003) for actinomycetes; pPICZ α and pPIC9 (Thermo Fisher Scientific) for *Pichia*, and pAO815 (Invitrogen Corporation), pAUR101 (Takara Bio Inc.), pAUR123 (Takara Bio Inc.) and pAUR316 (Takara Bio Inc.) for fungi.

[0250] In another aspect, the present disclosure also relates to a method of expressing the variant Bhr-PETase in the host cell. In another aspect, the present disclosure also relates to a method of making the variant Bhr-PETase comprising culturing the host cell under conditions wherein said variant Bhr-PETase is produced, and recovering said variant Bhr-PETase.

[0251] The culture of a transformed organism may be performed in the medium which can be a nutritive medium of the transformed host cell without affecting the transformation of the variant Bhr-PETase. Such a medium comprises an appropriate carbon source, nitrogen source, inorganic salt, natural organic nutrient and the like. As a carbon source, glucose, fructose, glycerol, sorbitol, organic acids can be used individually or in combination. The concentration of the carbon source is not particularly limited and may be 1 to 10 %. As a nitrogen source, ammonium, urea, ammonium sulfate, ammonium nitrate, ammonium acetate and the like can be used individually or in combination of two or more members thereof. As an inorganic salt, salts such as monopotassium phosphate, dipotassium phosphate, magnesium sulfate, manganese sulfate and ferrous sulfate can be used. In addition, as an organic nutrient source having growth-promoting effects of the bacteria to be used, peptone, meat extract, yeast extract, corn steep liquor and casamino acids can be used and furthermore, a small amount vitamins and nucleic acids may be contained in the medium.

5. PETase Formulations and Uses

[0252] As will be appreciated by those in the art, the formulation of the variant Bhr-PETases of the invention depends on its end use and the associated conditions. Suitable formulations for the variant Bhr-PETases of the invention include liquid formulations, dried formulations (including spray dried formulations), powdered formulations, granular formulations, and pelleted formulations. Bhr-PETases can also be formulated as “embedded in PET particles” for natural degradation.

[0253] In some embodiments, the enzyme composition (i.e., polypeptide compositions) of the present invention can be in any form suitable for use, such as, for example, a crude fermentation broth with or without cells removed, a cell lysate with or without cellular debris, a semi-purified or purified enzyme composition, or a host cell, as a source of the enzymes.

[0254] In some embodiments, the enzyme composition may be a dry powder or granulate, a non-dusting granulate, a liquid, a stabilized liquid, or a stabilized protected enzyme. Liquid enzyme compositions may, for instance, be stabilized by adding stabilizers such as a sugar, a

sugar alcohol or another polyol, and/or lactic acid or another organic acid according to established processes.

[0255] In some embodiments, the dosage of the polypeptide composition of the invention and other conditions under which the composition is used may be determined on the basis of methods known in the art.

[0256] The above compositions are suitable for use in PET biodegradation, PET biocycling, PET upcycling, and/or PET surface modification processes.

[0257] In some embodiments, the above compositions are used to degrade pretreated PET. PET pretreatment may be performed before the enzymatic degradation step. Commonly used PET pretreatment can be broadly classified into a) mechanical pretreatment, b) thermo-mechanical pretreatment, and c) chemical pretreatment. The mechanical process may involve grinding of the PET film into particles less than about 500 μm post sieving. This process could be combined with immersing the PET film into liquid nitrogen mainly for ease of the process of grinding. The advantage of this type of mechanical pretreatment may be enzyme accessibility due to reduced particle size. In the thermo-mechanical degradation, the main-chain scission reactions may affect the stability of the cyclic oligomers. In the thermo-mechanical pretreatment, highly crystalline PET flakes may be amorphized at very high temperatures ($> 260^{\circ}\text{C}$) using extruder equipped with melt pump and later micronized to particle size of less than 500 μm . An example of a commercial method of pretreating PET is by using an extrusion cast process, namely molten polymer cast onto chilled rolls of PET to solidify. The thermo-mechanical pretreatment may lower the crystallinity as well as particle size of the industrial grade PET allowing accessibility of the enzymes to depolymerize it. In case of chemical pretreatment, ionic liquid, strong acid, base, solvents etc. can be used to reduce crystallinity or to change the surface structure of the PET to facilitate access of the enzymes to further depolymerize it to its monomers.

[0258] The two significant stumbling blocks in recycling plastics irrespective of using chemical or biological method may be material variability and the costs associated with identifying and separating waste plastics into recognizable grade ranges. Different types and grades of plastics differ in densities and molecular weights. The advantage of knowing the properties of a particular plastic that is in the recycling process would make it easier to determine its value and durability when put into valuable second use applications. But, the process of sorting is a major hindrance from the standpoint of resource utilization, time

management, and cost effectiveness. Hence, when looking at biological mitigations to overcoming the roadblocks associated with differential plastic sorting, depolymerization of mixed plastics using robust enzymes that has broad substrate specificity becomes the utmost priority. Mixed plastic is a term that covers all non-bottle plastic packaging sourced from the domestic waste stream, and it includes rigid and flexible plastic items of various polymer types and colours that are typically found in the household waste bin. The mixed plastic refers to a mixture of different plastics. Various polymer types may refer to PET and/or analog of PET, PET-like or PET substitute derived biologically or chemically. Examples of analog of PET, PET-like or PET substitute include but are not limited to Polybutylene terephthalate (PBT), Polycarbonate (PC), Polycaprolactone (PCL), Polyethylene Furanoate (PEF) and High Density Polyethylene (HDPE). Bhr-PETase alone or in conjunction with other accessory enzyme (s) can revolutionize the biological method of depolymerization of mixed plastics. The process of enzymatic depolymerization of mixed plastics as opposed to chemical methodologies may be environmentally safer and capable of retaining the market value of the second use applications. Such biological treatments can also be combined with mild chemical or thermomechanical pre-treatment to achieve higher depolymerization efficiencies.

[0259] After PET pretreatment and enzymatic degradation, products may be recycled and generate other valuable chemicals. In one embodiment, TPA (terephthalic acid) is purified using an industry relevant process, and the process also may produce sodium sulphate, a chemical commonly used in the detergent, paper, and glass industries. In another embodiment, recycled TPA is used as the starting material to synthesize virgin PET. PET synthesized from recycled TPA may demonstrate similar properties, such as average molecular weight and intrinsic viscosity, as PET synthesized using petrochemical TPA. In one embodiment, bottles blown from recycled PET exhibits similar mechanical property and better lightness value than regular PET bottles.

[0260] In some embodiments, the present invention provides a method of preparing enzyme cocktail comprising the variant Bhr-PETase as described herein with other PET degrading accessory enzymes and downstream MHETase to produce a PET degrading enzyme cocktail for the efficient turn-over of pretreated PET.

EXAMPLES

Example 1: PET Hydrolase Gene Selection, Synthesis and Cloning

[0261] Multiple novel PET hydrolases were selected based on bioinformatics analysis and were synthesized by GeneWiz (<https://www.genewiz.com/en/>). The synthesized genes were cloned into the pET28b (+) vector (Millipore Inc., Catalog # 69865). Three hydrolases (Lcc-PETase, Bhr-PETase, Is-PETase) were selected for further purification and characterization.

Example 2: Preparation of PET Hydrolases Produced by *Escherichia coli* in 250ml Shake flask

[0262] The BL21(DE3) Chemically Competent *E. coli*, (ThermoFisher Scientific, USA: Catalogue #C600003) containing recombinant hydrolase-encoding genes from single colonies were inoculated into individual culture tubes containing 5mL lysogeny broth (LB broth) with 1% glucose and 50 µg/mL of Kanamycin. The cultures were grown overnight at 30°C, 200 rpm and 85% humidity. 1mL of overnight culture was transferred into shake flasks containing 50mL of terrific broth (TB broth) with 50 µg/mL Kanamycin. The flasks were then incubated for 2-2.5 hrs at 37°C, 250 rpm and 85% humidity. Pre-induction OD was measured at 2-2.5 hrs and if OD₆₀₀ reached around 0.6-0.8, induction with IPTG (Isopropyl β-D-1-thiogalactopyranoside) was carried out to obtain final concentration of 0.5 mM IPTG in flasks. The flasks were then incubated for 18-24 hrs at 30°C, 250 rpm and 85% humidity all except Is-PETase which was grown at 16°C. The supernatants and lysates were transferred to 50mL centrifuge tubes and stored at -20 °C prior to activity assay. A total of three candidates (Lcc-PETase, Bhr-PETase, Is-PETase) were grown based on the procedure herein.

Example 3: Ni-Column Purification of the His-tag PET Hydrolases Produced from Shake flasks

[0263] Each sample was concentrated to 15-20 ml before using Thermo Scientific HisPur™ Ni-NTA Spin Columns (catalog number 88226). The three PET hydrolases (Lcc-PETase, Bhr-PETase, Is-PETase) were purified from the cell culture. The protocol was followed as per manufacturer's guidelines. Multiple elution was done in 250mM Imidazole and based on

protein band intensity from the eluents in various fractions (measured using gel quantification), a stock solution of combined fractions was used for the desalting step.

Example 4: Desalting of purified PET Hydrolases Produced from Shake flasks

[0264] Using one time use Thermo Scientific Zeba™ Spin Desalting Columns, 7K MWCO (catalog number 89892), imidazole and NaCl were removed from Ni Purified enzyme. Desalting protocol was followed according to manufacturer's guidelines. Desalted enzyme was then quantified to determine protein concentration (g/L).

Example 5: Thermostability testing of *Escherichia coli* Produced PET

Hydrolases using BHET (Bis (2-Hydroxyethyl) terephthalate) as a substrate

[0265] Normalized PET hydrolase proteins of each candidate were subjected to a 1.5 hr challenge at either 30°C, 50°C, or 70°C. Enzymes kept on ice for the same time served as unchallenged condition. Post challenge, into Costar Deep wells plate, 250 µL of 10 mM BHET (Bis (2-Hydroxyethyl) terephthalate), 62.5 µL of unchallenged and challenged enzyme, and 170 µL of 0.1M sodium phosphate buffer, pH 8 was added. The plates were incubated at 50°C for 2 hours. After 2 hours, the samples were centrifuged at 4,000 rpm for 2 minutes. The supernatants were analyzed using High Performance Liquid Chromatography (HPLC) to determine the amount of TPA produced in our reactions. Zorbax Eclipse Plus C18 (Rapid Resolution HD 2.1x50mm 1.8-Micron) column with part number 959757-902 was used along with guard column part number 82175-901. Flow rate of 0.6 mL/minute with column temperature of 35°C was set up for running in HPLC. The gradient method was used to detect Terephthalic Acid (TPA) with mobile phase 1 consist of water with 0.1% trifluoroacetic acid and mobile phase 2 consist of Acetonitrile with 0.1% trifluoroacetic acid. % residual of each PET hydrolase was calculated by the following formula: (MHET + TPA produced at challenged condition) / (MHET + TPA produced at unchallenged condition) * 100.

[0266] The thermostability results of the three candidates (Lcc-PETase, Bhr-PETase, Is-PETase) are shown in Figure 4. Under the current experimental condition, Is-PETase is the most unstable candidate that loses 90% activity at 50°C. Bhr-PETase and Lcc-PETase retain ≥ 80% residual activity at all temperature tested.

Example 6: Large scale PET Film Assay to Evaluate *Escherichia coli*
Produced PET Hydrolases

[0267] These experiments were carried out in 250 ml glass bottles. Two types of PET substrate were used in these experiments. A) Amorphous PET film was obtained from Goodfellow (Catalog # ES301445) with 0.25 mm thickness. PET film was cut using paper trimmer into 1 x 30 cm strips, and then cut again to approximately 1 x 0.25 cm strips. Strips of PET film were grinded into fine powder with mechanical grinder. B) Highly crystalline PET powder > 40% crystallinity was obtained from Goodfellow (Catalog #ES306031/1). 2-10g/L PET powder loading was done in 0.1M sodium phosphate buffer, pH 8.0 to a final volume of 50mls. Various doses of normalized proteins of the three candidates were used for evaluation at either 65°C or 72°C with 200 rpm shaking. The reactions were continued for anywhere between 6-144hrs. At various timepoints the bottles were sampled, and the reactions were analyzed using High Performance Liquid Chromatography (HPLC) to determine the amount of TPA produced in our reactions. Zorbax Eclipse Plus C18 (Rapid Resolution HD 2.1x50mm 1.8-Micron) column with part number 959757-902 was used along with guard column part number 82175-901. Flow rate of 0.6 mL/minute with column temperature of 35°C was set up for running in HPLC. The gradient method was used to detect Terephthalic Acid (TPA) with mobile phase 1 consist of water with 0.1% trifluoroacetic acid and mobile phase 2 consist of Acetonitrile with 0.1% trifluoroacetic acid.

[0268] Results for PET assay are summarized in Figures 5 and 6. Both in amorphous and >40% crystalline PET, Bhr-PETase outperforms Lcc-PETase. Results further suggests that Lcc-PETase is less thermostable compared to Bhr-PETase irrespective of substrate type. In comparison to Bhr-PETase which can depolymerize > 90% amorphous PET at both 65°C and 72°C at an enzyme loading of 2.4 mg/g PET (Figure 5), depolymerization activity of Lcc-PETase at higher doses is significantly lower (data not shown). Figure 6 further suggests that Bhr-PETase cannot fully degrade highly crystalline PET and might require additional pretreatment or accessory enzymes to do so.

[0269] Overall, Bhr-PETase was identified as the best performing PET hydrolases among all that have been evaluated. Therefore, Bhr-PETase was selected as a target for further improvement.

Example 7: Design and Construction of Bhr-PETase collections

[0270] To further improve the activity and thermostability of Bhr-PETase, multiple G1 variant collections were designed based on sequence and structure analysis. The design includes one to multiple specific mutations per variant. The G1 variant collections were subsequently constructed on Bhr-PETase G1P (wild type Bhr-PETase, Generation 1 Parent) using standard site-directed mutagenesis methods and subsequently cloned into the pET28b (+) vector (Millipore Inc., Catalog # 69865) for production in *Escherichia coli*.

[0271] To further improve the activity and thermostability of Bhr-PETase, multiple G2 variant collections were designed based on sequence and structure analysis. The design includes one to multiple specific mutations per variant. The G2 variant collections were constructed on Bhr-PETase G2P (Generation 2 Parent, i.e. G1P with amino acid substitution S27L) using standard site-directed mutagenesis methods and subsequently cloned into a relevant vector for production in a desired host.

Example 8: HTP growth: Preparation of Bhr-PETase G1P, G1 variants, G2P and G2 Variants in Microtiter Plates

[0272] The BL21(DE3) Chemically Competent *E. coli*, (ThermoFisher Scientific, USA: Catalogue #C600003) containing recombinant Bhr-PETase encoding genes from single colonies were inoculated into individual wells of 96 well plates containing 180 µl lysogeny broth (LB broth) with 1% glucose and 50 µg/mL of Kanamycin. The cultures were grown overnight at 30°C, 200 rpm and 85% humidity. 20 µL of overnight culture was transferred from each well into 96 wells plate containing 380 µL of terrific broth (TB broth) with 50 µg/mL Kanamycin. The plates were then incubated for 2-2.5 hrs at 37°C, 250 rpm and 85% humidity. Pre-induction OD was measured at 2-2.5 hrs and if OD₆₀₀ reached around 0.6-0.8, induction with IPTG (Isopropyl β-D-1-thiogalactopyranoside) was carried out to obtain final concentration of 0.5 mM IPTG in wells. The plates were then incubated for 18-24 hrs at 30°C, 250 rpm and 85% humidity. The supernatants were transferred to round bottom plates and stored at -20 °C prior to activity assay.

[0273] The host containing recombinant Bhr-PETase encoding genes from single colonies were inoculated into individual wells of 24 or 96 well plates containing relevant medium. The cultures were then grown overnight at 30°C, 200 rpm and 85% humidity. Overnight culture was subcultured into 24 or 96 wells plate containing relevant medium. The plates

were then incubated at 30°C, 200 rpm and 85% humidity. Culture was induced at every 24 hours with respective inducer for up to 120 hours. The supernatants were transferred to round bottom plates at desired harvesting time and stored at -20°C prior to activity assay.

Example 9: HTP PET Film Assay to Evaluate Bhr-PETase G1P, G1 variants, G2P and G2 Variants Activity/Thermostability

[0274] For total activity testing, amorphous PET film was obtained from Goodfellow (Catalog # ES301445) with 0.25 mm thickness. PET film was cut using paper trimmer into 1 x 30 cm strips, and then cut again to approximately 1 x 0.25 cm strips. Strips of PET film were grinded into fine powder with mechanical grinder. PET powder was loaded into 96-well plates using resin loader. Approximately 8-9 mg of PET powder were dispensed in each of the 96 costar deep well. Into costar deep well containing PET powder, 1.4 mL of 0.1M sodium phosphate buffer was added, pH 8.0. 100 µL of enzyme was dispensed into costar deep wells, the plates were sealed, and then incubated at 65°C for 72 hours. After 72 hours, plates were centrifuged at 4,000 rpm for 2 minutes. Into costar round bottom plates, 180 µL of above reaction was transferred and 20 µL of in-house prepared Is-MHETase was added. The plates were incubated at 50 °C for 30 minutes. After 30 minutes, the plates were centrifuged at 4,000 rpm for 2 minutes. Reaction mixture was diluted to linear assay detection using sodium phosphate buffer, pH 7.2 in Costar Deep with addition of 50 µL of 10 mM EDTA, and 50 µL of 10 mM FeSO₄. Incubate the reaction in dark for 10 minutes and centrifuge plates at 4,000 rpm for 2 minutes before transferring 200 µL of reaction into black clear-bottom fluorometric plate. Endpoint reading was measured after 10 minutes with 328 nm for excitation and 421 emission for TPA (Terephthalic Acid) activity.

[0275] For thermostability testing, 150 µL of enzyme was transferred into PCR plates. The plates were incubated at 86 °C for 3 hours. After 3 hours, 100 µL of enzyme was transferred into costar deep well plates containing 1.4 mL of 0.1M sodium phosphate buffer, pH 8.0, and 8-9 mg/well of grinded PET powder. Plates were at 65°C for 72 hours. After 72 hours, plates were centrifuged at 4,000 rpm for 2 minutes. Into costar round bottom plates, 180 µL of above reaction was transferred and 20 µL of Is-MHETase was added. The plates were incubated at 50 °C for 30 minutes. After 30 minutes, the plates were centrifuged at 4,000 rpm for 2 minutes. The reactions were analyzed using High Performance Liquid Chromatography (HPLC) to determine the amount of TPA produced in our reactions. Zorbax

Eclipse Plus C18 (Rapid Resolution HD 2.1x50mm 1.8-Micron) column with part number 959757-902 was used along with guard column part number 82175-901. Flow rate of 0.6 mL/minute with column temperature of 35°C is set up for running in HPLC. The gradient method was used to detect Terephthalic Acid (TPA) with mobile phase 1 consist of water with 0.1% trifluoroacetic acid and mobile phase 2 consist of Acetonitrile with 0.1% trifluoroacetic acid.

[0276] The results are summarized in Figures 7, 8 and 9. All G1 variants showed improved total activity and/or thermostability than 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. All G2 variants showed further improved total activity than Bhr-PETase G2P.

CLAIMS

1A. 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.

1B. 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.

1C. 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.

2. The composition according to claim 1, wherein said amino acid substitution 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.

3. The composition according to claim 1, wherein said amino acid substitution 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.

4. The composition according to claim 1, wherein said amino acid substitution is at an amino acid position(s) selected from the group consisting of 8, 126, 137, 165, 169, 172, 191, 192 and 197.

5. The composition according to claim 1, 2, 3 or 4, wherein said amino acid substitution is selected from the group consisting of S27L, S27F, S27H, S27T, S27W, S1A, S1G, S1M, S1R, N2E, N2F, N2L, N2R, N2S, Q5E, N9A, N9E, N9S, R12K, S13L, S13R, A14K, A14S, L15I, T16E, T17A, T17C, T17G, T17H, T17I, T17K, T17L, T17M, T17N, T17Q, T17R, T17S, D18R, P20D, P20E, P20I, P20Q, P20T, F21W, F21Y, S22A, S22I, S22K, S22P, S22R, S22V, V23L, V23T, A24D, A24G, A24H, A24N, A24S, A24T, A24V, T25A, T25F, T25H, T25Q, T25R, T25V, Y26C, Y26K, Y26L, Y26T, R30K, S32K, S32M, S32Q, S32Y, V33G, V33Q, V33T, S34R, V40T, G46E, G46L, G46N, G46R, G46S, T48N, T48S, L49G, G53A, I54V, A55C, A55I, A55L, A55M, A55T, A55V, M56I, M56L, S57C, S57E, S57F, S57I, S57L, S57M, S57T, S57V, Y60A, Y60H, Y60I, A62T, A68F, L70M, R72P, L74W, H77Q, I82F, I82L, I82M, V83I, V83L, V83T, N85D, N87F, N87H, N87I, N87K, N87L, N87M, N87Q, N87R, N87V, N87W, N87Y, S88K, S88T, L90F, L90K, L90Y, F92G, F92I, F92K, F92L, F92N, F92Q, F92V, F92Y, A97C, A97E, A97F, A97G, A97L, A97P, A97Q,

A97S, A97T, A97V, S98A, S98D, S98E, S98L, S98M, S98N, S98Q, S98T, S98V, S101A, S101C, S101D, S101F, S101H, S101K, S101L, S101M, S101N, S101Q, S101R, S101V, S101W, S101Y, A102V, N105D, R108C, R108E, R108H, R108K, R108N, R108P, R108Q, R108S, R108T, R108V, T109A, T109F, T109G, T109K, T109L, T109N, T109R, T109Y, S110D, S110G, S110H, S110K, S110N, S110R, S113A, S113K, S113N, S113P, S113Q, S113R, S113T, S113Y, A114K, A114L, A114S, A114V, A117F, A117G, A117L, A117N, A117Q, A117S, A117T, A117Y, L119I, L119M, A121S, N122A, N122E, N122H, N122P, N122R, N122S, A125S, A127M, A127S, A127V, A135G, T136A, T136M, T136S, T136V, R138E, R138L, I139A, I139T, S140A, Q142D, Q142E, Q142H, Q142L, Q142W, I143N, I143R, T145S, K147F, K147G, K147N, K147Q, G149A, G149C, G149D, G149N, G149S, G149T, G149V, V150I, V150L, T153L, H156N, T157A, T157G, D158E, D158I, D158K, D158L, T160K, T160Q, T160R, T160S, T160V, F161V, F161W, N162E, N162H, N162P, N162R, T163I, T163S, P164E, P164H, P164N, P164R, P164S, P164T, Q167I, Q167T, Q167V, V170L, E173R, A174K, A174R, V177A, P179Q, S181A, S181C, S181R, Q182T, A184C, A184G, A184S, I185A, I185E, I185G, I185L, I185Q, I185R, I185S, 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, A216T, A216V, I217S, S218A, V219F, V219I, V219K, V219L, V219R, T221S, I222L, S223A, S223C, M225L, L227R, W228F, V229C, V229I, V229L, N231L, N231Q, N231S, R236C, R236E, R236H, R236K, R236Q, Q237R, N241P, V242T, N243P, A246D, A246K, A246S, A246T, D249I, D249M, D249N, D249S, D249T, F250I, F250L, F250V, F250Y, R251A, 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.

6. The composition according to claim 5, wherein 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.

7. The composition according to claim 5, wherein 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.

8. The composition according to claim 5, wherein 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.

9. A 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.

10. A 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 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,
M56I/I82F/R108T/I139A/F161W/T194S/D203N/N241P, M56I/S88T/R108T/N190S/L227R,
N204A/Q237R, N9A/D18R/M56I/N85D/L119I/N254R,
N9A/S22A/G46S/M56I/R72P/F92Q/L119I/T221S/M225L, N9A/S22A/Q167T,
N9A/V40T/L49G/I54V/R108T/V150I/D203N/T221S/M225L, N9S/T25H,
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/I54V/R72P/F92Q/V150I/V200L,
S22A/L49G/M56I/V83T/S101L/R108T/L119I/A127M/T194S, S22I/Y26K,
S27T/I82L/P213N, S27T/T48S/I82L/F92Y/S252T,
S27T/T48S/I82L/L90F/A135G/S140A/I143N/T145S/P213N,
S27T/T48S/I82L/S140A/I143N/G149A, S32Y/A62T,
S88T/R108T/V150I/A184S/D203N/T221S/M225L/N243P, S98Q/A209V,
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/M56I/V150I/S218A/V219K,
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, V177A/A216L, 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,
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, L90F/F92G/T109K/A125S/A174K,
S32K/L90F/F92L/T109K/A114K/Q142W,
V23T/S32Q/N87I/L90F/F92Y/S101N/A114V/Q142W/A174R/D203R/I222L/V229I,
S32Q/F92G/A114V/D203V, 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,
T17A/S32M/Y60H/Q142L/D203V/I222L,
R12K/Y60H/L90F/S101R/A114V/A117N/D158E,
T17S/D18R/L90Y/F92G/A114V/A117Q/D203R,
L90Y/F92L/S101A/S110R/A125S/Q142W/A174R/A216P, S32M/S110R/A125S/D158E,
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,
P20T/F21W/Y60I/S101A/T109K/A174K,
T17C/L90Y/F92L/S101N/T109L/S110R/A125S/A174K/D203R,
T17Q/L90F/F92G/S101M/A117Q/T136A/D158I/A174R,
V23L/L90F/F92G/A125S/D203V/I222L, T17C/L90Y/F92L/A125S/T136V/D158E/A174K,
V23L/S32Q/Y60I/A117Q/T136A/A174K/V229C, V23T/L90F/F92Y/D203V,
R12K/A24V/Y60H/A174R, T17G/P20T/Y60I/F92G/D203V,
L90Y/F92L/S110R/A125S/D158E/D203V,
T17Q/L90Y/F92L/T109L/T136A/D158E/A174R/D203V/V229C,
R12K/V23T/A24V/S32Q/F92G/D158L/A174R/D203R,
S32M/N87Y/L90F/F92G/A174K/D203V,
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,
F92G/A125S/T136A/D158E/A216P, N87R/F92G/A117N/D203V/A216P,
R12K/A14K/F92G/S110R/A117N/A125S/A174R/D203R,
S32Q/N87F/L90F/F92G/S101M/A114V/A117Q/D203V,
T17Q/A24V/N87M/F92G/A114K/A117N/D158E/D203R/I222L,
N2R/S32Q/L90F/F92G/Q142L/D203V,
A14K/S32Q/L90F/F92G/A114K/A117N/A174R/D203V/A216P/I222L,
R12K/P20T/D158E/D203R/I222L, N2R/A14K/T17A/L90F/F92G/S101H/A174R/D203V,
T17G/A24V/L90F/F92G/Q142W, S32K/L90Y/F92Y/S101A,
V23T/N87I/F92G/A125S/T136A/D158L/D203R, T17S/N87L/V229I,
A24T/L90F/F92L/A125S/T136A/D203V,
R12K/A14K/F92G/T109R/T136A/Q142L/D158E/A174K/D203R/I222L,
A24V/S32Q/Y60A/T136A, D18R/S32Q/N87H/L90Y/F92G/S101K/Q142L/D158I/A174R,
T17A/F21W/S32K/N87F/L90F/F92Y/S101Q/T136A/D203R,
A24V/L90F/F92G/A114V/A117N/T136V/Q142L/D203V/V229C,
T17Q/L90Y/F92G/S101N/T109L/S110R/T136V/Q142L/D203V/A216P,
S32Q/L90Y/F92L/T109K/A125S/T136V,
Y60H/N87K/A114K/A117Q/D158E/A174K/A216P,
P20T/L90Y/T109K/T136A/D158I/D203R, T17L/Y60A/D203V,

N2R/S32Q/L90Y/F92Y/T109K/D203V,
T17H/A24V/S32Q/L90Y/F92L/S101H/A114K/D158I/A174R/D203V,
N2R/S32M/F92Y/T109K/S110R/I222L, 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,
F21W/L90F/F92G/S101A/A114V/A125S/T136V/Q142L/D203V/A216P,
P20T/F21W/S32K/F92G/A125S/V229I, 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/Parent, 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,
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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,
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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,
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S27L/R12K/V23L/L90F/F92L/A114K/T136A/D158E/D203V/I222L, S27L/L90F/F92G,
S27L/A14K/P20T/S32Q/L90Y/A125S/D203V,
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S27L/T17S/L90F/F92Y/S101K/A127S/T136V/N204R/N211I/V229I/N231S,
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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,
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, S27L/F92G/A127S/A174R/S223A/V229I/N231S/R236C,
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 S27L/A24H/G46E, S27L/T17H/S101Q/T136V/Q142L/V229C and
 S27L/V23T/A24V/I222L/V229C.

11. The composition according to claim 10, wherein 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, M56I/I82F/R108T/I139A/F161W/T194S/D203N/N241P, M56I/S88T/R108T/N190S/L227R, N204A/Q237R, N9A/D18R/M56I/N85D/L119I/N254R, N9A/S22A/G46S/M56I/R72P/F92Q/L119I/T221S/M225L, N9A/S22A/Q167T, N9A/V40T/L49G/I54V/R108T/V150I/D203N/T221S/M225L, N9S/T25H, 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/I54V/R72P/F92Q/V150I/V200L,
S22A/L49G/M56I/V83T/S101L/R108T/L119I/A127M/T194S, S22I/Y26K,
S27T/I82L/P213N, S27T/T48S/I82L/F92Y/S252T,
S27T/T48S/I82L/L90F/A135G/S140A/I143N/T145S/P213N,
S27T/T48S/I82L/S140A/I143N/G149A, S32Y/A62T,
S88T/R108T/V150I/A184S/D203N/T221S/M225L/N243P, S98Q/A209V,
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/M56I/V150I/S218A/V219K,
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.

12. The composition according to claim 10, wherein 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.

13. The composition according to claim 10, wherein 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,
 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, L90F/F92G/T109K/A125S/A174K,
S32K/L90F/F92L/T109K/A114K/Q142W,
V23T/S32Q/N87I/L90F/F92Y/S101N/A114V/Q142W/A174R/D203R/I222L/V229I,
S32Q/F92G/A114V/D203V, 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,
T17A/S32M/Y60H/Q142L/D203V/I222L,
R12K/Y60H/L90F/S101R/A114V/A117N/D158E,
T17S/D18R/L90Y/F92G/A114V/A117Q/D203R,
L90Y/F92L/S101A/S110R/A125S/Q142W/A174R/A216P, S32M/S110R/A125S/D158E,
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,
P20T/F21W/Y60I/S101A/T109K/A174K,
T17C/L90Y/F92L/S101N/T109L/S110R/A125S/A174K/D203R,
T17Q/L90F/F92G/S101M/A117Q/T136A/D158I/A174R,
V23L/L90F/F92G/A125S/D203V/I222L, T17C/L90Y/F92L/A125S/T136V/D158E/A174K,
V23L/S32Q/Y60I/A117Q/T136A/A174K/V229C, V23T/L90F/F92Y/D203V,
R12K/A24V/Y60H/A174R, T17G/P20T/Y60I/F92G/D203V,
L90Y/F92L/S110R/A125S/D158E/D203V,
T17Q/L90Y/F92L/T109L/T136A/D158E/A174R/D203V/V229C,
R12K/V23T/A24V/S32Q/F92G/D158L/A174R/D203R,
S32M/N87Y/L90F/F92G/A174K/D203V,
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,

F92G/A125S/T136A/D158E/A216P, N87R/F92G/A117N/D203V/A216P,
R12K/A14K/F92G/S110R/A117N/A125S/A174R/D203R,
S32Q/N87F/L90F/F92G/S101M/A114V/A117Q/D203V,
T17Q/A24V/N87M/F92G/A114K/A117N/D158E/D203R/I222L,
N2R/S32Q/L90F/F92G/Q142L/D203V,
A14K/S32Q/L90F/F92G/A114K/A117N/A174R/D203V/A216P/I222L,
R12K/P20T/D158E/D203R/I222L, N2R/A14K/T17A/L90F/F92G/S101H/A174R/D203V,
T17G/A24V/L90F/F92G/Q142W, S32K/L90Y/F92Y/S101A,
V23T/N87I/F92G/A125S/T136A/D158L/D203R, T17S/N87L/V229I,
A24T/L90F/F92L/A125S/T136A/D203V,
R12K/A14K/F92G/T109R/T136A/Q142L/D158E/A174K/D203R/I222L,
A24V/S32Q/Y60A/T136A, D18R/S32Q/N87H/L90Y/F92G/S101K/Q142L/D158I/A174R,
T17A/F21W/S32K/N87F/L90F/F92Y/S101Q/T136A/D203R,
A24V/L90F/F92G/A114V/A117N/T136V/Q142L/D203V/V229C,
T17Q/L90Y/F92G/S101N/T109L/S110R/T136V/Q142L/D203V/A216P,
S32Q/L90Y/F92L/T109K/A125S/T136V,
Y60H/N87K/A114K/A117Q/D158E/A174K/A216P,
P20T/L90Y/T109K/T136A/D158I/D203R, T17L/Y60A/D203V,
N2R/S32Q/L90Y/F92Y/T109K/D203V,
T17H/A24V/S32Q/L90Y/F92L/S101H/A114K/D158I/A174R/D203V,
N2R/S32M/F92Y/T109K/S110R/I222L, 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,
F21W/L90F/F92G/S101A/A114V/A125S/T136V/Q142L/D203V/A216P,
P20T/F21W/S32K/F92G/A125S/V229I, 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/Parent, 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,
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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,
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S27L/V23L/L90Y/F92G/A127S/S193K/N204R/V219I/R236C/R255L,
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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,
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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.

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

15. An expression vector comprising the nucleic acid of claim 14.
16. A host cell comprising the expression vector of claim 15.
17. The host cell according to claim 16, wherein the cell is bacteria, yeast or fungi.
18. A method of making a variant Bhr-PETase enzyme comprising culturing the host cell of claim 16 or 17 under conditions wherein said variant Bhr-PETase enzyme is produced, and recovering said variant Bhr-PETase enzyme.
19. 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.
20. The method according to claim 19, wherein the mechanical pretreatment comprises grinding of the PET into particles.
21. The method according to claim 19, wherein the thermo-mechanical pretreatment comprises extruding the PET at a temperature configured to amorphize and reduce crystallinity of the PET.
22. The method according to claim 19, 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.
23. A method of degrading PET, comprising contacting the PET with the variant Bhr-PETase enzyme of any one of claims 1-13.
24. The method according to claim 17, further comprising pretreating the PET according to the method of any one of claims 19-22.
25. The method according to claim 23 or 24, wherein the method degrades the PET in a mixed plastics composition.
26. The method according to claim 25, wherein the plastics composition comprises analog of PET, PET-like or PET substitute derived biologically or chemically.
27. The method according to claim 25, wherein the plastics composition comprises at least one selected from the group consisting of Polybutylene terephthalate (PBT), Polycarbonate (PC), Polycaprolactone (PCL), Polyethylene Furanoate (PEF), and High Density Polyethylene (HDPE).

28. The method according to any one of claims 23-27, wherein the method excludes sorting plastics to select PET from a mixture of plastics.