CLAIMS

We claim:

1. An engineered deoxyribose-phosphate aldolase comprising a polypeptide sequence having at least ~~85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,~~ 95%~~, 96%, 97%, 98%, 99%, or more~~ sequence identity to SEQ ID NO~~S~~: 2~~, 6, and/or 466,~~ or a functional fragment thereof, wherein said engineered deoxyribose-phosphate aldolase comprises ~~at least one~~ a substitution ~~or substitution set~~ in said polypeptide sequence of X145C/R/K, and wherein said engineered deoxyribose-phosphate aldolase has at least 1.2 fold increased activity on (R)-2-ethynyl-glyceraldehyde as compared to the wild-type polypeptide sequence of SEQ ID NO:2, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2~~, 6, and/or 466~~.

~~2. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:2, wherein said engineered deoxyribose-phosphate aldolase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 10/47/66/141/145/156, 2, 6, 9, 10/47/88/156, 13, 31, 46, 47, 47/134/141/212, 66, 66/88/112/134/141/143/145/212, 71, 72, 88, 94, 102, 104, 112, 116, 133, 133/173/204/235/236, 134, 145, 145/173, 147, 173, 184, 189, 197, 203, 204, 207, 226, 235, 235/236, and 236, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 2.~~

~~3. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO:6, wherein said engineered deoxyribose-phosphate aldolase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 2, 2/6/10/66/88/102/184/235/236, 2/6/102/141/203/235, 2/10/102/235/236, 2/47/66/71/141/184/203/235/236, 2/47/71/88/203/235/236, 2/47/71/141/197/235/236, 2/88/141/235/236, 2/203/235/236, 5, 6/203/235/236, 9, 10/47/66/184/197/236, 10/47/66/235/236, 10/47/71/184/203/235/236, 13, 13/46, 13/46/66/94, 13/46/66/94/184/204, 13/46/66/133/134/184, 13/46/66/184, 13/46/112/134/184, 13/46/133/184, 13/66/94, 13/66/94/184, 13/66/112/133/134/184/204, 13/66/112/133/134/204, 13/66/112/184, 13/66/133/134/184/204, 13/66/184/204, 13/94/133/184, 13/94/184, 13/94/184/204, 13/133/134/184, 13/133/134/204, 13/134/204, 13/184, 27, 40, 46/66/94/112, 46/66/112/133/134/184, 46/66/133/134, 46/66/133/184/197, 46/66/133/197, 46/66/134/184/197/204, 46/66/184, 46/112/133, 46/112/133/134/204, 46/133/204, 46/197/204, 47/66/71/88/203/235/236, 47/66/71/141/184, 47/66/88/184/235, 47/66/88/203/235/236, 47/66/141/235/236,~~

47/66/184/197/203/235/236, 47/66/184/197/236, 47/71/88/184/203/235, 47/71/88/184/203/236, 47/71/141/184/203/235, 47/71/184, 47/71/184/197/236, 47/71/184/235, 47/71/184/236, 47/88/102/141/235/236, 47/88/203/235/236, 47/88/235/236, 47/102/184/197/235/236, 47/102/203/235/236, 47/141/184, 47/141/184/236, 47/184/236, 47/203/235, 47/203/235/236, 47/203/236, 47/235/236, 62, 66, 66/88/102/184/197/203/235/236, 66/88/197, 66/88/235, 66/88/235/236, 66/94, 66/94/112/133/184, 66/94/112/184/204, 66/102/184/235/236, 66/112/133/134/197, 66/112/133/197, 66/112/134/197, 66/112/184, 66/133/184, 66/133/197, 66/184, 66/197/203/235, 84, 88, 88/102/141/184/235/236, 88/184, 88/184/197/203/235/236, 88/184/197/235/236, 88/184/203/235, 88/184/236, 88/197/235/236, 88/236, 94/112/184/197, 94/133/184, 96, 102/141/203, 102/184/203/236, 102/184/236, 102/197/235, 112, 112/133/134, 112/133/184/204, 112/134/197, 114, 115, 120, 127, 132, 133, 133/134/184, 141/184/203/235/236, 141/184/235, 141/197, 141/203/235/236, 141/236, 146, 148, 184, 184/203, 184/203/235, 184/203/236, 184/235/236, 184/236, 197/235, 203/235/236, 203/236, 204, 218, 235, 235/236, and 236, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 6.

4. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said polypeptide sequence has at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 466, wherein said engineered deoxyribose-phosphate aldolase comprises at least one substitution or substitution set in said polypeptide sequence at one or more positions selected from 71/88/94/133, 71/133, and 133, and wherein the amino acid positions of said polypeptide sequence are numbered with reference to SEQ ID NO: 466.

5. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said engineered deoxyribose-phosphate aldolase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered deoxyribose-phosphate aldolase variant set forth in Table 3.1, 4.1, and/or 6.1.

6. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said engineered deoxyribose-phosphate aldolase comprises a variant engineered polypeptide provided in Table 3.1, 4.1, and/or 6.1.

7. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said engineered deoxyribose-phosphate aldolase comprises a polypeptide sequence that is at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or more identical to the sequence of at least one engineered deoxyribose-phosphate aldolase variant set forth in SEQ ID NO: 2, 6, and/or 466.

~~8. The engineered deoxyribose-phosphate aldolase of Claim 6, wherein said engineered deoxyribose-phosphate aldolase is a variant engineered polypeptide set forth in SEQ ID NO: 6 and/or 466.~~

~~9~~2. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said engineered deoxyribose-phosphate aldolase comprises a polypeptide sequence that is at least ~~85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%,~~ 95%~~, 96%, 97%, 98%, 99% or more~~ identical to the sequence of at least one engineered deoxyribose-phosphate aldolase variant set forth in the even numbered sequences of SEQ ID NOS: ~~2~~6, 12, 42, 104, or 140-478.

~~10~~3. The engineered deoxyribose-phosphate aldolase of Claim 1, wherein said engineered deoxyribose-phosphate aldolase comprises a polypeptide sequence forth in the even numbered sequences of SEQ ID NOS: ~~2~~6, 12, 42, 104, or 140-478.

~~11. The engineered deoxyribose-phosphate aldolase of any of Claims 1-10, wherein said engineered deoxyribose-phosphate aldolase exhibits at least one improved property compared to wild-type *Shewanella halifaxensis* deoxyribose-phosphate aldolase.~~

~~12. The engineered deoxyribose-phosphate aldolase of Claim 11, wherein said improved property comprises improved activity on a substrate.~~

~~13. The engineered deoxyribose-phosphate aldolase of Claim 12, wherein said substrate comprises R-2-ethynyl-glyceraldehyde.~~

~~14. The engineered deoxyribose-phosphate aldolase of Claim 11, wherein said improved property comprises improved thermostability.~~

~~15~~4. The engineered deoxyribose-phosphate aldolase of ~~any of~~ Claim~~s~~ 1~~-14~~, wherein said engineered deoxyribose-phosphate aldolase is purified.

~~16~~5. A composition comprising at least one engineered deoxyribose-phosphate aldolase provided in ~~any of~~ Claim~~s~~ 1~~-15~~.

~~17~~6. A polynucleotide sequence encoding at least one engineered deoxyribose-phosphate aldolase of ~~any of~~ Claim~~s~~ 1~~-15~~.

~~18~~7. An engineered polynucleotide sequence encoding at least one engineered deoxyribose-phosphate aldolase of Claim 1, wherein said polynucleotide sequence comprises at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 1, 5, and/or 465, wherein the polynucleotide sequence of said engineered deoxyribose-phosphate aldolase comprises at least one substitution at one or more positions.

~~19~~8. A engineered polynucleotide sequence encoding at least one engineered deoxyribose-phosphate aldolase of Claim 1 comprising at least 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or more sequence identity to SEQ ID NO: 1, 5, and/or 465, or a functional fragment thereof.

~~20~~9. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~17-19~~7, wherein said engineered polynucleotide sequence comprises a sequence forth in the odd numbered sequences of SEQ ID NOS: 1-477.

~~21~~10. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~17-20~~7, wherein said polynucleotide sequence is operably linked to a control sequence.

~~22~~11. The engineered polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~17-21~~7, wherein said engineered polynucleotide sequence is codon optimized.

~~23~~12. An expression vector comprising at least one polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~17-22~~6.

~~24~~13. A host cell comprising at least one expression vector of Claim ~~23~~12.

~~25~~14. A host cell comprising at least one polynucleotide sequence of ~~any of~~ Claim~~s~~ ~~17-22~~6.

~~26~~15. A method of producing an engineered deoxyribose-phosphate aldolase in a host cell, comprising culturing the host cell of Claim ~~24 and/or 25~~13, in a culture medium under suitable conditions, such that at least one engineered deoxyribose-phosphate aldolase is produced.

~~27~~16. The method of Claim ~~26~~15, further comprising recovering at least one engineered deoxyribose-phosphate aldolase from the culture medium and/or host cell.

~~28~~17. The method of Claim ~~26 and/or 27~~16, further comprising the step of purifying said at least one engineered deoxyribose-phosphate aldolase.