a subject who has been provided with at least one composition comprising at least one engineered polypeptide having phenylalanine ammonia-lyase (PAL) activity as provided herein) is able to eat a diet that is less restricted in its methionine, phenylalanine and/or tyrosine content than diets required by subjects who have not been provided at least one composition as provided herein. In some embodiments, the composition provided to the subjects comprises a pharmaceutical composition, while in some alternative embodiments, the composition comprises a dietary/nutritional supplement. The present invention also provides treated subjects, wherein the subject has been administered at least one composition and/or pharmaceutical composition comprising at least one engineered polypeptide having phenylalanine ammonia-lyase (PAL) activity as provided herein. In some embodiments, the subject is an animal selected from primates, rodents, and lagamorphs. In some additional embodiments, the subject is a mouse. In some further embodiments, the subject is a human. In still some further embodiments, the subject is a human infant or child, while in some alternative embodiments, the subject is a human adult or young adult. I

[0062] The present invention also provides uses of the compositions comprising at least one engineered polypeptide having phenylalanine ammonia-lyase (PAL) activity provided herein.

BRIEF DESCRIPTION OF THE DRAWINGS

[0063] Figure 1 provides an alignment of wild-type PAL protein sequences: *Anabaena variabilis* PAL (NCBI YP_ 324488.1 (SEQ ID NO:4)); *Nostoc punctiforme* phenylalanine/histidine ammonia lyase "NpPHAL" (NCBI YP_001865631.1 (SEQ ID NO:30); *Rivularia sp.* histidine ammonia-lyase "RspHAL" (NCBI YP_007056096.1 (SEQ ID NO:31); *Oscillatoria sp.* histidine ammonia-lyase "Osp HAL" (NCBI YP_07108482.1(SEQ ID NO:32); and *Gloeocapsa sp.* histidine ammonia-lyase "GspHAL" (NCBI YP_007127054.1) (SEQ ID NO:33).

[0064] Figure 2A shows the reduced sensitivity to proteolysis (expressed as chymotrypsin and trypsin tolerance) as compared to wild-type AvPAL tested at pH 7.0 for Variant No. 22 (SEQ ID NO:8), Variant No. 30 (SEQ ID NO:6) and Variant No. 36 (SEQ ID NO:10) as further described in Example 4.

[0065] Figure 2B provides a graph showing an increased tolerance to acidic pH as compared to wild-type AvPAL tested at pH 4.0 to 5.2, for Variant Nos. 22, 30 and 36 as further described in Example 4.

[0066] Figure 3 provides K_M results for wild-type PAL and Variant No. 36.

[0067] Figure 4 provides data showing the amino acid specificity of wild-type PAL and Variant No. 36.

[0068] Figure 5 provides results showing the relative stability of wild-type PAL and Variant No. 36 exposed to human chymotrypsin and trypsin.

[0069] Figure 6 provides results showing the relative stability of wild-type PAL, and Variant Nos. 36, 42, and 43 exposed to porcine pancreatic extract.

Table 2-1.
Relative Activity of PAL Variants When Unchallenged (U-C), Protease-Challenged (P-C), and Acid-Challenged (A-C).

Variant #	U-C	P-C	A-C	Amino Acid Differences Relative to SEQ ID NO:4
10	+	+	++	A39V/A91V/Y158H/S180A/K195E/A256G/S399N/ L407V/Y459F/T463N/K522N/T524S
11	+	+	++	A39V/A91V/Y158H/S180A/K195E/A256G/ N290G/S399N/Y459F/T463N
12	+	++	++	A39V/A91V/Y158H/S180A/K195E/T243I/A394V
13	+	++	++	A39V/A91V/Y158H/K195E/T243I/A256G/A394V/ S399N/N474Q/K522Y/T524S
14	+	++	++	A39V/A91V/Y158H/S180A/K195E/I245L/S399N/ L407V/Y459F/T463N/N474Q/T524S
15	+	++	+	H307M
16	+	+	++	A39V/A91V/Y158H/S180A/T243L/A394V/S399N/ T463N/K522F
17	+	+	++	A91V/N474Q
18	++	+	++	S180A/K195E
19	++	+	++	A91V/Y158H/T243I/A256G/N290G/S399N/L407V/ Y459F/ T463N/N474Q/K522N/T524S
20	++	++	++	A39V/Y158H/S180A/S399N
21	++	++	++	A91V/N270K
22	++	+++	++	H307G
23	++	++	+	N453G
24	++	++	++	H307Q
25	++	++	+	L257W
26	++	+	+++	A91V/Y158H/I245L/A256G/S399N/Y459F/T463N/ K522Y/T524S
27	++	++	+	F443H
28	++	++	++	A91V/Y158H/S180A
29	++	++	+	A91Y/ Y158H/ K195E/ S399N
30	++	++	+++	A39V/ A91V/ A256G/ N290G/ A394V/ S399N/ P404A/ L407V/K522Y/ T524S
31	++	++	+	L257A
32	++	+	+++	Y158H/K195E/T243L/A256G/A394V/S399N/ L407V/N474Q/ K522F/T524S
33	++	++		L349M
34	+++	+++	+	I326F
35	+++	+++	+++	T460G/ P528L

1. Relative activity was calculated as activity of the variant/activity of SEQ ID NO:4 (encoded by SEQ ID NO:3).
2. Variant # 22 has the polynucleotide sequence of SEQ ID NO:7 and polypeptide sequence of SEQ ID NO:8, and Variant # 30 has polynucleotide sequence of SEQ ID NO:5 and polypeptide sequence of SEQ ID NO:6.
3. + = 0.1 to 1.0 relative activity over wild-type AvPAL;
++ = >1.0 to 2.0 relative activity over wild-type AvPAL; and
+++ = >2.0 relative activity over wild-type AvPAL.

Table 2-4.
Relative Activity of PAL Variants Unchallenged (U-C), Protease-Challenged (P-C), and Acid-Challenged (A-C).

Variant #	U-C	P-C	A-C	Mutations Relative to Variant #30
53	+	+	+	D303R
54	+	+	++	E308A
55	+	+	+	E308D
56	++	++	+	G256A
57	+	++	+	H307A
58	+	+++	+	H307D
59	+	++	+	H307E
60	+	++	+	H307F
61	+	+++	+	H307G
62	+	+	+	H307I
63	+	+	+	H307L
64	++	+++	++	H307M
65	+	++	+	H307N
66	++	++	++	H307R
67	+	+	+	H307Y
68	+	+++	+	R305L
69	+	+++	+	R305M
70	+	+++	+	R305Q
71	++	++	+	V91A/G256A
72	+	++	+	Y304H
73	+	+	+	Y304W
74	+	+	+	C503K
75	+	+	+	C503Q
76	+	+	+	C565A
77	+	+	+	C565G
78	+	+	+	C565I
79	+	+	+	C565K
80	+	+	+	C565L
81	+	++	+	C565N
82	+	+	+	C565P
83	+	+	+	C565T

1. Relative activity was calculated as activity of variant/ activity of Variant No. 30 (Variants 53-73) or SEQ ID NO:4 (encoded by SEQ ID NO:3) (Variants 74-83.)

2. + = > 0.5 to 1.5 relative activity over Variant 30; ++ = > 1.5 to 3 relative activity over Variant 30; and +++ = > 3 to 10 relative activity over Variant 30.

The Claims defining the invention are as follows:

1. An engineered polypeptide comprising: a) an amino acid sequence having at least 90% sequence identity to reference sequence SEQ ID NO:4, where said amino acid sequence comprises an amino acid residue difference at position H307G/Q/M; and wherein said engineered polypeptide further comprises at least one or more of the following substitutions A39V, T54K, G59R, S73K, A112C, R134Q, A91V, Y158H, S180A, K195E, Q240R/W, T243I/L, I245L, A256G, L257W/A, N270K, N290G, Y304H, R305M, E308Q, I326F, L349M, D353A/N, L364Q, A394V, S399N, N400K, P404A, L407V, F443H, N453G, Y459F, T460G, T463N, N474Q, E509L, Q521K/S, K522Y/F/N, T524S, P528L, S546R, and P564G/L/M, when optimally aligned with the polypeptide of SEQ ID NO:4, and wherein said engineered polypeptide b) exhibits an improved property selected from i) reduced sensitivity to proteolysis, ii) increased tolerance to acidic pH, iii) reduced immunogenicity, or a combination of any of i), ii), or iii), as compared to the reference sequence SEQ ID NO:4.

2. The engineered polypeptide of Claim 1, wherein the improved property is selected from reduced sensitivity to proteolysis and/or increased tolerance to acidic pH.

3. The engineered polypeptide of Claim 1, wherein the engineered polypeptide has at least 90% sequence identity to reference SEQ ID NO:4.

4. The engineered polypeptide of Claim 1, wherein said engineered polypeptide is resistant to proteolysis, acid stable, and/or deimmunized.

5. The engineered polypeptide of Claim 4, wherein said engineered polypeptide is resistant to proteolysis by at least one digestive tract enzyme, wherein said engineered polypeptide is resistant to proteolysis by chymotrypsin, trypsin, carboxypeptidases, and/or elastases.

6. The engineered polypeptide of Claim 1, wherein said engineered polypeptide is deimmunized.

7. The deimmunized engineered polypeptide of Claim 6, wherein said polypeptide comprises an amino acid sequence having at least 90% sequence identity to SEQ ID NO:8.

8. The engineered polypeptide of Claim 1, wherein said polypeptide is purified.

9. A polynucleotide sequence encoding at least one engineered polypeptide of Claim 1.

10. The polynucleotide sequence of Claim 9, wherein said polynucleotide sequence is operably linked to a control sequence.

11. The polynucleotide sequence of Claim 9, wherein said polynucleotide sequence is codon-optimized.

12. An expression vector comprising at least one polynucleotide sequence of Claim 9 and at least one control sequence.

13. The expression vector of Claim 12, wherein said control sequence is a promoter.

14. A host cell transformed with at least one expression vector of Claim 12.

15. A method of producing an engineered polypeptide in a host cell comprising culturing a host cell comprising at least one expression vector of Claim 12, under suitable culture conditions, such that at least one engineered PAL polypeptide is produced.

16. The method of Claim 15, further comprising recovering at least one engineered polypeptide from the culture and/or host cells.

17. The method of Claim 16, further comprising the step of purifying said at least one engineered polypeptide.

18. A composition comprising at least one engineered polypeptide of Claim 1.

19. The composition of Claim 18, wherein said composition is a pharmaceutical composition.

20. The composition of Claim 19, wherein said composition is suitable for the treatment of phenylketonuria.

21. The pharmaceutical composition of Claim 20, wherein said composition is suitable for oral administration to a human.

22. The pharmaceutical composition of Claim 21, wherein said composition is in the form of a pill,

tablet, capsule, gelcap, liquid, or emulsion.

23. The pharmaceutical composition of Claim 22, wherein said pill, tablet, capsule, or gelcap further comprises an enteric coating.

24. The pharmaceutical composition of Claim 20, wherein said composition is suitable for parenteral injection into a human.