isolated from plants that grow in tropical rainforests. Although most of them share no sequence homology or structural similarity, thaumatin shares extensive similarity at the protein sequence level with certain non-sweet proteins found in other plants. No sweet-taste modifying proteins are known from fungi.

[0007] There remains a need in the art to produce new low or zero calorie sweeteners with improved tastes from natural sources. There remains a need in the art to economically produce such sweetening compositions from potential sources of the same, particularly from ascomycetes fungal species.

SUMMARY

[0008] The disclosure relates to newly identified fungally-derived sweet proteins, and to the genes and cDNA encoding said proteins, also called *MYD*/Myd herein. More particularly, the disclosure relates to newly identified sweet-tasting proteins, to the genes and cDNA encoding said proteins, and to methods of using such proteins, genes, and cDNA in the modulation of the taste of foods. The disclosure provides in particular a DNA sequence encoding a novel sweet protein identified herein as *MYD1* and the corresponding polypeptide Myd1 (also referred to as mycodulcein). Myd1 is the first sweet-tasting protein identified from fungi. Myd1 reduces the sourness, bitterness or astringency of foods and drinks and additionally Myd1 has an activity to enhance the taste of foods and drinks, namely a taste-modifying activity.

[0009] The disclosure provides a polynucleotide (e.g., isolated polynucleotide) encoding a polypeptide having sweet-taste modulation activity, wherein the polynucleotide sequence encodes a polypeptide selected from the group consisting of (a) a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, and SEQ ID NO:75; (b) a polypeptide having at least 80% sequence identity to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; and (c) a polypeptide sequence modified from the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID

NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 by deletion, insertion, substitution, or addition of no more than 24 amino acids. In one aspect, the present disclosure refers to an isolated polynucleotide encoding a polypeptide having sweet-taste modulation activity, wherein the polynucleotide sequence encodes a polypeptide selected from the group consisting of: (a) a polypeptide sequence selected from the group consisting of SEQ ID NO: 3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, and SEQ ID NO:75; (b) a polypeptide having at least 80% sequence identity to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; and (c) a polypeptide sequence modified from the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17 by deletion, insertion, substitution, or addition of no more than 24 amino acids.

[0009a] In one aspect, the present disclosure refers to an isolated polynucleotide wherein the polynucleotide sequence comprises a polynucleotide selected from the group consisting of: (a) the nucleic acid sequence set forth in SEQ ID NO:2; and (b) a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:2, wherein the polynucleotide encodes a polypeptide having sweet-taste modulation activity.

[0010] In one example, the polypeptide sequence of the polypeptide having sweet-taste modulation activity is the polypeptide sequence set forth in SEQ ID NO:3, or a polypeptide sequence having at least 80% sequence identity to SEQ ID NO:3. In another example, the polypeptide encoding a polypeptide having sweet-taste modulation activity is not the polypeptide of SEQ ID NO:3.

[0011] In another example, the polypeptide sequence comprises amino acid residues 1-11, 17-32, 39, 40, 45-67, 73-100, and 110-121 of SEQ ID NO:3.

[0012] The disclosure also provides a polynucleotide (e.g., isolated polynucleotide) selected from the group consisting of: (a) a polynucleotide comprising the nucleic acid sequence set forth in SEQ ID NO:2 with at least one substitution modification; (b) a polynucleotide comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence set forth in SEQ ID NO:2, wherein the polynucleotide is not the polynucleotide of SEQ ID NO:2, and (c) a polynucleotide comprising (i) the nucleic acid sequence set forth in SEQ ID NO:2 and (ii) a nucleotide sequence encoding a histidine tag, wherein the polynucleotide encodes a polypeptide having sweet-taste modulation activity.

[0013] In one example, the polypeptide sequence of the polypeptide having sweet-taste modulation activity comprises SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, or SEQ ID NO:75. In particular, the polypeptide sequence of the polypeptide having sweet-taste modulation activity comprises SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO; 66, or SEQ ID NO: 68.

[0014] In a particular example, the polynucleotide comprises the nucleic acid sequence of SEQ ID NO: 23, SEQ ID NO: 25, SEQ ID NO: 29, SEQ ID NO: 37, SEQ ID NO: 41, SEQ ID NO: 43, SEQ ID NO: 45, SEQ ID NO: 49, SEQ ID NO: 51, SEQ ID NO: 53, SEQ ID NO: 57, SEQ ID NO: 59, SEQ ID NO: 61, SEQ ID NO; 63, SEQ ID NO: 65, or SEQ ID NO: 67.

[0015] The polynucleotide encoding the polypeptide having sweet-taste modulation activity is optionally operably linked to a heterologous regulatory element. Additionally or

alternatively, the polynucleotide sequence further encodes a protein tag or label. The protein tag is optionally an affinity tag, The protein is optionally a histidine tag.

[0016] In one example, the polynucleotide comprises SEQ ID NO: 20, which corresponds to the coding sequence for His tagged mycodulcein in *E. coli* (wherein residues 364-381 correspond to an optional His tag sequence). SEQ ID NO: 20 is codon-optimized for expression in *E. coli*. In another example, the polynucleotide comprises SEQ ID NO: 22, which corresponds to the coding sequence for His tagged mycodulcein in *S. cerevisiae* (wherein residues 364-381 correspond to an optional His tag sequence). SEQ ID NO: 22 is codon-optimized for expression in *S. cerevisiae*. The corresponding polypeptide of SEQ ID NO: 21 corresponds to the His-tagged mycodulcein protein (wherein residues 122-127 correspond to the optional His tag sequence), which polypeptide sequence is the same for expression in *E. coli* and *S. cerevisiae*.

[0017] An expression cassette comprising the polynucleotide and a vector comprising the polynucleotide, as well as a host cell transformed with the vector is provided. In one aspect, the present disclosure refers to an expression cassette comprising any one of the isolated polynucleotides disclosed herein. In another aspect, the present disclosure refers to an expression cassette comprising any one of the polynucleotides disclosed herein. In one aspect, the present disclosure refers to a vector comprising any one of the isolated polynucleotides disclosed herein. In another aspect, the present disclosure refers to a vector comprising the polynucleotide disclosed herein. In one aspect, the present disclosure refers to a host cell transformed with the vector disclosed herein. A method of producing a protein having sweet-taste modulation activity, comprising culturing the host cell in a medium under conditions that result in producing the protein having sweet-taste modulation activity also is provided. In one aspect, the present disclosure refers to a method of producing a protein having sweet-taste modulation activity, comprising culturing the host cell of disclosed herein in a medium under conditions that result in producing the protein having sweet-taste modulation activity.

[0018] The disclosure includes a polypeptide (e.g., isolated polypeptide) comprising a polypeptide sequence having at least 80% sequence identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; wherein the polypeptide optionally contains at least one substitution or modification relative to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally further comprises a protein tag, particularly a histidine tag, and wherein the polypeptide has sweet-taste modulation activity. In one aspect, the present disclosure refers to an isolated polypeptide comprising a polypeptide sequence having at least 80% sequence identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally contains at least one substitution modification relative to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, or wherein the polypeptide optionally further comprises a histidine tag, and wherein the polypeptide has sweet-taste modulation activity.

[0019] In one example, the polypeptide comprises SEQ ID NO:3 or polypeptide sequence with at least 80% sequence identity to SEQ ID NO:3. In another example, the polypeptide is not the polypeptide of SEQ ID NO:3.

[0020] In yet another example, the polypeptide comprises amino acid residues 1-11, 17-32, 39, 40, 45-67, 73-100, and 110-121 of SEQ ID NO:3.

[0021] In one example, the polypeptide (e.g., isolated polypeptide) comprises a polypeptide sequence selected from the group consisting of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, and SEQ ID NO:75, wherein the polypeptide optionally is not SEQ ID NO: 3. In particular, the polypeptide comprises the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO; 66, or SEQ ID NO: 68.

[0022] The disclosure provides a composition, comprising a combination of (a) a product for oral administration, wherein the product is not *Mattirolomyces terfezioides* truffle, and (b) a sweetening composition comprising the polypeptide, wherein the combination has enhanced sweet taste compared to the product for oral administration. In one example, the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. In another example, the polypeptide has a polypeptide sequence having at least 80% sequence identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; wherein the polypeptide optionally contains at least one substitution modification relative to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally is not the polypeptide of SEQ ID NO:3, wherein the polypeptide optionally further comprises a histidine tag, wherein the polypeptide has sweet-taste modulation activity. For example, the polypeptide in the sweetening composition comprises the amino acid sequence of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, or SEQ ID NO:75 and optionally does not consist of SEQ ID NO:3. In particular, the polypeptide comprises the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO; 66, or SEQ ID NO: 68.

[0023] In one example, the disclosure provides a sweetening composition comprising a polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally is a polypeptide other than the polypeptide of the amino acid sequence of SEQ ID NO:3.

[0023a] In one aspect, the present disclosure refers to a composition, comprising a combination of (a) a product for oral administration, wherein the product is not *Mattirolomyces terfezioides* truffle, and (b) a sweetening composition comprising an isolated polypeptide, wherein: (i) the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; or (ii) the polypeptide is any one of the polypeptides disclosed herein; wherein the combination has enhanced sweet taste compared to the product for oral administration.

[0024] The disclosure provides a method for modulating the taste of a product for oral administration, comprising combining the product for oral administration with an effective amount of a sweetening composition comprising the polypeptide, wherein the product for oral administration is not *Mattirolomyces terfezioides* truffle, and wherein the combination has enhanced sweet taste compared to the product for oral administration. In one example, the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. In another example, the polypeptide has a polypeptide sequence having at least 80% sequence identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; wherein the polypeptide optionally contains at least one substitution modification relative to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally is not the polypeptide of SEQ ID NO:3, wherein the polypeptide optionally further comprises a histidine tag, wherein the polypeptide has sweet-taste modulation activity. For example, the polypeptide in the sweetening composition comprises the amino acid sequence of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, or SEQ ID NO:75 and optionally does not consist of SEQ ID NO:3. In particular, the polypeptide comprises the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO; 66, or SEQ ID NO: 68.

[0024a] In one aspect, the present disclosure refers to a method for modulating the taste of a product for oral administration, comprising: combining the product for oral administration with an effective amount of a sweetening composition comprising an isolated polypeptide, wherein: (i) the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; or (ii) the polypeptide is any one of the polypeptides disclosed herein, wherein the product for oral administration is not *Mattirolomyces terfezioides* truffle, and wherein the combination has enhanced sweet taste compared to the product for oral administration.

[0025] The product for oral administration can be a food, a beverage, a dietary supplement composition, or a pharmaceutical composition. Examples of food products include, but are not limited to, baked goods; sweet bakery products, pre-made sweet bakery mixes for preparing sweet bakery products; pie fillings and other sweet fillings, gelatins and puddings; frozen desserts; yogurts; snack bars; bread products; pre-made bread mixes for preparing bread products; sauces, syrups and dressings; sweet spreads; confectionary products; and sweetened breakfast cereals. Examples of beverage product include but are not limited to carbonated beverages; non-carbonated beverages; and beverage concentrates.

[0026] The disclosure also provides a method of purifying a polypeptide having sweet-taste modulation activity comprising (a) obtaining a composition comprising the polypeptide, and (b) purifying the composition via hydrophobic interaction chromatography (HIC) followed by size exclusion chromatography (SEC). In one example, the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17. In another example, the polypeptide has a polypeptide sequence having at least 80% sequence identity to a polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; wherein the polypeptide optionally contains at least one substitution modification relative to the polypeptide sequence selected from the group consisting of SEQ ID NO:3, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17, wherein the polypeptide optionally is not the polypeptide of SEQ ID NO:3, wherein the polypeptide optionally further comprises a histidine tag, wherein the polypeptide has sweet-taste modulation activity. For example, the polypeptide comprises the amino acid sequence of SEQ ID NO:71, SEQ ID NO:72, SEQ ID NO:73, SEQ ID NO:74, or SEQ ID NO:75 and optionally does not consist of SEQ ID NO:3. In particular, the polypeptide comprises the amino acid sequence of SEQ ID NO: 24, SEQ ID NO: 26, SEQ ID NO: 30, SEQ ID NO: 38, SEQ ID NO: 42, SEQ ID NO: 44, SEQ ID NO: 46, SEQ ID NO: 50, SEQ ID NO: 52, SEQ ID NO: 54, SEQ ID NO: 58, SEQ ID NO: 60, SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO; 66, or SEQ ID NO: 68.

[0026a] In one aspect, the present disclosure refers to a method for purifying a polypeptide having sweet-taste modulation activity comprising: (a) obtaining a composition comprising the polypeptide, (b) purifying the composition via hydrophobic interaction chromatography (HIC) followed by size exclusion chromatography (SEC), wherein: (i) the polypeptide comprises an amino acid sequence having at least 80% sequence identity to a polypeptide selected from the group consisting of SEQ ID NO:3, SEQ ID NO:7, SEQ ID NO:8, SEQ ID NO:9, SEQ ID NO:10, SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, SEQ ID NO:15, SEQ ID NO:16, and SEQ ID NO:17; or (ii) the polypeptide is any one of the polypeptides disclosed herein.

[0027] Other aspects and examples of the disclosure will be apparent on review of the figures, detailed description and non-limiting examples herein.

BRIEF DESCRIPTION OF THE FIGURES

[0028] Figure 1 shows a predicted three dimensional structure of Myd1 based on sequence data using the PHYRE2.0 protein folding prediction tool.

[0029] Figure 2 shows a Coomassie-stained SDS-PAGE gel of proteins obtained from fractions of partially purified *M. terfezioides* gleba.

[0030] Figure 3 shows a SDS-PAGE gel, Coomassie stained, of purification steps of SEQ ID NO:21 expressed in *E. coli*. lane 1: molecular weight standards; lane 3, crude lysate; lane 4, flow through fraction from HisPur™ Ni-NTA; lane 5, wash 1; lane 6, wash 2; lane 7, wash 3; lane 8, elution fraction.

[0031] Figure 4 shows the concentration-response functions for sweet taste for mycodulcein, aspartame, thaumatin, rebaudioside A. Data are plotted as the proportion (*p*) of responses occurring on the 200 mM sucrose-associated ("sweet") target. Each data point in the curves for mycodulcein, aspartame, thaumatin, rebaudioside A was calculated as the average across 32 replicates and averaged across 16 replicates for the sucrose curve; error bars are SEM. Points for water and sucrose controls were similarly calculated as the average across 128 and 64 replicates, respectively. Curves were fit by non-linear regression.

[0032] Figure 5A shows a comparison of mycodulcein predicted tertiary structure to the known crystal structures of thaumatin (PDB: 1RQW), monellin (PDB: 2O9U), brazzein (PDB: 1BRZ), and chicken egg white lysozyme (PDB: 1LSN) (protein data base), showing that mycodulcein has predicted tertiary structure similarity to other known sweet proteins, all containing antiparallel beta-sheets with an alpha-helix parallel to the beta sheets..

[0033] Figure 5B shows a representation of SEQ ID NO:3 predicted secondary structure superimposed on putative secondary structure motifs and location of point mutations within each motif.

[0034] Figure 6 shows results of mutant his-tagged mycodulcein compared with each other and with his-tagged non-mutant mycodulcein, equalized to equal protein concentration as measured by ELISA, for sweetness intensity, time of onset of sweetness perception, and for duration of sweetness perception.