

SUMMARY

[0005a] In one aspect, the present disclosure provides for a host cell comprising a transgene encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT comprises an amino acid sequence comprising SEQ ID NO: 2.

[0005b] In another aspect, the present disclosure provides for a method of producing gastrodin in a host cell, the method comprising culturing the host cell in cell culture medium comprising 4-hydroxybenzyl alcohol, wherein the host cell expresses a transgene that encodes a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT comprises an amino acid sequence - comprising SEQ ID NO: 2.

[0005c] In another aspect, the present disclosure provides for a vector comprising a nucleic acid encoding a uridine 5'-diphospho-glucosyltransferase (UGT) for converting 4-hydroxybenzyl alcohol into gastrodin, wherein the uridine 5'-diphospho-glucosyltransferase (UGT) comprises SEQ ID NO: 2.

[0005d] In another aspect, the present disclosure provides for a method of making a transgenic host cell, the method comprising introducing a vector into a host cell, the vector comprising a nucleic acid encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT comprises an amino acid sequence comprising SEQ ID NO: 2.

[0006] In an example, the present disclosure provides for a host cell including a transgene encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino acid sequence identity to SEQ ID NO: 2, and wherein the amino acid sequence of the heterologous UGT includes one or more of: M at position 88 of SEQ ID NO: 2; F, Y, or W at position 119 of SEQ ID NO: 2; F, Y, or W at position 145 of SEQ ID NO: 2; L or M at position 149 of SEQ ID NO: 2; M at position 198 of SEQ ID NO: 2; and F at position 383 of SEQ ID NO: 2.

[0007] In another example, the disclosure provides for a host cell including a transgene encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino acid sequence identity to SEQ ID NO: 28, and wherein the amino acid

sequence of the heterologous UGT includes one or more of: M at position 93 of SEQ ID NO: 28; F, Y or W at position 129 of SEQ ID NO: 28; F, Y or W at position 150 of SEQ ID NO: 28; L or M at position 154 of SEQ ID NO: 28; M at position 203 of SEQ ID NO: 28; and F at position 391 of SEQ ID NO: 28.

[0008] In another example, the disclosure provides for a method of producing gastrodin in a host cell, the method including culturing the host cell in cell culture medium including 4-hydroxybenzyl alcohol, wherein the host cell expresses a transgene that encodes a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino acid sequence identity to SEQ ID NO: 2, and wherein the amino acid sequence of the heterologous UGT includes one or more of: M at position 88 of SEQ ID NO: 2; F, Y, or W at position 119 of SEQ ID NO: 2; F, Y or W at position 145 of SEQ ID NO: 2; L or M at position 149 of SEQ ID NO: 2; M at position 198 of SEQ ID NO: 2; and F at position 383 of SEQ ID NO: 2.

[0009] In another example, the disclosure provides for a method of producing gastrodin in a host cell, the method including culturing the host cell in cell culture medium including 4-hydroxybenzyl alcohol, wherein the host cell expresses a transgene that encodes a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino

acid sequence identity to SEQ ID NO: 28, and wherein the amino acid sequence of the heterologous UGT includes one or more of: M at position 93 of SEQ ID NO: 28; F, Y or W at position 129 of SEQ ID NO: 28; F, Y or W at position 150 of SEQ ID NO: 28; L or M at position 154 of SEQ ID NO: 28; M at position 203 of SEQ ID NO: 28; and F at position 391 of SEQ ID NO: 28.

[0010] In another example, the disclosure provides for a vector including a nucleic acid encoding a gastrodin synthase for converting 4-hydroxybenzyl alcohol into gastrodin, wherein the gastrodin synthase can have at least about 75% amino acid sequence identity to SEQ ID NO: 2.

[0011] In another embodiment, the disclosure provides for a vector including a nucleic acid encoding a gastrodin synthase for converting 4-hydroxybenzyl alcohol into gastrodin, wherein the nucleic acid can have at least about 75% amino acid sequence identity to SEQ ID NO: 28.

[0012] In another example, the disclosure provides for a method of making a transgenic host cell, the method including introducing a vector into a host cell, the vector including a nucleic acid encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino acid sequence identity to SEQ ID NO: 2, and wherein the amino acid sequence of the heterologous UGT includes one or more of: M at position 88 of SEQ ID NO: 2; F, Y or W at position 119 of SEQ ID NO: 2; F, Y or W at position 145 of SEQ ID NO: 2; L or M at position 149 of SEQ ID NO: 2; M at position 198 of SEQ ID NO: 2; and F at position 383 of SEQ ID NO: 2.

[0013] In another example, the disclosure provides for a method of making a transgenic host cell, the method including introducing a vector into a host cell, the vector including a nucleic acid encoding a heterologous uridine 5'-diphospho-glucosyltransferase (UGT) operably linked to a promoter, wherein the heterologous UGT includes an amino acid sequence having at least 75% amino acid sequence identity to SEQ ID NO: 28, and wherein the amino acid sequence of the heterologous UGT includes one or more of: M at position 93 of SEQ ID NO: 28; F, Y or W at position 129 of SEQ ID NO: 28; F, Y or W at position 150 of SEQ ID NO: 28; L or M at position 154 of SEQ ID NO: 28; M at position 203 of SEQ ID NO: 28; and F at position 391 of SEQ ID NO: 28.

[0014] In another example, the disclosure provides for a pharmaceutical composition including gastrodin, wherein said gastrodin is produced by a transgenic plant or plant cell, fungal cell, yeast cell, insect cell, or bacterial cell.

BRIEF DESCRIPTION OF THE DRAWINGS

[0015] The foregoing will be apparent from the following more particular description of example embodiments, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale, emphasis instead being placed upon illustrating embodiments.

[0016] **FIG. 1** shows transformation of 4-hydroxybenzyl alcohol into gastrodin. The use of UDP-glucose sugar transferase (UGT) enzyme, GeUGT, to catalyze the transfer of glucose onto the 4-hydroxy group of 4-hydroxybenzylalcohol using UDP-glucose as the glucose donor is described. The reaction produces gastrodin and UDP as a byproduct.

[0017] **FIG. 2** shows that GeUGT is more efficient than the previously described AsUGT at converting 4-HBA into gastrodin. After 48-hour incubation of 4-HBA with *E. coli* strains containing either GeUGT or AsUGT, 4-HBA conversion was measured by HPLC analysis. GeUGT exhibited total conversion of 4-HBA to gastrodin, unlike AsUGT, which did not completely convert the 4-HBA in the media. Standards were run in growth media and used to identify both 4-HBA and gastrodin in the tested strains.

[0018] **FIG. 3** shows a total of 11 UGTs identified as potentially capable of converting 4-HBA into gastrodin. In addition to GeUGT, 11 previously described UGTs were discovered to have gastrodin synthase activity, which is an activity not previously reported for these enzymes. These enzymes were assayed as before with GeUGT, and their activity was compared after 24h and 48h.

[0019] **FIG. 4** shows a sequence alignment of GeUGT (SEQ ID NO: 2), AsUGT (SEQ ID NO: 26), and the 11 additional gastrodin synthase enzymes described (SEQ ID NOs: 4, 6, 8, 10, 12, 14, 16, 18, 20, 22, and 24) as well as a consensus sequence (SEQ ID NO: 28). Sequence analysis reveals that residues M88, F119, I139, F145, L149, M198, and F383 of GeUGT are almost completely unique to this enzyme, and could potentially explain the highly active nature of this enzyme.

[0020] **FIG. 5A** shows an image of the GeUGT active site and identified amino acid residues. **FIG. 5B** shows an image of the AsUGT active site and identified amino acid residues.