Supplementary Materials: Synthesis of Mono- and Di-Glucosides of Zearalenone and α-/β-Zearalenol by Recombinant Barley Glucosyltransferase HvUGT14077

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Figure S1. Kinetic analysis of HvUGT14077. All assays were performed at 37 °C, 100 mM Tris/Cl pH 7.5. Kinetic assays with UDP-glucose were done with 25 μM zearalenone. Saturation curves with zearalenone, α-zearalenol and β-zearalenol were determined with 10 mM UDP-glucose. Product formation was quantified by LC-MS/MS. Activity with kaempferol was determined with the UDP-Glo assay from Promega with 1 mM UDP-glucose. Since data regression with the Haldane model was not possible, no fitted curve is displayed in this case. Inhibition by UDP was determined at 10 mM UDP-glucose and 25 μM zearalenone.
**Figure S2.** High resolution tandem mass spectrometric product ion scan of the tentatively identified kaempferol-tri-glucoside in positive electrospray ionization mode at a collision energy of 15 eV.

**Figure S3.** High resolution tandem mass spectrometric product ion scan of the tentatively identified quercetin-tri-glucoside in positive electrospray ionization mode at a collision energy of 15 eV.
Figure S4. Synthesis of zearalenone-16-glucoside (ZEN-16-G) with HvUGT14077 (1.25 mg·mL⁻¹), different concentrations of a β-glucosidase from Lactobacillus brevis and sucrose synthase AtSUS1 (1.25 mg·mL⁻¹) for UDP-glucose regeneration. Sucrose was added to 100 mM. Time point “0 h” indicates the initial concentrations in the batch (91% zearalenone-14-glucoside, ZEN-14-G; 7.3% ZEN-16-G and 1.3% unconverted zearalenone, ZEN) used for this conversion.
Figure S5. Synthesis of zearalenol-16-glucoside (ZEN-16-G) with HrUGT14077 (1.25 mg·mL⁻¹), a β-glucosidase from Lactobacillus brevis (24 μg·mL⁻¹) and sucrose synthase AtSUS1 (1.25 mg·mL⁻¹) for UDP-glucose regeneration. Sucrose was added in different concentrations. Time point “0 h” indicates the initial concentrations in the batch (91% zearalenone-14-glucoside, ZEN-14-G; 7.3% ZEN-16-G and 1.3% unconverted ZEN) used for this conversion.
**Figure S6.** High resolution tandem mass spectrometric product ion scan of zearalenone-14,16-di-glucoside in negative electrospray ionization mode at a collision energy of 10 eV.

**Figure S7.** High resolution tandem mass spectrometric product ion scan of the tentatively identified $\alpha$-zearalenol-14,16-di-glucoside in negative electrospray ionization mode at a collision energy of 10 eV.

**Figure S8.** High resolution tandem mass spectrometric product ion scan of the tentatively identified $\beta$-zearalenol-14,16-di-glucoside in negative electrospray ionization mode at a collision energy of 10 eV.
Figure S9. Topology of pCA02. pCA02 is a derivative of pKLD116 [1] which in turn is a derivative of pET21a. pCA02/pKLD116 allow expression of fusion proteins with N-terminal His-tag, maltose binding protein (MalE gene), TEV recognition site and the C-terminal target protein. pCA02 contains the multiple cloning site of the pET21 vector series. The plasmid map was created with Benchling (https://benchling.com/).

References


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