Glycosylation of Xanthohumol by Fungi

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A screening test on 29 microorganisms for transformation of xanthohumol led to the selection of twelve fungal strains. One of them, *Beauveria bassiana* AM278, converted xanthohumol into a glucosylated derivative. This product was identified as xanthohumol 4′-*O*-β-D-4‴-methoxyglucopyranoside.

Key words: Xanthohumol, Biotransformation, *Beauveria bassiana*

Introduction

Xanthohumol [3′-(3,3-dimethylallyl)-2′,4′4-trihydroxy-6′-methoxychalcone] is the main prenylflavonoid of hop (*Humulus lupulus* L.) (Cannabaceae). Hops, the female inflorescences, are traditionally used in the brewing industry to add bitterness, aroma and flavour to beer. They are also a very rich source of bitter acids and prenylflavonoids. However, the best source of xanthohumol is the residue of supercritical carbon dioxide hops extraction, named spent hops. This hop processing waste product is free of the bulk of the bitter acids, and it is cheap.

Xanthohumol has recently received attention due to its various biological activities. It has been shown to be a potential anticancer agent (Colgate *et al*., 2007; Guerreiro *et al*., 2007). In addition, it exhibits properties such as antimutagenic and antioxidant (Miranda *et al*., 2000), antiviral (Buckwold *et al*., 2004), among them anti-HIV-1 (Wang *et al*., 2004), and has been also patented as a drug for osteoporosis (Tobe *et al.*, 1997).

Microbial transformation of xanthohumol has been poorly documented so far (Herath *et al.*, 2003; Kim and Lee, 2006). The aim of the present work is to generate novel microbial metabolites of xanthohumol.

Materials and Methods

Microorganisms

Fungi used in the study were purchased from Institute of Biology and Botany of the Medical Academy of Wrocław, Poland (indexed AM), Department of Forest Pathology of the Agricultural University of Kraków, Poland (indexed ARK), Department of Chemistry of the Wrocław University of Environmental and Life Sciences, Poland (indexed KCh), Department of Biotechnology and Food Microbiology of the Wrocław University of Environmental and Life Sciences, Poland (indexed PM), and National Collection of Agricultural and Industrial Microorganisms, Budapest, Hungary (indexed NCAIM) and Coopers Brewery, Regency Park, Australia (commercial yeasts, Australian real ale).

Cultivation of fungi

The fungi were maintained on agar slants and grown on a Sabouraud medium consisting of 3% glucose and 1% peptone. The cultures were shaken at 25 °C in 100-ml Erlenmeyer flasks with 25 ml of the medium in the screening studies and in 300-ml Erlenmeyer flasks with 100 ml of the medium in the preparative-scale transformation.

Conditions for transformations

Transformation experiments were performed in the dark under the conditions described above. In the screening experiments 10 mg of xanthohumol, dissolved in 0.5 ml of acetone, were added to the cultures and the reaction mixtures were incubated for 7 d. The preparative-scale fermentations were carried out with 30 mg of xanthohumol dissolved in 2 ml of acetone, and the reactions were continued for 6 d. All the experiments were per-

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formed in duplicate and with a control (the substrate in a sterile growth medium).

Reaction work-up and product analysis

In the screening experiments the fermentation media were extracted with 15 ml of ethyl acetate in one portion, whereas in the preparative-scale transformation they were extracted twice with 25 ml of ethyl acetate. The extracts were evaporated, and the residue dissolved in methanol and analyzed by TLC and HPLC. TLC was carried out on Merck silica gel 60, F254 (0.2 mm thick) plates. HPLC was performed on a Waters 2690 Aliance chromatograph with a Waters 996 photodiode array detector (detection at 280 and 370 nm wavelength) using an analytical HPLC column (Waters Spherisorb 5 μm ODS2, 4.6 × 250 mm) at a flow rate of 1 ml/min. A linear solvent gradient from 40 to 60% aqeous MeCN containing 1% HCOOH over 40 min was used. The product of xanthohumol transformation by *Beauveria bassiana* was separated by column chromatography on silica gel 60 (230–400 mesh, Merck) using chloroform/methanol (9:1 v/v) as eluent. The NMR spectra (^{1}H NMR, ^{13}C NMR, DEPT 135°, ^{1}H-^{1}H NMR, ^{1}H-^{13}C NMR) were recorded on a DRX 600 Bruker Avance instrument in DMSO-d_6.

Xanthohumol

It was isolated from supercritical carbon dioxide-extracted hops ("Marynka", crop 2005), obtained from Production of Hop Extracts of Fertilizer Institute, Puławy, Poland. Spent hops were immersed in ethyl acetate, sonificated and extracted. The extract was filtered, the solvent evaporated and the residue chromatographed over Sephadex LH-20 with methanol as eluent. – ^{1}H NMR: δ = 14.62 (1H, *s*, 2′-OH), 10.58 (1H, *s*, 4-OH), 10.08 (1H, *s*, 4′-OH), 7.73 (1H, *d*, *J* = 15.5 Hz, Hα C=O), 7.64 (1H, *d*, *J* = 15.5 Hz, Hβ C=O), 7.53 (2H, *d*, *J* = 8.5 Hz, H-2, H-6), 6.80 (2H, *d*, *J* = 8.4 Hz, H-3, H-5), 6.05 (1H, *s*, H-5′), 5.10 (1H, *t*, *J* = 6.8 Hz, H-2″), 3.83 (3H, *s*, O–Me), 3.10 (2H, *d*, *J* = 6.9 Hz, H-0″), 1.66 (3H, *s*, H-4″), 1.56 (3H, *s*, H-5″). – ^{13}C NMR: δ = 192.1 (C=O), 165.0 (C-2′), 162.8 (C-4′), 160.9 (C-6′), 160.3 (C-4), 142.9 (C-β), 130.3 (C-3″), 130.1 (C-2, C-6), 126.5 (C-1), 124.2 (C-α), 123.4 (C-2″), 116.4 (C-3, C-5), 107.8 (C-3′), 104.9 (C-1′), 91.4 (C-5′), 56.1 (C6′–O–Me), 25.9 (C-5″), 21.5 (C-1″), 18.1 (C-4″). Spectral data of the yellow-orange crystals obtained were in agreement with the literature ones (Stevens *et al.*, 1997).

*Xanthohumol 4′-O-β-*D*-4‴-methoxyglucopyranoside*: ^{1}H NMR: δ = 14.21 (1H, *s*, 2′-OH), 10.15 (1H, *s*, 4-OH), 7.76 (1H, *d*, *J* = 15.5 Hz, Hα C=O), 7.72 (1H, *d*, *J* = 15.5 Hz, Hβ C=O), 7.61 (2H, *d*, *J* = 8.6 Hz, H-2, H-6), 6.86 (2H, *d*, *J* = 8.6 Hz, H-3, H-5), 6.39 (1H, *s*, H-5′), 5.44 (1H, *m*, *J* = 5.5 Hz, H-2‴), 5.32 (1H, *d*, *J* = 5.6 Hz, H-3‴), 5.19 (1H, *t*, *J* = 6.8 Hz, H-2″), 5.04 (1H, *d*, *J* = 7.8 Hz, H-1‴), 4.85 (1H, *d*, *J* = 5.4 Hz, H-6‴), 3.93 (3H, *s*, O–Me), 3.69 (1H, *m*, H-6‴), 3.52 (1H, *m*, H-4‴), 3.51 (1H, *m*, H-6‴), 3.47 (3H, *s*, C-4‴–O–Me), 3.45 (1H, *m*, H-3‴), 3.35 (1H, *m*, H-0″), 3.32 (1H, *m*, H-2‴), 3.13 (1H, *dd*, *J* = 13.8, 6.6 Hz, H-0″), 3.0 (1H, *t*, *J* = 9.0 Hz, H-5‴), 1.74 (3H, *s*, H-4″), 1.61 (3H, *s*, H-5″). – ^{13}C NMR: δ = 193.0 (C=O), 163.6 (C-2′), 161.5 (C-4′), 160.9 (C-6′), 160.6 (C-4), 143.8 (C-β), 130.6 (C-3″), 131.2 (C-2, C-6), 126.4 (C-1), 124.1 (C-α), 123.2 (C-2″), 116.5 (C-3, C-5), 110.0 (C-3′), 106.7 (C-1′), 100.2 (C-0‴), 91.0 (C-5′), 79.8 (C-5‴), 77.1 (C-3‴), 76.5 (C-4‴), 74.0 (C-2‴), 60.9 (C-6‴), 60.2 (C-4‴–O–Me), 56.5 (C-6′–O–Me), 26.0 (C-5″), 21.7 (C-1″), 18.2 (C-4″).

Results and Discussion

In order to select the microorganisms capable of transforming xanthohumol we performed screening tests on 29 fungal strains. The results are presented in Table I.

TLC and HPLC demonstrated that 12 of the tested fungi metabolized the substrate. Two of them, *Cunninghamella japonica* and *Penicillium notatum*, belong to the genera earlier reported as being capable of xanthohumol transformation (Kim and Lee, 2006). *Aspergillus flavus* and *Botrytis cinerea* are known to transform other prenylated flavonoids (Tahara *et al.*, 1997). In our experiments we observed that *Aspergillus ochraceus* transformed xanthohumol, however, a *Botrytis cinerea* culture did not – the unchanged substrate was recovered.

For scale-up studies we have chosen the fungus *Beauveria bassiana*, because it showed the highest transformation efficiency (Fig. 1).

From the 6-day transformation extract we isolated a metabolite identified as xanthohumol 4′-*O*-β-D-4‴-methoxyglucopyranoside (Fig. 2).

Several remarkable differences were observed in the ^{1}H and ^{13}C NMR spectra of this compound, when compared with xanthohumol. Thirteen pro-

Table I. Screening for xanthohumol transforming fungi.

Microorganism	*
Fusarium equiseti AM15	+
Poria placenta ARK2213	–
Verticillium sp. AM424	–
Candida viswanathi AM120	–
Spicaria fusispora AM136	+
Mortierlla vinaceae AM149	+
Absidia glauca AM177	+
Botrytis cinerea AM235	–
Beauveria bassiana AM278	+
Stemphylium botryosum AM279	+
Absidia cylindrospora AM336	+
Aphanocladium album AM417	–
Spicaria divaricata AM423	–
Pezicula cinnamomea ARK15753	+
Mucor hiemalis AM450	–
Aspergillus ochraceus AM456	+
Saccharomyces cerevisiae AM464	–
Cunninghamella japonica AM472	+
Pleurotus ostreatus AM482	–
Laetiporus sulphurens AM515	–
Laetiporus sulphurens AM525	–
Trametes versicolor AM536	+
Penicillium notatum KCh904	+
Saccharomyces brasiliensis NCAIM Y-1223	–
Saccharomyces pasterianus NCAIM Y-1244	–
Zygosaccharomyces bailli PMI67	–
Candida parapsilopsis NCAIM Y-1011	–
Candida kefyr NCAIM Y-1070	–
Brewer's Yeast Real Ale Coopers Brewery	–

* Capability of transformation of xanthohumol: (+) capable; (–) incapable.

ton signals and seven carbon ones, corresponding to a sugar group were observed in the region ranging from δ_H 3.0 to 5.44, and from δ_C 60.2 to 100.2. The low-field shift of H-5′ in the ^{1}H NMR spectrum of the product (δ 6.39) compared to xanthohumol (δ 6.05) suggests a substitution of the hydroxy group at C-4. ^{1}H-^{1}H NMR and ^{1}H-^{13}C NMR experiments allowed to establish unambiguously the sugar moiety structure. The lack of the 4‴-OH signal and the presence of a new singlet of three protons at 3.47 ppm indicated the methoxy group at C-4‴. Additionally, in the ^{1}H-^{13}C NMR spectrum, we observed the correlation of this signal with the signal at 60.2 ppm, typical for methoxy carbon atoms. Our data correspond very closely to those described in the literature for 4′-*O*-β-glucopyranoside, which was obtained from xanthohumol in a *Penicillum chrysogenum* culture (Kim and Lee, 2006). Glycosylation and methylation of phenolic OH-groups are common mammalian metabolic features (Nookandeh *et al.*, 2004; Yilmazer *et al.*, 2001a, b). However, in microbial systems this reaction is rare and the reports are limited. In our previous investigations, we observed the glucosylation of naringenin in the analogical position, leading to prunin, by *Beauveria bassiana* (Huszcza *et al.*, 2007). This species is known as a highly regioselective bioreagent (Grogan and Holland, 2000). It is questionable whether flavonoids are more effective in the human body as free aglycones or as the whole molecules. This is specific for

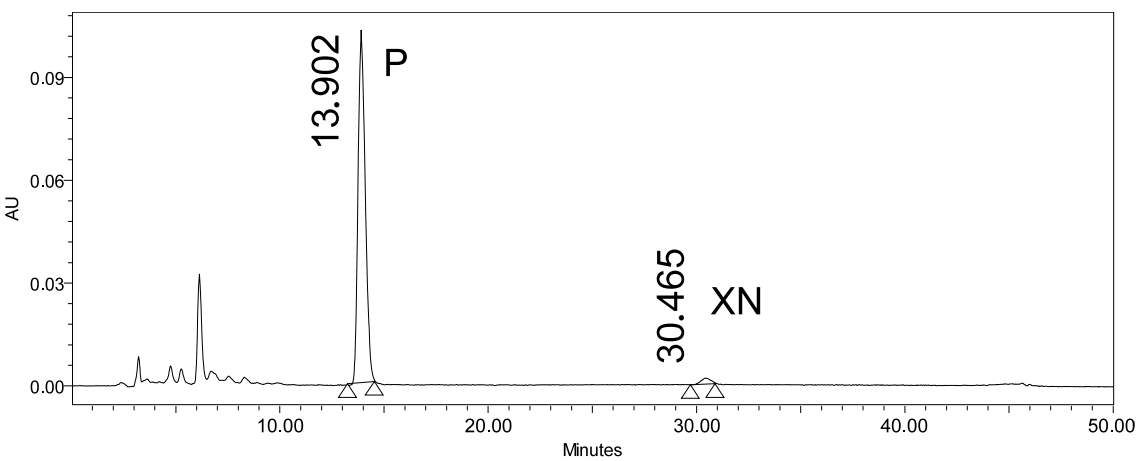

Fig. 1. HPLC chromatogram of the extract of 6-day-old *Beauveria bassiana* transformation medium (λ = 370 nm). XN, xanthohumol; P, product of biotransformation.

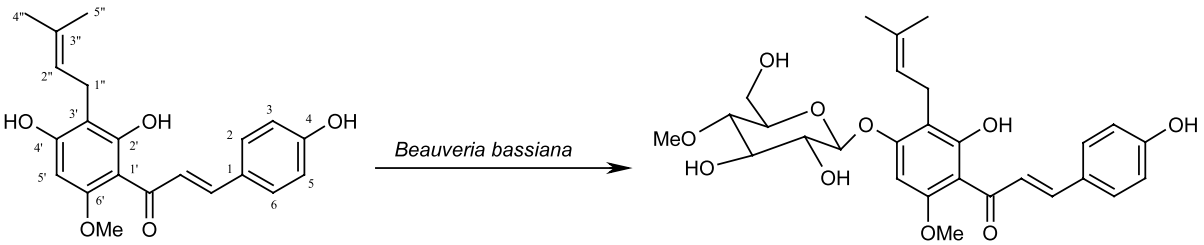

Fig. 2. Transformation of xanthohumol in *Beauveria bassiana* culture.

a particular flavonoid and for its biological activity (Hodek *et al.*, 2002).

Conclusions

Beauveria bassiana proved to glycosylate xanthohumol, the main prenylflavonoid of *Humulus lupulus*. With respect to the growing interest in this natural compound of important biological activity, this result is a noteworthy contribution to research in this area.

Buckwold V. E., Wilson R. J. H., Nalca A., Beer B. B., Voss T. G., Turpin J. A., Buckheit III R. W., Wei J., Wenzel-Mathers M., Walton E. M., Smith R. J., Pallansch M., Ward P., Wells J., Chuvala L., Sloane S., Paulman R., Russel J., Hartman T., and Ptak R. (2004), Antiviral activity of hop constituents against series of DNA and RNA viruses. Antiviral Res. **61**, 57–62.

Colgate E. C., Miranda C. L., Stevens J. F., Bray T. M., and Ho E. (2007), Xanthohumol, a prenylflavonoid derived from hops, induces apoptosis and inhibits NF-kappaB activation in prostate epithelial cells. Cancer Lett. **246**, 201–209.

Grogan G. J. and Holland H. L. (2000), The biocatalytic reactions of *Beauveria* spp. J. Mol. Cat. **9**, 1–32.

Guerreiro S., Monteneiro R., Martins M. J., Calhau C., Azevedo I., and Soares R. (2007), Distinct modulation of alkaline phosphatase isoenzymes by 17β-estradiol and xanthohumol in breast cancer MCF-7 cells. Clinical Biochem. **40**, 268–273.

Herath H. M. W., Ferreira D., and Khan J. A. (2003), Microbial transformation of xanthohumol. Phytochemistry **62**, 673–677.

Hodek P., Trefil P., and Stiborova M. (2002), Flavonoids – potent and versatile biologically active compounds interacting with cytochromes P450. Chem. Biol. Interact. **139**, 1–29.

Huszcza E., Bartmańska A., and Klessen S. (2007), Screening for the naringenin transforming fungi. Proceedings of the 9th International Commodity Science Conference, Poznań, Poland, pp. 817–822.

Kim H. J. and Lee I. S. (2006), Microbial metabolism of the prenylated chalcone xanthohumol. J. Nat. Prod. **69**, 1522–1524.

Miranda C. L., Aponso G. L. M., Stevens J. F., Deinzer M. L., and Buhler D. R. (2000), Prenylated chalcones and flavanones as inducers of quinine reductase in mouse Hepa lclc7 cells. Cancer Lett. **149**, 21–29.

Nookandeh A., Frank N., Steiner F., Ellinger R., Schneider B., Gerhäuser C., and Becker H. (2004), Xanthohumol metabolites in faeces of rats. Phytochemistry **65**, 561–570.

Stevens J. F., Ivanic M., Hsu V., and Deinzer M. L. (1997), Prenylflavonoids from *Humulus lupulus*. Phytochemistry **44**, 1575–1585.

Tahara S., Tanaka M., and Barz W. (1997), Fungal metabolism of prenylated flavonoids. Phytochemistry **44**, 1031–1036.

Tobe H., Kitamura K., and Komiyama O. (1997), Pharmaceutical composition for treating osteoporosis. Patent US 5, 679, 716.

Wang Q., Ding Z. H., Liu J. K., and Zheng Y. T. (2004), Xanthohumol, a novel anti-HIV-1 agent purified from hops *Humulus lupulus*. Antiviral Res. **64**, 189–194.

Yilmazer M., Stevens J. F., and Buhler D. R. (2001a), *In vitro* glucuronidation of xanthohumol, a flavonoid in hop and beer, by rat and human liver microsomes. FEBS Lett. **491**, 252–256.

Yilmazer M., Stevens J. F., Deinzer M. L., and Buhler D. R. (2001b), *In vitro* biotransformation of xanthohumol, a flavonoid from hops (*Humulus lupulus*), by rat liver microsomes. Drug Metab. Dispos. **29**, 223–231.