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Intestinal Bacterium *Eubacterium cellulosolvens* Deglycosylates Flavonoid *C*- and *O*-Glucosides

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Eubacterium cellulosolvens **cleaved the flavone *C*-glucosides homoorientin and isovitexin to their aglycones luteolin and apigenin, respectively. The corresponding isomers, orientin and vitexin, or other polyphenolic *C*-glucosides were not deglycosylated.** ***E. cellulosolvens*** **also cleaved several *O*-coupled glucosides of flavones and isoflavones to their corresponding aglycones.**

Certain forage crops contain large amounts of polyphenols, including tannins, which may affect rumen fermentation by inhibiting the growth and activity of the ruminal microbiota (8, 10, 13). Currently, bioactive polyphenolic plant compounds are studied with respect to their suitability as enhancers of animal health, in particular, that of ruminants (11). Health effects could be achieved by favorably modulating the gut fermentation or by inhibiting pathogens. The latter effect has come into focus since the addition of antibiotics to animal feed was banned in 2006 in the European Union. Polyphenolics could also serve as substrates for ruminal bacteria, in particular, when sugars are attached. Several intestinal bacterial species from animals and humans are able to release sugar moieties from flavonoids, and a few may even degrade the flavonoid basic structure (1, 2, 12). Based on the observed biological activities, the resulting flavonoid aglycones and their ring cleavage products have been proposed to mediate some of the health effects reported for the parent flavonoids in mammals (5, 12, 16).

One of the fodder plants very rich in polyphenols is the legume tagasaste (*Chamaecytisus proliferus*), which has been investigated in more detail. The polyphenolic fraction of tagasaste, which has no negative effects on rumen fermentation, contains more than 70% glycosides of the flavones apigenin and luteolin (6). It has been assumed that these flavones are mainly present in the form of their *C*-glucosides (e.g., vitexin, orientin) and hypothesized that the glucose moiety could be released and fermented by rumen bacteria (6). In contrast to polyphenolic *O*-β-glucosides that may also be cleaved by mammalian glycosidases (5), the deglycosylation of *C*-glucosides appears to be exclusively catalyzed by bacterial enzymes.

Eubacterium cellulosolvens, an anaerobic cellulolytic bacterium, has been isolated from the rumen of sheep and cows as well as the gut of mice and rabbits (4, 7, 15). It appears to be a prominent ruminal bacterium in cattle (14). While the binding and degradation of cellulose by *E. cellulosolvens* have been studied in detail, nothing has been known of the cleavage of polyphenolic glycosides by this species. Here, we demonstrate that *E. cellulosolvens* is capable of deglycosylating certain flavone *C*-glucosides and of hydrolyzing *O*-glucosides of flavones and isoflavones.

Conversion of polyphenolic *C*-glucosides. *E. cellulosolvens* ATCC 43171^T was grown in 800 μl modified Reinforced Clostridial Medium (RCM_{mod}) supplemented with 200 μM of the respective *C*-glucoside at 37°C for 144 h under strict anoxic conditions as described previously (3). *C*-Glucosides and bacteria incubated separately in RCM_{mod} served as controls. Samples taken after 0, 24, 48, 72, 96, and 144 h were processed and analyzed by reversed-phase high-performance liquid chromatography–diode array detection (RP-HPLC/DAD) according to previously described methods (3). All incubations were carried out in triplicate, and each sample was extracted and analyzed once. *E. cellulosolvens* was tested for the conversion of homoorientin, orientin, isovitexin (all from PhytoLab, Vestenbergsreuth, Germany), vitexin (Roth, Karlsruhe, Germany), mangiferin, carminic acid (both from Sigma-Aldrich, Munich, Germany), and puerarin (LKT Laboratories, St. Paul, MN) (Fig. 1). Of these *C*-glucosides, only homoorientin (luteolin 6-*C*-glucoside) and isovitexin (apigenin 6-*C*-glucoside) were converted by *E. cellulosolvens* (Table 1). Most parts of both compounds were used up within 24 h of incubation. While homoorientin was deglycosylated within this time period to equimolar amounts of luteolin, the incubation of isovitexin with *E. cellulosolvens* resulted in the formation of only 26% of the corresponding aglycone apigenin based on the applied substrate. The amount of apigenin further increased to maximally 44% after 96 h of incubation. Luteolin and apigenin were not further degraded. The results show that only the glucose moiety attached to the C6 of flavones was removed by cleavage whereas that at C8 as present in orientin (luteolin 8-*C*-glucoside) and vitexin (apigenin 8-*C*-glucoside) remained unaffected. The C6 position is characterized by two adjacent hydroxyl groups, which appear to be essential for deglycosylation. However, neither the xanthone *C*-glucoside mangiferin nor the anthraquinone *C*-glucoside carminic acid was cleaved although adjacent hydroxyl groups were present. This indicates that additional structural features of the aglycone also play a role. However, so far the possibility cannot be excluded that the observed differences reflect differences in the transport of the individual compounds.

For comparison, the bacterial strain CG19-1 was incubated with selected *C*-glucosides using conditions identical to those described above for *E. cellulosolvens*. The strictly anaerobic strain CG19-1, which was recently isolated from human feces, deglycosylates puerarin and other aromatic *C*-glucosides (3). Moreover, strain CG19-1 cleaves the aglycones luteolin and apigenin, which

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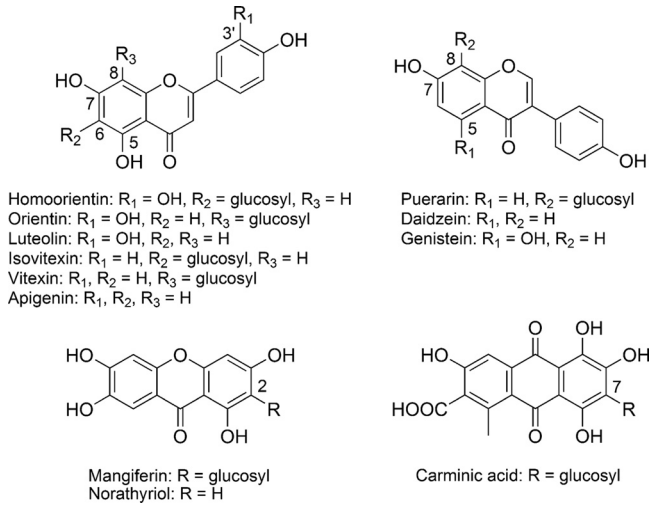

FIG 1 Structures of compounds used in the study.

result from deglycosylation of the corresponding flavone *C*-glucosides (3). In the present study, homoorientin, vitexin, and mangiferin were completely converted within 24 h to approximately equimolar amounts of 3-(3,4-dihydroxyphenyl)propionic acid, 3-(4-hydroxyphenyl)propionic acid, and norathyriol, respectively. These activities of strain CG19-1 confirmed previous work (3). The newly tested flavone *C*-glucosides orientin and isovitexin were completely transformed within 24 h of incubation to approximately equimolar amounts of 3-(3,4-dihydroxyphenyl)propionic acid and 3-(4-hydroxyphenyl)propionic acid, respectively. This indicates efficient deglycosylation of both *C*-glucosides as observed for their isomers, homoorientin and vitexin.

Thus, strain CG19-1 is capable of deglycosylating a more complex set of *C*-glucosides than *E. cellulosolvens*. However, one of the tested compounds, carminic acid, was not converted by either of the two organisms. This is probably due to its structural characteristics, which include a nonheterocyclic central ring and a second keto group on this ring. The difference observed between the two species may be explained by differences in substrate specificity of the *C*-deglycosylating enzymes or by differences in the uptake of the compounds. *E. cellulosolvens* and strain CG19-1 are only remote relatives within the *Clostridia*. While the former species is a member of the *Eubacteriaceae*, strain CG19-1 belongs to the *Lachnospiraceae*. The 16S rRNA gene sequence of *E. cellulosolvens* ATCC 43171 (accession number X71860) shows 90% identity to that of strain CG19-1 (accession number FJ711049).

The mechanism of *C*-deglycosylation by *E. cellulosolvens* and strain CG19-1 has yet to be clarified. Puerarin deglycosylation by another human intestinal bacterium was recently shown to occur by hydrolytic cleavage, yielding daidzein and glucose (9). *E. cellulosolvens* could take advantage of the released glucose moiety as a substrate for fermentation (15), whereas strain CG19-1 does not degrade glucose or other carbohydrates (3).

Conversion of flavonoid *O*-glucosides. The ability of *E. cellulosolvens* to convert *O*-coupled flavonoid glycosides was tested as described above using the flavone derivatives luteolin 5-*O*-glucoside, luteolin 3′-*O*-glucoside (both available from previous work) (3), luteolin 7-*O*-glucoside, and apigenin 7-*O*-glucoside as well as the isoflavones daidzein 7-*O*-glucoside and genistein 7-*O*-glucoside (all from Roth, Karlsruhe, Germany). The aglycone structures of the compounds are given in Fig. 1. *E. cellulosolvens* deglycosylated all of the tested compounds to their corresponding aglycones except luteolin 5-*O*-glucoside. The conversion rates differed, as reflected by the percentage of the substrate converted after 24 h of incubation (Table 1). Luteolin 7-*O*-glucoside, luteolin 3′-*O*-glucoside, apigenin 7-*O*-glucoside, and genistein 7-*O*-glucoside were completely used up after 48 h, and that result was accompanied by increased product formation, while 32% of the initial daidzein 7-*O*-glucoside concentration was still present at this time point. The findings suggest that the glucose moiety attached to the hydroxyl group in the C5 position of the flavone structure prevents its cleavage or its uptake into the cell. The lack of the C5 hydroxyl group in daidzein 7-*O*-glucoside led to delayed deglycosylation and/or transport. It can be concluded that the free C5 hydroxyl group plays an important role in the cleavage and/or uptake of flavonoid *O*-glucosides, probably mediated by formation of a hy-

TABLE 1 Substrate depletion and metabolite formation within 24 h and 48 h of incubations with *Eubacterium cellulosolvens*[a]

Substrate	% substrate depletion		Metabolite	% metabolite formation	
	Within 24 h	Within 48 h		Within 24 h	Within 48 h
C-Glucosides					
Homoorientin	79.9 ± 0.9	78.8 ± 1.2	Luteolin	82.9 ± 8.1	76.3 ± 11.9
Orientin	−2.7 ± 0.9	−8.1 ± 1.6	ND	NA	NA
Isovitexin	75.8 ± 2.3	90.7 ± 3.5	Apigenin	25.9 ± 5.2	27.8 ± 5.4
Vitexin	0.4 ± 6.7	−0.4 ± 8.4	ND	NA	NA
Mangiferin	2.3 ± 1.6	4.2 ± 1.4	ND	NA	NA
Puerarin	2.8 ± 1.6	3.0 ± 3.3	ND	NA	NA
Carminic acid	8.3 ± 0.4	10.6 ± 1.4	ND	NA	NA
O-Glucosides					
Luteolin 5-*O*-glucoside	1.5 ± 1.4	−0.9 ± 2.1	ND	NA	NA
Luteolin 7-*O*-glucoside	84.2 ± 1.7	98.2 ± 0.3	Luteolin	71.6 ± 2.4	85.7 ± 6.0
Luteolin 3′-*O*-glucoside	100.0 ± 0	100.0 ± 0	Luteolin	62.4 ± 1.9	63.4 ± 0.9
Apigenin 7-*O*-glucoside	90.1 ± 0.5	97.6 ± 0.2	Apigenin	38.3 ± 6.6	44.4 ± 4.3
Daidzein 7-*O*-glucoside	34.7 ± 5.6	67.7 ± 2.1	Daidzein	28.5 ± 1.9	53.3 ± 2.5
Genistein 7-*O*-glucoside	77.2 ± 2.1	100.0 ± 0	Genistein	73.4 ± 6.3	98.0 ± 1.3

[a] Values are means of triplicate incubations ± SD. ND, not detected; NA, not applicable.

drogen bond with the carbonyl group at C4. All of the *O*-glucosides tested with *E. cellulosolvens* in the present study are converted by the human intestinal strain CG19-1 to their aglycones (in the case of the isoflavones) or even to the corresponding hydroxyphenylpropionic acids by cleavage of the resulting flavones (3).

In conclusion, *E. cellulosolvens* may benefit from the deglycosylation of flavonoid *O*- and *C*-glucosides by fermentation of the liberated glucose moiety. The resulting aglycones, which are not further degraded by *E. cellulosolvens*, may affect both the intestinal microbiota and the host organism. Thus, it is supposed that *E. cellulosolvens* contributes to the bioactivation of polyphenolic compounds of fodder plants in livestock.

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