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Transformation of plant isoflavones into bioactive isoflavones by lactic acid bacteria and bifidobacteria

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ABSTRACT

Isoflavones are usually found in nature in their glycosilated or methylated forms, and should be hydrolysed to become bioavailable and physiologically active. The deglycosylation of isoflavone *C*-glycosides and *O*-glycosides and the demethylase activity were studied in a selection of lactic acid bacteria (LAB) and bifidobacteria by assessing the degree of transformation of the pure precursor compounds, daidzin, genistin, puerarin, formononetin and biochanin A into daidzein or genistein. Only one *Bifidobacterium* strain and two *Enterococcus* strains hydrolysed the *C*-glycosidic bond of puerarin, while deglycosylation of *O*-glycosides daidzin and genistin was observed in all the tested strains. Demethylation of biochanin A and formononetin was observed in the most of LAB and bifidobacteria. Besides, the subsequent metabolites dihydrodaidzein and dihydrogenistein where produced by many of the strains via daidzein and genistein. In this work, we show the potential of LAB and bifidobacteria as part of functional foods because of their ability to transform plant isoflavones into their bioactive forms.

1. Introduction

Isoflavones are naturally occurring compounds found in plants mainly in legumes, among which soy (*Glycine* max) is the main source in human diet (Dixon, 2004). They are classified as phytoestrogens because their structures resemble that of estrogen and have a weak affinity for the estrogen receptor (Vaya & Tamir, 2004). Consumption of isoflavones in the form of soy products or supplements is associated with protective effect against cancer (Fournier, Erdman, & Gordon, 1998; Messina, Kucuk, & Lampe, 2006), cardiovascular disease (Erdman, 2000), osteoporosis (Messina & Messina, 2000) and menopausal symptoms (Han, Soares, Haidar, de Lima, & Baracat, 2002), and on cognitive function (Kritz-Silverstein, Von Muhlen, Barrett-Connor, & Bressel, 2003).

The two major isoflavones in soybeans are daidzin and genistin, which are the *O*-glycoside conjugates of daidzein and genistein (Wang & Murphy, 1994). Other sources of isoflavonoids with increasing presence are supplements based on legumes such as red clover (*Trifolium pretense*) and kudzu root (*Pueraria lobata*). Red clover contains biochanin A and formononetin, which are methoxylated derivatives of genistein and daidzein (Wu, Wang, & Simon, 2003). On the other hand, kudzu is rich in puerarin, a *C*-glycoside of daidzein (Jin, Nishihata, Kakiuchi, & Hattori, 2008; Wu et al., 2003). The biological activity of isoflavones is usually attributed to the aglycones, daidzein and genistein, because they are absorbed faster than the glycosylated or methoxylated forms, and have more estrogenic and antioxidant activities (Kayano et al., 2012; Kuiper et al., 1998; Park, Shin, Bae, Lee, & Kim, 2006).

Additionally, bioactive aglycones are extensively conjugated to form *O*-glucuronides during and after absorption through the gut barrier (Wu, Kulkarni, Basu, Zhang, & Hu, 2011). The deconjugation of these compounds is also necessary for their actions to be physiologically relevant *in vivo* (Rowland et al., 2003) and for the formation of metabolites of interest such as equol (Setchell, Brown, & Lydeking-Olsen, 2002).

The conversion of dietary isoflavones occurs in the gut and is attributed to a great extent to the intestinal microbiota. After the formation of the isoflavone aglycones, they are further metabolized by the intestinal bacteria into dihydrodaidzein (DHD) and dihydrogenistein (DHG). DHD is the precursor of metabolites of interest such as *O*-desmethylangolensin (*O*-DMA) and equol; and DHG can be further metabolized into 5-hydroxy equol or 6 hydroxy *O*-DMA. Formation these bioactive isoflavones varies among individuals, probably because of differences in the microbiota composition, emphasizing the importance of isolating and studying the bacteria responsible for the bioconversions described above (Figs.1 and 2). The *O*-glycosides (daidzin and genistin)

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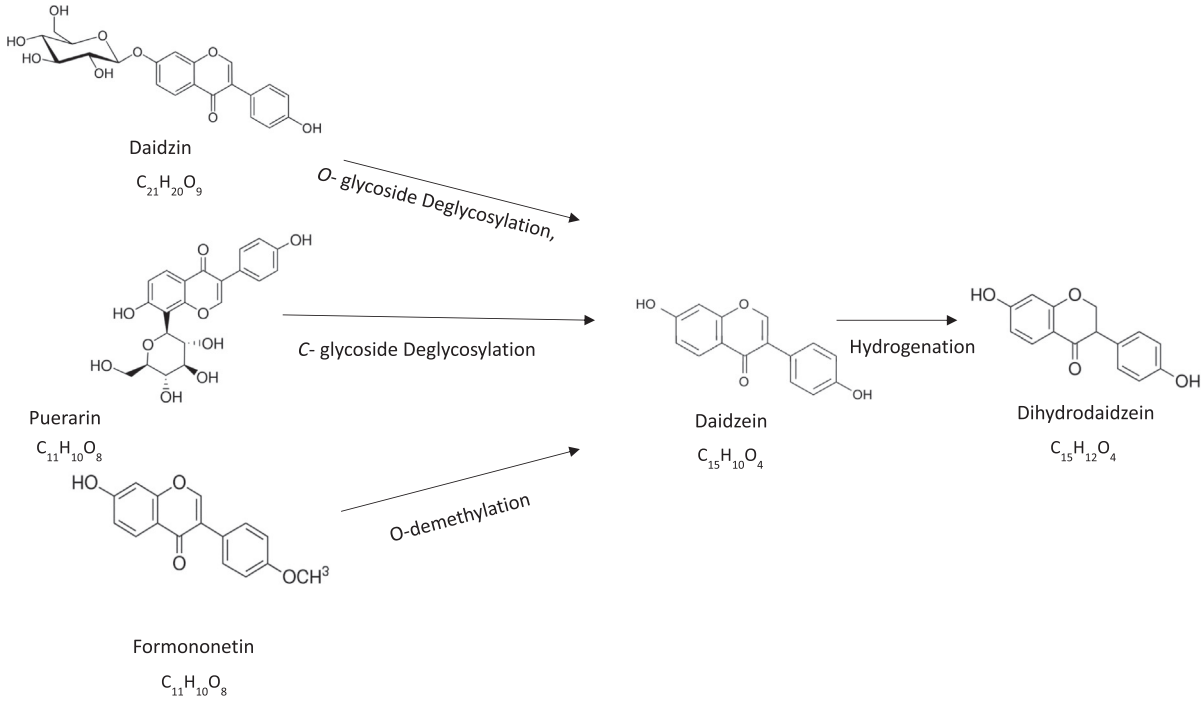

Fig. 1. Transformation of daidzin, puerarin and formononetin into daidzein and dihydrodaizein.

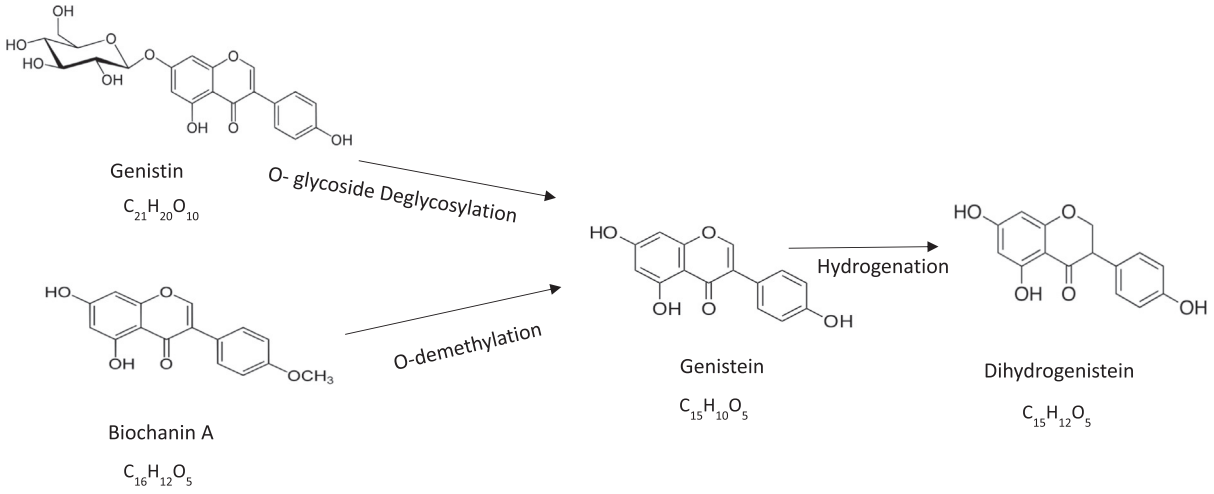

Fig. 2. Transformation of genistin and biochanin A into genistein and dihydrogenistein.

may be hydrolysed by human glycosidases, activity that is also widespread among genera of intestinal microbiota (Gaya, Peirotén, Medina, & Landete, 2016; Park et al., 2006). On the contrary, breakage of the *C*-glycoside bond seems to be more difficult, it is not present in all the individuals and only a few intestinal bacteria able to metabolize puerarin into daidzein have been identified (Braune & Blaut, 2016; Kim, Lee, & Han, 2015). Conversely, although demethylation of formononetin and biochanin A into daidzein and genistein has been described to occur in the gut (Setchell et al., 2001), only two intestinal bacterial strains capable of perform this bioconversion have been described (Hur & Rafii, 2000; Kim et al., 2015).

In this work, we searched for the mentioned biotransformation activities within a selection of lactic acid bacteria and bifidobacteria, of intestinal and dairy origin, with previously tested glycosidase activity on soy extracts (Gaya, Peirotén, et al., 2016). Deglycosylation of *C*-glycoside and demethylation was studied in parallel with the already *O*-glycosidase activity detected, this time using as substrate the pure compounds daidzin, genistin, puerarin, formononetin and biochanin A. Moreover, the ability to deconjugate *O*-glucuronides was also analyzed. The aim of the work was to find bacteria able to transform plant isoflavones into bioactive compounds, strains that could be used later in functional foods and/or as probiotics (Braune & Blaut, 2011; Landete et al., 2015; Riciputi et al., 2016).

2. Material and methods

2.1. Bacterial growth conditions

A total of 92 strains of LAB and bifidobacteria previously tested for transformation of isoflavone extracts (Gaya, Peirotén, et al., 2016) were subjected to a screening of β-glucuronidase activity (Table 1). A subset of 18 strains (Table 2) able to transform isoflavone extracts (Gaya, Peirotén, et al., 2016) was subjected to further analysis of their ability to transform pure isoflavone compounds.

Lactobacillus and *Enterococcus* were routinely cultivated under anaerobic conditions at 37 °C in MRS broth (Oxoid, Ltd. Basingstoke,