METABOLISM OF SOY ISOFLAVONES BY BACTERIA OF INTESTINAL ORIGIN

Lucía Vázquez*, Ana Belén Flórez, and Baltasar Mayo

Departamento de Microbiología y Bioquímica, Instituto de Productos Lácteos de Asturias (IPLA-CSIC), Paseo Río Linares, s/n, 33300-Villaviciosa, Asturias, Spain

*lucia.vazquez@ipla.csic.es

INSTITUTO DE PRODUCTOS LÁCTEOS DE ASTURIAS

CONSEJO SUPERIOR DE INVESTIGACIONES CIENTÍFICAS

Introduction

Epidemiological evidence suggests that high intake of soy foods or purified soy isoflavones is associated with less intense menopause symptoms and reduced risk of a number of hormone-mediated diseases. Isoflavones from diet are mostly found as glycoside conjugates (daidzin, genistin and glycitin). The bioavailability and activity of these molecules increase through metabolic reactions performed mostly by bacteria from the human gastrointestinal tract producing aglycones (daidzein, genistein, glycitein), which can be converted into fully-active compounds or inactive metabolites (Clavel and Mapesa, 2013). Among isoflavone metabolites, equol from daidzein is the compound having the strongest estrogenic and antioxidant activity (Figure 1) (Sánchez-Calvo et al., 2013). However, only 30-60% of humans are capable of producing this compound, and it may be only these subjects who fully benefit from soy or isoflavone intake.

Equol biosynthesis seems to take place through the consecutive action of three conserved reductases via dihydrodaidzein and tetrahydrodaidzein intermediates (Schröder et al.,2013). During the last decades, a number of bacterial strains capable of producing equol have been identified from human and animal sources; these mainly belong to the family *Coriobacteriaceae*. However, because of the nutritionally-fastidious and strict anaerobic conditions required by bacteria from human and animal gut, those populations and hence the biochemical pathways involved in equol production are still poorly characterized.

Objective

The aim of this study was to assess the metabolism of the two majority soy isoflavones (daidzein and genistein) in faecal samples, and their derived faecal cultures from menopausal woman with a positive or negative equol-production phenotype.

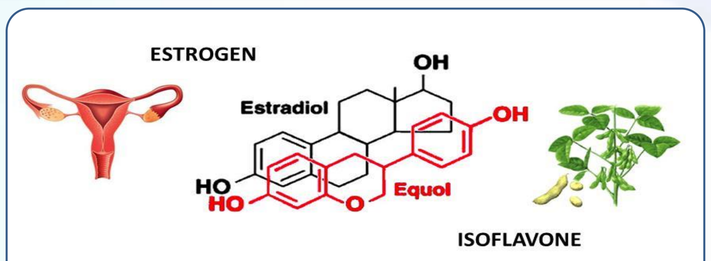

Figure 1.- Comparison of the structure of the isoflavone equol and estradiol molecules.

Methodology

Sampling. Faecal samples from eight women, three of whom had an equol production phenotype (Guadamuro et al., 2015) , were homogenized and plated on a general medium for colon bacteria.

Analysis of isoflavone-derived metabolites. Faecal homogenates, bulk cultures and isolated colonies from the counting plates were incubated in GAM+0.5% arginine, supplemented with either daidzein or genistein (final concentration each of 100 µM). Daidzein, genistein, dihydrodaidzein, dihydrogenistein and equol in cultures were detected and quantified measured by an ultra-high performance liquid chromatography (UHPLC) method.

Detection and quantification of genes involved in equol production. Microbial DNA was purified from cultures and used in conventional and real-time PCR (qPCR) analyses in order to identify and quantify reductase genes involved in equol formation from daidzein using primers based on equol-producing microorganisms in databases.

Results

Results from the UHPLC analysis of the faecal slurry or bulk cultures showed that equol was only present in cultures supplemented with daidzein and inoculated with faeces provided by equol-producing women (Table 1). Daidzein and genistein were recovered from the un-inoculated samples in varying amounts (67-81% of the added compound). And, no isoflavones or their metabolites were ever detected in control cultures without added isoflavones. Respect to the cultures, when the medium containing daidzein was inoculated with *Slackia isoflavoniconvertens* DSM 22006, the entire amount of added daidzein was converted into equol. However, the transformation of daidzein into equol was never complete when inoculated with faeces, and variable amounts of daidzein and dihydrodaidzein were recovered from these cultures (Table 1).

Table 1.- Cycle threshold (Ct) values obtained by qPCR for tetrahydrodaidzein reductase (*tdr*) and dihydrodaidzein reductase (*ddr*) genes and isoflavone metabolites measured by UHPLC in the faecal slurry cultures.

Faecal sample	GAM-Arg with[a]	qPCR amplification from faecal cultures		Isoflavone metabolites in faecal cultures (in µM)				
		Ct (*tdr*)	Ct (*ddr*)	Daidzein	Dihydrodadizein	Genistein	Dihydrogenistein	Equol
Equol producers:								
W3.3	Control	25.28 ± 0.07	26.07 ± 0.14	-	-	-	-	-
	DZEN	23.57 ± 0.17	24.60 ± 0.08	43.03	28.28	-	-	10.99
	GTEN	26.30 ± 0.02	26.84 ± 0.04	-	-	24.42	22.36	-
W8.1	Control	25.34 ± 0.38	23.47 ± 0.17	-	-	-	-	-
	DZEN	22.32 ± 0.31	21.10 ± 0.07	49.45	0.87	-	-	10.69
	GTEN	21.65 ± 0.43	20.78 ± 0.17	-	-	-	0.41	0.15
W18.1	Control	28.67 ± 0.55	-	-	-	-	-	-
	DZEN	29.56 ± 0.43	-	54.36	2.27	-	-	11.01
	GTEN	28.95 ± 0.20	-	-	-	57.46	2.15	-
Equol non-producers:								
W1.3	Control[a]	-	-	-	-	-	-	-
	DZEN	-	-	69.54	-	-	-	-
	GTEN	-	-	-	-	16.30	0.94	-
W5.3	Control	-	-	-	-	-	-	-
	DZEN	-	-	42.22	37.80	-	-	-
	GTEN	-	-	-	-	15.40	23.95	-
W7.3	Control	22.52 ± 0.15	23.24 ± 0.16	-	-	-	-	-
	DZEN	25.61 ± 0.07	26.35 ± 0.04	80.32	-	-	-	-
	GTEN	24.86 ± 0.01	25.70 ± 0.04	-	-	2.48	83.17	-
W15.3	Control	25.81 ± 0.04	26.90 ± 0.02	-	-	-	-	-
	DZEN	25.41 ± 0.09	26.26 ± 0.10	72.03	1.14	-	-	-
	GTEN	25.62 ± 0.11	26.54 ± 0.04	-	-	5.81	50.80	-
W17.1	Control	-	-	-	-	-	-	-
	DZEN	-	-	72.37	9.19	-	-	-
	GTEN			-	-	47.77	5.98	-
Culture controls:								
S. isoflav[b]	Control	nd	nd	-	-	-	-	-
	DZEN	nd	nd	-	-	-	-	100.00
	GTEN	nd	nd	-	-	45.76	6.69	0.79
GAM-Arg[c]	Control	nd	nd	-	-	-	-	-
	DZEN	nd	nd	81.18	-	-	-	-
	GTEN	nd	nd	-	-	67.00	-	-

Variable amounts of genistein and its derived metabolite dihydrogenistein were also recovered from cultures when this isoflavone was added. The exception was the faecal culture from W8, in which no genistein (detection limit 15.17 nM) and only a small quantity of dihydrogenistein (0.41 µM), was recorded (Table 1).

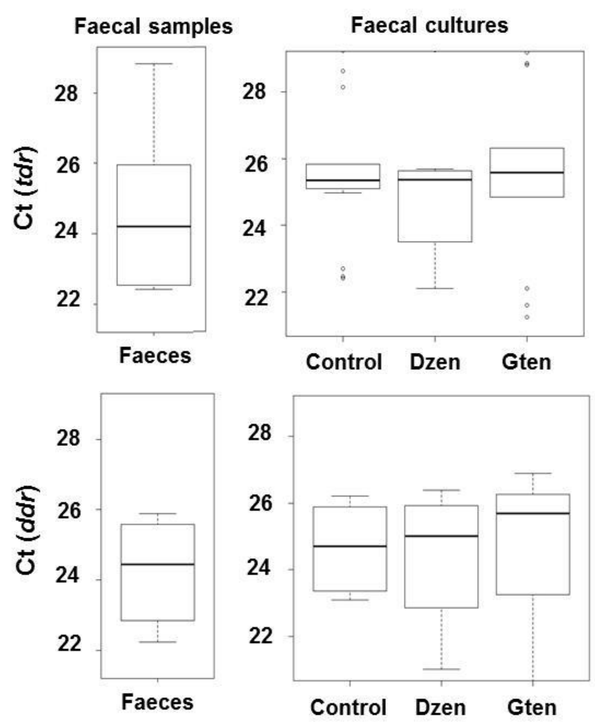

Figure 2.- Box diagramm of the Wilconson test for related samples of the Ct values for *tdr* and *ddr* genes in faecal samples and in their derived faecal cultures of control, and daidzein (Dzen) and genistein (Gten) supplemented cultures.

The genes involved in equol formation from daidzein were detected and quantified by conventional and qPCR approaches. The *tdr* gene was detected in the faeces and derived cultures of all three equol-producing women, while the *ddr* gene was only amplified in the faecal samples of two out of the three producing women, suggesting the presence in the non-amplified sample of reductase genes unrelated to those known (Table 1). When positive amplification of *tdr* and *ddr* was observed, equivalent copy numbers of these two genes were recorded (as judged from the Ct values) on DNA from faeces and cultures (Figure 2), suggesting they are located in the same genetic element, as in *S. isoflavoniconvertens* DSM 22006 (Schröder et al., 2013).

Surprisingly, positive amplification for both *tdr* and *ddr* genes was also observed in DNA from faeces and slurry cultures of two non-equol-producing women (W7 and W15). The presence of non-functional genes or the conversion of daidzein into downstream metabolites other than equol, could account for this apparent contradiction. In addition, as daidzein and genistein are chemically similar, the presence of daidzein-related genes involved in genistein metabolism may also be possible, which would explain the large amount of dihydrogenistein recovered in the faecal cultures of these two women (Table 1).

Conclusions

- Equol was only present in cultures derived from equol-producing women.
- Variable amounts of genistein and dihydrogenistein were scored in faecal cultures.
- Genes involved in equol production (*tdr* and *ddr*) were detected in similar amounts in DNA from faeces and faecal cultures.
- Reductases encoded by *tdr*- and *ddr*-related genes might also be involved with the metabolism of genistein.
- The biological significance of the presence/absence of *tdr* and *ddr* in the metabolism of isoflavones is currently under study.

References

- **Clavel, T., and Mapesa J. O.** (2013). In, *Handbook of Natural Products*. Ramawat, K. G., and Merillon, J. M. (Eds.). Chapter 94. Springer-Verlag, Berlin.
- **Sánchez-Calvo, J. M., et al.** (2013). *Phytochemistry Reviews* 12, 979-1000.
- **Guadamuro, L., et al.** (2015). *Frontiers in Microbiology* 6, 777
- **Schröder, C., et al.** (2013). *Applied and Environmental Microbiology* 79, 3494-3502.

Acknowledgements

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