**Influence of different lignan compounds on enterolignan
production by *Bifidobacterium* and *Lactobacillus* strains**

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Abstract

Enterolignans are polyphenols derived from the microbial metabolism of dietary lignans. They are considered phytoestrogens because they have estrogenic/antiestrogenic activity that confers them significant benefits to human health when they reach significant concentrations in plasma. Hence, there is a great interest in studying the bacteria involved in enterolignan production. In the present study, three bifidobacterial strains were found capable of producing low levels of enterodiols from lignan extracts. These enterodiols-producing bifidobacteria and another three *Lactobacillus* strains previously identified as enterolignans producers yielded both enterodiols and enterolactone from pure secoisolariciresinol (SECO) but not from matairesinol. In addition, the formation by all the strains of dihydroxyenterodiols as part of SECO metabolism was observed. In this work, we have demonstrated how the main groups of bacteria with probiotic potential, *Bifidobacterium* and *Lactobacillus*, are able to produce enterolignans. The production capacity of enterolignans and the metabolic route used are also shown and it is common in both groups of microorganisms. In addition to the high capacity for the transformation of secoisolariciresinol diglucoside (SDG) into SECO, we have demonstrated for the first time how strains of *Bifidobacterium* and *Lactobacillus* are capable of carrying out the complete metabolic pathway, transforming the SDG and SECO into enterolactone. They are the first microorganisms in which this capacity is demonstrated. The isolation and identification of bacteria able to produce enterolignans is of biotechnological interest, because of their potential application in functional foods and as probiotics.

44 **Keywords:** Lignans; food; entediol; enterolactone; bifidobacteria; lactobacilli

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1. Introduction

Phytoestrogens, which have similarities in chemical structure to mammalian estrogens (Farooq, 2015), are polyphenols found in plants or derived from plant precursors (Milder et al., 2005; Valsta et al., 2003). Five kind of phytoestrogens could be found, namely isoflavones, ellagitaninns, lignans, stilbenes and coumestans (Landete et al., 2016). Lignans are the most abundant phytoestrogens in the occidental diet, as they are present in high concentrations in oilseeds (flax, soy, grapeseed, sesame) and whole grain cereals and flours, as well as in some fruits and vegetables, coffee, tea and wine (Durazzo et al., 2013; Landete, 2012). Lignans such as secoisolariciresinol (SECO) and its precursor secoisolariciresinol diglucoside (SDG) are the most abundant lignans found in the diet. Moreover, other lignans such as matairesinol (MAT), and the SECO precursors pinoresinol and lariciresinol, can also be found in high concentration in some plant foods (Landete, 2012; Milder et al., 2005).

Dietary lignans are of interest because they have been proposed to play a role in the prevention of cardiovascular disease and cancer, especially breast and prostate cancer (Seibold et al., 2014; Vanharanta et al., 2003; Yoder et al., 2015). These health effects have been related to the presence of high concentrations of enterodiols (END) and enterolactone (ENL) in human plasma (Aarestrup et al., 2013; Piller et al., 2006; Vanharanta et al., 1999), while the intake of a lignan-rich diet has not shown so evident association. END and ENL are enterolignans produced from dietary lignans by intestinal microbiota and are much more bioavailable than their lignan precursors (Clavel, Borrmann, et al., 2006; Clavel et al., 2005). Enterolignans exert oestrogen agonism and antagonism (Mueller et al., 2004), show enzyme-inhibiting properties and have antioxidant activities higher than dietary lignans (Kitts et al., 1999). So, dietary

lignans have limited biological properties because of their low bioavailability (Landete et al., 2016; Quartieri et al., 2016) and must be converted into enterolignans to exert their beneficial effects.

Enterolignan production by intestinal microbiota is quite extended among individuals, although the variability in the amounts produced (Gaya, Medina, et al., 2016) could be attributed to differences in the microbiota composition. To date, scientific publications have reported that plant lignans can be converted by a consortium of intestinal bacteria into END and ENL (Wang et al., 2010; Woting et al., 2010; Xie et al., 2003). *Eggerthella lenta* is able to dehydroxylate dihydroxyenterodiol (DHEND) to produce END (Clavel, Henderson, et al., 2006; Jin, Zhao, et al., 2007); and several clostridia and *Ruminococcus* spp. strains have been described in the dehydrogenation of END into ENL (Clavel, Henderson, et al., 2006; Clavel et al., 2007; Jin, Kakiuchi, et al., 2007). Recently, we described the obtaining of enterolignans from flax extracts by the action of sole strains, specifically *Bifidobacterium adolescentis* INIA P784 able to produce END (Gaya, Peirotén, et al., 2017) and the simultaneous production of END and ENL by *Lactobacillus gasseri* and *Lactobacillus salivarius* strains (Bravo et al., 2017).

In the present work we extended the study of the enterolignan production from lignan extracts to other strains of bifidobacteria, together with the analyses of enterolignan metabolism from pure lignans (SECO and MAT) by the selected *Bifidobacterium* and *Lactobacillus* strains. Given that lignans are common in Western diet and the health effects of enterolignans, bacteria able to transform dietary lignans into enterolignans would have great biotechnological potential.

2. Material and methods

2.1. Bacterial strains and growth conditions

Bifidobacterium and *Lactobacillus* strains of human origin tested in this work are listed in Table 1. Bifidobacteria were cultivated under strict anaerobic conditions at 37°C in RCM broth (BD, Le Pont de Claix, France). Lactobacilli were grown under strict anaerobic conditions at 37°C in MRS (Oxoid Ltd., Basingstoke, United Kingdom).

2.2. Chemicals and solvents

The HPLC grade standard compounds SECO, MAT, END and ENL were purchased from Merck KGaA (Darmstadt, Germany). Methanol, acetic acid and acetonitrile were of HPLC grade (LabScan, Gliwice, Poland). LinumLife EXTRA, a lignan extract from flax seeds, were provided by Frutarom Netherlands BV (Veenendaal, the Netherlands).

2.3. Screening of *Bifidobacterium* strains producing enterolignans

Bifidobacterium strains were inoculated 0.1 % in 10 ml of BHI medium (Biolife, Milan, Italy) containing 0.5 g/L of L-cysteine (Merck KGaA), sterilized at 121 °C for 20 min, and supplemented with flax extracts (2 g/L) dissolved in DMSO (Merck KGaA). The pH of the broth was adjusted to 7.4 with NaOH. When present, the amount of DMSO in the broth did not affect bacterial growth. *Bifidobacterium* strains with the polyphenols were incubated in sealed jars (Oxoid Ltd) under anaerobic conditions using AnaeroGen sachets (Oxoid Ltd.) at 37 °C for 5 days.

BHI broth with flax extracts without bacteria was used as control. The control was incubated in sealed jars under anaerobic conditions at 37 °C for 5 days. All experiments were done in duplicate and the results were reproducible.

2.4. Analysis of enterolignan production from pure lignan compounds

The metabolism of SECO and MAT was analysed in the *Bifidobacterium* strains that were able to produce END from flax extract, and the three *Lactobacillus* strains that previously had showed END and ENL production from lignan extracts (Bravo et al., 2017). Besides them, two *Bifidobacterium* strains unable to produce enterolignans from flax extract were also tested. The strains were inoculated 0.1 % in 10 ml of BHI medium (Biolife, Milan, Italy) containing 0.5 g/L of L-cysteine and SECO (5 mM) or MAT (5 mM). The pH of the broth was adjusted to 7.4 with NaOH. The inoculated broths supplemented with flax extract and pure compounds were incubated in sealed jars (Oxoid) under anaerobic conditions using AnaeroGen sachets (Oxoid) at 37 °C. Non inoculated BHI broths with SECO or MAT were used as controls. All experiments were done in duplicate and the results were reproducible.

2.5. Identification and quantification of lignans by HPLC-ESI/MS analysis

Extraction of lignans was performed according to Gaya, Arqués, et al. (2016). All samples were analysed by HPLC-ESI/MS. Separation of phenolic compounds was achieved on a reverse phase Nova-Pak C18 column (300 mm × 3.9 mm, 4 µm) (Waters, Barcelona, Spain) and the analytical conditions were based on those described by Gaya, Medina, et al. (2016). Mass spectra were obtained using a LC-MS Agilent 1200 (Palo

Alto, CA, USA) chromatography system equipped with a quaternary pump (G1311A), a degasser (G1322A), an thermostated autosampler (G1367B), a thermostated column compartment (G1316A), a photodiode array detector (G1315B), and a quadrupole mass spectrometer (QTOF Agilent G6530A) with an electrospray ionization (ESI) interface and Masshunter Data Acquisition and Qualitative Analysis (B.40.0) as control software. Other ESI/MS parameters were as follows: range acquisition 100–1000 m/z, gas temperature 350 °C, gas flow 10 L/min, nebulizer 45 psig, sheath gas temperature 350 °C, sheath gas flow 11 L/min, capillary voltage 3500 V, and fragmentation voltage 120 V. The mass spectrometer operated in the negative ion mode.

Quantification of SECO, MAT, END and ENL was made using the external standard calibration curves, with commercial standards. SDG was not quantified, though their areas were measured by HPLC-ESI/MS, and DHEND could be quantified by the calibration curves of the most similar compounds END.

3. Results

3.1. Screening of enterolignan production from flax extract by Bifidobacterium strains

We analysed the metabolism of lignans by 20 bifidobacterial strains belonging to 10 different species and selected from INIA culture collection for its biotechnological and probiotic properties. The screening for lignan metabolism was carried out in the first place on flax extract, containing as main lignans SDG and SECO. An increase of SECO with respect to control was observed in 14 out of the 20 strains tested (Table 3), with the highest formation of SECO corresponding to *Bifidobacterium pseudocatenulatum* INIA

P946. Three *Bifidobacterium* strains of three different species produced END from flax extract: *Bifidobacterium bifidum* INIA P466, *Bifidobacterium catenulatum* INIA P732 and *Bifidobacterium pseudolongum* INIA P2, with no apparent relationship between increment of SECO and END formation.

3.2. Enterolignan production from pure compounds by *Lactobacillus* and *Bifidobacterium* strains

The *Bifidobacterium* and *Lactobacillus* strains able to produce enterolignans from flax extract were subjected to further analysis using the pure lignans SECO and MAT. Additionally, two strains that had not produced END from lignan extract were also incubated with the pure compounds.

The six strains that had previously showed enterolignan production from flax extract, showed production of END and ENL from SECO and the formation of the intermediate compound DHEND (Table 4, Figure 1 and 2). The production of ENL from MAT and the transformation of SECO into MAT described elsewhere (Heinonen et al., 2001) were not observed in any of the tested strains. On the other hand, the two bifidobacteria non-producing END from lignan extract and the control did not show production of enterolignans or DHEND from any of the compounds tested.

Of the metabolised SECO, more than 96 % was transformed into ENL by the six strains, resulting in lower concentration of DHEND and END (Table 2). The concentration of DHEND was approximately 3-fold higher than that of END for all the enterolignan-producing strains. No strain consumed the whole of SECO, being *B. catenulatum* INIA

P732 and *Lb. gasseri* INIA P508 the strains which consumed the greatest percentage of SECO (58.28 % and 53.22 % respectively) (Table 4).

In concordance with the greatest amount of SECO metabolised, the strain that yielded the highest concentration of ENL was *B. catenulatum* INIA P732 followed by *Lb. gasseri* INIA P508. The reduction of SECO could be correlated with the increase of ENL in the producing strains.

4. Discussion

Lignans are phytoestrogens common in Western diet, and the presence of enterolignans in plasm has been linked to the prevention of cardiovascular disease and colon, prostate and breast cancer (Aarestrup et al., 2013; Piller et al., 2006; Seibold et al., 2014; Vanharanta et al., 1999; Yoder et al., 2015). Enterolignans are produce by the intestinal microbiota and the interindividual differences in their production could be linked to variability in the microbiota composition. Therefore, the identification and use of bacteria for the biotransformation of lignan extracts into END and ENL is of high interest.

Recently we described the obtaining of END from flax extracts by the action of *B. adolescentis* INIA P784 (Gaya, Peirotén, et al., 2017), and three *Lb. salivarius* and *Lb. gasseri* strains able to produce END and ENL also from flax extracts (Bravo et al., 2017). In the present work, we extended the study including more bifidobacteria and exploring the metabolism with pure lignans.

Deglycosylation of the SDG present in flax extracts into SECO is the first step towards the formation of enterolignans. Several of the bifidobacteria tested showed the ability to increase the content of SECO of the flax extracts, accordingly to the frequency of this

trait found in bifidobacteria (Gaya, Peirotén, et al., 2017). Nevertheless, the ulterior metabolism towards the formation of enterolignans was only observed on three of the tested bifidobacteria, with the detection of low amounts on END. As opposed to the previous findings on *Lb. salivarius* and *Lb. gasseri* (Bravo et al., 2017), none of the bifidobacteria were able to produce ENL from flax extracts, and they produced END in lower amounts than those *Lactobacillus* strains. Interestingly, the ulterior incubation of these strains with pure SECO revealed the production of high levels of ENL, as well as the detection of DHEND and END, in both the *Lactobacillus* and the *Bifidobacterium* selected strains. The different behavior of bifidobacteria and lactobacilli when incubated with flax extract could be explained by a more inhibited growth of the bifidobacteria when the extract was present but not in presence of pure SECO, that could explain the lower production of END and the lack of production of ENL. The most of the pure SECO used by the strains was transformed into ENL, this was also observed with the intestinal microbiota of adults and infants (Gaya, Medina, et al., 2016; Gaya, Sánchez-Jiménez, et al., 2017), which produced much more ENL than END or even only produced ENL.

The other pure lignan tested, MAT, has been described as intermediate in the route of transformation of SDG and SECO by intestinal bacteria (Quartieri et al., 2016), where MAT is dehydroxylated to obtain ENL. However, we did not observe ENL production from MAT by any of the *Lactobacillus* or *Bifidobacterium* strains producing enterolignans from SECO. The levels of MAT were not affected because of the incubation with the tested strains, and so it can be inferred that these strains lack the capability of metabolizing this compound.

Both the three *Bifidobacterium* and the three *Lactobacillus* strains producing enterolignans from flax extract increased the production of these compounds, especially

of ENL, when the substrate was pure SECO instead of flax extract. This suggests that a higher availability of SECO facilitated the production of ENL. Although an increase on the deglycosylation of pure SDG into SECO has been described not to influence the enterolignan production by intestinal microbiota (Quartieri et al., 2016), other studies have showed a relationship between higher SDG transformation from lignan extracts and enterolignan production by intestinal microbiota (Gaya, Medina, et al., 2016; Gaya, Sánchez-Jiménez, et al., 2017). Differences between pure SDG used by Quartieri et al. (2016) and the SDG present in flax extracts (Gaya, Medina, et al., 2016; Gaya, Sánchez-Jiménez, et al., 2017) must be considered, incubation of strains with pure SDG could facilitate its subsequent metabolism.

Metabolism from pure SECO revealed that certain *Lactobacillus* and *Bifidobacterium* strains have an active lignan metabolism, far superior to that found with flax extracts. The greater availability of pure SECO facilitated their subsequent metabolism. However, the production of END and ENL from pure SECO is not viable due to the high cost. The combination of strains capable of producing high concentrations of SECO from SDG, such *B. pseudocatenulatum* INIA P946, together with any of the *Bifidobacterium* and *Lactobacillus* strains capable of transforming SECO into ENL would be of great interest in the development of functional foods containing enterolignans.

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Table 1. Bacterial strains tested in this work.

Strain	Origin	Reference
<i>B. adolescentis</i> INIA P549	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. animalis</i> INIA P490	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. animalis</i> INIA P913	adult faeces	INIA culture collection
<i>B. bifidum</i> INIA P466	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P12	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P367	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P763	breast-fed infant faeces	Peirotén et al., 2018
<i>B. breve</i> INIA P776	child faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P732	breast-fed infant faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P765	adult faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P825	child faeces	Peirotén et al., 2018
<i>B. dentium</i> INIA P883	adult faeces	INIA culture collection
<i>B. infantis</i> INIA P593	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. infantis</i> INIA P722	breast-fed infant faeces	Peirotén et al., 2018
<i>B. infantis</i> INIA P728	breast-fed infant faeces	Peirotén et al., 2018
<i>B. longum</i> INIA P9	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. longum</i> INIA P750	breast-fed infant faeces	Peirotén et al., 2018
<i>B. pseudocatenulatum</i> INIA P846	child faeces	Peirotén et al., 2018
<i>B. pseudocatenulatum</i> INIA P946	breast-fed infant faeces	INIA culture collection
<i>B. pseudolongum</i> INIA P2	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. gasseri</i> INIA P508	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. salivarius</i> INIA P448	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. salivarius</i> INIA P183	breast-fed infant faeces	Rodríguez et al., 2012

Table 2. Characteristics of lignans and enterolignans detected in the present work.

Compound	Formula	HPLC-ESI/MS	
		[M-H] ⁻ (<i>m/z</i>)	Retention time (min)
SDG	C ₃₂ H ₄₆ O ₁₆	685.3	9.7
SECO	C ₂₀ H ₂₆ O ₆	361.2	17.2
MAT	C ₂₀ H ₂₂ O ₆	357.1	25.0
DHEND	C ₁₈ H ₂₂ O ₆	333.1	26.6
END	C ₁₈ H ₂₂ O ₄	301.1	20.8
ENL	C ₁₈ H ₁₈ O ₄	297.1	27.1

*Secoisolariciresinol diglucoside (SDG), secoisolariciresinol (SECO), matairesinol (MAT), dyhydroxyenterodiol (DHEND), enterodiol (END), enterolactone (ENL)

Table 3. Metabolism of lignans from flax extracts (LinumLife EXTRA) after incubation with a collection of 20 *Bifidobacterium*.

<i>Bifidobacterium</i> strains	Production of*	
	SECO (mM) ¹	END (mM) ²
<i>B. adolescentis</i> INIA P549	n.i.	n.d.
<i>B. animalis</i> INIA P490	0.571 ± 0.027	n.d.
<i>B. animalis</i> INIA P913	0.837 ± 0.214	n.d.
<i>B. bifidum</i> INIA P466	n.i.	0.008 ± 0.002
<i>B. breve</i> INIA P12	0.596 ± 0.047	n.d.
<i>B. breve</i> INIA P367	1.195 ± 0.091	n.d.
<i>B. breve</i> INIA P763	n.i.	n.d.
<i>B. breve</i> INIA P776	1.143 ± 0.245	n.d.
<i>B. catenulatum</i> INIA P732	0.826 ± 0.154	0.002 ± 0.001
<i>B. catenulatum</i> INIA P765	n.i.	n.d.
<i>B. catenulatum</i> INIA P825	0.346 ± 0.114	n.d.
<i>B. dentium</i> INIA P883	0.052 ± 0.036	n.d.
<i>B. infantis</i> INIA P593	n.i.	n.d.
<i>B. infantis</i> INIA P722	n.i.	n.d.
<i>B. infantis</i> INIA P728	0.037 ± 0.018	n.d.
<i>B. longum</i> INIA P9	0.021 ± 0.011	n.d.
<i>B. longum</i> INIA P750	n.i.	n.d.
<i>B. pseudocatenulatum</i> INIA P846	0.501 ± 0.087	n.d.
<i>B. pseudocatenulatum</i> INIA P946	6.254 ± 2.561	n.d.
<i>B. pseudolongum</i> INIA P2	0.217 ± 0.087	0.011 ± 0.001

*Concentrations determined by means of HPLC ESI/MS and expressed as mean ± standard deviation. ¹Secoisolariciresinol (SECO) as increment respect to control without bacteria (0.092 mM), n.i. no increment. ²Enterodiol (END), n.d. no detected

Table 4. Concentrations of lignan metabolism compounds after incubation with SECO (5 mM).

Strains	Compound (mM)*			
	SECO	DHEND	END	ENL
<i>B. bifidum</i> INIA P466	3.325 ± 0.632	0.025 ± 0.010	0.008 ± 0.001	1.734 ± 0.174
<i>B. catenulatum</i> INIA P732	2.089 ± 0.467	0.041 ± 0.012	0.021 ± 0.002	2.321 ± 0.098
<i>B. pseudolongum</i> INIA P2	3.829 ± 0.541	0.018 ± 0.002	0.007 ± 0.001	0.973 ± 0.071
<i>Lb. gasseri</i> INIA P508	2.314 ± 0.245	0.017 ± 0.009	0.006 ± 0.001	2.147 ± 0.049
<i>Lb. salivarius</i> INIA P448	2.943 ± 0.651	0.024 ± 0.006	0.008 ± 0.001	1.813 ± 0.111
<i>Lb. salivarius</i> INIA P183	3.521 ± 0.214	0.019 ± 0.002	0.010 ± 0.001	1.242 ± 0.087
<i>B. breve</i> INIA P12	4.873 ± 0.132	n.d.	n.d.	n.d.
<i>B. infantis</i> INIA P728	4.965 ± 0.041	n.d.	n.d.	n.d.

*Concentrations determined by means of HPLC ESI/MS and expressed as mean ± standard deviation, secoisolariciresinol (SECO), matairesinol (MAT), dyhydroxyenterodiol (DHEND), enterodiol (END), enterolactone (ENL).

1 **Table 1.** Bacterial strains tested in this work.
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Strain	Origin	Reference
<i>B. adolescentis</i> INIA P549	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. animalis</i> INIA P490	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. animalis</i> INIA P913	adult faeces	INIA culture collection
<i>B. bifidum</i> INIA P466	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P12	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P367	breast-fed infant faeces	Rodríguez et al., 2012
<i>B. breve</i> INIA P763	breast-fed infant faeces	Peirotén et al., 2018
<i>B. breve</i> INIA P776	child faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P732	breast-fed infant faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P765	adult faeces	Peirotén et al., 2018
<i>B. catenulatum</i> INIA P825	child faeces	Peirotén et al., 2018
<i>B. dentium</i> INIA P883	adult faeces	INIA culture collection
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<i>B. longum</i> INIA P750	breast-fed infant faeces	Peirotén et al., 2018
<i>B. pseudocatenulatum</i> INIA P846	child faeces	Peirotén et al., 2018
<i>B. pseudocatenulatum</i> INIA P946	breast-fed infant faeces	INIA culture collection
<i>B. pseudolongum</i> INIA P2	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. gasseri</i> INIA P508	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. salivarius</i> INIA P448	breast-fed infant faeces	Rodríguez et al., 2012
<i>Lb. salivarius</i> INIA P183	breast-fed infant faeces	Rodríguez et al., 2012

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Table 2. Characteristics of lignans and enterolignans detected in the present work.

Compound	Formula	HPLC-ESI/MS	
		[M-H] ⁻ (<i>m/z</i>)	Retention time (min)
SDG	C ₃₂ H ₄₆ O ₁₆	685.3	9.7
SECO	C ₂₀ H ₂₆ O ₆	361.2	17.2
MAT	C ₂₀ H ₂₂ O ₆	357.1	25.0
DHEND	C ₁₈ H ₂₂ O ₆	333.1	26.6
END	C ₁₈ H ₂₂ O ₄	301.1	20.8
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*Concentrations determined by means of HPLC ESI/MS and expressed as mean ± standard deviation, secoisolariciresinol (SECO), matairesinol (MAT), dyhydroxyenterodiol (DHEND), enterodiol (END), enterolactone (ENL).

Figure captions

Figure 1. Identification of secoisolariciresinol (SECO) and products of its metabolism by *Lb. salivarius* INIA P448. HPLC-ESI/MS chromatograms showing production of dyhydroxyenterodiol (DHEND), enterodiol (END) and enterolactone (ENL), and remaining SECO.

Figure 2. Identification of secoisolariciresinol (SECO) and products of its metabolism by *B. catenulatum* INIA P732. HPLC-ESI/MS chromatograms showing production of dyhydroxyenterodiol (DHEND), enterodiol (END) and enterolactone (ENL), and remaining SECO.

Figure 3. Enterolignan metabolism observed in *Bifidobacterium* and *Lactobacillus* strains. Secoisolariciresinol diglucoside (SDG) is transformed into secoisolariciresinol (SECO) by means of deglycosylation reaction (1). Dyhydroxyenterodiol (DHEND), enterodiol (END) and enterolactone (ENL) are produced afterwards by means of demethylation (2), dehydroxylation (3) and dehydrogenation (4) reactions. Neither production of matairesinol (MAT) from SECO nor production of ENL from MAT were detected.

Figure1

Figure2

Figure3