

New real-time quantitative PCR method for detecting and quantifying equol-producing bacteria in faeces

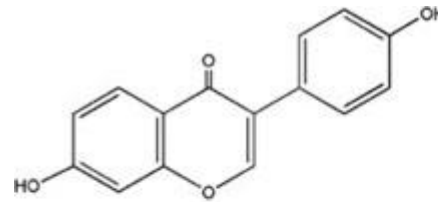


Lucía Vázquez, Ana Belén Flórez, Lucía Guadamuro and Baltasar Mayo
Instituto de Productos Lácteos de Asturias (IPLA)

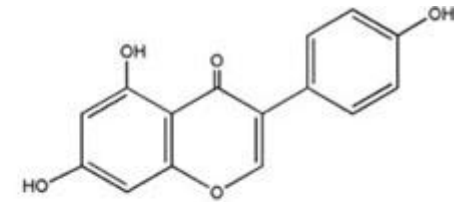
SOY ISOFLAVONES



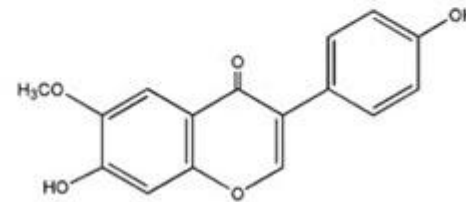
Main isoflavones in soy:



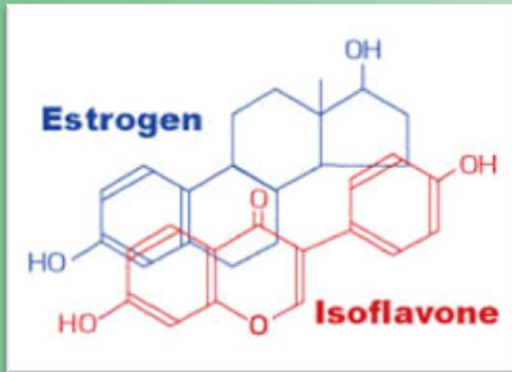
Daidzein



Genistein

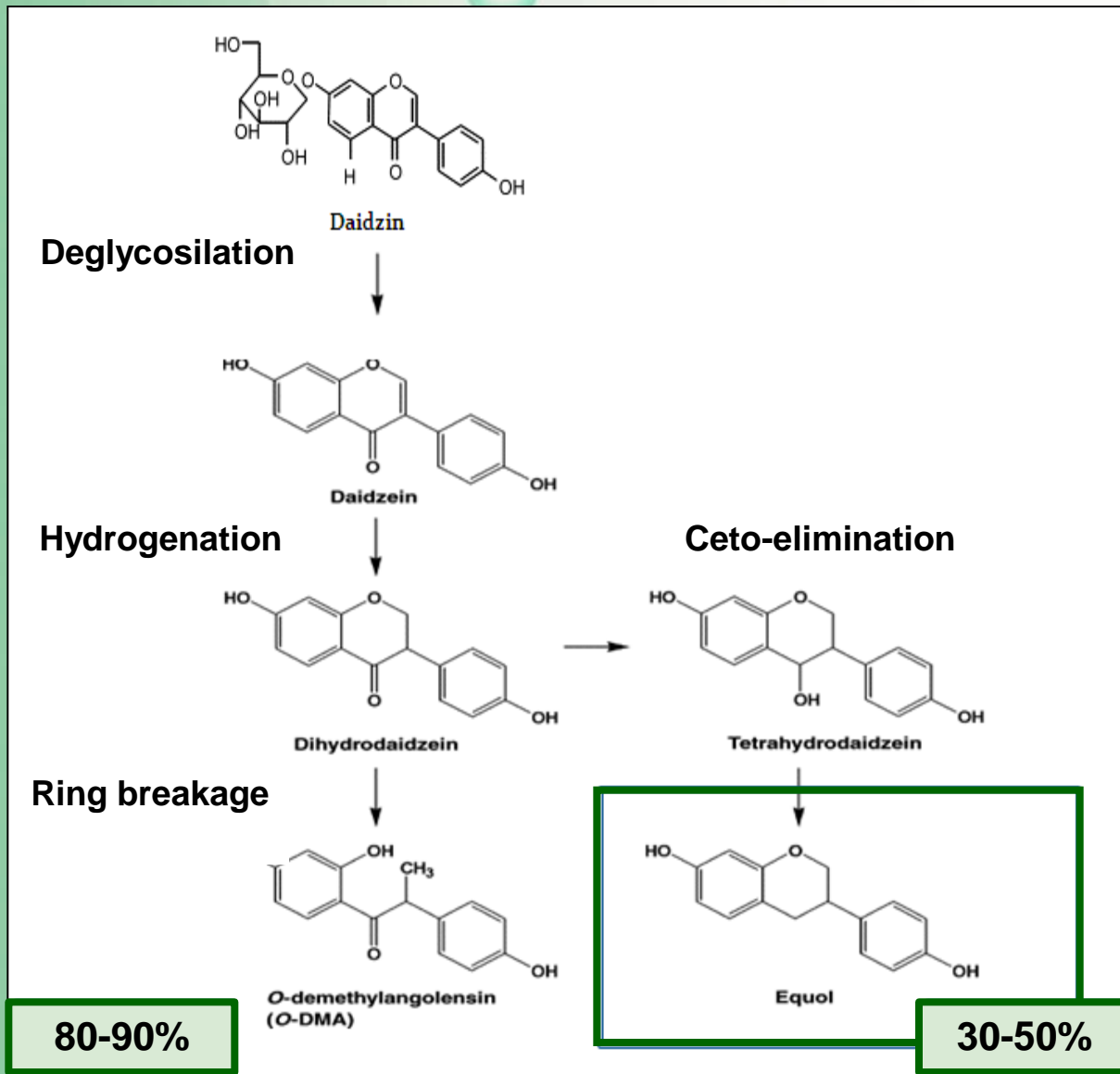


Glycitein

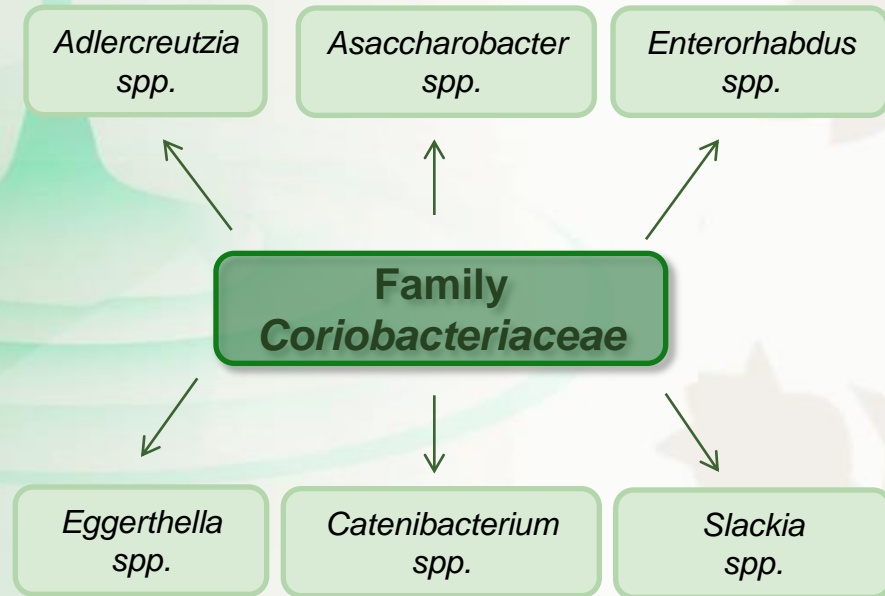
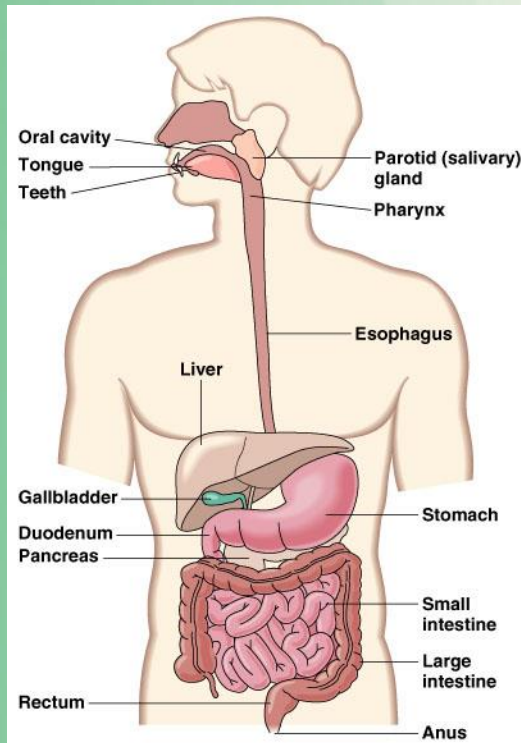


- Reduce menopause symptoms
- Decrease risk for a number of chronic diseases, such as osteoporosis, CV and neurodegenerative diseases and cancer
- Substitute Therapy Hormone Substitution (THS)
- In Spain, 35% of menopausal women follow this isoflavone phytotherapy

ISOFLAVONE METABOLISM



HUMAN GUT MICROBIOTA

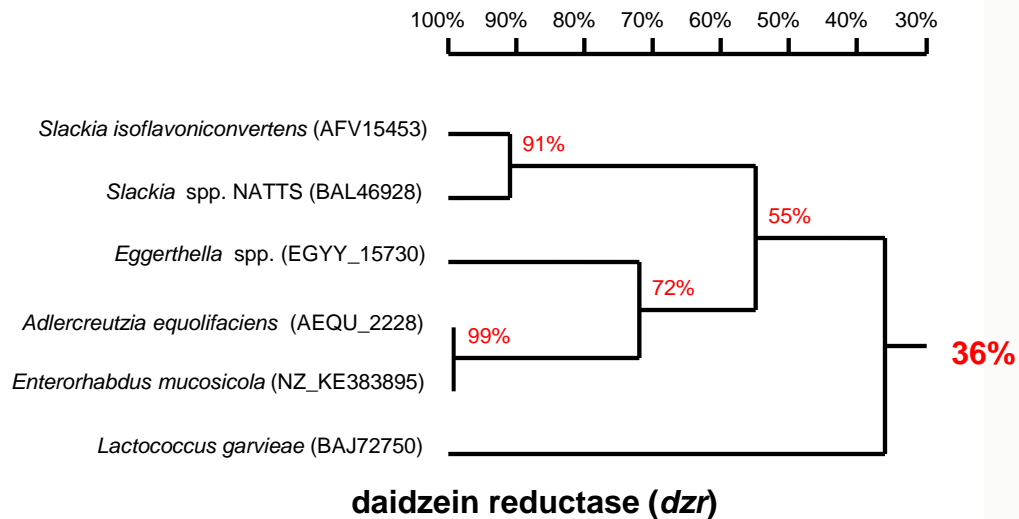
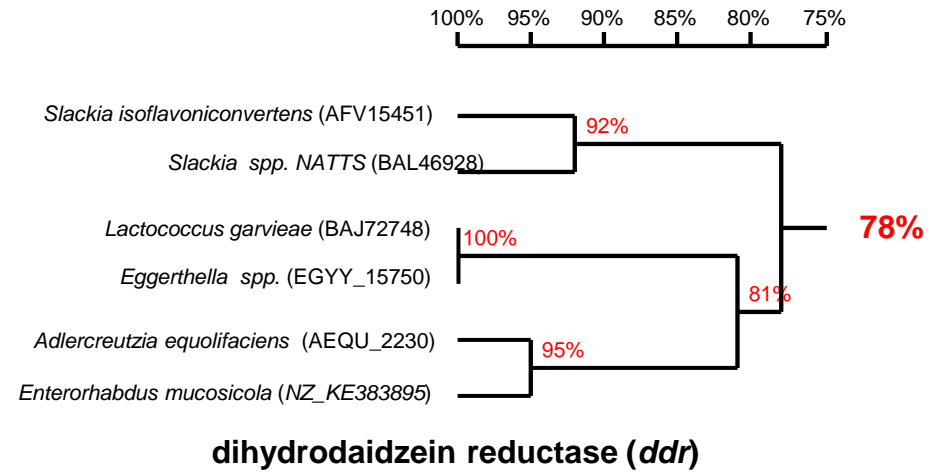
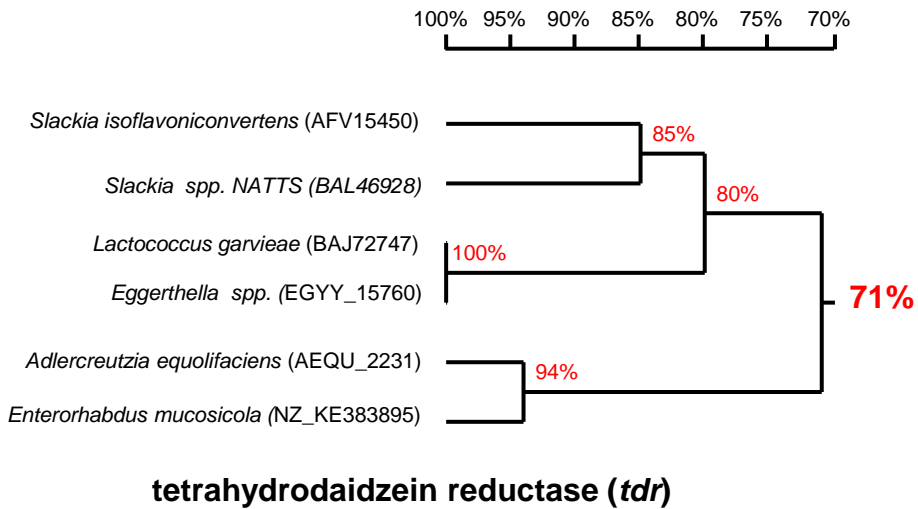


Complex and with great individual variability and diversity due to genetics, age, health state, diet, etc.

OBJETIVES

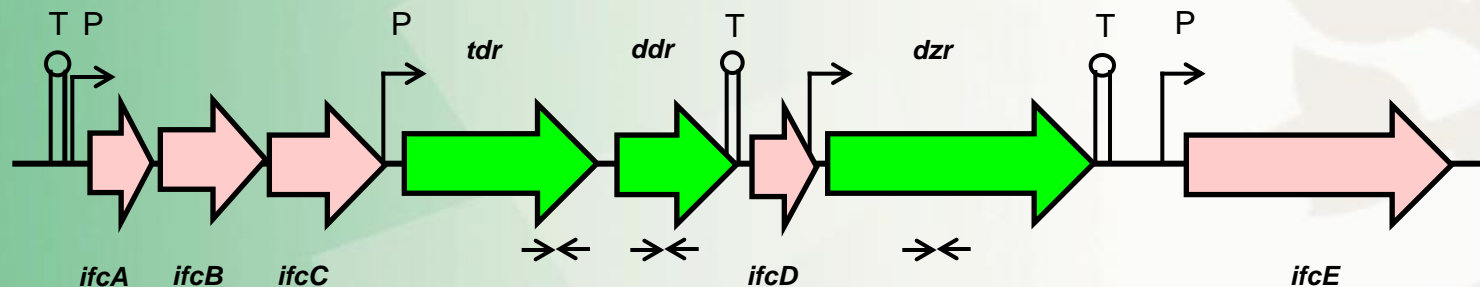
- To develop a method for tracking equol-producing bacteria
- Based on Real-Time PCR
- Targeting genes involved in equol formation

Homology of equol-related genes



qPCR primers

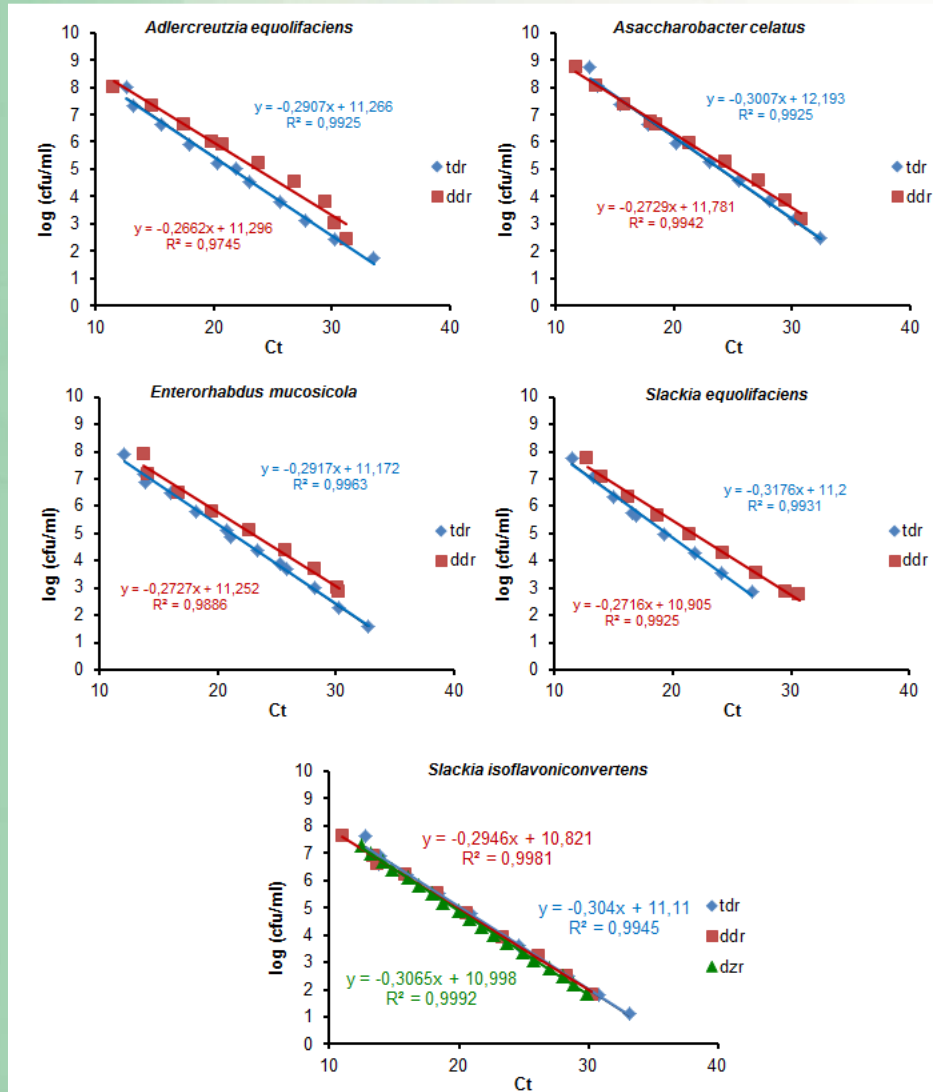
Oligonucleotide primer	Sequence (5'-3')	Position*	Efficiency (R ²)
tdr.qPCR-F	RTYAACGGCRAYATGCAGGT	<i>tdr</i> (1279-1298)	0.9934
tdr.qPCR-R	GGMAYYTCCATGTTGTAGGA	<i>tdr</i> (1372-1391)	
ddr.qPCR-F	CTCGAYCTSGTSTACAACGT	<i>ddr</i> (421-440)	0.9892
ddr.qPCR-R	GARTTGCAGCGRATKCCGAA	<i>ddr</i> (607-626)	
dzr.qPCR-F	GAAGCTTGATATGGACGACT	<i>dzr</i> (669-688)	0.999
dzr.qPCR-R	GGAATATGCACCTGTTCT	<i>dzr</i> (854-872)	



Equl gene cluster of *Slackia isoflavoniconvertens* DSM 22006^T
 Opposite arrows indicate position of the primers

Specificity of qPCR primers

Standard curves for *tdr*, *ddr* and *dzr* genes

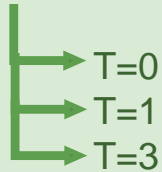


Detection limit:
 10^2 cfu/g of faeces.

qPCR assays of faecal samples

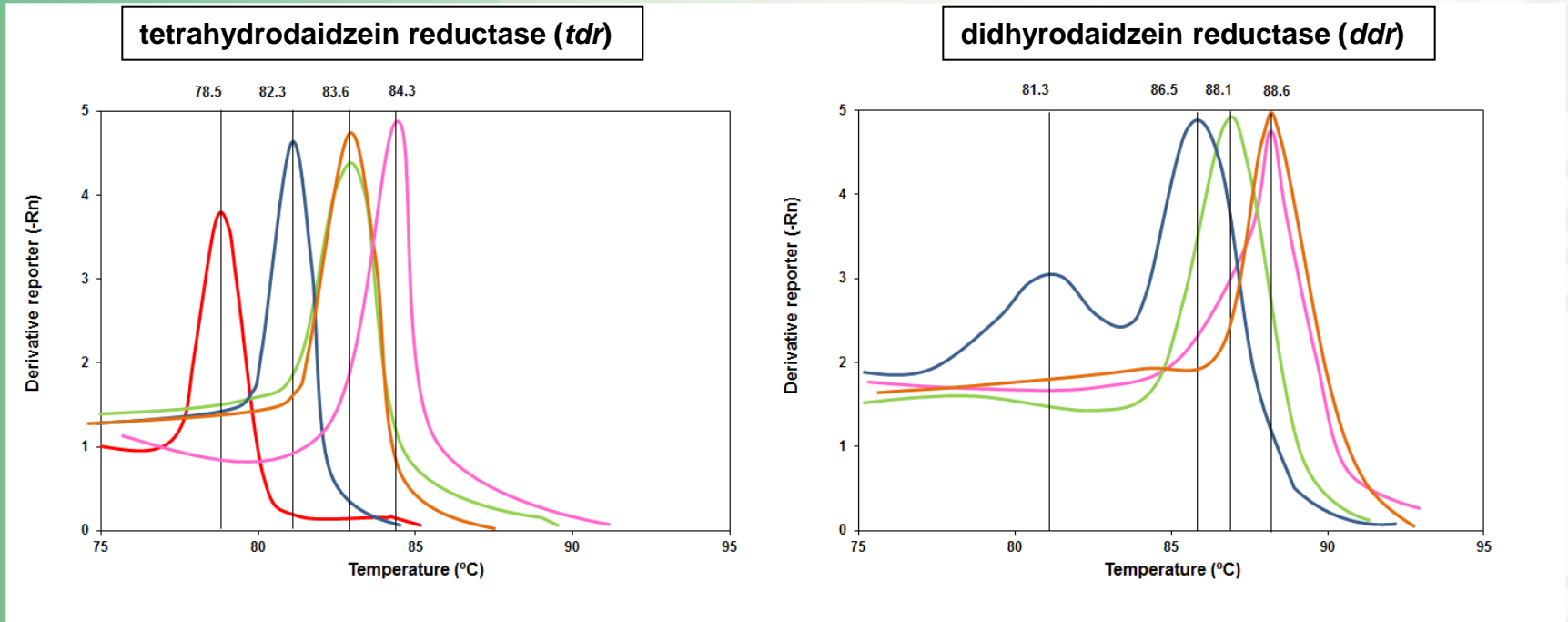
18 menopausal women

Three sampling point:



Women	Sample (time) ^a	qPCR amplification of total microbial DNA from faeces			
		Ct (<i>tdr</i>)	Log ₁₀ (ufc/ml) ±SD	Ct (<i>ddr</i>)	Log ₁₀ (ufc/ml) ±SD
Equol producers:					
W3	0	25.95 ± 0.01	3.57 ± 0.54	25.45 ± 0.05	4.17 ± 0.58
	1	23.75 ± 0.70	4.23 ± 0.53	25.00 ± 0.40	4.30 ± 0.58
	3	24.98 ± 0.18	3.86 ± 0.54	24.90 ± 0.04	4.32 ± 0.58
W8	0	22.53 ± 0.14	4.60 ± 0.52	22.25 ± 0.03	5.06 ± 0.55
	1	22.86 ± 0.22	4.50 ± 0.52	22.53 ± 0.19	4.98 ± 0.55
	3	22.53 ± 0.22	4.60 ± 0.52	21.67 ± 0.04	5.22 ± 0.55
W18	0	28.17 ± 0.57	2.90 ± 0.56	-	<2
	1	24.80 ± 0.22	3.92 ± 0.54	-	<2
	3	26.02 ± 0.25	3.55 ± 0.54	-	<2
Equol non-producers:					
W1	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W2	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W4	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W5	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W6	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W7	0	22.49 ± 0.08	4.61 ± 0.52	23.42 ± 0.05	4.73 ± 0.56
	1	23.40 ± 0.02	4.34 ± 0.53	24.30 ± 0.13	4.49 ± 0.57
	3	23.57 ± 0.13	4.29 ± 0.53	24.60 ± 0.01	4.41 ± 0.57
W9	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W10	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W11	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W12	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W13	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W14	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W15	0	24.16 ± 0.12	4.11 ± 0.53	25.77 ± 0.15	4.09 ± 0.52
	1	23.22 ± 0.08	4.39 ± 0.53	26.28 ± 0.25	3.94 ± 0.52
	3	23.91 ± 0.16	4.18 ± 0.53	27.18 ± 0.14	3.70 ± 0.53
W16	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2
W17	0	-	<2	-	<2
	1	-	<2	-	<2
	3	-	<2	-	<2


Analysis of the Melting Temperature



Tm of some amplicons was rather different to those of positive strains

Equol producing women:

W3 

W8 

W18 

Equol non-producing women:

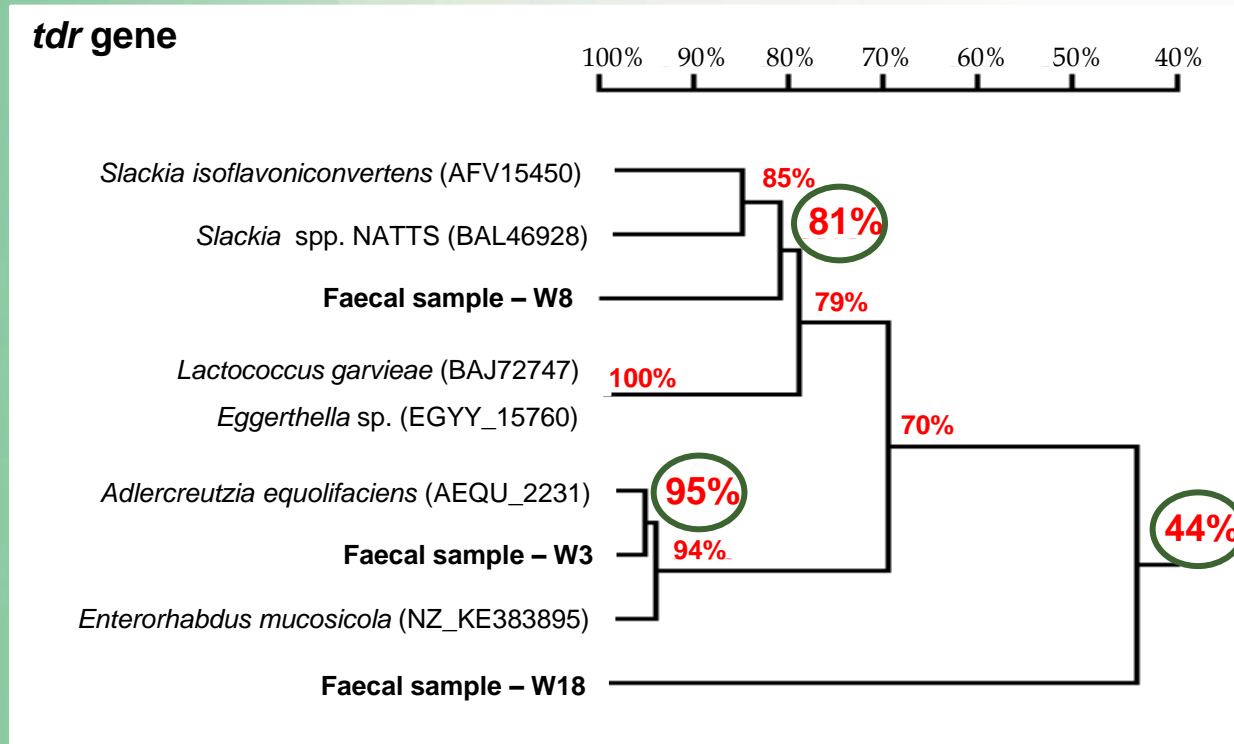
W7 

W15 



There might be genes involved in equol production different to those already characterized.

Homology of sequenced amplicons from faeces



A limited homology to the sequences of equivalent genes already characterized.

CONCLUSIONS

- 1.- We developed a highly specific, sensitive and reliable qPCR assay for detection and quantification of equol-producing bacteria in faecal samples
- 2.- The qPCR assay provides a tool for the evaluation of strategies aimed to increase endogenous formation of equol
- 3.- The biological significance of the presence/absence of *tdr* and *ddr* genes in isoflavone metabolism and equol production deserves further study

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