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


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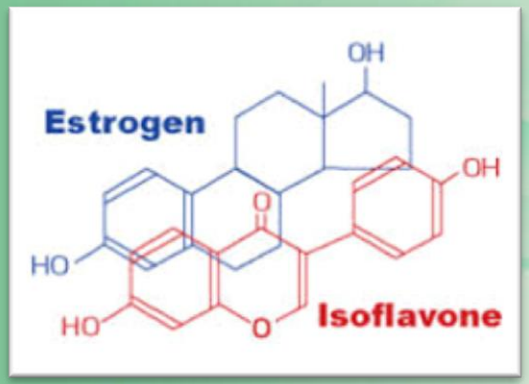
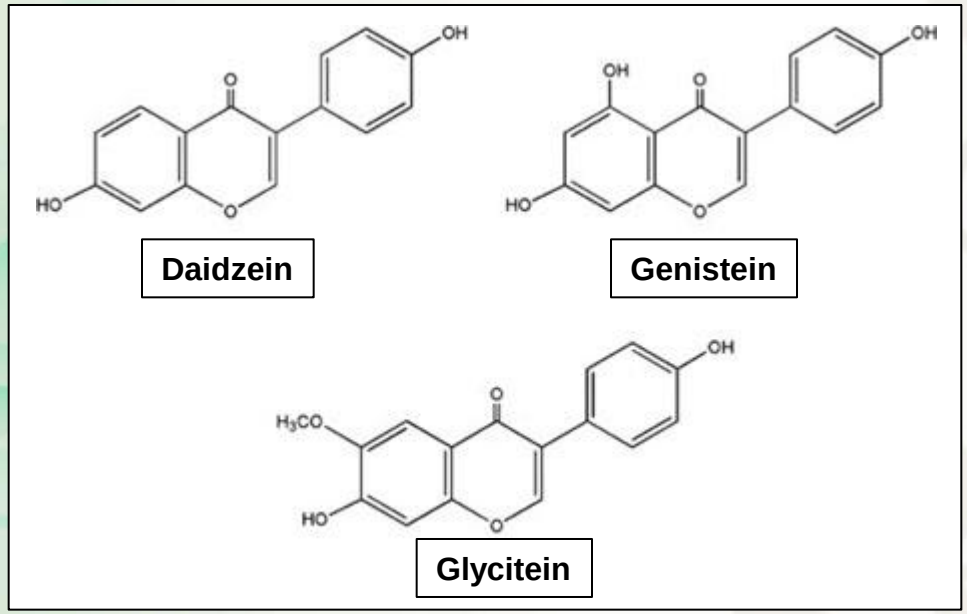
# SOY ISOFLAVONES

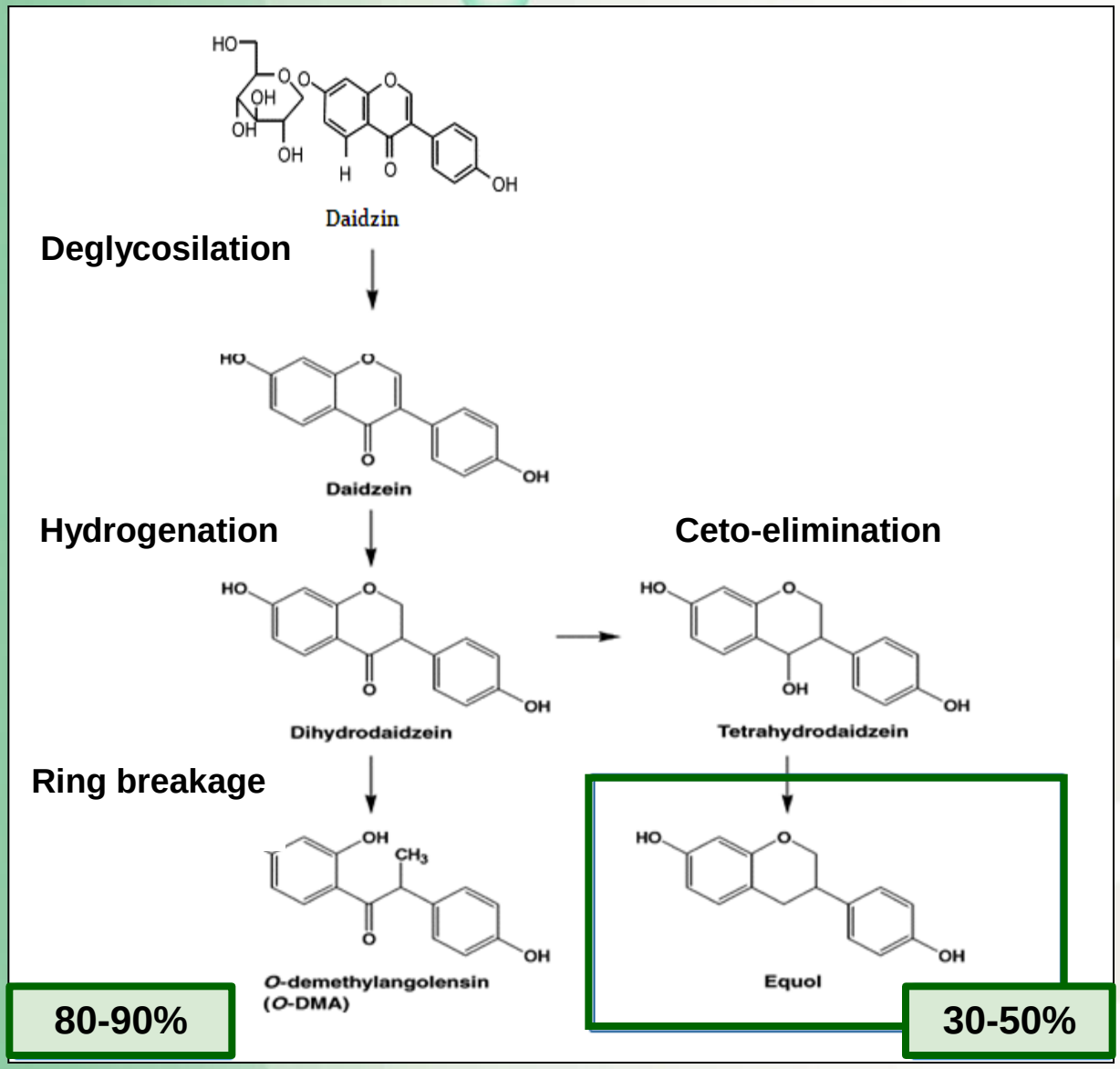



**Main isoflavones in soy:**

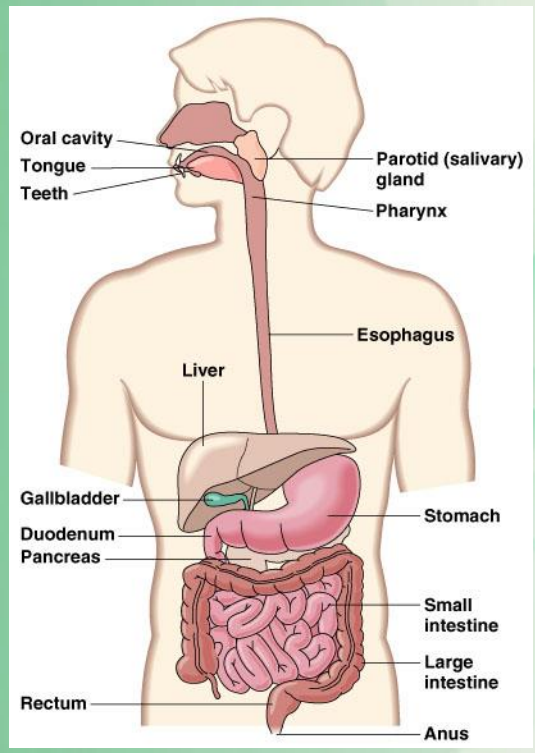


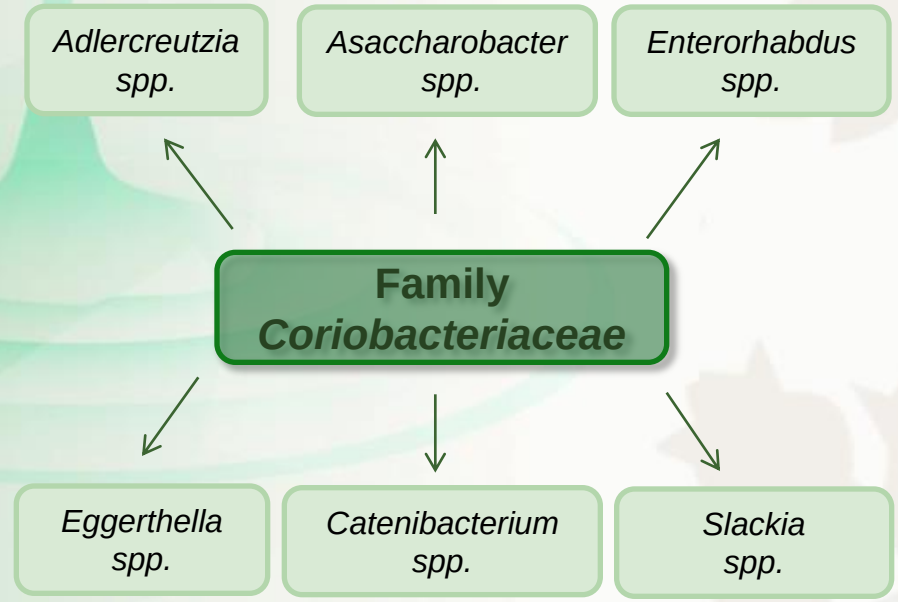

- Reduce menopause symptoms
- Decrease risk for a number of chronic diseases, such as osteoporosis, CV and neurodegenerative diseases and cancer
- Substitute Therapy Hormone Substitution (THS)
- In Spain, 35% of menopausal women follow this isoflavone phytotherapy

# ISOFLAVONE METABOLISM



# HUMAN GUT MICROBIOTA



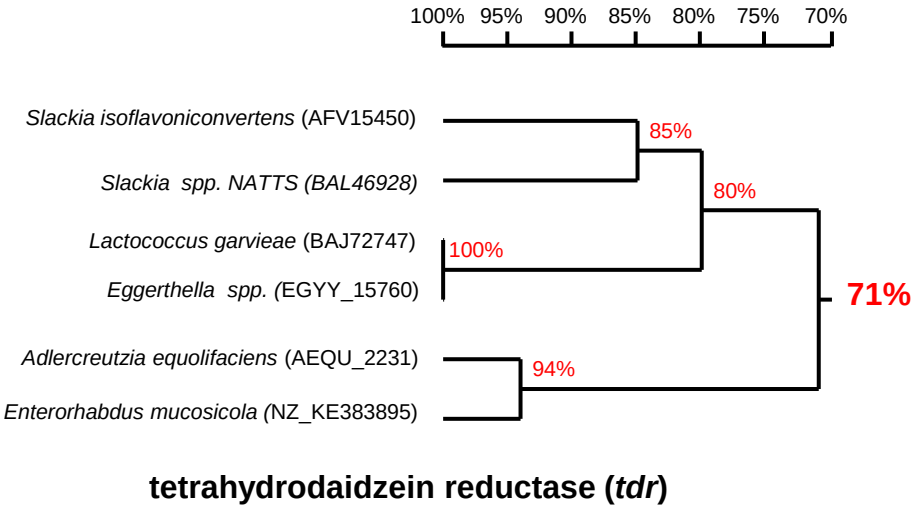




Complex and with great individual variability and diversity due to genetics, age, health state, diet, etc.

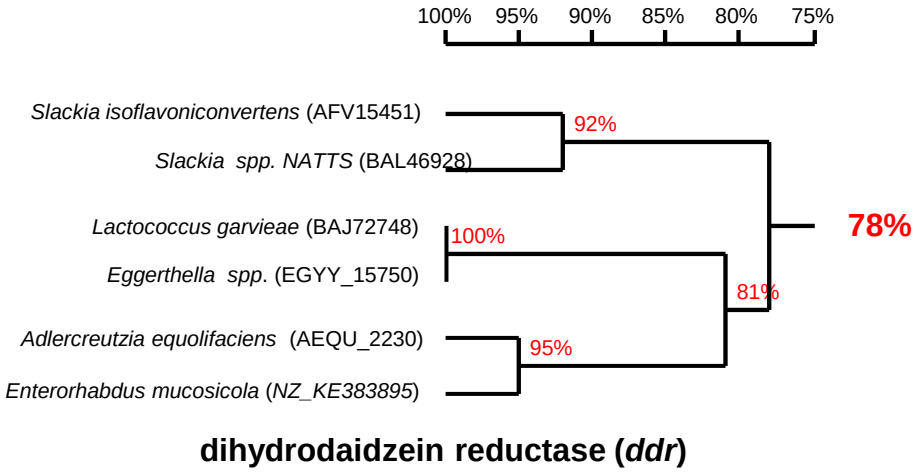
# OBJETIVES

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

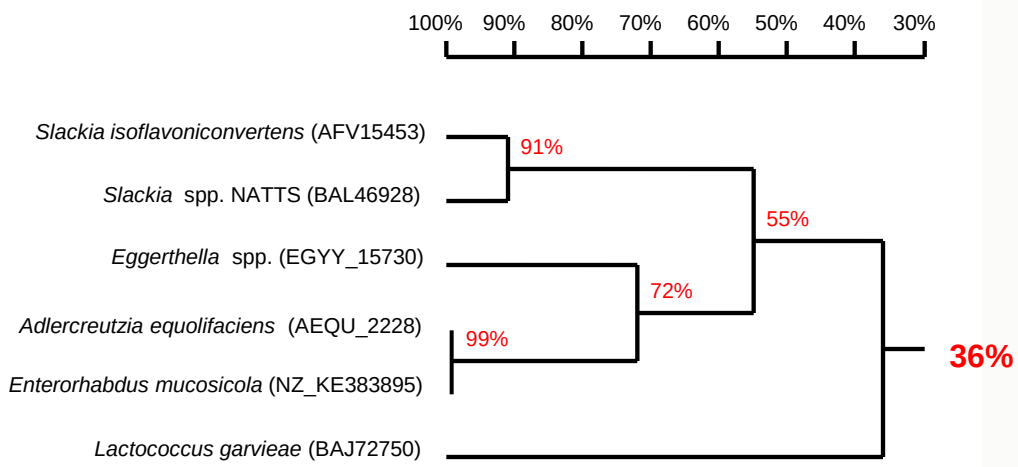
# Homology of equol-related genes




**tetrahydrodaidzein reductase (*tdr*)**



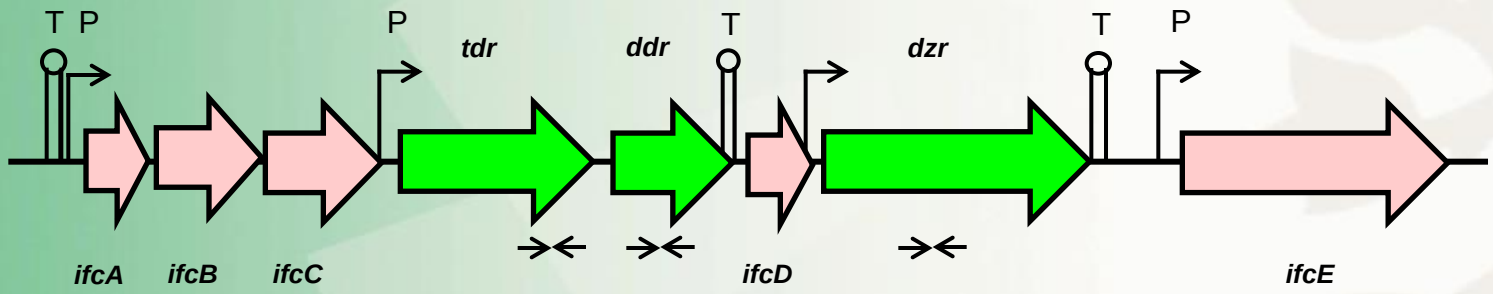

**dihydrodaidzein reductase (*ddr*)**




**daidzein reductase (*dzr*)**

# qPCR primers

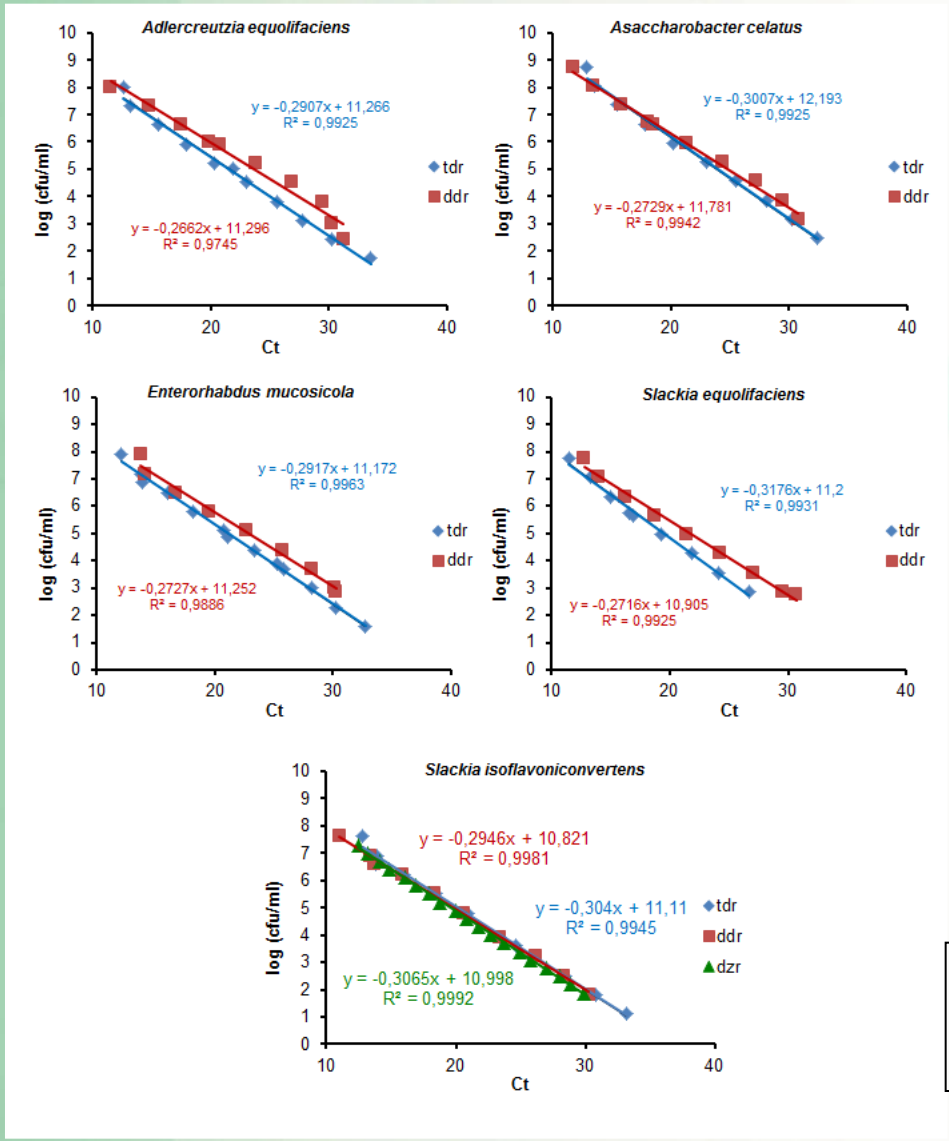
| Oligonucleotide primer | Sequence (5'-3') | Position* | Efficiency ($R^2$) |
|---|---|---|---|
| tdr.qPCR-F | RTYAACGGCRAYATGCAGGT | *tdr* (1279-1298) | 0.9934 |
| tdr.qPCR-R | GGMAYYTCCATGTTGTAGGA | *tdr* (1372-1391) | |
| ddr.qPCR-F | CTCGAYCTSGTSTACAACGT | *ddr* (421-440) | 0.9892 |
| ddr.qPCR-R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




Equol 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**

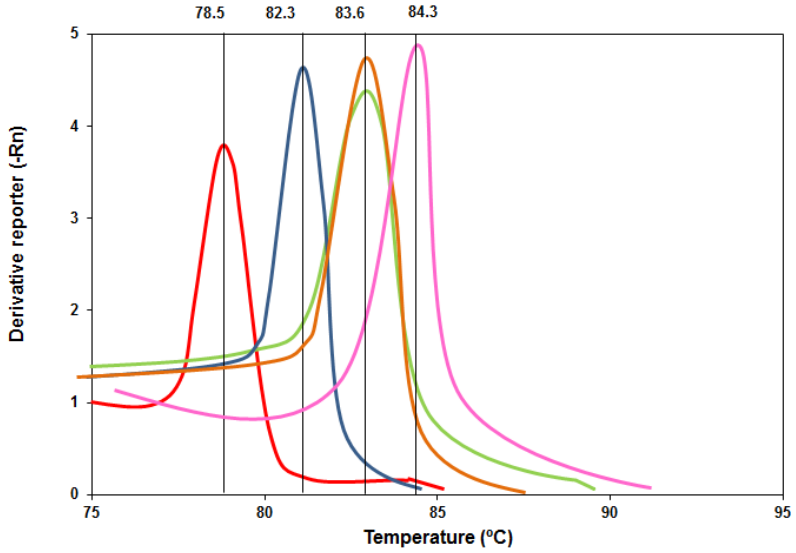
**Three sampling point**:

- T=0
- T=1
- T=3

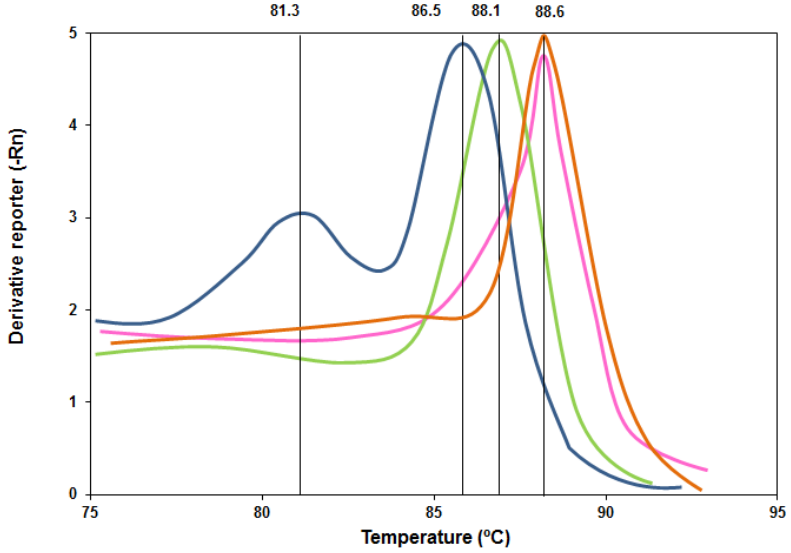
| Women | Sample (time)[a] | qPCR amplification of total microbial DNA from faeces | | | |
|---|---|---|---|---|---|
| | | Ct (*tdr*) | $Log_{10}$ (ufc/ml) ±SD | Ct (*ddr*) | $Log_{10}$ (ufc/ml) ±SD |
| **Equol producers:** | | | | | |
| **W3** | 0 | 25.95 ± 0.01 | 3.57 ± 0.54 | 25.45 ± 0.05 | 4.17 ± 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 | | |
| | 1 | 24.80 ± 0.22 | 3.92 ± 0.54 | - | <2 |
| | 3 | 26.02 ± 0.25 | 3.55 ± 0.54 | | |
| **Equol non-producers:** | | | | | |
| **W1** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W2** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W4** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W5** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W6** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **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 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W10** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W11** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W12** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W13** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W14** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **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 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |
| **W17** | 0 | | | | |
| | 1 | - | <2 | - | <2 |
| | 3 | | | | |

# Analysis of the Melting Temperature

**tetrahydrodaidzein reductase (*tdr*)**




**didhyrodaidzein reductase (*ddr*)**




**Equol producing women:**

**W3**

**W8**

**W18**

**Equol non-producing women:**

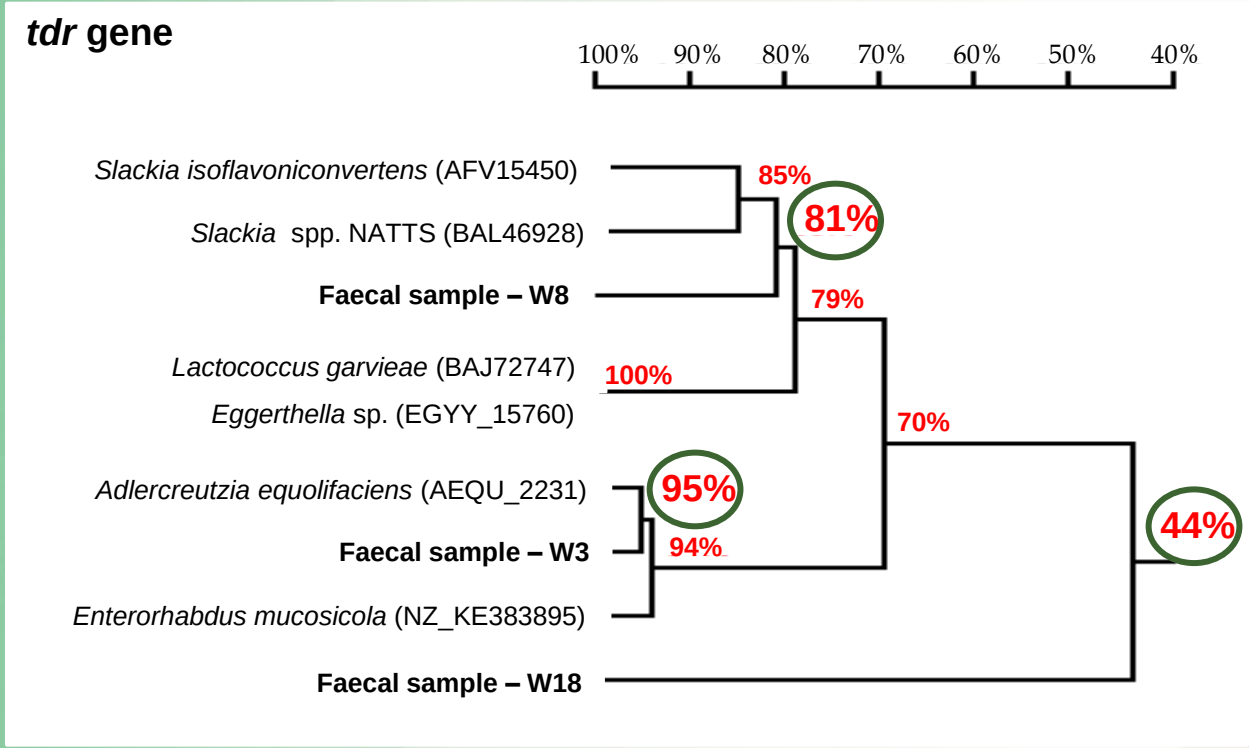
**W7**

**W15**

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

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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