

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

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Introduction and objectives

Epidemiological evidence suggests that high intake of isoflavones is associated with reduced menopause symptoms and decreased risk for a number of chronic diseases. Isoflavone-mediated effects appear to be driven by hormonal and antioxidant activities. Among isoflavone metabolites, equol from daidzein is the compound having the strongest estrogenic and antioxidant activity. However, only 30-60% of humans are capable of producing this compound. Conceivably, these subjects are the only that could fully benefit from isoflavone consumption.

Nearly all equol-producing microbes isolated so far fall into the family *Coriobacteriaceae*. This family includes a series of newly described species, which are difficult to isolate from a vast majority of accompanying microbes. Consequently, the aim of this study was to develop a real-time quantitative PCR (qPCR) method, which would allow identification and quantification of equol-producing bacteria by targeting genes involved in equol synthesis.

Materials and Methods

Publically-available genome sequences of equol-producing bacteria in the NCBI database were downloaded and the genes involved in equol production aligned by using Clustal Omega. Primers based on conserved regions of dihydrodaidzein reductase (*ddr*) and tetrahydrodaidzein reductase (*tdr*) genes were designed by using Primer Express Software v2.0. Efficacy and specificity of the primers were evaluated using as a template DNA from equol-producing and non-producing intestinal bacteria.

Results and Discussion

The designed primers showed high specificity and sensitivity with purified DNA from positive control bacteria. To demonstrate validity and reliability, the primers were then applied to detect and quantify equol-producing organisms in total DNA purified from faecal samples of equol-producing and non-producing women. Among a set of 18 women, three had been previously considered equol producers based on excretion of this molecule in urine. The *tdr* gene was detected in all equol producing women at a calculated level of about 4-5 log₁₀ copies per gram of faeces. However, the *ddr* gene could only be amplified from samples of two out of the three equol-positive women. To our surprise, positive amplification was also obtained for both *tdr* and *ddr* genes from DNA samples of two equol non-producing women.

This novel qPCR tool provides a technique for monitoring the numeric relationships of gut microbiota components with equol. The biological significance of the presence/absence of *tdr* and *ddr* genes in isoflavone metabolism phenotypes in humans is currently under study.