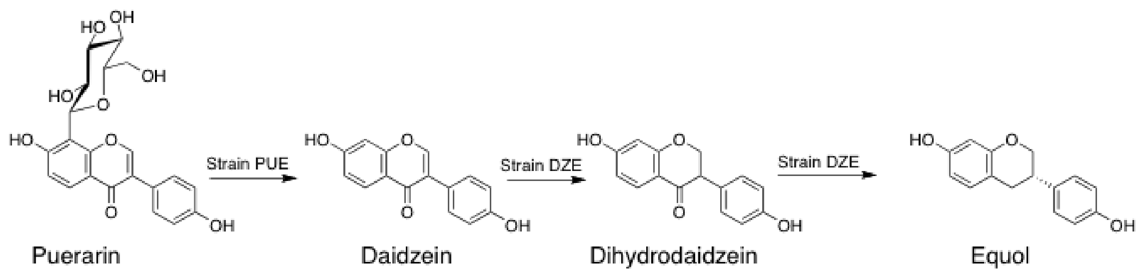
Puerarin



Metabolic processes of puerarin by human intestinal bacteria

代謝実験

腸内細菌代謝

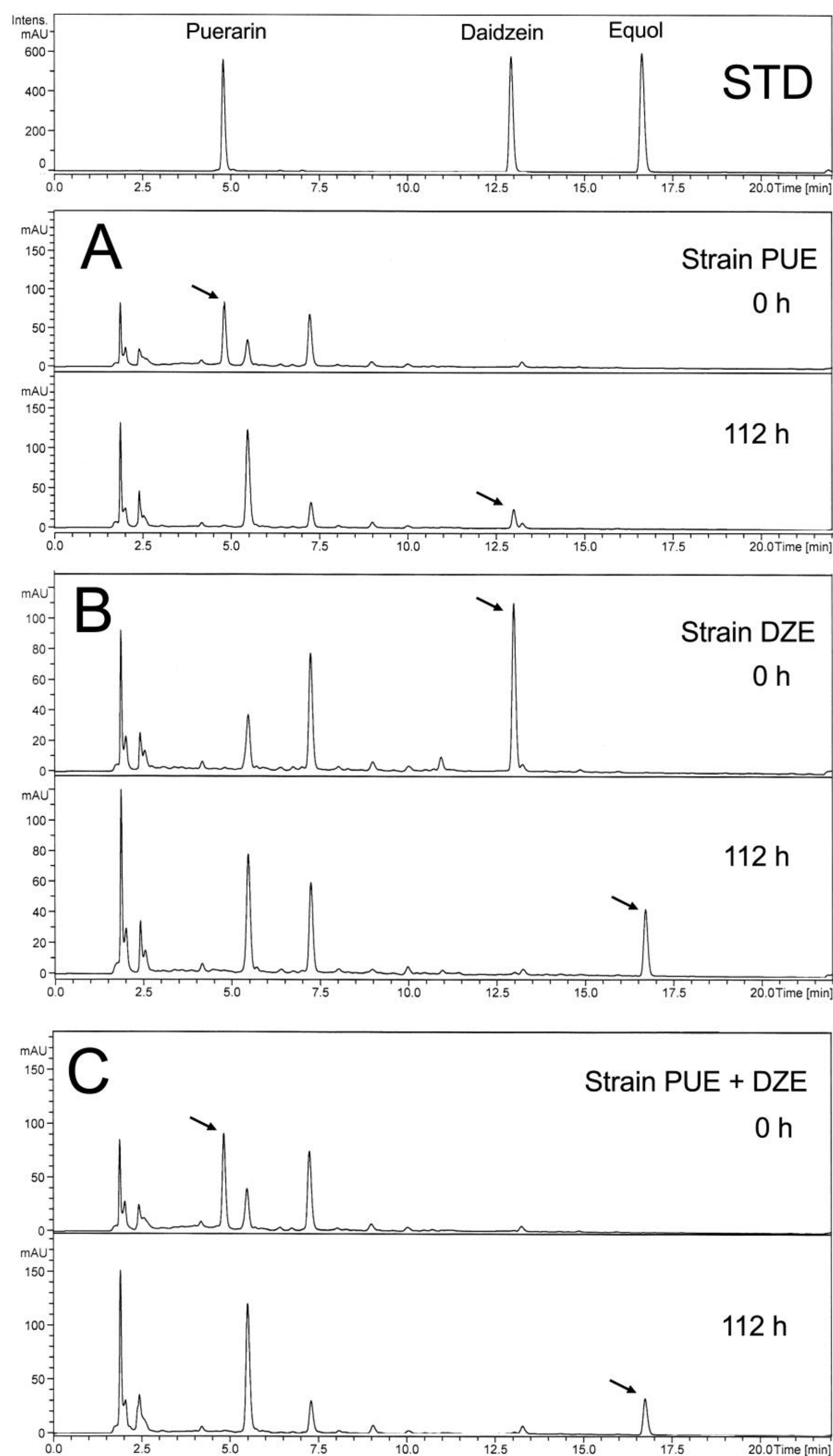


Fig. 1 Transformation of puerarin by human intestinal bacterial species, strains PUE and DZE (*Slackia equolifaciens* sp. nov.), analyzed by HPLC

A, transformation of puerarin to daidzein by strain PUE; B, transformation of daidzein

to equol by strain DZE; C, transformation of puerarin to equol by a mixture of strain PUE and DZE. Other peaks except puerarin, daidzein, and equol were identified as ingredients of GAM broth through analysis of incubation samples in the absence of substrates. STD, standards.

Bacterial Incubation and analysis of metabolites

Each bacterium was picked up from GAM agar plates and inoculated into 2 ml of GAM broth. When turbidities (540 nm) of bacterial suspensions reached 1.96±0.09 optical density (O.D.) (strain PUE) and 0.30±0.02 O.D. (strain DZE), a 100-µl portion of the precultured bacteria was inoculated into 2 ml of GAM broth with substrates. After incubation, a 100-µl aliquot was removed and extracted three times with 200 µl of BuOH. After evaporation of the BuOH *in vacuo*, the residue was dissolved in 0.3 ml of MeOH. The MeOH solution was filtered through a 0.2-µm membrane filter, and a 10-µl portion was injected onto a column for HPLC analysis.

LC/MS Analysis HPLC was performed on an Agilent 1100 system (Agilent Technologies, Waldbronn, Germany) with a photodiode array detector and Agilent 1100 series binary pump, and an Esquire 3000plus mass spectrometer (Bruker Daltonic GmbH, Bremen, Germany) coupled with an ESI interface and an ion trap mass analyzer. Analysis of puerarin, daidzein, and equol was performed under the following conditions: column, TSK-gel ODS-80Ts (Tosoh Co., Tokyo, Japan, 4.6 mm × 150 mm); mobile phase, H_2O (solvent system A) and CH_3CN (solvent system B) in a gradient mode (B from 15 to 50% in 20 min); flow rate, 1.0 ml/min; detection, UV 280 nm; temperature, 30˚C. High-purity nitrogen was used as dry gas at a flow rate at 10 L/min, dry temperature at 360˚C. Helium was used as nebulizer at 50 psi. The electron spray ionization (ESI) interface and mass spectrometric parameters were optimized to obtain maximum sensitivity.

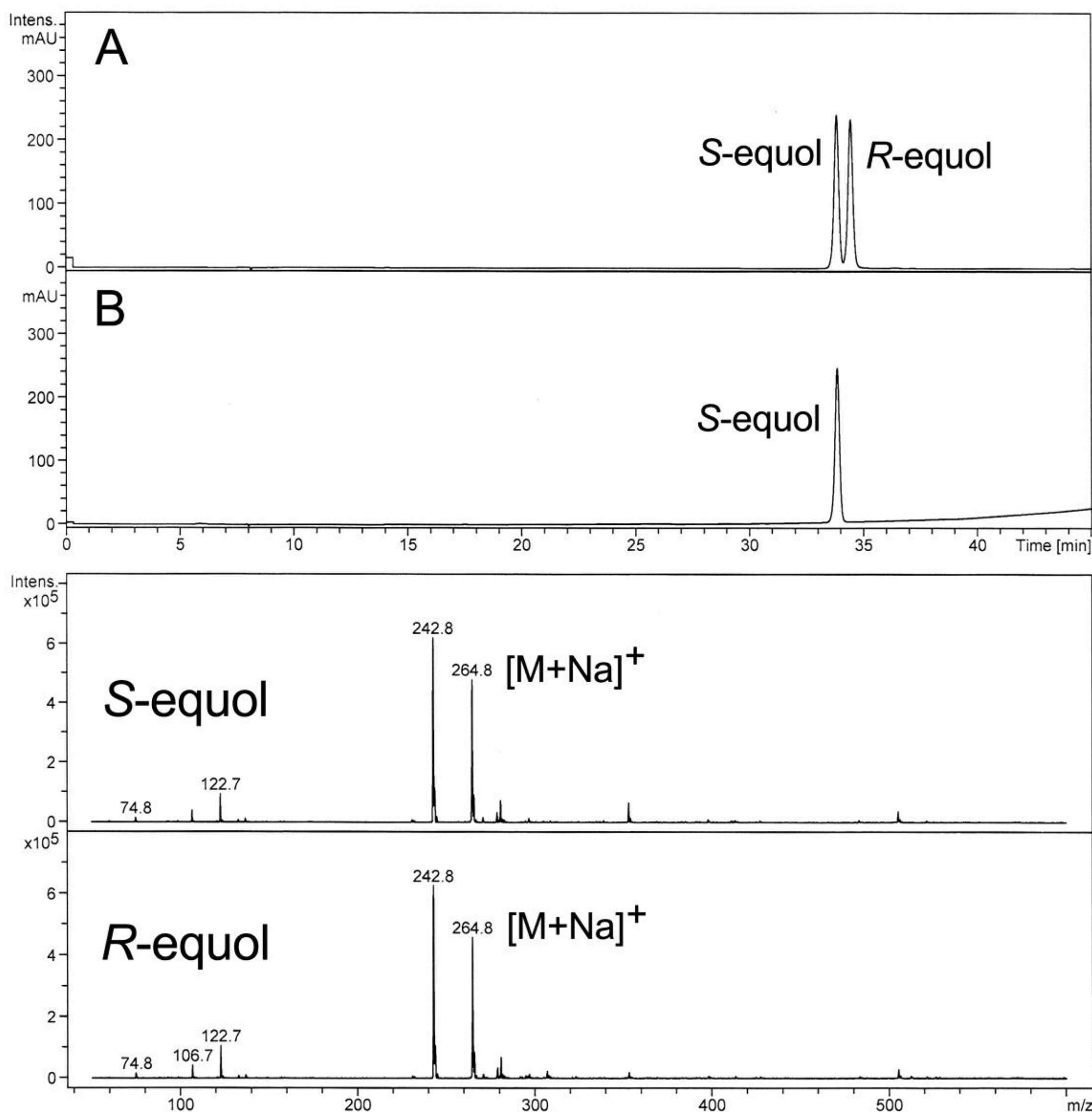


Fig. 2 HPLC elution profiles and ESI-MS spectra of chemically synthesized (±)-equol and biosynthesized (-)-equol.

A, synthesized (±)-equol; B, biosynthesized (-)-equol. ESI-MS spectra of biosynthesized (-)-equol were identified with those of synthesized (-)-equol. Analysis of *R*- and *S*-equols was performed under the following conditions: column, chiral CD-Ph (Shiseido, Tokyo, Japan, 4.6 mm × 250 mm); mobile phase, 0.1 % TFA (solvent system A) and CH_3CN (solvent system B) in a gradient mode (B from 20 to 70 % for 50 min); flow rate, 0.5 ml/min; detection, UV at 280 nm; temperature, 30˚C.

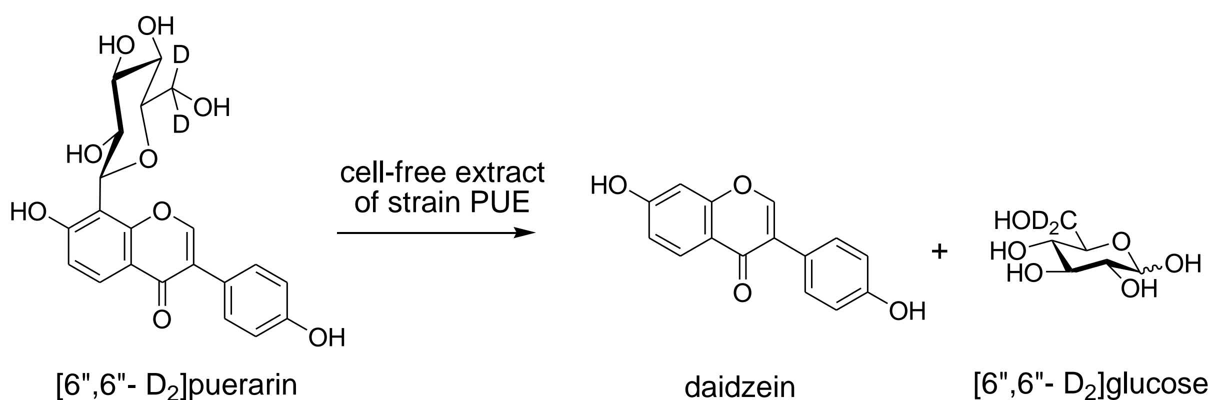


Fig. 3　Mechanism of the cleavage of a *C*-glucosyl bond of puerarin by a human intestinal bacterium strain PUE

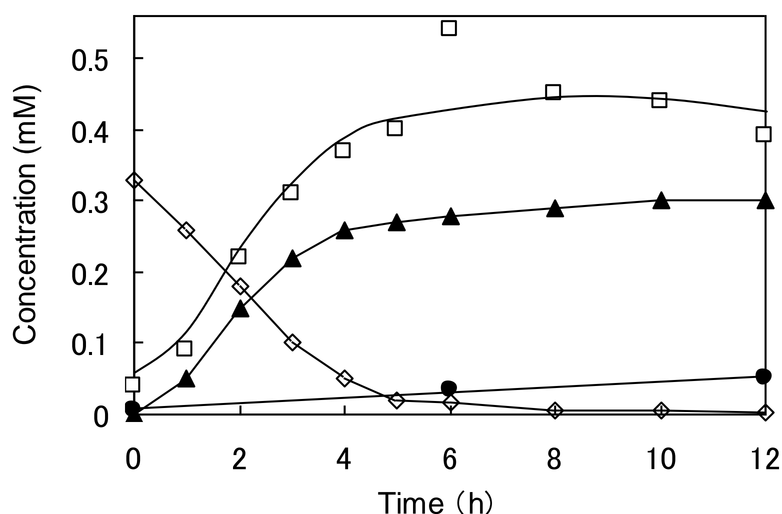


Fig. 4　Time course of conversion of puerarin (◇) to daidzein (□) and glucose (▲) by a cell-free extract (CFE) of strain PUE

A solution of 0.3 mM puerarin and 1 mM $MnCl_2$ was added to a CFE of strain PUE. To the glucose of CFE (●) were added neither puerarin nor $MnCl_2$.

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