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Quercetin introduces strand breaks into bacterial DNA

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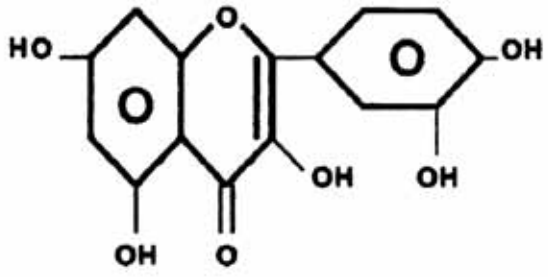
Quercetin (3,3',4',5,7-pentahydroxyflavone, CAS No. 117-39-5) conjugated with sugars is widely distributed in plant kingdom (Scheme 1).

Scheme 1

STRUCTURE OF QUERCETIN



3,3',4',5,7 - PENTAHYDROXYFLAVONE
Mol.wt:302.2
$C_{15}H_{10}O_7$

It is the main component of diet flavonoids, consumed daily in about 1 g amount mostly in vegetables [1]. In human lower intestinal tract, quercetin glycosides are hydrolysed by bacteria and free quercetin is released [2]. In contrast to glycosides, free quercetin is mutagenic in the Ames assay [3 - 5]. Some tests performed *in vitro* and *in vivo* in mammalian cell systems suggest that quercetin is genotoxic [6 - 8]. It also was reported to induce bladder and intestinal tumors in rats [9]. However, the mechanism of genotoxic activity of quercetin still remains obscure.

The aim of this work was to check whether quercetin and its metabolites formed in the presence of microsomal enzymes of fraction S9 introduce the SOS-inducing DNA lesions and, if they do, whether the induction results from single-strand breaks in DNA.

Induction of the SOS response was monitored in bacterial test as the expression of one of SOS genes, *sfiA* gene [10]. In the bacterial strain used, *E. coli* K-12 PQ37 *uvrA*, *sfiA* gene is fused with *lacZ*, a structural gene for β-galactosidase [11, 12]. The effects of quercetin were studied both without and with metabolic activation. Quercetin was used at the concentration range of 2.5 - 60 μg/ml, which was far from being toxic to bacteria (i.e. 100 μg/ml).

The results presented in Fig. 1 indicated that quercetin and probably its metabolites were able to induce the SOS system in PQ37 *uvrA*, strain although the effects were poorly expressed.

To study the ability of quercetin and its metabolites to produce single strand breaks in DNA we used *E. coli* strain MD322-PQ37 *serB*, *leu*$^+$, *dnaC* 28. Strain MD322 is derivative of PQ37 *uvrA* strain with termosensitive mutation in gene *dnaC*. In double mutant *dnaC (Ts) uvrA* the SOS response at nonpermissive temperature (42°C) may be induced only by agents producing DNA breaks, since neither excision repair or reinitiation of DNA replication is possible [13, 14].

We found that both quercetin and its metabolism products induced the SOS response at permissive temperature in the strain MD322-PQ37, but the induction factor (IF) for metabolites of quercetin was much higher than that measured in the SOS-Chromotest (see Figs. 1 and 2). At nonpermissive temperature IF remained higher only for metabolites (Fig. 2).

We suggest that the above phenomenon could result from oxidation of quercetin metabolites, which is known to lead to formation of H_2O_2

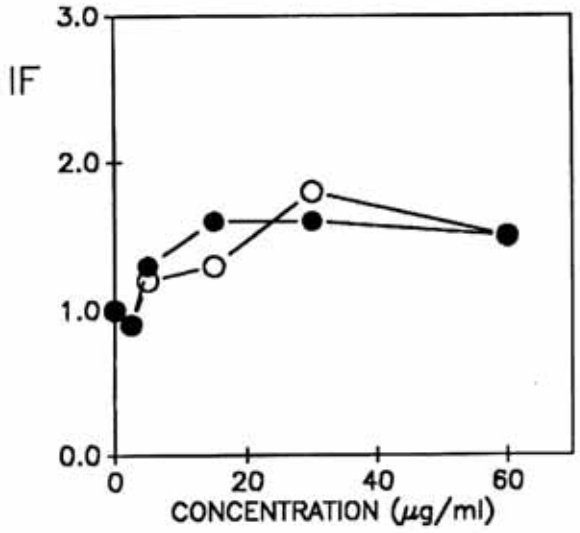


Fig. 1. Induction of the SOS-system in E. coli strain PQ37 by quercetin and its metabolites.
The recipe was prepared according to original protocol [11, 12]. Briefly, bacteria at the concentration of 5×10^7/ml were incubated till A_{600} reached about 0.2, then diluted with 10 vols. of fresh L medium and treated with increasing concentrations of quercetin with (●) or without (○) S9 mix (10% S9) for 2 h at 37°C.The induction factor (IF) is defined as the ratio of the β-galactosidase activity in the samples to which quercetin was added to that in the samples devoid of this compound

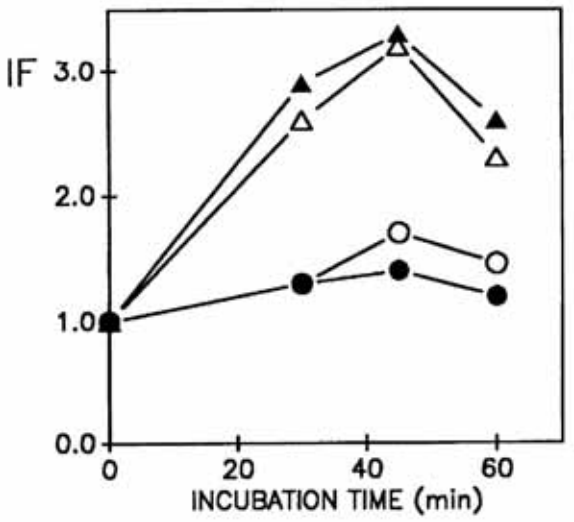


Fig. 2. Induction of β-galactosidase activity in E. coli strain MD332 by quercetin and its metabolites at permissive or nonpermissive temperature.
MD332 *dnaC, uvrA6, sfiA :: Mvd (Aplac)* was a gift from B. Salles, Toulouse (France). The strain was grown in M9 minimal medium supplemented with 0.5% casamino acids and 1 μg/ml thiamine [16]. Bacteria at the concentration of 5×10^7/ml were incubated for 70 min at 30°C (or 42°C), then treated with 50 μg/ml of quercetin (with or without S9 mix) for 1 h and kept at either temperature throughout the experiment. The results represent the average of at least 3 experiments. Quercetin without S9 mix at 30°C (○) or 42°C (●). Quercetin with S9 mix at 30°C (Δ) or 42°C (▲)

and free radicals [7, 15] and, eventually, to DNA breaking.

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