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Evaluation of phenolics and sugars as inducers of quercetinase activity in *Penicillium olsonii*

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Abstract

Quercetinase is produced by various filamentous fungi when grown on rutin as sole carbon and energy source. We investigated on the effect of 10 phenolics and two sugars, structurally related to substrates and products of the rutin catabolic pathway, on the induction of a quercetinase activity in *Penicillium olsonii*. Neither the sugars (glucose and rhamnose, two constituents of rutin), nor phenolics such as protocatechuic acid, salicylic acid, 4-hydroxy-benzoic acid and phloroglucinol were inducers. Rutin (maximum activity 150 nmol/min/mL after 5 days), quercetin (70 nmol/min/mL, 3 days), phloroglucinol carboxylic acid (60 nmol/min/mL, 3 days), 2-protocatechuoylphloroglucinolcarboxylic acid (50 nmol/min/mL, 5 days), 2,6-dihydroxy-carboxylic acid (90 nmol/min/mL, 7 days) and 2,4-dihydroxy-carboxylic acid (30 nmol/min/mL, 7 days) were demonstrated to be quercetinase inducers. We propose that rutin, quercetin and 2-protocatechuoyl-phloroglucinol carboxylic acid, the product of the reaction catalysed by quercetinase, act as inducers after their catabolic transformation in phloroglucinol carboxylic acid.

Keywords: Quercetin-2,3-dioxygenase; Quercetinase; *Penicillium olsonii*; Enzyme induction; Rutin catabolism; Phloroglucinol carboxylic acid

1. Introduction

Quercetinase (quercetin 2,3-dioxygenase, E.C. 1.13.11.24) is part of a set of enzymes produced extracellularly by filamentous fungi, like *Aspergillus* and *Penicillium*, when grown on rutin as sole carbon and energy source (Scheme 1) [1–3]. It catalyses the oxidative transformation of flavonols into a depside and carbon monoxide [1,4]. Although an iron containing quercetinase has been characterised recently in *Bacillus subtilis* [5,6], quercetinases are copper containing dioxygenases [7,8] that belong to the vast superfamily of cupins [9]. This enzyme is particularly interesting from the chemical point of view [10,11] since it catalyses the breakdown of two carbon–carbon bonds and the release of carbon monoxide. Crystal structures are now available for both copper and iron containing quercetinases [11,12]. Despite these outstanding structural progresses, the enzyme as a whole has been poorly studied. Although it has been shown that quercetinase synthesis is inducible by rutin [13], to the best of our knowledge no specific studies devoted to quercetinase induction have been reported until today.

We have recently described an optimisation of the extracellular quercetinase production by *Penicillium olsonii* grown on a rutin based medium [14]. Although this

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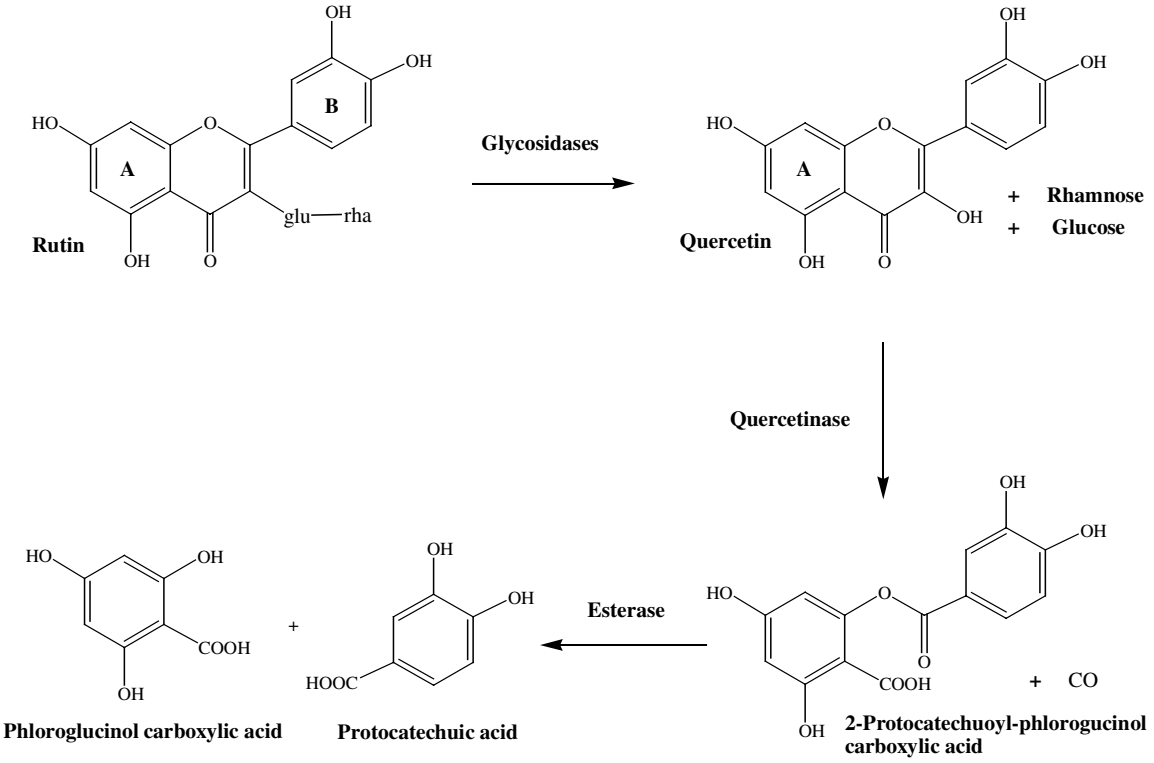
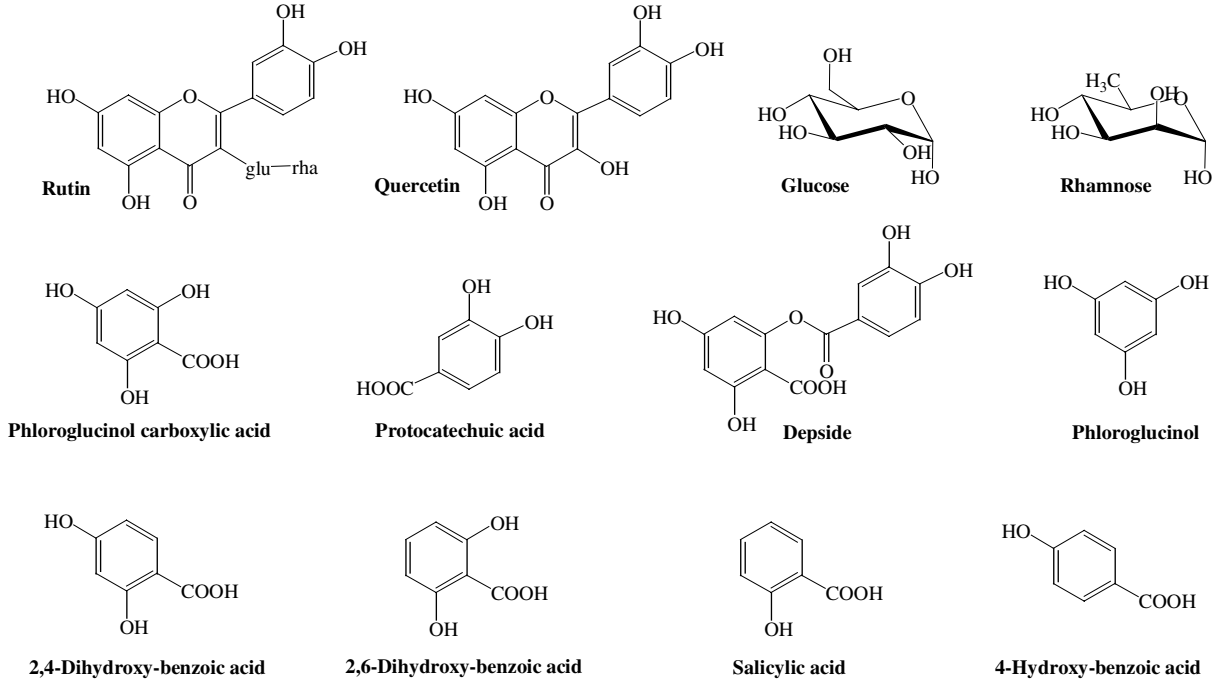


Scheme 1. Catabolic pathway of rutin in various *Aspergillus* and *Penicillium*.



Scheme 2. Chemicals studied as potential quercetinase inducers.

study lead to a sixfold increase in quercetinase production, the yield of purified enzyme was still low. In order to better understand the fate of quercetinase production by *P. olsonii*, we investigated the effect of two sugars and 10 phenolics (Scheme 2) representative of compounds of the rutin catabolic pathway on quercetinase induction.