Partial purification of a ß-rutinosidase from *Rhodococcus erythropolis* DSM 44534 and degradation experiments with different flavonoids as substrates

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Introduction: Glycosidases are enzymes which have various functions in nature including degradation of biomass, antibacterial defense strategies, pathogenesis mechanism and normal cellular functions. The *ß*-rutinosidase is a glycosidase which hydrolyse rutin into quercetin and rutinose. It discriminate both: the sugar part and the flavonol aglycone part of its substrate. According to the literature the *ß*-rutinosidase has been isolated from fungi as *Penicillium rugulosum* [1] and bacteria like *Corynebacteria sp.* [2], but for the genus *Rhodococcus* it is still unknown. Therefore it would be interesting to elucidate the rutin catabolic pathway in the *Rhodococcus erythropolis* DSM 44534.

Methods: In this study the *ß*-rutinosidase was purified from *R. erythropolis* cells, which were cultivated in a modified minimal salt medium supplemented with rutin, dissolved (in dimethyl sulfoxide), as carbon source at a final concentration of 0.1 mM. The cultures were incubated over 10 days at 28°C and 160 rpm in a horizontal shaker. The protein was purified using 60 % ammonium sulfate precipitation at 4°C. The crude protein was dialyzed against 10 mM sodium phosphate buffer pH 6 before isoelectric focusing (IEF) with ampholyte pH-value 3-10. The activity test was performed with rutin as substrate at 37°C for 2h. The quercetin concentration was measured by reversed phase high performance liquid chromatography with diode array detector.

Results: 3.6 g of wet weight cells were obtained from a 3 L culture. These cells were used to prepare a crude extract according to the methodology. The crude protein had a concentration of 8.1 mg/ml (145 mg total protein) after the dialysis step. The biotransformation system was made with the IEF fraction 7-11 with a pH-value of 5.22, 5.52, 6.22, 6.40 and 6.72 respectively (Fig. 1). The highest level of quercentin was observed in the fraction 10 and 11 after 2 h with a concentration of 51 µg/ml (Fig. 2). The addition of ampholyte to the sample did not transform rutin into quercentin (blank experiment).

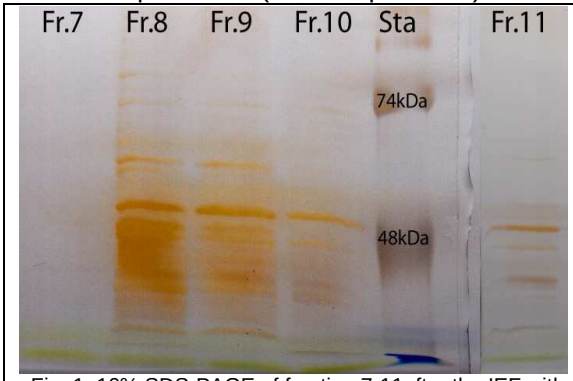

Fig. 1: 10% SDS-PAGE of fraction 7-11after the IEF with prestained broad range standard

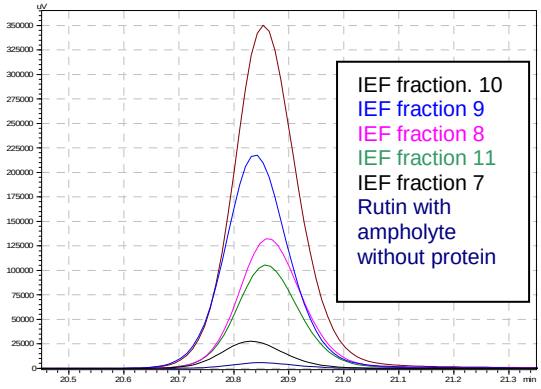

Fig. 2: HPLC-Chromatogram of rutin transformation into quercentin with IEF fraction 7-11 after 2h incubation

Conclusion: We have elucidated an enzyme with rutinosidase or rutinase activity from *R. erythropolis* DSM 44534. The partially pure enzyme was induced in *R. erythropolis* cells having quercentin as major product. Understanding gained from this work will be useful in studying the degradation and modification of natural products from microorganisms

Literatur:

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