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Research Article

Rutin Degrading Enzyme from *Fusarium croockwellen* MTCC-2084

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Abstract

The rutin degrading enzyme was secreted by *Fusarium croockwellen* MTCC-2084 in liquid culture medium which was specific for releasing the disaccharide rutinose from the rutin with no release of glucose, rhamnose and isoquercetin. The conversion of rutin to quercetin was optimal at pH 5.0 and 65º C temperature in the presence of 0.5% dimethyl sulfoxide. The crude enzyme does not hydrolyzed hesperidin and naringin. On the basis of these result it was concluded that the crude enzyme from *F. croockwellen* MTCC -2084 is hydrolyzed rutin in to quercetin and rutinose which is rare bioactive compounds.

Keywords: Biotransformation; Rutin; Degrading Enzyme; *Fusarium croockwellen*; Quercetin

Introduction

Rutin(3,3',4',5,7-pentahydroxy favone-3-β-L-rhamnoglucoside) and naringin are major flavonoid glycosides of some plants mainly buckwheat [1], tobacco [2], and fruit such as tomato, grapes, apple and citrus fruit [3-7]. Waste products of the agro and fruit juice producing industry contains large amounts of rutin, hesperidin and naringin, which represent inexpensive starting material for the synthesis of aglycons with potentially interesting biological and pharmacological activities and for preparation of rutinose [8]. Rutinosidase is an enzyme which catalyzes the cleavage of glyosidic bond of rutin to generate quercetin and rutinose [9]. Rutinosidase have been reported in the fungi *Aspergillus flavus* and *Penicillium rugulosum* [10]. The bacterium *Pseudomonas viridiflava* [11], the plant *Fagopyrum tataricum* [12], *Pyrococcus furiosus* [13] and rat small intestine. Quercetin (3,3',4',5,7 penta hydro xylavone) is a flavonol with multifaceted therapeutic applications. It is one of the most common and abundant flavonoids in plant kingdom. In nature it is present in glycosylated form and its extraction direct from plants is difficult. Presently, it is prepared by acid hydrolysis of rutin but do not give pure quercetin and are not ecofriendly. Quercetin has many beneficial health effects including antioxidant, anti-inflammatory, anti-cancer [14,15]. Moreover, during the conversion of rutin to quercetinrutinose is produced as a side product (Figure 1). This disaccharide (6-O-α-L-rhamnopyranosyle-β-D-gucopyranose) has potential application in cosmetics but so far it has not been available for research and practical applications. In the present study, secretion and biochemical properties of rutin degrading enzyme from *F. croockwellen* MTCC-2084 was determined. The quercetin and rutinose were produced from rutin hydrolysis catalyzed by this enzyme.

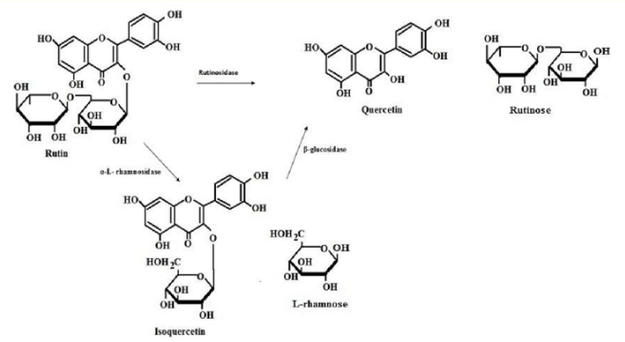

Figure 1: Hydrolysis of rutin to quercetin by Rutinosidase (rutin degrading enzyme).

Materials and Methods

Chemicals

L-rhamnose, rutin, quercetin, naringin, hesperidin, andβ- glucosidasewere purchased from Sigma Chemical Company, St. Louis (USA).An α-L-rhamnosidasewas purified in the laboratory from culture filtrate of *F. moniliforme* MTCC-2088. All other chemicals were either from Merck Limited Mumbai (India) or from s. d. – fine CHEM limited Mumbai (India) and were used without further purifications.

Fungal strains

The fungal strain *F. croockwellen* MTCC-2084 was procured from Microbial Type Culture Collection Center and Gene Bank, Institute of Microbial Technology, Chandigarh (India) and maintained in the laboratory on the agar slant as mentioned in the MTCC catalogue-2000.

Secretion of enzyme

The secretion of Rutinosidase by the fungal strain was studied in the liquid culture growth medium having composition: $CaCl_2$ 1.0 g, $MgSO_4.7H_2O$ 3.0 g, KH_2PO_4 20.0g, $N(CH_2COONa)_3$ 1.5 g, $MnSO_4$ 1.0g, $ZnSO_4.7H_2O$ 0.1g, $CuSO_4.5H_2O$ 0.1g, $FeSO_4.7H_2O$ 0.1g, H3BO3 10.0mg, sucrose 40.0g, ammonium tartarate 8.0 g. water (MilliQ)1000 ml. One ml of the spore suspension (spore density 6 x 105 spores/ml) from the agar slant was inoculated aseptically into the liquid culture medium (25ml) kept in 100 ml culture flask which also contained 0.5% of rutin as inducers. The experiments were performed in triplicates. The culture flasks were incubated in a Biological Oxygen Demand (BOD) incubator at 30°C and the fungal strain was allowed to grow under stationary condition. One ml of the growth medium were withdrawn at the regular intervals of 24 h, were filtered through Millex syringe filters (0.22µm) and were analyzed for the presence of Rutinosidase activity as given below.

Enzyme assay

The activity of Rutinosidase was assayed using rutin as the substrate and monitoring the liberation of quercetin by TLC using reported method [10]. The assay solution contained 1 mL of 20m Mrutin in 50% (v/v) dimethylsulfoxide (DMSO), 2mL of 0.1 M citrate-phosphate buffer pH 5.0 and 1 mL of enzyme solution. The reaction solution was incubated at 60ºC for 20 min, the reaction was stopped by addition of 1mL of 25% trichloroacetic acid followed by 1mL of 0.5 M Na_2CO_3.The release of quercetin was monitored by TLC (acetone/water/formic acid = 30:1:1, v:v:v) and spots was visualized in iodine chamber. One unit of Rutinosidase (U) was defined as the amount of enzyme that produces 1 µmol of quercetin per min under these assay conditions.

Effect of pH and temperature on the activity of enzyme

The effect of pH and temperature on enzyme activity was determined using rutin as substrate. The enzyme activity was measured at 60ºC in citrate-phosphate buffer pH was adjusted between 4.0-7.0 and in glycin –HCl buffer at pH 2.5-3.5. The temperature optimum was measured in 50 mM citrate-phosphate buffer pH 5.0 in the temperature range 40-70ºC. The enzyme activities were measured under standard assay conditions as described above. All assays were done in triplicate.

Substrate specificity

Rutin, naringin and hesperidin (20 mM) were dissolving in 1 mL dimethylsulphoxide, 2 mL of 100 mM citrate – phosphate buffer,pH5.0. 1 mL of crude enzyme sample was added to the suspension and the mixure was incubated at 60ºC. The reaction was monitored by TLC (acetone/water/acetic acid = 15:1:1, v:v:v). Three set of experiment were done, in the first step solution of naringin, hesperidin and rutin were treated with crude enzyme and monitor the release of rhamnose. In second set of experiment, A solution of rutin was treated with enzyme and monitor the release of quarcetin. In third set of experiment solutions of rutin ware treated with α-L-rhamnosidase, β- D –glucosidase and crude enzyme sample and monitor the release of rhamnose, glucose and rutinose.

Results and Discussion

Enzyme secretion

The secretion of Rutinosidase in the liquid culture growth medium amended by the addition of the rutin is shown in Figure 1. It is obvious from the figure 2 that the peak value (0.0018 IU/mL) of enzyme secreted in the liquid culture medium containing 0.05% rutin are maximum on 7th day after incubation of the fungal spores. In order to enhance the secretion of Rutinosidase from *F. croockwellen* MTCC-2084in its liquid culture growth medium, the effects of different concentration of rutin in liquid culture growth medium are determined. The peak value (0.0174 IU/mL) of enzyme secreted in the liquid culture medium containing0.08% is maximum on 7th day after incubation of the fungal spores (Figure 3). Thus, the peak value of the enzyme secreted per mL in case of medium amended by 0.08% rutin is approximately 10 times higher than in the blank growth medium. The reason for such a high induction of Rutinosidase in the presence of rutin is difficult to find because not much is known about the structure and regulation of Rutinosidase. Therefore, for the purification of the enzyme, fungal strain was grown in the medium amended with 0.08%rutin.

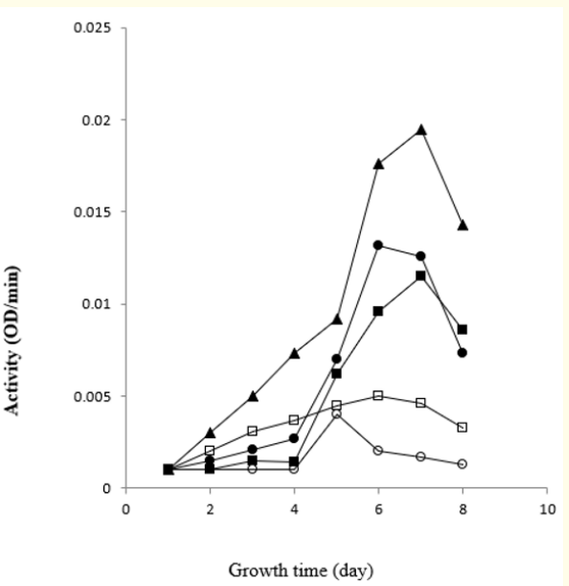

Figure 2: Secretion of enzyme in liquid culture growth media amended with 0.05% inducers.

Rutin (closed triangle), Naringin (closed square), Hesperidin (closed circle), rhamnose (open circle), Control (open square).

pH and temperature optima

The optimum pH of Rutinosidase secreted by *F. croockwellen* MTCC-2084have reported at pH 5.0 (Figure 4) and enzyme was stable between 3.0 to 7.0. The enzyme showed its highest activity

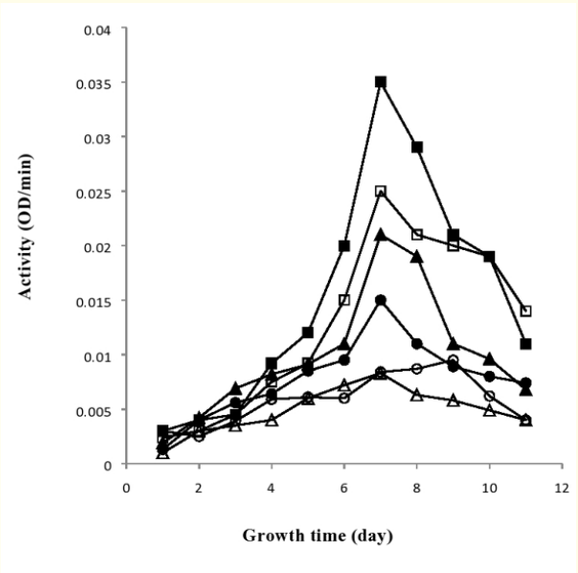

Figure 3: Secretion of enzyme in liquid culture growth media using different concentration of rutin. 0.02% (closed triangle), 0.04% (open circle), 0.06%(closed circle), 0.08% (open square), 0.1% (closed square), 0.12% (open triangle).

atoptimum Temperature 65º C (Figure 5) while it was stable below 4.0ºC. The reported pH optima for Rutinosidase are in the range 4-7 and temp optima 50ºC though one have been reported [10] pH optimum at 2.2. Most of Rutinosidase have pH optima at pH 5.0 (Hyun., *et al.* 2005). Thus the pH optimum of the Rutinosidase secreted by *F. croockwellen* MTCC-2084 5.0 lies in the range of pH optima for the reported in the literature [10].

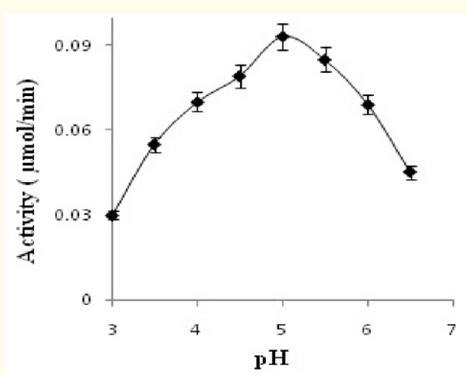

Figure 4: pH profile of the enzyme: The enzyme activity was measured at 60ºC in citrate-phosphate buffer pH was adjusted between 4.0-7.0 and in glycin –HCl buffer at pH 2.5-3.5.

Application of enzyme

The substrate specificity of this enzyme was examined with TLC for detecting sugar released. The enzyme hydrolyzed rutin to quercetin but not the others. Three set of experiment were done, the

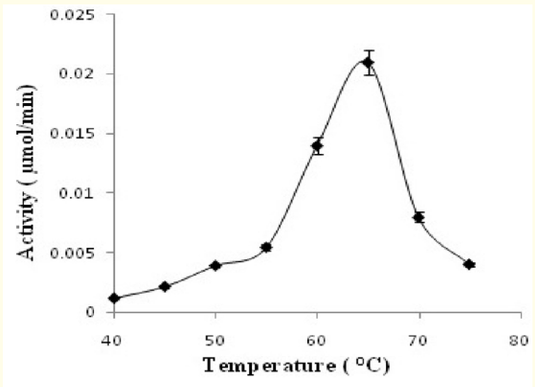

Figure 5: Temperatures profile of the enzyme: The temperature optimum was measured in 50 mM citrate-phosphate buffer pH 5.0 in the temperature range 40-70ºC.

results of first set of experiment shown in figure 6. In figure 3 lane 1 and 3 shows no enzymatic conversion of naringin and hesperidin, lane 2 shows that crude enzyme hydrolyzed rutin and release their rutinose (α-L-rhamnopyrano-β-D- glucoside) Rf value is 0.49. The result of second set of experiment shown in figure 7, lane 1 shows spot of untreated reaction mixture of rutin, lane 2 shows reaction mixture of rutin treated with crude enzyme sample and lane 3 shows the spot of reference sample of quercetin. The Rf value of reference quercetin (0.74) and rutin hydrolyzed sample ware same (0.75). Figure 8 shows third set of experiment, lane 1 shows the spot of rhamnose, lane 2 shows spot of rutinose and lane 3 shows spot of glucose which were released by hydrolyzed of rutin solutions by α-L-rhamnosidase, Rutinosidase and β-D-glucosidase respectively. It is obvious from these figures the enzyme secreted by *F. croockwellen* MTCC-2084 active at pH 5.0 and 65ºC were inferred as Rutinosidase. Several enzymes with rutinosidase activity have been reported in plants and bacteria[9,10,16-19]. Only few reports of fungal Rutinosidase are available in literature [19].

Figure 6: Studies on the release of rutinose from rutin.
Lane 1: Naringin solution treated with enzyme.
Lane 2: Rutin solution treated with Enzyme.
Lane 3: Hesperidin Solution treated with enzyme.

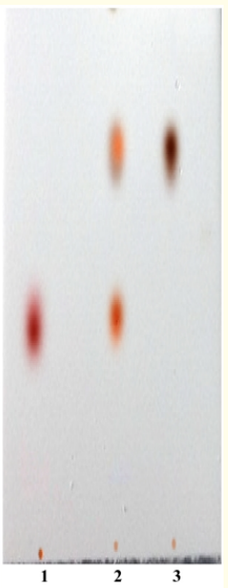

Figure 7: Studies on the release of quercetin from rutin.
Lane 1: Untreated rutin solution.
Lane 2: Rutin solution treated with enzyme.
Lane 3: Reference sample of quercetin.

Figure 8: Studies on the hydrolysis of rutin by
Lane 1: α-L- rhamnosidase,
Lane 2: Rutinosidase,
Lane 3: β-D- Glucosidase.

In this communication reports the rutin degrading enzyme (Rutinosidase) from *F. croockwellen* MTCC-2084 was characterized and applied to produce quercetin and rutinose from rutin. Activity of enzyme using rutin as substrate was determined at pH 5.0 and 65ºC temperature. Very few studies done on fungal Rutinosidase involve in the biotransformation of rutin to quercetin. Further studies are needed to isolate, identify and purify more fungal strains which secrete Rutinosidase which contribute to biotransformation of rutin to quercetin.

Conclusion

On the basis of these result it was concluded that the crude enzyme from *F. croockwellen* MTCC -2084 is hydrolyzed rutin in to quercetin and rutinose which is rare bioactive compounds.

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Conflict of Interest

The authors declare no conflict of interest.

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