



THERMOSTABLE INULINASE PRODUCTION BY *RHIZOPUS HOMOTHALLICUS*

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Introduction. Fructose and fructo-oligosaccharides (FOS) are fast emerging as important ingredients in the food and pharmaceutical industry. Fructose is considered as a safe alternative sweetener to sucrose because its beneficial effects in diabetic patients, increases the iron absorption in children and has a higher sweetening capacity. On the other hand, FOS are source of dietary fiber and have bifidogenic effect⁽²⁾. Both can be obtained from inulin, a reserve carbohydrate found in plants, by enzymatic hydrolysis. However, inulin presents limited solubility at room temperature, thus, the industrial production of fructose and FOS is carried out at 60 °C. Therefore, there is a growing interest to isolate and characterize thermostable inulinases. The aim of this work was to evaluate the capacity of Mexican fungal strains isolated from diverse environments to produce potential thermostable inulinases.

Methods. *Rhizopus homothallicus* var. *rhizopodiformis* was selected in a previous study as the best inulinase producer from a total of 27 strains belonging to the genera *Aspergillus*, *Penicillium*, *Rhizopus*, *Rhizomucor* and *Thermomyces*. This strain was cinetically evaluated for inulinase production under submerged culture using Czapek Dox medium with inulin as a sole carbon source. The crude enzyme extract was obtained to evaluate inulinase activity using DNS method. The effect of temperature on the enzyme was investigated at pH 5.0 in 0.2 M acetate buffer over a temperature range of 60-80 °C. For determination of thermal stability, the enzyme was incubated for variable durations (0 to 60 min). The residual activity was estimated under standard assay conditions after incubation intervals and expressed as relative activity.

Results. *Rh. homothallicus* var. *rhizopodiformis* 13a showed its maximal enzymatic activity (7.97 U/mL) at 60 h with an optimal temperature for inulin hydrolysis of 60 °C. In Table 1, optimal temperatures are showed for other inulinases.

Table 1. Optimal temperatures from inulinases produced by different microorganisms

Optimal temperature	Microorganism
35 – 45 °C	<i>Penicillium janczewskii</i> ⁶ <i>Rhizopus</i> spp. TN-96 ⁵
50-55 °C	<i>Kluyveromyces marxianus</i> ⁴
60 °C	<i>Pichia guilliermondii</i> ³ <i>A. fumigatus</i> ²

When thermal stability was evaluated it was found that only 13 % of inulinase activity was lost over 6 h at this temperature. However, enzymatic extract also showed stability at 70 °C retaining 57 % of its original activity after 6 h. Finally, when extract was incubated at 80 °C it lost 61 % of the original activity at 6 h. In other studies with inulinases, it was found that inulinase produced by *Aspergillus fumigatus* lost 22 % of its original activity after 3 h at 60 °C⁽²⁾ while *Aspergillus ficuum* retained 74 % and only 22 % after 2 h at 60 and 70 °C, respectively⁽¹⁾.

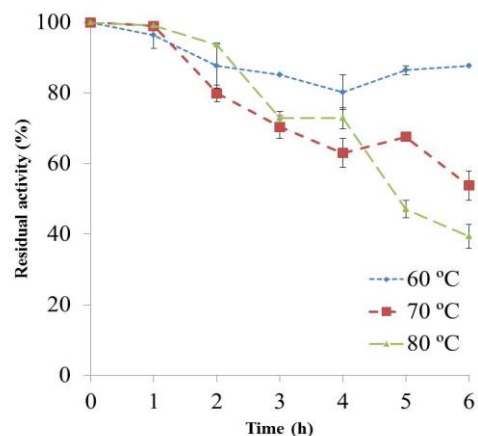


Fig.1. Thermal stability of enzymatic extract from *Rhizopus homothallicus* at 60, 70 and 80 °C for 6h.

Conclusions. It was possible to identify *Rhizopus homothallicus* as thermostable inulinase producer using inulin as a sole carbon source.

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