HACETTEPE JOURNAL OF BIOLOGY AND CHEMISTRY
Volume 35, Issue 2, June 2007
Research Article
Hacettepe J. Biol. & Chem., 2007, 35 (2), 89-92

Effect of Some Medium Components on Lactase Production from *T. Viride ATCC 32098*

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Abstract

T.viride was used to produce extracellular lactase in different media. Effect of various carbon sources (lactose, glucose, galactose, sucrose, maltose, fructose and xylan) on enzyme production was investigated. Among nitrogen sources, $(NH_4)H_2PO_4$ was concluded to be the best nitrogen source, followed by $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$. In addition, increasing $(NH_4)H_2PO_4$ concentration to 0.250 M increased enzyme production. Finally, additional glucose was not effective on lactase production.

Key Words: carbon source, lactase, nitrogen source, *Trichoderma*

Introduction

Lactase (EC 3.2.1.23) hydrolyses lactose into glucose and galactose. This enzyme plays an important role especially in dairy industry. Lactose is a substance found in milk and milk products such as whey, a by-product of cheese production whose disposal is a problem from environmental point of view. Therefore, lactase enzymes are widely used in the dairy industry to obtain new products. This enzyme is also used in toxicity tests and pharmaceutical industries [1-3]. Lactase can be obtained from fungal, bacterial, or yeast sources [4-6].

Literature on similar studies reveals that the rate of enzyme production is enhanced when suitable carbon and nitrogen sources were added to the medium. In this respect, an attempt has been made in this study to optimize the medium composition such as carbon and nitrogen sources to increase lactase production from *T. viride ATCC 32098.*

Materials and Methods

Microorganisms: *Trichoderma viride ATCC 32098* was obtained from Tubitak Marmara Research Center, Turkey. *T.viride ATCC 32098* was preserved on potato dextrose agar slants at +4°C and transferred to fresh slants (incubated at 30 °C in 4 5 days).

Media and inoculation procedure: Medium described by Fiedurek and Ilczuk [7] was used with some modifications for growth and enzyme production. The composition of this medium was (as gl^{-1}); 10.0 lactose, 1.5 peptone, 1.0 yeast-extract, 1.0 KH_2PO_4, 7.0 $(NH_4)H_2PO_4$, 1.0 $MgSO_4.7H_2O$ and 0.3 $CaCl_2$. Enzyme production was carried out in 50 mL/250 ml flasks. The medium was adjusted to pH 5 before autoclaving (121ºC, 1.5 atm, 15 min) for sterilization. One ml spore suspensions containing 15.10^6/ml was inoculated into 50 ml growth media.

Biomass determination: The amount of growth in cultures was calculated as dry weight with filtration method [8].

Analytical determinations: Activity of lactase was measured by the hydrolysis of 2.5 mg/ml o-nitrophenyl-β-D-galactopyranoside (ONPG) as substrate, which was prepared in 0.1 M sodium-acetate buffer with pH 5. This lactase assay was carried out according to method described by Reczey et al. [9].

The cell-free supernatant obtained by centrifugation (7,200 rpm, 15 min) was used for determining extracellular lactase activity. The assay mixture that contained 1 ml of ONPG (ICN) as substrate and 0.2 ml enzyme sample was incubated at 50°C for 5 min. The reaction was terminated by addition 1 ml of 10% sodium carbonate into the reaction tube. The o nitrophenol released was estimated spectrophotometrically by absorbance at 420 nm using Jenway, 6105 UV-vis spectrophotometer. The amount of o-nitrophenol was calculated from the standard curve.

One unit of enzyme activity was described as the amount of enzyme producing 1 µmole of o nitrophenol in 1 ml medium at 50ºC in 1 min. Enzyme

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activity is expressed as U/ml. For specific activity calculations, the amount of protein was determined by Lowry Method [10]. Enzyme activity is expressed as U/mg protein.

Determination of lactase activity depending on growth: The differential enzyme synthesis rate was described as the enzyme activity per cell and was calculated as the ratio of the enzyme activity to growth rate.

Effect of medium components: Effect of seven different carbon sources and six different nitrogen sources on lactase production was investigated. Lactose, which is the substrate in the media, was replaced with 1% glucose, galactose, sucrose, maltose, fructose and xylan separately. Following, different nitrogen sources, namely $(NH_4)_2SO_4$, NH_4Cl, $NaNO_3$, $(NH_4)_2HPO_4$ and KNO_3 were added separately (0.05 M equivalent) to the growth media instead of $(NH_4)H_2PO_4$, which is nitrogen source in the reference media. For screening carbon and nitrogen sources, incubation was carried out at 30ºC for 8 days on a rotary shaker (150 rev min^{-1}). Lactase activity and growth measurements were done in triplicate. For investigation of effect of nitrogen concentrations, growth was carried out in the medium containing 0.025-0.400 M $(NH_4)H_2PO_4$.

Finally, in order to investigate the additional glucose in the culture media on lactase synthesis, two parallel experiment sets were used. The first set was the basic media and the other was containing 0.1% glucose as carbon sources. Consequently, *T.viride ATCC 32098* were grown on lactose and lactose plus glucose as carbon sources for comparison purposes.

Incubations were carried out at 30ºC for 8 days on a rotary shaker (150 rev min^{-1}). Lactase specific activity, growth and differential synthesis rates were calculated.

Results and Discussion

To study the effect of different sources of carbon on the production of lactase, glucose, galactose, sucrose, maltose, fructose, and xylan were separately added to the medium. Although the growth rates were within close proximity, the lactase-specific activity was found to be higher in the medium containing lactose as compared to those of other sources of carbon (Figure 1).

Equal amounts of different sources of nitrogen have been added to growth media and the effect of these different sources of nitrogen on the production of lactase has been examined. In the medium prepared by the addition of $(NH_4)H_2PO_4$, lactase activity was detected to be highest. While it has been observed that lactase activity is substantially high in media containing $(NH_4)_2HPO_4$ and $(NH_4)_2SO_4$, lactase activity has been quite low in those containing NH_4Cl, KNO_3, and $NaNO_3$. A look at the growth rates for different nitrogen sources has not indicated significant differences (Figure 2).

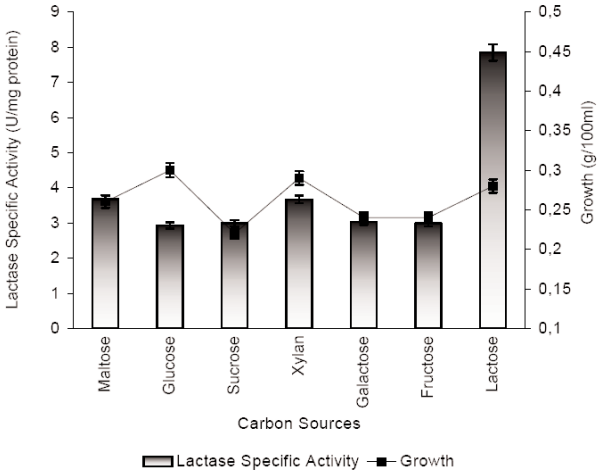

Figure 1. Effect of different carbon sources on lactase production.

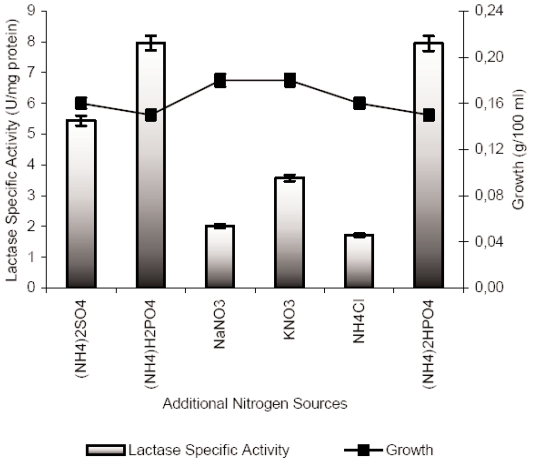

Figure 2. Effect of different nitrogen sources on lactase production.

It was observed that, when produced in media containing $(NH_4)H_2PO_4$ at concentrations between 0.025-0.400 M, an increase in lactase activity was observed up to the concentration of 0.250 M $(NH_4)H_2PO_4$. It was determined that at higher concentrations of $(NH_4)H_2PO_4$, there was no significant difference in lactase activity. As mentioned previously, it was observed that the concentration of 0.250 M $(NH_4)H_2PO_4$ was suitable and that higher concentrations did not affect enzyme activity. An increase in production is expected with increased

amounts of nitrogen added to the production medium. In our study conducted in parallel with this expectation, it was observed that there was an increase in production in relation with increasing nitrogen concentrations (Figure 3).

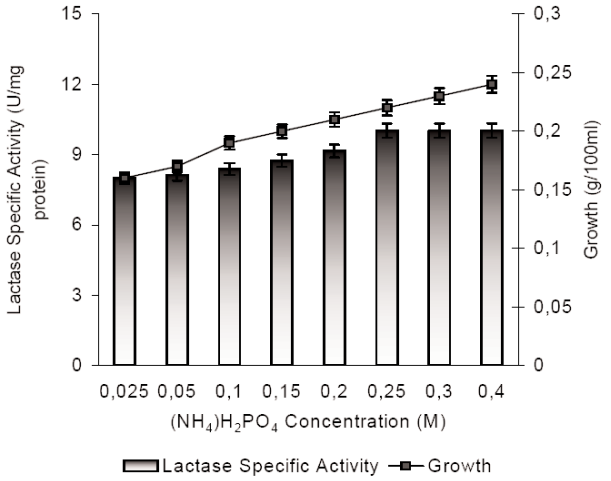

Figure 3. Effect of different $(NH_4)H_2PO_4$ concentrations on lactase production.

Following, 0.1% glucose was added to the production medium as an additional carbon source, but no increase in lactase activity was observed (Figure 4).

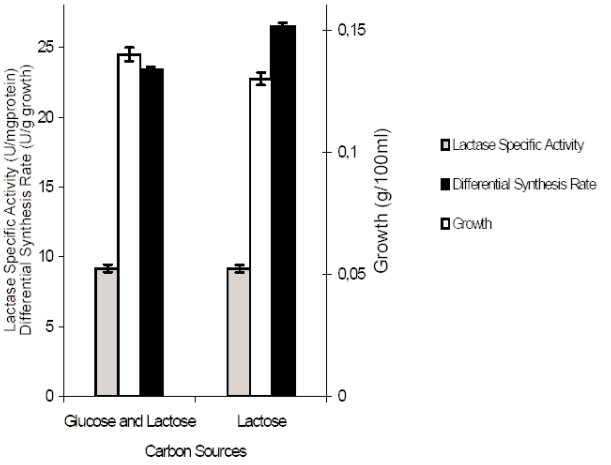

Figure 4. Effect of additional glucose on lactase production.

Trichoderma sp. were found to be effective sources for different enzymes [11-13], but not many studies on efficient lactase production from T*richoderma sp.* could be found in literature. Previously, *T.viride ATCC 32098* has been found to produce high lactase activity [14]. It is of great importance to select the appropriate sources of nitrogen and carbon to be used in the media within the scope of studies on the production of industrial enzymes. For this reason, the effect of the addition of different sources of carbon and nitrogen on the production of lactase in growth media has been examined.

In a study conducted with *A.nidulans*, the lactase activity was found to be higher in lactose containing medium in parallel with our findings. In this study, it was also indicated that galactose has a positive effect on enzyme activity [15]. In a study carried out with *Auerobasidium pullulans*, it was concluded that lactose is a stronger inducer for enzymes than are other sugars [16].

In our study, it was observed that activity is low in all carbon sources except lactose. Under circumstances where there exist easily metabolized carbon sources, the microorganism prefers this carbon source and may not be able to realize lactase responsible for lactose hydrolysis at high rates. This can also be explained by the suppressing effect of glucose on lactase synthesis. It is probable that sugars other than lactose also cause suppression in a similar manner. In addition, given xylan's more complex carbon source, media containing xylan also demonstrate low lactase activity. When growth in this medium is studied, the existence of high rates of growth indicates to the probability that the microorganism may have tended toward the synthesis of enzymes it could have utilized for this carbon source. It is already known that species of *Trichoderma* are efficient producers of xylanase [12,13]. However, in a study conducted by Ulezlo et al. [17], it has been determined that xylan is an optimal carbon source in the production of lactase, hence its inclusion in carbon sources taken into examination.

In a study utilizing *P.notatum* and similar to ours, the most suitable nitrogen sources for lactase production have been identified to be $(NH_4)_2HPO_4$ and $NH_4H_2PO_4$, with the lactase activities in growth media containing NH_4Cl, $NaNO_3$, and KNO_3 significantly low [5]. In yet another study conducted with *Thermoanaerobium*, it has been determined that ammonium phosphate is the optimal nitrogen source for lactase production [17].

In addition to the above conclusions bearing similarities with our findings, it has been observed that studies with fungi utilize $NaNO_3$ as the additional nitrogen source [18,19]. Moreover, a study including *Auerobasidium pullulans* has obtained high lactase activity when $NaNO_3$ was used [16].

In addition to the selection of the appropriate source of nitrogen, determination of the suitable concentration of the nitrogen source is also important to increase enzyme production. According to the results of our study, it was understood that the nitrogen source and its concentration is greatly effective in

the production of lactase and that lactase activity may increase by as much as 26%.

When glucose was added to the production medium as an additional carbon source, it was expected that the small amount of glucose added would increase production and be consumed rapidly. However, it was determined in the study that the additional glucose added to the growth medium did not help an increase in lactase production. Owing to restricted glucose not leading to an increase in lactase production in this medium containing lactose, the use of additional glucose is not preferred due to the additional cost. Literature survey demonstrates that lactose is the only carbon source used in lactase production [18,20]. In the presence of a rapidly metabolized carbon source like D glucose the growth rate on glucose was high.

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