Detection of Lipase Production from Newly Isolated *Trichoderma Citrinoviride*

Yeni İzole Ediilmiş *Trichoderma Citrinoviride*'den Lipaz Üretiminin Saptanması

Research Article

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ABSTRACT

The production of lipase is aimed from a new fungal source in this study. Fungus was isolated from soil locality Kocaeli. It was determined as *Trichoderma citrinoviride* by the analysis of 18S rRNA sequence. Various parameters and media components were investigated for production of lipase. Glucose and peptone were found to be most suitable carbon and nitrogen source, respectively. To determine the suitable oil as carbon source, various oils were added to the production medium. Olive oil was found to be the optimal oil for lipase production from *Trichoderma citrinoviride*. pH 5.5, temperature 30°C and incubation time for 4 days were found to be optimal incubation conditions for lipase production. We also determined lipase yield from *Trichoderma citrinoviride* which is produced in molasses medium as an alternative carbon source.

Key Words
Lipase, *Trichoderma citrinoviride*, microbial lipase production.

ÖZ

Bu çalışmada yeni izole edilmiş bir fungustan lipaz üretimi amaçlanmıştır. Fungus Kocaeli bölgesinden topraktan izole edilmiştir. 18S rRNA analizi sonucu *Trichoderma citrinoviride* olarak saptanmıştır. Lipaz üretimi için çeşitli parametreler ve besiyeri bileşenleri araştırılmıştır. Sırasıyla, glikoz ve pepton en uygun karbon ve azot kaynağı olarak bulunmuştur. Karbon kaynağı olarak uygun yağı tespit edilmesi için, üretim ortamına çeşitli yağlar eklenmiştir. Zeytinyağının *Trichoderma citrinoviride*'den lipaz üretimi için en uygun yağ olduğu bulunmuştur. pH 5.5, sıcaklık 30°C ve 4 günlük inkübasyon süresi lipaz üretimi için en uygun inkübasyon koşulları olarak bulunmuştur. Alternative karbon kaynağı olarak melas ortamında üretilen *Trichoderma citrinoviride*'den lipaz verimi de tespit edilmiştir.

Anahtar Kelimeler
Lipaz, *Trichoderma citrinoviride*, mikrobiyal lipaz üretimi.

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INTRODUCTION

Enzymes are defined as biological catalysts that accelerate chemical reactions and allow the maintenance of biological activity, also can be used repeatedly [1]. More before enzymes described they were used in food and textile production [2]. The commercially most widely used enzymes are carbohydrases, proteases and lipases [3]. Lipases (triacylglycerol acylhydrolases, EC. 3.1.1.3) catalyze hydrolysis of triacylglycerol to free fatty acids and glycerol [4]. The use of lipases is becoming more popular in food industry pharmaceutical industry and in cleaning products [5]. Also biodiesel can be synthesized via lipase-catalyzed transesterification [6,7].

It is possible to produce lipase enzyme from fungi that isolated from; oil contaminated soils, waste oils from plants, dairy industry, seeds and perishable food [5]. Lipase production from different fungal sources is possible; Aspergillus, Rhizopus, Mucor, Penicillium, Geotrichumand and Trichoderma [7-9].

Trichoderma is a fungus that has a filament structure, grows very rapidly and can found in any kind of soil, manure or rotting plants. Because of its competitive structure, Trichoderma is predominantly concentrated in the soil. Trichoderma frequently isolated from forest and agricultural soil. Also such as many plant pathogens fungi lives on decaying organic material [8,10-12].

In recent years assessment of waste from the sugar factory, have an important place in lipase production studies. Molasses occurs during the processing of sugar beet to obtain a dark brown colloidal effluent. It is a dark brown colloidal waste. 4 kg molasses occurs in every 100 kg of processed sugar beet [13].

Various studies show that Ülker and colleagues produced extracellular lipase from Trichoderma harzianum, Kashmiri et.al. isolated lipase from Trichoderma viride and Krastanov et.al. isolated laccase from Trichoderma longibrachiatum [8,14,15].

In this paper describes for the first time, the characterization of a novel extracellular lipase from *Trichoderma citrinoviride* that isolated from soil in İlimtepe/Kocaeli, Turkey.

MATERIALS and METHODS

In this study *Trichoderma citrinoviride* that isolated from soil (ilimtepe/ Kocaeli/Turkey) was used for lipase source. After isolation Bioeks Medical and Biotechnology Research Systems isolated DNA accordance with the protocol by Bioseepdy DNA isolation kit® and 18S rRNA sequence analysis has determined the fungi as *Trichoderma citrinoviride*. The microorganism matched with *Trichoderma citrinoviride*(accession number: EU280098.1, 99%).

To determination lipolytic activity, inoculated on tributyrin agar(1% agar, 0.5% peptone, 3% yeast extract) Many researchers have used tributyrin agar for determination microorganisms lipolytic activity. The microorganisms that having lipase enzyme, create a zone while during reproduction [16-18].

Lipase Production Medium

After the determination of the lipase enzyme the organism was cultured in 100 mL of basal mineral medium that Hatzinikolau et.al. described [19]: ((g/L): 12 NaH_2PO_4, 2 KH_2PO_4, 0.330 $CaCl_2.2H_2O$, 0.030 $ZnSO_4.7H_2O$, 0.030 $MgSO_4.7H_2O$, 0.005 $FeSO_4.7H_2O$), and 1 ml olive oil added as a carbon source, in a 250 mL conical flask shaken at 150 rpm at 30°C and pH setted to 5.5.

Determination of Growth Curve

To determinate *Trichoderma citrinoviride* growth curve we inoculated it in lipase production medium for ten days. Each day lipase production medium media was filtered through pre-weighted filter paper (Whatman No. 1) to extract the biomass. Thus measuring the dry weight the growth curve occurred.

Lipase Activity Assay

The filtrate of the lipase production medium used for enzyme source. Titrimetric assay performed for measuring the lipolytic activity as Sugihara described [20].

1 ml olive oil, 4.5 ml 50 mM acetate buffer 0.1 ml 0,5 M $CaCl_2$ and 1 ml filtrate has added for incubation area. Distilled water was added instead of filtrate to prepare blind tube. After 30 minutes incubation time, 20 ml of ethanol added for reaction stop. After this step both 50 mM KOH tube was added until the

pH rises to 10.5. After titration, amount of expended KOH formulated to calculate lipase activity,

(50xexpended KOH)/(30 (incubation time))=U/ml

Special activity was calculated by dividing the lipase activity to dry weight.

Determination of the Optimal Incubation Time, pH and Temperature

Trichoderma citrinoviride inoculated in lipase production medium and incubated it for ten days. Each day lipase activity measured to find optimal incubation time. To find out optimal pH and temperature, lipase production medium was setted between pH 3-9 and incubation temperature was setted between 10-40°C.

Effect of Carbon and Nitrogen Source

Sunflower oil, soybean oil, corn oil and hazelnut oil were used as carbon source instead of olive oil. In addition, it added various carbon sources were added to lipase production medium; glucose, galactose, fructose, lactose, maltose and sucrose. After this step, added optimal oil and sugar source to detect together effects.

To determinate optimal nitrogen source in addition to peptone, we added ammonium sulfate, urea, yeast extract, casein, ammonium oxalate, ammonium nitrate, ammonium carbonate and proteose peptone, described by Sugihara and colleagues [20].

Lipase Production in Molasses Medium

The cost of production media for lipase production and to evaluate this food industrial waste, *Trichoderma citrinoviride* was inoculated in media that include only molasses. To examine the effect of molasses to lipase production, we diluted molasses in different proportions. Accordingly, the total molasses rate is prepared to 1%, 2%, 3%, 4% and 5%. 1 ml of our culture inoculated in production media. Lipase activity and optimal molassesration were determined after incubation.

RESULTS and DISCUSSION

After ten days incubation, and each day measuring the dry weight, growth curve occurred. (Figure1). In first five days *Trichoderma citrinoviride* grown increasingly, after then grow has been decreased.

Optimal Incubation Time, pH and Temperature

After ten days incubation and measuring lipase activity we determined highest activity on 4th day, after then lipase activity started to decrease. Thence in this study optimal incubation time was regarded as 4th day (Figure 2). And remaining study incubated for 4 days.

The effect of hydrogen ion concentration of production medium for lipase activity of *Trichoderma citrinoviride* was studied. At pH 5 lipase activity measured 9.29 U/ml, and at pH 6 it measured 8.13 U/ml. These values is the highest rates in different pH levels. When we compare the

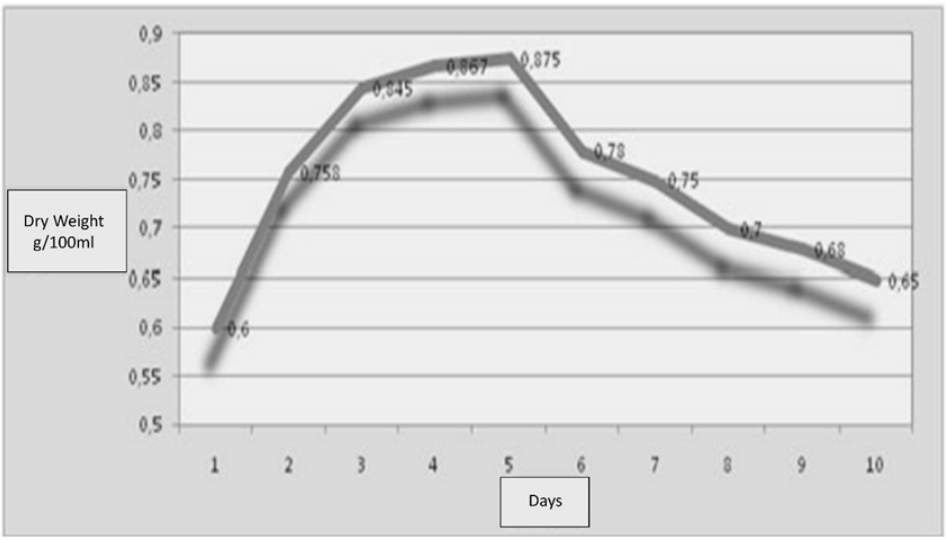

Figure 1. Trichoderma citrinoviride growth curve.

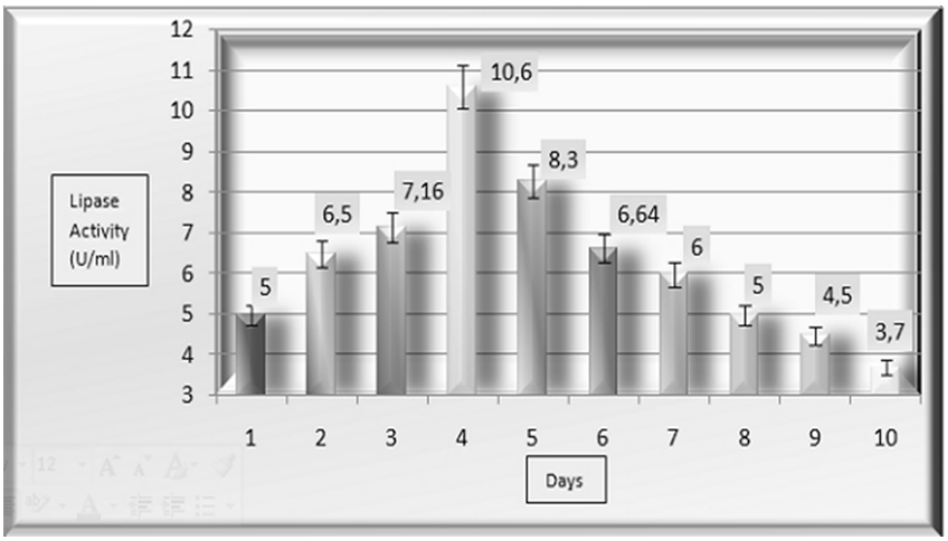

Figure 2. Effect of incubation time on lipase activity.

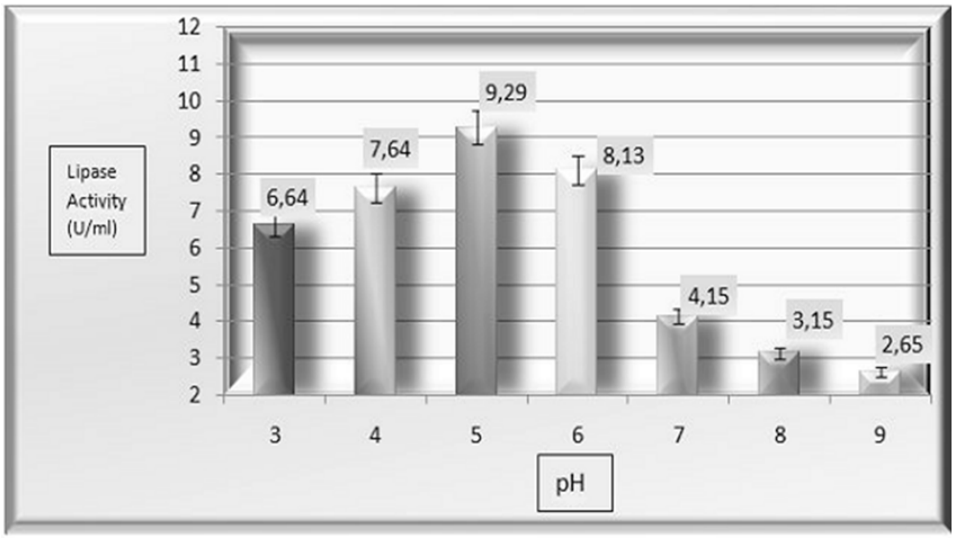

Figure 3. Effect of pH on lipase activity.

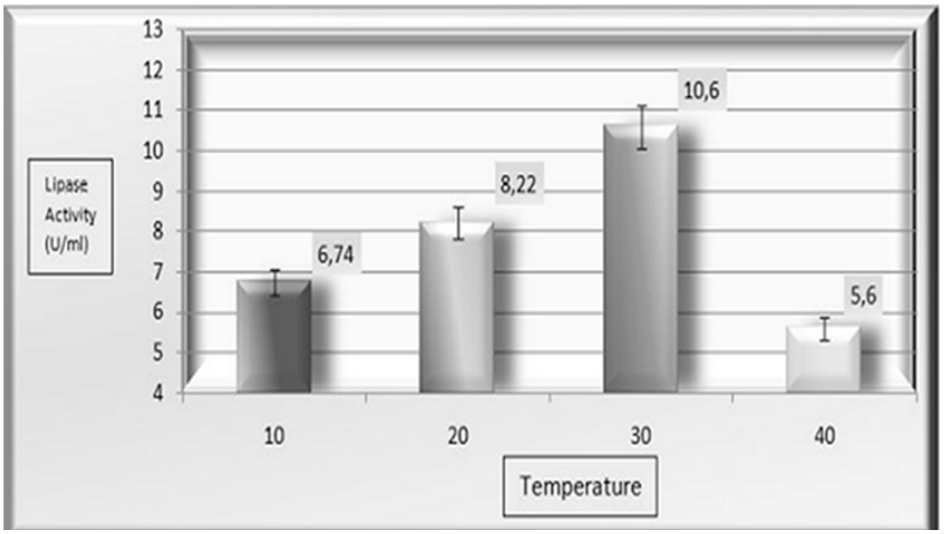

Figure 4. Effect of temperature on lipase activity.

results with the beginning conditions we saw that pH 5.5 (10.6U/ml) is the optimum pH for lipase production from *Trichoderma citrinoviride* (Figure 3). And optimum temperature for lipase production was founded as 30°C (Figure 4).

Highest activity was observed in the medium that includes olive oil, and we found out that lowest lipase activity is in the medium that includes corn oil (Fig. 5). We also detected that glucose is most suitable carbon source(Figure 6) and peptone is most suitable nitrogen source for lipase activity from *Trichoderma citrinoviride* (Figure 7).

Finally we detected lipase activity under the optimum conditions we determined for lipase activity (Carbone source glucose, and olive oil, nitrogen source: peptone, pH: 5.5, temperature: 30°C). The activity was detected 13.68 U/ml. (Figure 8).

We determined the highest lipase activity at 4th day of the incubation. We compare our results with other studies; Kashmiri et.al. detected that 50 hours is optimum incubation time for *Trichoderma viride* in their study [14]. Açıkel et al. find out optimum incubation time is 5 day for Rhizopus

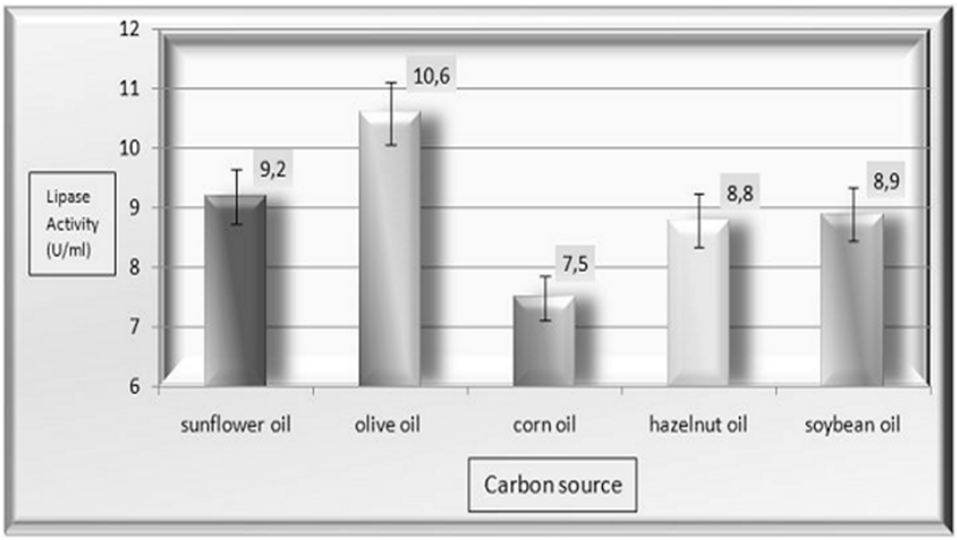

Figure 5. Effect of carbon source (oil varieties) on lipase activity.

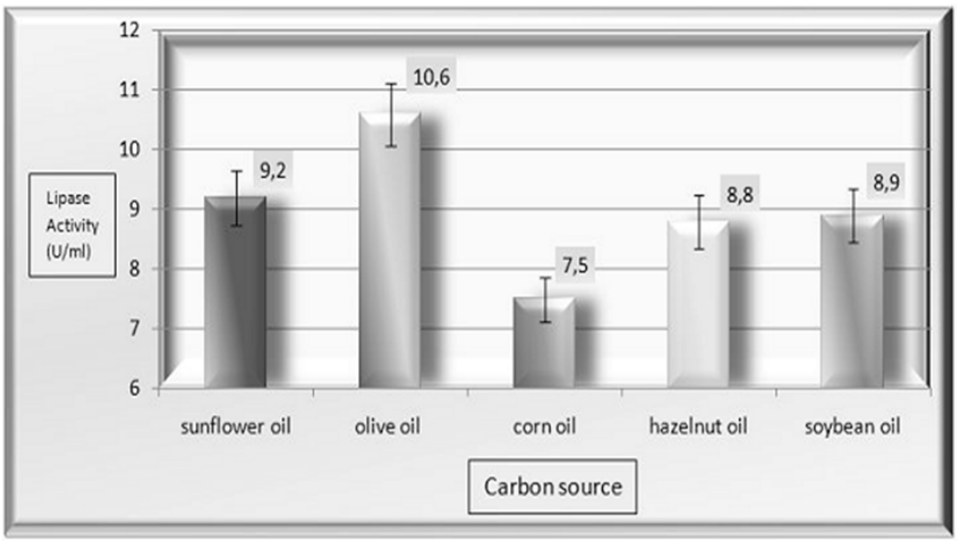

Figure 6. Effect of carbon source (sugar varieties) on lipase activity.

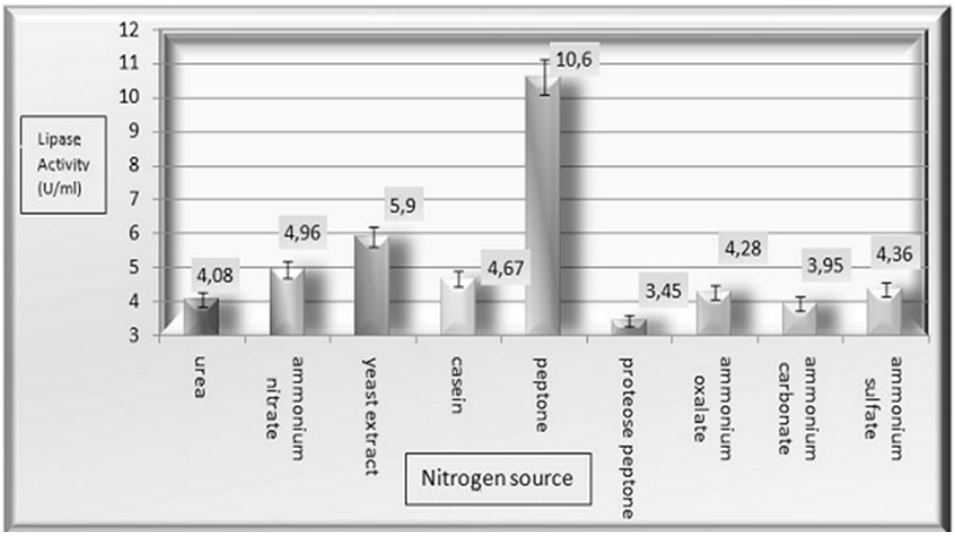

Figure 7. Effect of nitrogen source on lipase activity.

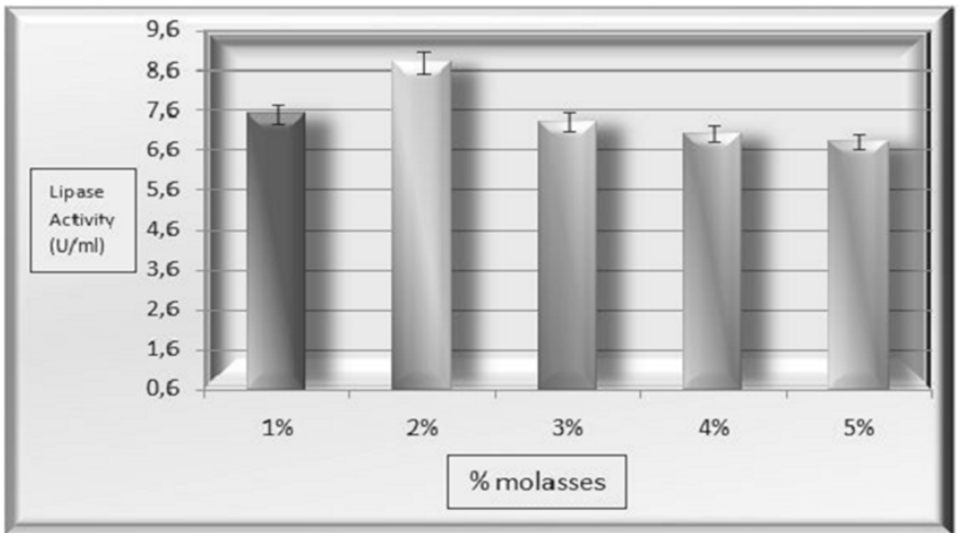

Figure 8. Lipase activity in molasses medium.

delemar [21], Ulker et al. detected the 7th day is optimum incubation time for lipase activity from Trichoderma harziamum [8].Optimum lipase activity was determined at pH 5. Decrease was observed at lipase activity in alkaline pH, acidic pH is more suitable for *Trichoderma citrinoviride* lipase activity. Kumar et.al. find out the optimum pH 8.5, for al alkaliphilic Bacillus coagulans [22].

Optimum incubation temperature was founded 30°C. Considering the cleaning industry; optimum 30°C for lipase activity is very useful especially in terms of energy saving. In lower temperature (10-20°C) despite the reduction, the activity was observed substantially. Especially in detergent formulation cold active lipase is used for cold washing also reduces the wear and tear of textile fibers. Toscano et.al. determined that Trichoderma harzianum lipase activity had started to decrease after 50°C [23].

The oils that added to the growth medium have not any direct effect on *Trichoderma citrinoviride* reproduction. But it is observed that there is a significant effect on lipase activity.The growth medium that added olive oil as carbon source

showed the highest lipase activity. Sunflower oil hazelnut oil and soybean oil has showed close activity. The medium with corn oil as carbon source was showed the lowest activity. We also found out that glucose is most suitable carbon source for lipase activity from *Trichoderma citrinoviride*. And galactose is not convenient for lipase production. Peptone was found to have a remarkable effect on lipase activity. Various nitrogen sources except peptone, caused a decrease in lipase activity.

There has been shown slightly decline of lipase activity after using molasses for production *Trichoderma citrinoviride*. But when considering the costs, it was concluded that molasses can be used for Trichoderma production.

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