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# Optimization of Culture Conditions and Environmental Factors of Dextranase Enzyme Produced by *Paecilomyces lilacinus*

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**Abstract**

Dextranase (α-1,6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme which hydrolizes the α-1,6 glucosidic linkages in dextran, found in higher plants, mammalian tissues, fungi and bacteria. 17 fungal strains were screened for their ability to produce dextranase enzyme. *Paecilomyces lilacinus* was selected as the best fungal source to dextranase production among them. The production of dextranase from *P. lilacinus* was investigated and a number of factors affecting enzyme production, i.e. cultivation time, temperature, initial pH and rotation speed were optimized. Maximum dextranase production was obtained in 7 day cultures at 100 rpm. Optimum pH and temperature for dextranase production were pH: 6 and 30°C, respectively.

## INTRODUCTION

Dextranase (α-1,6-D-glucan, 6-glucanohydrolase; E.C.3.2.1.11) is an enzyme which hydrolizes the α-1,6 glucosidic linkages in dextran, found in higher plants, mammalian tissues, fungi and bacteria [1]. The enzyme has several applications in medicine and sugar industry because of its hydrolizing capacity of dextran. The presence of dextran has many negative effects in sugar processing levels such as sucrose loss, increased viscosity of process syrups and poor recovery of sucrose. Using dextranase can solve many problems in sugar processing due to microbial dextran deposits. Because dextranase can hydrolyze or inhibit the synthesis of glucans, it can be used in treatment of dental plaque. It is also used for preparing low molecular weight dextran and cytotoxic dextran-conjugate and dextranase is showed as an enhancer of antibiotic activity in endocarditis [2-5]. Moreover, dextranase has gain attention because of directed synthesis of isomaltooligosaccharides which have been shown to exhibit prebiotic effects. One of the different ways of obtaining isomaltooligosaccharides is digestion of dextran polymer with dextranase enzyme [6,7].

Although dextranase has been reported from many fungi, most of these organisms are low producers. In this study, various fungi were screened to find the

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best dextranase producer and the culture conditions for dextranase production from *P. lilacinus* were optimized.

## MATERIALS AND METHODS

### Microorganisms and Media

17 different fungi were used in order to find the best dextranase producer. Fungal strains used in this study were; *Ascospheara apis*, *Gibberella fujikuroi, Beauveria bassiana, Penicillium* sp., *Fusarium* sp., *Fusarium acuminatum, Fusarium culmorum, Paecilomyces lilacinus, Funalia trogii, Phanerochaete chrysosporium, Trichoderma viride, Trichoderma viride* A2, *Trichoderma harzianum* D2, *H. insolens, Alternaria alternata, Aspergillus niger, Rhizopus sp.* obtained from the culture collection of Hacettepe University, Department of Biotechnology and UBI. They were maintained on PDA and slants were kept at 4°C. Transfers were made at 1 month intervals.

### Enzyme production

The medium used for all fungi to provide enzyme production consisted of 10.0 g/L dextran, 1.0 g/L $NaNO_3$, 1.0 g/L $K_2HPO_4$, 0.25 g/L $MgSO_4.7H_2O$, 0.5 g/L KCl, 0.01 g/L $FeSO_4.7H_2O$ and 1.0 g/L yeast extract [8-11]. After determining the best dextranase producing fungi, the basal medium which contained 10.0 g/L dextran, 2.0 g/L $NaNO_3$, 2.0 g/L $K_2HPO_4$, 2.0 g/L yeast extract was used to optimization studies [12]. For enzyme production, a spore suspension of $2.10^5$ spores/mL from routine subcultures was used to inoculate Erlenmeyer flasks containing enzyme production medium. Cultures were grown for 7 days at 30°C in a rotatory shaking (150 rpm). The content of each flask was filtered and the filtrate was clarified by centrifugation at 6000 g for 20 min. The supernatant was assayed for protein and enzyme activity. To determine biomass in terms of dry weight, fungal pellets were dried until constant weight. The dry weight of the biomass was determined as gram per 50 mL.

### Measurement of Dextranase Activity

Dextranase activity was analyzed with the 3,5-dinitrosalicylic acid (DNS) method by assaying the reducing sugars released during a 25 min reaction (2% (w/v) dextran, 0.1 M sodium acetate buffer, pH 5.0, 50°C). The absorbance was read at 550 nm using a Jenway, 105 uv/vis. spectrophotometer. The amount of reducing sugar was calculated from the standard curve based on the equivalent glucose. One enzyme activity unit (U) was defined as the amount of enzyme that liberates one micromole of glucose per min reaction under assay conditions [13,14]. The protein estimation was determined by the method of Lowry et al [15]. The results of the analysis are the mean values of three separate experiments.

### Effects of environmental conditions on enzyme production

In order to understand the effect of environmental conditions on dextranase production, modifications of the culture conditions were made as described in the text. Cultivation time, temperature, initial pH and rotation speed (rpm) were investigated.

## RESULTS AND DISCUSSION

The aim of this study was to find strains of fungi for the production of dextranase with potential industrial applications. One of these strains was selected for further studies. The 17 selected fungi were screened in the presence of dextran. Among the strains examined, *Paecilomyces lilacinus* was selected as the best dextranase producer (Figure 1). As shown in Figure 1, most of the strain produced negligible amount of dextranase only. Therefore, optimization studies continued with the fungus *Paecilomyces lilacinus* hereafter.

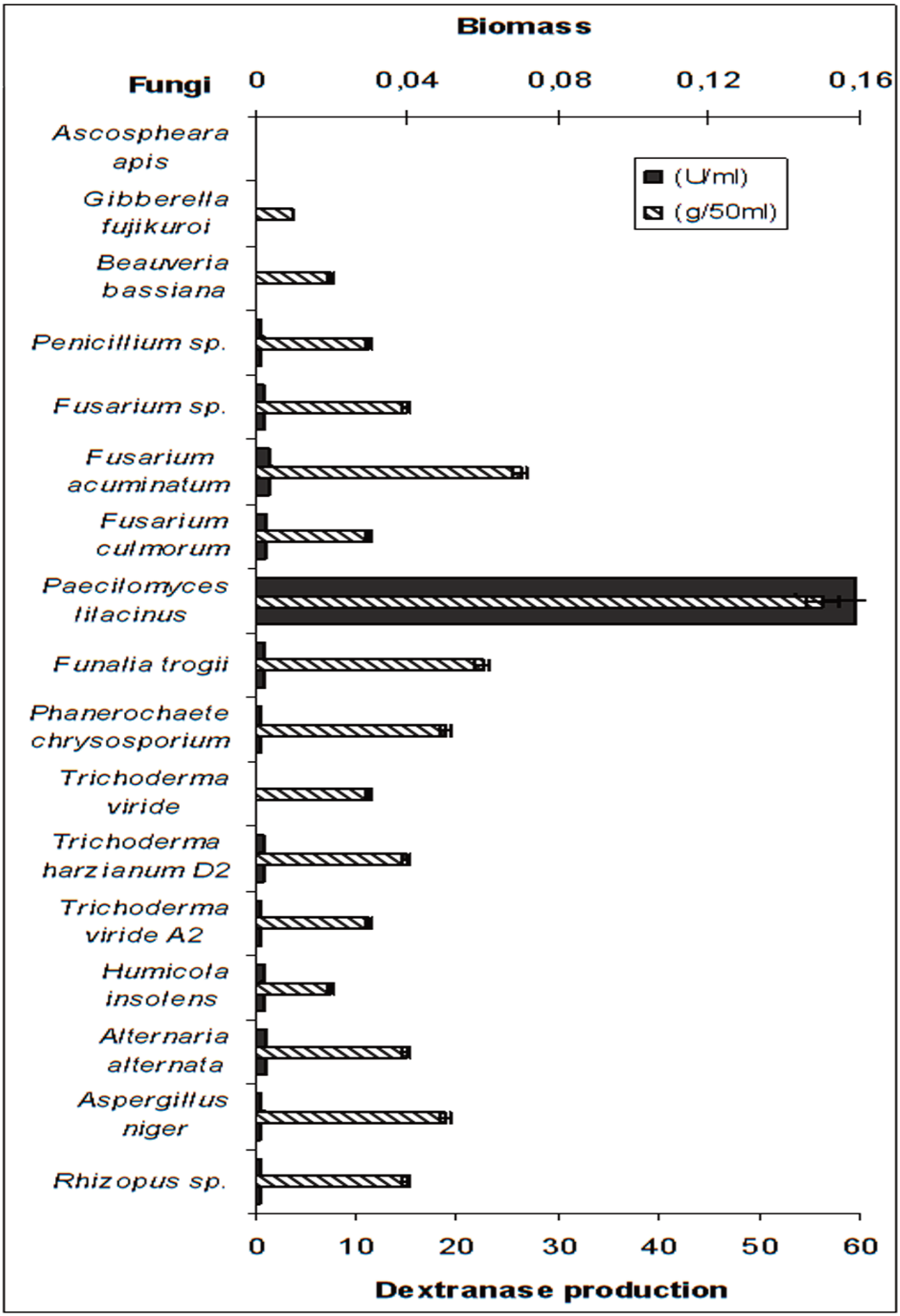


Figure 1. Screening of different fungi for dextranase production. Biomass and specific dextranase activity were determined in culture filtrate. Results represent the means of three separate experiments. Standard deviations are shown on the graphs.

**Cultivation time**

The time course of biomass and dextranase production by *Paecilomyces lilacinus* in basal medium is displayed in Figure 2. Maximum dextranase activity was reached after 7 days; thereafter the enzyme activity declined. Besides dextranase production, fungal biomass generation and total protein were investigated during cultivations. Accordingly, the highest fungal biomass production was at 7$^{th}$ day (Figure 2) for *P. lilacinus*. After that, there was no more increase in the biomass. The results of this study show that reaching the maximum cell mass is important for the dextranase production of the *P. lilacinus*. The enzyme activity showed as specific dextranase activity (U/mg prot).

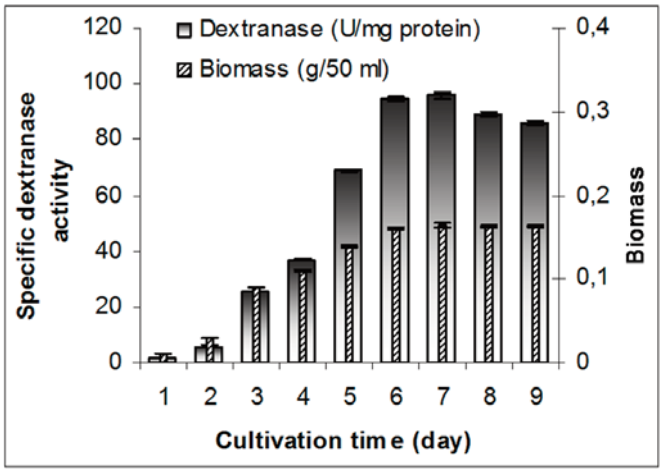


Figure 2. Effect of the incubation period on dextranase production by *Paecilomyces lilacinus*. Biomass and specific dextranase activity were determined in culture filtrate. Results represent the means of three separate experiments. Standard deviations are shown on the graphs.

**Effects of temperature**

To establish the effect of growth temperature, dextranase activity, fungal biomass generation, and total protein content were determined at different growth temperatures ranging from 20 to 35°C while other circumstances were stable. The results indicate that 30°C seems favor to dextranase production (Figure 3). 30°C is also known as the optimal incubation temperature for fungi. Similar observations were reported for the production of dextranase by various fungi [10,16,17]. Although there was not an important change in the range of 20 to 30, there was a sharp decrease at 35°C both in dextranase production and fungal biomass. Certain temperatures which are not suitable for the microorganisms could affect the microbial metabolism and cause low production, therefore, studies are attended at 30°C after that.

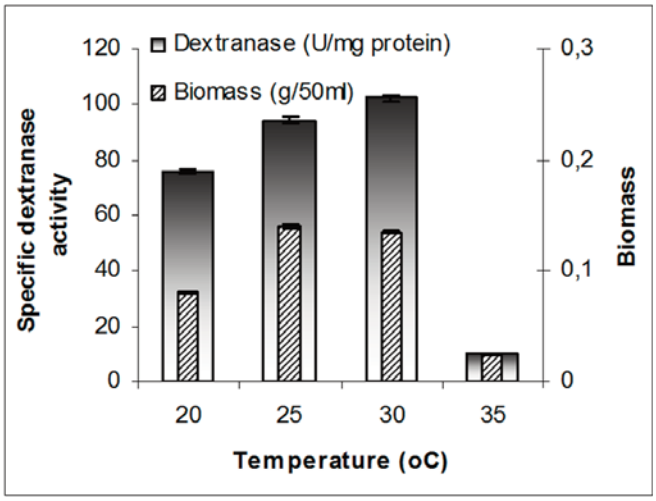


Figure 3. Effect of the temperature on dextranase production by *Paecilomyces lilacinus*. Biomass and specific dextranase activity were determined in culture filtrate. Results represent the means of three separate experiments. Standard deviations are shown on the graphs.

**Effects of Initial pH**

Figure 4 shows the dextranase activities of cultures with different initial pH values. When the initial pH value of the basal medium is higher than 6.0, the development of the fungus is stimulated (Figure 4). The production of dextranase is favored by neutral-low pH values than high. The maximum dextranase production was observed at initial pH of the basal medium of 6. Initial pH is the parameter which depends on both the microorganism and the product. Reported optimal initial pH values for dextranases produced by fungi were similar to our results from 5.5 for *Fusarium* sp. and 5.6-6.0 for *Penicilium lilacinum* [18,19].

**Effects of rotation speed (rpm)**

In order to understand the effect of the rotation

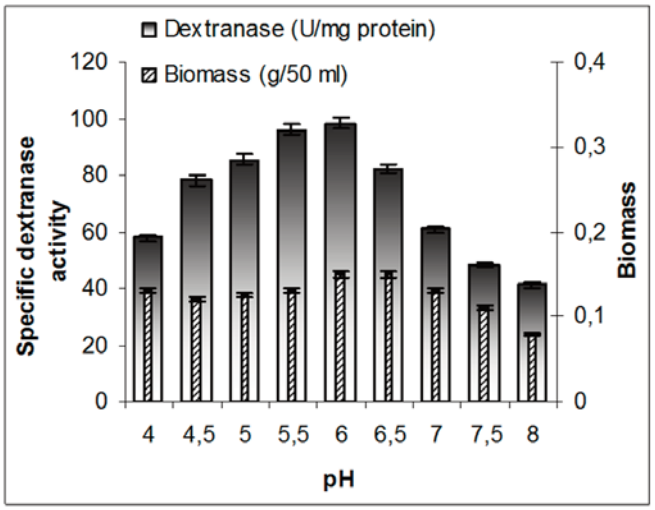


Figure 4. Effect of initial pH on dextranase production by *Paecilomyces lilacinus*. Biomass and specific dextranase activity were determined in culture filtrate. Results represent the means of three separate experiments. Standard deviations are shown on the graphs.

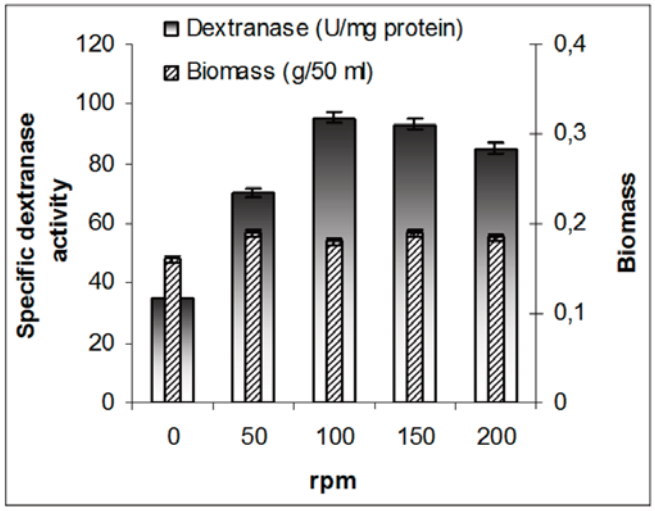


Figure 5. Effect of the rotation speed on dextranase production by *Paecilomyces lilacinus*. Biomass and specific dextranase activity were determined in culture filtrate. Results represent the means of three separate experiments. Standard deviations are shown on the graphs.

speed, cultures incubated at different conditions as static and the altered rpm values. When the culture was incubated at static conditions, enzyme production was low and the appearance of biomass was different than the agitated cultures. In static cultures, biomass was formed at the surface, therefore contact of nutrients and oxygen was low. So that the agitated cultures produced more enzyme than the static cultures as it is expected. Maximum enzyme production was observed at 100 rpm (Figure 5). It can be claimed that agitated conditions are more favorable for dextranase production than static conditions. Reported results were varied in the range of 100-200 rpm in literature [8,19-21].

In this study, screening of 17 fungal strains resulted in selection of *P. lilacinus*, which synthesizes dextranase acting equally well at pH 6, 100 rpm, 30°C, 7 days incubation. The value of the dextranase activity from *P.lilacinus* in this study (113.7 U/mg proteins in culture filtrate) is higher than many of fungi including *Penicillium funiculosum*, *P. notatum*, *Fusarium moniliforme*, *Aspergillus carneus* [8,9,12,22,23]. Although dextranolytic enzymes gain much attention, there is little research on the type culture *P. lilacinus* in terms of dextranase production. Therefore, this *P. lilacinus* strain is of interest for further studies.

## ACKNOWLEDGEMENT

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