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AMYLOLYTIC ACTIVITIES OF FUNGI SPECIES ON THE SCREENING MEDIUM ADJUSTED TO DIFFERENT pH

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ABSTRACT:

The purpose of this study was to determine the amylolytic activities in different fungal species on the medium with different pH values. In the study, thirty-eight fungal species were screened by using a plate culture method on Czapek-Dox Agar medium with different pH values for the determination of amylase production. Among the tested fungal species; four species on medium adjusted to pH 4.0, ten species on medium with pH 4.5, nine species on medium with pH 5.0, four species on medium with pH 5.5 and one species on medium with pH 6.0 showed higher amylolytic activity. While *Chaetomium sp*. on medium adjusted to pH 4.0-4.5 showed high rates of amylolytic activity, *Alternaria citri* on medium adjusted to pH 4.5-5.0 showed high rates of amylolytic activity. *Cladosporium cladosporoides* on medium adjusted to pH 5.0 showed amylolytic activity however they didn't show it at the other levels of pH. Seven fungi species did not show any amylolytic activity on the medium with different pH values. The selection of suitable fungi species for amylase production depends upon environmental conditions, especially upon medium pH.

Keywords: Amylase, fungi, pH

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ÖZET

Bu çalışmanın amacı, değişik küf türlerinin farklı pH a sahip besiyerlerinde amilolitik aktivitelerinin belirlenmesidir. Çalışmada 38 küf türü, değişik pH a sahip Czapek-Dox Agar besiyerinde agar kültür metodu kullanılarak amilaz üretimi yönünden tarandı. Test edilen küf türlerinden; dört tür pH'ı 4.0 olan besiyerinde, on tür pH'ı 4.5 olan besiyerinde, dokuz tür pH'ı 5.0 olan besiyerinde, dört tür pH'ı 5.5 olan besiyerinde ve bir tür pH' ı 6.0 olan besiyerinde yüksek amilolitik aktivite gösterdi. *Chaetomium sp*. pH 4.0-4.5 olan besiyerlerinde yüksek amilolitik aktivite gösterirken, *Alternaria citri* pH 4.5-5.0 olan besiyerlerinde yüksek oranlarda amilolitik aktivite gösterdi. *Cladosporium cladosporoides* pH'ı 5.0 olan besiyerinde amilolitik aktivite gösterirken diğer pH düzeylerinde amilolitik aktivite göstermedi. Yedi küf türü farklı pH lı besiyerlerinde herhangi bir amilolitik aktivite göstermedi. Amilaz üretimi açısından uygun olan küf türlerinin seçimi çevresel şartlara özelliklede besiyeri pH' ına bağlıdır.

Anahtar kelimeler: Amilaz, küf, pH

1. INTRODUCTION

Amylases are starch degrading enzymes employed in the starch processing industries for the hydrolysis of polysaccharides such as starch into simple sugar constituents by degrading 1-4 linkage of starch. These enzymes have found numerous applications in commercial processes, including thinning and liquefaction of starch in the alcohol, brewing and sugar industries (Sanghvi et al., 2011). In addition to, amylases have many applications in bread and baking industry, starch liquefaction and saccharification, textile desizing, paper industry, detergent industry, analysis in medical and clinical chemistry, food and pharmaceutical industries (Metin et al., 2010).

Nowadays, amylases (α-amylases, β-amylases and glucoamylases) represent one of the most important enzyme groups within the field of biotechnology (Bansode, 2010). Starch hydrolytic enzymes comprise 30 % of the world's enzyme consumption (Sanghvi et al., 2011).

Recent discoveries on the use of microorganism as sources of industrially relevant enzymes have led to on increased interest in the application of microbial enzymes in various industrial processes

(Varalakshmi et al., 2009). Today a large number of microbial amylases are available commercially and they have almost completely replaced chemical hydrolysis of starch in starch processing industry (Gupta et al., 2003).

Amylase can be obtained from several fungi, yeast, bacteria and actinomycetes; however, especially fungi, have gained much attention because of the availability and high productivity of fungi, which are also amenable to genetic manipulation (Sidkey et al., 2011). Many fungi had been found to be good sources of amylolytic enzymes. A perusal of the literature indicates that amylases of fungal origin are more stable than those of bacterial origin (Sanghvi et al., 2011).

The selections of a suitable fungi species for amylase production depend upon environmental conditions. The growth requirements for fungi may vary from strain to strain. Fungi grow over a wide range of pH conditions and must thus be able to tailer gene expression to the particular pH of their growth environment. Filamentous fungi vary in pH requirements (Shafique et al., 2009).

Medium optimization for maximum enzyme production is an important step for its commercial usage (Tanyıldızı and Ozer, 2011). In the present study we screen to detect amylase producing fungi and the effect of pH for better production of the enzyme by using plate culture method.

2. MATERIALS AND METHODS

2.1. Microorganisms

A total of 38 fungal isolates used in this study were provided by the fungi collection of Arda Vocational School, Trakya University. 32 fungal isolates were identified to the species level, and 6 were identified to the genus level only.

2.2. Screening Media

Czapek-Dox Agar (CDA) was used as solid medium for screening. The screening procedure for amylase was based on a plate culture method which uses soluble starch (2 %) as the sole carbon

source. Medium contained (g/L): soluble starch 20; $NaNO_3$ 1; K_2HPO_4 1; $MgSO_4$ 0.5; $FeSO_4$ 0.01; agar 15 (Balkan and Ertan, 2005). The pH of the medium was adjusted from 4.0 to 7.0 with 1M NaOH or HCL. All the ingredients listed were sterilized at 121 ^oC for 15 min.

2.3. Screening Method

Each fungus was streaked onto the plates screening media. The plates were incubated at 27 ^oC. Depending on the formation of colonies, on the 5^{th}, 6^{th} and 10^{th} days of incubation, amylase activities were detected as clear zones after exposure to iodine (1% w/v). The ability of starch degrading activities of fungi was estimated in terms of diameter of clear zone (DCZ)/diameter of fungus colony (DFC) ratios by centimeter ruler (Balkan and Ertan, 2005; Balkan et al., 2010; Vaseekaran et al., 2010).

3. RESULTS

Among the tested fungi species, *Alternaria dianthicola*, *Fusarium sp.*, *Eupenicillium anatolicum* and *Cladosporium chlorocephalum* on medium adjusted to pH 4.0; *Aspergillus parasiticus, Aspergillus niger, Aspergillus fumigates, Alternaria alternate, Aspergillus oryzae, Aspergillus wentii, Drechslera australiensis, Phoma sp., Penicillium solitum* and *Ulocladium chartarum* on medium adjusted to pH 4.5; *Acremonium sordidulum, Aspergillus ostianus, Aspergillus flavus, Dreschlera dematoidea, Gibberella fujikuori, Penicillium chrysogenum, Penicillium brevicompactum, Penicillium herquei* and *Trichotecium roseum* on medium adjusted to pH 5.0; *Aspergillus sclerotiorum, Aspergillus sp.*, *Dendriphion comosum* and *Penicillium citrinum* on medium adjusted to pH 5.5; *Epicoccum sp.* on medium adjusted to pH 6.0 showed high rates of the amylolytic activity. While *Chaetomium sp*. on medium adjusted to pH 4.0-4.5 showed high rates of amylolytic activity, *Alternaria citri* on medium adjusted to pH 4.5-5.0 showed high rates of amylolytic activity. *Cladosporium cladosporoides* on medium adjusted to pH 5.0 showed amylolytic activity however they didn't show it at the other levels of pH. *Arthrinium phaeospermum, Cladosporium herbarum, Cladosporium sphaerospermum, Drechslera state of Cochliobolus spicifer, Penicillium citreonigrum, Phoma glomerata, Stemphylium sp.* did not show amylolytic activity on medium (Table 1).

Table 1. Amylolytic activities of fungi species and genera.

	4.0	4.5	5.0	5.5	6.0	6.5	7.0
	DCZ/ DFC	DCZ/ DFC	DCZ/ DFC	DCZ/ DFC	DCZ/ DFC	DCZ/ DFC	DCZ/ DFC
Species and Genera							
Acremonium sordidulum [c]	1.16	1.33	**1.35**	1.2	1.19	1.1	1.09
Alternaria alternata [b]	1.35	**1.55**	1.33	1.29	1.26	1.25	1.15
Alternaria citri [g]	1.1	**1.16**	**1.16**	1.15	1.13	1.08	1.04
Alternaria dianthicola [a]	**1.17**	1.13	1.1	1.06	1.06	1.06	1.04
Arthrinium phaeospermum [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Aspergillus flavus [c]	1.30	1.38	**1.45**	1.37	1.35	1.33	1.30
Aspergillus fumigatus [b]	1.1	**1.21**	1.15	1.15	1.1	1.06	1.06
Aspergillus niger [b]	1.07	**1.19**	1.07	1.06	1.06	1.03	1.03
Aspergillus oryzae [b]	2.3	**2.63**	2.2	2.16	1.85	1.78	1.71
Aspergillus ostianus [c]	1.27	1.32	**1.39**	1.28	1.21	1.16	1.16
Aspergillus parasiticus [b]	1.24	**1.8**	1.3	1.17	1.16	1.14	1.12
Aspergillus sclerotiorum [d]	1.1	1.1	1.13	**1.16**	1.12	1.08	1.07
Aspergillus wentii [b]	1.14	**1.47**	1.4	1.38	1.37	1.17	1.11
Cladosporium chlorocephalum [a]	**1.25**	1.17	1.16	1.15	1.14	1.1	1.04
Cladosporium cladosporoides [c]	0.0	0.0	**1.40**	0.0	0.0	0.0	0.0
Cladosporium herbarum [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Cladosporium sphaerospermum [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Drechslera state of *Cochliobolus spicifer*[*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Dendriphion comosum [d]	1.27	1.3	1.3	**1.51**	1.17	1.06	1.02
Drechslera australiensis [b]	1.16	**1.18**	1.11	1.09	1.06	1.04	1.02
Dreschlera dematoidea [c]	1.30	1.39	**1.4**	1.27	1.1	1.08	1.06
Eupenicillium anatolicum [a]	**1.27**	1.25	1.23	1.15	1.15	1.11	1.05
Gibberella fujikuori [c]	1.07	1.07	**1.36**	1.2	1.06	1.06	1.05
Penicillium brevicompactum [c]	1.1	1.29	**1.31**	1.11	1.10	1.09	1.05
Penicillium chrysogenum [c]	1.32	1.41	**1.71**	1.53	1.52	1.34	1.29
Penicillium citreonigrum [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Penicillium citrinum [d]	1.25	1.29	1.38	**1.43**	1.38	1.35	1.34
Penicillium herquei [c]	1.17	1.2	**1.21**	1.18	1.18	1.13	1.1

Table 1 cont.

Penicillium solitum [b]	1.5	**1.84**	1.52	1.43	1.36	1.3	1.29
Phoma glomerata [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Trichothecium roseum [c]	1.34	1.34	**1.71**	1.21	1.20	1.18	1.06
Ulocladium chartarum [b]	1.13	**1.24**	1.17	1.06	1.06	1.04	1.04
Aspergillus sp. [d]	2.0	2.30	2.41	**2.58**	2.5	1.86	1.75
Chaetomium sp. [f]	**1.07**	**1.07**	1.04	1.02	0.0	0.0	0.0
Epicoccum sp. [e]	1.07	1.08	1.1	1.13	**1.15**	1.08	1.04
Fusarium sp. [a]	**1.24**	1.22	1.13	1.13	1.08	1.05	1.01
Phoma sp. [b]	1.31	**1.44**	1.33	1.23	1.18	1.14	1.09
Stemphylium sp. [*]	0.0	0.0	0.0	0.0	0.0	0.0	0.0

DFC: Diameter of fungus colony **DCZ:** Diameter of clear zone Fungus with high amylolytic activity on medium adjusted to pH 4.0[a] ; pH 4.5 [b]; pH 5.0 [c] ; pH 5.5 [d]; pH 6.0 [e]; pH 4.0-4.5[f] ; pH 4.5-5.0 [g]; the fungi which did not show amylolytic activity [*].

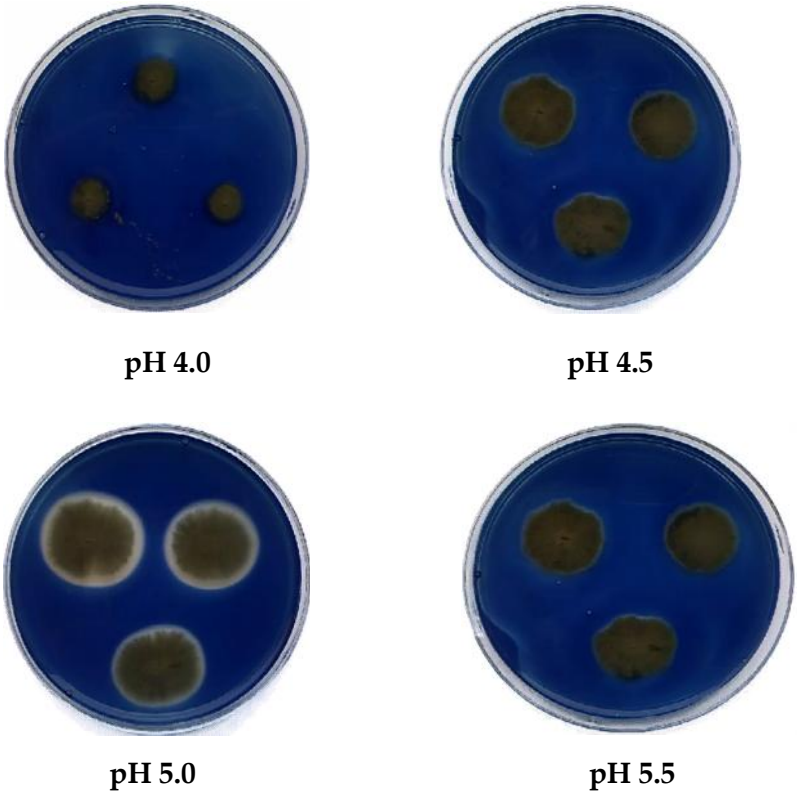

Figure 1. Amylolytic activitiy of *Cladosporium cladosporoides* on the screening medium adjusted to different pH.

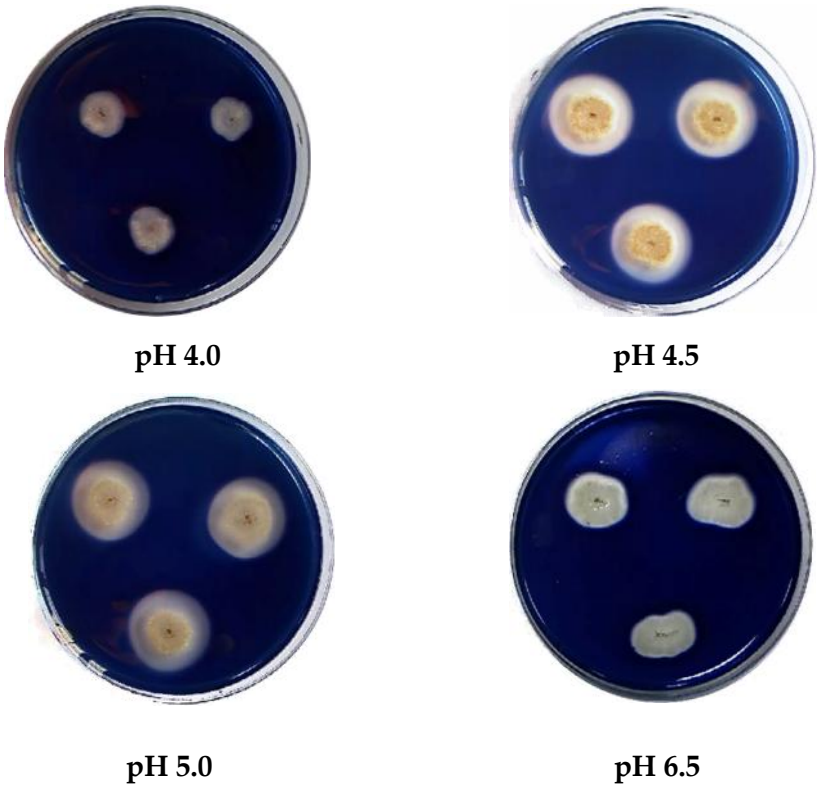

Figure 2. Amylolytic activitiy of *Aspergillus wentii* on the screening medium adjusted to different pH.

pH 5.0 **pH 6.0**

Figure 3. Amylolytic activitiy of *Acremonium sordidulum* on the screening medium adjusted to different pH.

4. DISCUSSION

Production of amylase is highly dependent on starch hence, it have maximum contribution. Starch is ubiquitous and is on easily accessible source of energy for microbial growth (Bansode, 2010). The use of plate culture method containing starch as a sole carbon source is a simple and rapid way to screen amylolytic microorganism. Most screening methods devised for the detection of amylolytic micro-organism involve growing them on solid media containing soluble starch and testing for starch hydrolysis either by flooding the plates with iodine solution or 95% (v/v) ethanol (Akpan et. al., 1999).

Among the physical parameters, the pH of the growth medium plays an important role by inducing morphological change in the organism and in enzyme secretion (Fooladi and Sajjadian, 2010). The pH change observed during the growth of microbes also affects product stability in the medium (Gupta et al., 2003). Different fungal species were tested for amylase production by starch hydrolysis test on medium adjusted to different pH values. The ability of starch hydrolysis activities of fungi was estimated in terms of DCZ/DFC ratios. The size of DCZ/DFC ratio was affected by the pH of the medium (Table, Figure 1, 2, 3). The result of this study clearly reflects that pH value of medium has the ability to affect the enzyme production by fungi. Similar findings were also reported by Akpan et al., (1999).

Except one, all of the fungi showed amylolytic activity between pH 4.0-5.5. It has been reported that widely used industrial fungal amylases are active in the acidic medium (Gouda and Elbahloul, 2008; Shafique et al., 2009). Our results are consistent with this report. The size of DCZ/DFC ratio decreased at pH values above 6.0 (Table).

Abdullah et al. (2011) has reported that further increase in the pH reduced the enzyme production, as enzymes are usually very sensitive to minor changes in pH. Any increase or decrease in H^+ ion concentration has significant effect on the growth of mycelium and hence, on the enzyme excretion as well. In this study, significantly, amylolytic activity of *Cladosporium cladosporoides* matches with this report (Figure 1). While this fungus has showed amylolytic activity on screening medium adjusted to pH 5.0, it has not done so in any other mediums.

Aspergillus oryzae is widely used for the production of numerous hydrolytic enzymes such as alpha amylase, glucoamylase, protease etc. (Abdullah et al., 2011). In this study, while the highest DCZ/DFC ratio was found in *Aspergillus oryzae*, the lowest DCZ/DFC ratio was found in *Chaetomium sp.* This ratio cannot in any way be correlated quantitatively with the amount of enzyme produced. Therefore, the isolation of enzyme producers using starch plates can only be partially selected (Shafique et al., 2009). But, the pH values showing maximum activity of amylases produced by *Aspergillus niger* (Shafique et al., 2009), *Penicillium chrysogenum* (Balkan and Ertan, 2005), *Aspergillus flavus* (Bakri et al., 2009), *Aspergillus oryzae* (Abdullah et al., 2011), *Alternaria alternate* (Shafique et al., 2010), *Penicillium citrinum* HBF62 (Metin et al., 2010), *Penicillium brevicompactum* (Balkan and Ertan 2010), *Aspergillus sclerotiorum* (Yagar et al., 2008) are reported as following 4.5; 5.0; 5.0; 4.5; 5.5; 5.0; 5.0, respectively. These results match with the pH values determined for the amylolytic activity of this fungi species in our study (Table). Amylase is a pH sensitive enzyme. Therefore, the selection of optimal pH is essential for the production of amylase (Bakri et al., 2009). Amylolytic activities that are determined on the screening mediums adjusted to different pH of fungi will form the

basis for further studies in future regarding industrial applications. Thanks to these results, it can be said that in the future studies using these fungi, there won't be a need to determine pH value where enzyme shows maximum activity. Thus it can be suggested that work force and the cost of experiments can be reduced with the help of this study.

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