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Araştırma Makalesi / Research Article

AMYLOLITIC ACTIVITIES OF DIFFERENT FUNGI SPECIES IN THE SCREENING MEDIUM CONTAINING DIFFERENT RAW STARCH

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Abstract; *Thirty-nine fungal species were screened for the production of extracellular amylase hydrolyzing raw starch using a plate culture method. Czapek-Dox Agar containing different raw starch (corn, wheat, potato and rice) was used as culture medium for screening. Among these, thirteen, twelve, seven and five fungi showed higher amylolytic activity on solid medium containing raw wheat starch, raw rice starch, raw potato starch and raw corn starch, respectively. Two fungi did not show any amylolytic activity.*
Keywords: *Amylase, fungi, raw starch hydrolysis, screening medium.*

Farklı Ham Nişasta İçeren Tarama Besiyerlerinde Farklı Fungus Türlerinin Amilolitik Aktiviteleri

Özet; *Otuz dokuz fungus türü ham nişastayı hidroliz eden amilazları üretebilmeleri açısından Petri kültür metodu ile tarandı. Karbon kaynağı olarak farklı ham nişastaları içeren Czapek-Dox Agar ham nişastayı hidroliz eden amilaz üreticilerinin taranması için kullanıldı. Bunlar arasında on üç, on iki, yedi ve beş fungus sırası ile ham buğday, pirinç, patates ve mısır nişastalarını içeren besiyerlerinde en yüksek amilolitik aktiviteyi gösterdi. İki fungus ham nişasta içeren besiyerlerinde amilolitik aktivite göstermedi.*
Anahtar Kelimeler: *Amilaz, fungus, ham nişasta hidrolizi, tarama besiyeri*

Introduction

Starch is the most abundant form of storage polysaccharide in plants and constitutes an inexpensive source for production of syrups containing glucose, fructose or maltose, which are widely used in the food industries (Goyal et al., 2005). In starch granules, the molecules are densely packed in a polycrystalline state with intra and intermolecular bonds and are hence insoluble in cold water and often resistant to chemicals and enzymes (Hamilton et al., 1999).

In the course of conventional enzymatic liquefaction, slurry containing 15-35 % starch is gelatinized, where it is heated to 105 ^{o}C to physically disrupt the granule and open the crystalline structure for the enzyme action. This increases the viscosity of the slurry by 20-fold, and therefore makes mixing and pumping difficult. The gelatinized starch is liquefied with thermostable alpha-amylase, and in then saccharificated with glucoamylase at a much lower temperature of 50-60 ^{o}C. The whole process requires a high-energy input, which increases the production cost of inverted sugar products (Uthumporn et al., 2010).

In recent years, the importance of enzymatic saccharification of raw starch without heating has become well recognized, mainly from the view points of energy savings and effective utilization of the biomass, thereby reducing the cost of starch processing. This has generated a worldwide interest in the gelatinization and can directly hydrolyze the raw starch in a single step, and that too at a moderate temperature much below the gelatinization temperature (Goyal et al., 2005).

Raw starch digesting amylases have been reported from different fungal (Abe et al., 1998; Morita and Fukuoka, 1997; Marlida et al., 2000; Matsubara et al., 2004), bacterial (Hayashida et al., 1998; Lin et al,. 1998) and yeast strains (Nagasaka et al., 1995).

Many microorganisms are capable of producing extracellular amylases. However, only few fungi have been reported to be producers of active amylases capable of degrading raw starch. A search for additional enzyme producers from new sources was needed for effective raw starch saccharification (Sasaki et al., 1986).

Our primary research aim here was the find novel fungal amylases which efficiently hydrolyzed different raw starches such as corn, wheat, potato and rice for practical use in the starch industry.

Materials and Methods

Microorganisms

Fungal species used in the study were provided by the fungi collection of Arda Vocational School, Trakya University.

Screening Media

Czapek-Dox Agar (CDA) was used as solid medium for screening of amylolytic activity on various raw starches by fungi. Medium contained (g/L): raw starch 20; $NaNO_3$ 1; K_2HPO_4 1; $MgSO_4$ 0.5; $FeSO_4$ 0.01; agar 15. The pH of the medium was adjusted to 5.0. All the ingredients listed were sterilized at 121 ^oC for15 min., except raw starch, which was sterilized separately by dry heating in an oven at 140 ^oC for 1 hr and added aseptically in the sterilized medium.

Screening Method

Each fungus was streaked onto the plates screening media. The plates were incubated at 30 ^oC for five days. Raw starch digesting activities were detected as clear zones after exposure to iodine (1% w/v). Diameters of the clear zones and fungal colonies were evaluated by millimeter ruler.

Results

Thirteen fungi on raw wheat starch containing medium, twelve fungi on raw rice starch containing medium, seven fungi on raw potato starch containing medium, five fungi on raw corn starch containing medium showed high rates of the amylolitic activity. Two fungi did not show amylolitic activity on medium containing raw starch. Aspergillus oryzae, Acremonium sordidulum, Aspergillus sclerotiorum ve Arthrinium phaeospermum on medium containing raw corn starch, Aspergillus sp. raw potato and on raw rice starch containing medium, Aspergillus fumigatus on medium containing raw corn and wheat starch, Eupenicillium anatolicum on medium containing raw corn and potato starch, Aspergillus wentii and Trichothecium roseum on medium containing raw corn, potato and rice starch did not show any the amylolitic activity (Table1).

Discussion

The use of plate culture method containing starch as a sole carbon source is a simple and rapid way to screen amylolytic microorganism. The ability of raw starch degrading activities of fungi were estimated in terms of diameter of clear zone (DCZ)/diameter of fungus colony (DCF) ratios (Figure1).

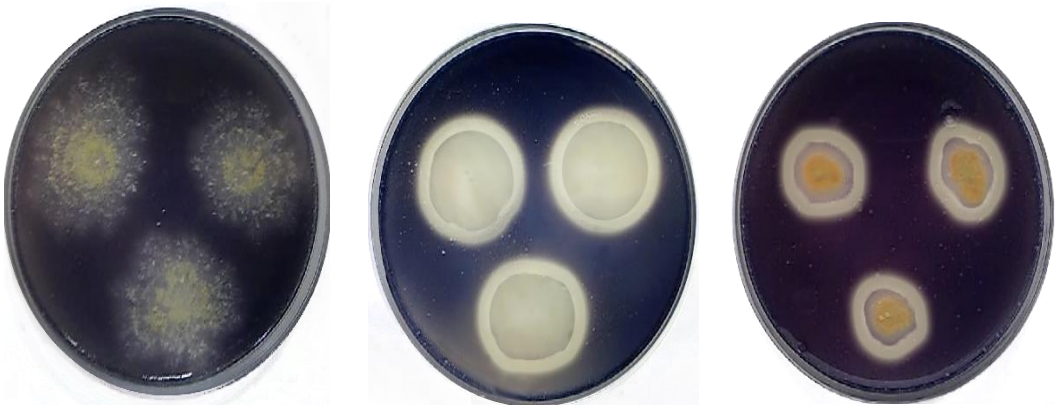
Table 1 Amylolytic activities of fungi species.

	Raw corn starch			Raw wheat starch			Raw potato starch			Raw rice starch		
Fungi	**DCZ (cm)**	**DCF(cm)**	**DCF/ DCZ**	**DCZ (cm)**	**DCF (cm)**	**DCF/ DCZ**	**DCZ (cm)**	**DCF (cm)**	**DCF/ DCZ**	**DCZ (cm)**	**DCF (cm)**	**DCF/ DCZ**
Fusarium sp. [e]	4.1	0.0	0.0	4.3	0.0	0.0	4.8	0.0	0.0	4.7	0.0	0.0
Cochliobolus spicifer'in [e]	4.3	0.0	0.0	4.4	0.0	0.0	4.4	0.0	0.0	5.4	0.0	0.0
Penicillium griseofulvum [b*]	1.1	1.7	1.54	1.0	1.8	1.8	1.1	1.4	1.27	1.0	1.9	1.9
Dreschlera dematoidea [a*]	3.2	3.7	1.15	3.2	3.9	1.21	3.0	3.5	1.16	3.7	4.1	1.10
Alternaria dianthicola [b*]	3.7	4.5	1.21	3.9	4.5	1.15	4.0	4.8	1.20	3.8	4.7	1.23
Arthrinium phaeospermum [b]	2.9	0.0	0.0	2.7	3.6	1.33	2.5	3.5	1.40	2.2	3.2	1.45
Alternaria citri [c*]	3.5	4.2	1.2	3.5	4.1	1.17	4.0	4.4	1.1	3.2	3.8	1.18
Rhizopus sp. [a*]	1.0	1.3	1.3	1.6	2.5	1.56	1.4	1.8	1.28	1.2	1.8	1.50
Phoma glomerata [b*]	3.2	3.6	1.12	3.0	3.3	1.1	3.0	3.3	1.1.	3.0	3.5	1.16
Phoma sp. [c*]	2.0	2.8	1.4	2.2 .	3.0	1.36	2.1	3.0	1.42	2.9	3.6	1.24
Penicillium citrinum [b*]	2.9	3.1	1.06	2.5	2.9	1.16	2.7	3.0	1.11	2.5	3.1	1.24
Aspergillus ostianus [d*]	1.7	2.3	1.35	2.9	3.5	1.20	2.7	3.4	1.25	2.4	3.0	1.25
Aspergillus sclerotiorum [c]	2.5	0.0	0.0	2.5	2.9	1.16	1.4	2.7	1.92	1.7	2.7	1.58
Epicoccum sp. [c*]	2.5	3.3	1.32	3.5	4.0	1.14	2.7	4.0	1.48	3.3	4.3	1.30
Aspergillus fumigatus [c*]	2.4	3.1	1.29	1.8	2.0	1.11	2.0	2.9	1.45	3.0	3.6	1.2
Dendriphion comosum [b*]	3.7	4.3	1.16	4.0	5.2	1.3	4.1	4.7	1.14	3.4	4.5	1.32
Alternaria alternate [b*]	3.5	3.9	1.11	3.3	3.7	1.12	3.4	3.7	1.08	3.5	4.2	1.2
Penicillium herquei [c*]	2.5	2.8	1.12	2.7	3.2	1.18	2.3	2.8	1.21	2.5	3.9	1.2
Stemphylium sp. [a*]	3.5	4.1	1.17	3.0	4.0	1.33	3.7	4.2	1.13	3.2	4.2	1.31
Gibberella fujikuori [a*]	4.2	4.5	1.07	3.8	4.8	1.26	4.2	4.6	1.09	4.0	4.6	1.15

Aspergillus flavus [a*]	3.2	3.8	1.18	3.2	4.8	1.5	3.2	3.6	1.12	3.1	3.9	1.25
Penicillium solitum [a*]	1.5	2.0	1.33	0.9	1.3	1.44	1.5	1.6	1.06	1.4	2.0	1.42
Penicillium brevicompactum [b*]	3.1	3.9	1.25	3.2	3.5	1.09	2.5	2.7	1.08	2.7	3.6	1.33
Aspergillus parasiticus [a*]	3.4	3.7	1.08	3.4	4.0	1.33	2.8	3.2	1.14	2.9	3.4	1.17
Penicillium chrysogenum [c*]	3.0	3.3	1.1	2.9	3.2	1.1	2.4	3.0	1.25	2.3	2.8	1.21
Cladosporium chlorocephalum [b*]	2.1	2.2	1.04	2.2.	2.3	1.04	2.0	2.2	1.10	2.5	2.9	1.16
Cladosporium herbarum [d*]	2.1	2.5	1.19	2.3	2.5	1.08	2.1	2.4	1.14	2.5	2.7	1.08
Cladosporium sphaerospermum [d*]	1.2	2.3	1.91	2.1	2.2	1.04	2.2	2.6	1.18	1.9	2.2	1.15
Eupenicillium anatolicum [a]	2.5	0.0	0.0	2.2	2.7	1.22	2.5	0.0	0.0	2.3	2.5	1.08
Cladosporium cladosporoides [d*]	2.1	2.3	1.09	2.0	2.1	1.05	2.0	2.1	1.05	2.3	2.4	1.04
Aspergillus fumigatus [b]	2.4	0.0	0.0	2.5	0.0	0.0	2.4	2.7	1.12	2.8	3.5	1.25
Aspergillus sp. [a]	1.5	1.7	1.13	2.4	3.2	1.33	2.3	0.0	0.0	2.1	0.0	0.0
Trichothecium roseum [a]	3.5	0.0	0.0	2.7	4.2	1.55	3.5	0.0	0.0	3.5	0.0	0.0
Aspergillus wentii [a]	1.9	0.0	0.0	2.4	3.1	1.29	2.1	0.0	0.0	2.2	0.0	0.0
Acremonium sordidulum [b]	3.4	0.0	0.0	3.2	3.7	1.15	2.7	3.2	1.18	2.4	3.1	1.29
Aspergillus oryzae [a]	1.4	0.0	0.0	1.5	3.0	2.0	1.1	1.4	1.27	1.2	1.7	1.41
Drechslera australiensis [c*]	2.9	3.8	1.31	2.8	3.9	1.39	2.4	3.9	1.62	3.2	4.3	1.34
Ulocladium chartarum [a*]	3.6	4.0	1.11	3.5	4.0	1.14	3.7	4.1	1.10	3.6	4.0	1.11
Fusarium concolor [b*]	4.3	5.5	1.27	4.3	5.5	1.27	4.5	6.0	1.33	3.5	5.5	1.57

DCF: Diameter of fungus colony **DCZ:** Diameter of clear zone
Amylolytic activity of fungus after 5 day at 30 ^{o}C on: raw wheat starcha; raw rice starchb; raw potato starchc; raw corn starch mediumd; the fungi which did not show amylolytic activity e; the fungus which show amylolytic activity on all raw corn, wheat, potato and rice medium*.

In the media containing raw potato starch as a sole carbon source seven fungal species showed amylolytic activity higher than other mediums did. Thirty fungi showed higher amylolitic activity in the media containing other raw starch (rice, wheat and corn) as the sole carbon source. Two fungal species did not show amylolytic activity (Table1). In the screening media, the raw wheat and rice starches were hydrolyzed more than the raw potato and corn starches (Table1). This study showed that raw starches hydrolyzed by amylase were significantly dependent on the starch source. This agrees with earlier reports of Okolo et al. (1995) and Marlida et al. (2000). Hydrolysis of raw starches is not only a unique property of the enzyme but that this depends on the type of starch, granule size and viscosity of the raw starch (Marlida et al., 2000).



a b c

Figure. Amylolytic activities in the screening media (a)*Trichothecium roseum* (raw potato starch without amylolytic activity)(b)*Penicillium herquei*(raw wheat starch with amylolytic activity)(c)*Aspergillus oryzae* (raw rice starch with amylolytic activity)

Several Aspergillus species are known to produce raw starch digesting amylase for cereal starches (Abu et al., 2005) but few have been known to be active towards root or tuber starches (Abe et al., 1988; Okolo et al., 1995). In the study, nine Aspergillus species were tested. While seven of these hydrolyzed cereal starches superior to tuber starch, the other two (Aspergillus sclerotiorum and Aspergillus fumigatus) hydrolyzed tuber starch better than cereal starches (Table1). This clearly demonstrates the unique characteristic of these Aspergillus species.

It is more difficult for amylases to act on raw starch granules than on gelatinized starch. Previous studies indicated that the saccharification of raw starch by amylolitic enzymes might be related to the extent of adsorption of an enzyme to the starch granules (Itkor et al., 1989). According to Leloup et al. (1990) there are several steps involved in the enzymatic reaction which are; (1) the diffusion to the solid surface, (2) the adsorption of the enzyme and finally (3) the occurrence of the catalysis. The adsorption step is essential prior to the subsequent catalytic activity. Therefore the enzyme needs to pass across the boundary between the aqueous phases and solid phases before attaching to the granule. The penetration of hydrolyzing enzymes and other large molecules, however, is restricted and only possible through pores or channels (Oates, 1997).

It was reported that the adsorption rates of Penicillium brevicompactum amylase on raw starches (corn, rice, wheat) were in order rice>corn>wheat (Balkan and Ertan, 2010). In our study, raw starches hydrolysis of amylase produced by Penicillium brevicompactum was in order rice>corn>wheat. Previous studies (Iefuji et al., 1996) indicated that the saccharification of raw starch by amylolitic enzymes might be related to the extent of adsorption of an enzyme to the starch granules. Our results match with this statement. However Balkan and Ertan (2010), adsorption-desorption process used in the enzyme purification procedure because the enzyme connected strongly to raw rice starch. Raw starch hydrolysis associated with adsorption so the results that we find in the purification steps will form the basis in our further studies. It can be said that in the future these may help us in the matter of time and economy.

In this study, the best fungus which showed amylolytic activity on all raw corn, wheat, potato and rice starch medium was Penicillium griseofulvum. The raw starches hydrolysis of amylase produced by P.griseofulvum was in order rice>wheat>corn> potato. It can be suggested that P. griseofulvum may be a potential source of amylase for biotechnological applications. Performances of the fungus could be improved by further investigation in larger scaled operation and by mutagenic methods.

References

- ABE JI, BERGMANN FW, OBATA K, HIZUKURI S. Production of the raw starch digesting amylase of Aspergillus sp. K-27, *Applied Microbiology and Biotechonology*. 27: 447-450, 1998.

- ABU EA, ADO SA, JAMES DB. Raw starch degrading amylase production by mixed culture of Aspergillus niger and Saccharomyces cerevisae grown on sorghum pomace, *African Journal of Biotechnology*. 4: 785-790, 2005.

- BALKAN B, ERTAN F. The production of a new fungal α-amylase degraded the raw starch by means of solid-state fermentation. *Preparative Biochemistry Biotechnology*. 40: 213–228, 2010.
- GOYAL N. GUPTA JK, SONI SK. A Novel Raw Starch Digesting Thermostable α-Amylase From Bacillus sp. I-3 and Its Use in the Direct Hydrolysis of Raw Potato Starch. *Enzyme and Microbial Technology*. 37: 723–734, 2005.
- HAMILTON LM, KELLY CT, FOGARTY WM. Purification and Properties of the Raw Starch-Degrading α-Amylase of Bacillus sp. IMD 434. *Biotechnology Letters*. 21: 111–115, 1999.
- HAYASHIDA S, TERAMOTO Y and INOUE T. Production and characteristics of raw potato starch digesting α-amylase from Bacillus subtilis 65. *Applied and Environmental Microbiology*. 6: 1516-1522, 1998.
- IEFUJI H, CHINO M, KATO M, IIMURA Y. Raw Starch Digesting and Thermostable α-Amylase From the Yeast Cryptococcus sp. S-2: *Purification, Characterization, Cloning and Sequencing. Biochemical Journal*. 318: 989–996, 1996.
- ITKOR P, SHIDA O, TSUKAGOSHI N and UDAKA S. Screening for raw starch digesting bacteria. *Agricultural Biological Chemistry*. 53: 53-60, 1989.
- LELOUP VM, COLONNA P. and RING SG. Studies on probe diffusion and accessibility in amylose gels. *Macromols*. 23:862-866, 1990.
- LIN LL, CHYAU CC and HWEI HSU W. Production and properties of a raw starch degrading amylase from the thermophilic and alkaliphilic Bacillus sp. TS-23. *Biotechnology and Applied Biochemistry*. 28: 61-68, 1998.
- MARLIDA Y, SAARI N, HASSAN Z and RADU S. Improvement in raw sago starch degrading enzyme production from Acremonium sp. endophytic fungi using carbon and nitrogen sources. *Enzyme and Microbial Techonology.* 27: 511-515, 2000.
- MATSUBARA T, AMMAR YB, ANINDYAWATI T, YAMAMOTO S, ITO K, IIZUKA M and MINAMIURA N. Molecular cloning and determination of the nucleotide sequence of raw starch digesting α-amylase from Aspergillus awamori KT-11. *Journal of Biochemistry and Molecular Biology.* 4: 429-438, 2004.
- MORITA H, FUKUOKA YF. High spesific activity of raw-starch digesting glucoamylase producing Rhizopus sp. A-11 inliquid culture. *Starch.* 49: 293-296, 1997.
- NAGASAKA Y, MURAKI N, KIMURA A, SUTO M, YOKOTA A, TOMITA F. Cloning of Corticium rolfsii glukoamylase cDNA and its expression in Saccharomyces cerevisiae. *Applied Microbiology and Biotechnology*. 44: 451-458, 1995.
- OATES CG. Towards an understanding of starch granule structure and hydrolysis. *Trends in Food Science and Technology*. 8:375-382, 1997.
- OKOLO BN, EZEOGU LI, MBA CN. Production of raw starch digesting amylase by Aspergillus niger and Bacillus alvei grown on native starch sources. *Journal of the Science of Food Agriculture*. 69: 109-115, 1995.
- SASAKI H, KUROSAWA K, TAKAO S. Screening of microorganisms for raw starch saccharifying enzyme production. *Agricultural Biological Chemistry*. 50:1661-1664, 1986.
- UTHUMPORN U, ZAIDUL ISM, KARIM AA. Hydrolysis of granular starch at sub-geletinization temperature using a mixture of amylolitic enzymes. *Food and Bioproducts Processing*. 88:47-54, 2010.