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Utilization of Whey Amended with Some Agro-industrial By-products for the Improvement of Protease Production by *Aspergillus terreus* and its Compatibility with Commercial Detergents

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Abstract: *Aspergillus terreus* a local wild fungal isolate was tested for the production serine alkaline protease (E.C. 3.4.1.14) when grown on whey amended with some agro-industrial by-products. The addition of 30 % whey increased the activity to 2.25 units /ml, the addition of 40% cane molass yield 3.40 units/ml, the addition of 30% beet molass give 2.84 units/ml, the addition of 1% glutofeed give 2.88 units/ml while, the addition of 0.5% of ragee elkon give 2.94 units/ml. The optimum conditions for enzyme production was 40 ºC , six days of incubation and pH 7.0. the enzyme was pH stable at a wide range of pH and also thermostable at temperatures 40-90 ºC. the enzyme was found to compatible with certain local detergents and suitable for detergent applications.

Key words: Alkaline protease, Agro-industrial by-products- *Aspergillus terreus,* compatibility with detergents.

INTRODUCTION

Proteases is an important group of enzymes in both physiological and commercial fields especially in the food processing, laundry detergents and other chemical and pharmaceutical industries[4,11,15]. Microbial proteases dominate the commercial applications[29,24]. In recent years there has been interest in proteases from thermophiles which were expected to produce thermostable enzymes because thermal denaturation is a common cause of enzyme inactivation[12,15]. Production of proteases from certain thermophilic and thermotolerant fungi has been investigated[23,10,1,17,20,13,27,15]. Utilization of cheap by-products is cost effective[27].

MATERIALS AND METHODS

Fungus: The fungus was isolated from soil samples butcheries at Zagazig, Sharkia, Egypt on Casein-agar medium[26]. Pure cultures were maintained on Yeast-glucose agar medium[6]. and identified by Dr. Samson, Centraalbureau Voor Schimmel Cultures Baarn, Netherlands; as *Aspergillus terreus.* The effect of temperature on mycelial growth of the fungus revealed that it is a thermotolerant mesophile.

Diary and Agro-industrial by-products: These are whey, cane & beet molasses, glutofeed and ragee elkon. They are easily obtained.

Cultivation and culture conditions: Modified Czapek's broth in which casein 0.6 % replaced $NaNO_3$ (pH 7.0) was used as a basal medium. The effect of addition of agro-industrial by-products; whey, cane molasses, beet molasses, corn meal, gluten (2.3 % N), gluten (11.36 % N) and ragee elkon was investigated. The test fungus was grown in triplicate sets of 250 ml Erlenmyer flasks, each receiving 50 ml broth for 6 days at 40º C.

Enzyme assay: This was achieved using the method adopted by[17]. The reaction mixture contained 1 ml of enzyme solution and 5 ml of 0.6% casein in 0.1 M phosphate buffer (pH 8.0) and was incubated at 45º C for 60 min. The reaction was stopped by adding 5 ml of 5% trichloroacetic acid and centrifuged at 2 x 10^3 g for 10 min. protein was determined in the supernatant by Lowry method[14]. using tyrosine as a standard. A control containing 1 ml of the boiled enzyme was similarly treated. One unit of the activity was defined as the amount of enzyme producing a change of absorbance equivalent to 1µg of tyrosine min^{-1} under assay conditions.

Protein determination: Protein in the enzyme solution was estimated by the procedure of[14]. employing bovine serum albumin as a standard.

The pH and thermal stability of the enzyme: The pH stability of the enzyme after incubation after 15 and 60

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min. at pH (5 - 10) was investigated and heat stability after incubation for 15 and 60 min. at (40 - 90º C) were also studied.

Compatibility of the enzyme with some commercial detergents: The compatibility of protease enzyme from *A. terreus* with the following detergents (0.7 % w/v); savo, hattric, ariel, persil, tide and general after incubation with (0.15 %) enzyme solution for 60 min. with each of these detergents at 30, 60, 90 ºC. A treatment without detergent was used as control then the residual activity of protease in each case was determined.

RESULTS AND DISCUSSION

The aim of this paper is the study the utilization of certain available cheap diary and agro-industrial by-products to motivate the protease production by the experimental fungus *Aspergillus terreus.*

Whey, cane & beet molasses, glutofeed and ragee elkon are by-products of cheese, corn starch and rice industries respectively were used to enhance protease production. These by-products are renewable and produced annually in great quantities and usually disposed to the environment causing pollution.

Due to governmental environmental pressures, it is suitable for the economic point of view to recycle such cheap by-products in other industries.

These by-products were kindly analyzed for total nitrogen, carbohydrates and lipids in the National Search Center, Dokki, Cairo, Egypt. Under the supervision of the German-Egyptian Cooperation by the authorities of the project of plant nutrition in Egypt. The analyses of these by-products are shown in Table (1).

Proteases are used primarily in the detergent industry and in the dairy industry. However, other areas in which proteases are used include the pharmaceutical industry, the leather industry, the manufacture of protein hydrolysate, the food industry, the cinema film industry and waste processing[3,27,15].

Production of proteases by thermophilic and thermotolerant fungi has been reported by many investigators[23,10,1,17,20,13,27,15]. The effect of addition of whey, cane molass, beet molass, glutofeed and ragee elkon on protease production was studied using different concentrations.

Addition of whey to optimized medium for *Aspergillus terreus* increase the production of protease production up to 30 % (Table 2) then decreased; which observed through the activity (2.25 units / ml) and specific activity (2.74 units / mg).

The addition of cane molass increased the production of protease by *A. terreus* up to 40% then decreased Table 3 where the activity reached 3.40 units / ml and the specific activity 3.09 (units / mg protein).

The results obtained revealed that generally the addition of beet molass also increased protease production by by *A. terreus* up to 40 % then decreased (Table 4) where activity reached 2.45 (Units / ml) and the specific activity 3.40 (Units / mg protein).

The optimum temperature for mycelial growth of *Aspergillus terreus* was found to be 40º C (Fig. 1).

According to[7]. *A. terreus* is a thermololerant mesophile.[17]. recorded that maximum production of protease from *Thermoasus aurantiacus* var *cevisporus* occurred at 50º C.[19]. reported that 45º C was the optimum temperature for protease production by *Thermoas thermophilum*[30]. reported that optimum temperature for synthesis of thermostable protease from thermophilic strain of *Aspergillus* sp was 40º C.

Maximal protease was obtained after six days of incubation for *A. terreus* as shown in (Fig.2).

Similar results were obtained by[19]. who found that *Thermoas thermophilum* maximum production was achieved after six days of incubation.

It is worthy to mention that the incubation period was 48 h for maximum protease production from *Aspergillus flavus* by solid state fermentation[16]. and also for protease production from *Conidiobolus coronatus* in skaked cultures[25].

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The effect pH value on the production of protease by *A. terreus* was studied and was found to reach its maximum at pH 7.0 then decreased as shown in Fig. 3, the activity was 1.05 (U/m) and specific activity (2.52 U/mg protein) stated that growth at neutral and alkaline protease. The pH of the culture medium greatly affects the availability of certain metallic ions, permeability of membranes and enzymatic activity.

The properties of alkaline proteases from different taxa of fungi were intensively studied[23,28,22,21,16,20,18,8,25,9]. The effect of pH value on the enzyme stability could be distinguished experimentally by pre-incubating the enzyme at pH range 5 – 10 for 60 min. after which the original pH was restored and the residual activity was estimated. The results Fig. 4 show that protease of *A. terreus* is stable over a wide range of pH especially in the alkaline side. The optimum pH was 8.0.

Table 1: Analysis of some dairy and agro-industrial by-products.

By-product	Total nitrogen (%)	Total carbohydrates (%)	Total lipids (%)
Whey	3.7	5.3	0.3
Cane molass	0.011	3.8	0.0
Beet molass	0.022	5.2	0.0
glutofeed	11.36	1.25	11.5
Ragee elkon	1.60	2.0	10.9

Table 2: Effect of whey on the production of protease by *Aspergillus terreus*

Treatment% (v/v)	Protein (mg/ml)	Protease activity (Units/ml)	Specific activity (Units /mg protein)
Control (c) 0.0	0.51	1.23	2.41
Whey (w) 100	0.91	1.40	1.54
20	0.94	1.96	2.09
30	0.82	2.25	2.74
40	0.88	2.10	2.39
50	0.92	1.89	2.05

Table 3: Effect of cane molass on the production of protease by *A. terreus*

Treatment % (v/v)	Protein (mg/ml)	Protease activity (Units/ml)	Specific activity (Units /mg protein)
Control (c) 0.0	0.81	2.23	2.70
20	0.93	2.75	2.96
30	0.98	2.84	2.90
40	1.10	3.40	3.09
50	1.00	2.68	2.68

Table 4: Effect of beet molass on production of protease by *Aspergillus terreus*

Treatment % (v/v)	Protein (mg/ml)	Protease activity (Units/ml)	Specific activity (Units /mg protein)
Control (c) 0.0	0.83	2.24	2.71
20	0.85	2.52	2.96
30	0.68	2.80	4.11
40	0.72	2.45	3.40
50	0.75	2.59	3.45

Table 5: Effect of glutofeed on production of protease by *Aspergillus terreus*

Treatment % (v/v)	Protein (mg/ml)	Protease activity (Units/ml)	Specific activity (Units /mg protein)
Control (c) 0.0	0.82	2.23	2.70
0.2	0.52	1.19	2.29
0.4	0.61	1.23	2.02
0.6	0.64	1.94	3.03
0.8	0.74	2.76	3.73
1.0	0.78	2.88	3.69
1.2	0.72	2.32	3.22

Table 6: Effect of ragee elkon on production of protease by *Aspergillus terreus*

Treatment % (v/v)	Protein (mg/ml)	Protease activity (Units/ml)	Specific activity (Units /mg protein)
Control (c) 0.0	0.81	2.23	2.70
1.0	0.78	1.89	2.42
2.0	0.80	2.24	2.80
3.0	0.84	2.80	3.33
4.0	0.89	2.87	3.22
5.0	0.90	2.94	3.27
6.0	0.92	2.94	3.19
7.0	0.94	2.94	3.12

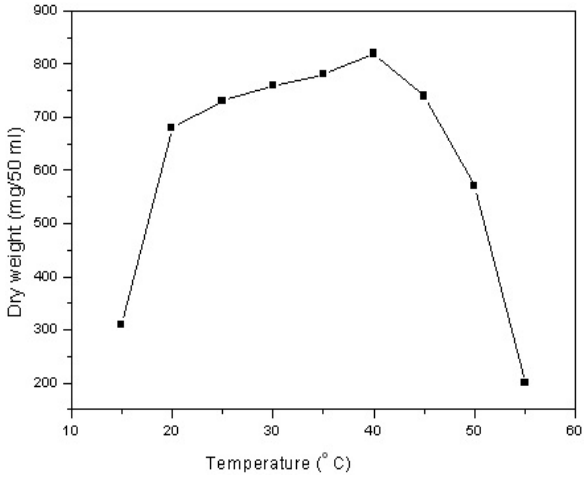

Fig. 1: Effect of culture temperature on mycelial growth of *Aspergillus terreus*

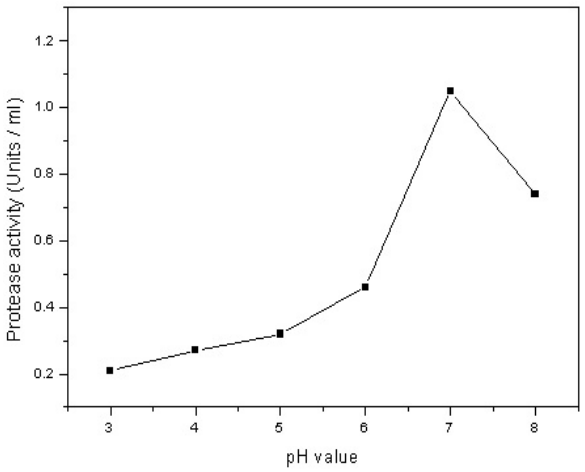

Fig. 3: Effect of pH value on the production of protease by *A. terreus.*

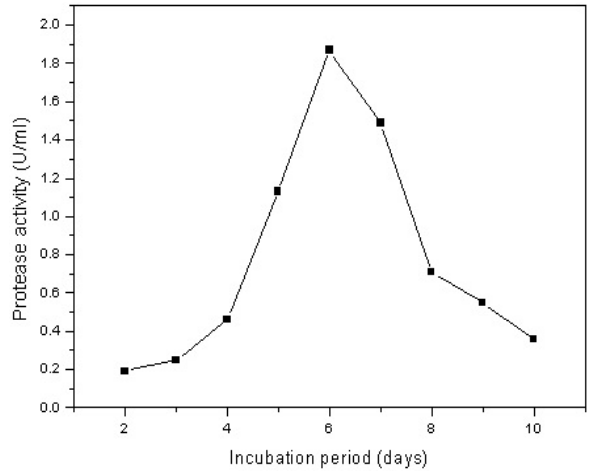

Fig. 2: Effect of incubation period on protease production of *Aspergillus terreus*

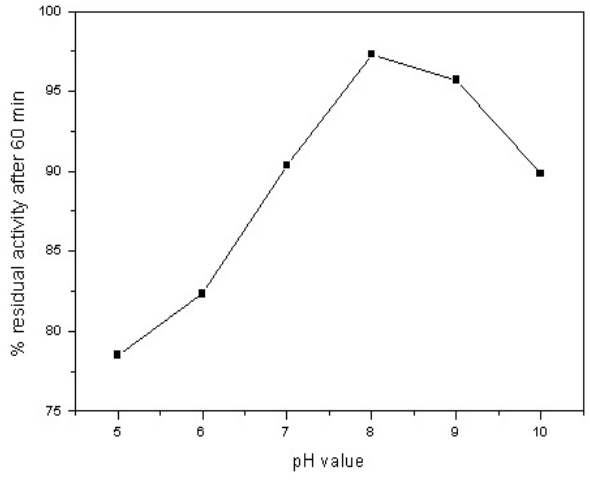

Fig. 4: pH stability of protease of *A. terreus.*

Thermal stability of the protease of *A. terreus*, was investigated by pre-incubating the enzyme solution at temperature range 40 – 90 ºC for 60 min. After which the enzyme was assayed. The optimum temperature was 40 ºC (Fig. 5).

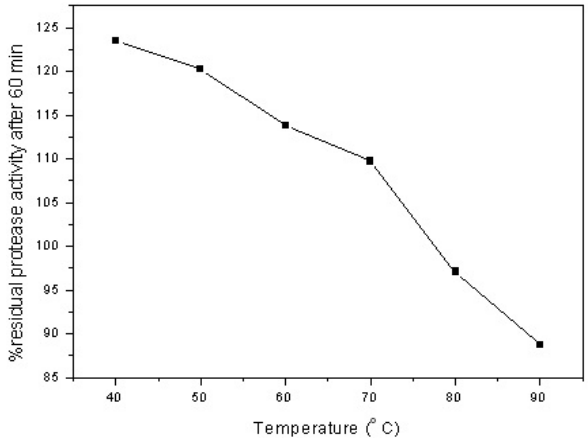

Fig. 5: Thermal stability of protease of *A. terreus*.

Some enzymes are suitable for detergent formulation designed for home laundry in Egypt using heated or tap water. Protease detergents remove blood, milk, sweat, greases from clothes. The proteases effectively breakdown the polypeptide chain in the protein into soluble peptides and amino acids that can be removed with wash[2]. Proteases described for enzyme detergents are fairly non-specific serine proteases[5,2715,]. The results Table (7) revealed that there is compatibility between protease produced by *A. terreus* and commercial detergents which can be arranged as follows: Tide, general, persil, hattric, savo and finally aerial, where the decrease in the enzyme activity ranged from 18.0% in tide to 47% in aerial.

Table 7: Compatibility of protease from *A. terreus* with certain commercial detergents.

Some Local	Temperature (ºC)	Residual activity (%) after 60 min
Savo	30	71.00
	60	70.00
	90	68.00
Hattric	30	81.80
	60	76.30
	90	71.40
Ariel	30	63.00
	60	56.00
	90	53.00
Persil	30	78.80
	60	77.00
	90	75.00
Tide	30	83.70
	60	83.00
	90	82.70
General	30	82.00
	60	81.00
	90	80.00

Conclusion: The previous properties of protease produced by *Aspergillus terreus* i.e pH and thermal stabilities and inexpensive production show that it is an alkaline protease and make it a good candidate for employing in detergent formulation.

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