International Journal of Advanced Biotechnology and Research(IJBR)
ISSN 0976-2612, Online ISSN 2278–599X,
Vol5, Issue4, 2014, pp790-794
http://www.bipublication.com

Synthesis and Characterization of Lipolytic Enzymes in Seed Deterioration by Seed Borne Fungi

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[Received 02/12/2014, Accepted-25/12/2014]

ABSTRACT:
Alternaria alternata, Aspergillus flavus, A. niger, F. oxysporum, Penicillium digitatum, the five dominant fungi were studied for lipase synthesis. These fungi synthesized lipase in Czapek medium supplemented with soybean powder and soybean oil separately. The fungi synthesized more enzymes in soybean powder supplemented medium over soybean oil supplemented medium. The enzyme production was affected by the pH and temperature. The optimum pH was found to be in the range of 5.0 to 6.5 and 45°C to 50 °C was optimum temperature.

Keywords: Fungi, soybean, lipase

[I] INTRODUCTION

The deterioration of grain in our country is approximately 10% of total production. Beside fungi, these losses can be attributed the unsanitary storage state and elevated dampness level of seeds or absorption of moisture during storage. These condition poses suitable conditions for deterioration of seed especially in pulses and the losses can be attributed to the enzyme synthetic ability of fungi associated. Seed-borne fungi cause losses in terms of seed quality and quantity in most of the crops.

The associated fungi also reduce the germination and storability of the seed. They are responsible for seed rot, seedling blight, root/stem rot, foliar infection as well as pod blight diseases [1], [2], [3].

Seeds of many pulses crop are known to harbour large amount of mycoflora. Seed borne microorganisms affect farm produce in the field as well as in storage. In several cases such mycoflora is found to affect adversely the seed germination, vigour and quality and quantity [4], [5].

Seed deterioration due to mycoflora is common feature leading to loss of viability and numerous fungi develop on stored seed [6].

Soybean seeds are rich in oil the deterioration of soybean seed may be related to ability to synthesis of lipase [7]. Lipases catalyze the hydrolysis of fats and mono-and di-glycerides to free fatty acids and glycerol. The synthesis of lipases is affected by many factors such as pH, temperature, carbon and nitrogen [8].

Many genera as *Penicillium, Rhizopus, Aspergillus* and *Fusarium* are known to synthesize lipases.

To investigate this aspect fungi associated with soybean was isolated and the dominant fungi were screened for the production of lipases.

[II] MATERIALS AND METHODS

2.1. Isolation of fungi

Untreated seeds were obtained from various sources – breeders, retailer, farmers etc. these seeds were assessed for presence of fungi using standard blotter method as recommended by International Seed Testing Association [9], [10],[11].

2.2. Culture and Lipase production medium

The associated fungi were maintained on Czapek medium-broth supplemented with oil and Triton X 100 instead of sucrose as carbon sources. Czapek medium-broth supplemented with 1% oil and Triton X 100 instead or 1% soybean powder was used as enzyme production medium. The pH was adjusted to 6.0 by adding dilute 0.1N HCl in all cases[12].

2.3. Preparation of enzymes

Fifty ml of medium was poured in 250 ml flasks and was inoculated with 0.5 ml of spore suspension of the fungi. The flasks were incubated for 27±2°C for 10 days. Flasks were drawn after regular time interval and the contents were filtered through Whatman No. 1.

The culture filtrate was collected, centrifuged at 5000 rpm for 15 minutes to remove spore and suspended matter. The filtrate was dialysed against running tap water for 24 hours. The partially purified solution was used as crude enzyme source and was stored at 4 °C for further use.

2.4 Measurement of Lipase Activity

The crude enzyme obtained was assayed for determination of its activity. titrimetric method with slight modifications [13] was used for the assay. The mixture of soybean oil and tween-80 (1%) was used as substrate.

10ml of substrate was taken in conical flask and 4ml of 0.1M sodium phosphate buffer (pH 7.0), 0.5ml of CaCl2, 1ml of enzyme was added. The total contents were incubated at 37˚C in water bath for 20 mins with frequent shaking for every 5-10min.

The reaction was terminated by addition of 20ml of acetone:ethanol mixture (1:1 v/v). The total contents were titrated against 0.1N sodium hydroxide (NaOH) using phenolphthalein as an indicator, until the appearance of pale pink colour as end point.

The enzyme activity was determined as One unit of enzyme was defined as the free acid released / unit time / gm of protein.

The lipase activity was carried out by estimating the free fatty acid content in blank and in the test after the incubation of oil substrate with enzyme by titrimetric method. The titrimetric method for determination of the enzyme activity yielded lipase activity of 2.74U/ml/min.

[III] RESULTS

The Fungi are known for their capacities to synthesize a variety of enzyme depending upon availability of substrate. The isolated fungi were maintained on seed meal agar and the most dominant were used for assessment of lipase production by these fungi. A series of experiments were undertaken to assess the ability of fungi to degrade oil present in the soybean seed by secretion of lipase.

Alternaria alternata, A. tenuis, Aspergillus flavus, A. niger, A. terreus, Chaetomium globosum, Curvularia lunata, Fusarium moniliforme, F. oxysporum, Macrophomina phaseolina, Penicillium citrinum, P. digitalis, Verticillium albo-atrum and few non sporulation forms were isolated form the seeds. Among them the most dominant in terms of their radial growth on the plate, they were selected for further studies. *Alternaria alternata, Aspergillus flavus, A. niger, F. oxysporum, Penicillium digitatum* were selected.

These selected fungi were grown on soybean powder containing medium. The 8 days old culture filtrate was used as crude enzyme source.

Table 1. Production of lipase enzyme by dominant fungi on seed powder medium

Age of culture filtrate(Days)	lipase activity (U/ml)				
	AA	*AF*	*AN*	*FO*	*PD*
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.02	0.00	0.00	0.00
3	0.00	0.04	0.02	0.03	0.00
4	0.02	0.06	0.05	0.05	0.02
5	0.03	0.08	0.06	0.06	0.04
6	0.05	0.11	0.11	0.10	0.07
7	0.9	0.14	0.12	0.11	0.10
8	0.11	0.17	0.13	0.12	0.12
9	0.11	0.17	0.13	0.12	0.12
10	0.11	0.17	0.13	0.12	0.12

AA:*Alternaria alternata*, ***AF:****Aspergillus flavus*, ***AN:****Aspergillus. niger*, ***FO:****Fusarium oxysporum*, ***PD****:Penicillium digitatum*

All the selected fungi synthesizes lipase in both the media. The synthesis increased with increase in time of incubation in both the media; however the amount of enzymes varied. Maximum enzymes were secreted in soybean powder medium (Table no. 1) followed by Czapek agar with soybean oil containing medium (Table no. 2). The quantity of enzyme secreted in soybean powder medium increased during the study period, the maximum amount was detected on 8th day. It was 0.11 U/ml by *Alternaria alternata*, 0.17 U/ml *Aspergillus flavus*, 0.13U/ml by *A. niger*, 0.12U/ml by *F. oxysporum* and *Penicillium digitatum.* In Czapek agar with soybean oil containing medium the synthesis of enzymes increased up to 7 days and thereafter it remained constant. The synthesis was 0.07 U/ml by *Alternaria alternata*, 0.10 U/ml *Aspergillus flavus* and *A. niger* 0.12U/ml by *F. oxysporum* and 0.11U/ml by *Penicillium digitatum.*

Table 2. Production of lipase enzyme by dominant fungi on Czapek agar with Soybean oil

Age of culture filtrate (Days)	lipase activity (U/ml)				
	AA	*AF*	*AN*	*FO*	*PD*
1	0.00	0.00	0.00	0.00	0.00
2	0.00	0.00	0.00	0.00	0.00
3	0.01	0.03	0.02	0.02	0.01
4	0.02	0.05	0.03	0.05	0.02
5	0.03	0.06	0.05	0.06	0.04
6	0.05	0.08	0.08	0.11	0.08
7	0.07	0.10	0.10	0.12	0.11
8	0.07	0.10	0.10	0.12	0.13
9	0.07	0.10	0.10	0.12	0.13
10	0.07	0.10	0.10	0.12	0.13

AA:*Alternaria alternata*, ***AF:****Aspergillus flavus*, ***AN:****Aspergillus. niger*, ***FO:****Fusarium oxysporum*, ***PD****:Penicillium digitatum*

In the study of effect of temperature and pH on the synthesis of lipase enzymes, it was found that 5.0 to 6.5 (Table no. 3) was optimum pH and 45°C to 50 °C (Table no. 4) was optimum temperature.

[IV] DISCUSSION

The assessment of soybean in storage for the quantities of total oil content present revealed that there was reduction in total oil content in soybean seeds. The dominant fungi associated were mostly internal fungi. This indicates the role of fungi in deteriorating total oil content and quality by secretion of lipase. Similar results were reported in case of lipase production in SSF using A. Niger, with the use of Wheat bran and soyabean meal are as substrates, 2.9 U/ml was the observed lipolytic activity[14].

Table 3. Effect of pH on production of lipase enzyme by dominant fungi soybean powder medium

pH	lipase activity (U/ml)				
	AA	*AF*	*AN*	*FO*	*PD*
3.5	0.09	0.08	0.10	0.09	0.08
4.0	0.10	0.09	0.11	0.10	0.09
4.5	0.11	0.11	0.12	0.11	0.11
5.0	0.12	0.12	0.13	0.12	0.12
5.5	0.12	0.17	0.14	0.12	0.13
6.0	0.12	0.18	0.14	0.12	0.13
6.5	0.10	0.18	0.14	0.10	0.11
7.0	0.08	0.08	0.12	0.08	0.08
7.5	0.05	0.05	0.10	0.05	0.05
8.0	0.02	0.03	0.05	0.02	0.03
8.5	0.03	0.00	0.02	0.03	0.00
9.0	0.00	0.00	0.00	0.00	0.00
9.5	0.00	0.00	0.00	0.00	0.00
10.0	0.00	0.00	0.00	0.00	0.00
10.5	0.00	0.00	0.00	0.00	0.00

AA:*Alternaria alternata*, ***AF:****Aspergillus flavus*, ***AN:****Aspergillus. niger*, ***FO:****Fusarium oxysporum*, ***PD****:Penicillium digitatum*

This indicates the lipolytic abilities of the dominant fungi associated with the soybean. The study of the dominant fungi for synthesis of lipase revealed that they were ardent producers of lipases. So the deterioration of seed can be attributed to the lipolytic ability of fungi. Similarly the decrease in total oil content indicates their utilization as substrate by these fungi.

Table 4. Effect of Temperature on production of lipase enzyme by dominant fungi soybean powder medium

Temp (°C)	lipase activity (U/ml)				
	AA	*AF*	*AN*	*FO*	*PD*
20	0.08	0.07	0.08	0.07	0.07
25	0.10	0.09	0.11	0.09	0.09
30	0.11	0.11	0.12	0.10	0.11
35	0.12	0.12	0.13	0.11	0.12
40	0.12	0.17	0.14	0.12	0.13
45	0.12	0.18	0.14	0.12	0.13
50	0.10	0.18	0.14	0.10	0.11
55	0.09	0.12	0.12	0.08	0.08
60	0.04	0.05	0.08	0.05	0.05
65	0.03	0.03	0.05	0.02	0.03

AA:*Alternaria alternata*, ***AF:****Aspergillus flavus*, ***AN:****Aspergillus. niger*, ***FO:****Fusarium oxysporum*, ***PD****:Penicillium digitatum*

The results indicates that *Alternaria alternata, Aspergillus flavus, A. niger, F. oxysporum, Penicillium digitatum* were found to play decisive role in altering nutritive value. The growth of these fungi caused significant deterioration in the quality of the seeds. This clearly suggests well equipped enzyme make up of these fungi to degrade and utilize any kind of storage chemical present in the seeds. However, the degree of enzyme production was found to be variable among the mycoflora. This may be related with their adaptation potential which might be different in these fungi. Similar type of reports regarding production of enzymes in seed borne pathogens has been reported in literature [15], [16], [17].

[V] CONCLUSION

Lipase enzyme was synthesized by *Alternaria alternata, Aspergillus flavus, A. niger, F. oxysporum, Penicillium digitatum*, the five dominant fungi isolated from soybean seeds. Parameters such as incubation period, temperature, pH were studied. The optimum pH was found to be in the range of 5.0 to 6.5 and 45°C to 50 °C was optimum temperature.

FINANCIAL DISCLOSURE:
Authors are grateful to UGC, New Delhi for financial support in the form of UGC major research project wide sanction no. 38-202/2009(SR) 24 December 2009

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