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ORIGINAL ARTICLE

Studies on the Mycoflora of Oil Cakes And characterization of Amylase

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ABSTRACT
The role of oil cakes in maintaining soil beneficial biota and suppressing harmful pathogens is also widely recognized in different soil as well as different ecosystems. Annual growth in oil cake production is projected to average 2.3% annually over the decade to 2010. Oil cakes especially non-edible in nature are more viable option to meet this demand significantly as edible oil cakes cannot be utilized for agriculture purposes . Utility of oil seed cakes are recognized in different spheres.In this studies concentrate the isolation and identification of mycoflora of Neem, Illupai, Coconut, Sesame and Groundnut oil cake and which biomass and amylase production characteristic features were carried out.
Keywords: Mycoflora, Amylase

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INTRODUCTION

Oil cakes are considered as suitable nutrient supplements for arable crops considering its rich nutrient composition. The use of different non edible oilcakes towards nutrient supplementation, pest management and other agricultural practices are well known from ancient times itself but its importance is more emphasized in recent times. There are several reports describing production of various enzymes using oil cakes as substrate in solid-state fermentation (SSF), or as supplement to the production medium. Oil cakes are ideally suited nutrient support in SSF rendering both carbon and nitrogen sources and reported to be good substrate for enzyme production using fungal species. The enzyme production could be further enhanced by optimisation of physiological and biological conditions. Lipase (Benjamin and Pandey, 1996), α-amylase (Ramachandran*et al.,* 2004a,b), phytase (Sabu*et al.,* 2002; Bogar*et al.,* 2003; Ramachandran*et al.,* 2005; Roopesh*et al.,* 2006), protease (Sandhya*et al.,* 2005; Sumantha*et al.,* 2005) and glutaminase (Kashyap*et al.,* 2002) are some of the enzymes produced using oil cakes as nutrient source.
Bacitracin biosynthesis was reported in SSF using media containing defatted oil cakes (SBC, SuOC) by *Bacillus licheniformis*(Farzana*et al.,* 2005).Two major strong antioxidants from fermented SOC were purified and identified as known sesaminoltriglucoside and sesaminoldiglucoside, however, their results also demonstrated that the fermentation process with *B. circulans*YUS-2 was highly effective to gain the extraction effciency of the sesaminolglucosides (Ohtsuki*et al.,* 2003).
In this studies isolation, identification, biomass production, the ability of α- amylase and effectiveness pH and UV an α – amylase Production of fungal organisms were carried out from the selected oil cakes such as neem, illupai, coconut, sesame and groundnut oil cakes.

MATERIALS AND METHODS

Isolation enzymatic microorganisms
Extracellular amylase producing fungal species grown well in oil bearing feeds and oil cakes which are rich in oils and fats. These enzymatic degrading fungal species growing on the oil cake of Neem, Illupai, Coconut, Sesame and Groundnut which were selected for the present study.

Isolation of fungal from oil cake

Oil cake (10g) was taken in a 250ml conical flask containing 100ml sterile distilled water. The flask was shaken on an electric shaker to get a homogenous suspension and serial dilutions of the soil sample which as 10^{-1}, 10^{-2} and 10^{-3} were prepared. One ml of 10-3 dilutions was plated in petridishes containing PDA medium. The pH of the medium was adjusted to 5.6. Streptomycin sulphate ($100mg^{-1}$) was added to the medium to prevent the bacterial growth. The plates were incubated at 25±2°C for five days and the fungi appearing on the medium were recorded.

$$\text{Population of fungi } g^{-1} = \frac{\text{Mean number of propagules in dilution plate}}{\text{Wt. of the dry soil}} \textbf{ X } \text{Dilution factor}$$

Identification Fungi

Mycelium of the fungi isolated were stained with Lactophenol cotton blue and then thoroughly washed with distilled water for the removal of additional staining. The slides were observed under the Nicon microscope. The fungi were identified by using standard manuals, such as Manual of soil fungi (Gillman, 1957), DematiaceousHyphomycetes (Ellis, 1971), More DematiaceousHyphomycetes (Ellis, 1976) and Hyphomycetes (Subramanian, 1971).

Enzymes studies

The amount of α-amylase was produced from dominated fungal strains,Which estimation from isolated Fungal Culture by Peter Bernfield (1955).

Determination of Amylase activity of isolated fungal cultures

Determination of Amylase activity of isolated fungal cultures were carried out by using following reagents: Sodium acetate buffer, 0.1 M; pH 4.7, Starch 1% solution, DinitroSalicyclic acid reagent (DNSA),40 % Rochelle salt solution and Maltose solution.

Effect of pH on activity The effect of pH on the enzymatic activity of protease was studied by measuring caseinolysis in buffers (0.2 M) with different pH. The buffers used were Tris-HCI (pH 7.5-9.0), carbonate-bicarbonate (9.0-10.5) and phosphate-NaOH (10.5-12.0). Reactions were carried out at 40°C for 10 min.

Effect of temperature on activity

The effect of temperature on the activity of proteases was studied in glycine-NaOH buffer 0.2 M (pH 10.0), using casein as substrate. Proteases was tested at different temperatures ranging from 30-70°C and 30-65" respectively. Reactions were carried out for 10 min.

Effect of inhibitors on activity

The effects of inhibitors on the activity of the enzymes were studied. The enzymes were incubated with various inhibitors (Sigma) at 1mm and 10mm concentrations for 15 min at 37°C, and the residual activities were determined. Activity of the controls not containing any inhibitors wasalso determined.

Effect of metal ions on activity

The effect of various metal ions on the enzyme activity was studied. The enzymes were incubated with various metal ion sources (1 mM) in 20 mMTris-HCI buffer (pH 9.0) at 37°C for 15 min and the enzyme activity was determined. The enzyme activity in buffer without any of these metal ion sources (control) was also determined.

Effect of pH on stability

pH stability of proteases was studied. The enzymes were incubated in buffers (0.2 M) with different pH, for 24 h at 25°C. The buffers used were acetate (pH 5 and 6), phosphate (pH 7 and8), glycine-NaOH (pH 9 and 10) and phosphate-NaOH (pH 11 and 12). Activities were determined before and after incubation. The percentage of activities remaining was calculated.

RESULTS

The neem oil cake spoiling fungal strains were isolated and identified, they were *Aspergillus flavus ,Aspergillus fumigatous, Asperfillusniger, Fusariumsps, Penicilliumsps, Trichodermaharzianum*and *Alternariaalaternata*(Table-1).

From Illupai oil cake were isolated and identified, *Aspergillus flavus , Aspergillus fumigatous, Asperfillus niger, Fusarium sps, Penicillium sps, Trichoderma harzianum* and *Alternaria alaternata* (Table-2).

The Coconut oil cake spoiling fungal strains were isolated and identified, they were *Aspergillus flavus ,Aspergillus fumigatous, Asperfillus niger, Fusariums ps, Penicillium sps, Trichoderma harzianum* and *Alternaria alaternata* (Table-3).

From Sesame oil cake were isolated and identified, they were *Aspergillusflavus , Aspergillus fumigatous, Asperfillusniger, Fusarium sps, Penicilliumsps, Trichoderma harzianum, Alternariaalaternata, Chactomiumglobosum, Curvularialunata, Dactylosporium macropus, Humicolafuscoatra* and *Mucormuced o*(Table-4).

From Groundnut oil cake were isolated and identified, *Aspergillus flavus , Aspergillus fumigatous, Asperfillus niger, Fusarium sps, Penicilliumsps, Trichodermaharzianum, Alternaria alaternata, Chactomium globosum, Curvularialunata, Dactylosporium macropus, Humicola fuscoatra, Mucormucedo, Torulaherbarum* and *Trichoderma viride* (Table-5).

Among the all organisms, the dominant fugal flora of oil cakes decaying fungal strains was selected for the production of amylase.

Effect of pH

To study the effect of pH on the production of amylase, protease and lipase were elucidated with different range viz., 3, 4, 5, 6, 7 and 9 were selected. The pH adjusted using appropriate buffer. Out of these pH, only 9 was inhibited the enzyme activity. These enzyme showed active range between at pH 3 – 4 (Table - 6)

Effect of UV radiation

Generally exposure time factor play a main role for the production of amylase, protease and lipase. Higher amount of these enzymes harvested from the lower radiation time but higher time gave minimum amount of enzymes. (Table - 7).

Table 1: Monthly variation in the population of soil fungi (number of colonies x 10^{-3} g^{-1} dry wt. of the soil) in the Neem oil cake

S. No	Name of the fungi	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Frequency (%)
1	*Aspergillusflavus*	8.5	-	6.9	6.0	5.5	6.2	4.8	-	-	37.9
2	*Aspergillusfumigatous*	3.9	4.8	8.2	3.6	5.4	6.8	3.9	-	4.8	41.2
3	*Aspergillusniger*	9.8	5.3	11.5	5.3	5.6	5.9	5.6	-	5.2	54.2
4	*Fusariumsps.*	4.3	4.6	-	-	4.3	9.2	7.7	-	6.8	36.9
5	*Penicilliumsps.*	3.7	4.7	-	7.7	-	-	6.8	5.5	-	28.4
6	*Trichodermaharzianum*	5.2	12.9	-	4.1	-	7.9	-	6.8	6.2	43.1
7	*Alternariaalaternata*	5.2	5.2	-	-	4.8	-	-	4.6	-	19.8

Table-2: Monthly variation in the population of soil fungi (number of colonies x 10^{-3} g^{-1} dry wt. of the soil) in the Illupai coil cake

S. No	Name of the fungi	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Frequency (%)
1	*Aspergillusflavus*	5.8	-	4.2	3.3	2.8	3.5	2.1	-	-	21.7
2	*Aspergillusfumigatous*	1.2	2.1	5.5	0.9	2.7	4.1	1.2	-	2.1	19.8
3	*Aspergillusniger*	7.1	2.6	8.8	2.6	2.9	3.2	2.9	-	2.5	32.6
4	*Fusariumsps.*	1.6	1.9	-	-	1.6	6.5	5.0	-	4.1	20.7
5	*Penicilliumsps.*	1.0	2.0	-	5.0	-	-	4.1	2.8	-	14.9
6	*Trichodermaharzianum*	2.5	10.2	-	1.4	-	5.2	-	4.1	3.5	26.9
7	*Alternariaalaternata*	2.5	2.5	-	-	2.1	-	-	1.9	-	9.0

Table 3: Monthly variation in the population of soil fungi (number of colonies x 10^{-3} g^{-1} dry wt. of the soil) in the coconut oil cake

S. No	Name of the fungi	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Frequency (%)
1	*Aspergillusflavus*	10.1	-	8.5	7.6	7.1	7.8	6.4	-	-	47.5
2	*Aspergillusfumigatous*	5.5	6.4	9.8	5.2	7.0	8.4	5.5	-	6.4	54.2
3	*Asperfillusniger*	11.4	6.9	13.1	6.9	7.2	7.5	7.2	-	6.8	67.0
4	*Fusariumsps.*	5.9	6.2	-	-	5.9	10.8	9.3	-	8.4	46.5
5	*Penicilliumsps.*	5.3	6.3	-	9.3	-	-	8.4	7.1	-	36.4
6	*Trichodermaharzianum*	6.8	14.5	-	5.7	-	9.5	-	9.4	7.8	53.7
7	*Alternariaalaternata*	6.8	6.8	-	-	6.4	-	-	6.2	-	26.2

Table-4: Monthly variation in the population of soil fungi (number of colonies x 10^{-3} g^{-1} dry wt. of the soil) in the Sesame oil cake

S. No	Name of the fungi	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Frequency %
1	*Aspergillusflavus*	5.8	-	4.2	3.3	2.8	3.5	2.1	-	-	21.7
2	*Aspergillusfumigatous*	1.2	2.1	5.5	0.9	2.7	4.1	1.2	-	2.1	19.8
3	*Asperfillusniger*	5.1	4.2	-	5.1	3.8	5.5	-	4.1	5.5	33.3
4	*Fusariumsps.*	3.2	2.7	3.1	-	2.7	4.1	2.1	-	-	17.9
5	*Penicilliumsps.*	2.5	2.1	-	1.9	-	2.7	1.8	1.5	1.1	13.6
6	*Trichodermaharzianum*	-	-	-	2.7	2.2	1.1	5.2	3.1	4.2	18.5
8	*Alternariaalaternata*	2.2	-	1.5	-	2.8	-	1.9	1.5	1.3	11.2
7	*Chactomiumglobosum*	3.1	3.2	2.2	2.1	1.8	1.5	-	-	-	13.9
9	*Curvularialunata*	2.8	2.7	2.1	-	2.9	-	-	-	1.5	12.0
10	*Dactylosporiummacropus*	3.8	2.2	-	1.1	-	-	1.0	-	-	8.1
11	*Drechslerarostrata*	1.2	-	1.8	-	1.6	1.3	-	-	1.1	7.0
12	*Leptostromaactaea*	1.3	-	1.3	-	-	1.3	-	-	1.2	5.1

Table-5: Monthly variation in the population of soil fungi (number of colonies x 10^{-3} g^{-1} dry wt. of the soil) in the groundnut oil cake

S. No	Name of the fungi	Oct	Nov	Dec	Jan	Feb	Mar	Apr	May	Jun	Frequency %
1	*Aspergillusflavus*	7.8	5.3	2.9	8.7	11.2	12.5	-	-	10.5	15.9
2	*Aspergillusfumigatous*	-	2.7	-	3.5	3.9	3.2	2.8	2.1	-	18.2
3	*Asperfillusniger*	3.1	2.8	-	2.7	3.5	4.1	-	2.8	2.5	20.9
4	*Fusariumsps.*	2.7	2.9	2.8	3.1	0.8	7.2	10.3	8.2	-	38.0
5	*Penicilliumsps.*	-	1.8	1.2	-	-	1.7	-	1.5	-	6.2
6	*Trichodermaharzianum*	-	-	-	-	2.7	2.2	1.5	1.3	1.2	8.9
8	*Alternariaalaternata*	1.8	1.6	-	-	1.9	2.5	-	-	-	7.8
7	*Chactomiumglobosum*	3.2	-	-	2.5	1.1	1.8	-	1.9	-	10.5
9	*Curvularialunata*	2.1	1.8	1.3	-	1.5	-	1.2	1.0	-	8.9
10	*Dactylosporiummacropus*	1.5	-	-	1.2	-	1.3	1.5	-	1.8	7.3
11	*Drechslerarostrata*	2.8	2.7	-	2.5	-	1.5	-	-	1.8	11.3
12	*Leptostromaactaea*	1.3	-	-	1.1	-	-	1.8	-	1.1	5.3
13	*Torulaherbarum*	1.3	-	-	1.1	-	-	-	1.2	-	3.6
14	*Trichodermaviride*	1.8	-	-	1.9	1.5	-	1.2	-	-	6.4

Table-6 : Dry weight and enzyme produced by four fungi

S.No	Name of the organisms	Day of incubation	pH	Dry weight (mg)	Amylase (µg/ml)
1	*Aspergillusniger*	7	3.5	186	10
		14	3.5	358	12
		21	3.5	212	8
2	*Aspergillusflavus*	7	4.0	150	11
		14	4.0	375	14
		21	4.0	197	9
3	*Mucorsps*	7	4.0	186	8
		14	4.0	385	12
		21	4.0	167	9

Table-7: Effect of UV radiation on dry weight and enzyme produced by four fungi

S.No	Name of the organisms	Day of incubation	Exposure Minute	pH	Dry weight(mg)	Amylase (µg/ml)
1	*Aspergillusniger*	14	0	3.5	298	8
			5	3.5	175	6
			10	3.5	249	13
			15	3.5	336	14
			20	3.5	241	8
			25	3.5	132	4
			30	4.0	81	2
2	*AspergillusFlavus*	14	0	4.0	170	11
			5	4.0	141	7
			10	4.0	212	27
			15	4.0	295	22
			20	4.0	165	14
			25	4.0	118	7
			30	4.0	92	2
3	*Mucorsps*	14	0	4.0	76	13
			5	4.0	168	8
			10	4.0	243	21
			15	4.0	389	25
			20	4.0	265	16
			25	4.0	142	9
			30	4.0	112	3

DISCUSSION

Oil cakes are rich in fiber, protein and energy contents. They offer potential benefits when used as substrates in developing bioprocesses for the production of organic chemicals and biomolecules. Studies using them for the production of industrial enzymes have shown promising results. Mixed substrate fermentation has been more advantageous for such applications. While edible oil cakes are used as feed source and protein hydrolysate, some of the non-edible cakes find its application as biocontrol agents. Also, use of oil cakes offers good alternative to traditional applications by their exploitation in the production of environmentally friendly green bio fuel. Another key point to be noted is that the bioprocess utilising oil cakes is attractive due to relatively cheaper vailability of the oil cakes throughout the year, making it even more favourable when economics is considered. Fungi produce a vast array of enzymes covering the whole groundnut of catalytic ability.

Aspergillus sps., *Penicillium roqueforti, Rhizopusdelemar, Candida rugosa, Candida cylindriacea, Mucorsps, Rhizomucormiehei* are the chief contaminating microbes from oil cakes. A survey of commercial enzymes revealed that *Aspergillus* were highly selective for short chain acids and alcohols (Pandey*et al*., 1999). In this study, the dominant fugal flora of Neem, Illupai, Coconut, Sesame and Groundnut cakes decaying fungal strains were selected for the production of amylase.

In this study, we selected oil cakes Neem, Illupai, Coconut, Sesame and Groundnut containing fungal strains were *Aspergillus flavus ,Aspergillus fumigatous, Asperfillusniger, Fusariumsps, Penicilliumsps, Trichoderma harzianum, Alternaria alaternata, Chactomium globosum, Curvularia lunata, Dactylosporiummacropus, Humicolafuscoatra, Mucormucedo, Torulaherbarum* and *Trichodermaviride*

SUMMARY

The isolated and identified fungal species from five types of oil cakes they were*Aspergillus flavus ,Aspergillus fumigatous, Asperfillusniger, Fusarium sps, Penicillium sps, Trichodermaharzianum, Alternariaalaternata, Chactomium globosum, Curvularialunata, Dactylosporiumma cropus, Humicola fuscoatra, Mucormucedo, Torulaherbarum* and *Trichodermaviride*

Among the dominant three species such as *Aspergillus niger, Aspergillus flavus* and Mucorsps they were used to produce amylase. The effect of pH and UV radiation play a pivotal role in the extraction of enzymes.

REFERENCES

1. Benjamin, S. and Pandey, A., 1996.Lipase production by *C. rugosa*on copra waste extract.*Indian J. Microbiol.*,**45** : 452 - 456.
2. Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Francis, F., Nagy, V., Szakacs, G. and Pandey, A., 2004a. Coconut oil cake – a potential raw material for the production of a-amylase.Bioresour.*Technol.*, **93 :**169 - 174.

3. Ramachandran, S., Patel, A.K., Nampoothiri, K.M., Chandran, S., Szakacs, G., Soccol, C.R. and Pandey, A., 2004b.Alpha amylase from a fungal culture grown on oil cakes and its properties. Brazilian Arch. *Biol. Technol*., **47 :** 309 - 317.
4. Sabu, A., Sarita, S., Pandey, A., Bogar, B., Szakacs, G. and Soccol, C.R., 2002. Solid-state fermentation for production of phytase by *Rhizopusoligosporus*.. Appl. Biochem. Biotech. - Part A, Enzyme Eng. *Biotechnol.,* pp. 251-260.
5. Bogar, B., Szakacs, G., Pandey, A., Sabu, A., Linden, J.C. and Tengerdy, R.P., 2003. Production of phytase by *Mucorracemosus*in solid-state fermentation.Biotechnol.Progr.**19** : 312 - 319.
6. Ramachandran, S., Roopesh, K., Nampoothiri, K.M., Szakacs, G. and Pandey, A., 2005.Mixed substrate fermentation for the production of phytase by *Rhizopus*spp. using oilcakes as substrates.*Process Biochem*.,**40 :** 1749 - 1754.
7. Roopesh, K., Ramachandran, S., Nampoothiri, K.M., Szakacs, G. and Pandey, A., 2006. Comparison of phytase production on wheat bran and oilcakes in solid-state fermentation by *Mucorracemosus.Bioresour.Technol.*.**97:** 506 - 511.
8. Sandhya, C., Sumantha, A., Szakacs, G. and Pandey, A., 2005. Comparative evaluation of neutral protease production by *Aspergillusoryzae*in submerged and solid-state fermentation.*Process Biochem*., **40 :** 2689 - 2694.
9. Sumantha, A., Sandhya, C., Szakacs, G., Soccol, C.R. and Pandey, A., 2005. Production and partial purification of a neutral metalloprotease by fungal mixed substrate fermentation. Food Technol*. Biotechnol.,***43** : 313 - 319.
10. Kashyap, P., Sabu, A., Pandey, A., Szakacs, G. and Soccol, C.R., 2002. Extracellular L-glutaminase production by *Zygosaccharomycesrouxii*under solid-state fermentation.Process Biochem.**38** : 307 - 312.
11. Farzana, K., Shah, S.N., Butt, F.B. and Awan, S.B., 2005. Biosynthesis of bacitracin in solid-state fermentation by *Bacillus licheniformis*using defatted oil seed cakes as substrate.*Pakistan J. Pharm. Sci*, **18** : 55 - 57.
12. Ohtsuki, T., Akiyama, J., Shimoyama, T., Yazaki, S.I., Ui, S., Hirose, Y. and Mimura, A., 2003. Increased production of antioxidativesesaminolglucosides from sesame oil cake through fermentation by *Bacillus circulans*strain YUS-2. 2003. Biosci. Biotechnol.Biochem.**67** : 2304 - 2306.
13. Gillman, S.C., 1957. A manual of soil fungi, Revised 2nd edition, Oxford and IBH Publishing Company (Indian reprint), Calcutta, Bombay, New Delhi.
14. Ellis, M.B., 1971. Dematiaceoushyphomycetes.Commonwealth Mycological Institute, Kew, U.K.
15. Ellis, M.B., 1976. More DematiaceousHyphomycetes. CAB International Mycological Institute, Kew, UK. 507 pp.
16. Subramanian,C.V.,1971.HyphomycetesAnaccout of Indian species except Cercosporae. ICAR, New Delhi.
17. Bernfeld, P., 1955. Amylaseαand β, In Methods in Enzymology (Colowick SP, Kaplan NO, ed.), Academic Press Inc, New York **1:** 149-158.
18. Pandey,A., Benjamin, S.,Soccol, C.R., Nigam, P., Krieger, N. and Soccol, V.T., 1999. Therealm of microbial lipase in biotechnology, *Biotechnol.Appl. Biochem*.,**29** :119-131.

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