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Lipase Activity of Two Seed-Borne Fungi of Sesamum (*Sesamum indicum* LINN.)

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ABSTRACT. *Macrophomina phaseolina* and *Phoma nebulosa* secreted lipase which varied with the medium. *M. phaseolina* produced more lipase than *P. nebulosa*. Sesamum seed meal caused stimulation of lipase production. The lipase secreted by both organisms showed maximum activity at pH 5.0. The optimum temperature for activity of lipase secreted by *M. phaseolina* was found to be 40 °C, while for that of *P. nebulosa* it was 30 °C.

Microbial lipases hydrolyze triglycerides to fatty acids and glycerol which can easily be assimilated. Studies of fungal lipases are comparatively recent (Sridhar and Ou 1973; Benzonana 1974; Jensen 1974; Kinesella and Hwang 1974; Arends *et al.* 1975; Sharma and Chauhan 1976; Das and Benerjee 1979). Ksandopulo (1974) and Chandra *et al.* (1980) studied the effect of different fats on lipase activity of some of the fungi. The lipolytic microorganisms play an important role in reducing total seed oil and increasing free fatty acids (Lalithakumari *et al.* 1971*a*,*b*; Rancadori *et al.* 1971). During an extensive survey for mycoflora associated with sesamum (*Sesamum indicum* LINN.) seeds *Macrophomina phaseolina* and *Phoma nebulosa* were found to cause serious damage to sesamum seeds which is one of the richest source of oil (50 %). Hence, an attempt was made to assay the lipase-producing capacity which may throw some light on their biology.

MATERIALS AND METHODS

Macrophomina phaseolina (TASSI) GOID and *Phoma nebulosa* (PERS. *ex* S.M. GRAY) Berk isolated from surface-disinfected sesamum seeds were employed. The lipase activity of these fungi was assayed on synthetic liquid medium A (peptone 20 g, glucose 10 g, yeast extract 5 g, sodium chloride 5 g in 1 L distilled water) with different additions, thus groundnut seed meal for B, castor seed meal for C, and sesamum seed meal for D. Seed meal was prepared by grinding the seeds at 0.5 % level. The medium pH was adjusted to 5.0 with 0.1 M HCl. Twenty-five mL of the medium was dispersed in 100-mL Erlenmeyer conical flasks and sterilized at 103 kPa for 30 min. Flasks were inoculated with 7-d-old cultures of *M. phaseolina* and *P. nebulosa* and incubated at room temperature (27 ± 2 °C) for 15 d. The lipase activity was assayed after 5, 10 and 15 d of incubation. The cultures were

TABLE I. Lipase activity of two seed-borne fungi on different media

Medium[a]	Days of incubation	*M. phaseolina*			*P. nebulosa*		
		pH	dry mass[b]	lipase[c]	pH	dry mass	lipase
A	5	5.5	27.0	5	7.0	29.6	9
	10	7.5	18.0	13	7.5	26.0	14
	15	8.2	14.7	20	8.0	16.5	16
B	5	7.5	26.5	12	5.5	20.0	5
	10	8.0	28.0	20	7.0	26.5	8
	15	8.0	29.8	35	8.0	21.0	10
C	5	5.2	20.5	5	7.5	30.8	14
	10	7.0	26.0	10	8.0	20.5	9
	15	8.0	19.8	25	8.0	17.4	7
D	5	7.0	30.0	20	6.5	23.6	8
	10	7.5	21.5	25	7.5	21.5	10
	15	7.5	19.0	40	8.0	18.0	12

[a] See *Materials and Methods*.
[b] Mycelial growth per 2 mL of culture broth.
[c] Activity expressed in units per 2 mL of culture broth (1 unit corresponds to 0.1 mL 50 mM NaOH being required to neutralize free fatty acids liberated in 3 h).

harvested on dried and weighed Whatman filter paper No. 42 and dried a-60—70 °C for 2 d to determine the biomass of the fungus. The culture filtrate thus obtained was centrifuged at 1800 ***g*** for 15 min and dialyzed over night and served as an enzyme.

Lipase activity was determined by the method suggested by Urs *et al.* (1962) with some modifications. The reaction mixture consisted of 2 mL triacetin, 2 mL enzyme solution, 5 mL citrate—phosphate buffer (pH 8.0) and 1 mL toluene, and incubated at room temperature for 3 h. The reaction was terminated with 25 mL of absolute ethanol and titrated against 50 mM NaOH using 1 % ethanolic phenolphthaleine as indicator. The activity was calculated from the difference between the control and experimental titre value expressed in units. 0.1 mL of 50 mM NaOH required was considered as 1 unit of enzyme activity.

An attempt was made to characterize the enzyme by studying the influence of enzyme concentration, substrate concentration, pH changes and temperature of reaction mixture on enzyme activity.

RESULTS AND DISCUSSION

The lipase production increased with the increase in age of the fungus (Table I). The maximum lipase activity was noted after 15 d of incubation in both fungi. *M. phaseolina* was more efficient in lipase production.

Groundnut seed meal supplementation resulted in a stimulation of lipase production with *M. phaseolina*, while it inhibited *P. nebulosa*. On the other hand, castor meal medium inhibited the production of lipase by *M. phaseolina*. The unfavourable action of castor seed meal may be due to the presence there of the highly unsaturated ricinoleic acid. *M. phaseolina* proproduced maximum lipase in sesamum seed meal supplemented medium, suggesting its suitability for growth and perpetuation of the fungus. On the

TABLE II. Effect of enzyme concentration, substrate concentration, pH and temperature on lipase activity of two seed-borne fungi

Quantity		*M. phaseolina*	*P. nebulosa*
Enzyme concentration (in mL)	0.5	5	5
	1	10	6
	2	19	15
	3	21	24
	4	28	32
Substrate concentration (in mL)	1	14	12
	2	20	15
	3	13	18
	4	9	19
	5	9	20
pH	4	28	31
	5	30	35
	6	22	20
	7	20	17
	8	19	15
	9	14	13
	10	11	12
Temperature (°)	20	10	13
	25	15	14
	30	20	18
	35	25	15
	40	28	13

other hand, *P. nebulosa* was inhibited by sesamum seed meal. Stimulation of lipase production by *Rhizopus deimar* was also noted by Iwai and Tsujisak (1974). On the other hand, Eitenmiller *et al.* (1970) and Chandra *et al.* (1980) working with *Penicillium roqueforte* and *Aspergillus wintii* respectively noted inhibition of lipase production in the presence of lipids.

Growth of the fungi was not different on different media. The pH of the media was found to shift to the alkaline side. There was no correlation between vegetative growth and lipase production.

The lipase activity of both the organisms increased with the increase in enzyme concentration. Similarly with the increase in substrate concentration, an increase in lipase activity was noted with the *P. nebulosa* secreted enzyme whereas lipase of *M. phaseolina* showed an increased activity only up to 2 mL substrate concentration which decreased at higher concentrations. This may be due to substrate repression.

Lipase secreted by the present fungi was most active at pH 5.0. Similarly, Chopra *et al.* (1981) noted maximum lipase activity at pH 5.0 secreted by *Mucor racemosus*. Increased activity of lipase secreted by *M. phaseolina* was noted with an increase in temperature up to 40 °C. The optimum temperature for lipase activity of *P. nebulosa* was found to be 30 °C. The higher temperature optimum of lipase secreted by *M. phaseolina* may represent an ecological advantage.

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