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ISOLATION AND IDENTIFICATION OF MUSTARD SEED CAKE DETOXIFYING MICROORGANISMS*

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Abstract. Microorganisms capable of growing on mustard seed cake were isolated from air and identified. Three strains of mold (*A. niger*, *A. flavus and Mucor mucedo*) and two of bacteria (*Bacillus subtilis* and *Bacillus subtilis* var *niger*) grew well when mustard seed cake was the only source of carbon. *Aspergillus flavus* also hydrolysed Sinigrin (a toxic substance present in the cake).

Pakistan is among the major producers of rape and mustards eeds being the third in the wolrd. The cake left after extraction of oil contains up to 44.1% crude protein[1] and has good amino acid make up; but is not consumed by monogastric animals because it has a thioglucoside (Sinigrin). Several Chemical and enzymic methods[2–8] of elminating sulphur compound have been reported. Staron[9] studied detoxification of rapeseed with various microorganisms (bacteria, yeast, fungi). It was reported that six of those gave satisfactory results. Microorganisms capable of utilizing mustard-seed cake as the sole source of carbon were isolated, identified and used for detoxification of mustard-seed cake at PCSIR Laboratories, Lahore.

Material and Methods

Isolation of Microorganisms. Petri dishes containing Sauton agar media in which mustard-seed cake was the sole source of carbon were exposed to air and then incubated for 3–5 days at $30° \pm 0.5°C$. The colonies which appeared on the plates, were picked up and then subcultured on the slants of the above medium. The growth from the slants was transferred again to petri dishes, by streaking method, for purification of the cultures. These strains were then subjected to identification and taxonomical studies.

The microorganisms were also propagated on Sauton medium in which Sinigrin (0.7 g/litre) was the only source of carbon.

Composition of the Medium. (a) Mineral solution at pH 6.8: KH_2PO_4, 1.0 g: $MgSO_4.7H_2O$, 0.5 g; KCl, 0.2 g; $CaCl_2$, 0.2 g; $FeSO_4.7H_2O$, 0.03 g; $ZnSO_4.7H_2O$, 0.01 g and $CuSO_4.5H_2O$, 2.0 mg, made up with distilled water to 1000 ml, (b) mustard-seed cake 35 g/l and (c) urea 4.0 g/l.

For identification work, the molds and bacterial cultures were subcultured on Czapek agar and nutrient agar medium respectively.

Cultural and Microscopic Characteristics of Aspergillus niger *Culture No. NRRL* A-22, 121.

Medium. Czapek agar.

Age. Five days.

Colony characteristics (incl. dia., character of growth, coloration, extent of sporulation, etc.). Dia 4–5 cm, basal mycelium white, moderately, compact, sporulating areas black. Conidiophores abundance, except for the margin of the colonies.

Conidial State

Head. Quantity Present: Abundance, borne on long conidiophores, black, round, size min 500, mean 550, and max 600 μ.

Vesicle. Size: Average dia 70, min 65, and max 77 μ. Portion covered by sterigmate.

Sterigmata. Primary size: 35×45 by 7.5–10.5 μ; secondary size 8–12 by 7–8 μ.

Conidia. Form: Globose, hyaline, warted spore bodies. Size: Average dia 4–5, min 6, and max 8 μ.

Stalk. Average 5.0, max 6.0 mm. Dia below heads 30–40 μ, colour hyaline.

Cultural and Microscopic Characteristics of Aspergillus flavus *Culture No. NRRL* A-22, 122.

Medium. Czapek agar

Age. Five days.

Colony characteristics (incl. dia, character of growth, coloration, extent of sporulation, etc.). Dia 4–5 cm, basal mycelium white moderately, compact, sporulating areas green. Conidiophores abundance, except, for the margin of the colonies.

Conidial Stage

Head. Quantity Present: Abundance, borne on long conidiophors, green round, min 130, mean 150, and max 170 μ.

Vesicle. Average dia 25, min 30, and max 35 μ. Portion covered by sterigmata. Sterigmata primary size 7–10×3.5-4 μ.

Conidia. Subglobose, spinulose, average dia 5, min 4.5 and max 6 μ.

Stalk. Average 3.0 and max 3.4 mm. Dia below head 25–40 μ, hyaline.

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Cultural and Microscopic Charcteristics of Mucor mucedo *Culture No. NRRL* A-22, 123.

Medium. Czapek agar.
Age. Four days.

Temperature. 30°C.

Colony. Height, filling the petri dish; rate of growth fast.

Colour. Colony Colour: White becomes black after sporulation; reverse colour white.

Sporangiophores. Origin, from the hyphae; colour white; marking and septations: coenocytic, erect, unbranched; height, and width: 5–6 mm, rhizoids and stolons, no rhizoids and stolons.

Sporangia. Colour including colour changes in the maturing of the sporangium: white changing to black in the maturing of the sporangia; deliquesing, round 100–200, single celled, abundance, erect.

Columellae. Dome shaped, white collerette; size 50–80 μ.

Sporangiospores. Elliptical, smooth, hyaline; size 6–12 long × 3–6 μ wide.

Chlamydospores Oidia.

Zygospores. Markings: Warts, spherical, black; position in colony, scattered, heterothallic, 90–200 μ in dia.

Results and Discussion

Taxonomy of Molds.[10,11] Taxonomical studies of the pure cultures were based on microscopic examination and the cultural characteristics. The results are as under:

Three strains were isolated from Czapek agar medium. These closely resembled with the characteristics of *Aspergillus niger*, *Aspergillus flavus*, and *Mucor mucedo* strains.

Six strains of bacteria were subjected to microscopic examination and biochemical tests for identification according to the diagonostic Table 1.[12,31]

Biochemical Tests

Morphology. Small thin homogeneous bacilli measuring 1 × 3 μ in 24-hr agar cultures. Somewhat thickend and longer on glucose agar.

Motility. Sluggishly motile in young cultures.

Staining Properties. Gram positive.

Spore Formation. Spores are formed early appearing within 24 hr on plain and glucose agar.

Agar Slant. Luxurient growth, membranous growth along line of inoculation later spreading out over entire surface of agar. The surface is usually dry and hard. It becomes soft and smeary.

Agar Stab. No liquefaction, membranous growth on the surface of the agar.

Agar Colonies. Rough growth with wrinkles.

Broth. Single isolation pellicles appear on the surface in 24 hr. Medium becomes turbid in first 24 hr but later clears. Scum is precipitated as a whole in about 10 days. This scum formation is a characteristic of *B. subtilis*

The details of all the tests are reported in the Table 1.

Aspergillus flavus could hydrolyse Sinigrin whereas others gave negative results (Table 2).

TABLE 1. IDENTIFICATION OF BACTERIA.

Locally isolated microorganisms	Morphology	Motility	Staining properties	Spore formation	Pellicle formation	Glucose	Arabinose	Mannitol	Starch	VP test	Urease test	Kosers citrate test	Catalase test	Growth at 65°C
Bacillus subtilis 1–5	1 × 3 μ elliptical single or chains	Motile	Gram +ve	Spore former	+	–	–	$+^{4}$	+	+	$+^{2}$	+	+	–
Bacillus subtilis var *niger** 6·5	1 × 3 μ mostly single or in small chains	″	Gram +ve	″	+	–	–	$+^{4}$	+	+	$+^{3}$	+	+	–

* Medium having tyrosine becomes black due to the growth of this bacteria.

Table 2. The Hydrolytic Effect of Different Microorganisms on Sample of the Pure Thioglucoside[1] (Sinigrin).

Microorganism	Growth on Sauton medium (Sinigrin was the only carbon source)	Allyl isothiocyanate
Control	—	—
Bacillus subtilis var *niger*	—	—
Aspergillus niger	—	—
Aspergillus flavus	+	+
Mucor mucedo	—	—

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