

RESULTS

Sl. No.	Carbonyl compound	Molecular formula of hydrazones	Melting point (°C)	% Chlorine	
				Found	Required
1.	Acetone	.. $C_{11}H_{12}O_4N_3Cl$	288 (Decomp.)	12.56	12.43
2.	Acetophenone	.. $C_{16}H_{14}O_4N_3Cl$	290 (Decomp.)	10.65	10.21
3.	Ethyl Methyl Ketone	.. $C_{13}H_{14}O_4N_3Cl$	284 (Decomp.)	11.65	11.85
4.	Salicylaldehyde	.. $C_{15}H_{12}O_5N_3Cl$	286 (Decomp.)	9.51	10.15
5.	Acetaldehyde	.. $C_{10}H_{10}O_4N_3Cl$	282 (Decomp.)	13.59	13.07
6.	<i>p</i> -Dimethyl amino benzaldehyde	.. $C_{17}H_{17}O_5N_4Cl$	272 (Decomp.)	8.89	9.42

For preparing the hydrazones, the hydrazide (0.4 gm) was dissolved in ethyl alcohol (70 ml) with gentle warming. The carbonyl compound (0.4 gm) was added to the warm solution of the hydrazide and refluxed for 30 minutes, on a hot water-bath. On cooling to 0°C, coloured crystalline hydrazones were obtained, these were filtered at the pump, washed with small quantity of alcohol and recrystallised from either methanol or ethanol.

Formaldehyde did not react with methyl 2-methoxy-3-nitro-5-chlorobenzohydrazide to give hydrazones. A drop of hydrochloric acid was also added to the reaction mixture to catalyse the hydrazone formation but with no avail.

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ISOLATION OF THERMOPHILIC FUNGI FROM SOILS*

IMPORTANCE of temperature as one of the environmental factors affecting the microflora of a habitat qualitatively and quantitatively has been well documented^{2,4,7}. Although its direct influence on the prevalence of fungi has been worked out, its usage as a technique indirectly to isolate thermophilic and thermotolerant fungi has been confined to a few habitats such as composts, bird's nests and other self-heating materials^{1,5,6,8-10,11}. The present report deals with the isolation of such fungi from rice-cultivated soils around Madras.

Soil samples from two rice-fields were analysed for the flora using dilution and soil plate methods. Poured plates were kept in desiccators and incubated at 45°, 55° and 60°C. Three replicates were maintained for each sample and the fungal counts made on the seventh day. Martin's rose Bengal agar and Emerson's Yeast extract-phosphate-starch-sulphate (YpSs) agar were used in this study. Pure cultures were maintained and the fungi identified according to Cooney and Emerson³.

Table I shows the species of thermophilic fungi isolated from the soils by the two techniques. Of the ten species isolated, dilution plate method registered six species while soil plate nine, thereby showing the efficiency of the latter. Also, *Aspergillus fumigatus* has consistently been isolated from both the soil samples, irrespective of the increase in the incubation temperature. Only one species, viz., *Torula thermophila* was isolated by soil-plate method at the incubation temperature of 55°C whereas others occurred at 45°C. No fungus was recorded at 60°C.

Thermophilism, initiated by Mische⁹, has been studied by different investigators in detail to understand mycofloras of diversified habitats. Maheswari⁸,

TABLE I
Thermophilic fungi isolated from soils

Fungi	Techniques	
	Dilution plate	Soil plate
<i>Phycomycetes</i> :		
<i>Mucor pusillus</i> Lindt.	—	+
<i>Ascomycetes</i> :		
<i>Thermoascus aurantiacus</i> Miehe	+	+
<i>Chaetomium thermophile</i> var. <i>thermophile</i> La Touche	—	+
<i>C. thermophile</i> var. <i>dissitum</i> La Touche	+	+
<i>Deuteromycetes</i> :		
<i>Aspergillus fumigatus</i> Fres.	+	+
<i>Humicola grisea</i> var. <i>thermoidea</i> Traaen	+	+
<i>H. insolens</i> Cooney and Emerson	—	+
<i>H. lanuginosa</i> (Griffin and Maublanc) Bunce	+	—
<i>Torula thermophila</i> Cooney and Emerson	—	+
<i>Malbranchea pulchella</i> var. <i>sulphurea</i> Miehe	+	+
Total	6	9

while working with composts, dung and other sources for isolating thermophilic fungi emphasized that work on different soil samples would greatly enhance the validity of this indirect method of isolating fungi that are dependent upon a specific environmental factor, i.e., temperature. Present work has indeed shown that this selective method is useful in isolating fungi quite varied from the normal flora isolated by incubation at room temperature¹¹, suggesting that this method could, by itself, be used as a technique to isolate fungi of specific group from soils that aid in the decomposition of cellulose and other organic matter in soil along with other common cellulolytic fungi.

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NITROGEN FIXATION BY A SALT AND ALKALI TOLERANT STRAIN OF *AZOTOBACTER CHROOCOCCUM*

In earlier studies on asymbiotic nitrogen fixation in calcareous soil of Pusa (North-Bihar), a positive significant correlation between *Azotobacter* population and the amount of nitrogen fixed in different types of soils was observed (Rai *et al.*, 1975)¹. Soil properties including soluble salts were also found to influence microbial population in these soils (Rai *et al.*, 1972-73)². Moreover, the *Azotobacter* population in calcareous saline-alkali soils of North-Bihar, covering several districts has been found to be relatively depressed (Anon., 1975)¹.

For augmenting soil nitrogen in saline and alkali soils, a strain of *Azotobacter chroococcum* growing at pH 9-0 with 0.2% salt (NaCl) was isolated from calcareous saline-alkali soil of Pusa Farm, by enrichment technique. Its nitrogen fixing capacity was determined in Ashby's mannite solution at different pH with and without added salt (Table I). The culture was tested for enrichment of N in soils of varying characteristics (Table II) and the gain of N fixed was determined.

A perusal of the data indicated that the culture was effective during a wide pH range (6.2 to 8.8). It was capable of tolerating salinity ranging from 35 to 195 mg/100 g soil. Similarly it flourished