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Studies on Thermoascus aurantiacus Miehe

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Thermoascus aurantiacus has been shown to be widely distributed in India on various substrates. Its growth characters at three different temperatures and five pH values have been studied. Its carbon and nitrogen requirements have been investigated at two different temperatures. Preliminary investigations showed that none of the isolates included in the present study are pathogenic.

INTRODUCTION

The genus *Thermoascus* was created in 1907 by Miehe with *T. aurantiacus* Miehe as the type species. It has been seldom reperted and „it too appears to be the rarer of thermophiles" (Cooney, Emerson 1964). Its general rarity was also emphasized by Crisan (1959) and Pore and Larsh (1967). Only in a few instances the fungus was reported and in almost all of them it was isolated at 45°C. According to Cooney and Emerson (1964) it has a maximum growth temperature of 55°C. Physiological characters of this fungus remained largely unknown. Earlier investigations by Noack (1912, 1920) on the physiology of this fungus were aimed at understanding the basis of thermophily. Tendler et al. (1967) studies the interaction of temperature and nutritional environment on the production of exoenzymes and antibiotics complex in an unidentified species of *Thermoascus* and *Humicola*.

During the course of investigations on thermophilic fungi *T. aurantiacus* has been repeatedly isolated from various substrates colected from different parts in India (Table 1). Its morphological characters, nutritional requirements, effect of pH and temperature on growth and sporulation have been investigated. Preliminary investigations on the pathogenicity of the isolates are also presented.

Table 1

Isolation of *Thermoascus aurantiacus* at 60°C from various habitats in India

Habitat	Place	Number of strains isolated
1. Bat droppings	Vijayawada	2
2. Dust	Poona	12
3. Compost		
a. Agriculture	Hyderabad	3
	Bhopal	2
b. Industrial	Gwalior	12
	Poona	8
c. Municipal	Gwalior	1
	Poona	4
	Chandigarh	3
4. Rabbit dung	Poona	2
5. Soil		
a. Drainage canal	Poona	4
b. Irrigated land	Hyderabad	3

MATERIAL AND METHODS

Samples were collected into presterilized tubes. A sufficient amount of sterile distilled water was added to each tube, mixed well, and 0.5 ml of the supernatant liqid was used to inoculate previously set YpSs agar plates contaning 30 ppm tetracycline HCl. Each plate pontained 25 ml of the agar medium. To prevent excessive drying the plates were sealed with adhesive tape and were incubated at 60°C (±0.5). When discrete colonies just appeared each colony was transferred to a fresh YpSs agar plate, sealed and incubated at 60°C for further observations. From these, single ascospore cultures were developed and were used in all further experiments. Spore suspension was made by adding aseptically 5 ml of sterile distilled water on to an abundently sporulating culture grown on YpSs agar slant for ten days at 37°C. Spores were scraped off with a sterile needle and transferred to a 250 ml Erlenmayer flasks containing 50 ml sterile distilled water. Effect of pH was studied by aseptically transfering 3 mm agar discs cut from a well grown culture on to YpSs agar plates of pH 2, 4, 6, 7, 9 and 10 prepared in buffor solutions. In order to obviate the difficulty of agar setting at pH 2, the agar medium was adjusted to this pH after sterilization by aseptically adding a predetermined quantity of 1N sterile HCL. All experiments were carried out in duplicate. The cultures were allowed to grew for 7-8 days

at 37,45 and 60°C. The diameter of the colonies as a measure of growth was recorded at 12 hr intervals. Similarly the effect of temperature was also studied but the agar plates used were at pH 6.

Carbon nitrogen requirements were studied at two different temperatures — 37 and 60°C. The basal medium consisted of glucose 5 gms., potassium nitrate 3.5 gms., potassium dihydrogen phosphate 1.75 gms., magnesium sulphate 0.75 gms., distilled water 1000 cc. All carbon compounds were used at 2% level. The amount of nitrogen compounds used was equivalent to that present in the basal medium. Different compounds of carbon and nitrogen and the basal medium devoid of either carbon or nitrogen were prepared in double concentration required. All these media were sterilized for fifteen minutes at 120°C. 15 ml of each compound was aseptically transferred into 100 ml of Erlenmayer flasks containing an equivalent amount of the basal medium. The pH of these combined solutions was aseptically adjusted to pH 6 by adding 2N NaOH or HCl. Analytical grade chemicals were used throughout the experiments; 0.5 ml of the seed was used to inoculate the flasks. The inoculated flasks were incubated at 37° and 60°C. After ten days of incubation the cultures were harvested by useing Whatman filter paper previously dried to constant weight in an hot air even maintained at 60°C. The final pH of the filtrates was determined by using a pH-meter. The mycelial mats were washed twice with distilled water and dried to a constant weight in a hot air even for 24 hrs. An analytical balance was used to determine the mycelial dry weight. On the basis of number of spores present in the low power field microscope the degree of sporulation was graded as −absent, + poor, ++ moderate, +++ good, ++++ excellent.

Animal inoculation experiments were carried out on swiss albino mice weighing 18-20 gms. Spore suspension was made in physiological saline from a heavily sporulating culture slant and was adjusted to give a spore cout of 7×10^8 cells/ml. 1 ml of this was injected intraperitonially into the mice. Triplicated were used for each isolate. A control group received only saline. The animals were observed for 6 wooks at 5 day intervals and at the end of which they were sacrificed, each organ was separated, macerated and plated out to reiselate the organism.

OBSERVATIONS

On YpSs agar at 45°C colonies rapidly growing, prostrate, cover 90 mm petriplate in 72 hrs., white at first, slowly turn cinnamon brown and arachnoid with the formation of cleistothecia: diffusible pigment absent; creamy to cinnamon brown; mycelium smooth, septate, hyaline 10-22×1.1 μm in size. Conidia oval to subglobose, terminal smooth,

hyaline, one celled 9.9-22.0×8.8-12.0 µm; cleistothecia develop in the temperature range of 37-45°C, simple or gregarious, hyaline at first turn cinnamon brown on maturation, 210-350×215-380 µm; asci numerous, subglobose to pear shaped, smooth, hyaline, octosporous, 12.0-13.0×10-13.0 µm; ascospores oblongate subglobose, pale yellow to hyaline, finely striated, one celled 6.6-7.7×4.4-5.5 µm, peridium dark reddish brown and parenchymatus.

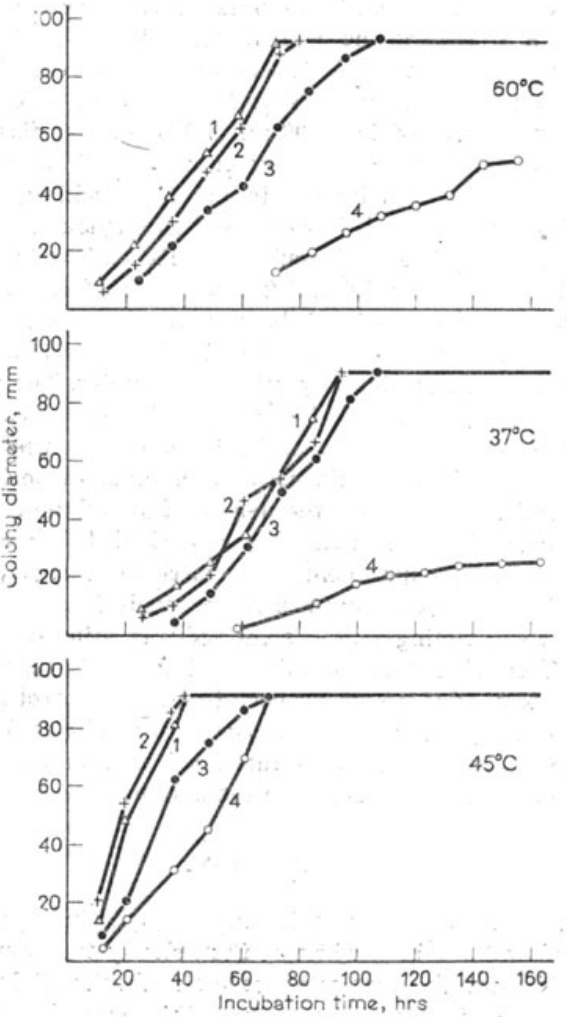

Fig. 1. Effect of pH on growth of *T. aurantiacus* at different temperatures
1 — pH 4, *2* — pH 6, *3* — pH 7, *4* — pH 9

Habitat: Mushroom compost and various other substrates (Table 1). Isolation number: 202.

T. aurantiacus grew from 37-60°C with an optimum temperature of 45°C. On either side of this temperature growth was markedly effected. At 45°C it grew to 90 mm in 40 hrs. while it took 80 and 68 hrs. at 37 and 60°C respectively (Fig. 1). Similarly sporulation was also varied with incubation temperature. Conidia developed throughout the growth temperature although extent of conidiation varied at different temperatures. Above 45°C it remained predominantly conidial and cleistothecial formation was either completely inhibited or small white cottony knots appear marking the initiation of fruit bodies, but failed to develop further. At 37°C cleistothecia abundantly formed succeeding the conidial formation. At 45°C both conidia and cleistothecia developed.

It grew from pH 4-9 with an optimum pH range of 4-6. No growth occurred on either side of this range. Under optimum conditions of pH (4-6) and temperature (45°C) maximum growth (90 mm in diam) occurred in 40 hrs. With the increase in pH towards alkalinity growth was progressively restricted up to pH 9 and stopped at pH 10. At an alkaline pH like 9 the onset of growth was delayed and the lag phase was found to be temperature dependent. At 37°C growth occurred after 56 hrs. and at 60°C after 60 hrs.

No withstanding with the temperature of incubation asexual spores developed at all the pH values studied although the extent of conidiation varied with temperature. Thus from pH 4-9 conidia were abundant at 60°C, moderate at 37 and 45°C. Similarly sexual spores developed in this range of pH but the onset and maturation varied. They developed in 40 hrs. at pH 4 and 60 hrs. at pH 9. They matured in 75 hrs. at pH 6-9 and in 96 hrs. at pH 4 (Fig. 2).

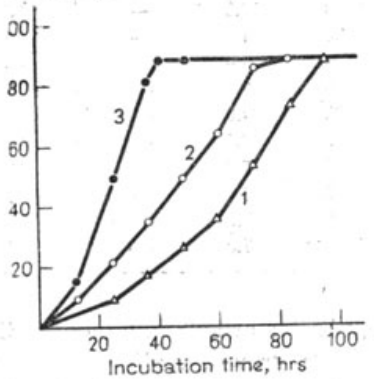

Fig. 2. Effect of temperature of growth of *T. aurantiacus* at the optimus pH
1 — 37°C, *2* — 60°C, *3* — 45°C

In general growth was better at 60°C than at 37°C. Of all the carbon sources investigated glucose supported best growth and sporulation at both the temperatures. Growth was poor on all the organic acids used and also on L (+) arabinose, L (+) rhamnose, sucrose, lactose, raffinose, inulin, dulicitol, mannitol, and sorbitol. Sporulation was poor with lactose and absent on other sugars. Growth and sporulation were moderate on soluble starch and maltose at both the temperatures. Glycerol was not utilized. D (+) xylose and galactose supported moderate growth and poor sporulation (Table 2).

Table 2

Showing dry mycelial weight, final pH and sporulation of *T. aurantiacus* on media containing different carbon compounds at 2% level

Carbon compound	Dry mycelial weight in mg	pH	Sporulation	Dry mycelial weight in mg	pH	Sporulation
	60°C			37°C		
L (—) Arabinose	10	7.0	—	5	6.5	—
D (+) Xylose	50	7.3	—	35	7.0	±
L (+) Rhamnose	20	6.0	±	20	6.0	±
D (—) Glucose	75	7.2	+++	60	7.0	++++
D (—) Fructose	35	6.5	++	20	7.0	++
D (—) Galactose	45	7.0	+	35	7.0	+
D (—) Mannose	50	7.3	—	40	7.0	±
L (—) Sorbose	25	7.0	—	15	7.0	±
Sucrose	5	7.0	—	10	7.0	±
Maltose	35	8.0	++	30	6.0	++
Lactose	10	6.8	+	10	6.4	+
Soluble starch	45	7.0	+++	40	7.0	++++
Dextrin	30	6.0	—	15	7.0	—
Inulin	20	7.0	—	10	7.0	—
Glycerol	—	—	—	—	—	—
Dulicitol	15	7.0	—	5	7.0	—
Mannitol	25	7.0	—	20	7.0	—
Sorbitol	15	6.5	—	10	6.0	—
Oxalic acid	15	7.0	—	10	6.5	—
Citric acid	10	7.0	—	10	6.5	—
Tartaric acid	5	7.0	—	5	6.0	—
Control	Trace	7.0	—	5	6.5	—

Ammonium nitrate, nitrites of potassium and sodium, DL-methionine, were found unsuitable for growth and sporulation. Best growth and sporulation was observed on urea, nitrates of sodium, potassium and calcium, ammonium tartrate and glutamic acid. Poor growth and sporu-

lation occurred in a medium containing magnesium nitrate, ammonium sulphate, L (—) glycine and peptone. On thiourea, L-serine, DL-serine, DL-phenylalanine, DL-leucine, DL-alanine and ammonium acetate growth was poor and sterile (Table 3).

None of the mice inoculated showed any pathological symptoms. Attempts to reisolate the organism from different tissues were also unsuccessful.

Table 3

Showing dry mycelial weight, final pH and sporulation of *T. aurantiacus* on media containing equivalent amounts of different nitrogen compounds

Nitrogen compound	Dry mycelial weight in mg	pH	Sporulation	Dry mycelial weight in mg	pH	Sporulation
	60%			37%		
Potassium nitrate	50	6.5	+++	40	6.5	++
Sodium nitrate	55	6.8	+++	50	6.3	++
Ammonium nitrate	—	—	—	—	—	—
Magnesium nitrate	10	6.0	±	10	6.4	±
Calcium nitrate	55	6.5	±	60	6.0	±
Potassium nitrite	Trace	7.0	—	Trace	7.0	—
Sodium nitrite	Trace	7.0	—	Trace	7.0	—
Ammonium chloride	40	6.0	+	30	6.0	+
A. sulphate	20	5.0	±	10	5.0	±
A. bicarbonate	15	6.0	+	15	6.2	+
A. tartrate	80	6.0	+++	55	6.0	+++
A. acetate	10	7.6	—	10	8.0	—
A. dihydrophosphate	40	7.0	++	25	7.2	++
A. phosphate	10	7.0	±	10	7.3	±
DL-valine	55	7.0	+++	50	7.0	+++
DL-alanine	20	6.8	—	25	6.5	—
L-Valine	45	7.0	++	45	7.0	++
DL-Leucine	25	6.5	—	20	6.8	—
DL-Phenylalanine	15	6.2	—	15	6.0	—
L-glycine	15	7.0	±	15	7.2	±
DL-methionine	—	—	—	—	—	—
DL-serine	15	6.8	—	10	6.5	—
L-serine	10	6.0	—	5	6.3	—
DL-aspartic acid	30	7.5	±	30	6.0	±
L-glutamic acid	55	6.0	+++	30	6.5	+++
L-asparagine	30	7.0	+	30	7.5	++
Urea	80	8.5	++++	70	8.0	++++
Thiourea	10	5.5	—	10	5.5	—
Peptone	20	7.6	+	15	7.6	+
Control	Trace	7.0	—	Trace	7.0	—

DISCUSSION

T. aurantiacus was reported to be one of the rarer of thermophiles (Cooney, Emerson 1964; Pore, Larsh 1967; Crisan 1959). In the present investigation several strains of this thermophile were isolated from several places and different habitats like dust, bat droppings, compost, rabbit dung, soil from irrigated land and a drainage canal. It was predominent in the dust samples collected in Poona and industrial compost from Gwalior. A small number of strains were also isolated from rabbit dung, bat droppings, and agricultural and manicipal compost. Thus it is evident that *T. aurantiacus* is widely distributed in India. It is also observed that at 60°C white colonies appear earlier than at 37°C and this may be due to less competition at elevated temperatures and also its true thermophilic nature. The fallure of the earlier investigators to isolate this obligate thermophile may be due to the low incubation temperatures used.

It grew from 37-60°C and pH 4-9 with an optimum temperature at 45°C and pH 4-6. The range of temperature which allowed sporulation was narrower than the range of temperature for growth (Cochrane 1959). However in the present investigation conidia developed throughout the growth temperature although extent of conidiation varied with temperature. Thus with increase in temperature from 37-45°C there was an increase in the conidia formed and above which it remained predominently in conidial form upto 60°C. At 37°C cleistothecia formed in abundance. At 45°C a relatively smaller number of cleistothecia formed. At 60°C either cleistothecial development was completely inhibited or small white cottony knots appeared marking the initiation of fruit bodies but failed to develop further. Thus similar to mesophiles (Klebs 1900; Cochrane 1958) its temperature limits for sexual reproduction are narrower than for its asexual reproduction. Similar observations were also reported for its asexual reproduction. Similar observations were also reported for *Stilbella thermophila* (Al-Hassan, Fergus 1966). Temperature induced variation in the size of asci and ascospores was reported for some of the mesophilic fungi (Barnett, Lilly 1950). In the present study no such variation was observed.

It grew in a wide range of pH from 4-9. Acidic pH favoured better growth and sporulation than alkaline or neutral pH. Best sporulation and growth was observed in the pH range of 4-6. Incubation temperature was reported to influence the pH optimum and also pH range in fungi (Barnett, Lilly 1950). However in the present study no shift was observed either in the range of pH or its optimum. But growth rate varied with incubation temperature. At optimum temperature and pH

4 it grew to 90 mm in 40 hrs. while it took 92 hrs. at 37°C and 80 hrs. at 60°C. At pH 9 and 45°C the growth rate was nearly four times grater than 37°C and two times than at 60°C. Alkaline pH induced a lag phase in growth and it was found to be temperature dependent. At pH 9 onset of growth was delayed until 56 hrs. at 37°C and 60 hrs. at 60°C.

Asexual spores developed in the entire range of pH suitable for growth. Conidial development was delayed with increase in pH towards alkalinity. Under acidic conditions of pH (4-6) cleistothecia developed in 40 hrs. at 45°C while at alkaline pH (9) they appeared after 60 hrs. Similarly at 37°C fruiting bodies appeared in 70 hrs. of incubation at pH 4-6,82 hrs. at pH 7 and 104 hrs. at pH 9.

In general the utilization of different carbon compounds was better at 60°C than at 37°C. None of the pentoses studied were found to be suitable for sporulation although D (+) xylose supported good growth at 60°C and moderate at 37°C. Glucose among hexoses, maltose among disaccharides and soluble starch among polysaccharides supported better growth and sporulation. These results are in agreement with the earlier observations (Reese 1946; Rege 1927; Sahm, Chapman 1976; Subrahmanyam, Thirumalachar 1977). Utilization of lactose varied with different thermophilic fungi. *Torula thermophila* Cooney and Emerson was reported to grow well on lactose (Subrahmanyam 1977) while *Papulaspora thermophila* Fergus showed poor growth (Sahm, Chapman 1976). Growth and sporulation of *T. aurantiacus* were poor at either temperature used. It failed to utilize glycerol. Growth was poor and non-sporulating on all the organic acids studied.

Ammonium nitrate, nitrates of potassium, sodium, DL-methionine were found unsuitable for its growth and sporulation. *Humicola lanuginosa* (Griff. et Maubl.) Bunce and *Penicillium dupontii* were also unable to sporulate on DL-methionine (Mayer Glenn 1970). Good growth and sporulation were observed on urea, nitrates of potassium, sodium, calcium, ammonium tartrate and glutamic acid, Premabai and Rao (1960) observed that *H. lanuginosa* and *P. dupontii* grew well on L-glutamine. Thiourea and L-glycine were reported to support good growth in *Sporotrichum thermophile* Apinis (Subrahmanyam, Thirumalachar 1977). However *T. aurantiacus* showed poor and sterile growth.

Pore and Larsh (1967) isolated a pathogenic strain of *T. aurantiacus* from broanchial washings of a patient. In view of this finding, animal inoculation experiments were conducted and the results have shown that none of the isolates included in the present study were pathogenic.

SUMMARY AND CONCLUSION

T. aurantiacus has been shown to be widely distributed in India. It can easily be isolated from most of the substrates if higher incubation temperatures like 50°C and above are used. It has a wide range of temperature with an optimum at 45°C. Temperature range for growth and asexual reproduction are the same while sexual reproduction occurred in the temperature range of 37-45°C. Higher temperatures like 55 and 60°C are unsuitable for cleistothecial formation. It grew and sporulated well in the acid range of pH at all the temperatures used. It can tolerate alkaline pH up to 9. In general utilization of various carbon and nitrogen compounds was better at 60°C than at 37°C. Glucose, maltose, soluble starch among carbon compounds and nitrates of potassium, sodium, calcium, ammonium, tartrate and glutamic acid among nitrogen sources supported good growth and sporulation. None of the isolates studied were found pathogenic.

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Studia nad Thermoascus aurantiacus Miehe

Streszczenie

Badano wpływ czynników klimatycznych oraz odczynu gleby na *Thermoascus aurantiacus*. Żaden ze szczepów tego szeroko rozpowszechnionego w Indii grzyba nie był patogenem.