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Short Communication

pH and temperature optima for growth and sporulation in some common fungi from city waste

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Relationships between the growth of certain fungi isolated from city waste and pH and temperature were examined by two methods. The tested isolates showed their maximum growth and sporulation at different pHs while temperature requirements were the same (28°C), except for *Humicola grisea* (43°C). *Cladosporium herbarum* and *H. grisea* showed double pH optima. The ranges of pH and temperature for sporulation were more limited than those for the vegetative growth. Although all the tested isolates showed wide tolerances to pH and temperature, the degree of tolerance varied with the isolates. A considerable change from the initial pH of the liquid medium was noted at the end of the experiment.

Key Words——city waste; fungal growth; pH; temperature.

The environment in which organisms exist exerts a profound effect upon them and often dictates their behaviour. The microbial population of a habitat is influenced either singly or in combination by a number of biotic and abiotic factors. City waste provides a complex habitat supporting an abundant and extremely diverse population of microorganisms. There is considerable evidence that ecological conditions play an important role in microbial activity (Hudson, 1968; Rai et al., 1981; Jaitly, 1982, 1987), of which hydrogen ion concentration and temperature are very important in determining the growth, activity and composition of organisms (Alexander, 1961; Agnihotri, 1963, 1964; Mukerjee, 1966). Individual species may have lower or higher tolerance to these factors than the general fungal community (Jaitly, 1982, 1987). Hegarty and Curran (1985) observed that maximum wood decay by most of their isolates was in the temperature and pH ranges of 15–25°C and 5–8, respectively.

Investigations have been conducted on pH and temperature optima of fungi from various habitats, but no report is available on these lines for the fungi from city waste. Furthermore, because of authors' interest in biodegradation and enzymatic hydrolysis of composting material, it was essential to find the optimal pH and temperature for the experimental fungal isolates.

Effects of pH and temperature on the growth and sporulation of some common fungi from city waste were evaluated by a diameter measurement method because this has been shown to be of sufficient reliability (Brancato and Golding, 1953; Jaitly, 1982, 1987). Dry weight measurements were also taken (Jaitly, 1987).

Czapek-Dox agar was prepared in citrate-phosphate buffer of different pH values ranging from 3.0 to 8.0 at intervals of 0.5 unit. Sterilized medium was poured in sterilized Petri dishes (10 cm) and single point central inoculations were made with 2 mm discs of freshly grown mycelium of the test fungus. Three replicates of each isolate were grown at a constant temperature of 29±1°C. Plates were regularly examined at 24-h intervals for seven days. Two diameters were measured at right angles to each other as a measure of the average diameter for each colony. Isolates were examined for sporulation regularly. In the second method, 25 ml of Czapek-Dox medium of required pH values was poured in 100-ml Erlenmeyer flasks and autoclaved at 15 lbs pressure for 15 min. The flasks were inoculated with 0.5 ml of homogenous spore suspension of the test fungus containing 10^7 spores/ml. The flasks were incubated at 29±1°C. Two replictes were taken for each. After seven days of incubation, the biomass obtained was filtered through preweighed Whatman filter paper No. 1 and dried for 24 h at 80°C. Final weight was taken to calculate the dry weight of fungal biomass.

Test fungi were grown on Czapek-Dox medium at the optimum pH for their growth at different temperatures in the same way as stated for pH.

The results of pH-growth relationship from colony diameter measurements were in agreement with those of the dry weight measurements. Tested isolates showed their maximum growth at different pHs (Table 1). After an initial lag phase, the increase in diameter was more or less linear for all the tested fungal isolates (Fig. 1) at pH near to their optima. The isolates grew optimally in the acidic to neutral pH range but also showed tolerance to alkaline pH. Slow growth was observed at pH 3.0 for all the isolates. Maximum growth for all the tested isolates

Table 1. Effect of pH on growth of some fungi after seven days (dry weight measurement).

Fungus	Dry weight in mg*									
pH**	3.0	3.5	4.0	4.5	5.0	5.5	6.0	6.5	7.0	8.0
Alternaria humicola Oudemans	130 −	150 −	175 ++	230 +++	360 ++++	290 +++	240 ++	200 −	100 −	110 −
Aspergillus niger van Tieghem	120 +	180 +	240 ++	180 +++	210 +++	230 +++	320 ++++	200 +++	180 ++	100 −
Aspergillus terreus Thom	390 ++	440 +++	520 +++++	535 +++++	580 +++++	530 +++++	510 ++++	470 +++	420 ++	350 ++
Cladosporium herbarum (Pers.) Link ex Gray	150 −	190 −	290 +	330 ++	450 ++	480 +++	580 +++++	560 +++++	390 +++	510 ++
Curvularia lunata (Wakker) Boedijn	180 −	185 +	450 ++++	360 ++++	340 +++	330 +++	325 ++	210 ++	260 −	250 −
Humicola grisea Traaen	320 −	330 +	300 ++	445 +++	490 ++++	380 +++	360 ++	380 ++	480 ++	350 ++
Myrothecium roridum Tode ex Fr.	30 −	45 −	75 −	80 +	140 ++	160 ++	190 +++	90 +	50 −	45 −
Penicillium chrysogenum Thom	190 −	200 +	210 ++	230 ++	245 ++	260 +++	350 ++++	380 ++++	230 +++	260 ++

* Growth on Czapek-Dox medium.
** Citrate phosphate buffer.
+, − Degree of sporulation.

was obtained at pH between 4.0 and 6.5. *Humicola grisea* Traaen and *Cladosporium herbarum* (Pers.) Link ex Gray showed double pH optima, one in the acidic range (pH 5.0 and 6.0, respectively) and the other at neutral and alkaline pH i.e., pH 7 and pH 8, respectively. Microscopic examination of tested isolates revealed that the pH range for sporulation is narrower than that for the vegetative growth. Normally, excellent sporulation was obtained between pH 4.0 and 5.5. All the tested isolates were observed to grow at pH 8.0 with varying colony diameter, indicating that they have different abilities to tolerate extreme pH. A considerable change from the initial pH of the liquid medium was noted (Table 3).

Temperature requirements were same for all the tested fungal isolates, except for *H. grisea* (Table 2). After an initial lag phase, colony diameter increased linearly with time for all the isolates (Fig. 2). Tested fungal isolates showed optimum growth at 28°C. However the mycelial growth of *H. grisea* was maximum at 43°C. Most of the fungi showed lower growth at below 23°C and above 33°C. Sporulation was also maximum in the temperature range of 28-33°C.

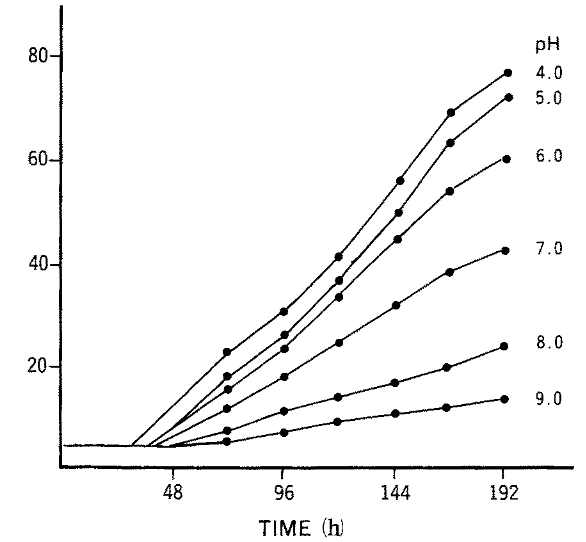

Fig. 1. Cardinal growth of *Curvularia lunata* on Czapek-Dox agar medium. In relation to pH.

The two methods used to determine growth in relation to pH or temperature were satisfactory and yielded similar results. At extremes of pH and temperature, the isolates grew only after a long incubation period. The existence of microorganisms in the waste materials is affected by a number of environmental factors such as pH, temperature, aeration, moisture, organic matter etc., of which pH and temperature are the most important (Hegarty and Curran, 1985; Pearce and Malajczuk, 1990; Bowerman and Goos, 1991). All the tested isolates showed their pH optima in the range of 4.0 to 6.5. This might be due to the presence of high concentrations of organic matter which decomposes to form different nitrogenous and carbonaceous substances, thus lowering the pH of waste material. These studies are in agreement with those performed by Jaitly (1987). Alexander (1971) also stated that the application of fertilizers containing nitrogenous substances leads to the formation of acids by fungi on one hand and inhibition of the growth of bacteria and actinomycetes on the other hand.

Tested isolates showed some tolerance to alkaline pH but their optimum growth lay in the lower range. This is in agreement with the results obtained by several workers (Wolf et al., 1950; Brewer, 1960; Rai et al., 1970, Jaitly, 1987). In the present investigation, *C. herbarum* and *H. grisea* showed double pH optima. Biomodal curves have also been obtained by several other workers (Jones and Irvine, 1972; Jaitly, 1987).

Table 2. Effect of temperature on growth of some fungi after seven days (dry weight measurement).

Fungus	Temperature °C	Dry weight in mg*					
		23	28	33	38	43	48
Alternaria humicola		200 ++	310 +++	230 ++	220 ++	190 +	140 −
Aspergillus niger		190 ++	450 ++++	330 +++	240 +++	210 ++	180 ++
Aspergillus terreus		140 ++	550 +++++	460 ++++	370 +++	180 ++	070 +
Cladosporium herbarum		240 ++	420 ++++	380 ++++	320 +++	270 ++	230 −
Curvularia lunata		250 +++	540 +++++	490 ++++	430 +++	320 ++	180 +
Humicola grisea		150 −	250 +++	280 +++	320 ++++	390 ++++	310 +++
Myrothecium roridum		110 ++	270 +++	190 +++	180 ++	120 ++	100 +
Penicillium chrysogenum		230 ++	380 +++	350 +++	310 +++	270 ++	210 ++

* Growth on Czapek-Dox medium at optimum pH.
+, − Degree of sporulation.

Table 3. Shift in the initial pH of basal medium.

Initial pH	Final pH values after 7 days of growth							
	Alternaria humicola	*Aspergillus niger*	*Aspergillus terreus*	*Cladosporium herbarum*	*Curvularia lunata*	*Humicola grisea*	*Myrothecium roridum*	*Penicillium chrysogenum*
3.0	5.0	4.5	5.0	5.5	4.0	4.5	4.0	4.0
3.5	5.0	4.5	5.0	5.5	5.0	4.5	4.0	4.0
4.0	5.5	5.0	5.8	5.5	5.0	5.5	5.0	4.4
4.5	6.0	5.5	6.0	5.8	5.5	5.5	5.0	4.6
5.0	6.0	6.0	6.0	6.2	5.0	6.0	5.5	4.6
5.5	6.0	6.0	6.0	6.5	5.5	6.5	5.5	4.6
6.0	6.0	6.5	6.0	6.5	5.5	6.5	5.5	4.6
6.5	6.5	6.5	6.5	6.5	6.0	6.5	6.0	4.8
7.0	7.0	6.8	6.8	6.5	6.0	6.8	6.0	5.0
8.0	7.5	7.0	7.0	6.0	6.5	6.8	6.5	5.0

Fungi are known to alter the pH of medium in which they grew. The present results also showed a change in the initial pH of the basal medium. Generally the lower pH values became higher and the higher became lower, while those in the vicinity of pH 7.0 did not shift significantly to either side (Table 3).

In the present study the species grew after a long incubation period at the extreme temperatures. Many workers (Tansey, 1972; Jaitly, 1982) suggested that a long lag phase can be expected at incubation temperatures differing from that of the inoculum. Evans (1971) concluded that a short incubation period may not reflect the true cardinal temperatures for growth. During the present investigation, all the tested isolates showed their maximum growth at 28°C, except *H. grisea*, which showed optimum growth at 43°C, indicating that the species is of thermotolerant nature. The ranges of pH and temperature for sporulation were lower than those for vegetative growth (Jatilty, 1982, 1987).

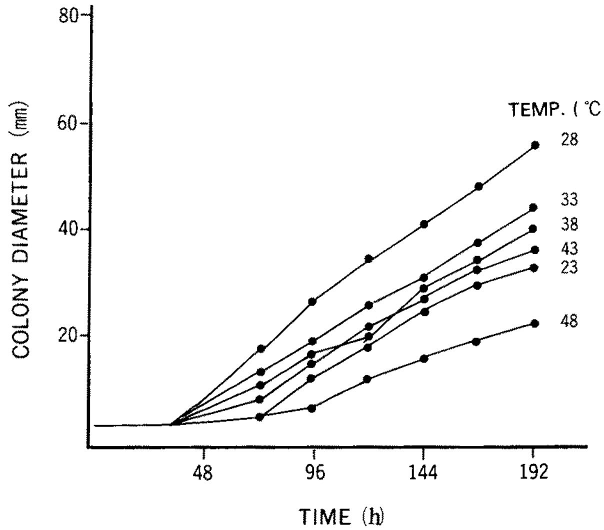

Fig. 2. Cardinal growth of *Curvularia lunata* on Czapek-Dox agar medium. In relation to temperature.

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