thermophilic actinomycetes. In this report, we describe a modified combination of these methods for preferential isolation of thermophilic actinomycetes.

Forty soil samples from eight localities were collected from the vicinity of Pune and surrounding area. The samples were uniformly suspended in sterile physiological saline (10 g in 90 ml) and were subjected to three different methods of isolation, namely the DC[3], HTPI[4] and a combination of both these methods, modified as detailed below:

The soil suspension was centrifuged for 20 min in a REMI T23 centrifuge at 3000 r.p.m. (RCF 604 × G at the surface and 1610 × G at the bottom of the cuvette). One ml aliquot of the centrifuged suspension was pour-plated in triplicate using glucose yeast extract agar medium of pH 6.5. The plates were subjected to pre-incubation at 110°C for 10 min. After the pre-incubation, the plates were incubated at 55°C in a humidified incubator for 48 hr. The morphology of colonies developing on the isolation plates of all the three methods was visually examined and ascertained microscopically. The colony counts were noted as belonging to bacteria and actinomycetes. None of the methods tried yielded mold growth. The thermophilic actinomycetes isolated belonged to *Streptomyces*, *Thermoactinomyces*, *Thermomonospora*, *Micromonospora* and *Micropolyspora* species. The mean actinomycete counts (MAC) and the mean bacterial counts (MBC) obtained by the three methods are shown in table 1. In each of the methods, the MAC differed significantly from the MBC as obtained by comparing the two sample means at 1 % level of significance. This was confirmed by using the statistical formula:

$$|\bar{x} - \bar{y}|/\{[S_1^2/(n_1 - 1)] + [S_2^2/(n_2 - 1)]\}^{1/2}$$

where $\bar{x}$, $\bar{y}$ are the mean of the first and second series of counts, respectively n_1 and n_2 are number of observations in the first and second series respectively, $S_1 = \Sigma(x_1 - \bar{x})^2/n_1$ and $S_2 = \Sigma(y_1 - \bar{y})^2/n_2$. It was evident from the results that the DC + HTPI combination method was superior to the other two methods so far as the isolation of thermophilic actinomycetes was concerned. The reason for such a preferential isolation may be that the differential centrifugation helped the separation of actinomycetes spores and high temperature pre-incubation favoured the growth of thermophilic actinomycetes, at the same time preventing bacterial growth.

Table 1 *Mean thermophilic actinomycetes and bacterial counts*

Mean counts per gram of soil	DC method	HTPI method	DC + HTPI method
Mean thermophilic actinomycete count (MAC)	3.125×10^2	8.750×10^2	11.250×10^2
Mean bacterial count (MBC)	13.625×10^2	1.940×10^2	1.000×10^2

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INFLUENCE OF DESICCATION STRESS IN A XEROPHILIC THERMOPHILE *HUMICOLA* SP

M. K. MAHAJAN, B. N. JOHRI and R. K. GUPTA*
*Department of Microbiology and *Department of Biological Sciences, College of Basic Sciences and Humanities, G. B. Pant University of Agriculture and Technology, Pantnagar 263 145, India.*

It has long been recognized that a fundamental difference exists between the water economy of higher and lower plants. The terms 'homoiohydrous' and 'poikilohydrous' have been used to describe this condition in higher and lower plants respectively. Unlike the former, poikilohydrous plants also absorb water from vapours present in the atmosphere. Whereas considerable work has been done regarding the response of water stress in higher plants, algae, bryophytes and lichens, relatively little is known about the biochemical changes due to desiccation stress in fungi[2,3]. It is known that in higher plants sugar and proline content increases while phospholipids decrease with increasing water stress[4]. However, no such studies exist with fungal systems. Since thermophilic moulds

Table 1 *Relative water content (RWC) and biochemical changes in osmophilic Humicola sp after 12 hr of exposure to various relative humidities*

RH (%)	RWC (%)	Glucose (mg/g total carbohydrates)	Sterols (mg/g total lipids)	Phospholipids (mg/g total lipids)	Free proline (mg/g dry wt)
100 (control)	101.0 ± 3.0	13.9 ± 1.1 (100)	242.0 ± 20.0 (100)	214.0 ± 21.0 (100)	0.77 ± 0.09 (100)
95	71.5 ± 1.5	15.7 ± 1.3 (112.9)	257.0 ± 15.0 (105.8)	205.0 ± 23.0 (95.7)	0.92 ± 0.09 (119.4)
75	39.5 ± 2.0	20.3 ± 1.6 (146.0)	429.0 ± 25.0 (176.9)	188.8 ± 17.0 (88.2)	1.23 ± 0.09 (159.7)
50	21.0 ± 0.4	25.7 ± 1.7 (184.8)	451.0 ± 25.0 (186.0)	112.5 ± 17.3 (52.6)	1.65 ± 0.07 (214.2)

Each value is the mean of three replications with ± S.E. Figures in parentheses represent values of percentage change over the control.

are exposed to stress under field conditions, an isolate recovered from sand dune sample was examined for biochemical changes during the water stress.

A xerophilic (osmophilic) isolate of a thermophilic Humicola was recovered on dichloran glycerol medium (DG-18) from sand dune samples collected from Osian and Hindon region of Rajasthan. Suitable suspension of dune sample was spread on DGM and plates incubated at 45°C for 8 days in a BOD incubator. Individual colonies were checked for purity, observed microscopically as also for osmotolerance on sucrose-containing medium (10–50%). *Humicola* sp required a minimum of 20% sucrose for growth but showed optimum extension of mycelium at 40 and profuse growth at 50% level. One g of 10-day-old mycelial mat was exposed to 100 (control), 95, 75 and 50% relative humidity (RH) for 1, 2, 4, 6, 8 and 24 hr in specially designed glass chambers; RH was maintained as described by Gupta[6]. One set of fungal sample was used to measure the relative water content (RWC)[7] and the second set to estimate glucose[8,9], phospholipids[10], sterols[11] and proline[12].

Results of RWC and various biochemical changes after different desiccation treatments are presented in table 1. At all the treatments, RWC was considerably greater at 95% RH than at 75 and 50% RH. Glucose content increased markedly with increasing desiccation treatment; at 50% RH glucose level increased by 84.8% over the control. It is known that starch is hydrolyzed to sugars due to desiccation in higher plants[4]. However, in fungi, glycogen is the reserve food material. It would therefore appear that increased glucose level reflects the hydrolysis of glycogen to glucose.

Phospholipids decreased considerably with increasing desiccation stress (table 1). This trend is known to operate in higher plants[4]. Williams and Chamman[13] concluded that practically all the phospholipids in a cell were located in the membranes so that a measure of the total phospholipids represented a measure of total membranes present. It is quite likely therefore that desiccation stress may have affected membrane integrity in *Humicola* sp.

Proline content increased considerably with the lowering of RH; the level was 114-fold greater at 50% RH than the control (100% RH). This was not surprising since similar increases have been reported in higher plants. The amount of sterols in *Humicola* sp increased markedly with increased desiccation stress. It is known that with increased cholesterol content, the permeability of artificial membrane to water is decreased[14]. Thus, a change in sterol content due to desiccation stress will reflect alterations in membrane permeability.

Further studies on desiccation stress in drought sensitive species are now under way to understand the adaptive mechanisms in thermophilic fungi.

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HELMET DEVELOPMENT IN *DAPHNIA CEPHALATA* KING UNDER LABORATORY CONDITIONS

S. MANIMEGALAI, K. VENKATARAMAN* and S. KRISHNASWAMY
*School of Biological Sciences, * School of Energy, Environment and Natural Resources, Madurai Kamaraj University, Madurai 625021, India.*

CYCLOMORPHOSIS is a seasonal change in the morphology of many planktonic crustaceans and has been described in several species of *Daphnia*[1-4]. It has been reported that the increase in helmet size in various species of zooplankton may be due to a rise in temperature[5-7] or turbulence[8-11] or a chemical released by a predator[12,13].

In Madurai (Lat: 9°53′ N; Long: 78°E), four species of helmeted *Daphnia* were recorded along with a non-helmeted species[14]. Among these four helmeted species, *Daphnia cephalata* King has a large cephalic expansion. It is abundant in nature and co-occurs with a round-headed *Daphnia similis* Claus. An invertebrate predator, *Anisops bouvieri* is also common in these ponds where the daphnids co-occur.

Grant and Bayley[12] and Kruger and Dodson[13] reported that a chemical substance released by the predator is responsible for helmet growth in daphnids. In the present study, to test the above hypothesis, we performed a few experiments. Gravid females of both *D. cephalata* and *D. similis* were cultured individually in a plastic container (50 ml) and the filtered pond water was used as the medium to study the life history following the method of Venkataraman[15]. About thirty *A. bouvieri* were taken and ground in 30 ml of distilled water centrifuged at 3000 rpm for 10 min. The supernatant was taken and 0.5 ml of this extract of *A. bouvieri* was added to one litre of the filtered pond water and used as medium for culturing the daphnids. This medium was changed daily. The body size, helmet size, width and the number of eggs were measured following the method of Hebert[7]. The results were analyzed statistically using ANOVA[16].

It was observed that the helmeted *D. cephalata* can produce only helmeted youngone and not the round-headed form and *vice versa*. An increase was noted in total length, head length and head width between the control and experimental forms; however, no significant change was noticed in H/C ratio (table 1).

In the present study the clutch size of *D. similis* is larger than that of *D. cephalata* (figure 1). One possible

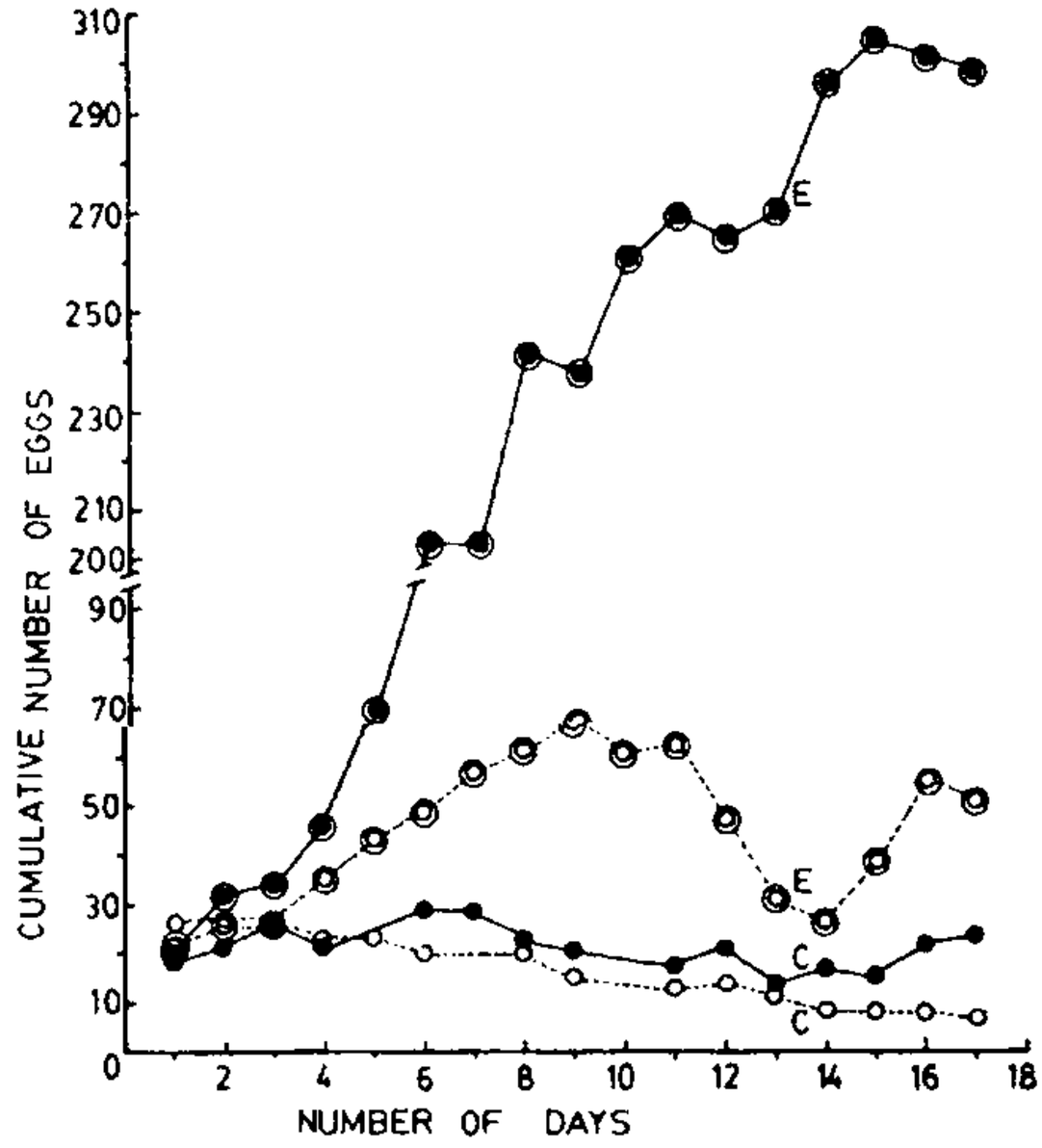

Figure 1. Fecundity of *D. similis* and *D. cephalata* in relation to number of days. (E—experimental; C—control; solid line—*D. similis*; dotted line—*D. cephalata*).