Folia Microbiol. **49** (1), 37–40 (2004) **http://www.biomed.cas.cz/mbu/folia**

Vegetative Survival and Reproduction under Submerged and Air-Exposed Conditions and Vegetative Survival as Affected by Salts, Pesticides, and Metals in Aerial Green Alga *Trentepohlia aurea*

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Received 13 June 2003
Revised version 9 September 2003

ABSTRACT. *Trentepohlia aurea* vegetative cells do not survive submerged conditions for more than 5 months, but can survive air-exposed conditions for more than 1 year. Disintegration and rapid death of algal cells was observed to a higher extent under submerged than air-exposed condition. Under submerged conditions *T. aurea* did not form any sporangium while prolific formation occurred under air-exposed conditions. Under submerged conditions algal cells formed few-celled, filamentous, cytoplasmic type setae. Vegetative cells were resistant to some extent to various levels of salt (NaCl, ≤0.8 mol/L), pesticides (DDT, 2,4-D or captan, 2000 ppm) and 'heavy' metals (zinc or nickel, 200 ppm; cobalt, ≤100 ppm.)

Literature data on the effect of various environmental factors such as submerged culturing, pesticides or 'heavy" metals on the vegetative survival and reproduction in any aerial green alga are relatively scarce. Some work was done in this direction on aquatic green algae *Pithophora oedogonia*, *Cladophora glomerata*, *Rhizoclonium hieroglyphicum* and *Scenedesmus bijuga* and aquatic blue-green algae *Anabaena iyengarii*, *Westiellopsis prolifica* and *Nostochopsis lobatus*, showing an adverse effect of the effectors and the organisms (Agrawal and Singh 1999*a*,*b*, 2002; Agrawal and Misra 2002; Fathi 2002; Agrawal and Pal 2003). Here we report on the influence of submerged and air-exposed conditions on the vegetative survival and reproduction, and that of the presence of salt (NaCl), pesticides (DDT, 2,4-D or captan), and 'heavy' metals (Ni, Co, Zn) on the vegetative survival of the strictly aerial green alga *Trentepohlia aurea*.

MATERIAL AND METHODS

Algal material. *Trentepohlia aurea* (L.) MARTIUS thallus was collected while growing attached to rock surface and tree bark in Darjeeling (India) in mid-December 2001 (Fig. 1A). The thallus was filamentous, irregularly branched and was brownish-red. Filaments were observed to be in vegetative condition; they did not have any sporangium when collected.

Vegetative survival and reproduction under submerged and air-exposed conditions. Masses of algal thallus about 5 mm in diameter were inoculated into liquid Bold's basal medium (Nichols and Bold 1965; pH adjusted prior to autoclaving to 7.5), stirred twice a day, completely sunk in the medium after 3 d of inoculation. The cells were kept in the culture chamber at 25 °C and light intensity of ca. 30 μmol m^{-2} s^{-1} from daylight fluorescent tubes for 16 h a day.

Masses of algal thallus were also kept air-exposed in the same culture chamber in an open polythene bag as collected. The mean relative humidity of the culture chamber during all experiments was 47–55 %.

Filaments under submerged and air-exposed conditions were assessed periodically up to 12 months to determine vegetative survival and formation of sporangium (if any). Percent vegetative survival was determined by counting the number of living vegetative cells relative to dead vegetative cells (looking hyaline, empty or having ruptured wall and showing disintegration) out of about 6000–7000 vegetative filament cells counted from each of three replicates. Under submerged conditions no sporangia developed but vegetative cells developed few-celled, tapering, filamentous, cytoplasmic-type setae (Fig. 1B,C). The percent formation and/or disintegration of setae was determined by counting the number of those vegetative cells which possess the setae in intact form relative to all other living vegetative cells of the filaments counted. Setae were shed off and disappeared in aged cultures since they are thin-walled.

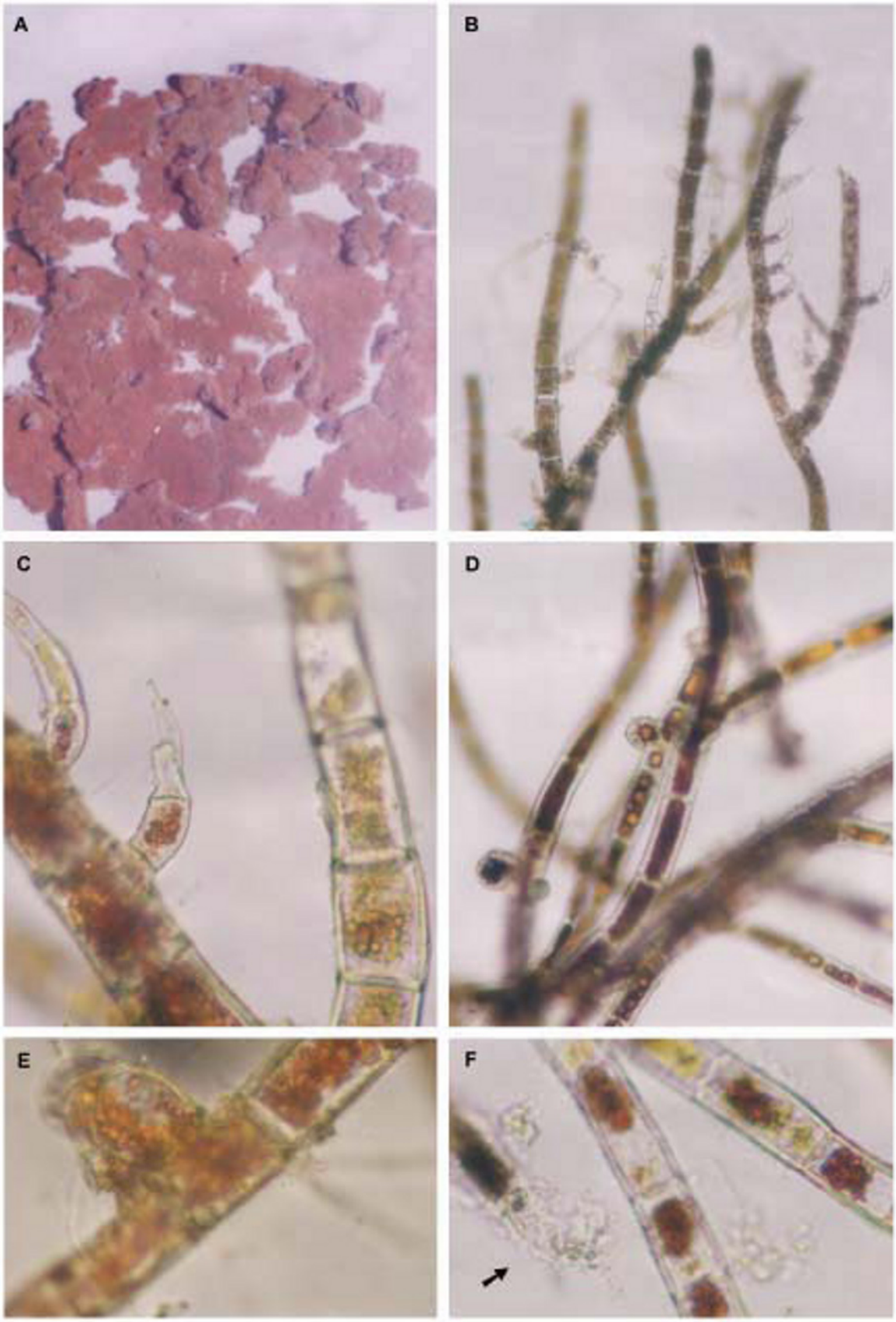

Fig. 1. *Trentepohlia aurea.* **A**: Thallus; ×l; **B**: algal filaments in basal medium showing development of filamentous-type setae from vegetative cells; ×400; **C**: setae; ×1600; **D**: vegetative cells of the filaments showing development of sessile sporangia during air-exposure; ×600; **E**: sporangium showing disintegration; ×1600; **F**: vegetative filaments showing death and disintegration of vegetative cells (*arrow*); ×1000.

Under air-exposed conditions the vegetative cells developed sessile sporangia (Fig. 1D). The percent sporangium formation and/or disintegration was determined by counting the number of vegetative cells possessing the sporangium in intact form relative to all other living vegetative cells of the filaments having no sporangium. The sporangia disintegrated when aged (Fig. 1E).

To take all such readings, the submerged filaments were assessed as such, while those lying exposed to air were first inoculated into a basal medium for 1 d and then assessed in order to clearly distinguish living and dead cells.

Vegetative survival as affected by salt, pesticides or 'heavy' metals. Algal material was separately inoculated into a liquid basal medium containing the following effectors: 0.2–1 mol/L NaCl (99 %; *Merck*, India), 500–2000 ppm 1,1,1-trichloro-2,2-bis(4-chlorophenyl)ethane ('dichlorodiphenyltrichloroethane'; DDT; 10 %; supplied from Government stock, India), 2,4-dichlorophenoxyacetic acid (2,4-D; 86 %; *Tropical Agro*, India), captan (a broad spectrum fungicide belonging to the phthalimide group; 50 %; *Rallis*, India), 25–200 ppm nickel sulfate (99 %; *Merck*, India), cobalt dinitrate (97 %; *BDH*, India) or zinc sulfate (99 %; *S.D. Fine Chemicals*, India). In all cases, pH of the medium was adjusted to 7.5 prior to autoclaving. The conditions for liquid culture were the same as indicated earlier. The vegetative survival of the alga was determined as usual 10 d after inoculation.

RESULTS AND DISCUSSION

Vegetative survival and reproduction under submerged and air-exposed conditions. Most of submerged vegetative cells died within 5 months of inoculation in liquid basal medium including those having supply of essential extrabiotic nutrients (Fig. 1F, Table I). However, under air-exposed conditions about 10 % of filament algal cells survived for more than 12 months (Table I). Submergence led to a rapid cell death and disintegration. Howland (1929) reported that *T. aurea* can withstand long periods of drying without appreciable change. Geitler (1923) has pointed out that *Trentepohlia* spp. vegetative cells closely resemble the resting stages of other algae in possessing a rich hematochrome content. Resistance to desiccation of this alga can also be linked with its resistance to salt (Table II). Lamellation of *Trentepohlia* spp. cell wall (West and Hood 1911) can also explain the protection of these algae against desiccation; the wall was found to be of cellulosic nature (West and Hood 1911) and not to contain sporopollenin (Good and Chapman 1978).

Table I. Influence of submerged conditions and air exposure on vegetative survival (VS), sporangium formation and/or disintegration (SP) and seta formation and/or disintegration (ST; all in %[a]) in T. aurea

Time months	Submerged[b]		Air exposure[c]	
	VS	ST	VS	SP
0	100	0	100	0
⅓	100	9.5	100	15
⅔	100	10	100	17
1	83	6	100	14
2	59.5	1	92	4.5
3	31	0	78	1.5
4	12.5	0	70	0.5
5	3	0	67	0
6	0	–	62	0
9	–	–	40.5	0
12	–	–	11	0

[a]Means of three replicates; for details *see Material and Methods*.
[b]SP was not observed. [c]ST was not observed.

Vegetative *T. aurea* cells under submerged condition did not develop any sporangium; however, within 10 d of inoculation in liquid medium under submerged conditions they developed few-celled, tapering, filamentous, cytoplasmic-type setae, which are probably modified branches (Fig. 1B,C; Table I). Most of them were shed off and disappeared under submerged conditions within 1–2 months after they appeared. They were light colored and thin-walled compared with vegetative cells.

About 15 % vegetative cells of *T. aurea* filaments developed sessile sporangia within 10 d under air-exposed conditions (Fig. 1D, Table I). Dryness was found to induce the formation of cysts in terrestrial algae *Vaucheria geminata* (Stahl 1879) and *Protosiphon botryoides* (Moewus 1935). *T. aurea* sporangia disintegrated under the same conditions within 2–3 months after being formed (Fig. 1E, Table I). No filamentous setae developed under air-exposed conditions. Sporangia did not release any swarmer if submerged in water.

Table II. Influence of NaCl (0.2–1 mol/L); DDT, 2,4-D or captan (all three 500–2000 ppm), nickel, cobalt or zinc (25–200 ppm) ions present in liquid basal medium on percent of vegetative survival (*second lines*) in *T. aurea*[a]

NaCl				Nickel			
0.2	0.5	0.8	1	25	50	100	200
66	20.5	4.5	0	89	78	50.5	22.5
DDT				**Cobalt**			
500	1000	1500	2000	25	50	100	200
77.5	61	56	45.5	66	37	8.5	0
2,4-D				**Zinc**			
500	1000	1500	2000	25	50	100	200
94	85	70.5	62	96	89	71	58
Captan							
500	1000	1500	2000				
60	51	42	33.5				

[a]10 d after inoculation; controls represent 100 % vegetative survival; mean of three replicates.

Vegetative survival as affected by salt, pesticides or 'heavy' metals. Vegetative *T. aurea* cells exhibited limited NaCl resistance (they tolerated to some extent even 0.8 mol/L NaCl; Table II). Besides tolerating dryness (Table I) and salt excess they were also resistant to a high amount of pesticides (DDT, 2,4-D or captan at 2000 ppm, nickel and zinc at 200 ppm and cobalt at 100 ppm levels; Table II). In contrast, aquatic green algae *Pithophora oedogonia*, *Cladophora glomerata* and *Rhizoclonium hieroglyphicum*, aquatic blue-green algae *Anabaena iyengarii*, *Westiellopsis prolifica* and *Nostochopsis lobatus* were relatively sensitive at various levels to dryness, pesticides and 'heavy' metals (Agrawal and Singh 1999*a*,*b*, 2002; Agrawal and Misra 2002).

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