GROWTH AND DEVELOPMENT OF *TRENTEPOHLIA ODORATA* IN CULTURE

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ABSTRACT

Trentepohlia odorata, a filamentous green alga, grows profusely and imparts an orange color on walls of many high-rise apartment buildings in Singapore. Since bulk cultures are needed in the screening of possible chemicals to control infestation, variations in the growth and development of the alga in the laboratory under different physico-chemical conditions were investigated.

Bold's medium either as a liquid or solidified with agar was suitable substrate for the culture of the alga. An acidic growth medium (pH 4.5-6.5) favored the formation of sporangia that developed into yellowish-green colonies, whereas a basic medium (pH 7.5-9.5) produced green colonies and no sporangia. An agar concentration of 0.7% as well as the addition of thiamine to the liquid basal medium enhanced the production of sporangia developing into numerous daughter colonies after two months. High relative humidities increased colony growth and promoted the formation of yellow colonies of cylindrical cells.

On walls of buildings, the cells are elliptical to barrel-shaped, bright orange, and possess few sessile sporangia. In cultures where moisture and nutrients are not limiting, cells are narrow and elongated, with the filaments radiating from a central mass, and the sporangia are of the sessile and pedicellate types.

INTRODUCTION

Trentepohlia is a filamentous green alga easily recognized by its characteristic orange color due to an abundance of carotene in oil globules in its cells. The alga is common in the tropics, with a few species even in the temperate and sub-arctic areas. Five species have been recorded in Sin-

gapore (Johnson 1978), the more conspicuous being *T. aurea* (L.) Martius and *T. odorata* (Wigg.) Wittr. The former is commonly found on tree trunks and surfaces of rocks, forming short streaks of orange to green filaments. In the shade, the filaments may be green while under full sunlight they may turn orange. The latter forms a crustose layer on concrete surfaces and building walls, especially those of the high-rise apartment buildings, giving the surface a distinct tinge of orange (Wee and Lee 1981). *T. odorata* is of particular interest in Singapore as its proliferation on surfaces of building hastens their repainting. Hence the use of paints containing an effective anti-algal chemical may reduce infestation. Unfortunately, anti-algal paints are not as commonly available as those with anti-fungal additives. Faint manufacturers may claim that their anti-fungal products are as effective on algae as on fungi, but this has yet to be proven. Screening tests for potential algicides, undertaken locally (Ngiam et al. 1973, Ngiam and Yong 1975) as well as elsewhere (Drioko and Crylly 1974, Fitzgerald 1964) utilized blue-green algae rather than *T. odorata,* the major organism colonizing on bare walls in Singapore. Natural populations of the alga are, however, insufficient if large-scale screenings are to be undertaken. It was therefore necessary to mass-produce the alga in the laboratory for screening purposes. The following report gives an account of the influence of the pH and nutrient strength of the culture medium, vitamin supplements, and relative humidity on the growth and development of *T. odorata* under laboratory conditions. The study, hopefully, will also provide basic information necessary for controlling infestation.

MATERIALS AND METHODS

Plant Materials

Samples of 7. *odorata* were scraped from walls of buildings around the former Bukit Timah Campus of the University of Singapore. A portion of the scrapings was examined under the microscope and the morphological features were noted. The rest was inoculated in Bold's basal medium contained in 125 ml Erlenmeyer flasks (Nicholas and Bold 1965). These flasks were placed under continuous lighting for three to four weeks to allow the *Trentepohlia,* together with any other algae, to proliferate. The *Trentepohlia* colonies were then isolated and reinoculated a number of times in the same medium until pure cultures were obtained. In all experiments, an inoculum of two colonies of equal diameter from the stock culture was introduced into each flask.

pH of Culture Medium

Bold's medium after autoclaving had a pH of 6.5 which served as the

control. Media of pH 4.5, 5.5, 7.5, 8.5 and 9.5 were prepared by adding dilute sodium hydroxide or hydrochloric acid to autoclaved Bold's medium.

Agar Concentration

T. odorata was also cultured in Bold's medium with various agar concentrations of 0, 0.1, 0.3, 0.5, 0.7, 1.0, 1.3, and 1.5%. To vary the agar concentration, the procedure of Hunter et al. (1966) was followed, i.e., the agar and the mineral medium of double strength were prepared separately, autoclaved, cooled to 50°C, and then mixed.

Strength of Culture Medium

Bold's basic medium of 0, 25, 50, 75, 100, and 200% strength were prepared by varying the amounts of distilled water added to the basic nutrients. The 100% or full strength medium served as the control and 0% was plain distilled water.

Vitamins, namely thiamine hydrochloride (Vitamin B_1), d-biotin (Vitamin H), and Vitamin B_{12} were used to supplement the culture medium. In all cases, the vitamins were first dissolved in distilled water as stock solutions, autoclaved, and then added to make concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, and 0.6 mg/1 in the case of thiamine, and 0.2, 0.5, 1.0, 1.5, 3.0, and 6.0 mg/1 for the other two vitamins.

Saturated solutions of different chemicals were used to vary relative humidities (Clayton 1967). These solutions were placed in petri dishes and a glass slide with the alga was placed above, supported by a watch glass inside. The petri dish was sealed with paraffin to maintain a constant relative humidity within the dish. The dish was placed on the laboratory bench at ambient temperature and standard lighting for 12 hours followed by 12 hours of darkness. Each was replicated thrice, using algal colonies of more or less similar size. Readings of length and breadth of cell as well as length of sporangia were made after a three-week culture period.

RESULTS

T. odorata consists of a series of subspherical to barrel-shaped to elliptical cells joined end to end in short chains. The cells are 9-17 um long and 7-15 μm wide with smooth walls of about 1 μm thick. The prostrate filaments are more developed than the erect, and in many cases only the former are found. Sporangia are sessile, terminal or intercalary, spherical to oblong in shape, and are 10-25 μm long.

When grown in either liquid or solid culture, the filaments became cylindrical and elongated. These filaments radiated from the central core of

original subspherical to elliptical cells and branched profusely. In liquid medium, the colonies were circular and compact, showing a heterotrichous condition. The typical cells developing in culture were longer than broad, measuring 29-58 μm long and 30-5 μm wide. Under the light microscope, the cells contained less carotene than those collected from walls. The chloroplasts appeared as parietal bands although discoid forms were also observed.

In culture, sessile and pedicellate sporangia developed laterally and are generally bigger than those observed in the natural habitat. The mature sporangia possessed a characteristic beak-like protrusion at the apical end.

pH of Culture Medium

The optimum pH was 7.5 in liquid culture, based on colony size, relative abundance of new colonies formed, and dimensions of apical cells (Table 1). Generally, growth was better with increasing pH. Sessile sporangia were noted between pH 4.5 and 6.5, these being terminal at pH 4.5 and 5.5, but lateral and confined to the older cells toward the center of the colony at pH 6.5 (Table 2). At the normal pH of 6.5, the cells were cylindrical and the colony profusely branched. The few small oil globules containing carotene were clustered at the center of the cells. With decreasing pH, the cells became barrel-shaped, then elliptical, and accumulation of carotene in the cells increased. On the other hand, increasing pH reduced **the** carotene content and the chloroplasts became prominent bands.

Table 1. Growth of *T. odorata* in liquid medium at different pH after 6 weeks

pH	Mean increase in diameter of colony (mm)	Mean size of apical cells (μm)*	
		Width	Length
4.5	0.05	9	29 (20-35)
5.5	0.75	4	35(17-46)
6.5	1.10	3	46 (32-58)
7.5	1.20	3	55 (40-70)
8.5	1.15	5	60 (46-70)
9.5	1.15	5	60 (46-70)

*n = 50.

Strength of Culture Medium

Dilution of Bold's medium depressed the growth of the alga, increased its carotene content, and caused a reversion of the characteristic cylindrical forms of actively growing cells to the elliptical shape of cells found naturally on concrete walls (Table 3). At nutrient strengths of 75, 100, and 200%, growth appeared normal, the cells remained green, and their shape was characteristically cylindrical. When the normal concentration (100%) of the

Table 2. Morphological characteristics of *T. odorata* grown in liquid medium at different pH after 6 weeks

pH	Cell shape	Morphological characteristics*		
		Color of filament	Sporangia	Carotene
4.5	elliptical (++) cylindrical (+)	yellow-green	sessile and terminal (++)	(+++)
5.5	elliptical (+) barrel-shaped (++) cylindrical (+)	yellow-green	sessile; terminal and lateral (++)	(+++)
6.5	cylindrical (+++)	yellowish-green (profuse branching)	sessile, usually lateral (+)	(++)
7.5	cylindrical (+++)	light-green	(-)	(++)
8.5	cylindrical (+++)	green	(-)	(+)
9.5	cylindrical (+++)	dark-green	(-)	(-)

*(-) absent; (+) sparse; (++) common/intense; (+++) very common/very intense.

Table 3. Cell shape and colony color of *T. odorata* in different strengths of Bold's liquid medium after 6 weeks

Concentration of Bold's medium	Cell shape*	Colony color
0%	elliptical (++) sub-spherical (+++)	amber
25%	elliptical (++) cylindrical (+)	yellow
50%	barrel-shaped (+) cylindrical (++)	yellow
75%	barrel-shaped (+) cylindrical (++)	yellow-green
100%	cylindrical (+++)	green
200%	cylindrical (+++)	green

*(+) sparse; (++) common; (+++) very common.

medium was doubled, the green colonies consisted of cells packed with chloroplasts, and the presence of carotene was hardly visible. Transferring the alga from the diluted 25 and 50% culture media to normal strength caused the cells to become green within four days and newly formed cells were cylindrical.

Agar Concentration

The mean number of colonies after eight weeks increased from 33 in the liquid medium (0%) to 390 in the medium with 0.7% agar (Table 4). Similarly, the mean number of sporangia formed per colony increased with higher agar concentrations up to 0.7%. At 1-1.5% agar concentrations, when the medium was solid, the mean number of colonies decreased. The mean number of sporangia per colony similarly showed a decline in the 1.3 and 1.5% agar media. At concentrations of 0.1-0.5%, the sporangia were sessile and confined to the central region of the colony while sessile and pedicellate sporangia developed all over the colony at agar concentrations of 0.7%; and above.

Growth characteristics also varied with different agar concentrations. In liquid and 0.1% agar media, the colonies were spherical and grew suspended in the medium. At a higher concentration of 0.3%, the colonies ranged from spherical to circular and flat, with the former suspended in the medium and the latter growing on the surface. At concentrations of 0.5% and above, the alga grew only on the surface of the medium with the circular colonies close to one another and the filaments radiating delicately from the center of each colony. In solid media of 1.3 and 1.5%, the circular colonies developed a heterotrichous habit with erect filaments growing from the center.

Table 4. Effects of agar concentrations on the growth of *T. odorata* after 8 weeks

% Agar	State of substratum	Mean no. of colonies	Sporangial no. per colony	Colony shape and formation
0	Liquid	33	8	Spherical, suspended in medium
0.1	Liquid	119	2	Spherical, suspended in medium
0.3	Gel	220	18	Spherical, suspended in medium and circular on surface of medium
0.5	Semi-solid	303	28	Circular and compact, on surface of medium
0.7	Semi-solid	390	87	Circular and compact, on surface of medium
1.0	Firm	14	70	Circular and compact, on surface of medium
1.3	Hard	10	12	Circular and compact, with aerial filaments
1.5	Hard	8	19	Circular and compact, with aerial filaments

Vitamins

The addition of thiamine hydrochloride to liquid medium increased algal wet weight through the enhancement of sporangia] formation which produced more colonies (Table 5). Most of the sporangia were intercalary or terminal in position, d-biotin and vitamin B_{12} did not affect the growth of the alga. The number of daughter colonies did not increase with the presence of either vitamin in the medium.

The addition of any of the three vitamins did not apparently affect cell size and shape. However, it was noted that d-biotin and Vitamin B_{12} at the higher concentrations enhanced carotenogenesis since the culture appeared more yellowish than those at lower concentrations. In the absence of vitamins, cultures were green.

Table 5. Effects of thiamine on growth of *T. odorata* after 6 weeks

Thiamine conc. (mg/1)	No. of sporangia	Mean no. of colonies	Mean wet weight of colonies (mg)	Cell length range (μ)*
0	0	25	30.0	26-49(35)
0.1	3	26	43.4	29-46(41)
0.2	10	34	122.2	38-46 (35)
0.3	12	42	140.6	32-46 (38)
0.4	24	76	234.5	41-49(44)
0.5	39	72	154.0	26-41 (35)
0.6	26	48	142.0	38-46(41)

*Figures in parentheses are means of 50 cells measured from the first to fifth cell after the apical cell.

Relative Humidity (RH)

Growth of the colony increased with increasing relative humidities (Table 6). In the absence of moisture in the ambient air or at 0% RH, colonies failed to form new cells nor increase in size, and turned uniformly green after three weeks. Branching of the filaments was sparse and the cells of the peripheral filaments were cylindrically elongated, a shape typical of the alga under liquid culture.

At 35% RH, branches developed from the peripheral filaments. The elliptical cells of the branches contained chloroplasts and numerous small oil globules filled with carotene to impart a light color to the colony (Table 6). At 55% RH, more profuse and longer branches were formed whose cells were longer and cylindrical but subspherical to oval at terminals. At higher humidities, the new cells were cylindrical and much elongated. The yellowish colonies had older central cells packed with carotene-containing

Table 6. Effects of relative humidity (RH) on the growth and morphology of *T. odorata* after 3 weeks

RH(%)	Mean increase in diameter of colony (μ)	Color of colony	Shape of new cells	Sporangial origin
0	0	Green		
32	30	Light yellow	Elliptical	
55	50	Pale green	Elliptical and cylindrical	
81	200	Yellow	Cylindrical	Intercalary
95	250	Yellow	Cylindrical	Intercalary and apical
100	1100	Yellow	Cylindrical	Lateral and apical

oil globules and younger peripheral cells with smaller globules and a less intense carotenoid color. At 95 and 100% RH, most of the apical cells of the peripheral filaments rounded off into sporangia. Many of these apical sporangia were empty, indicating that the zoospores had been liberated as confirmed by the presence of ten new colonies under an RH of 100%.

DISCUSSION

T. odorata is commonly found growing on walls of high-rise buildings in Singapore, especially in areas where there is an excessive runoff of rain water. The presence of the alga imparts an orange tinge to infested surfaces which turn blackish with the presence of blue-green algae. Although *T. odorata* is a green alga it appears orange due to the abundance of carotene which masks the chlorophyll. Carotene is regarded by Senn (1911) as a food reserve since its concentration declines when nutrients are readily available and growth is active. When moisture and nutrients are limiting, the alga accumulates carotene. Also, under shade conditions, the pigment may be completely lacking and the alga appears green (Fritsch 1971).

it has also been suggested that carotene protects the chlorophyll against phyto-oxidation under conditions of high light intensity (Griffiths et al. 1955). Thus, the accumulation of carotene by *T. odorata* growing on walls of buildings enables the alga to survive such an exposed habitat. The ability of the alga to withstand long dry periods also helps in its adaptation to a habitat where moisture is only available during rainy months. The presence of numerous, highly refractile fat granules of various shapes and sizes within the cells would also contribute to the alga's ability to survive drought (Fritsch 1916, Piercy 1917). The complete absence or paucity of large vacuoles in the protoplasm may likewise enable the cell to withstand dessication (Fritsch

1922). The ability of the cell walls to rapidly absorb atmospheric moisture, as observed in *T. aurea* (Howland 1929), is also another adaptation to dry conditions.

Geitler (1923) earlier pointed out that the appearance of cells of *Trentepohlia* under moisture stress and when nutrients are limiting closely resembles the resting stages of many other algae. This observation is supported by the morphological changes in the cells of *T. odorata* when cultured in mineral nutrients. The shortly stringed elliptical cells give rise to branched filaments of elongated cells, giving the impression of germinating resting cells. The newly formed colony, ball-like in liquid medium and circular in solid, takes a yellow-green appearance as neither carotene nor chlorophyll is the predominant pigment. In liquid culture and under decreasing pH, the cells revert to the natural elliptical shape and have a high carotene content. A reduction in relative humidity resulted in a similar reversion.

In its natural habitat where growth is extremely slow, *T. odorata* is usually sterile. The alga, with its short chain of elliptical cells reproduces vegetatively by cells which readily separate. During periods of rain, detached cells are washed down the surface as indicated by the characteristic vertical orange striations on walls infested with the alga. During dry periods, the detached cells are dispersed by wind as fine dust. Sporangia are rarely formed in nature but, when present, are sessile, terminal, or intercalary in position. In laboratory cultures, sessile and pedicellate sporangia are commonly formed and occur laterally. At pH 5.5 and 4.5 when cells revert to the elliptical shape, the sporangia formed are of the terminal sessile type rarely observed in nature.

The addition of thiamine to the liquid medium also enhances sporangia] production, hence, the formation of daughter colonies. However, the production of sporangia and daughter colonies is less than that in a medium with 0.7% agar. The necessity of vitamins for the growth of various groups of algae has been reported. A few members of Cyanophyta are auxotrophs with an absolute requirement for vitamin B_{12} (Van Baalen 1961). In *Ochromonas,* a Chrysophyta, auxotrophy for various vitamins (thiamine, biotin, B_{12}) is common (Provasoli 1958). Members of the Pyrrophyta, especially the marine dinoflagellates are similarly known to require vitamin B_{12} (Bold and Wynne 1978). Among the Chaetophorales (Chlorophyta), the first report of a member requiring an external source of vitamin is *Draparnaldiopsis* (Johnstone 1977). The positive response of *T. odorata* to thiamine (but not to biotin and B_{12}) is another record for the Chaetophorales.

The great increase in the number of daughter colonies in a semi-solid medium (with 0.7% agar) can be attributed to the zoospores requiring something solid to attach to prior to germination. These zoospores have been observed to swarm for some time after liberation from the sporangium. They

then come to rest, attach their anterior end to the surface of the glass slide, and rotate for a while before they withdraw their flagella and then germinate. The substratum partly solidified with 0.7% agar is presumably hard enough for the zoospores to attach to and soft enough for them to disperse around, thus providing a larger area for the zoospores to swarm and subsequently get attached prior to germination. When the medium is solid (with 1% agar), the zoospores would not be able to penetrate the surface for swarming.

T. odorata can be cultured easily in the laboratory using Bold's medium either in a liquid or solidified with agar. The alga prefers slightly alkaline (pH 7.5) environment although it can grow within a wide range of pH This preference for an alkaline environment is not surprising. In nature it colonizes whitewashed building walls as well as painted surfaces where the pH is distinctly alkaline.

The addition of 0.4 ppm thiamine enhanced growth and stimulated the production of sporangia which developed into numerous daughter colonies. Culturing the alga in a semi-soil medium (with 0.7% agar) also promoted the formation of daughter colonies.

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