CHRY5 2004

Exploratory investigations of palmella-stage formation in the Synurophyceae

by James L. Wee*, Alicia M. James & Craig S. Hood

Department of Biological Sciences, Loyola University, 6363 St. Charles Ave., New Orleans, Louisiana 70118-6195, USA. E-mail: wee@luc.edu

with 3 figures and 1 table

Abstract: Flagellated cells and statospores are acknowledged life history stages in the Synurophyceae. Although palmella stages were reported by Paechter & Conrad early in the twentieth century and Andersen documented the stage with light micrographs in his description of the Synurophyceae, palmella-stage formation in the class remains poorly understood. Therefore, a series of exploratory laboratory culture experiments were designed to investigate the incidence of palmella-stage formation among species, observe the process of palmella-stage formation, test the role of desiccation by air and salt in palmella-stage formation, and whether cells in palmella stages could be induced to re-emerge as flagellated cells. DV-IV was used as the culture medium in liquid cultures in re-emergence experiments as well as with 1% agar to test for palmella-stage formation. Palmella-stage colonies were induced in all 17 strains of Synura and Mallomonas examined and generally occurred after ca. 7–9 days incubation. No variation was observed in palmella-stage formation among ten isolates, as observed with light microscopy. The number of palmella-stage colonies formed on a plate increased significantly if at least 50% of the periphery of the plate was scaled. In all three of the strains examined, palmella-stage colonies formed in the presence of 0, 0.5, 1.0 and 2.0 ppm sodium chloride, but the number of colonies decreased as the salt concentration increased. Low numbers of flagellated cells of S. weire were observed in 100 mL cultures inoculated with a palmella-stage colony after one week, even in media lacking nitrogen or phosphorus. It is postulated that palmella-stage formation can be a life history stage utilized by syncryophytes for dispersal by semi-aquatic animals. Also, the gross morphological similarity between palmella stage colonies and the vegetative colony morphology of Tessellaria volvicina and Synura lapponica is discussed.

Key words: Synurophyceae, palmella stage, life history, desiccation, dispersal, Synura, Mallomonas

Introduction

The Synurophyceae are golden-brown flagellates closely related to the Chrysophyceae but placed into a separate class based upon the absence of chlorophyll c2, differences in flagellar root systems and a number of other characters (Andersen 1987, Andersen et al. 1999). A layer

* Corresponding author

of siliceous scales with approximately bilateral symmetry overlays the cells (Anderson 1987), and scale morphology forms the basis for species-level taxonomy in the group (e.g. Wee 1997). In plankton samples the Synurophyceae occur as biflagellated cells joined at the posterior to form colonies or as unisexuals where one flagellum usually is highly reduced. Other life history stages and processes are recognized for the Synurophyceae (e.g. vegetative cell division, stiatoспорes, palpemella stages, sexual fusion, naked amoeboid states), but are not commonly observed in field-collected samples (Sandgren & Flanagan 1986, Anderson 1987, Kristiansen 2001). Although dispersal mechanisms for the class are unknown (Kristiansen 1996, 2001), one report describes Synura sp. grown in media washed from waterfowl suggesting dispersal by waterfowl (Schilichting 1960).

In general, sexual reproduction occurs by simple cell division in a longitudinal plane beginning at the flagellated end of the cell (see Kristiansen 2002), increasing colony size in colonial forms by the addition of new cells. Sexual reproduction occurs via hologametic isogamy (Sandgren & Flanagan 1986). In unicellular species (e.g. Mamilomonas), isogametes resembling vegetative cells fuse to form a diploid zygote (reviewed in Kristiansen 2002). Sexual reproduction has been investigated in laboratory culture experiments only in Synura petersonii Korshikov (Sandgren & Flanagan 1986). In this colonial species a cell detaches from a parent colony forming a male gamete and fuses with a female gamete still attached to its colony. Both male and female gametes appear identical to a vegetative cell. The resulting zygote develops into a stiato spor, and individual colonies can produce multiple stiato spor cultures (Sandgren & Flanagan 1986). Very low frequencies of homothallic stiato spor production have been observed in S. petersonii cultures (Sandgren & Flanagan 1986, reviewed in Sandgren 1988).

The term “palmella stage” as used here follows Fritsch (1935), Christensen (1980) and Graham & Wilcos (2000). The term often is used synonymously with “palmelloid state or phase” to refer to those temporary algal life history stages where cells lose their flagella, sometimes settling on a substrate. Subsequently, they divide repeatedly while simultaneously producing a common envelope that envelopes the cells. Later, cells from a palmella stage will reacquire flagella and assume the flagellated growth form. The term should not be confused with those algal species living in a “palmellloid habit or growth form” (sometimes referred to only as “palmelloids”) where non-flagellated vegetative cells are retained permanently in a gelatinous matrix and only reproductive cells are flagellated (for a discussion see Fritsch 1935, p. 15; 41; Christensen 1980, p. 83; Graham & Wilcos 2000, p. 161).

In the Synurophyceae, the palmella stage is among the least documented and most poorly understood of all the life history stages, even being referred to as unimportant (Sandgren 1988). Palmella-stage descriptions for Synura and Mamilomonas were reported early in the twentieth century by Pascher (1912) and Conrad (1922) based upon field samples. Subsequently, Anderson (1987) illustrated the stage with light micrographs from biphasic soil-water cultures and observed that palmella stages could be collected from lake sediments and damp soils along shorelines. Given the paucity of information on palmella stages in the Synurophyceae, a series of exploratory culture experiments were designed to investigate the incidence of palmella-stage formation among a number of clonal isolates in the Synurophyceae, observe the formation process, test the role of desiccation and as an environmental trigger and whether cells in palmella stages could be induced to re-emerge as flagellated cells. Specifically, the roles of air and sodium chloride as environmental and physiological desiccants were used to test induction of palmella-stage.

Materials and methods
All experiments used liquid inocula spread on agar plates. The preliminary experiment inves-
tigated the incidence of palmella-stage formation after lengthy incubation among a number of
clonal isolates in the Synurophyceae. Experiment 1 used a single Synura strain to examine the
role of desiccation from air in palmella-stage formation by varying the number of holes in the
petri plate lids. In experiment 2 the formation of palmella stages was observed with light microscopy in several strains. The role of physiological desiccation was explored in ex-
periment 3 by adding varying levels of sodium chloride to the agar. Palmella-stage cells were also
added to liquid culture media to determine if flagellated cells would re-emerge from pal-
melia stages.

DV IV (Andersen et al. 1997) was used in all liquid cultures as well as in preparing stand-
ard 1 % agar plates for inducing palmella stages. One mL of rapidly growing cells from unial-
gal, clonal Synura or Mallomonas liquid cultures (Table 1) was spread evenly across the agar
plates and incubated at 18 °C and 4-11 μmol photons - m² - s⁻¹. All cell counts were con-
ducted with a hemocytometer counting cell. When cell concentrations were too low to provide
reliable data in liquid cultures, a 5 mL aliquot of the homogenized culture was fixed in acid-
difyed Lugol's solution (Wei 1983) and concentrated 5x by settling before counting.

In the preliminary experiment, the plates were sealed with Parafilm and incubated for 16
weeks. To test the role of air in drying cultures (experiments 1 & 2), the degree of desiccation
was controlled by sealing a portion of the plate margin with Parafilm exposing 0, 10, 50, 100%
of the periphery. New palmella-stage growth was recorded every day. For experiments
using salt as a physiological desiccant, NaCl was added at 0, 0.5, 1, or 2 ppm to the DV IV/
agar mixture prior to autoclaving and the entire plate periphery was sealed. Treatments in air
desiccation experiments were replicated five times and four times in the sodium chloride des-
iccation experiment. The treatments in experiment 2 were not replicated. The Quade test, a
non-parametric randomized block method, was used for statistical analysis for experiment 1
(Conover 1999; Quade 1972, 1979). One and two-way ANOVAs used for statistical analysis
in experiment 3 were conducted using the software package BIOSTAT 3.2 (1996, Applied
Biostatistics, Inc.).

Experiments investigating the induction of flagellated cells from palmella stages were con-
ducted in liquid cultures. The experimental treatments were designed by varying DV IV com-
ponents (replete or lacking one of the following: vitamins, trace metals, sodium metallicate, nitrogen as ammonium chloride and sodium nitrate, sodium glycercophosphate). One palmel-
la-stage colony of Synura usell Stein was added to each flask and incubated as described above.

Results and discussion

In a preliminary experiment where plates were incubated for 16 weeks, palmella stages were
observed (Figure 1) in all 17 strains studied, including seven species each of both Synura and
Mallomonas (Table 1). During visual examination of the plates in the course of the experi-
ment, it was noted that the culture medium liquid was absorbed by the agar before colored
palmella-stage colonies began to appear. This suggested a relationship between desiccation
and palmella-stage formation and was used to formulate experiments 1 and 2. These results
support the premise that palmella-stage formation is common and widespread in the Syn-
urophyceae under appropriate conditions.

The role of air as a desiccant for inducing palmella stages was explored in experiment 1
with a single strain of S. usell Stein et. Korschikov (Table 1). Palmella-stage colonies first
appeared after seven days in the treatment with 50% of the plate's periphery exposed to air
and after eight days in all other treatments (Figure 2). The number of palmella-stage colonies
Table 1. List of algal strains used in palmaella stage experiments. Prelim. Exp. refers to preliminary experiments (see text). Strain designations: W - J. L. Wee’s personal culture collection, W65 isolated by J. L. Wee, Aas - personal designation of Robert Andersen, CCMP - Provasoli-Guillard National Culture Center of Marine Phytoplankton, CHR - Loras College Freshwater Diatom Culture Collection isolated by David Czarnecki, FW - University of Washington Culture Collection isolated by Craig Sandgren, SAG - Sammlung von Algenkulturen der Universität Göttingen.

<table>
<thead>
<tr>
<th>Taxon Name</th>
<th>Strain</th>
<th>Strain</th>
<th>Prelim.</th>
<th>Exp 1</th>
<th>Exp 2</th>
<th>Exp 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Synura sphaegnicola Korsh.</td>
<td>W 1</td>
<td>And 3198</td>
<td>X</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. peterseni Korsh.</td>
<td>W 2</td>
<td>Sandgren 2 (Sandgren &amp; Platanin 1986)</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mallomonas papillosa Harris &amp; Bradley</td>
<td>W 0</td>
<td>CCMP 476</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. annulata (Bradley) Harris</td>
<td>W 14</td>
<td>CCMP 474</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aculeata Stein em. Korsh.</td>
<td>W 17</td>
<td>CHR-10</td>
<td>X</td>
<td>X</td>
<td>X</td>
<td></td>
</tr>
<tr>
<td>M. cuvata Ivanov em. Krieger</td>
<td>W 41</td>
<td>FW 644</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. aculeata Stein em. Korsh.</td>
<td>W 50</td>
<td>CCMP 870</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. maffeiipina (Petersen &amp; Hansen) Peterfi &amp; Morreu</td>
<td>W 52</td>
<td>CCMP 887</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. razilis Dürrschmidt</td>
<td>W 55</td>
<td>CCMP 477</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. razilis Dürrschmidt</td>
<td>W 56</td>
<td>CCMP 478</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. razilis Dürrschmidt</td>
<td>W 57</td>
<td>CCMP 479</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Synura sp.</td>
<td>W 65</td>
<td>Pool near Yarra River, Heidelberg, Victoria, Australia</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. adams Harris &amp; Bradley</td>
<td>W 67</td>
<td>CCMP 1783</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. akvokorsa Rutten in Pascher</td>
<td>W 68</td>
<td>SAG 54.88</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. splendens (G.S. West) Playfair em. Croome, Dürrschmidt &amp; Tyler</td>
<td>W 70</td>
<td>CCMP 1782</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. echinulata Korsh.</td>
<td>W 73</td>
<td>SAG 15.92</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>S. cartipinna (Petersen &amp; Hansen) Asmund</td>
<td>W 74</td>
<td>SAG 29.92</td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

peaked at day ten for treatments with 0%, 10%, and 50% exposure. The treatment with 100% exposure (i.e. none of the plate’s periphery was sealed) formed the largest number of palmaella-stage colonies on day eight which was the first day the stage was observed in this treatment. The Quade test results showed that numbers of palmaella-stage colonies formed in 0% and 10% air exposure treatments during the experiment were significantly different (and higher) than the 50% and 100% air exposure treatments (0.01 < P < 0.05, for application and interpretation of the Quade test, see Conover 1999). Presumably, those plates with greater exposure to air dried more rapidly, causing some cells to die before they can form palmaella-stage
colonies. From these results and the visual observations described above, we hypothesize that as the plates started to dry out, cells responded with loss of flagella, and palmella-stage colonies began to form by day 8. As the experiment continued, optimal conditions for colony formation were reached (day 10 for most treatments).

Experiment 2 was designed to test whether differences in palmella-stage formation could be observed with light microscopy (DIC, bright field) among different species of Synurophyceae. Strains of five species each of Symploca and Mallomonas (Table 1) were inoculated onto plates sealed on the periphery in varying degrees with Paraffin, as described above. No
difference was observed in the way the palmaella stage developed among the cultures. During the first three days, the cells were flagellated and motile in all treatments, as they would be found in nature. On day four the process of palmaella-stage formation would begin. No flagellated cells were observed at this point in the 100% exposure plates, whereas a few motile cells occurred in the other treatments. Following day four, the cells lost their flagella and settled onto the agar. It could not be determined whether they swam to the agar surface and lost their flagella or the flagella were lost first and then the non-motile cells settled to the substrate. When the cells were first observed to be non-motile, they had lost their flagella but had not necessarily formed the palmaella stage. On day five in all treatments, cloudiness of unknown origin appeared on the agar, possibly due to the loss of the flagella or the sloughing of the scales. By day six and seven, cells in all treatments had settled on the agar. The completely formed palmaella stage (i.e., non-flagellated cells enclosed in mucilage) was observed first on day eight or nine. At this time the cells appeared robust, healthy and continued dividing after the palmaella stage was formed, as indicated by the presence of a flat surface separating recently divided sister cells. Sister cells also appeared as enclosed in the same mucilage and surrounded by what is interpreted here as the same scale layer (Figure 1).

The goal of experiment 3 was to investigate whether physiological desiccation could induce or facilitate palmaella-stage formation. Although Synura and Mallomonas are freshwater organisms, they have been observed at salinities as high as 2.0 ppt in southeast Louisiana, USA (Wee, unpublished). Therefore, this experiment utilized sodium chloride to determine whether a physiological desiccant would increase the incidence of palmaella-stage formation.
in culture. Two strains of Mallomonas and one strain of Synura (Table 1) were used, and the number of palmella-stage colonies were counted after nine days of incubation, the time when the maximum number of new palmella-stage colonies occurred in previous experiments.

Palmella-stage colonies formed in all salt concentration treatments, with fewer colonies formed at higher salt concentrations (Figure 3). The two-way ANOVA results were highly significant for species ($F = 10.9, df = 2, P < 0.0002$) and salt concentrations ($F = 37.1, df = 3, P < 0.0001$). One-way ANOVAs testing for differences in colony formation within a taxon among treatments also were statistically significant for each of the three species. Multiple comparisons showed that species responded with statistically significant (and higher) palmella-stage colony formation at the 0 ppt salt treatment compared to other salt concentrations. Comparisons of species responses within a salt concentration treatment showed that the three species responded differently in the 0 ppt and 0.5 ppt treatments, with M. papillosa Harris & Bradley forming more colonies than the other two taxa. If NaCl had induced colony formation, more colonies would have been formed at the higher salinity treatments. Therefore, these results are not consistent with the hypothesis that the palmella stage could be triggered by the physiological effects of desiccation caused by increasing salt concentration. However, it is striking that although the Synurophyceae are not usually found in nature at salinities of 2 ppt, the taxa examined in this study were able to live long enough to develop the palmella stage at these higher salinity levels.

Fig. 3. Formation of palmella-stage colonies by three taxa in response to four salt concentration treatments. Mean colonies formed at the end of nine days incubation are plotted. Two-way ANOVA showed that there were statistically significant responses by both species ($F = 10.9, df = 2, P < 0.0002$) and salt concentration ($F = 37.1, df = 3, P < 0.0001$).
Induction of flagellated vegetative cells from palmaella-stage cells was accomplished, al-
though the experiments investigating the conditions necessary for induction were inconclu-
sive. Initially, the vegetative palmaella-stage induction experiments were performed at small
volumes (2 ml. maximum) in 24-well culture plates. However, after two weeks, none of the
pumella-stage cells regained their flagella and vegetative growth form. Subsequent feasibility
experiments indicated that vegetative stages could be induced in larger volumes when a single
S. novella palmaella-stage colony was placed into 100 ml of DY-IV in 250 ml Erlenmeyer
flasks. This led to an experiment that consisted of a control (i.e. replete culture medium) and
experimental treatments omitting key nutrients (i.e. vitamins, trace metals, Si, P, N) from liq-
uid DY-IV cultures. Small numbers of flagellated vegetative cells or colonies were observed
in at least some replicates of each experimental treatment. However, when the experiment was
terminated after one week, cell counts yielded values too low for reliable statistical interpre-
tation and resources were not available to repeat the experiment for a longer duration. How-
ever, it was clear that flagellated, vegetative cells could be induced from palmaella-stage cells
and that the induction warrants further investigation.

To summarize, palmaella-stage formation was observed in the experiment all species examined, suggesting it is ubiquitous in Sympyr and Mallomonas. The mechanism of palmaella-stage formation as observed by light microscopy is quite similar among those Sympyr and Mallomonas species examined (i.e. cells stressed by exposure to air lose their flagella, settle on the substrate and produce a copious gelatinous matrix which appears to move the scales away from the cell sur-
face). Palmaella-stage cells form readily under physiological stress from high salt concentra-
tions, suggesting that palmaella stage formation is not a fragile process. These results support
the hypothesis that the palmaella stage can be a significant component in the life history in
Sympyraceae. As viewed with the light microscope, the copious mucilage that separates the scales from the cell in the palmaella stage in the taxa examined is reminiscent of the morphology of two
other members of the Sympyraceae, Fessellaria volvocina Playfair and Stoma lapponica Skuja (compare fig 2 with Wee 2001, fig 2; Tyler et al. 1989, fig 1). In the flagellated vege-
tative stage of these two species, the scales surround the entire colony in a thick gelatinous
layer (reviewed in Wee 1997) and do not form an imbricate layer adnate on the cell membrane
as in the vegetative cells of all other Sympyr and Mallomonas species investigated to date. Fur-
thermore, recent phylogenetic evidence suggests that S. lapponica and T. volvocina are ances-
17 taxa examined in this study readily formed palmaella stages suggests the formation of this
life history phase could be a general feature of the Sympyraceae. Together these two as-
pects, (1) the commonality of palmaella stage formation in the Sympyraceae and (2) colony
morphology resembling the ancestral S. lapponica and T. volvocina linked to the palmaella-
stage morphology, lead to the speculation that palmaella-stage formation is an ancestral or ple-
siomorphic condition in the class. Furthermore, the hypothesis is proposed here that the pal-
maella stage is an intermediate developmental or evolutionary step between the colony
morphology of S. lapponica and T. volvocina and the adnate, imbricate scale morphology of
the other species of Sympyr and Mallomonas.

The role of the palmaella stage in the life history of the Sympyraceae still is unclear, but
may be involved in moving species to new geographic localities. Although Sympyraeae species
have been cultured from waterfowl feathers (Schlichting 1960), dispersal mechanisms for
Sympyraceae and Sympyraceae are poorly understood, and discussions emphasize the stat-
ospore as the dispersal agent (reviewed in Kristiansen 1996, 2001). However, statospores are
rarely observed in plankton-net collections and their presence in the water column may be so
seldom that dispersal via statospores might be a fortunate event. If so, the most significant
role of statospores in synephyte life histories may be as a perennation stage within the
same water body (e.g. Sundgren 1988). Based upon the experimental conditions and results presented here, the primary obstacle to palmaella stages as a dispersal agent is that it takes days for the stage to develop. Still, the possibility exists that stages could form in the feathers of waterfowl or the fur of semi-aquatic animals where frequent exposure to water via swimming or wading as well as capillary action might create a sufficiently moist environment for flagellated cells to form palmaella stages and be transported to another water body. A heavy gelatinous matrix surrounding cells, such as algae, could be expected to provide a buffer to desiccation if caught on the body of waterfowl or semi-aquatic animals and also would function as a sticky substance for adhering cells to animals or other transportable surfaces. For example, palmaella stages could form on floating vegetation that then acts as rafts for transporting algae on the water surface via the wind and currents where they would be released to colonize new water bodies. Small-bodied floating vegetation (e.g. Lemnaceae, Azolla, Salvinia etc.) with palmaella-stage colonies attached could provide additional insula
tion to desiccation when caught up by feathers or fur.

Acknowledgments

Funding was provided by a Richard Frank Grant from the Loyola Student Government to AMJ, as well as a grant from the Louisiana Board of Regents – Board of Regents Support Fund (contract no. LEQSF (2002-04)-RD-A-25) and the Mullaly Biology Endowed Fund at Loyola University to J.W. We would like to thank Dr. Stephen Sciarano of Loyola University for assistance with some of the statistical analyses.

References


