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Effect of growth rate on unicell production in two strains of *Scenedesmus* (Chlorophyta)

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P. SIVER AND F. TRAINOR. Effect of growth rate on unicell production in two strains of *Scenedesmus* (Chlorophyta). *Phycologia*, **22**, 127–131.

Two strains of *Scenedesmus* were examined in laboratory culture in a variety of growth conditions in an attempt to stimulate unicell production. The UTEX strain 614 did not form unicells at any time, even though others have recently implicated growth rate as a factor involved in unicell production. *Scenedesmus* strain 170, a known unicell producing organism, develops a unicellular stage in response to available ammonium and internal carbohydrate level. At constant growth rate, from approximately 5 to 100% unicells were produced by varying the nutrient base. Many spine-bearing members of this genus develop the unicell stage. At present a nutritional demand appears to be the common factor in triggering unicell production, but not growth rate.

INTRODUCTION

The question of unicell production in *Scenedesmus* (Chlorophyta) has plagued phycologists for almost 100 years. A unicell is a very distinct stage in the *Scenedesmus* life history. It is formed when the products of a recently divided cell fail to join and form a colony prior to release from the parent cell (Trainor, Cain & Shubert, 1976). Additionally, before release, the individual cells of spine-bearing species produce two or more spines at the pole of each unicell. Wolle (1887) first described unicells as a possible stage in a typical life history. Working with xenic cultures, some no doubt containing several species of algae, Chodat (1926) investigated unicell production in the spine-bearing species of the genus and also believed that this was a significant stage in a life history. However, because Chodat's cultures were sometimes bialgal, most of his work had to be confirmed in later studies.

Trainor & Hilton (1963) initiated a study of morphogenesis in the genus *Scenedesmus*, using established culturing techniques. Since that time, we have learned that even though many isolates produce such a unicell stage, a large number do not. Furthermore, unicells can be formed by both the spine-bearing and non-spine-bearing members of the genus. Pseudounicells can be produced in several ways (Fig. 1). First, a colony may fragment. With any spine-bearing member of this genus, this rarely happens. (If a 4-celled colony of *S. quadricauda* fragmented, it would have two 'unicells' with one spine at each of the apices and two spine-less 'unicells'. Upon close examination these could not be confused with true unicells.) Second, unicells may be mistakenly reported if the observer views a colony end on. Third, the last remaining cell of a colony, after the other cell(s) had divided and released their progeny (Fig. 1), might occasionally be tallied as a unicell, and recorded with that population (Gavis *et al.*, 1979). At all times good microscopy is needed for accurate enumeration. Finally, there is a nutritional basis to the formation of unicells in most strains examined (Trainor & Rowland, 1968; Siver & Trainor, 1981), but we have not yet seen a unifying principle.

Gavis *et al.* (1979), working with nitrogen-limited chemostat cultures grown under continuous light at 20°C, reported that unicells in the UTEX 614 strain of *Scenedesmus* were formed primarily at low growth rates. Fewer than 4% unicells (see their Fig. 1) were reported under all experimental conditions. These data were in direct contrast with work using other species (Trainor & Shubert, 1974; Swale, 1967; Siver & Trainor, 1981), as well as with previous observations we had made with the same 614 strain of *Scenedesmus*. In morphological (Trainor & Shubert, 1974; Trainor *et al.*, 1976) and EM studies (Bisalputra, 1965 or Trainor & Massalski, 1971) with UTEX 614 a true unicellular stage was never observed.

We will indicate why it is important to distin-

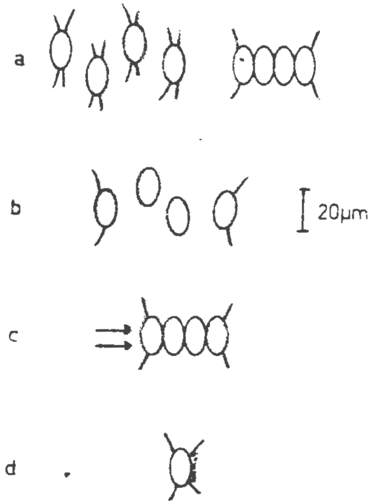

Fig. 1. Unicells and pseudounicells in *Scenedesmus*. *True unicells:* (a) formed during the division cycle. When four new cells fail to join, each produces several spines. Thus a 4-celled colony is equivalent to four unicells. *Pseudounicells:* (b) theoretical single cells if a colony, such as figured in (a), fragmented. (c) A pseudounicell might be tallied if a colony were observed in end view, with cells one above the other. The observation would be from an end view, in the direction of the arrows. (d) One cell remaining in what was a 2-celled colony.

guish between true unicells and pseudounicells; these two forms develop under entirely different physiological conditions and represent distinct stages in any life history. Eventually, in dealing with the taxonomy of these organisms it will be important to recognize the various morphological types in a polymorphic species, especially stages observed under natural conditions, and to understand their method of production.

MATERIALS AND METHODS

Two axenic strains of *Scenedesmus* were used in this study, UTEX 614 and strain 170. The first strain was chosen because it was studied by Gavis *et al.* (1979); the latter strain was selected because it is a known unicell producer, and some degree of control of this stage has been achieved.

Both organisms were grown in Bristol's medium (Siver & Trainor, 1981); strain 170 was cultured in both soil extract and Bristol's with various additives (Table 1). UTEX 614 was grown in Swan Lake Water (SLW), which has often been used in attempts to duplicate natural conditions (Trainor, 1976). Swan Lake is on the University of Connecticut campus.

Experimentation was conducted at 22°C, at photon flux densities of 60 or 100 μmol m^{-2} s^{-1} (16:$\overline{8}$ h photoregime) with a daily replenishment of nutrients. Both organisms were grown in a daily transfer system. With UTEX 614, we worked with an initial cell concentration of 10^5 cells ml^{-1}; cell concentrations soon adjusted to the carrying capacity of the two culture solutions used (see results). With strain 170, a sufficient aliquot of the culture was replaced daily to re-establish the initial concentration of 3.5×10^4 cells ml^{-1} (Siver & Trainor, 1981). Growth was measured as cell doublings per day.

Table 1. The mean doublings/day and percentages of unicells for *Scenedesmus* strain 170 grown in five media.

Medium	Doublings per day		% Unicells
	Mean	s.d.	
Bristol's	1.50	0.23	7
Bristol's + GA	1.51	0.30	7
Bristol's + NH_4^+	1.51	0.36	21
Bristol's + NH_4^+ + GA	1.51	0.155	71
Bristol's + NH_4^+ + GA + Vitamin B_{12}	1.61	0.154	93

Cells were grown at 22°C, 60 μmol m^{-2} s^{-1} and a 16:$\overline{8}$ h photoregime. Additions of ammonium, glycolic acid and vitamin B_{12} were 7.8, 39 and 0.5 mg l^{-1} respectively. Cells were transferred daily and the percentages of unicells were means for populations on day 4. An analysis of variance showed no significant (0.05 significance level) difference among the mean growth rates for the various media. However, there were significant (0.05 significance level) differences in unicell production between any two media except between Bristol's and Bristol's + GA (ANOVA and Tukey q).

RESULTS

Scenedesmus strain UTEX 614

This strain was examined for one week in a daily transfer system, at two different light intensities, in both Bristol's medium and Swan Lake Water (SLW). In all observations, using a 1:1 daily transfer there was approximately one doubling of the population daily. However, Bristol's medium supported 3.5× more cells than Swan Lake

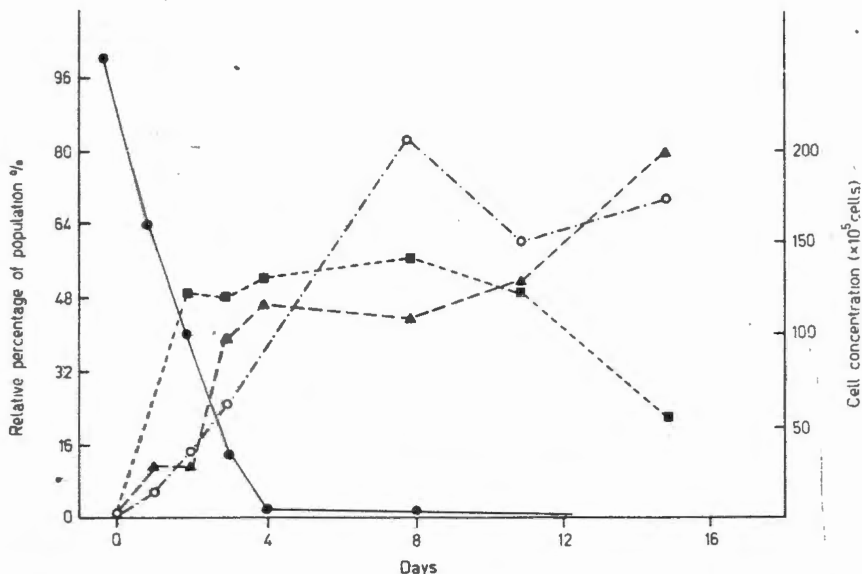

Fig. 2. The percentage of the population of *Scenedesmus* strain 170, grown in soil extract, but not transferred, expressed as unicells (●) and colonies. In time the population shifted to 2-celled (▲) and 4-celled colonies (■). The cell number (○) is expressed as cells per ml.

water. Also at the higher light intensity (100 μmol m^{-2} s^{-1}), SLW had sufficient nutrients to support 60% more organisms than at 60 μmol m^{-2} s^{-1}.

Unicells were not observed. Two-celled (few) and 4-celled colonies were found in Bristol's medium, while colonies with four or eight cells developed in SLW.

Scenedesmus strain 170

When grown in five media (Table 1), at 22°C, 60 μmol m^{-2} s^{-1} with daily transfer, mean growth rate ranged from 1.5 to 1.61 doublings per day (Table 1). However, mean unicell concentration ranged from 7% to 93%. There was *no significant difference* (0.05 significance level) among the mean growth rates, but there were significant differences in unicell production between any two media, with the exception of Bristol's and Bristol's with glycolic acid (ANOVA and Tukey *q*).

To further investigate the relationship between growth rate and unicell formation, *Scenedesmus* strain 170 was grown in four media at four different temperatures. There were significant differences (0.05 significance level) for both growth rates and unicell production between the middle (22–23°C and 28–29°C) and the extreme (15–17°C and 30–31°C) temperature ranges. For each medium, the greatest percentage of unicells was formed at the highest growth rates. In addition, within each temperature range the largest percentages of unicells were formed in two media, namely Bristol's-ammonium-glycolic acid or Bristol's-ammonium-glycolic acid-vitamin B_{12}.

When soil extract (SE) was inoculated with 100% colonies of *Scenedesmus* strain 170 and transferred daily, the population became 90% unicellular; the transformation took two days. It remained almost completely unicellular as long as daily transfers were maintained. Two doublings per day were observed in these experiments using SE. When daily transfers were terminated the unicell level dropped to less than 2% within four days (Fig. 2).

DISCUSSION

We confirmed most previous observations with UTEX 614 (Bisalputra, 1965; Trainor & Massalski, 1971), namely that it is not a producer of true unicells. Despite the fact that Gavis *et al.* (1979) discussed unicell production with this strain, we observed no unicells among the thousands of cells counted. We believe it unlikely that these differences in results are due to use of Woods Hole MBL medium in the 1979 study,

simply because UTEX 614 is not a unicell producer.

There is a great deal of variability among strains of this organism, and after a careful review of the literature, it was observed that there is quite a difference among strains in their ability to develop a unicellular stage. When 614 data are compared with information on other spine forming *Scenedesmus* species, UTEX 614 is found to be considerably larger than most species, one which grows at a slower rate than most, and a species which can form only the colonial stage. Also, there are several mechanisms involved in unicell formation, but there is no evidence that growth rate is one of these (see below).

In the absence of diagrams, one cannot be certain that Gavis *et al.* (1979) really observed a unicellular stage. Firstly, the percentages of unicells reported were 4% or lower. Secondly, they included in their enumeration some colonies which had released progeny from all but one cell. This colony was designated a 'unicell'. Thirdly, orientation of colonies on the counting slides may have compounded the problem. Colonies of most species of *Scenedesmus* are observed with all cells in a plane parallel to the microscope slide surface. On the other hand, UTEX 614 has a more random orientation; quite commonly one sees the end cell of a colony, with the other cell(s) perpendicular to the plane of the microscope stage. Such an organism can be tallied only after waiting for the specimen to move, or after gently tapping the slide. It is our opinion that Gavis *et al.* (1979) were enumerating what we have referred to as pseudounicells earlier in this paper. Until Gavis *et al.* (1979), there had been no reports of a relationship between growth rate and unicell formation in *Scenedesmus* (Swale, 1967; Trainor & Rowland, 1968; Shubert & Trainor, 1974; Overbeck & Stange-Bursche, 1966).

Growth rate, especially low growth rate, was not a factor involved in unicell production in any strain we have examined (Table 1, 614 data, unpublished data with other strains). Our results with *Scenedesmus* 170 clearly show that at constant growth rate, a very wide range of unicell percentages can be achieved (Table 1). In addition, when grown under conditions yielding lower growth rates, either at lower temperatures or when daily transfer was stopped and a fresh supply of nutrients was not available (Fig. 2), the percentages of unicells dropped significantly. Swale (1967), who originally isolated 170, observed that unicells developed during the exponential phase of growth when a fresh soil water bottle was inoculated. Trainor (1971) grew 170 in a defined dilute medium and used a daily transfer system. The orgnaism produced 100% colonies in the dilute medium, but was transformed to 100% unicells after two days upon the addition of soil extract. He concluded that a nutritional factor(s) in the soil extract stimulated unicell production. It appeared that the soil extract factor could be used up, for the effect lasted only temporarily, depending on number of cells in the inoculum and the amount of soil extract present. Unicell percentages increase upon addition of ammonium and an organic carbon source to a defined medium (Table 1) (Siver & Trainor, 1981). Nitrogen, as ammonium, was the main factor involved in unicell formation. Temperature and light regimes were indirectly related, depending on their involvement with the nitrogen nutrition (Siver & Trainor, 1981).

Even if the 1–4% 'unicells' reported by Gavis *et al.* for UTEX 614 were true unicells, results represented in their Fig. 1 do not show any trend towards increased unicell production under low growth rates. Colony formation and growth conditions will be analysed in a later paper.

In conclusion, *Scenedesmus* UTEX 614 does not have a unicellular stage. In those unicell producing strains which have been examined in detail to date, nutrition (e.g. presence of ammonium, soil extract or phosphate) appears to be the most important factor in stimulating formation of unicells. Low growth rates are not involved.

Eventually, we may find a master mechanism explaining control of the unicell stage in all strains, but at present we have a somewhat complicated nutritional picture. Essential to our being able to piece together the complete explanation and understand the mechanisms involved in achieving various developmental stages in *Scenedesmus* will be the abilities of investigators to describe accurately all of the morphological expressions of individual strains.

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