Evidence of Genetic Recombination in *Cyanidium caldarium*

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Introduction

Cyanidium caldarium has attracted the interest of biologists since it was first described by Tilden in 1896. This thermoacidophilic alga has an optimum growing temperature of 45° C. and has been collected from the margin of hot springs where the acidity may fall below pH 2. In recent years there has been considerable research on *C. caldarium*, with more than one-hundred papers dealing entirely or in part with this organism published world-wide during the five-year period ending 1984.

Much attention has been given to the classification of *C. caldarium*. Mary Belle Allen has traced the early attempts in the classification of this organism (1). She notes that Tilden in 1896 first considered it a blue-green alga, calling it *Chroococcus varius*, but later thought it better classified a green alga, naming it *Protococcus botryoides f. caldaria*. She further notes that Geitler in 1936 proposed a new genus of Cyanophyta for the alga and named it *Cyanidium caldarium*. Her own position in 1959 held it to be an anomalously-pigmented chlorophyte. Recent advances in technology have made possible many biochemical findings that are an aid in taxonomic studies. Continuing improvements in electron microscopy have made possible more detailed morphological observations. Still, the taxonomic position of this organism remains in question as reported by Ford in 1984 (3). Ford cites recent literature in support of three different taxonomic positions 1) that *C. caldarium* is an anomalously-pigmented rhodophyte, 2) that it is a transitional form between the Cyanophyta and Rhodophyta, and 3) that it is a colorless, eukaryotic alga with an endosymbiotic cyanelle.

Electron microscopic studies of *C. caldarium* reported by Ford (1984) reveal the presence of a definite nucleus, chloroplasts, mitochondria, ribosomes, vacuoles, and other fine details. De Luca, *et al.* have reported endospore formation (2). Moreover, differences in cell size, organelles, inclusions, and storage products led them to consider the complex to consist of two species.

C. caldarium is characterized by chlorophyll *a*, carotenoid pigments, and two phycobilins, phycocyanin and allophycocyanin. In 1960 Nichols and Bogorad reported the isolation of several UV-induced mutants of this alga that varied in pigment formation, including two used in this study (4). These are mutant III-C which synthesizes chlorophyll *a* and the carotenoid pigments but no measurable phycobilins, and GGB, a chlorophyll-less mutant producing only the carotenoid pigments and the phycobilins. Each is readily distinguishable in its coloration from the other and from the dark-green color of the wild-type cell.

The authors know of no reports of genetic recombination in this species. The availability of these two mutants so easily identified by their distinct colorations seemed ideal material with which to attempt matings to determine if recombinations do occur.

Materials and Methods

Wild-type cells of *C. caldarium* and cells of the mutants III-C and GGB were grown separately in plates in the dark at 43° C. for 10 to 14 days on an agar medium previously described by Nichols and Bogorad (5). Plate cultures of the three types were then washed with a liquid medium into separate 250 ml flasks, 80 ml per flask. The cell suspensions were shaken at room temperature over fluorescent light until pigmented. Suspensions of III-C and GGB were mixed and the mixture redistributed

equally between the original flasks. These mixed-cell suspensions as well as unmixed suspensions of the mutants and suspensions of wild-type cells were then shaken over light for an additional 7 to 10 days.

Cell counts of the suspensions were determined using a hemocytometer and dilutions prepared to provide from 100 to 300 cells per 0.5 ml of the diluted suspension. Agar medium plates were inoculated with 0.5 ml aliquots of the diluted suspensions and the plates spun. The cultures were incubated at 43° C. in the dark for 10-14 days at which time nonpigmented colonies of cells could be seen distributed over the surface of the agar medium. The plates were then placed under fluorescent light at room temperature until pigmentation of the colonies permitted identification of the cell types.

Results

Following pigmentation of the cells, plates of the diluted, mixed-cell suspensions, exhibited three kinds of colonies. The majority of the colonies showed the pigmentation characteristic of III-C or GGB-type cells. Some colonies on each plate were, however, unlike either III-C or GGB, showing the pigmentation characteristic of wild-type cells. The percent of colonies showing the dark-green coloration typical of wild-type cells, hereinafter called hybrid colonies, was variable, ranging generally from 8 to 12 percent. Numerous mixed-cell suspensions were diluted and plated during the course of the investigation; all showed similar results.

Dilutions of unmixed III-C and GGB cell suspensions were also plated. When pigmented the colonies were of the parent cell types only, and showed no evidence of wild-type colonies.

Cells of each of the three differently pigmented colonies found on plates inoculated with the mixed-cell suspension were transferred to separate plates and cultured to provide sufficient cells for spectrophotometric analysis. Whole-cell suspensions of each type were run on a Beckman DU-7 spectrophotometer interfaced with an Apple II computer which drove a Bausch and Lomb DMP-29 digital plotter. The absorption spectrum of cells of a hybrid colony is shown in Figure 1, and is seen to be similar to the absorption spectrum of intact wild-type cells of this organism as shown here and as previously reported (5). The absorption maximum at 680 nm identifies chlorophyll *a*, while the absorption at 625 nm identifies the phycobilins. These observations are presented as possible evidence of genetic recombination between the two parent cells, III-C and GGB. Further studies are continuing to determine possible segregation of the pigmentation characteristics in subcultures of the hybrid.

Conclusion

Genetic recombination in *C. caldarium* could provide an additional approach to the ultimate classification of this organism. Most efforts in its classification have dealt with ultramicroscopic studies of the cell's fine structure or biochemical studies of its pigments, storage products, and metabolism. Knowledge of the nature of recombination whether by conjugation, syngamy, or other means, and the extent to which control of pigment formation is nuclear or extrachromosomal, or shared between the two, could assist in its classification.

The possibility of inducing genetic recombination between variously pigmented mutants of *C. caldarium* could also provide a means of securing further information of the synthetic pathway of pigment formation.

Literature Cited

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