Isolation and Characterization of Some UV-Induced Mutants of *Chlorella vulgaris* Prings.

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Clones of new UV-induced mutants of *Chlorella vulgaris* are reported, including two strains darker green than the wild type. The isolated strains were characterized as to relative UV survival and general mutability. Significant stimulation of growth after low doses of UV irradiation was obtained. A taxonomic criterion distinguishing *C. vulgaris* and *C. pyrenoidosa* was recognized. Diameter measurements of cells grown under uniform environmental conditions differentiated *C. vulgaris* from *C. pyrenoidosa,* and various mutant clones within an initial isolation. Various histochemical and aberrant cytological features of mutant strains were observed. Thin-layer chromatography of extracted pigments demonstrated several that differed from those of the normal cells.

MUTATIONS, SPONTANEOUSLY occurring in natural populations or artificially induced, are abrupt changes in the genetic makeup of the organism, manifesting themselves phenotypically as altered biosynthetic capabilities through succeeding generations. These changes may result in detectably altered physiology and morphology. Metabolites having no counterpart in normal cells may be found.

Chlorella is especially suitable for mutation studies because it has a haploid nuclear complement, short life cycle, and is autotrophic and unicellular.

A naturally occurring mutant, *Chlorella rubescens*, was reported by Chodat in 1929 (1). Beijerinck (2) obtained yellow and colorless colonies of *C. variegata* in 1940. The work of Beadle and Tatum (3) influenced Davis (4) to investigate some mutant strains of *Chlorella* to elucidate the photosynthetic process, while Granick (5) studied the protoporphyrin compounds of *Chlorella* mutants involved in the biosynthesis of chlorophyll. Butler (6) isolated several UV-induced mutants of *C. pyrenoidosa* and characterized them as to starch synthesis, plastid formation, cell size, and changes in pigmentation. In 1963 Kvitko (7) used X-ray irradiation to induce a large number of different types of mutants and reported a synthetic classification for them. This classification included photosynthetic, nonphotosynthetic, biochemical, and gross morphological mutants. Zakharov (8) reported the isolation of several auxotrophic mutants of *Chlorella* by replicating and characterized two of them as arginine and thiamine deficient strains.

Previous reports on the study of selected *Chlorella* mutants have not included a procedure for the isolation of pure clones. Initial isolations were assumed to be uniform strains. This report characterizes some new UV-induced mutant clones and compares them with their corresponding wild type. In addition to changes in pigmentation other phenotypic observations were made to test the possibility of multiple mutations occurring simultaneously in the same strain. Adaptive effects of UV irradiation and increased mutability were considered.

A survey of the literature (9–14) and consideration of the slow growth rate previously mentioned prompted examination of cytological and histochemical methods which require only small amounts of material for intensive study. Flaumenhaft *et al.* (15) applied histochemical stains to normal and deuterated *Chlorella* and *Scenedesmus* cultures to study differences in carbohydrates, lipids, proteins, nuclear material, and population cell size.

Davis (4) reported absorption spectra of total extracted pigments of *Chlorella* mutants, but did not separate the pigments. Strain (16) isolated the chlorophylls of normal and deuterated *Chlorella* and *Scenedesmus* while Allen (17)

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investigated various carotenoid fractions of a normal strain of *C. pyrenoidosa* and some selected mutants. To understand the biochemical changes occurring in abnormally pigmented mutants, the individual pigments should be considered. Since populations of pure clones were obtained, variations in pigment production could be attributed to the genetic constitution of the strains considered. Elucidation of the pigment changes which occurred further characterized the various mutants studied.

EXPERIMENTAL

Materials and Methods—Wild type cultures of *C. vulgaris* (13402-R. W. Krauss strain Pringsheim 211/11a.) and *C. pyrenoidosa* (ATCC 11469-R. W. Krauss strain Pringsheim 211-8b) were used. Mutants were produced entirely by UV irradiation of this *C. vulgaris* strain. Both strains were subcultured on glucose medium every 10 days. Mutants were subcultured on glucose medium periodically to maintain them in the logarithmic growth phase.

Cultures were grown on Myers' (13) salt medium modified by deletion of his "possible micronutrients" and use of sequestered iron. Glucose medium contained in addition to salts, 1.5% glucose at pH 5.5. In early growth experiments a complete medium was used consisting of Myers' salt solution, 1.5% glucose, 1.5% casein hydrolysate, and 0.5% yeast extract. Since growth was better on glucose medium, complete medium was discarded.

All cultures were grown under aseptic conditions. To ensure sterility, plates, slants, and liquid media were incubated for 3–4 days at 32° before inoculation.

Growth Conditions—All cells were grown in a specially designed growth room with the temperature and humidity continuously controlled and recorded. The mean temperature was 23.6 ± 0.5° with a range of 22.2–25°. The relative humidity was maintained at 50% ±5%.

Light was supplied to the growing cells by 21 fluorescent lamps adjusted to 121.9 cm. (48 in.) above the table surface.[1] Photometer readings at table top level with new lamps were recorded as 500 ftc. Cultures were given continuous light.

Petri dishes and slants held in wire racks were placed on the table top with the colonies facing the light source. Liquid medium was placed on a horizontal shaker regulated to give twenty-four 500-ml. flasks 150 r.p.m., thus providing aeration. No additional means of introducing sterile air or carbon dioxide was used.

Growth Rate—Growth rate was determined by daily measurements of packed cell volume, culture turbidity, or cell counts in a hemocytometer. The cells were harvested by scraping or washing solid media or centrifuging liquid cultures.

Cell Size—Diameter measurements of cell populations in the logarithmic growth phase mounted in water were taken with a calibrated ocular micrometer. The diameters of approximately 1,000 cells were measured for each strain to provide an adequate sampling to ensure statistical validity.

Irradiation—All irradiations were accomplished by a single 15-w. germicidal lamp[2] giving off most of its energy at the wavelength of 2537 Å. An individual lamp was used for 100 hr. and then discarded. The lamp was permanently situated in a fluorescent fixture suspended 30.5 cm. (12 in.) above the table top with a white cardboard shield around the lamp to protect the investigator. The UV irradiation/sec./sq. cm. was standardized.[3]

The cells to be irradiated, in the logarithmic growth phase, were washed from slants and serially diluted. Aseptic aliquots of the dilutions were counted in a hemocytometer and the dilution representing 1×10^4 cells/ml. was used. One-milliliter aliquots were spread to a monocellular layer on the surface of sterile glucose medium Petri dishes.

The inoculated plates were then placed under the irradiation lamp and the covers removed to allow UV penetration. Any contaminated plates were discarded. Initial experiments indicated that a 30-sec. dose of UV irradiation produced no mutations, even with replica plating screening procedures, while 5-min. irradiation produced apparently discolored mutants.

Of the surviving colonies, obvious mutants were aseptically transferred to glucose medium slants and allowed to multiply. The isolations surviving this transfer were labeled with letters of the alphabet (16 of the 55 isolations survived). To obtain pure clones, each isolation was serially diluted. Aliquots containing 1 cell/ml. were transferred to glucose plates and allowed to grow. Colonies of each of the 16 types were labeled with numerical subscripts and continued through at least three subinoculations before consideration as established mutants (Table I). At least 75 days of growth were needed to obtain enough cells of these pure strains to begin characterization.

Survival, Lethality, and Mutation—The mutation index for a strain was defined as the percentage of colonies demonstrating a color difference from the cells on the control plate (0 UV) per 100 colonies counted in the population of surviving irradiated cells. Such indexes were determined for each of the six major mutant strains investigated as well as the two wild types.

Survival (survival capacity) is defined as the mean number of surviving colonies receiving a particular dose of UV compared with the mean number of colonies on the respective control plate for each strain (× 100%).

Lethality is defined as 100% minus the survival capacity.

Histochemical Methods—Cells of the various strains in the late logarithmic phase on slant cultures were mounted in water. The various histochemical stains were applied directly to the slide. These stains included the periodic acid-Schiff test for complex carbohydrates (18), acridine orange for mucin (19), ruthenium red for pectin (18), sudans III and IV for lipids (20), mercuric bromophenol blue for proteins (21), and iron-hematoxylin for nuclear material (22). After a suitable time for these various colorimetric reactions had elapsed observations were made microscopically. Care was taken to treat

[1] General Electric F 6 T 6 Cool White lamps.

[2] General Electric lamp No. G15T8.

[3] Weston Electronic Instrument Corp., model 703 type 3.

TABLE I—OBSERVATIONS OF COLONIES AND CELLS OF MUTANT STRAINS OF *Chlorella* GROWING ON GLUCOSE AGAR MEDIUM

Strain	Color of Colony	Spreading Characteristics on Agar Surface	Gelatinous Sheath	Cellulose Wall	Chloroplast Structure	Pigment Differentiation	Cell Divisions	Spore Formation
C. vulgaris	Green	Thickly covers entire surface	Normal	Normal	Normal	Normal	Normal	Normal
C. pyrenoidosa	Green	Thickly covers entire surface	Normal	Normal	Normal	Normal	Normal	Normal
A-1	Golden-green	Thickly covers entire surface	—	—	—	—	—	—
A-2	Light-green	Thickly covers entire surface	Thinner	Less prominent	—	—	—	—
A-3	Light-green	Thickly covers entire surface	Thicker	—	—	—	Unequal	—
C-1	Yellow	Film over surface	—	—	Irreg. granular	Red spots on chloroplast	—	—
C-4	Golden-brown	Covers entire surface	Thinner	Not visible	Irreg. granular	No chlorophyll visible	—	—
C-5	Yellow-orange	Covers entire surface	—	—	—	—	—	—
C-7	Golden-brown	Covers entire surface	—	—	—	Discrete spots on chloropl.	—	—
C-8	Golden-brown	Covers entire surface	—	—	—	—	—	Increased over normal
C-9	Golden-brown	Covers entire surface	Thicker	Crenulated	—	Discrete spots on chloropl.	—	—
C-10	Golden-brown	Covers entire surface	None evident	More prominent	Cytopl. ridges	No chlorophyll visible	—	—
C-11	Yellow	Film over surface	Thinner	Crenulated	—	—	—	—
E-4	Dark-green	Covers entire surface	Thinner	Less prominent	—	Darker green	—	—
E-5	Dark-green	Covers entire surface	Thicker	Crenulated	—	Darker green	Unequal	—
F-4	Yellow-green	Covers entire surface	Thinner	Crenulated Less prominent	—	—	—	—
H-3	Light-green	Covers entire surface	—	—	—	—	—	—

the mutants and wild types concurrently with each stain as well as to compare the unstained counterpart for each strain.

Chromatography and Quantitation of Pigments—Initial paper and column chromatographic separation of plastid pigments proved unsatisfactory for this work. Thin-layer chromatography (23) provided finer separation, less tailing, and the rapidity necessary to avoid photocatalysis of the various constitutents.

Commercial sources of carotenes were satisfactory, but extraction of chlorophylls and xanthophylls of leaf tissue were necessary to obtain standards for chlorophylls A and B and the xanthophyll group. Action spectra of the leaf pigments provided proof of identity and purity.

Cells representing 1-month growths in the logarithmic phase (several successive cultures) for each strain were harvested by washing and centrifugation. They were combined and then frozen. When needed for use the frozen cells were thawed, treated with liquid nitrogen for 15 min., then extracted exhaustively with acetone. The pigment-solvent mixtures were combined for each strain and extracted exhaustively with petroleum ether (b.p. 30–60°). The petroleum ether fraction was evaporated in a vacuum desiccator to a minimal volume and quantitatively transferred to 10-ml. volumetric flasks and brought to final volume. Aliquots of these solutions were spotted on activated Silica Gel G plates, and developed in the following solvent mixture: petroleum ether–benzene–absolute ethanol (40:15:4) according to the method of Schaltegger (23). After development the solvent front was marked; the spots were outlined; notations were made as to color and R_f values; and comparisons of various compounds were made.

Elution and spectrophotometric determination of the separated pigments were attempted. The slow growth rate of the various strains and low concentrations of pigments limited further comparative quantitative work.

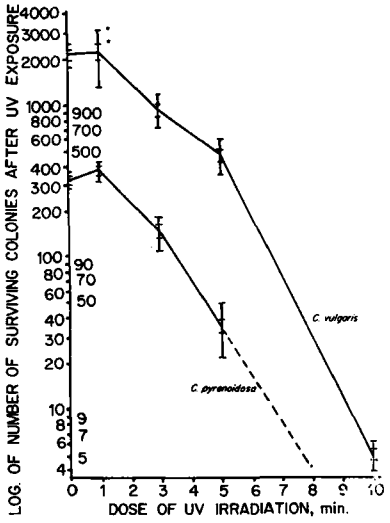

Fig. 1—Survival of C. vulgaris *and* C. pyrenoidosa *after exposure to UV irradiation under standardized conditions. Key: *, wider deviations are standard deviation; narrower deviations are standard error of the mean.*

RESULTS

Macroscopic Observations—The major discernible macroscopic feature differentiating the mutants from the wild types was color (Table I). Mutants C-1 and C-11 distributed themselves across the agar surface of glucose medium slants as a thin film while the other mutants and normal cells formed a thick layer. All mutants and the wild types were shiny while growing on glucose medium.

Test for Glucose Dependency—When the cells from each strain were inoculated on both glucose medium and salt medium, it was found that all of the strains were capable of growth on salt medium. All of the mutants were therefore classified as photosynthetic mutants according to Kvitko's classification system (7).

Comparative Survival After UV Exposure—Strains *C. vulgaris*, *C. pyrenoidosa* A-1, A-2, C-1, C-11, E-4, and H-3 were each exposed to 0, 1, 3, 5, and 10 min. of UV light and their relative survival compared (Fig. 1 and Table II). In all of the strains except E-4 there was a slight increase in survival over that of the control at low dosages of UV, though high doses produced the expected decrease. Mutant E-4 decreased in survival even at low doses of UV while its survival after high doses was greatly reduced. Strains H-3, A-2, *C. pyrenoidosa* were significantly more resistant to the lethal effects of UV than the wild type ($p < 0.01$ in Table II).

Mutation Indexes—With increasing doses of UV the mutation rate of the respective strains became

TABLE II—RELATIVE SURVIVAL OF SELECTED STRAINS OF *Chlorella* AFTER 1-MIN. AND 5-MIN. EXPOSURES TO UV IRRADIATION

Strain	Percent Increase, 1-min. UV[a]	Probability That This Culture Has Same Survival Capacity as its Control	Index of Survival, 5-min. UV	Probability That This Culture Has Same Survival Capacity as *C. vulgaris*
C. vulgaris	15.59	$p < 0.01$	11.59	$p = 1.00$
C. pyren.	6.33	$p > 0.40$	22.22	$p < 0.01$
A-1	0.21	$p > 0.20$	5.98	$p < 0.10$
A-2	15.59	$p < 0.02$	52.15	$p < 0.01$
C-1	10.84	$p > 0.20$	9.77	$p > 0.20$
C-11	12.93	$p > 0.2$	4.94	$p < 0.05$
E-4	−11.49	$p > 0.2$	7.43	$p > 0.2$
H-3	20.62	$p < 0.005$	23.47	$p < 0.005$

[a] Surviving colonies at 0-min. UV—surviving colonies at 5-min. UV/surviving colonies at 0-min. UV × 100%.

TABLE III—RELATIVE MUTATION INDEXES[a] OF SELECTED STRAINS OF *Chlorella* AFTER 3-MIN. AND 5-MIN. EXPOSURE TO UV IRRADIATION

Strain	Mutation Rate[b] After Exposure to 3-min. UV, %	Probability That This Culture Has Same Mutation Rate as *C. vulg.*	Mutation Rate After Exposure to 5-min. UV, %	Probability That This Culture Has Same Mutation Rate as *C. vulg.*
C. vulg.	0.04	$p = 1.00$	3.93	$p = 1.00$
C. pyren.	0.24	$p < 0.01$	2.83	$p > 0.4$
A-1	6.40	$p < 0.005$	22.22	$p < 0.005$
A-2	2.63	$p < 0.005$	6.11	$p < 0.20$
C-1	14.00	$p < 0.005$	19.23	$p < 0.005$
C-11	0.20	$p < 0.005$	37.17	$p < 0.005$
E-4	—	—	63.31	$p < 0.005$
H-3	15.43	$p < 0.005$	32.47	$p < 0.005$

[a] Mutation index = probability of pigmented mutant/probability of any mutation $\simeq$ constant. [b] Number of colonies (per 100 colonies counted) having a color other than that of the control plate, respectively, for each strain.

greater (Table III). All of the mutants had mutation rates significantly higher than the wild type. At 5-min. UV the differences in mutability were not statistically significant. The mutation rates of *C. pyrenoidosa* and *C. vulgaris* were not statistically different at the 1% level.

Size Measurements—The mean diameters of all of the mutants were found to be larger or smaller than the normal cells ($p < 0.05$–0.005), but with no uniform pattern. The normal cells had a smaller range of size than the mutants. The strains were arranged from smallest to largest (Table IV). Mutant E-5 had the greatest standard deviation and demonstrated unequal divisions of the cells. Contrary to the report of Retovsky (11), note should be taken that *C. vulgaris* and *C. pyrenoidosa*, under uniform environmental conditions, can be differentiated by the criterion of cell size ($p < 0.005$, Table IV). Comparison of cell size variation about the means of particular clones within an initial isolation (C and E but not A) demonstrated significant differences. If initial isolations were pure clones such variations would not be expected under uniform environmental conditions.

Histological Observations—The periodic Schiff reaction demonstrated the presence of complex carbohydrates in the walls of all cells, but this test as well as the acridine-orange test did not demonstrate any significant difference between the mutants and the normal strains. Ruthenium red did not differentiate *C. vulgaris* from *C. pyrenoidosa* as reported by Kessler (18), but did differentiate mutants A-1, C-5, and C-11 as having no pectin present. In all strains the mucilaginous envelope appeared to contain the greatest concentration of lipids. Mutants A-1 and A-2 appeared to contain less lipids than any of the others.

No significant differences could be found in the protein and nucleic acid contents of the respective strains.

Cytology of Irradiated Cells—Cytological observations indicated differences in the gelatinous envelope, the cell wall, chloroplast structure, cell divisions, formation of spores, appearance of the protoplast, and pigment differentiation. The aberrations from the normal are summarized in Table I.

Chromatography and Comparison of Pigments—*Chlorella vulgaris* had a yellow carotene at R_f 0.93 but *C. pyrenoidosa* had a yellow compound at R_f 0.82. Mutants A-2, A-1, E-4, and H-3 had orange, red, yellow-orange, and red-orange spots, respectively, at R_f values of 0.82, 0.82, 0.87, and 0.90. No carotene compounds were observed in C-1 and C-11 (Fig. 2).

Mutant E-4 was visibly darker green than *C. vulgaris*. On the TLC plate it contained 5 chlorophyll spots instead of the 4 found in the other strains tested. Mutant C-1 demonstrated no chlorophylls, and C-11 had only minute amounts of 3 chlorophylls, though the poor growth rate of these strains and the quantity of compounds available for extraction were probably responsible. Mutants A-1, A-2, E-4, and H-3 produced a reddish spot at R_f 0.25 while both wild type strains had none (Fig. 2).

TABLE IV—CELL SIZE (DIAMETER) OF NORMAL AND MUTANT *Chlorella* STRAINS

Cell Type	Number of cells	Mean Diameter, μ	$\pm$ *SD*	*SEM*	Probability
C-1	912	6.45	±1.92	0.04	0.005
C-8	1313	7.80	±1.91	0.05	0.005
C-10	1068	8.16	±2.14	0.01	0.005
C-9	688	8.22	±1.69	0.11	0.05
A-2	1298	8.35	±1.46	0.05	0.005
A-1	1202	8.45	±1.53	0.05	0.005
C. vulg.	1127	9.02	±1.89	0.06	—
C-5	1243	9.07	± .82	0.02	0.01
E-4	1153	9.10	±1.65	0.05	0.005
C-7	903	9.18	±2.08	0.09	0.05
C-11	1327	9.39	±2.12	0.06	0.005
C. pyr.	1333	9.42	±2.07	0.09	0.005
H-3	944	9.51	±2.12	0.07	0.005
E-5	1382	10.10	±2.20	0.06	0.005

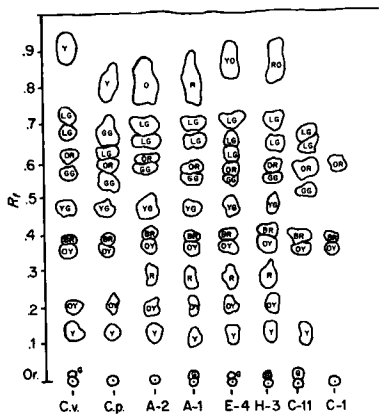

Fig. 2—Scale representation of thin-layer chromatogram of pigments extracted from Chlorella *mutants. Key: G, green; R, red; O, orange; OP, orange-pink; GG, gray-green; BR, brown-red; OR, orange-red; LG, light green; OY, orange-yellow; Y, yellow.*

DISCUSSION

With the minor modifications noted, Myers' (13) medium appears to be highly satisfactory for mutation studies with *Chlorella* species. Addition of yeast extract and casein hydrolysate did not enhance growth as might be expected, but actually reduced the volume of packed cells obtained.

All of the strains were able to grow on the salt medium but growth on glucose medium was faster and resulted in larger volumes of packed cells. Because of the decreased growth rate of the mutant strains relative to *C. vulgaris*, glucose medium was used to stimulate growth. No error in carbohydrate metabolism could be found in any of the mutant strains.

Redford and Myers (24) reported a slight increase in survival capacity of normal *C. vulgaris* irradiated with low doses of UV but they did not statistically document their finding. When the increase of the mean survival at 1-min. UV exposure was compared with the controls for each strain, respectively, it was found that *C. vulgaris* ($p < 0.01$) had a statistically significant increase while *C. pyrenoidosa* did not ($p > 0.4$). *C. pyrenoidosa* had a significantly larger survival from its control at 5-min. UV than did *C. vulgaris* over its control ($p < 0.01$).

Taken together, these two phenomena may be useful to differentiate the two species taxonomically (Table II).

This growth increase of *C. vulgaris* at low doses of UV irradiation may have practical applications for mass production of *Chlorella* as a source of food or other industrial uses. To enhance the growth and vitality of "seed cultures" for each batch, low doses of UV light may be significant. Low doses of UV to *Chlorella* represent rather high doses of UV light for bacterial contaminants and such doses may help ensure a more aseptic culture than would otherwise be expected.

Mutants A-2, H-3, and *C. pyrenoidosa* were more resistant to the lethal effects of 5-min. UV exposure than the normal cells ($p < 0.01$) based on survival percentage at 5-min. UV (Table II). It is evident that in the process of selecting mutants for color differences from the normal, some strains were selected which, upon further irradiation, had the ability to survive additional exposure better than their controls. All of the mutant strains had a mutation rate greater than the normal strains. As the lethality of the UV irradiation increased for the strains, the respective mutation rates (after 5-min. UV) also increased.

Retovsky (11) reported that the mean diameter of *Chlorella* cells in nature, *i.e.*, not grown under uniform conditions, did not provide an adequate taxonomic criterion for differentiating the two wild type species. With the qualification of growth under uniform conditions significantly different mean cell diameter distributions could be obtained. On several bases Soeder (25) concluded that *C. pyrenoidosa* should be reclassified. In view of the large spread of cell diameter measurements about the mean reported in this work for *C. pyrenoidosa* compared with *C. vulgaris*, Soeder's view is further strengthened (see Table IV).

Kessler (18) reported a distinction between *C. vulgaris* and *C. pyrenoidosa* using ruthenium red. Such a distinction could not be made in this work, but mutants A-1, C-5, and C-11 demonstrated a lack of pectins by this method. Mutants A-1 and A-2 appeared to contain less lipid than the other strains. Possible biochemical errors were indicated.

The glucose-dependency test described previously (1) indicated no carbohydrate metabolic error present. The PAS reaction verified this result.

Most of the morphological differences described by previous workers were macroscopic colony changes. The unequal division of mutant cells in certain strains is noteworthy. The characteristic thinner or not-evident gelatinous sheath (A-2, C-11, E-4, C-10, C-4, and F-4) has a rough correlation with the characteristically less prominent or not-evident wall (A-2, E-4, C-4, C-9, and F-4). Strains A-2, E-4, C-4, and F-4 had both characteristics in common and differ in that C-9 had a prominent gelatinous sheath with a nonprominent wall. It may be surmised that the genetic mechanism governing wall thickness may have some control over the gelatinous sheath. Both processes include glucose residues, polyuronides, and polysaccharides. It should be noted also that C-10 had a more prominent wall than *C. vulgaris*, but also had no thin gelatinous sheath evident.

The location of discrete chlorophyll spots appears to be distinctive for the C strains (C-1, C-4, C-7, and C-9) although not universal for all of the C strains. All of the other characteristics described appear to be distinctive for individual strains (Table I).

Excellent separation of pigments was obtained by application of thin-layer chromatography. The presence of compounds with R_f values similar to the carotenes of *C. vulgaris* provides substantial theoretical interest. The fact that spots did occur implies that some kind of carotene was formed. The determination of whether these spots represent the build-up of a precursor from a genetic block or an entirely new substance produced by the cell could not be ascertained because the relatively slow growth rate of the strains restricted this work.

Mutant E-4 appeared darker green to the naked eye. On the thin-layer plate E-4 had an extra chlorophyll spot. In almost every test E-4 demon-

strated difficulty adjusting to a new environment. This behavior might be explained in part if E-4 were synthesizing an inactive form of chlorophyll.

Mutants C-1 and C-11 had the slowest growth rate. Upon examination by TLC, fewer chlorophyll spots were found than would be expected. It is postulated that chlorophylls were present, however, because both C-1 and C-11 were photosynthetic on salt agar.

It is noted that less than 50 mg. of cells (fresh weight) of each strain was available for cell extraction.

SUMMARY

Thirteen UV-induced strains of *C. vulgaris* are reported. Especially interesting were mutants E-4 and E-5 which were darker green than normal to the eye.

This research represents an attempt to characterize pure mutant clones by use of a variety of methods.

Selected mutants were tested for susceptibility to UV damage and mutability. All of the mutants were found to be more mutable than the normal but several mutants were more resistant to the damage of UV irradiation than the normal.

A new taxonomic criterion for distinguishing the two species of *C. vulgaris* and *C. pyrenoidosa* became apparent. The increase in survival capacity over controls (0-min. UV for each strain) and the percent decrease after 5-min UV were statistically documented.

It was suggested that irradiated seed cultures of *Chlorella* cells may enhance the initial growth rate and help sterilize cultures for industrial algal growth.

Chlorella vulgaris and *Chlorella pyrenoidosa* could be differentiated by cell size when grown under uniform environmental conditions.

Clones within particular initial isolations demonstrated significant differences in cell size from the normal.

C. vulgaris and *C. pyrenoidosa* were not distinguished with ruthenium red but certain mutants could be distinguished from the normals with this stain.

Cytologic aberrations were observed including those of the sheath, cellulose wall, chloroplast, pigmentation, patterns of cell division, and spore formation.

TLC demonstrated that the mutants produced certain pigments with similar R_f values to the normal pigments but of different color, and also produced several pigments apparently not synthesized by the normal.

Mutant E-4, visibly darker green to the naked eye, chromatographed an extra chlorophyll spot when compared with the normal.

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Keyphrases

Chlorella vulgaris mutants–UV—induced
UV irradiation—*Chlorella* cultures
Mutation indexes—*Chlorella*
Cells, *Chlorella*—size, growth rate
TLC–separation, plastid pigments