NOTES

MITOMYCIN-INDUCED VARIANTS OF *BLASTOCLADIELLA EMERSONII*: ALTERATIONS IN γ PARTICLE CONTENT, FLAGELLATION, AND PIGMENTATION

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Aspects of the taxonomy, morphology, physiology, biochemistry, and development of the water mold *Blastocladiella emersonii* CANTINO & HYATT[1] have been reviewed[2,3]; its motile cells are being increasingly employed[4,5,6,7] for experimental studies. Wild type *B. emersonii* can produce four kinds of plants designated[1] as orange (O), ordinary colorless (OC), late colorless (LC), and resistant sporangial (RS); a fifth type, possibly only a developmental intermediate[8] magnified by continued cultural selection, was labeled[9] "pseudo RS". Zoospores produced by these plants are generally motile, uniflagellate, and uninucleate. Bi- or multi-flagellate spores, formed only rarely under the usual culture conditions, presumably result from incomplete cleavage at sporogenesis. Although transient cytoplasmic bridges can form between motile cells[10], true sexual fusions have never been observed. Thus, *B. emersonii* is apparently asexual. Yet, a good argument can be made[3] that the O phenotype is a male plant, its terminal cell corresponding to the orange, male gametangium of *Allomyces*. Two naturally-occurring mutants[11] of *B. emersonii* that produced only orange plants containing γ carotene (thus equivalent to pure male strains, albeit non-functional ones) were lost some time ago. To continue our work on sex and phenotypic variation in *B. emersonii*, attempts are being made to induce it to mutate to the orange phenotype; this is a preliminary report on some of our results.

Materials and Methods

Individual O plants were randomly isolated from the wild type and various lines subcultured *via* zoospores for 5 generations. No increase beyond that reported previously[1] was obtained in the incidence of the O phenotype, and stable mutants with a significantly higher incidence of orange plants did not appear.

Subsequently, wild type RS zoospores were dispersed (*ca.* 15/cm^2) on modified PYG agar (PYGM: 0.1 % each of peptone and yeast extract, 0.3 % glucose, and 1.0 % agar, pH 6.8 before autoclaving) containing mitomycin at geometrically-increasing concentrations from 0.1 mcg/ml to 100 mcg/ml, and incubated at *ca.* 22°C for 7 days. No growth occurred above 3.13 mcg/ml, and maximum viability occurred below it.

Results and Discussion

For populations grown on 3.13 mcg mitomycin/ml, viability was *ca.* 3 %. About 20 % of the mature plants (0.6 % survival rate for the inoculated spores) released spores when transferred to water; the rest were RS plants. The discharged spores were cultured on PYGM at *ca.* 22°C. From this population, a new strain (MC-3) was established by selective subculture *via* spores from O plants. MC-3 produced more O plants than wild type during the first five generations. The growth rate of MC-3 thalli, and the rate at which O plants discharged spores *in situ*, was much lower than for wild type OC plants. After the 5th generation, a colorless clone appeared; its subculture led to establishment of a stable albino mutant, Ma-1. Its distinguishing features (Table 1) were:

1) Ma-1 plants exhibited high viability; when allowed to release spores *in situ*, many 2nd generation thalli developed around the parent plant to yield sizeable clones. Such colonies were always colorless; they never contained orange or brown (*i. e.* O or RS) phenotypes.

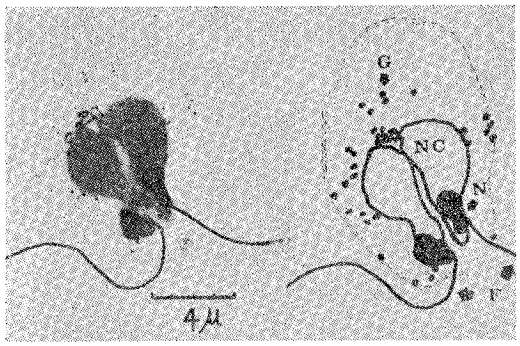
2) Populations of Ma-1 discharged *in situ*

Table 1. Comparative zoospore sizes and γ particle numbers, color, responses to $NaHCO_3$, and generation times for wild type, previously-described mutants, and the present mitomycin-induced variants of *B. emersonii*. All values are from the present study except those in parentheses which are from previously published work.

	MC-3M		Ma-1	Wild-Type				BEM[11]	Strain 9[8]
Phenotype	"Big" O	"Small" O	LC	OC	LC	RS	O	O	OC(?)
Size of zoospores in μ	8~9×11~12	Two types: 4×6 & 7×9	7×9	7×9	(7×9)[1]	7×9	4~5×6~7*	(4×6)[11]	(7×9)[8]
Ave. number γ particles/spore**	40~50	Two classes: 8 & 17~22	16.0	12.1 (12.5)***	(15.5)	12.0 (12.5)	8.1 (7.5)	(8)[12]	12.0
Generation time on PYGM (hrs.)****	48	96	26~32	18	24(?)	72	24		19~20
Growth on $NaHCO_3$:									
10^{-2} M	+	+	−	+	+	+	+	(−)[11]	(−)[8]
10^{-3} M	+	+	+	+	+	+	+		(+)[8]

* However, they are *ca.* 7×9 μ in the O plants of another strain of *Blastocladiella* (BSS)[11].
** Spores fixed with 2% osmic acid and stained with 0.25% alcoholic methylene blue for γ particles according to MYERS and CANTINO, unpublished data.
*** These values in parentheses for wild type spores are from CANTINO and HORENSTEIN[12] for reference controls.
**** Hours after inoculation when plants began to produce orange color and/or released spores *in situ*.

Fig. 1. Representative biflagellate spore with two nuclear caps and two nuclei; 60~80% of the swarmers liberated from "Big" O plants of the Orange variant strain, MC-3M, were of this type. The interpretive drawing identifies the γ particles (G), approximate cell boundary (plasma membrane, -----), flagella (F), nucleus (N) and nuclear cap (NC), some of which are not clearly visible in the photograph.



between 26 and 23 hours after inoculation.

3) Ma-1 was less sensitive to mitomycin than either wild type or MC-3.

4) Ma-1 was less sensitive to $NaHCO_3$ than will type.

5) Ma-1 was more resistant to high (37°C) and low (0°C) temperature than wild type.

6) Spores of Ma-1 contained an average of 16.0 or more γ particles. Thus, the whole population of Ma-1 resembled the few[1] LC plants normally found in wild type populations.

Another new strain (MC-3M), which differed from both MC-3 and Ma-1, was also established, as follows: Spores from O plants of MC-3 were grown on 3.13 mcg mitomycin/ml (survival rate, 0.6 %) and then subcultured *via* O plants for 20 generations. O-Spores were then treated again with mitomycin over the original range of concentrations. This time, 12.5 mcg mitomycin/ml was needed to yield 0.6 % survival rate, and O plants therefrom were again subcultured successively on PYGM. This strain is still "unstable", but it has been maintained exclusively *via* the many O plants it always produces; it has now (Dec. 1, 1969) been carried through some 30 generations. The important features of MC-3M (see Table 1, Fig. 1) follow:

1) Viability of MC-3M zoospores was similar to that of wild type OC spores; under our present conditions, *ca.* 500~1,000 mature 2nd-generation plants were produced per parent plant.

2) At *ca.* 96 hours after inoculation, 30 % to 90 % of the growing thalli in the population were O plants.

3) These O plants consisted of two phenotypes, "small" plants and "big" plants; their orange color appeared *ca.* 48 hours and 96 hours, respectiviely, after inoculation.

4) Some of the O plants then discharged automatically *in situ* (*i. e.*, after *ca.* 48 and 96 hours, respectively), but others did not release spores even after 4 weeks or more; these finally died. However, if such non-discharging O plants were transferred to water, up to 50 % released spores. There seemed to be a correlation, as yet ill-defined, between age of plants and capacity for discharge.

5) Many O plants produced zoospores with two or more flagella (and, accordingly, with correspondingly increased sizes), presumably the results of incomplete cleavage during sporogenesis. In particular, biflagellates constituted a consistent majority (*ca.* 60~80 %) of zoospores released from "big" O plants (Fig. 1).

6) Some O plants, however, released biflagellate zoospores which contained two nuclear caps but retained the dimensions of uniflagellate spores.

7) Zoospores released from "small" O plants of MC-3M were of two kinds: one averaged 8 γ particles/cell, the other 17~22 γ particles/cell. Zoospores released from "big" O plants averaged 40~50 γ particles/cell. Thus, with respect to γ particles, zoospores of strain MC-3M fell into three phenotypic groups.

Although uncertainty[13] remains about their *in vivo* action, the mitomycins seem to inhibit DNA synthesis[14,15]. The γ particles in *B. emersonii* are Feulgen-positive (R. B. MYERS and E. C. CANTINO, unpublished data), and thus may be DNA organelles; they are multiplied many times during the growth of a single generation of the fungus. The possible significance of the increased number of these γ particles, as well as the occurrence of two nuclear caps, in the spores of MC-3M, and the derivation of the biflagellate condition from this normally-uniflagellate fungus, will be further evaluated and discussed when we succeed in stabilizing the behavior of this interesting strain of *B. emersonii*.

Summary

From populations of *B. emersonii* grown in peptone-yeast extract-glucose media containing mitomycin at 3.13 mcg/ml and 12.5 mcg/ml, one stable albino mutant (Ma-1) and two unstable orange variant strains (MC-3 and MC-3M), respectively, were established by selection. Their characteristic generation times and responses to $NaHCO_3$, as well as the size and γ particle content of their motile cells, were measured and compared with the features of the wild type.

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