SENSITIVITY OF THE SPORES OF *BLASTOCLADIELLA EMERSONII* AND RELATED FUNGI TO ANTIBIOTICS AND SOME OTHER DRUGS

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The MIC of 48 antibiotics and other antifungal agents was established for the wild type and six mutants of *Blastocladiella emersonii*, as well as *B. britannica*, *B. simplex*, *B. cystogena*, and the related *Allomyces macrogynus*. Meiospores and mitospores of *A. macrogynus* differed from RS and OC spores of *B. emersonii* primarily in their responses to tetracycline, trichomycin, mitomycin C, antimycin A, and actinomycin, as well as bis(dimethylthiocarbamoyl)disulfate (T-1) and acriflavin (T-13). The four wild type species of *Blastocladiella* differed from one another primarily in their responses to mitomycin C, actinomycin S, chloramphenicol, endomycin, chlortetracycline, and trichomycin, as well as pentachloronitrobenzene (T-7), 2,4-dichloro-6-(2-chloranylyno)-S-triazine (T-8), 2,2′-methylene bis(3,4,6 trichlorophenol) (T-9), 3,4,4′-trichlorocarbanilide (T-4), malachite green (T-12), and T-13. The wild type *B. emersonii* differed from its albino mutants primarily in its response to antimycin A, T-4, and (T-8). The Blastocladiaceae were also compared with two yeasts and *Staphylococcus aureus* in their response to various toxicants.

There are few reports in the literature dealing with the *in vivo* sensitivity of uniflagellate fungi to antibiotic substances and other toxicants. As part of ongoing attempts[1,2,3] to induce mutations and to alter the γ-particles in *Blastocladiella emersonii* CANTINO *et* HYATT, the effect of various drugs upon it and upon some of its relatives was tested. In this report, we present the comparative sensitivities of *B. emersonii* and some mutants thereof, several other species of *Blastocladiella*, and the gametophytes and sporophytes of *Allomyces*, to some forty antibiotics and other fungicidal agents. Background material on these organisms can be found in recent reviews.[4,5]

Materials and Methods

Wild type *B. emersonii*, passed through its resistant sporganial (RS) stage every three weeks to ensure strain stability, and *B. britannica* HORENSTEIN *et* CANTINO, were descendents from the original isolates[6,7]. *B. cystogena* COUCH *et* WHIFFEN, and *B. simplex* MATTHEWS, were kindly sent to us by Dr. RALPH EMERSON (from his stock cultures Nos. 54-2 and

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48-8, respectively) in March, 1970. *Allomyces* (*Euallomyces*) *macrogynus* (EMERSON) EMERSON *et* WILSON, was a derivative of EMERSON's Burma 1 Da strain; it was subcultured by one of the authors (E.C.C.) from EMERSON's collection in Berkeley, California, in 1948; its meiosporangia, kept dry since that time (*ca.* 22 years), were used to start the gametophyte and sporophyte cultures tested in this report. The several albino mutants of *B. emersonii* were: a UV-induced albino[8], Strain 9; a mitomycin-induced strain, Ma-1, and a spontaneous mutant, Albino-1, both previously described[1,2,3]; and two other mitomycin-induced albinos, Ma-2 (similar to Strain 9) and Ma-3 (similar to Albino 1). *Candida albicans* and *Saccharomyces cerevisiae*, and the cultures of *Staphylococcus aureus*, were kindly provided by Dr. E. S. BENEKE and Dr. EVELYN SANDERS, respectively.

All organisms were subcultured every three weeks on Difco PYG or Difco YpSs agar, or on modified PYG (PYGM) agar containing only 1 % agar. To obtain spore suspensions from first generations "ordinary colorless" (OC) plants of wild type *B. emersonii*, spores from RS plants were put on PYGM at 23°C; after 16~20 hours, the ensuing OC plants were washed twice with 5 ml water, then flooded with 5 ml water again. After 10~20 minutes, spore suspensions were collected, chilled in an ice bath, and their cell densities determined with a Coulter Counter. Spore suspensions from RS plants of wild type *B. emersonii*, as well as thin-walled plants of *B. britannica* and *B. simplex* and the albino mutants of *B. emersonii*, were obtained by transferring 5~10 clones from stock cultures to 5 ml water and waiting until enough swarmers were released; for *B. cystogena*, populations of individual RS plants were used. All zoospore suspensions were chilled and counted as described above. The reference standards, *C. albicans*, *S. cerevisiae*, and *S. aureus*, were subcultured in PYG broth at 23°C for 48 hours. The following toxicants were employed for the experiments: bis(dimethylthiocarbamoyl)disulfate (T-1), N'-dichlorofluoromethylthio-N,N'-dimethyl-N-phenylsulfamide (T-2), *p*-dimethylaminobenzenediazo sodium sulfonate (T-3), 3,4,4'-trichlorocarbanilide (T-4), sodium-*p*-dimethylaminophenyldiazosulfonate (T-5), pentachlorophenol (T-6), pentachloronitrobenzene (T-7), 2,4-dichloro-6-(2-chloranylyno)-S-triazine (T-8), 2,2'-methylene bis(3,4,6 trichlorophenol) (T-9), 3-methyl-5-(*p*-chlorophenylsulfonyl) 3-methyl-5-(*p*-chlorophenylsulfonyl)-1,2,4 thiazole (T-10), 2-methoxyethylmercuric chloride (T-11), malachite green (T-12), acriflavin (T-13), 2-naphthyl-N-methyl-N-(3-tolyl)thionocarbamate (T-14), N-(1-naphthyl)-N-methyl-O-(2-naphthyl)thionocarbamate (T-15) and 4-morpholinecarboximidoyl guanidine (T-16). These toxicants were kindly given by Dr. Y. HASHIMOTO, Biological Research Laboratories, Nippon Soda Co. Ltd., in Kanagawa, Japan, Dr. M. AYA, Central Research Laboratories, Nippon Tokushu Noyaku Co. Ltd., in Tokyo, Japan.

The following antibiotics were used in the experiments: penicillin G, oxacillin, phenethicillin, methicillin, streptomycin, viomycin, kanamycin, crestomycin, streptovaricin, leucomycin complex, oleandomycin, spiramycin, erythromycin, helvolic acid, tuberactin, tetracycline, chlortetracycline, chloramphenicol, chloramphenicol (Enteromycin from Eambon, Italy), mitomycin C, carzinophilin, actinomycin S (actinomycin D), cycloheximide, blasticidin S, nystatin, amphothericin B, kasugamycin, polyoxin B, antimycin A, trichomycin, and endomycin. These antibiotics were obtained from Toyo Jozo Co. Ltd., in Shizuoka, Japan, Kyowa Hakko Co. Ltd., in Tokyo, Japan, and Sankyo Co. Ltd., in Tokyo, Japan. Some of the antibiotics were kindly given by Dr. T. YAMAGUCHI, Institute of Applied Microbiology, University of Tokyo, Japan and collected in Japan from the National Institute of Health, Tokyo, Japan by the authors.

Drugs were dissolved in small amounts of ethanol or acetone and diluted with water to desired levels. Final concentrations were obtained by making 1/20 dilutions of the drug solutions with molten (55°) agar media. For tests with *Blastocladiella* and *Allomyces*, 0.06~0.1 ml of spore suspension (10^5 spores/ml) was placed on *ca.* one cm^2 of the agar media; for the yeasts and bacteria, broth cultures were streaked on the test plates. Cultures were incubated at 23°, and examined and scored for growth after 36 hours and after 7 days.

Results and Discussion

The comparative sensitivity of the organisms to 17 potentially antifungal agents and 31 antibiotics (range: 10^{3}~10^{-4} mcg/ml) was established; the results, as minimum inhibitory concentrations (MIC) for growth, are tabulated in Tables 1 and 2.

It should be reemphasized, at this point, that inoculations were made with motile propagules for all the water molds tested; thus, the absence of detectable growth at a given concentration of a toxicant means that proliferation was arrested at a very early period in development, *i.e.*, either the spores were killed, their encystment blocked, or a stage in their germination inhibited by the toxicant being tested.

Comparative Sensitivity of Swarmers from Thick-walled and Thin-walled Cells of Wild Type *B. emersonii* and *A. macrogynus*

In this and subsequent discussions of comparative responses, we have adopted the the 'arbitrary' working definition that differences in sensitivity must be ten-fold or greater to qualify as being of major significance. On this basis, the swarmers from RS and OC plants of *B. emersonii* respond similarly to all antibiotics tested and all but one of the other toxicants; the exception is T-1, to which OC swarmers are 10 times more sensitive than RS swarmers.

On the other hand, the haploid *Allomyces* meiospores (from meiosporangia, *i.e.*, superficially equivalent to the RS cells of *B. emersonii*) and the diploid *Allomyces* mitospores (from mitosporangia, *i.e.*, superficially equivalent to the OC cells of *B. emersonii*) differ from the corresponding swarmers of *B. emersonii* in their contrasting response to toxicants. With respect to antibiotics, meiospores and mitospores show either no differences in reaction to related antibiotics such as streptomycin (miscoding) and the tetracycline-chloramphenicol-spiramycin-leucomycin group (ribosome site inhibitors), or insignificant (*ca.* 2-fold) differences for other ribosome site (blasticidin-S), elongation (cycloheximide), and RNA polymerase (actinomycin) inhibitors. They also respond similarly to inhibitors of DNA polymerase (mitomycin C), cell-wall synthesis (penicillin), and respiration (antimycin A) and display only small (*ca.* 2~5 fold) differences in response to polyene antibiotics (membrane function inhibitors). But, with respect to non-antibiotic toxicants, *Allomyces* meiospores are 10 and 20 times more sensitive than mitospores to T-13 and T-7.

The meiospores and mitospores of *Allomyces* are 1 N and 2 N respectively[9], while the RS- and OC-spores of *B. emersonii* are of an unknown genotype[5]. Although their differential sensitivities to toxicants could be related to their differences in ploidy, the quetion is further complicated by the following kind of comparison.

Comparison of the Responses of RS and OC Swarmers from *B. emersonii* with those of Meiospores and Mitospores of *A. macrogynus*

I. OC swarmers *vs.* mitospores. With respect to the antibiotics, three differences were ascertained. *Allomyces* mitospores are *ca.* 25 times more sensitive than *Blastocladiella* OC swarmers to tetracycline, but only 1/10 as sensitive to mitomycin C and actinomycin S. With respect to the other toxicants, there is one major difference: *Blastocladiella* is *ca.* 25 times more sensitive than *Allomyces* to T-1.

Table 1. The sensitivity[a] of some Blastocladiaceae and reference organisms to various toxicants

Compound[b]	Compound number	*B. emersonii* (from RS plants)	*B. emersonii* (from OC plants)	*B. simplex*	*B. britannica*	*B. cystogena*	Ma-1	Ma-1-45	Ma-2	Strain 9	Ma-3	Albino-1	*A. macrogynus* gametophyte	*A. macrogynus* sporophyte	*Candida albicans*	*Sacch. cerevisiae*
Bis(dimethylthiocarbamoyl)disulfate	T-1	1	0.1	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.1	0.1	2.5	2.5	2.5	2.5
N′-Dichlorofluoromethylthio-N,N′-dimethyl-N-phenylsulfamide	T-2	0.5	1	0.25	0.25	0.5	0.25	0.25	1	0.25	1	0.5	0.5	0.25	>1	>1
p-Dimethylaminobenzenediazo sodium sulfonate	T-3	50	50	50	50	50	250	250	50	50	100	100	100	250	250	>1000
3,4,4′-Trichlorocarbanilide	T-4	10	10	10	1	0.5	1	2.5	10	10	5	5	10	10	>1000	>1000
Sodium-*p*-dimethylaminophenyldiazosulfonate	T-5	10	10	10	10	10	25	10	10	5	10	5	10	10	>1000	>1000
Pentachlorophenol	T-6	0.25	0.25	0.25	0.1	0.1	0.1	0.25	0.25	0.25	0.25	0.25	0.25	0.25	>100	>100
Pentachloronitrobenzene	T-7	10	—	1	10	1	5	2.5	2.5	2.5	5	5	2.5	50	>1000	>1000
2,4-Dichloro-6-(2-chloranylyno)-S-triazine	T-8	5	10	0.5	0.5	0.5	0.25	0.5	1	0.25	10	5	10	10	>100	50
2,2′-Methylene bis(3,4,6-trichlorophenol)	T-9	0.5	0.1	0.1	0.25	0.05	0.1	0.1	0.25	0.1	0.25	0.25	0.25	0.1	>100	>100
3-Methyl-5-(*p*-chlorophenylsulfonyl)-1,2,4-thiazole	T-10	0.25	0.25	0.5	0.25	0.25	0.25	0.1	0.25	0.25	0.25	0.25	0.1	0.25	10	10
2-Methoxyethylmercuric chloride	T-11	5	2.5	2.5	1	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	2.5	50	25
Malachite green	T-12	0.25	0.5	0.25	0.05	0.01	0.1	0.1	0.5	0.25	0.25	0.25	0.1	0.1	1	1
Acriflavin	T-13	500	500	500	250	1[c]	500	500	500	500	500	500	10	100	100	100

a) MIC; *i.e.*, minimum concentration which inhibited growth (mcg/ml; average of 2 or more experiments); concentrations tested were 1,000, 500 and 250 mcg/ml and successive ten-fold dilutions thereof down to 0.01 mcg/ml.

b) Compounds which yielded essentially uniform responses with all the fungi tested were: 2-naphthyl-N-methyl-N-(3-tolyl)thiocarbamate (T-14), N-(1-naphthyl)-N-methyl-O-(2-naphthyl)thionocarbamate (T-15) and 4-morpholinecarboximidoyl guanidine (T-16), >1,000 mcg/ml (Ma-2, Ma-3, Strain 9, and *S. aureus* not included in test); chlorohexidine (T-17), >100 mcg/ml for all fungi and 0.5 mcg/ml for *S. aureus*.

c) Albino plants were produced at concentrations just permitting growth; *i.e.*, typical brown color of RS plants not developed.

II. RS swarmers *vs.* meiospores. With respect to the antibiotics, four differences were ascertained: *Allomyces* meiospores are *ca.* 25 and *ca.* 10 times more sensitive than *Blastocladiella* RS swarmers to tetracycline and trichomycin, respectively, but only 1/20 as sensitive to mitomycin C and antimycin A. With respect to other toxicants, there is one major difference: *Allomyces* is *ca.* 50 times more sensitive than *Blastocladiella* to T-13.

Table 2. The sensitivity[a] of some Blastocladiaceae and reference organisms to various antibiotics

Antibiotic[b] (mcg/ml)	*B. emersonii* from RS plants	*B. emersonii* from OC plants	*B. simplex*	*B. britannica*	*B. cystogena*	Ma-1	Ma-1-45	Ma-2	Strain-9	Ma-3	Albino-1	*A. macrogynus* gametophytes from meiospores	*A. macrogynus* sporophytes from mitospores	*C. albicans*	*S. cerevisiae*	*Staph. aureus*
Chloramphenicol[c]	25	25	25	5	2.5[d]	25	25	25	10	25	25	25	25	>100	>100	2.5
Chloramphenicol[e]	10	10	10	1	1[d]	2.5	10	5	10	10	10	10	5	>100	>100	1
Tetracycline[f,g]	25	25	25	25	5[d]	25	25	25	10	25	25	1	1	>100	>100	2.5
Chlortetracycline	25	—	50	10	1[d]	25	—	—	—	—	25	—	0.5～10	>100	>100	2.5
Mitomycin C	5	10	5	1	>100	25	5	50	5	5	5	100	100	500	>500	0.1
Carzinophilin (units/ml)	150	—	>150	>150	>150	>150	150	—	150	—	>150	—	> 50	>150	>150	—
Actinomycin S	2.5	2.5	2.5	25	25	1	1	1	1	2.5	2.5	10	25	>250	>250	0.1
Cycloheximide	0.25	0.25	0.25	0.1	0.05	0.25	0.1	0.1	0.1	0.1	0.25	0.1	0.25	>10	0.25	5
Blasticidin S[h]	0.5	0.5	2.5	0.5	0.5	1	1	0.5	2.5	1	2.5	1	0.5	>100	0.5	2.5
Nystatin	100	100	50	>100	25	100	>100	100	>100	>100	>100	25	>100	5	5	>100
Amphothericin B	500	—	500	500	500	500	—	500	—	—	500	—	500	50	50	—
Kasugamycin	50	—	500	500	500	500	—	500	—	—	500	—	500	>500	>500	—
Polyoxin B	25	25	25	25	25	25	25	25	25	25	25	25	25	>100	>100	>100
Antimycin A	5	25	1	1	1[d]	0.1[i]	0.1	0.1	0.1[i]	10	10	>100	>100	>100	>100	>100
Trichomycin	5	10	0.5	10	5	5	5	5	5	5	10	0.5	2.5	0.5	0.5	>10
Endomycin	5	5	5	5	0.5	1	1	1	1	5	5	2.5	5	>10	>10	>10

a) "Sensitivity" is defined in Table 1.

b) Antibiotics which yielded essentially uniform responses with all the fungi tested were: penicillins (penicillin G, oxacillin, phenethicillin, methicillin, streptomycin, viomycin, kanamycin, crestomycin, and streptovaricin), >100 mcg/ml for fungi and 0.05 to 0.25 mcg/ml for *S. aureus*; Leucomycin complex, oleandomycin, spiramycin, and erythromycin, 500 mcg/ml or more for the fungi and 2.5, 5.0, 2.5, and 0.5 mcg/ml, for *S. aureus*; helvolic acid andtu beractin, >500 and >1,000 mcg/ml, respectively, for the fungi. With the exception of penicillin, the foregoing antibiotics were not tested against Ma-3, Strain 9, and Ma-1-45.

c) A crystalline chloramphenicol from Sankyo Co. Ltd., Tokyo, Japan.

d) Albino plants; *i.e.*, typical brown color of RS plants not produced at highest concentration permitting growth.

e) Enteromycin from Eambon, Italy.

f) Chloramphenicol and the tetracycline group yielded somewhat variable results among the replicate experiments.

g) At concentrations just permitting growth of *B. emersonii*, tetracycline, chloramphenicol, and a number of the other antibiotics which interfere with DNA and/or RNA metabolism induce formation of variable quantities (45～98% of the populations) of RS plants; this phenotype is not ordinarily produced in the absence of these antibiotics nor in their presence at lower concentrations.

h) At concentrations just permitting growth, this substance induced large but variable increases in the incidence of the orange phenotype (0 plants) in populations of *B. emersonii*.

i) Sensitivity to antimycin A significantly higher than that indicated in an earlier report[2].

Comparative Responses among the Different Wild Type Species of *Blastocladiella*

I. *B. emersonii vs. B. cystogena.* These two species differ significantly with respect to several antibiotics; *B. emersonii* is 10~20 times more sensitive than *B. cystogena* to mitomycin C, kasugamycin, and actinomycin S, but only 1/10 to 1/25 as sensitive to chloramphenicol, endomycin, and chlortetracycline. With respect to other toxicants, *B. emersonii* is 1/10 as sensitive as *B. cystogena* to T-7, T-8, T-9; 1/20~1/25 as sensitive to T-4 and T-12; and only 1/500 as sensitive to T-13.

II. *B. emersonii vs. B. britannica.* In this case, *B. emersonii* differs significantly from *B. britannica* with respect to three antibiotics; it is 10 times more sensitive to actinomycin S, and kasugamycin, but only 1/10 as sensitive to one brand of chloramphenicol. With respect to other toxicants, it is only 1/10~1/20 as sensitive as *B. britannica* to T-8 and T-12.

III. *B. emersonii vs. B. simplex.* These two species differ significantly with respect to 4 substances: *B. emersonii* is 10 times more sensitive to kasugamycin, but only 1/10 as sensitive as *B. simplex* to trichomycin, T-7 and T-8.

IV. "Unique" differences which distinguish other species from *B. emersonii*. For purposes of this comparison, a "unique" difference is defined 'arbitrarily' as follows: the MIC for the species under consideration must differ by at least ten-fold from the MIC for all other species of *Blastocladiella*. On this basis, it was observed that:

(1) *B. cystogena* differs uniquely from *B. emersonii* in its sensitivity to mitomycin C, chlortetracycline, endomycin, T-13, and T-4. Additionally, at the highest concentrations of some drugs just permitting growth (see footnotes of Tables 1 and 2), *B. cystogena* does not produce the brown color usually associated with RS plants.

(2) *B. simplex* differs uniquely from *B. emersonii* in its sensitivity to trichomycin.

(3) *B. britannica* does not differ uniquely from *B. emersonii* in its sensitivity to any toxicant.

Comparative Responses of *B. emersonii* and its Albino Mutants

Among the mitomycin C-induced albino mutants (Ma-1, Ma-1-45, Ma-2, Ma-3), two exhibit some decreased sensitivity to mitomycin C and two do not. But, the only "significant" variation from wild type OC spores in response to antibiotics is the 50-fold increase in sensitivity to antimycin A displayed by Ma-1, Ma-1-45, and Ma-2. However, these mitomycin C-induced mutants do exhibit a broader range of altered responses to other toxicants: Ma-1, 10 and 40 times more sensitivity to T-4 and T-8; its temperature sensitive variant, Ma-1-45, an elevated (20-fold) sensitivity to only T-8; Ma-2, a 10-fold increase in sensitivity to T-8; and Ma-3 no significant changes in sensitivity.

The UV-induced mutant, Albino 9, displays 250- and 40-fold increases in sensitivity to antimycin A and T-8, respectively; the spontaneous mutant, Albino 1, shows a higher MIC for kasugamycin but no other significant alterations in its sensitivity.

In conclusion, employing the definition used in the preceding section, none of the foregoing albino mutants can be classified as uniquely different from any other in terms of altered sensitivities to toxicants.

Comparative Responses of the Blastocladiaceae, Yeasts, and *Staphylococcus aureus*

S. aureus is much more sensitive than *S. cereviseae* and *C. albicans* to most antifungal agents (T-4, T-6, T-7, T-8, T-10, and T-11); on these, the MIC for the Blastocladiaceae is similar to the MIC for the bacterium, not the yeasts. On the few antifungal agents (T-1, T-2 and T-3) to which the yeasts and *S. aureus* react similarly, the MIC for the Blastocladiaceae is generally lower. Thus, the motile cells of the water fungi appear to be exceptionally sensitive to almost all the antifungal agents tested, and, in their responses to them, more nearly resemble the reference prokaryote rather than the eukaryotes tested here.

With respect to the antibiotics, the results can perhaps best be highlighted in relation to the metabolic loci with which they presumably interfere (according to recent reviews[10]).

I. Membrane function. The MIC of nystatin and trichomycin for *S. aureus* is much higher than for yeasts; except for the meiospores of *Allomyces* and *B. cystogena*, the response of all the water molds to these antibiotics resembles that of *S. aureus*. On the other hand, the Blastocladiaceae are somewhat more sensitive to endomycin than either the bacterium or yeasts. If any generalization is possible, it would seem to be that the motile cells of water fungi more nearly resemble bacteria than yeasts in their responses to polyene inhibitors of membrane function.

II. Cell wall function. Penicillins display relatively little antifungal activity; their MICs for *S. aureus* are much smaller than those for yeasts. The water molds also display relatively high resistance to the penicillin group; in this respect, they resemble the yeasts.

III. Aerobic respiration. Antimycin A is not highly active against either the two yeasts or *S. aureus*; the Blastocladiaceae, on the other hand, are fairly sensitive to it.

IV. DNA and RNA polymerase activity. *S. aureus* is much more sensitive than the yeasts to inhibitors of DNA polymerase (mitomycin C) and RNA polymerase (actinomycin S). The MICs of these two substances for the water molds generally fall in a range between the yeast and bacterial MICs.

V. Ribosomes and protein synthesis. Most antibiotics that act at this site inhibit *S. aureus* strongly but the yeasts only weakly. Of these, the macrolide substances such as leucomycin A_8, spiramycin, oleandomycin, and erythromycin (binding at 50 S subunit; protein synthesis inhibition) and streptomycin (misreading) inhibit the water molds about as little as they do the yeasts. However, broad spectrum antibiotics such as chloramphenicol (binding to ribosome at 50 S subunit; peptide extension interference), tetracycline (interference with binding of amino-acyl s-RNA to ribosome complex) and chlortetracycline do show somewhat greater inhibitory activity with most water molds (*B. britannica* and *B. cystogena* excepted), thus placing them in an intermediate position between the yeasts and *S. aureus* in their responses. In addition, cycloheximide (peptide extension interference) and blasticidin S (binding at 50 S subunit; protein synthesis inhibition), both fairly active against the yeasts and *S. aureus*, are similarly active against most of the aquatic fungi.

From the foregoing digest and additional data in Table 2, one generalization can be made: Excluding the antibiotics interfering with RNA and DNA polymerases and peptide extension, the Blastocladiaceae seem to resemble *S. aureus* when they are responding to antibiotics that have relatively little antibacterial effect, while they more nearly resemble *Saccharomyces* and *Candida* in their responses to antibiotics that have little effect upon the yeasts.

General Conclusions

The intent of this report is simply to record the results of our initial screenings of various toxicants because of their potential use for investigations of zoospore encystment and germination, and the general developmental biology of *Blastocladiella*. The 'specific' early stages in the life history of the various fungi that were affected by antifungal substances have not yet been analyzed in any great detail. A few often-used antibiotics have previously been employed to interfere selectively with certain sequential events during sporogenesis[11] and spore germination[12] in *B. emersonii*. Some of the antibiotics tested herein may also prove to be similarly useful in analyzing other sequential phenomena, at different levels of organization, also known[13,14] to be associated with spore encystment in *B. emersonii*.

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