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Evaluation of Chemicals for Restricting Colony Spreading by a Xerophilic Mold, *Eurotium amstelodami*, on Dichloran–18% Glycerol Agar

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Twenty chemicals were screened for their effectiveness in restricting colony spreading by four strains of a xerophilic mold, *Eurotium amstelodami*, on dichloran–18% glycerol agar. Triton X-100, Triton X-301, Tergitol NP-7, and Tergitol 15-S-3 (each at 200 μg/ml) and 1,000 μg of sodium deoxycholate, 1 μg of iprodione, 0.1 μg of propiconazole, and 0.01 μg of Maxim per ml were judged to be most effective for restricting the rate of colony spreading.

Dichloran–18% glycerol (DG18) agar (8) (a_w, 0.955) was formulated for enumeration of xerophilic fungi in intermediate- and low-moisture foods. The medium is particularly useful for supporting colony development by *Wallemia sebi*, members of the *Aspergillus restrictus* series, and *Eurotium* species which do not grow well on enumeration media with higher a_w. Unfortunately, some *Eurotium* species grow too rapidly on DG18 agar, obscuring the growth of other xerophiles. The addition of sorbitol to dichloran rose bengal chloramphenicol agar retards colony development by *Eurotium amstelodami* Mangin, but hyphal color development is poor (4).

Various chemicals have been evaluated for their suitability for controlling the spread of fungal colonies. Fungicides (1, 7, 8), antibiotics (2, 6), dyes (5, 9), ammonium propionate (11), oxgall (12), and surfactants (3, 10, 13, 14) have been demonstrated with various degrees of success to control the rate of colony development by fungi representing a wide range of tolerance or requirement for a specific a_w. These chemicals inhibit various metabolic activities of molds, resulting in a reduced rate of mycelial growth. Surfactants, in particular, retard hyphal tip development and thus can be effective in controlling the spread of colonies on enumeration media. The investigation reported here was designed to determine the effects of 20 chemicals on the rate of colony spreading by *E. amstelodami*.

Four strains of *E. amstelodami* were studied. Strains FRR 475 and FRR 3020 were supplied by Ailsa Hocking at the Commonwealth Scientific and Industrial Research Organization, North Ryde, Australia; strains NRRL A-27649 and NRRL A-27651 were obtained from the USDA National Center for Agricultural Utilization Research, Peoria, Ill. Strains were grown in the dark on the surface of potato dextrose agar (pH 5.6) at 25°C for 13 to 15 days. Sterile 0.1% peptone was deposited on the surface of colonies, and conidia were dislodged by gentle rubbing with a sterile bent glass rod. Suspensions were filtered through sterile glass wool to facilitate removal of mycelial fragments. Resulting filtrates, essentially devoid of mycelia and adjusted to an optical density of 0.55 (600 nm), served as inocula for all studies.

DG18 agar was used as the basal medium for all studies. Twenty chemicals were analyzed for their effectiveness in controlling the rate of spread of *E. amstelodami* colonies in preliminary tests. Appropriate amounts of sterile 10% aqueous solutions of chemicals were added to sterile, molten (48 to 55°C) DG18 agar. Thirteen surfactants at concentrations of 1,000 and 5,000 μg/ml were examined: Triton X-100, X-301, and N-101 (Rohm and Haas Co., Philadelphia, Pa.); Teepol 610 and HB7 (Shell Chemical Co., New York, N.Y.); Span 20 and Span 80 (Sigma Chemical Co., St. Louis, Mo.); Tween 20 and 80 (Sigma); and Tergitol NP-7, NP-40, 15-S-3, and 15-S-40 (Union Carbide Corp., New York, N.Y.). Another surfactant, sodium lauryl sulfate, was tested at 500 and 1,000 μg/ml, as were sodium propionate and sodium deoxycholate. Oxgall (bovine bile, dried, unfractionated; Sigma) was tested at 1,000 and 5,000 μg/ml. The remaining chemicals analyzed were fungicides (500 and 1,000 μg of active ingredient per ml): ketoconazole (Sigma); Maxim [4-(2,2-difluoro-1,3-benz-dioxol-4-yl)-1H-pyrrole-3-carbonitrite] and propiconazole [1-([2-(2,4-dichlorophenyl)-4-propyl-1,3-dioxolan-2-yl]methyl)-1H-1,2,4-triazole] (CIBA-GEIGY Corp., Greensboro, N.C.); and iprodione [3-(3,5-dichlorophenyl)-*N*-(1-methyl-ethyl)-2,4-dioxo-1-imidazolidine-carboxamide] (Rovral 50WP; Rhone-Poulenc, Inc., Research Triangle Park, N.C.). The a_w of test formulations was 0.95.

In the first experiment, triplicate samples of suspensions of *E. amstelodami* diluted in 0.1% peptone were surface plated (0.1 ml) on DG18 agar containing test chemicals. Plates with 10 to 100 colonies were selected for evaluation. Colony diameters were measured after 6, 12, and 18 days of incubation at 25°C. Spreading was not controlled on DG18 agar containing Span 80, Tween 20 and 80, Tergitol NP-40 and 15-S-40, and oxgall. A reduced number of spreading colonies developed on agar containing Span 20. These chemicals were not further evaluated. No colonies were formed on agar containing Teepol 610 and HB7, sodium lauryl sulfate, or the test fungicides.

Chemicals exhibiting potential for controlling spreading of *E. amstelodami* and those which were toxic at the concen-

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TABLE 1. Diameters of colonies of *E. amstelodami* formed on DG18 agar containing various chemicals

Chemical	Concn (μg/ml)	Colony diameter (mm)[a] after:									
		3 days		5 days		7 days		10 days		14 days	
		Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
None (control)	0	4.4 (a)	2.9–5.4	11.8 (a)	8.8–14.8	20.7 (a)	15.5–22.7	36.5 (a)	33.9–39.9	45.7 (a)	42.0–49.1
Triton X-100	200	1.1 (d)	0.8–1.3	3.3 (fg)	1.7–4.3	5.9 (f)	4.2–7.3	10.3 (e)	8.3–12.0	15.9 (f)	14.4–17.0
Triton X-301	200	0.9 (d)	0.8–1.0	2.8 (g)	1.8–3.6	4.8 (g)	3.8–6.6	8.2 (f)	6.4–10.3	12.8 (g)	11.1–13.9
Tergitol NP-7	200	1.0 (d)	0.9–1.0	2.7 (g)	2.2–3.1	4.6 (g)	3.6–5.9	8.1 (f)	6.4–9.8	12.4 (g)	11.4–13.8
Tergitol 15-S-3	200	1.0 (d)	0.8–1.0	4.4 (de)	3.6–5.0	7.9 (e)	6.9–8.5	13.5 (d)	13.1–14.6	18.7 (e)	17.1–20.0
Sodium deoxycholate	1,000	1.3 (d)	0.4–1.0	3.7 (ef)	3.1–4.6	6.4 (f)	5.1–7.6	10.2 (e)	8.5–12.0	16.2 (f)	14.2–17.4
Iprodione	1.0	1.9 (c)	1.3–2.0	5.1 (cd)	4.1–5.7	9.0 (d)	8.1–10.0	16.4 (c)	14.0–18.6	25.0 (d)	22.6–30.3
Propiconazole	0.1	2.4 (b)	1.6–3.4	8.9 (b)	7.9–10.2	16.6 (b)	14.0–20.0	29.9 (b)	26.6–33.4	38.7 (b)	34.9–43.1
Maxim	0.01	2.3 (bc)	0.1–2.8	5.5 (c)	4.8–6.2	10.2 (c)	8.3–11.0	17.7 (c)	16.9–19.9	26.9 (c)	24.6–31.3

[a] Data represent values from three replicate trials performed in triplicate. Means were calculated by using four test strains. Mean values in the same column which are not followed by the same letter are significantly different ($P \leq 0.0001$) as determined by analysis of variance and Duncan's multiple range test. Ranges indicate minimum and maximum values obtained for the four test strains.

trations examined in the first experiment were evaluated at reduced concentrations in a second set of experiments. Teepol 610 and HB7 were each tested at concentrations of 10, 50, and 100 μg/ml, whereas the Triton surfactants, Tergitol NP-7 and 15-S-3, and sodium deoxycholate were each tested at 100, 500, and 1,000 μg/ml. Sodium propionate was examined at 1,000, 5,000, and 10,000 μg/ml, and the fungicides and sodium lauryl sulfate were each examined at concentrations of 5, 10, and 50 μg/ml. Three-point inoculations were done by three different people. The procedure consisted of submerging a sterile platinum needle into 0.1% peptone-water suspensions of conidia (optical density, 0.55 ± 0.02) and then piercing the surface of DG18 agar containing test chemicals. Three inoculations were applied to each plate. Colony diameters were measured after 6 and 12 days of incubation at 25°C. The Teepol surfactants did not control spreading. Irregularity of margins of colonies, which tended to spread between 6 and 12 days, was evident on agar containing sodium lauryl sulfate and sodium propionate. Ketoconazole caused colonies to appear translucent, making enumeration difficult. The other test chemicals had little effect on colony appearance.

A third three-point inoculum experiment was done with 500 μg each of Triton surfactants, Tergitol NP-7, and Tergitol 15-S-3 per ml; 1,000 μg of sodium deoxycholate per ml; and 1, 0.1, and 0.01 μg each of iprodione, propiconazole, and Maxim per ml. On the basis of an acceptable rate of colony development, colony form, and color observed in this experiment, a final experiment evaluating 200 μg each of Triton X-100, Triton X-301, Tergitol NP-7, and Tergitol 15-S-3 per ml and 1,000 μg of sodium deoxycholate, 1 μg of iprodione, 0.01 μg of propiconazole, and 0.01 μg of Maxim per ml was conducted. Colony diameters were measured after 3, 5, 7, 10, and 14 days at 25°C. Means and ranges (minimum and maximum) of colony diameters of the four test strains are presented in Table 1. Strains FRR 475, NRRL A-27649, and NRRL A-27651 responded similarly to each test chemical; strain FRR 3020 tended to form larger colonies on most media. Even after 3 days of incubation, mean diameters of colonies of the four strains formed on all test media were significantly ($P \leq 0.0001$) smaller than the mean diameters of colonies formed on DG18 agar containing no test chemicals (control). These significant differences continued throughout the 14-day monitoring period. The lack or occurrence of significant differences in diameters of colonies formed on any two test media should be interpreted only as a relative response to the concentration of the chemicals. It is likely that exhaustive experiments could be done in which concentrations of test chemicals could be adjusted such that no significant differences in colony diameter would occur on test media but all would yield significantly smaller colonies than those formed on DG18 agar without test chemicals.

The important observation is that the eight chemicals evaluated at the concentrations listed in Table 1 do retard spreading of *E. amstelodami* for an incubation period that would be sufficient to allow colony development by other normally more slowly growing xerophiles. However, the abilities of other xerophiles to tolerate the presence of these chemicals in DG18 agar are not known. Studies in our laboratory which are designed to determine the abilities of the four *E. amstelodami* strains examined in these experiments and 25 other strains of heat- and cold-stressed xerophilic fungi to resuscitate and form colonies on DG18 agar containing the test chemicals listed in Table 1 are under way.

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