EFFECT OF ANTIBIOTIC TREATMENT ON MYCELIAL GROWTH OF ISOLATES OF THE MOSQUITO PATHOGENIC FUNGUS *LAGENIDIUM GIGANTEUM* (OOMYCETES: LAGENIDIALES) FROM AUSTRALIA, COLOMBIA AND UNITED STATES

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ABSTRACT. Bacteria collected in mosquito breeding ponds were evaluated for resistance to streptomycin, chloramphenicol, penicillin and trimethoprim. Mycelial growth of *Lagenidium giganteum* isolates from Australia, United States and Colombia were evaluated in PYG media containing one antibiotic or a mixture of these compounds. Media containing chloramphenicol reduced mycelial growth of most of the isolates. The antibiotic mixtures and penicillin-streptomycin penicillin-trimethoprim did not significantly affect mycelial growth of the isolates; however, the later substantially reduced bacterial contamination.

Lagenidium giganteum Couch, a water mold of the Class Oomycetes, is a facultative parasite of mosquito larvae. Kerwin et al. (1990) concluded that this pathogenic fungus could be used safely for mosquito control operations. On different occasions between 1985 and 1987, Frances et al. (1989), isolated *L. giganteum* in Millaa Millaa, Australia. Recently, we have isolated and examined the pathogenicity to mosquito larvae of 5 new isolates of *L. giganteum* from 3 different areas in Colombia (Orduz, unpublished data). In all cases, the isolation methodology described by Brey and Remaudiere (1985) was followed; however, the normal bacterial inhabitants of these ponds were resistant to penicillin and in many cases to streptomycin, thus complicating the isolation procedures.

In the present study, a comparison of the effect of some antibiotics on the mycelial growth of *L. giganteum* isolates from Australia, United States and Colombia is presented, and a new antibiotic mixture for the isolation of *L. giganteum* from the Colombian tropics is given.

Lagenidium giganteum California (CA) was obtained from P. T. Brey, Institut Pasteur, Paris; North Carolina (NC), and Louisiana (LA) isolates were obtained from J. Kerwin, University of Washington, Seattle, Washington; the Australian isolate (ARSEF 2532) was obtained from A. W. Sweeney, Army Malaria Research Unit, New South Wales. These isolates and the Colombian (CIB) strains used in these experiments were maintained on sunflower seed extract (SFE) agar as described by Guzman and Axtell (1986) at room temperature (23 ± 3°C), and subcultured from dead larvae every 2–3 weeks.

Aquatic bacteria collected from 15 mosquito larval habitats belonged to 17 species and 7 genera. All bacterial species tested according to standard procedures (NCCLS 1984) were resistant to 10 units of penicillin; one species was resistant to 30 μg of chloramphenicol; 4 species were resistant to 50 μg of streptomycin; and 5 species were resistant to 1.25/23.75 μg of trimethoprim/sulphamethoxazole respectively (data not shown).

Lagenidium giganteum mycelial growth was measured 36 h after transferring 20 mm^2 SFE-agar discs containing mycelia of each isolate of *L. giganteum* to PYG (per liter, peptone 1.3 g, yeast extract 1.3 g and glucose 3 g) with antibiotics to a final concentration of 0.05% of each antibiotic or mixture of them, penicillin V 10,000 units (PYGP), streptomycin 10 mg/ml (PYGS), chloramphenicol 6 mg/ml (PYGC) and trimethoprim-3 mg/ml (PYGPT), and mixtures of penicillin-streptomycin (PYGPS), penicillin-chloramphenicol (PYGPC), penicillin-trimethoprim (PYGPT), and penicillin-streptomycin-chloramphenicol (PYGPSC). Ninety-six hours after fungal isolates were transferred to PYG with antibiotics, 4 mm diameter discs containing fungal mycelia were transferred to PYG without antibiotics, and measurements of radial growth were taken after 36 h. Data were analyzed by analysis of variance and means compared using Tukey's test (Systat, Statistics/Tukey).

At the concentrations tested, penicillin and streptomycin had no adverse effects on mycelial growth on any of the *L. giganteum* isolates. Radial growth ranged between 12.2 mm in isolate CIB 164-PDCH to 0.6 mm in CIB 79-MED. Growth of most of the isolates in PYGPS was not affected by these antibiotics; however, for isolates CIB 183-ARU and CIB 183-NUQ, growth was significantly reduced. Isolates growing in PYGPT were not significantly different from their respective controls except for isolate CIB 183-NUQ. In all isolates tested, growth in

PYGC, PYGPC, and PYGPSC was reduced compared with their controls except for isolates CIB 79-MED and 79-TDT growing in PYGC and isolate CIB 79-MED growing in PYGPC and PYGPSC. Isolate CIB 183-NUQ was the most susceptible to antibiotic treatments (Table 1).

When agar discs from each isolate, antibiotic treatment and controls were transferred to PYG-agar without antibiotics, definite growth trends were observed. After 36 h of transfer, growth of the isolates CA, ARSEF 2532, CIB 79-MED and 183-ARU did not differ from their controls. Length of mycelia in isolates CIB 163-PDCH, LA, NC, CIB 79-TDT and CIB 183-NUQ coming from treatments with chloramphenicol was significantly reduced. However, isolate CIB 183-NUQ, which was severely affected by treatments PYGC and PYGPSC, completely recovered and did not show any significant difference from its control (Table 1).

The lower rate of mycelial growth observed in some treatments after transferring the mycelia to the media without antibiotics could be due to the effect of antibiotic residues on the transferred mycelia. Antifungal activity has been demonstrated for some β-lactam compounds (Gottstein et al. 1971), for antibiotics acting by blocking protein synthesis (Roberts 1980), and for those which act by inhibiting folic acid synthesis (Lopes and Armond 1968).

The reduced bacterial contamination in the isolation medium PYGPT readily permitted the isolation of fungal mycelia. This isolation procedure was easier due to faster mycelial growth in all antibiotic treatments (range of radius 2.9–12 mm/36 h), with the exception of isolate CIB 79-MED (0.6 mm/36 h) compared with the one obtained from Brey and Remaudiere (1985), 10 mm/7 days, and in some cases to the isolation methodology described by Frances et al. (1989) (diam 25–30 mm/11 days), who used the antibiotics described by Rueda and Axtell (1991). Differences in mycelial growth pattern found by the Brey and Remaudiere study (1985) and Frances et al. (1989) could be due to the reduced space furnished by the test tubes used to isolate the fungus relative to the petri dishes used in these experiments. The petri dishes supply the fungi with more nutrients and space to grow, facilitating the isolation procedure.

Reduced bacterial contaminants and the highest growth rate showed by *L. giganteum* isolates in the treatment PYGPT, recommend it as a good medium for isolation of this fungus in Colombia and other parts of the world.

Table 1. Effect of 4 antibiotics, their mixtures and residual effect on the mycelial growth rate of *Lagenidium giganteum* strains from Australia (ARSEF), United States (NC, CA, LA) and Colombia (CIB).

	Radius of mycelial growth of *L. giganteum* isolates after 48 h*								
Treatment	NC	CA	LA	ARSEF 2532	CIB 163-PDCH	CIB 79-TDT	CIB 79-MED	CIB 183-ARU	CIB 183-NUQ
					With antibiotics				
PYG	3.5a	3.1a	4.0a	4.1a	3.0a	12.2a	0.7a	6.2a	5.2a
PYGP	3.2a	3.0a	3.7a	4.0a	2.7a	11.0a	0.6a	5.5ab	4.0ab
PYGS	3.0a	3.0a	3.7a	4.1a	2.7a	12.2a	0.7a	6.2a	4.5ab
PYGC	1.2a	0.8b	0.6b	0.7b	1.0a	4.2b	0.6a	1.5d	0.7c
PYGPS	3.1a	1.7a	3.9a	4.0a	3.0a	11.7a	0.5a	4.5b	3.2b
PYGPC	0.3b	0.7b	1.0b	0.4b	0.9b	3.2bc	0.1a	1.7cd	0.9c
PYGPT	2.7a	2.7a	4.0a	4.0a	2.9a	12.0a	0.6a	6.0a	3.5b
PYGPSC	0.4b	0.5b	1.3b	0.6b	0.5b	2.5c	0.1a	2.9c	0.9c
					After antibiotics				
PYG	4.2a	2.9a	4.5a	3.5a	4.5a	11.5a	2.2a	4.0a	2.7a
PYGP	4.5a	3.0a	4.5a	3.2a	4.7a	11.5a	0.9a	4.0a	2.7a
PYGS	4.9a	3.0a	3.5a	3.7a	4.0a	9.0b	1.5a	4.0a	2.2b
PYGC	3.2b	2.7a	2.5b	4.2a	1.0b	9.0b	1.2a	3.2a	4.5a
PYGPS	4.0a	1.5a	3.5a	3.7a	4.7a	10.0ab	1.5a	4.0a	2.5b
PYGPC	3.2b	3.0a	4.0a	3.0a	4.5a	7.2bc	2.2a	4.0a	2.0b
PYGPT	5.0a	3.0a	4.3a	3.2a	4.0a	10.0a	1.5a	3.7a	1.7b
PYGPSC	3.0b	3.0a	3.8a	3.5a	4.2a	7.0c	1.7a	4.0a	3.5a

* Measurements in millimeters. Mean values are the result of 2 tests with 4 replicates per treatment. Means followed by the same letter in each category (with antibiotics and after antibiotics) in each column are not significantly different (Tukey HSD, alpha = 0.05). CIB, Corporación para Investigaciones Biológicas.

PYG: Peptone, yeast extract, glucose; C; Cloramphenicol, P: Penicillin, S: Streptomycin, T: Trimethoprim.

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