A Microsyringe Method for Determining Concentration of Fungal Propagules

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ABSTRACT

Concentration of zoospores was determined by a microsyringe method without prior termination of spore motility. The method was applicable also to other types of fungal propagules and the population determined with this method had smaller standard deviation than the hemacytometer method.

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During our studies on zoospore behavior, we encountered difficulties in determining spore concentration with a hemacytometer because of spore motility. Termination of zoospore motility by heat or cold treatment or by staining usually resulted in rupture. Induction of zoospore encystment by shaking (6) was time-consuming and also caused bursting of some spores. Therefore, a microsyringe method was developed to overcome these difficulties and proved suitable for determining concentrations of other types of fungal propagules.

Sporangia of *Phytophthora palmivora* Butler and conidia of *Helminthosporium maydis* Nisik. & Miyake were obtained by growing each under continuous fluorescent light for 7 days at 28 C on V-8 juice agar (1, 2). Zoospores of *P. palmivora* were liberated from sporangia by incubating a sporangial suspension at 16 C for one hour. Sporangia were then separated from zoospores by passing the suspension through a 20-μ screen (Buckbee-Mears Co.). Chlamydospores of *P. palmivora* were produced by growing the fungus in 25 ml of papaya juice medium for 1 month (4), and were detached from mycelia by grinding the washed mycelial mats in an Omni-Mixer at 3,800 rpm for 1 min. The resulting suspension was filtered through two layers of cheesecloth. Chlamydospores in the filtrate were separated from the mycelial fragments by sedimentation in a test tube. Conidiation of *Alternaria tomato* (Cke.) Weber was induced by growing the fungus on V-8 juice agar at 28 C for 3 days with light followed by a 4-day incubation at 20 C (1). *H. maydis* and *A. tomato* were supplied by M. Aragaki. Microsclerotia of *Calonectria crotalariae* (Loos) Bell & Sobers were produced by growing the fungus in 25 ml of glucose-peptone medium (per liter: 20 g glucose, 0.2 g peptone) for 2 weeks at 28 C with light. The mycelial mats containing microsclerotia were washed three times with distilled water, and triturated in an Omni-Mixer at 9,200 rpm for 1 min. The suspension was filtered through two layers of cheesecloth. Microsclerotia in the filtrate were separated from mycelial fragments by sedimentation in a test tube. The following conidia were obtained from cultures on V-8 juice agar: *Penicillium frequentans* Westling, *Aspergillus fumigatus* Fresenius, and *Mucor ramannianus* Möller.

For determining propagule concentration, the volume-control sleeve attached to the plunger guide of a 10-μl microsyringe (Hamilton microliter syringe) was pre-adjusted to 2 μl. A 2-μl aliquot of diluted spore suspension was taken up from each sample and distributed in 2 1-μl drops on a glass slide and the number of propagules in each drop was counted. The total area of one drop of spore suspension was equal to approximately two microscopic fields at X10. The number of propagules per ml was calculated from the average number of propagules/μl. For easy counting, propagule suspensions should be diluted to ca. five propagules/μl for motile zoospores and 5 to 15 propagules/μl for other types of propagules. For comparing the microsyringe method with the hemacytometer method, propagule concentration in each sample was measured six times with each method, and the means and standard deviations were computed (5).

Menyonga & Tsao (6) terminated the motility of zoospores of *Phytophthora parasitica* by either staining or shaking before counting with a hemacytometer and found that shaking resulted in about 10% higher count than staining. A zoospore suspension of *P. palmivora* was shaken by a wrist-action shaker at 228 excursions/min for 10 min to induce zoospore encystment, and the spore concentration was determined by the microsyringe method. Apparently, shaking also caused bursting of zoospores. The zoospore concentration before shaking was about 50% higher than after shaking. Since motile zoospores can also be counted with the microsyringe method, this method is considered relatively simple and accurate.

Results obtained with the microsyringe method, and the hemacytometer method for different propagules from seven fungal species are shown in Table 1. For each fungal propagule the use of the microsyringe method resulted in smaller standard deviation than with the hemacytometer method. This is probably because the volume of propagule suspension measured by the microsyringe is relatively consistent, whereas the volume introduced into the counting chamber of a hemacytometer varies greatly. Moreover, rapid settling of larger propagules in the pipette may also contribute to the greater standard deviation of the hemacytometer method. Fukuki & Aragaki (3) eliminated the settlement problem by suspending conidia of *H. maydis* in a solution containing 50% sucrose and 0.2% Tween 20. Since only 2 μl was taken up and all the propagules in it were counted with the microsyringe method, results obtained with this method were not affected by the rapid settling.

The microsyringe method enables us to count

TABLE 1. Comparison of microsyringe method with the hemacytometer method for determining concentration of fungal propagules

Fungus	Type of propagules	Microsyringe method		Hemacytometer method	
		Mean ($\times 10^3$/ml)	Standard deviation	Mean ($\times 10^3$/ml)	Standard deviation
Penicillium frequentans	Conidia	12.5	1.38	23.9	2.42
Aspergillus fumigatus	Conidia	21.3	2.23	30.8	3.73
Mucor ramannianus	Conidia	16.6	1.59	27.3	3.07
Helminthosporium maydis	Conidia	15.5	2.75	20.7	2.90
Alternaria tomato	Conidia	17.3	2.98	17.2	5.69
Phytophthora palmivora	Sporangia	19.8	4.21	21.2	5.41
P. palmivora	Chlamydospores	10.2	1.88	9.9	2.93
Calonectria crotalariae	Microsclerotia	1.3	0.68	2.8	1.31

actively swimming zoospores and to count other types of fungal propagules with smaller standard deviation than the conventional hemacytometer method.

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