

A convenient and safe method to generate a large quantity of *Aspergillus parasiticus* spores.

In order to examine the spore pigments in strains of *Aspergillus parasiticus*, we developed a method to safely generate a large quantity of spores while conserving laboratory space, resources, and time.

Traditional methods employed to generate stock supplies of spores usually involve inoculating the surface of nutrient agar, in a flask or petri dish, with a defined number of spores (Pontecorvo et al. 1953. Adv. Gen. 5:141-238). The culture is placed at 30 C for 48 hrs to encourage mycelial growth then moved to room temperature. Asexual sporulation is evident after an additional two days with a maximum number of spores generated by ten days. The traditional vessels used for large scale spore production are 2.8 liter Fernbach flasks (surface area-SA = 285 cm²) and 15 cm petri dishes (SA = 175 cm²). Spores are harvested by placing buffer in the flask or dish and gently scraping the agar surface with a long handled glass rod. Initially the hydrophobic spores are difficult to wet and repeated mechanical agitation is required to harvest them. Finally, the spore suspension is collected, washed, and concentrated by centrifugation. During the protracted scraping step, spores can escape from the vessel thereby increasing the chances of laboratory contamination and human exposure to a concentrated spore inoculum. The method we describe eliminates the scraping step and employs easily stacked, reusable, non-breakable plastic containers with three times the surface area of a Fernbach flask. We feel that the risk of releasing a large quantity of spores while harvesting is greatly reduced.

Two liter, polystyrene Corning Roller Bottles (RBTL - catalogue number = 25240) are inoculated with 10(7) spores in 200 mls of 3% YGT agar (yeast extract 0.5%, glucose 2%, and trace elements) at 50 C using standard sterile technique. The caps are replaced, and the bottles rolled steadily in an ice bath until the agar has solidified along the inner surface (3 to 5 minutes). The caps are then replaced with sterile foam plugs to allow gas exchange and incubated as described above. The bottles may be placed in a vertical position or conveniently stacked on their sides during mycelial growth and sporulation. Normal mycelial growth initially proceeds within the agar and eventually breaks through the surface where asexual sporulation occurs. The spores are harvested by placing 200 mls of buffer inside the vessel, replacing the original plastic cap, and shaking vigorously. The spore solution is decanted, concentrated by low speed centrifugation, and washed in storage buffer. The spores are almost entirely free of contaminating mycelial debris and agar fragments.

Although inoculating pre-poured RBTLs with a spore solution may seem more convenient, the harvesting procedure is compromised in two ways. An additional filtration step is required to remove debris that is released from the mycelial mat growing on the agar surface, and bits of agar frequently plug the filters. When spores are inoculated with the molten agar, mycelial filaments infiltrate and effectively reinforce it. A surface mycelial mat leaves the agar fragile and susceptible to the shear forces required to wet the spores.

Table 1

<u>Spore Inoculation</u>	<u>Spore Yield per Culture Vessel</u>		<u>Factor Increase</u>
	<u>Fernbach</u>	<u>Roller Bottle</u>	
10 ⁴	5.1 +/- 1.4 x 10 ⁹	-----	
10 ⁷	4.7 +/- 0.2 x 10 ⁹	1.9 +/- 0.18 x 10 ¹⁰	4 x
3 x 10 ⁷	-----	1.65 +/- 0.61 x 10 ¹⁰	
<u>Surface inoculation in buffer</u>			
3 x 10 ⁷	-----	2.18 +/- 0.81 x 10 ¹⁰	

Table 1 indicates typical spore yields from Fernbachs and RBTLs inoculated with a variable number of spores. The initial spore inoculum did not play a statistically significant role in the final number of spores generated per flask or RBTL. The number of spores generated was roughly proportional to the available surface area.

In summary, the described procedure has a number of benefits over conventional methods for generating large quantities of spores. It is safer due to the unbreakable nature of plastic RBTLs and because spore harvesting can be done in a completely closed container. In addition, the RBTLs save space since they have approximately 3X the usable surface area compared to Fernbach flasks and they can easily be stacked on their side on a shelf.