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DENSITY GRADIENT FRACTIONATION OF MICROSI

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RESEARCH NOTE

A Density Gradient Method for Fractionating Microsporidan Spores

A simple method was needed to separate spores of Nosema algerae from the bodies of mosquitoes. The spores represent only a small fraction of the material from triturated mosquitoes. Contaminating materials include mosquito tissues, detritus adhering to the surface of the larvae (including bacteria and protozoa) and gut contents. Attempts to obtain clean spores by washing with water resulted in an unacceptably high loss of spores, which are not abundant to begin with. Furthermore, spore samples usually contain a mixture of mature and immature spores and it would be advantageous if mature spores could be isolated.

A simple, fast and reliable method was developed by using a density gradient made from Ludox (a colloid of 40% silica in a sodium hydroxide solution with a pH of 9.8 and a specific gravity of 1.303, Dupont). A continuous gradient was established using the gradient mixer described by Britton and Roberts (Sci. 131,32, 1960) modified by connecting the two chambers with a siphon rather than a hole at the bottom of the chambers. Five and one-half ml of Ludox was placed in one chamber and 5 1/2 ml of the diluent, distilled water, was placed in the other. A small bore plastic tube drained the Ludox chamber into a 12 ml cellulose nitrate ultracentrifuge tube. A magnetic stirrer mixed the Ludox with the diluent which flowed from the siphon as the Ludox chamber emptied. When both chambers were empty the resulting gradient was 40% silica on the bottom and almost pure water on top.

Mosquitoes were crushed in a glass tissue grinder then strained through an 80 mesh copper screen. The mixture passing through the screen was

centrifuged at 170 x G in a clinical centrifuge for 20 minutes and the supernatant discarded. The resulting residue was resuspended in about 1 ml of distilled water and carefully layered on top of the gradient. The gradient was centrifuged in a swinging bucket head at 16300 x G for 20 minutes at 4°C. Equilibrium was not achieved because further centrifugation would sediment silica particles (Pertoft, Biochem. Biophys. Acta 126, 294, 1966). The spore fraction was drawn off in a volume of about 1.5 ml with a syringe. This fraction was washed twice with distilled water to remove the silica and sodium hydroxide.

Additionally, the entire gradient was fractionated by puncturing the bottom of the cellulose nitrate tube with a 20 gauge hypodermic needle and collecting fractions of about .3 ml each. Each fraction was examined with phase contrast microscopy. A sample from each fraction was also dried on a microscope slide, fixed with methanol and stained with giemsa. Spores from the gradient were fed to first instar Anopheles stephensi larvae to determine their viability.

A cloudy band containing mature spores was found about 2/3 of the way from the top of the tube. The only contamination in this band was occasional pieces of host cuticle. Bacteria remained at or near the top of the gradient. Immature spores (appearing dark in phase contrast microscopy) were found from the top of the gradient down to the band containing the mature spores, with some overlap. Nothing was found below the mature spore region of the gradient.

In the early infection two peaks of spores were found, a peak of mature and a peak of immature spores (Fig. 1,A). The scarcity of spores with intermediate refraction and density (between the two peaks in Fig. 1,A) may indicate a rapid intraspore developmental event causing both an increase in density and

a change in refraction of the spores. Later in the infection mature spores increase in relative abundance (Fig. 1,B).

Mosquito larvae became infected after exposure to spores fractionated by Ludox. It has been demonstrated that high pH does not kill the spores of N. algerae (Alger and Undeen, J. Invert. Pathol. 13, 321, 1970).

Fig. 1,A. is a histogram of a spore distribution resulting from the density gradient centrifugation of triturated larvae eight days after exposure to an infective dose of *N. algerae*.

Fig. 1,B. is the results of a similar centrifugation of adult mosquitoes, which were infected in the first instar. These adults ranged in age from about 2 to 5 weeks when triturated.