RESEARCH NOTES

Continuous Axenic Culture of *Aphelenchoides* sp.

E. J. BUECHER[1], E. L. HANSEN[1], AND R. F. MYERS[2]

Successful axenic culture of nematodes has been achieved with certain free-living forms (5), the insect-parasitic *Neoaplectana carpocapsae* (6) and *N. glaseri* (E. L. Hansen, unpublished data), and the plant-parasitic *Aphelenchoides* sp. (7). Subsequent nutritional studies of the latter nematode utilized larvae from monoxenic culture (8). The following is a report on the response of *Aphelenchoides* sp. to several proteinaceous supplements in chemically defined media, and the establishment of continuous axenic culture.

In preliminary trials of various basal media and supplement combinations, *Aphelenchoides* sp. larvae were reared monoxenically on potato dextrose agar cultures of *Pyrenochaeta terrestris* and allowed to migrate from a small block of agar/mycelium placed on sterile water agar. These larvae were removed by micropipette; groups of eight larvae were inoculated into 10 × 75 mm tubes containing 0.15 ml aliquots of media and incubated at either 20 C or 23 C. Media consisted of *Caenorhabditis briggsae* maintenance medium (CbMM) (3) or *Aphelenchoides* maintenance medium (M-7) (7), supplemented v/v with various proteinaceous extracts. These included 10% and 1% yeast extract (1, 2) and 2% liver factor (8 mg/ml stock solution) plus 7.7% Ficoll (3). Chick embryo extract (Grand Island Biological Company) at 25% plus 10% human serum was tested only in CbMM. After 3 weeks incubation, populations of about 100 F_1 larvae were obtained in all media except that containing chick embryo extract and serum; this medium had a population of greater than 200, comprising F_1 adults and F_2 larvae. Heated liver extract at 10% in peptone-yeast medium (9) was also tested but was not satisfactory.

When 10 F_2 larvae from the chick embryo extract-serum supplemented medium were transferred to fresh medium, they matured and reproduced. The remainder of the culture inoculated into fresh medium on a glass wool column (5) produced a high population which has now been maintained for four months at 20 C as a continuous culture. Weekly harvests contain approximately 6,000 larvae and adults, and 25,000 eggs. Additional columns incubated at 20 C and 23 C have been initiated with portions of the harvests; another culture on glass wool was started with an inoculum of 100 eggs.

These continuous axenic cultures served as the inoculum source for re-evaluating several media. Washed eggs were used to avoid any possible nutritional carry over. Eggs were transferred into CbMM diluted to ½× with water and incubated at 20 C for 2–5 days. Newly hatched larvae were then transferred in groups of 5 or 8 in the routine assay procedure. The populations which developed from these axenically reared larvae after incubation for 3 weeks at 20 C and 23 C in several CbMM supplemented media are

Received for publication 25 November 1969.

[1] Clinical Pharmacology Research Institute, 2030 Haste Street, Berkeley, Calif. 94704. Supported by USPHS Grant A1-07359 TMP.

[2] Department of Entomology and Economic Zoology, Rutgers University, New Brunswick, N. J. 08903. Paper of the Journal Series, New Jersey Agricultural Experiment Station; supported in part by USDA Grant 12-14-100-9124(34).

TABLE 1. Populations[1] of *Aphelenchoides* sp. in axenic cultures in supplemented chemically defined medium, CbMM.

Supplement to CbMM	Population at 3 weeks	
	20 C	23 C
25% chick embryo extract + 10% human serum	105	1220
10% yeast extract + 10% human serum	12	36
25% chick embryo extract	71	670
10% yeast extract	5	9
10% human serum	6	10
2% liver factor + 7.7% Ficoll	24	50

[1] Cultures at 20 C were initiated with 5 larvae, except liver factor-Ficoll medium for which 8 larvae were used; cultures at 23 C were initiated with 8 larvae.

shown in Table 1. Chick embryo extract plus serum was again the best supplement; however, with other supplements populations were smaller than those produced when larvae reared upon *P. terrestris* mycelia had been used as the inoculum.

Development of large populations of *Aphelenchoides* sp. in a medium composed of commercially available ingredients, and establishment of continuous axenic cultures on glass wool columns will facilitate study of nutritional requirements of this nematode. In addition, successful continuous axenic culture of this *Aphelenchoides* sp. should stimulate interest in establishing axenic cultures of other stylet bearing nematodes.

LITERATURE CITED

1. BUECHER, E. J., AND E. L. HANSEN. 1969. Yeast extract as a supplement to chemically defined medium for axenic culture of *Caenorhabditis briggsae*. Experientia 25: 656.
2. BUECHER, E. J., E. L. HANSEN, AND T. GOTTFRIED. 1970. A nematode growth factor from baker's yeast. J. Nematol. 2:93–98.
3. BUECHER, E. J., E. L. HANSEN, AND E. A. YARWOOD. 1966. Ficoll activation of a protein essential for maturation of the free-living nematode *Caenorhabditis briggsae*. Proc. Soc. Exp. Biol. Med. 121:390–393.
4. BUECHER, E. J., G. PEREZ-MENDEZ, AND E. L. HANSEN. 1969. The role of precipitation during activation treatments of growth factor for *Caenorhabditis briggsae*. Proc. Soc. Biol. Med. 132:724–728.
5. HANSEN, E. L., AND W. S. CRYAN. 1966. Continuous axenic culture of free-living nematodes. Nematologica 12:138–142.
6. HANSEN, E. L., E. A. YARWOOD, G. J. JACKSON, AND G. O. POINAR, JR. 1968. Axenic culture of *Neoaplectana carpocapsae* in liquid media. J. Parasitol. 54:1236–1237.
7. MYERS, R. F. 1967. Axenic cultivation of plant parasitic nematodes. Nematologica 13:323.
8. MYERS, R. F. 1968. Nutrient media for plant parasitic nematodes. 1. Axenic cultivation of *Aphelenchoides* sp. Exp. Parasitol. 23: 96–103.
9. YARWOOD, E. A., AND E. L. HANSEN. 1968. Axenic culture of *Pelodera strongyloides* Schneider. J. Parasitol. 54:133–136.