RESEARCH NOTES

Oligidic Medium for Axenic Culture of *Aphelenchoides* sp.[1]

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A diet of 25% chick embryo extract (CEE) plus 10% human serum in *Caenorhabditis briggsae* medium (CbMM) was sufficient to rear the stylet-bearing nematode *Aphelenchoides* sp. (1). The medium, however, is complex and expensive and since Cryan *et al.* (2) demonstrated soya-peptone and yeast extract could be used in place of CbMM, experiments were designed to determine the efficacy of this mixture in combination with CEE for rearing *Aphelenchoides* sp.

Initially, 3 g each of Bacto Soytone (ST, Difco Laboratories, Detroit, Mich. 48200) and of yeast extract (YE, Nutritional Biochemicals Corp., Cleveland, Ohio 44100) were dissolved in 30 ml of distilled water. The solution was adjusted to *p*H 7.0 with 0.1 N KOH and autoclaved at 121 C, for 15 min. According to the manufacturer, CEE 50% (Cat. No. 511, Grand Island Biological Co., Grand Island, N.Y. 14072), is prepared by removing 10-day-old embryos from the egg and thoroughly grinding them in a blender. A calculated amount of GBSS (Gey's balanced salt solution) is added and this is then blended into a homogenous mixture. The material is allowed to extract for a specified time and is then centrifuged, with the supernatant being the extract. No contamination of the CEE has been observed and it does not require additional sterilization. CEE although listed at 50% strength by GIBCO is considered to be full strength, or 100%, when calculating dilutions used in this paper. Medium was compounded by aspectic combination of 3 ml ST-YE mixture (3%), 2.5 ml of CEE (25%) and 3.5 ml of sterilized distilled water; 0.9 ml of this mixture was aseptically dispensed into screw-top vials. Nematodes suspended in 0.1 ml of water were added and the tubes slanted for 15 days at 23 ± 1C. Six replications of each treatment were used in all experiments.

When 27 nematodes were incubated in the above mixture, an average of 686 were recovered and by substituting 15% or 20% CEE, 753 and 831 nematodes, respectively, were recovered. When ST-YE mixture was deleted leaving only 25% CEE, 97 nematodes were recovered; increasing CEE concentration above 25% also caused decreased reproduction.

Since 15% CEE and 3% each of ST and YE were sufficient for continuous cultivation of *Aphelenchoides* sp., axenic stock cultures were established on this medium to supply the nematodes used in further studies.

An experiment was designed to determine the optimum ratio of ST to YE. Table 1 indicates that a diet utilizing 3% ST—2% YE produced the highest population, but that it was not significantly different from either 2% ST—2% YE or 3% ST—1% YE. However, the percent of females was less in the latter treatment than in the former two.

In subsequent experiments, 3% ST—2% YE was held constant and the percent CEE varied. Results are reported in Table 2. The numbers of nematodes recovered grad-

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TABLE 1. Number of *Aphelenchoides* sp. recovered after 15 days at 23 C on 15% CEE and the indicated percentages of Soytone and yeast extract.†

Soytone %	Yeast Extract %	Mean Numbers††	Adult Females %	Mortality %
3	2	1918 a	34	13
2	2	1652 ab	36	12
3	1	1600 ab	19	12
1	2	1458 b	24	15
2	3	1436 b	28	13
0	3	1410 b	26	20
0	2	1394 b	23	12
1	3	1375 b	14	8
3	3	1264 b	16	12
1	1	1060	21	16
0	1	680	19	16
3	0	588	10	17
0	0	46	5	10

† Mean of six replications, each tube originally inoculated with 42 nematodes.

†† Means followed by the same letter are not significantly different at the 5% level of confidence by Tukey's (1953) test of significance (3).

ually increased with increasing amounts of CEE until at 8% concentration and above approximately the same numbers were recovered.

The final formulation of diet chosen was 3% ST and 2% YE, combined with 10% CEE for rearing *Aphelenchoides* sp. This species has now been in continuous axenic culture for the past year.

TABLE 2. Number of *Aphelenchoides* sp. recovered after 15 days at 23 C on 3% Soytone plus 2% yeast extract and the indicated percentages of chick embryo extract (CEE).

Percent CEE	Experiment I† Mean Numbers	Experiment II Mean Numbers
0	128	64
4	847	424
8	1124	702
10	—	784
12	—	726
14	—	690
16	1279	—
20	1274	—
24	1042	—

† Mean of 6 replications and each tube originally inoculated with 53 nematodes in Experiment I and 31 nematodes in Experiment II.

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