Cultivation of *Caenorhabditis briggsae* and *Turbatrix aceti* with Defined Proteins[1]

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Following the report that addition of hemin and cholesterol allowed reproduction of *Caenorhabditis briggsae* in media containing autoclaved bacteria (6), we showed that by addition of hemin certain commercially available proteins could be used successfully in the culture medium (1). We now report that when both hemin and cholesterol are added, certain completely characterized proteins are effective supplements to media for cultivation of *C. briggsae* and *Turbatrix aceti*.

Nematode response was estimated in terms of the time required for maturation of newly hatched larvae inoculated into duplicate tubes containing 0.20 ml of medium, and the population attained at 30 days. An inoculum of three larvae was used for the hermaphroditic *C. briggsae*; thirty larvae were used for the dioecious *T. aceti*, thus increasing the probability of mating. *C. briggsae* was incubated at 20 C and *T. aceti* at 23 C.

C. briggsae chemically-defined maintenance medium (CbMM) (5), compounded at 2× concentration and *p*H 6.0, was obtained from Grand Island Biological Company, Grand Island, New York, and pork insulin (low zinc) from Eli Lilly and Co., Indianapolis, Indiana. The protein from tobacco mosaic virus (TMV) was a generous gift.

Proteins were dissolved in water at approximately 5 mg/ml and the concentration was determined with the Folin-phenol reagent (7). Cholesterol was added by a modification of the technique of Cole and Krusberg (3). The protein solutions were aseptically lyophilized, then suspended in diethyl ether with or without cholesterol. After evaporation at room temperature the samples were reconstituted in water. TMV-protein was precipitated by freezing and thawing prior to lyophilization. Hemin chloride (Calbiochem, Los Angeles, California), 8 μg/ml, was added to the protein-supplemented medium. Water was added to bring the final concentration of CbMM to 1× and proteins to the indicated levels.

In CbMM with TMV-protein and only cholesterol added, nematodes did not mature (Table 1). However, when hemin alone was added, there was a low level of reproduction, and when both hemin and cholesterol were added, large populations developed.

At low concentrations of TMV-protein or insulin, the response of *T. aceti* was greater than that of *C. briggsae*. At low levels these proteins are largely in solution. The response of *C. briggsae* with TMV-protein was increased in some cases by prefreezing the complete medium (Table 1); with insulin, lowering the *p*H to 5.3 had the same effect. Both procedures produced a fine precipitate similar to that formed by activation methods with liver growth factor (2). At higher concentrations the proteins are highly precipitous and appear less favorable. The degree of precipitation is affected also by the levels of hemin and cholesterol and it is possible that with adjustment of these proportions an optimum degree of precipitation could be achieved to obtain the maximum response of both assay nematodes.

Insulin was included in these tests because

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TABLE 1. Growth of *Caenorhabditis briggsae* and *Turbatrix aceti* in chemically defined medium (CbMM) containing hemin, cholesterol, and defined proteins.

Medium			*C. briggsae*		*T. aceti*	
Protein (mg/ml)	Hemin (μg/ml)	Cholesterol (μg/ml)	Maturation Time (days)	Progeny at 30 days	Maturation Time (days)	Progeny at 30 days
TMV Protein						
6.5	8	0	10	30	—[a]	—
6.5	8	5	10	323	12	361
3.1	0	0	nm[b]	0	nm	0
3.1	0	5	nm	0	nm	0
3.1	8	0	12	47	16	24
3.1	8	5	10	462	14	124
0.4	0	5	nm	0	nm	0
0.4	8	5	16	103	11	233
0.4	8	0.6	13	86	11	340
0.1	8	0.5	15	27[c]	15	217
Insulin						
2.5	8	5	15	3	18	9
0.3	8	0.6	25	5	9	702
0.3	8	0.6	11	50[d]	—	—

[a] No test.
[b] nm, non-maturing.
[c] The complete medium was prefrozen to increase precipitation (2).
[d] The *p*H was adjusted to 5.3 to increase precipitation (2).

TMV-protein (4), is commercially available. The response of *T. aceti*, approximately 75% of that with liver growth factor, suggests that this might be a useful system for further investigation. By using insulin as the characterized defined protein, the role of the protein as well as the requirement for components of the basal defined medium could be determined.

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