In Vitro Cell. Dev. Biol.—Animal 34:629–630, September 1998

1071-2690/98 $05.00 + 0.00

Letter to the Editor

CULTURE OF MOSQUITO CELLS IN EAGLE'S MEDIUM

Dear Editor:

Aedes albopictus mosquito cells derived from the ATC-15 line of Singh (1967) have been extensively used in production of somatic cell mutants (Gerenday et al., 1997), expression of transfected genes (Fallon, 1989; Shotkoski and Fallon, 1994), and most recently, isolation and characterization of factors involved in the inducible immune response (Hernandez et al., 1994; Yoshiga et al., 1997). Although *Ae. albopictus* cells are often maintained in "insect" tissue culture media, such as the formulation of Mitsuhashi and Maramorosch (1964) which contains lactalbumin hydrolysate and yeastolate, the ability of mosquito cells to thrive in vertebrate tissue culture media under a standard 5% CO_2 atmosphere was noted in the original description of ATC-15 cells (Singh, 1967) and has facilitated comparative studies between mammalian and mosquito cells.

Apart from the serum component, vertebrate tissue culture media such as Eagle's medium are completely defined, a feature that facilitates development of selective conditions for recovery of somatic cell mutants and use of radioisotopes as metabolic precursors in biochemical studies. In particular, the C7-10 *Ae. albopictus* cell line used in current analyses was derived from cells that had been gradually adapted from the Mitsuhashi and Maramorosch (1964) medium to Eagle's (1959) vertebrate tissue culture medium supplemented with L-glutamine, nonessential amino acids, antibiotics, and 5% fetal bovine serum (Sarver and Stollar, 1977; reviewed in Fallon and Stollar, 1987). Preparation of this medium by combining Earle's salts, essential and nonessential amino acids, vitamins, glutamine, antibiotics, sodium bicarbonate, and fetal bovine serum has been detailed previously (Fallon, 1989). In this letter, we describe a simplified preparation protocol based on the commercially available powdered or liquid formulations shown in Table 1.

In a first application, we used a powdered Eagle's minimal essential medium (MEM) from Sigma Chemical Co. (St. Louis, MO) containing Earle's salts, L-glutamine, and nonessential amino acids (Table 1) as a base. Briefly, 10 g of the powdered Sigma medium was dissolved in 875 ml of distilled water with constant stirring. To arrive at equivalent concentrations of key components, relative to the earlier formulation (Fallon, 1989), additional nonessential amino acids and glutamine, vitamins, D-glucose, antibiotics, and sodium bicarbonate (Table 2) were added, as detailed in Table 3. After adjusting the pH to 6.8 with 1 *N* HCl or 1 *N* NaOH, the volume was adjusted to 1000 mL, and the medium was sterilized by filtration and stored as 95-ml aliquots at 4° C. Prior to use, heat-inactivated fetal bovine serum was added to a final concentration of 5%. When C7-10 cells were maintained in the Sigma-based medium (Table 3), cell growth measured with a Coulter electronic cell counter was indistinguishable from that in medium prepared from components as described previously (Fallon, 1989).

TABLE 1

COMMERCIAL FORMULATIONS AND CATALOG DESCRIPTIONS OF CULTURE MEDIA

Formulation	Description
Powder	Sigma M-0643: Eagle's Minimum Essential Medium (MEM); with Earle's salts, L-glutamine, and nonessential amino acids; without sodium bicarbonate
Powder	GIBCO BRL #61100 (MEM with Earle's salts and L-glutamine; without sodium bicarbonate
Liquid	GIBCO BRL #11095 (1 × MEM with Earle's salts and L-glutamine)
Liquid	GIBCO BRL #51200 (1 × MEM with Earle's salts; without phenol red, without L-glutamine)

TABLE 2

SOURCES AND CATALOG NUMBERS OF SUPPLEMENTS USED IN PREPARING CULTURE MEDIA

Component	Supplier
Distilled water	—
Sodium bicarbonate	(7.5% wt/vol; GIBCO BRL #25080)
MEM nonessential amino acids	(10 m*M* = 100X; GIBCO BRL #11140)
MEM vitamin solution	(100X; GIBCO BRL #11120)
L-glutamine	(200 m*M* = 100X; GIBCO BRL #25030)
Penicillin/Streptomycin	(10,000 U/ml; 10,000 µg/ml; GIBCO BRL #15140)
D(+)glucose (dextrose)	(10% wt/vol; GIBCO BRL #15023; Sigma G-7021)

TABLE 3

ADDITIONS TO MEDIA TO MAKE THEM COMPARABLE TO THOSE USED IN FALLON (1989)

Characteristic or ingredient	Source, catalog number, and amount			
	Sigma M-0643	GIBCO 61100	GIBCO 11095	GIBCO 51200
Weight or volume (prepackaged)	10 g	9.5 g	1 L	1 L
Distilled water	875 ml	875 ml	—	—
Glucose	10 ml	10 ml	10 ml	10 ml
Glutamine	10 ml	10 ml	10 ml	20 ml
Vitamins	10 ml	10 ml	10 ml	10 ml
Nonessential amino acids	10 ml	20 ml	20 ml	20 ml
Antibiotics	10 ml	10 ml	10 ml	10 ml
Sodium bicarbonate	29.3 ml	29.3 ml	—	—

As expected, comparable formulations of powdered or liquid media from GIBCO BRL (Grand Island, NY; Table 1), supplemented as detailed in Table 3, also supported identical growth rates of C7-10 cells. Finally, for studies with the steroid hormone 20-hydroxyecdysone, we have begun to maintain C7-10 cells in a medium formulated without phenol red (Berthois et al., 1986) (GIBCO #51200; Table 1), and containing fetal bovine serum (heat-inactivated at 56° C for 30 min) either untreated or made steroid-free by dextran-charcoal treatment. Particularly with dextran-charcoal-treated serum, growth rates in the phenol red-free medium are reduced and frequent refeeding is required. Because this phenol red-free preparation also lacks glutamine, additional glutamine is added as described in Table 3.

Commercial preparations of culture media are cost-effective and simple to use, save time used in preparation of medium from components, and reduce the possibility of batch-to-batch variation. Because comparison of the many available formulations of vertebrate media is tedious, we anticipate that the updated information provided here will supersede our earlier publication (Fallon, 1989) and facilitate use of mosquito cells by other investigators.

Acknowledgments

This work was supported by grants AI20385 and HD24869 from the National Institutes of Health and by the University of Minnesota Agricultural Experiment Station (publication # 981170005), St. Paul, MN.

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Karen M. Shih
Anna Gerenday
Ann M. Fallon[1]

Department of Entomology
University of Minnesota
1980 Folwell Avenue
St. Paul, Minnesota 55108

(Received 16 March 1998)

[1]*To whom correspondence should be addressed.*