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FISH CELL CULTURE: A NEW CELL LINE FROM *CYNOSCION NEBULOSUS*

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SUMMARY

A new fibroblast-like cell line, CyN, has been developed from muscle tissue of the marine fish *Cynoscion nebulosus* (the spotted weakfish). A subline, CyN-1, was characterized fully and carried through 100 subcultures. CyN-1 is heteroploid and has a modal chromosome number of 49. The line is susceptible to LT-1, lymphocystis, eastern equine encephalitis and vesicular stomatitis viruses. Optimal growth occurred at 25° to 34° C in growth medium adjusted to 0.150 M NaCl.

Key words: marine fish tissue culture.

INTRODUCTION

A cell line (designated CyN) was established from the spotted weakfish (*Cynoscion nebulosus*) by methods previously described (1). Explant cultures were established in 1974 using muscle tissue from the base of the caudal fin of a mature female fish captured the previous day and held on ice in the intervening period.

MATERIALS AND METHODS

Explants were placed in 30-ml plastic flasks (Falcon) in L-15 medium (2) supplemented with 15% fetal bovine serum, 5% human serum, penicillin (100 U per ml), streptomycin (100 μg per ml), Fungizone (0.25 μg per ml), and gentamicin (Schering) (0.1 mg per ml). The final NaCl concentration was adjusted to 0.150 M. The cultures were incubated at 26° C.

Sufficient outgrowth of cells developed from the explants to allow subculturing after 16 days. Subsequently, a total of 100 subcultures (at 26° C) were made; the average interval between the cultures was 1 week (exclusive of freeze-storage). Beginning at passage 80, human serum was omitted from the growth medium and fetal bovine serum reduced to 10%. Split ratios as high as 1:6 were able to be made even though at passage 60 the line had an absolute plating efficiency of only 4% at 26° C. All subcultures were made using a trypsin-versene dispersant (ATV) (3).

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RESULTS

In freeze viability studies, CyN cells at various passage levels were stored in ampuls in liquid nitrogen for periods ranging from 1 week to 3 years. The freeze medium consisted of growth medium containing either 10% glycerol or 5% or 10% dimethylsulfoxide as protectant. Cell survival was 70% to 90% at all passage levels for all storage periods.

Extensive karyological studies were performed on the cell line—the first at passage 1. At this passage, 85% of the cells observed had a chromosome number of 50 (the normal diploid number has not been reported) and all chromosomes were acrocentric. Beginning at passage 25, four sublines of CyN were established (CyN-1, CyN-2, CyN-3 and CyN-4) and maintained under identical conditions through 25 additional subcultures. Further karyological studies were done on all sublines at passages 44 through 50. The four sublines had modal chromosome numbers of 49, 56, 56 and 44, respectively, with no more than 25% of cells having a given modal number. The chromosome distribution of the CyN-1 subline is presented in Table 1. Some metacentric chromosomes and chromosomes with secondary terminal constrictions (satellites) appeared in all sublines by passage 50. Subline CyN-1 was selected for continued subculture, and it is this subline that is currently in passage 100.

CyN-1 is composed of typical fibroblast-like cells. A typical monolayer of these cells is presented in Fig. 1. The species origin of CyN-1 has

TABLE 1

CHROMOSOME DISTRIBUTION IN SUBLINES OF CyN CELLS

Sublines	No. Chromosomes																				Total No. Cells Examined
	29–40	41	42	43	44	45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62–85	
CyN (passage 1)	0[a]	0	0	1	1	0	2	3	5	6	187	8	4	1	0	1	1	0	0	0	0	0	0	220
CyN-1 (passage 44)	7	0	2	5	0	5	4	10	11	12	8	6	3	0	1	0	1	0	0	0	0	0	6	81
CyN-2 (passage 44)	0	0	0	0	0	0	0	1	0	1	4	3	3	3	4	9	13	0	3	0	3	4	11	62
CyN-3 (passage 50)	4	0	0	0	0	0	1	1	1	1	2	0	2	2	1	4	21	3	12	4	4	2	4	69
CyN-4 (passage 50)	6	2	0	1	7	3	1	1	1	0	0	4	3	1	1	0	0	0	0	0	0	0	6	37

[a] Number of cells.

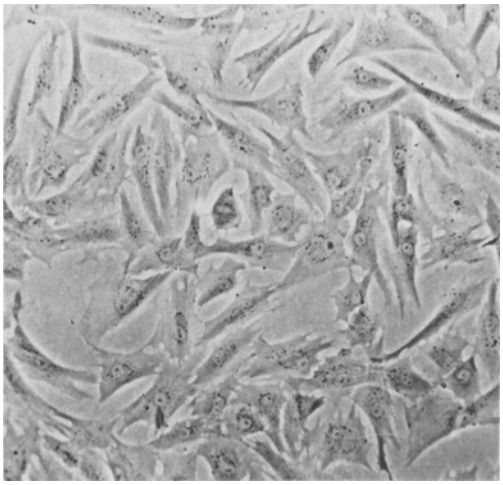

FIG. 1. CyN-1 cells (passage 100). Phase contrast. ×170.

been confirmed by the cytotoxic antibody dye exclusion test (4). Ten percent of CyN-1 cells remained viable following treatment with rabbit antiserum to muscle tissue of *C. nebulosa*, whereas 95% of cells from heterologous species (*Micropogon undulatus*) remained viable. Ninety-eight percent of CyN-1 cells treated with normal rabbit serum remained viable. Sterility tests (5, 6) indicated that the cell line at passage 100 contains no bacterial, fungal or mycoplasmal contaminants.

Numerous growth curves were established for CyN and the CyN-1 subline at various incubation temperatures and at various passage levels. Minimal growth occurred at 18° C and optimal growth occurred at 25° to 34° C. Growth occurred at temperatures as high as 36° C, but cells showed rounding and other signs of distress. Fig. 2 presents typical growth curves at 18° C, 27° C and 36° C for CyN-1 at passage 25.

Virus susceptibility studies were carried out on CyN-1 at passages 25 and 87. Viruses tested included LT-1 (7), lymphocystis (from three sources), eastern equine encephalitis (EEE), vesicular stomatitis (VSV), reovirus (type 2), poliovirus (type 1), vaccinia, coxsackievirus (B3), measles, herpes simplex and channel catfish viruses. Cells were inoculated with 100 $TCID_{50}$ of each virus as determined by titration in appropriate cell lines and incubated at 26° C, 34° C and 36° C. CyN-1 supported significant replication of EEE and VSV (3 log increases over inoculum), and at passage 25 cells infected with these viruses and incubated at 34° and 36° C showed signs of

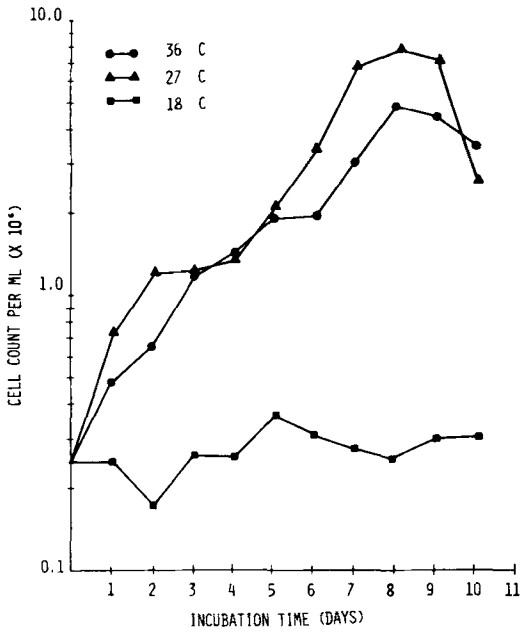

FIG. 2. Growth curves of CyN-1 cells (passage 25) at various temperatures of incubation.

cytopathology. LT-1 virus replicated to high titer and produced extensive cytopathology at all temperatures tested. The line also proved susceptible to lymphocystis virus isolated from lesions of *Bairdiella chrysura* (the silver perch) and *Micropogon undulatus* (the Atlantic croaker) but not to the lymphocystis virus available from the American Type Culture Collection (Leetown stain, isolated originally from *Micropterus salmoides*, the largemouth bass). Cytopathology in lymphocystis virus-infected cells began to develop within 3 days and was characterized by the appearance of very refractile, spindle-shaped cells enlarged to 2 to 3 times normal size. These cells showed intense staining with Giemsa.

DISCUSSION

CyN-1 is easily maintained, grows rapidly and produces monolayers that are very stable. Monolayers can be successfully subcultured after as long as 2 to 3 months at 26° C with a single change of medium. Its viral susceptibilities over a wide range of temperature offer considerable potential for virological studies. This cell line, in passages above 100, is available to all interested investigators.

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