クルマエビのリンパ様器官の初代培養

誌名	日本水産學會誌
ISSN	00215392
著者	伊丹, 利明 青木, 陽一郎 林, 健一
巻/号	55巻12号
掲載ページ	p. 2205-2205
発行年月	1989年12月

Short Paper

In Vitro Maintenance of Cells of Lymphoid Organ in Kuruma Shrimp *Penaeus japonicus*

Toshiaki Itami,* Yōichirō Aoki,* Ken-Ichi Hayashi,* Yan Yu,* and Yukinori Takahashi*

(Received March 30, 1989)

Outbreaks of vibriosis in kuruma shrimp *Penaeus japonicus* have been occurred throughout Japan and caused serious damage in kuruma shrimp culture. Major histopathological change of vibriosis was extensive necrosis by severe bacterial invasion mainly in the lymphoid organ.[1] But a function of the lymphoid organ in defense systems of kuruma shrimp has not been clarified. Therefore, as a preliminary attempt to reveal a function of the organ, maintenace of cells of lymphoid organ *in vitro* has been studied in various kind of media.

The components and their proportions in culture media were modified from Chen's[2] formula and Machii's.[3] One culture medium based on Chen's formula consisted of 45% synthetic medium, 18% calf serum, 27% muscle extract of kuruma shrimp, 10% blood homogenate of *Charybdis japonicus* and 6 g of NaCl in 1 *l* of medium. Muscle extract of kuruma shrimp was prepared from the same method as described by Chen *et al.*[2] Blood of *Charybdis japonicus* was drawn from the heart into syringe and homogenized after clotting. The homogenate was centrifuged at 15,000 rpm for 15 min at 4°C. Supernatant was diluted with the same volume of seawater, absorbed with activated charcoal and filtered with a 0.22 μm pore-sized membrane as described by Chen *et al.*[3] Synthetic media used here were Eagle's Minimum Essential Medium (Flow Laboratories), Leibovitz's L-15 medium (Flow Laboratories), double strength of L-15, RPMI 1640 medium (Whittaker M. A. Bioproducts) and 199 medium (Gibco Laboratories) as in Chen's formula.

Table 1. Evaluation of various media for maintenance of the cells of the lymphoid organ of kuruma shrimp

Medium number	Synthetic medium	Days after inoculation 1	3	5	7	Survival time (days)
1*	PRMI 1640	+	+	−	−	3
2*	L-15	+	+	++	++	38
3*	L-15 (X2)	+	+	++	++	42
4*	Eagle's MEM	+	+	++	++	40
5*	199	+	+++	+++	+++	54
6**	199	+	+++	+++	+++	54
7**	CMRL 1066	+	+++	+++	+++	30

* Composition of medium and balanced salt solution were Chen's formula.
** Composition of medium and balanced salt solution were Machii's formula.
−: Few tissue fragments or cells adhere to the flask surface.
+: A few tissue fragment or cells adhere to the flask surface.
++: Many tissue fragments or cells adhere and the tissue fragments are surrounded by the growth of cells extending outward from the tissue.
+++: Many tissue fragments or cells adhere and the tissue fragments are connected each other by the growth of cells extending outward from the tissue.

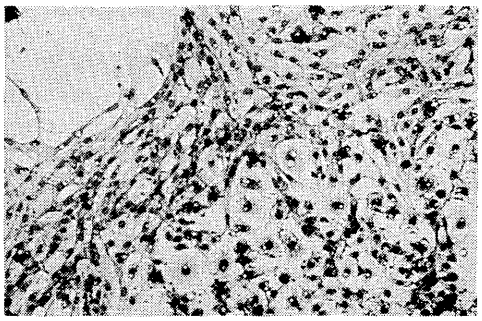

Fig. 1. Primary culture of the lymphoid organ of kuruma shrimp (100×, Giemsa staining). Culture was maintained for 8 days in the medium containing 199 medium.

The other medium according to Machii's formula consisted of 80% synthetic medium, which was either 199 medium or CMRL 1066 medium (Gibco Laboratories), and 20% calf serum plus 0.1 g of lactalbumine hydrolysate, 11 g of NaCl, 0.4 g of KCl, 3.0 g of $MgSO_4 \cdot 7H_2O$, 3.3 g of $MgCl_2 \cdot 6H_2O$, 0.9 g of $CaCl_2$ and 0.05 g of $NaH_2PO_4 \cdot 2H_2O$ in 1 *l* of medium.

The mean body weight of 5 kuruma shrimps used was 35 g. Shrimps were strilized by immersion in Isephor (Ise Chemical Industries Co., LTD) dissolved in seawater for 5 min and wiped with 70% ethanol. The lymphoid organ was removed from the shrimp aseptically and immersed three times in Chen's[2] or Machii's[3] balanced salt solution. Five pairs of rinsed whole lymphoid organ were pooled and minced. Tissue fragments were directly suspended in culture media in plastic culture dish (35×10 mm, Becton Dickinson Labware). The inoculated tissue fragments were incubated at 30°C and were observed daily under an inverted microscope, recorded the relative viability of cells from − to +++. Each culture medium was changed every 5–7 days.

In primary cultures of the lymphoid organ, epithelioid cells and fibroblastic cells were observed (Fig. 1). The effect of a various kind of synthetic medium on cell growth is shown in Table 1. The culture medium consisted of 199 medium or CMRL 1066 medium showed apparently better cell growth of the lymphoid organ than other media in 3–7 days after inoculation. Cells inoculated in the culture media containing 199 medium were maintained for up to 54 days. But a confluent monolayer cell sheet was not observed.

Further study is needed to clarify the function of the lymphoid organ and to establish the cell line derived from the lymphoid organ of kuruma shrimp for isolation of virus from shrimp.

References

1) S. Egusa, Y. Takahashi, T. Itami, and K. Momoyama: *Fish Pathology*, **23**, 59–65 (1988).
2) S. N. Chen, S. C. Chi, G. H. Kou, and I. C. Liao: *Fish Pathology*, **21**, 161–166 (1986).
3) A. Machii, K. T. Wada, M. Awaji, H. K. Nakamura, and S. J. Townsley: in "Invertebrate and fish tissue culture" (ed. by Y. Kuroda, E. Kurstak, and K. Maramorosch), Japan Scientific Societies Press, Tokyo, 1988, pp. 11–14.

* Department of Biology and Aquaculture, Shimonoseki University of Fisheries, Shimonoseki, Yamaguchi 759–65, Japan (伊丹利明, 青木陽一郎, 林 健一, 闔 愚, 高橋幸則: 水産大学校増殖学科).